Institute of Complex Systems (ICS)

Research in the Field of Biophysics and Soft Matter

Interim Report (2009-2011)
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Introduction

Institute of Complex Systems: Research in the Field of Biophysics and Soft Matter

Biophysics and soft matter are concerned with the qualitative and quantitative understanding of structure and dynamics of complex macromolecules and their assemblies at various levels up to living cells. They represent a very dynamic and rapidly growing area of transdisciplinary research for solving fundamental problems and questions at the interface between physics, chemistry, and biology. Knowledge and techniques created by cutting-edge research in this field is essential for sustained rapid progress in biotechnology and life sciences, as well as nanotechnology and material sciences.

The Institute of Complex Systems (ICS) was founded in January 2011 to bundle the disciplinary, methodological, and technological competences in soft matter and biophysics research at Forschungszentrum Jülich (FZJ) under a common roof. The ICS contributes with 8 departments to this research field, namely:

- ICS-1: Neutron Scattering (head: D. Richter)
- ICS-3: Soft Matter (head: J. Dhont)
- ICS-6: Structural Biochemistry (head: D. Willbold)
- ICS-7: Biomechanics (head: R. Merkel)
- ICS-8: Bioelectronics (head: A. Offenhäusser)

The highly multidisciplinary scientific infrastructure of ICS combines medium and large-scale experimental facilities, e.g. neutron scattering and ultra high field NMR spectroscopy, as well as a high-level laboratory science, like state of the art molecular and cell biological laboratories, a synthesis platform for polymers, colloids, and biomaterials together with a large variety of optical imaging and light scattering techniques, that mainly has been developed and built in-house. Furthermore, ICS has access to supercomputing and the Helmholtz Nanoelectronics Facility on the campus of FZJ.

ICS is tightly linked together with three further institutes of Forschungszentrum Jülich: Jülich Centre for Neutron Science (JCNS), Institute of Advanced Simulation (IAS), and Peter Grünberg Institute (PGI). JCNS is dedicated to the operation and development of neutron scattering instruments at national and international neutron sources. IAS targets the development and application of high-performance computing to understand complex systems. PGI focuses on research of electronic systems and phenomena, as well as their applications in information technologies. As three ICS directors substantially contribute with their research activities to the research fields of JCNS, PGI, and IAS, respectively, they are simultaneously directors of ICS and one of the above mentioned
institutes. These directors therefore head the following departments: Neutron Scattering of ICS and JCNS, Theoretical and Soft Matter Biophysics of ICS and IAS and Bioelectronics of ICS and PGI.

ICS conducts the Helmholtz Programme “Macromolecular Systems and Biological Information Processing” in the research field Key Technologies. Furthermore, three ICS departments contribute to a certain extent to one of the Helmholtz Programmes “Research with Photons, Neutrons and Ions” (PNI) and “Fundamentals and Future Information Technology” (FIT) in the research field Key Technologies, as well as “Function and Dysfunction of the Nervous System” (Neuro) in the research field Health.

BioSoft started as a new research programme in the research area Key Technologies within the programme-oriented funding of the Helmholtz Association in the funding period 2010-2014. Within this transdisciplinary research programme theoreticians and experimentalists from different fields of physics, biology, and chemistry are working in close cooperation. BioSoft ultimately aims at qualitative understanding of biological systems. Research performed in this programme is therefore focused on an in-depth biophysical investigation of key phenomena and processes of soft matter and biological systems to obtain a detailed understanding of biological processes from molecules to cells, emphasizing a physical approach. BioSoft comprises three topics where the scientific work is carried out.

Topic 1, Soft Matter Composites, aims at a quantitative and theoretical understanding of structures and mechanisms governing soft matter and related biological compounds. Topic 1 therefore addresses self-assembly and structure formation of soft and biological systems, investigates the flow properties and the effect of external constraints and fields and applies soft matter concepts to understand the complex machinery of biological systems.

Topic 2, Structural Biology, targets the characterization of basic molecular processes in living cells and the investigation of the molecular bases of diseases like AIDS and neurodegenerative disorders. It is devoted to the development and application of methods to investigate precisely three-dimensional structures and molecular mechanisms of biologically and medically relevant macromolecules involved in cellular processes like signalling pathways, protein synthesis, folding, misfolding, and aggregation, as well as protein-protein and protein-ligand interactions.

Topic 3, Physics of the Cell, strives for a comprehensive understanding of cellular information processing from the whole cell level down to molecular details. Topic 3 thereby studies mechanical and neuronal signals as two complementary pathways. Within mechano-signalling basic principles of mechano-sensation and mechano-response are studied. The focus in neuronal signalling is on molecular processes and feedback-loops affecting cellular signalling pathways, as well as fundamental processes of signalling in small designed artificial neuronal networks and in the retina.

Within these topics strong synergy effects are received by these three complementary research fields: Soft matter research aims to understand self-organisation, dynamics, and properties of macromolecular systems in terms of statistical physics concepts. Structural biology and cell biophysics seek to comprehensively understand the functions of biomacromolecules and their dynamic interplay in the living cell. However, major challenges for both fields exist at the mesoscopic length scale between single molecules and entire cell compartments. Decisive, at this length scale, is the behaviour of many, often weakly interacting macromolecules. As this is exactly the domain of soft matter research, major chances are seen for achieving a comprehensive understanding of cells by combining these three research fields. For PhD students and young
scientists the Programme BioSoft offers a broad interdisciplinary training within the framework of its
International Helmholtz Research School “Biophysics and Soft Matter”.
The Institute ICS-1/JCNS-1 performs research on a broad range of soft matter and bio-materials. The goal is always to understand properties and function on the basis of molecular structure and dynamics. Neutron scattering with its ability to reveal the structure and dynamics of large molecules in space and time is the basic method for our investigations. In order to use this technique at its highest potential, tailor made macromolecules, that are deuterated or protonated are synthesized in the chemistry group of the institute. Neutron scattering is complemented by a number of additional techniques that provide further information on mesoscopic or macroscopic scales, such as dielectric spectroscopy, rheology, dynamic and static light scattering, X-ray small angle scattering and other laboratory techniques. The potential of the institute is significantly enhanced by a large number of activities financed by third party funds.

The application and industrial processing of many soft condensed matter systems strongly depend on their rheological properties that are determined by the interactions and motions of the constituent structural units, such as chain molecules, aggregates, colloidal particles and surfactants. Their understanding is one of the great challenges of basic soft condensed matter science and would facilitate molecular design of new materials. Much of our research in polymer physics and soft colloidal systems may be subsumed under this head line. Topics are: hierarchical relaxation of branched polymers, the structure and dynamics of rings, selfhealing polymers, polymer nanocomposites and confinement. Self assembly is an overarching theme in soft matter science. We investigate the formation and equilibrium exchange kinetics in block copolymer micelles and study microemulsion polymer composite systems. The question of confinement near a wall or in nano pores combines fundamental interest with practical relevance such as enhanced oil recovery. Recently we have applied this large knowledge base towards the investigation of the functional dynamics of biomolecules in particular proteins. Using neutron spin echo spectroscopy, such motions maybe identified in space and time on the scale of the protein. In recent studies we found functionally important motions in alkoholdehydrogenase and phosphoglycerate kinase.
Large-scale domain motions of enzymes are often essential for biological function. Phosphoglycerate kinase (PGK) has a widely open domain structure with a hinge near to the active center between the two domains. Applying Neutron Spin Echo Spectroscopy (NSE) and Small Angle Neutron Scattering (SANS) we have investigated the internal domain dynamics. Structural analysis revealed that the holoprotein in solution seems to be more compact as compared to the crystal structure, but would not allow the functionally important phosphoryl transfer between the substrates, if the protein would be static. Domain movements as observed by NSE facilitate a close encounter of the key residues in the active center to build the active configuration. The observed dynamics shows that the protein has the flexibility to allow fluctuations and displacements that enable the function of the protein.

Proteins, and in particular multidomain proteins, share a structural complexity, which is also reflected in a complex dynamical behavior. A complete understanding of these biomolecules is possible only if we know the three-dimensional structures and their dynamical properties. Besides rather unspecific and fast stochastic local fluctuations on the picosecond timescale, large-scale rearrangements and movements of domains relative to each other have been investigated with various experimental and theoretical techniques, in particular for larger proteins with two or more domains. These domain movements often are directly related to numerous protein functions. The experimental elucidation of such motions is therefore an important element for our understanding of the underlying functional mechanisms.


SANS and neutron spin echo spectroscopy (NSE) together reveal information about the characteristic length- and timescales of protein motions. A configurational change due to substrate binding is evidenced by SANS measurements, showing thereby that solution and crystal structure are somewhat different, but both would not allow the functionally important phosphoryl transfer between the substrates, if the protein would be static.

NSE observes the Brownian dynamics of the protein. Large scale domain fluctuation dynamics was revealed additionally to the expected rotational and translational diffusion contribution. We found internal dynamics with a relaxation time around 60ns for PGK without substrate and a faster relaxation of 30ns and smaller amplitude if the substrates ATP and 3PG were bound to the protein. The found Q dependence of the
additional amplitude in the intermediate scattering function was compared to the displacement patterns of low frequency elastic normal modes as shown in figure 2. The amplitude is small at small Q, increasing to large Q with a plateau for PGK without substrate. With substrate the amplitude is smaller. The low frequency normal modes describe displacement patterns with the weakest part of the protein at places with largest deformation allowing large domain fluctuations. The weakest part of PGK is the hinge region shown in figure 1. The displacements along the normal mode coordinates describe our experimental results reasonably well as shown in figure 2. For the functional relevance of the observed PGK dynamics it is of particular interest whether the amplitudes of thermal fluctuations, as described in terms of the lowest normal modes, are large enough to facilitate the phosphoryl group transfer between the two substrates within the gap between the main domains. The transferred phosphoryl group is stabilized in trigonal bipyramidal configuration formed by the backbone nitrogen of Gly-376 and Gly-399 and the second nitrogen of the guanidinium group for Arg-39 (see figure 3). The binding of the substrate closes the cleft and reduces the distance of the important atoms. These distances are still too large in the closed conformation and would not allow the transfer of the phosphoryl group between the two substrates in a static protein structure. In particular the domain movements facilitate a closer encounter of the key residues/atoms in the active center to build the active configuration. Figure 3 shows the reduction of the distance between the main residues, which stabilize the transfer.

The observed dynamics shows, that the protein has the flexibility to allow fluctuations and displacements that seem to enable the function of the protein. Moreover, the presence of the substrates increases the rigidity, which is deduced from a faster dynamics with smaller amplitude. The catalytic mechanism of substrate binding and chemical activity is here divided in a part for substrate binding corresponding to the look and key model. An assumed induced fit - as the closing of the cleft due to the binding - is here not enough to allow activity, even if a more compact structure is observed as it was also measured in other studies. Only the here observed domain fluctuations allow the complete closing of the cleft to reach the catalytic configuration. This shows that dynamic processes have an important contribution to the functionality of proteins.

Dynamics of poly(ethylene oxide) confined in AAO nanopores

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Dynamics of poly(ethylene oxide) confined in cylindrical nanopores of anodic aluminium oxide was studied by neutron scattering. Local (segmental) dynamics has been investigated by time-of-flight and backscattering techniques. Rouse-like dynamics indistinguishable from that in the bulk within the uncertainties has been revealed in the low-Q limit. In the high-Q limit a slowing down of the dynamics with respect to the bulk behaviour evidences an effect of confinement. Measurements of the single-chain dynamics at larger time scale by neutron-spin echo demonstrate the first experimental microscopic evidence of the dilution of the entanglement density in a polymer melt under strong confinement. We discuss also the slowing down of the dynamics in the intermediate regime as compared to bulk polymer.

Effects of a solid surface on the structure and dynamics of polymers are a heavily studied subject nowadays [1]. Understanding the structure and dynamics of polymers closely adjusted to the confining surfaces (interface) and in the intermediate phase between the interface and the bulk phase (interphase) is important for applications in nanotechnology such as coatings for electrical devices, lubricants, and polymer nanocomposite materials. On the other side, investigation of the dynamics of polymer chains confined in a system of artificial obstacles allows to elucidate details of the tube hypothesis, a main ingredient for the theory of polymer rheology and dynamics. We aimed to explore the influence of the solid surface (confinement) on polymer dynamics at different time/length scale and the impact of possible interaction between the polymer and pore walls.

Well ordered nanoporous anodic aluminium oxide (AAO) templates have been used as a confining matrix. A two step anodization procedure proposed by Masuda et al. leads to the formation of domains with a regular hexagonal distribution of the cylindrical pores due to self-organization processes [H. Masuda, K. Fukuda, Science 268, 1466 (1995)].

The local dynamics of poly(ethylene oxide) (PEO) confined in anodic alumina oxide (AAO) nanopores has been investigated using backscattering and time-of-flight spectrometers [2]. These instruments allowed to explore the time window from picoseconds to a few nanoseconds and values of momentum transfer \( q = \frac{|k|}{\lambda} \) in the interval from 0.2 Å⁻¹ < \( Q < 1.9 \) Å⁻¹ corresponding roughly to the length scales from some nanometers down to tenth of nanometers. Moreover, by exploring two different sample orientations the identification of the components of motions parallel and perpendicular to the pore axis was possible. The obtained results have been compared with those for PEO in bulk at the same temperature.

We found the dynamics of PEO virtually indistinguishable from the bulk in the low Q-range showing no influence of the confinement on polymer dynamics [2]. Around \( Q = 1.4 \) Å⁻¹ an anisotropic slowing down has been observed under confinement. This slowing down is more pronounced in the direction perpendicular to the pore axis. This effect was attributed to the attractive interactions between pore walls and polymer segments within a ~1 nm layer. The important finding is that neutron scattering is sensitive to the surface interactions between polymer segment and pore walls.
Furthermore, we have applied NSE spectroscopy to directly observe at a molecular level the single-chain dynamics of a polymer confined in AAO pores with well-defined geometry [3]. These experiments provide the effective tube diameter and thereby the entanglement density in the confined polymer, which is not accessible by other techniques.

Two samples (PEO confined in AAO nanopores) were prepared with different ratios of the end-to-end distance and the pore diameter \(R_{ee}/d_{pore}\) corresponding to a weak and strong confinement. In both cases (Fig.2) at long times the data show finite plateaus reflecting confinement effects due to topological interactions with the surrounding chains. This plateau is typical for the polymer chains longer than the characteristic entanglement distance. The results could be well described by the reptation model [M. Doi and S. Edwards, The Theory of Polymer Dynamics (Clarendon, Oxford, 1986)] with an effective tube diameter as a model parameter.

First, we notice that for weakly confined PEO the bulk values for the effective tube diameter are reproduced (Fig.2a). On the other side, for a strongly confined polymer a 15% increase of the tube diameter was found (Fig.2b). The last result constitutes the first experimental microscopic evidence of the dilution of the entanglement density in a polymer melt under strong confinement.

We emphasize also the slowing down of a dynamics in the intermediate regime as compared to bulk polymer (Fig.2(a,b)). At the same time the segmental friction remains unchanged. This result suggests that certain dynamical modes of the polymer chain are affected by polymer-wall interactions and/or modified confinement in the interphase.

In conclusion, the investigation of the local dynamics demonstrates that only the surface layer is affected by confinement, taking into account attractive interaction between AAO surface and poly(ethylene oxide). At long times in the plateau region, for \(R_{ee} > d_{pore}\) we found an expanded entanglement network. In the intermediate time the Rouse spectrum obviously is affected by the confinement.

![FIG. 2: Single chain dynamic structure factor of the weakly confined chains \(M = 22.7 \text{ kg/mol}\) (a) and the strongly confined chains \(M = 443 \text{ kg/mol}\) (b) at the \(Q\)-values indicated. Dashed lines represent De Gennes curves fitting to the bulk PEO [4] with a tube diameter value of 52.6 Å. Solid lines in (b) are fits by De Gennes theory using bulk parameters, yielding a tube diameter value of 60.3 Å. The figure clearly demonstrates an increase of the tube diameter and a decrease of the entanglement density respectively in confinement as compared to the bulk polymer.](image_url)


Structure and dynamics of polymeric rings by neutron scattering

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We present the first microscopic approach to the relaxation mechanisms in ring polymers by means of Small Angle Neutron Scattering (SANS) and Neutron Spin Echo Spectroscopy (NSE). Experiments on Polyethylene oxide (PEO) rings ($M_w$=M₉) show a significantly more compact structure compared to the linear counterpart with same molecular weight. Furthermore, it could be shown from the combination with rheological measurements that both, the squared radii of gyration as well as the viscosities for the rings and the linear chains differ by a factor of ~2. Whereas this is in agreement with theoretical predictions, the - by the same factor - faster center of mass diffusion of the rings as measured by NSE is unexpected. NSE on larger rings ($M_w$>M₉) allowed to tackle also the segmental dynamics.

Relaxation processes in polymer systems are strongly dominated by the presence of chain ends, an observation that holds in particular for branched architectural polymers but nonetheless also for simple linear chains. Buzzword are arm retraction, hierarchical relaxation and path length fluctuations. The recently regained academic interest in polymeric rings, in which chain ends are fully lacking, is strongly related to today’s achievements in synthesizing almost defect-free model ring systems. Their structural and dynamical properties are at present one of the most attractive subjects in the quest to unravel polymer chain dynamics.

The intriguing question how cyclic polymers relax without contribution from chain ends is not yet solved. Here, Neutron scattering is of utmost importance to understand the underlying mechanisms from both structural and dynamic point of interest. Therefore the dynamics of two PEO ring polymers was studied for the first time by Neutron Spin-Echo Spectroscopy and structurally sustained by their form factor from Small Angle Neutron Scattering. Two molecular weights, $M_w$ = 1.8 kg/mol and $M_w$ = 5.3 kg/mol were studied which in the linear case would correspond to a non-entangled linear Rouse chain ($M_w$≈$M_e$, the entanglement molecular weight) and an only slightly-entangled chain, respectively. SANS evaluations on isotopic blends that consist only of ultra-pure architectures due to special separation techniques and accounting for the finite size and Gaussian statistics yielded an unrivalled comparison between linear and closed chains. Additionally, macroscopic measurements of the zero-shear viscosity at the same temperature were performed. Both experiments proved the extreme purity of the systems and showed that the squared radii of gyration ($R_G$) of linear-to-ring chains as well as the ratio of Newtonian viscosities ($\eta_0$) is 2, as expected from considerations for the ring closure and Rouse theory from the dynamical side.

**FIG. 1.** SANS data on the linear (circles) and ring (squares) melts for both molecular weights. The inset shows a Kratky plot for the smaller molecular weight case only.

Figure 1 shows the obtained SANS scattering profiles of the linear and the ring polymer for both
The respective form factors are well described with a multivariate Gaussian distribution approach while accounting for the closure relation. This allowed to extract the respective $R_G$ (Small rings: $R_G = 9.9 \, \text{Å}$, linear polymer, $R_G = 15 \, \text{Å}$; Larger rings: $R_G = 15 \, \text{Å}$, linear polymer, $R_G = 23 \, \text{Å}$). A quantitative analysis in the $Q R_G > 1$ region shows qualitatively distinct $Q$-dependences for both systems. Whereas the polymers effectively agree with Gaussian chain statistics for short and longer chains, the Kratky plot shown in the inset of Fig. 1 illustrates the different architecture of rings unequivocally. The shallow peak developing in the plot is indicative for an increased compact structure. From the $Q$-dependence one proves that ring chain dimension scales like $\sim N^{0.4}$ instead of $N^{0.5}$ for linear chains.

![Figure 2: S(Q; t) vs. t for the ring (squares) and linear (circles) at the lowest Q value ($Q = 0.05 \, \text{Å}^{-1}$) for both $M_w$. Solid lines are fits to the Rouse diffusion. Since for the linear chain with $M_w = 5 \, \text{kg/mol}$ and $M_w = 20 \, \text{kg/mol}$, the center-of-mass diffusion is an explicit violation of the Rouse model.](image)

In summary we have presented a first-time-ever combination of NSE and SANS results on ideally pure model ring polymers [1] which confirmed that i) cyclic polymers are expected to be more compact in the melt than the corresponding linear chains, ii) $R_G$ and the viscosity of the ring are both 2 times smaller than of the linear in the unentangled case (the larger ring to be completed), iii) the diffusion of rings is unexpectedly a factor of 2 faster than the linear reference and iv) for larger rings the modified Rouse model describes well the full $Q$ range of the coherent dynamic structure factor.

The next challenge is thus the further exploration of model rings with molecular weights well in the entanglement regime to address the question if rings rephase at all and how self-interpenetration influences the dynamic behaviour. Additionally, a better understanding of the mutual interaction of linear chains and ring polymers and of the implications for the respective dynamics of both components as was observed experimentally already on Polystyrene rings [2] remains a still difficult task to pursue.


Unified description of dielectric and viscoelastic long chain relaxation

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We present a new approach to a unified description of the dielectric end-to-end vector and the viscoelastic stress relaxation of entangled homopolymer melts in terms of the tube model.

Our comparison and quantitative analysis of carefully recorded viscoelastic and dielectric relaxation spectra revealed that the dielectric mode distribution is insensitive to the thermal constraint release process within experimental accuracy. The general validity was demonstrated on monodisperse polyisoprene (PI) for a wide range of molecular weights and on poly(butylene oxide) (PBO).

The full relaxation of long flexible linear polymer chains occurs on time scales encompassing many orders of magnitude ranging from picoseconds to seconds due to their tremendous configurational freedom. While the very fast local dynamics of the constituent monomeric units naturally depend on the specific chemistry, the overall global chain motion, which ultimately defines the macroscopic properties of real melts, obeys universal behaviour. Above the critical molecular weight $M_c$, polymer chains mutually interpenetrate and entangle, thereby severely restricting the possible chain motions to a laterally confined tube-like region as depicted in Fig 1.

![FIG. 1: In the tube model the motional freedom of the test chain (black) is constrained to the tube-like region (grey), which is formed by the entangledments of neighboring chains (red).](image)

In the most successful so-called tube model, originally developed by deGennes and Doi-Edwards, the confined motion was first modeled in a mean-field approach as a one-dimensional diffusion along the tube profile known as reptation.

In addition, two refinements have to be included to account for the less confined dynamics near the chain ends and the mutual equilibration by the equal motion of the surrounding chains. The former correction, known as contour length fluctuations (CLF), shortens the longest relaxation time needed for the chain to disentangle, i.e. escaping the tube. The latter one describes a softening of the tube due to constraint release (CR) and similarly accelerates the terminal relaxation. However, the exact time evolution equation for the coupled single- and many-chain motion yet remains a challenge for theoreticians as well as for state-of-the-art computer simulations.

On the experimental side these numerous relaxation phenomena are well accessible by the use of broadband measurement techniques like dynamic mechanical experiments, NMR or dielectric spectroscopy. However, the interpretation of the measured relaxation functions in terms of the tube model is not straightforward and is up-to-present an ongoing matter of discussion. For instance in the case of polyisoprene (PI), it is well known that both the dielectric and the mechanical spectroscopy detect the same global chain motion, yet, the measured frequency-dependent normalized viscoelastic and dielectric relaxation functions of entangled chains do not superimpose on each other [H. Watanabe, Macromol. Rapid Commun. 2001, 22, 127-75].

Taking into account that both experiments involve fundamentally different correlation functions, we proposed a new straightforward approach to concurrently describe the viscoelastic and dielectric relaxation of long linear chains by a single common theory using the same set of molecular parameters. It is based on the Likhtman-McLeish (LM) theory, which approximately solves the tube-model problem for viscoelastic stress relaxation by assuming that the many-chain motion (i.e. CR) is independent but self-consistently determined by the single chain motion (reptation, CLF) with an additional free “strength” parameter $c_{\text{f}}$. [A.E.Likhtman,T.C.R.McLeish, Macromolecules, 2002, 35, 6332-6343]. The full definition of our model including the formulas is given in our publication. [1].

In order to test our model we carefully recorded viscoelastic and dielectric data of a series of nearly monodisperse entangled polyisoprene samples over a
A wide range of molecular weights and on poly(butylene oxide) under identical conditions. All samples were synthesized and well characterized at the Forschungszentrum Jülich. The details on the experimental setup and on the data analysis can be found in ref [1].

Fig. 2 directly compares the measured normalized viscoelastic (top) and dielectric (bottom) spectra at the common reference temperature of 25°C. The thin dashed lines clearly indicate that the respective peak frequencies, which correspond to the inverse of the longest relaxation time due to reptation, do not coincide between both experiments. Moreover, the high-frequency side of the dielectric data all show a uniform powerlaw with a slope of -1/4 in contrast to the viscoelastic data. These apparent discrepancies reflect the differences in the respective correlation functions. While the viscoelastic stress relaxation is given by the isochronal orientational anisotropy of bond vectors S(t) along the chain, the dielectric normal mode relaxation directly reveals the end-to-end vector correlation function \( <\hat{E}(t) \hat{E}(0)> \) of the chain.

The black lines in Fig. 2 follow our proposed common model description based on the Likthman-McLeish theory using identical sets of molecular tube model parameters \( Z \) (number of entanglements) and \( \tau_e \) (Rouse time of an entanglement segment) but different strengths for the CR mechanism. While the fits to the viscoelastic data yield a finite value for \( c_\nu \) around 0.5, the dielectric data are modelled without the CR motion (by fixing \( c_\nu=0 \)). The remarkable agreement between the experimental data and our model description clearly evidences that the LM single-chain solution fully accounts for all the features of the dielectric end-to-end vector relaxation.

From our experimental results we conclude that the dielectric normal mode relaxation is insensitive to the constraint release mechanism. Hence, it allows an accurate observation and characterization of the pure single chain entanglement dynamics. Furthermore, we tested our model on a chemically and structurally different polymer, poly(butylene oxide) (PBO). Again, we were able to get a very good common description of viscoelastic and dielectric spectra, which demonstrates the universality of entanglement dynamics.

With our work we would like to stimulate further experimental and in particular theoretical work in order to verify our proposed model and to enhance the understanding on the competition of reptation with contour-length-fluctuations and the constraint-release mechanisms.

Conformation of polymer chains in the presence of non-attractive nanoparticles

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Polymer nanocomposites constitute a very important material class because of the wide range of tunability of their mechanical properties. A prerequisite for an enhanced understanding of the underlying microscopic mechanisms which lead to these unique properties is the knowledge of the polymer conformation in the presence of nanoparticles. We used small angle neutron scattering (SANS) to investigate the influence of the particles on the prevailing polymer conformations in order to resolve this issue in a model system with non-attractive polymer-particle interactions.

Reinforcement of polymers by inorganic particles has been known and used for a long time. Nevertheless a detailed molecular understanding of the microscopic mechanisms is still a challenge. With the availability of surface-modified nanosized fillers the research on well-defined polymer nanocomposites was greatly enhanced. In particular, attention has been dedicated to the investigation of the polymer conformation in the presence of nanoparticles. Unfortunately, existing experimental results mostly apply to specific polymer-particle systems instead of stating general physical implications.

For this reason we have chosen a “simple” model system in order to gain basic insights into the behaviour of polymers in the presence of nanoparticles. “Simple” in this case refers to the choice of constituents of the composite. As polymeric component poly(ethylene-alt-propylene) (PEP) was chosen, being a linear, hydrophobic polymer that has undergone thorough investigations in the past in the unfilled state. Two different molecular weights \( M_1 \approx 3 \mathrm{kg/mol} \) (PEP3k) and \( M_2 \approx 50 \mathrm{kg/mol} \) (PEP50k) with corresponding radii of gyration \( R_{g,1} \approx 2 \ \text{nm} \) resp. \( R_{g,2} \approx 8 \ \text{nm} \) were used in order to study the dependence on relative polymer-filler sizes. As nanoparticle component silica particles with hydrophobic surface were employed. Due to this indifferent hydrophobic particle surface, the resulting nanocomposite system features non-attractive polymer-particle interactions. Note, however, that it will be necessary to distinguish between intrinsic properties of the components and structural changes in the sample through the presence of the particles. This is also sketched in figure 1. The particles have an average radius \( r \approx 8 \ \text{nm} \). In this way, the regimes \( R_{g,1}/r << 1 \) and \( R_{g,2}/r \approx 1 \) were realized i.e. varying degree of confinement.

Whereas this radius ratio \( R_{g}/r \) is fixed in the experiments, the average particle-particle distance in a given composite varies strongly with the particle volume fraction \( \Phi_{\text{part}} \).

Fig. 1 Schematic sketch of the model system, consisting of core-shell nanoparticles and protonated (red) as well as deuterated (blue) polymer chains.

Nanocomposites at different filler fractions \( \Phi_{\text{part}} \) were prepared and measured by small angle neutron scattering (SANS) at the KWS-2 instrument in Garching (\( T = 150^\circ \text{C} \)). In order to access the polymer structure in the presence of the particles, the average scattering length density \( \rho_{\text{part}} \) of the latter is required for the appropriate choice of the ratio of protonated polymer (hPEP) versus deuterated polymer (dPEP) in the final samples. Therefore a thorough contrast matching study was performed in solution and revealed a core-shell-like structure of the particles, which had been neglected in literature so far. The final samples were then prepared such that the average polymer scattering length density \( \rho_{\text{hPEP}} \) matched the average \( \rho_{\text{part}} \). This fact excluded simultaneous contrast matching of both core and shell in the full Q-range. The resulting scattering data normalized to the polymer fraction (1-\( \Phi \)) are shown in figure 2 for both PEP molecular weights.

We will first address the regime of high Q, where no particle contributions to the scattering signal are visible and information about the polymer structure can be directly extracted. In this regime the unfilled polymer melts exhibit a constant slope characteristic for the PEP random walk chain statistics. The Q² dependence as seen in figure 2(b) for the PEP50k chains is characteristic of Gaussian statistics. The PEP3k composite data in figure 2(a), on the other hand, differ from that and follow roughly \( Q^{1.5} \), a result which could be explained at first glance through excluded volume interactions in the shortest PEP polymer.
This apparent deviation from ideal chain behaviour, however, is due to the finite length of the chain. The discrete, short-chain description of the Debye function is in full agreement with the data, i.e., the chain statistics on the segmental scale is not perturbed.

Furthermore, in the high Q regime the scattering intensities of two different nanocomposite samples 1 and 2 can be related to the statistical segment length \( \xi \) from comparing the respective plateau heights in a Kratky representation. Thus, coincidence of the scattering data normalized to the polymer volume fraction, as shown in figure 2(a) and inset, hints to an unchanged radius of gyration \( R_g(\Phi) = R_g(\Phi = 0) \) in the case of the short PEP3k chains. In the case of the longer PEP50k chains, the ratio of \( R_g(\Phi)/R_g(\Phi = 0) \) (cf. inset of figure 2(b)) shows that \( R_g(\Phi) \) slightly decreases with increasing filler content \( \Phi \). The minimal value is reached at \( \Phi = 0.50 \approx 0.89 R_g(\Phi = 0) \).

After the analysis of the high Q regime where particle scattering could be safely neglected, the whole experimental Q range can be reconsidered. Both in the PEP3k and PEP50k nanocomposites (figure 2(a) and (b)) a pronounced scattering peak evolves around \( Q_{\text{peak}} \approx 0.04 \text{ Å}^{-1} \). The peak height increases with increasing filler fraction \( \Phi \), whereas its position remains unchanged and corresponds to a dominant correlation length \( \xi = 2\pi/Q_{\text{peak}} \approx 16.0 \text{ nm} \). This is exactly the average nanoparticle diameter. In an ancillary SANS measurement at a high concentration of nanoparticles in toluene, an identical scattering peak was found. This information enabled us to quantitatively split the nanocomposite data from figure 2 into a independent contribution from particles and from polymer according to

\[
I_{\text{total}}(\Phi, Q) = I_{\text{part}}(\Phi, Q) + I_{\text{pol}}(\Phi, Q)
\]

Rescaling for the different contrast situation, this allows to isolate the polymer intensity from the total intensity. The so obtained results for \( I_{\text{pol}}(\Phi, Q) \) of the PEP3k composites are depicted in figure 3 for different \( \Phi \). Obviously the polymer scattering data and thus also the underlying polymer structure is not affected at all by the addition of the nanoparticles in this case.

The same procedure was also applied to the PEP50k nanocomposites. In that case, however, a scattering peak remained also in \( I_{\text{pol}}(\Phi, Q) \), indicative for a structural change in the polymer correlation. It may be either attributed to interstitial voids in the nanocomposites, which cannot be accessed by polymer chains, or eventually to new polymer density fluctuations, which could be induced by the presence of the nanoparticles. In particular, a disturbance of chain packing near particle surfaces is able to qualitatively explain the observations. The details of the precedent analysis can be found in ref. [1]. This work triggered forth-coming studies using Neutron Spin Echo spectroscopy to obtain the dynamic coherent single chain structure factor which measures the dynamics at the presently studied length scales.

In summary, the SANS experiments on our model system for non-attractive polymer nanocomposites have revealed the great relevance of the choice of relative polymer and particle length scales. For the PEP3k chains with \( R_g/r \ll 1 \), no changes were detected at all. On the contrary, in the PEP50k composites with \( R_g/r \approx 1 \) the particle presence clearly affected the polymer conformations. There the radius of gyration decreased with increasing \( \Phi \), and a scattering peak indicative of polymer density correlations or impenetrable voids in the sample was witnessed.

Ultrasoft colloid/polymer mixtures – making complex fluids look simple

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Binary mixtures of ultrasoft colloids and linear polymer chains were investigated by small-angle neutron scattering and liquid state theory. We show that experimental data can be described by employing recently developed effective interactions between the colloid and the polymer chains, in which both components are modeled as point particles in a coarse-grained approach, in which the monomers have been traced out. Quantitative, parameter-free agreement between experiment and theory for the pair correlations, the phase behavior and the concentration dependence of the interaction length is achieved.

Hard spheres or hard spheres with added linear polymer chains have been established in the past as model systems to investigate on a fundamental level the effective interactions and phase behaviour of soft matter. Building on these simple models, great advances have been made in the study of colloidal systems in general [K. N. Pham et al., Science 296 (2002) 104]. Mixtures of soft particles offer a much higher versatility with respect to their hard counterparts, both in terms of structural and rheological properties and of effective interactions [J. Mattson et al., Nature 462, (2009) 83]. However, an accurate comparison between theoretical predictions and experimental results for structural correlations in soft mixtures has taken much longer to come forward.

Our new theoretical approach eliminates systematically the rapidly moving degrees of freedom and focuses on the relevant slow degrees of freedom, a time-consuming and challenging task. Each complex macromolecule is replaced with a sphere of the appropriate size as schematically shown in Fig. 1. The challenge involves integrating the degrees of freedom that have been eliminated in the simplified systems as averages so that the characteristics of the substances are retained.

As experimental model system we study mixtures of star-like micelles (ultra-soft colloids) and linear polymer chains and provide a systematic and quantitative characterisation of structure factors and phase behaviour in terms of effective interactions. By combining small-angle neutron scattering (SANS) and liquid state theory, we measure and model the correlations between star-like micelles and linear chains. SANS measurements in core contrast allow a direct determination of experimental structure factors, \( S(Q) \), providing the basis for a comparison with the recently-developed theory, in which both components are modelled as point particles in a coarse-grained approach that traces out the monomeric degrees of freedom.

**FIG. 1:** Theory uses "coarse grained spheres" (bottom) to replace the complex macromolecules of the mixture of star polymers (yellow and blue) and linear polymers (red) shown on the top. That the simplified system retains all characteristics of the original substances was quantitatively validated by small angle neutron scattering experiments at D11, ILL.
A direct comparison without any adjustable parameters, i.e., using the quantities directly given by experiments, provides a very good agreement between experiment and theory for structure factors, phase behaviour and concentration dependence of the interaction length. The comparison is done for a broad range of concentrations and it brings forward the influence of the added chains on the larger star polymers.

Fig. 2 shows SANS intensities \( I(Q) \) as measured at D11 for selected concentrations below and around the overlap concentration \( c^* \approx 3\% \) total polymer volume fraction. To compare with theory, we set the interaction range to be given by the typical star size at low concentrations and above \( c^* \) we varied it according to well-established scaling laws; thus, the theory contained no free adjustable parameters. The agreement between experiment and theory for all other concentrations is very convincing. Position, height and width of first and second order peak of liquid-like experimental structure factors are indeed nicely described by theory. For direct comparison, theoretical \( S(Q) \) were multiplied by the experimental form factor \( P(Q) \) and convoluted with the instrumental resolution function. The inset in Fig. 2 demonstrates the strong weakening of the peak of the star-like micelle scattering intensity upon increasing the chain density at fixed star density.

To strengthen our quantitative comparison between experiment and theory, we also consider the phase behaviour of the system. Indeed, while \( S(Q) \) is only slightly affected by \( \xi = R^c_s / R^s_g \) with \( R^c_s \) being the radius of gyration of the linear chain and \( R^s_g \) the radius of gyration of the ultra-soft colloid varying the size ratio, the phase behaviour can be sensitively altered by a small change in the same. Therefore, the agreement in phase behaviour provides an additional consistency check to narrow down the values of \( \sigma_c, \sigma_s \) and \( \xi \) and to establish the correctness of the effective interactions that we have adopted. Experimentally, for all samples under investigation no phase separation was observed, and hence they all lie in the one-phase region, in agreement with the theoretical results for \( \xi = 0.30 \). In addition, we investigated the rheological properties of all samples by means of both steady and oscillatory shear measurements and find a transition to a solid (glassy) state around 29%. Indeed, the rheological properties of all investigated samples are in accordance with the theoretical phase diagram.

In summary, we have described structural and phase behaviour of binary mixtures of ultrasoft colloids and linear polymers. By combining rheology, small angle neutron scattering and liquid state theory we offer robust experimental evidence to the accuracy of the theoretical coarse-graining procedure. Without any adjustable parameter we find quantitative agreement between experiments and theory in a wide range of concentrations. Our work provides a comprehensive characterization of soft binary mixtures in terms of effective potentials and is therefore a successful benchmark in the study of complex soft matter systems [1].

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The Institute “Theoretical Soft Matter and Biophysics” investigates the structure and dynamics of macromolecular, supramolecular, and mesoscopic systems. This is an interdisciplinary research area, which encompasses statistical physics, materials science, chemistry, and biology. Our systems of interest include polymer solutions and melts, colloidal suspensions, amphiphilic systems, membranes, vesicles, and cells. In particular, composite systems are studied, ranging from colloids in polymer solutions to mixtures of surfactants and amphiphilic block copolymers.

A major focus is the hydrodynamic behaviour of complex fluids and biological systems, both in equilibrium and under flow conditions. The topics range from the rheology of star-polymer solutions to the flow behavior of blood and the dynamics of synthetic and biological microswimmers.

At ICS-2, a large variety of methods are applied. In fact, a combination of analytical and numerical methods is often required to successfully characterize the properties of these complex systems. In particular, simulation methods (Monte Carlo, molecular dynamics), mesoscale hydrodynamic simulation techniques (Multi-Particle Collision Dynamics, Dissipative Particle Dynamics), field theory, and perturbation theory are employed. Since the building blocks of soft matter systems often contain a large number of molecules, “simplified” mesoscale modeling is typically required, which is then linked to the molecular architecture.
Research Highlights

Friction and rheology of star polymers in solution

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Star polymers are interesting model systems for soft and ultra-soft colloids, because their properties can almost continuously be changed from that of flexible linear polymers to spherical particles with very soft pair interactions and ultimately almost hard spheres. The friction between star polymers depends on the drag velocity; for large drag velocities, the interaction is found to change from steric repulsion to apparent attraction. This behavior has important consequences for the rheology of semi-dilute and dense star-polymer solutions.

Introduction

Friction between macroscopic bodies has been studied for centuries. More recently, the microscopic processes responsible for friction and lubrication have come into focus, and have received broad attention. Much less is known, however, about the non-equilibrium behavior of mesoscopic, deformable particles sliding past each other under the effect of external forces. Such particles are ubiquitous in complex fluids and biofluids; droplets, capsules, vesicles, ultra-soft colloids and polymers are able to undergo large shape changes and have relaxation times comparable to the time scale of external perturbations. Hence, (dense) suspensions of such objects exhibit a much more complex non-equilibrium behavior than Newtonian liquids. This is reflected, for example, in the formation of shear bands when a suspension is exposed to shear flow.

Star polymers, which are composed of linear polymers linked to a common center by one of their ends, are particularly interesting because their properties can almost continuously be changed from that of flexible linear polymers to a spherical colloidal particle with very soft pair interactions [1]. Moreover, using synthetic and/or biopolymers, the size of the colloids can be varied from a few nanometers to micrometers. This renders them interesting candidates for applications as lubricants, inter alia, in synovial joints.

Non-equilibrium Friction between Dragged Star Polymers – Two stars are considered with \( f = 20, 40, \) and 60 arms of \( N_f = 40 \) monomers each [2]. The central beads of the stars are confined in steep harmonic potentials, which are displaced with constant velocity \( v_d \), see Fig.1. We characterize the influence of the external force on the conformations of the stars by the Prandtl number \( \text{Pe} = v_d \tau/\lambda_g \), where \( \tau \) is the equilibrium relaxation time of the polymer, and \( \lambda_g \) is its radius of gyration.

![Simulation snapshot of two star polymers which are dragged past each other with constant velocity \( v_d \) and constant vertical displacement \( R_d \).](image1)

At equilibrium, the stars exert a repulsive force \( F \) on each other, as shown by the bottom curve in Fig.2.

![Drag forces for the Prandtl numbers \( \text{Pe} = v_d \tau/\lambda_g = 0 \) (green, bottom curve), 0.3 (black curve), 0.7 (red curve), and 1.3 (blue, top curve). The distance is \( R/R_g = 0.48 \) and functionality \( f = 40 \). The inset shows the corresponding excess forces, i.e., the differences between the intermolecular forces at nonzero \( \text{Pe} \) and the equilibrium forces.](image2)
This simulation result is consistent with the well-known ultra-soft (logarithmic) interaction potential between two star polymers.

When the stars are dragged, they start to interact when \( \Delta x/R_0 > -3 \), see Fig. 2. The repulsive forces for \( \Delta x < 0 \) increase somewhat stronger than the equilibrium values. More importantly, however, the force-distance curve exhibits a qualitative different behavior for large \( \Delta x > 0 \); here, the force is always positive, which implies that the star polymers exhibit an apparent attraction [2]. The mechanism of this attraction can be understood by a detailed analysis of the time-dependence of the star conformations. As the simulations show, at higher Pedet numbers there is little interpenetration of the coronas for the considered arm lengths. Instead, the attraction arises from a symmetry breaking of the monomer density distribution with respect to the plane perpendicular to the \( xy \)-plane and containing both star centers [2].

**Rheology of Star-Polymer Solutions** - At high concentrations, a star polymer interacts with many of its neighbors simultaneously (see simulation snapshot [3] displayed in Fig.3); this enhances the deformation of single stars as compared to just two stars (as depicted in Fig.1). Due to the resulting strongly drag-velocity dependent friction between star polymers at high concentrations, the shear viscosity is expected to decrease strongly with increasing shear rate. Such shear-thinning behavior is even enhanced in case of very long polymer chains due to entanglement forces [4]. At relatively small shear rates, a significant number of entanglements are formed on approach of two stars (\( \Delta x < 0 \) in Fig.2), and disentanglement occurs when two stars have passed each other (\( \Delta x > 0 \)). This leads to a repulsion and attraction for negative and positive \( \Delta x \), respectively, and thus enhances the forces as plotted in Fig.2.

**FIG. 4:** Flow profiles of shear-banded states of a concentrated polybutadiene star-polymer solution in tetradecane, as measured by spatially resolved heterodyne dynamic light scattering [4]. The number on the right axis are the overall applied shear rates. The number of linear chains in a single star is 122, and each linear chain has a molecular weight of 72100 g/mol.

At high shear rates, entanglements have less time to develop so that the magnitude of friction forces decreases. This is the mechanism that leads to a very strong decrease of the viscosity with increasing shear rate. It is well-known that such a strong shear thinning leads to a flow instability that is commonly referred to as "shear banding" [5]. The stable flow profile is now a banded state, where two regions (the "bands") coexist. Each of the bands the shear rate is constant, independent of position, but differ for the two bands. Preliminary experiments [4] on solutions of star-polymers with large chains indeed show a banded flow profile, as can be seen in Fig.4. We plan more systematic experiments, to extent the approximate but analytical theory in Ref.[4], and to perform simulations at high concentrations and for longer chains where entanglements become important, in order to explain the experimentally observed flow behavior of star polymers.


The hydrodynamics of micro-swimmers

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The behavior of micro-swimmers is dominated by low-Reynolds number hydrodynamics, hydrodynamic interactions, and thermal fluctuations. We employ mesoscale hydrodynamics simulations to study the behavior of microswimmers near walls, and the collective dynamics of two or more microswimmers. Here, we focus on two types of swimmers, sperm and squirmers. The results show a strong hydrodynamic attraction in both cases.

Introduction – Both in soft matter and in biology, there are numerous examples of microswimmers and self-propelled particles. With a typical size in the range of a few nano- to several micro-meters, both low-Reynolds-number hydrodynamics and thermal fluctuations play an essential role in their swimming behavior. Well-known biological examples include sperm cells which are propelled by a snake-like motion of their tail, bacteria like E. coli which move forward by a rotational motion of their spiral-shaped flagella, and volvox algae which are propelled by the beating of numerous cilia on their surface. In soft matter systems, synthetic self-propelled particles have been designed to perform directed motion. Examples are bimetallic nanorods which are driven by different chemical reactions at the two types of surfaces.

In order to understand and predict the dynamical behavior of microswimmers, the following issues have to be addressed:

- How does a single microswimmer move in a fluid?
- How do two microswimmers interact, both sterically and hydrodynamically?
- How do microswimmers move near walls and in confinement?
- What is the collective behavior of many swimmers?
- Which aspects of swimming are generic, and which depend on the specific propulsion mechanism of a microswimmer?

We study the hydrodynamics of microswimmers by the Multiparticle Collision Dynamics (MPC) approach, a particle-based mesoscopic simulation technique. This approach has been shown to capture the hydrodynamic behavior of many types of complex fluids very well [1].

Sperm motion near walls – Both in soft matter and in biological systems, surfaces and walls are ubiquitous. For example, bacteria in wet soil, near surfaces or in microfluidic devices, or sperm in the female reproductive tract find themselves in strongly confined geometries. Already in 1963, Lord Rothschild found that sperm accumulate at surfaces. Thus surfaces strongly affect the dynamics of swimmers and self-propelled particles.

FIG. 1: Simulation snapshot of the trajectory and shape of a swimming sperm. In the bulk, chiral sperm swim on a helical path, which becomes a circular path at a wall.

Our sperm model consists of a spherical head, a passive, curved midpiece, and an actively beating tail with a sinusoidal beat pattern [2], see Fig.1. The curvature of the midpiece lies in a plane which is tilted with respect to the beating plane of the tail by an angle of π/3; this gives sperm a chiral shape, which is important for the swimming behavior in three dimensions. We investigate the swimming behavior in the bulk and near walls as we vary the midpiece curvature and elastic properties.

In the bulk, chiral sperm swim on a helical path, which becomes a circular path at a wall, as shown in Fig.1. For a flagellum, which is flexible enough that it can deform somewhat in reaction to hydrodynamic forces, we find that sperm always adhere to wall, independent of the midpiece curvature [2].

However, the radius \( R_W \) of the circular trajectory and the frequency \( \Omega_W \) of rolling motion (rotation around the long body axis) depends on the midpiece curvature \( c_0 \), as shown in Fig.2. For strongly curved midpiece, the steric hindrance with the wall is strong enough to completely suppress any rolling. The mechanism for wall adhesion is a combination of the hydrodynamic pusher interaction, and the elongated shape of sperm, which suppresses changes in orientation near a wall [2] (and may by itself already be sufficient to induce wall adhesion [3]).
The trajectories of two squirmers are shown in Fig. 3. In this example, the squirmers are started with parallel orientation (in the x direction) at a center-of-mass distance $\Delta z = 2\sigma$, with a horizontal displacement $\Delta x = 0$ (where $\sigma$ is the squirmer diameter) [5]. The same hydrodynamic interaction, which is discussed above for sperm, initially leads to attraction and convergence of the trajectories. However, thermal fluctuations of the swimmer orientation lead to symmetry breaking, and redirection of motion.

For very stiff flagella, the behavior is more complicated, because the helical shape of the bulk trajectory competes with the effective attraction to the wall.

For many sperm or flagella, we find that the same interactions which lead to wall adhesion now imply synchronization of the flagellar beat and the formation of large clusters [4].

**Hydrodynamic Squirmer Interactions** - Another class of microswimmers is almost spherical organisms that are propelled by active hair-like organelles (cilia) covering the body. On a mesoscopic length scale, the synchronized beating (metachronal waves) of the cilia can be mapped onto a spherical envelope, and its time average corresponds to a sphere with a steady prescribed tangential surface velocity. Moreover, these objects – called “squirmers” – may also serve as a simple generic model for other types of microswimmers, for example, diffusiophoretic particles. The surface velocity field of a squirmer is expanded in a series of Legendre polynomials, with amplitudes $B_n$. The first mode determines the swimming velocity $v$, with $v = 2B_1/3$; the second mode determines whether the squirmer is a pusher (with main propulsion in the rear part, $B_2 < 0$, similar to sperm), or a puller (with main propulsion in the front part, $B_2 > 0$), like *Claydomonas algae*. The parameter $\beta = B_2/B_1$ determines the strength of the hydrodynamic interactions.

We have investigated this interplay of hydrodynamic interactions and thermal fluctuations in more detail. The competition between swimming and diffusion is characterized by the Peclet number $Pe = v\sigma/D$, where $D = k_B T/(3\pi \eta)$ is the translational diffusion coefficient (with fluid viscosity $\eta$). Results for three different Peclet numbers, all with horizontal displacement $\Delta x = 0$, are shown in Fig. 4. For large $Pe$, the hydrodynamic attraction dominates, and the squirmers come very close or even touch, before short-range hydrodynamic forces or orientational fluctuations lead to a divergence of the trajectories [5]. For smaller $Pe$, fluctuations dominate and lead to a early divergence. It is important to notice that in all cases, hydrodynamic interactions are not strong enough to lead to bound squirmer pairs.

![FIG. 2: Trajectory radius $R_w$ and rolling frequency $\Omega_w$ of sperm adhered to a wall.](image)

![FIG. 3: Simulation snapshot of the trajectories of two squirmers (pushers, $\beta=3$) which interact hydrodynamically.](image)

![FIG. 4: Average distance of squirmer pairs (with $\beta=3$) as a function of scaled time for different swimming velocities. The corresponding Peclet numbers are $Pe=289$ (magenta dashed-dotted), 577 (red dotted), 866 (green dashed), and 1155 (blue solid). The error bars represent the standard deviation.](image)

**References**


Blood flow: Red-cell rheology and white-cell margination

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Blood, a suspension of blood cells and proteins in plasma, mediates and controls numerous physiological processes in the organism. We employ numerical modelling to study blood rheological properties and establish a connection between blood rheology, structure, and single cell dynamics. We also study white blood cell (WBC) margination in blood flow, which is an essential pre-condition for their proper functioning. The dependence of WBC margination on hydrodynamic interactions of blood cells with the vessel walls as well as on their collective behaviour and deformability is investigated for a wide range of flow and cell suspension conditions.

Introduction — Blood flow plays a fundamental role in a wide range of physiological processes in the organism including transport of the necessary elements and cells to and away from the body tissues, organism defence through immune and inflammatory response, hemodynamic resistance, and haemostasis. Blood consists of red blood cells (RBCs), predominant by volume, and a small fraction of other cells and proteins suspended in the plasma. RBCs constitute approximately 45% of the total blood volume, and therefore determine the rheological properties of blood.

We employ the dissipative particle dynamics method, a particle-based mesoscopic simulation technique, to model RBC suspension. A RBC membrane is represented by a triangular network [1] with viscoelastic spring connections in combination with out-of-plane elastic bending resistance, and constraints for constant membrane area and constant cell volume. Fitting of the model parameters is performed through a number of static and dynamic experiments on single RBCs [1] and no further adjustment is made for the RBCs in suspension.

Blood rheology — The blood viscosity is derived from simulations of RBC suspension under shear flow for shear rates in the range of 0.005 s⁻¹ to 1000.0 s⁻¹ and with/without aggregation interactions between RBCs [2]. In equilibrium, RBCs in human blood aggregate into structures called “rouleaux”, which resemble stacks of coins. Figure 1 shows the relative viscosity (blood viscosity normalized by the viscosity of the suspending medium) against shear rate \( \dot{\gamma} \) at physiological concentration 0.45. The model predictions are in excellent agreement with the blood viscosities measured in three different laboratories.

The model, consisting only of RBCs in suspension, clearly captures the effect of aggregation on the viscosity at low shear rates, and suggests that cells and molecules other than RBCs have little effect on the viscosity. The model also captures typical formation/destruction behaviour of rouleaux, which are formed of a number of RBCs at low shear rates and are completely dispersed at high shear rates.

Whole blood is believed to exhibit a yield stress, i.e. a threshold stress for flow to begin. Our simulations support this hypothesis, and show that a non-aggregating RBC suspension has zero yield stress, while aggregation interactions between RBCs lead to a non-zero yield stress with the value of about 0.0017 Pa, which is within the range of experimental measurements. We also provide the first quantitative estimates of the strength of aggregation forces between two RBCs to be in the range 3-7 pN, which has not been measured experimentally.

The non-Newtonian nature of blood emerges from the interactions between cells and from their properties and dynamics. The steep increase in viscosity of the aggregating blood at low shear rates is mainly due to the cell aggregation into rouleaux. The increasing size
of the rouleaux structures with decreasing shear rate also explains the existence of yield stress, since these structures result in an eventual “solidification” of the suspension. The shear thinning at intermediate regime of shear rates between 5 s⁻¹ and 200 s⁻¹, where RBC aggregation interactions can be neglected, is due to RBC deformation. At this range of shear rates RBCs attain on average a more spherical shape. In addition, the transition of some cells to the tank-treading motion further reduces the shear stresses.

A very similar model has been employed to predict the flow of RBCs through narrow capillaries [3]. The developed computational framework is general and can be employed to investigate other cell, vesicle, and capsule suspensions with potential usage in biology, medicine, and engineering. The suspension properties may be tuned to yield a desired behaviour by changing the solvent viscosity, material properties of suspended cells, and inter-cell aggregation interactions.

**White blood cell (WBC) margination** — WBCs perform various organism defence functions. In order to reach the sites of action (e.g., inflammation), first WBCs have to migrate to the vessel walls through a process called margination. The mechanism for WBC margination in microvessels is related to the presence of RBCs in blood. RBCs in microvessels migrate to the vessel centre, which is governed by cell-wall hydrodynamic interactions (often called lift force). WBC margination is believed to be a consequence of the competition between lift forces on RBCs and WBCs, where the lift force on a RBC is larger than that on a WBC due to the non-spherical discocyte shape and high deformability of RBCs.

Using a two-dimensional blood-flow model, we perform an extensive study of the dependence of WBC margination on RBC concentration (hematocrit, $H_\mathrm{r}$), flow rate, WBC deformability, and RBC aggregation [4]. In particular, we show that there is a pronounced effect of hematocrit on WBC margination, and provide new insights into the physical mechanism. We find a re-entrant behaviour, where margination first increases with increasing hematocrit, but then decreases again at higher hematocrits. Furthermore, changes in WBC deformability strongly affect margination since a deformed WBC may significantly depart from the spherical shape.

Figure 2 shows the probability of WBC margination (being not further than 0.5 $\mu$m away from the vessel wall) with respect to hematocrit and dimensionless shear rate. The strongest margination effect is observed for a certain range of intermediate hematocrits and shear rates. As expected at low $H_\mathrm{r}$, WBC margination is weak due to low concentration of RBCs. However, at high $H_\mathrm{r}$ values the simulated results seem to contradict the main hypothesis for WBC margination: a higher RBC concentration is expected to lead to more RBC crowding in the channel centre, and therefore to stronger WBC migration towards the wall.

To identify the physical mechanism responsible for this observation, we examined the RBC distribution around a marginated WBC. At low enough $H_\mathrm{r}$, the region downstream of the WBC near the wall is virtually void of RBCs, and it remains free of RBCs during most of the simulated time. As hematocrit is increased RBCs are found to often enter the region downstream of the WBC due to high RBC crowding. RBCs in that region move slower than the WBC next to the wall, so that a RBC may enter the gap between the WBC and the wall and force the WBC to move away from the wall. This “lift-off” mechanism for the WBC is different from the lift force due to cell-wall hydrodynamic interactions and is governed by the particulate nature of blood.

Our simulation results are also consistent with experimental observations that WBC adhesion occurs mainly in venules (not in arterioles) in the organism. The characteristic values of the dimensionless shear rate $\gamma^* = \gamma \tau$, where $\tau$ is the characteristic RBC relaxation time, in venules of a comparable diameter are approximately in the range $\gamma^* \approx 10^{-2}$, while in arterioles $\gamma^* \gtrsim 30$. Thus, efficient WBC margination and consequent adhesion are mainly expected in the venular part of a microvascular network.

Non-equilibrium polymer dynamics

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The structural and rheological properties of polymers in dilute and semidilute solution under shear flow are governed by the non-equilibrium relaxation times and are universal functions of the concentration dependent Weissenberg number. In dilute solution, hydrodynamic interactions determine the polymer dynamics, whereas they are screened in semidilute and concentrated solutions, which changes the nature of their tumbling motion. Mesoscale hydrodynamic simulations exploiting the multiparticle collision dynamics approach provide quantitative insight into the relevance of hydrodynamic interactions at various shear rates.

There is a long-standing interest in unraveling the microscopic dynamical properties of individual polymer chains in dilute and semidilute solution, driven by the desire to relate them to their macroscopic rheological characteristics. A hint on the complex behavior comes from non-equilibrium computer simulations of single polymers and fluorescence microscopy studies on single DNA molecules in shear flow, which reveal a remarkably rich dynamics [1]. Specifically in shear flow, a polymer chain exhibits large conformational changes and continuously undergoes stretching and compression cycles, denoted as tumbling, and never reaches a steady-state extension. The detailed temporal evolution depends upon the shear rate. These microscopic dynamical properties are tightly linked to the macroscopic rheological behavior of a polymer solution and give rise to phenomena such as shear-rate dependent viscosities and non-vanishing normal stress differences [1].

The characterization of the tumbling dynamics is an important step toward a microscopic understanding of the polymer dynamics. As it turns out, this is a non-trivial task because of the non-Markovian nature of the process. As is commonly accepted by now, tumbling is a cyclic but non-periodic process, which poses challenges for the calculation of a characteristic time [1,2].

The complex interactions in polymer solutions harm an analytical treatment. Here, computer simulations are essential to shed light on the rich and intricate dynamical behavior. We apply a hybrid mesoscale hydrodynamics simulation approach, which combines molecular dynamics simulations (MD) for the polymers with the multiparticle collision dynamics (MPC) method describing the solvent [1,3] to study the non-equilibrium stationary state properties of polymers under shear flow [1,4]. By this approach, we are able to address the question on the relevance of hydrodynamic interactions in non-equilibrium systems.

In large-scale simulations, we study the conformational, dynamical, and rheological properties of linear polymers with monomers in the range $N_m=50–250$ and over a broad range of concentrations from dilute to semidilute. Figure 1 shows typical polymer conformations, and Fig. 2 an example of the polymer density in the flow-gradient plane, where $c$ is the polymer concentration, $c^*$ the overlap concentration, $Wi_c=\gamma c$ the Weissenberg number, the shear rate $\gamma$, and $\tau(c)$ the concentration-dependent polymer end-to-end vector relaxation time at equilibrium. Evidently, the polymers are stretched along the flow direction and compressed along the gradient direction. Moreover, the polymers are strongly aligned with the flow. More importantly, in the stationary state, the conformational and rheological properties for various concentrations are universal functions of the Weissenberg number $Wi_c$, as shown in Fig. 3 for the deformation ratio $\delta G_{xx}/\delta G_{xx}$, the radius of gyration tensor component along the flow direction with its equilibrium value $\bar{G}_{xx}$. The alignment angle and the polymer contribution to

FIG. 1: Snapshot of a system of $N=800$ polymers of length $N_m=50$ ($c/c^*$) for the Weissenberg number $Wi_c=184$. For illustration, some of the polymers are highlighted in red.

FIG. 2: Monomer density distribution in the flow-gradient plane for the polymer concentration $c/c^*=10.38$ and $Wi_c=569$. The contour lines for the densities 0.1 (outer) and 0.5 (inner) are highlighted to emphasize the non-ellipticity of the shape.

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viscosity show a similar universal behavior. Hence, with increasing concentration, hydrodynamic interactions affect the conformational and rheological properties only via the increasing relaxation time $\tau(c)$, which is strongly dependent on hydrodynamic interactions at low concentrations.

The influence of hydrodynamic interactions is reflected in various other quantities. On the one hand, we find a concentration dependence of the distribution function of the angle $\varphi$ between the projection of the end-to-end vector onto the shear-gradient plane and the flow direction [4]. Comparing distribution functions at the same Weissenberg number at low and high concentrations, we find broader distributions at higher concentrations, thereby the angle of the maximum is unchanged. We attribute this dependence to screening of hydrodynamic interactions for concentrations $c/c^* > 1$. Brownian MPC simulations, i.e., simulations without hydrodynamic interactions, of polymers in dilute and semidilute solutions at very similar Weissenberg numbers yield identical distributions, which, in addition, agree with those of semidilute solutions of the same concentration and Weissenberg number in the presence of hydrodynamic interactions [4]. On the other hand, the end-to-end vector relaxation times and the polymer tumbling times $\tau_T$ exhibit a concentration dependence. A priori it is not evident how the non-equilibrium polymer relaxation time is related to the tumbling time and how such a tumbling time can be extracted from the simulation data. Various strategies have been proposed to extract $\tau_T$. In Ref. [1], we determine tumbling times from the time-dependent correlation function of the fluctuations of the radius of gyration tensor components along the flow and gradient direction.

These components are tightly linked and show short time correlations, which disappear for longer times due to the non-periodicity of the tumbling dynamics. Figure [4] shows tumbling times for various polymer lengths. Evidently, a universal, chain-length independent behavior is obtained at low concentrations. Hydrodynamic interactions are captured in the relaxation times. With increasing concentration, the tumbling times decrease and saturate at high concentrations, i.e., again a universal behavior is obtained. We attribute the different behavior to screening of hydrodynamic interactions for concentrations $c/c^*$.

FIG. 3: Deformation ratios $\dot{\varepsilon}_c$ as function of Weissenberg number. Open symbols correspond to systems with $N_w=50$ for $c/c^*=0.16$ (○), $c/c^*=1.6$ (△), and $c/c^*=2.08$ (□). Filled symbols denote results for $N_w=250$ with the concentrations $c/c^*=0.17$ (●), $c/c^*=2.77$ (▲), $c/c^*=5.19$ (●), and $c/c^*=10.38$ (■). The inset shows the same data as function of $W_i$.

FIG. 4: Tumbling frequencies $f = \tau(c)/\tau_T$ for dilute systems (red), and the concentrations $c/c^* = 1.635$ (blue) and 2.08 (green) as function of the Weissenberg number. The polymer lengths are $N_w=50$ (bullets), $N_w=70$ (squares), and $N_w=100$ (diamonds). The solid line is calculated by the analytical expression derived in Ref. [2].

References


Polymers and colloids in micro-channels

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The dynamics of colloids, polymers, and cells in microchannels is governed by the intricate interplay of conformational changes, fluid-mediated interactions, inhomogeneous flow profiles, and wall interactions. For semiflexible polymers under flow, cross-streamline migration leads to inhomogeneous density distributions. Externally actuated colloids induce flow in linear and curved micro-channels.

Introduction – Complex fluids – such as polymer solutions, colloidal dispersions, and suspensions of vesicles or cells – exhibit an intriguing flow behavior, particularly in confined geometries. The interplay of internal degrees of freedom, e.g., conformational changes of polymers or shape changes of vesicles and cells, combined with fluid-mediated interactions, inhomogeneous flow profiles, and wall interactions lead to novel and often unexpected effects. Insight into these aspects provides not only an understanding of phenomena for conventional applications such as polymer processing and blood flow, but also of emergent microfluidic devices with their micro- or nano-size length scales. In this context, the investigation and analysis of single molecules or cells is of particular interest, since on the one hand such knowledge is essential to develop and optimize micron- and nano-scale devices, while on the other hand the study of individual particles often provides a more detailed microscopic insight than can be obtained from an ensemble-based approach.

We study the hydrodynamics of polymers and colloids in microchannels by the Multiparticle Collision Dynamics (MPC) approach, a particle-based mesoscopic simulation technique. This approach has been shown to capture the hydrodynamic behavior of many types of complex fluids very well [1].

Semiflexible Polymers in Microchannel Flow – Polymer transport in the presence of a no-slip wall leads to cross-streamline migration. This effect has been observed in planar shear flow in the presence of walls as well as Poiseuille flows, as a formation of a depletion layer near the wall. We focus here on understanding the migration properties of semiflexible and stiff polymers in microchannel flows; this is important for the study of the flow behavior of many biopolymers, such as actin filaments, microtubules, and intermediate filaments. Since polymer conformations are determined by their rigidity, the migration behavior of semiflexible and rodlike polymers can differ significantly from that of flexible polymers.

![Simulation snapshots of polymer conformations close to the channel center (top row) and near the wall (bottom row), for Peclet number Pe = 360 and persistence lengths Lp/Lr = 0.5 (a),(d), 2.1 (b), 3.8 (c), 7.7 (diamond), 15.4 (triangle), and 30.8 (*).](image1)

![Radial monomer distributions for Peclet number Pe = 360 and persistence lengths Lp/Lr = 0.5 (+), 2.1 (bullet), 3.8 (square), 7.7 (diamond), 15.4 (triangle), and 30.8 (*).](image2)
For persistence lengths $L_p/L_r<8$, the density at the center of the channel decreases with increasing Péclet number Pe. In contrast, for stiffer polymers with $L_p/L_r>8$, the density at the channel center first increases with increasing Pe and then decreases at large Péclet numbers. Simultaneously, the wall-induced migration toward the channel center causes an increase in concentration at a finite distance from the center for all stiffnesses. For high flow rates, off-center density maxima are obtained for all stiffnesses, see Fig.2. For the flow rate $Pe = 360$, we observe a decrease in the depletion zone adjacent to the wall with increasing stiffness for $L_p/L_r < 8$ and a reversion of the trend at larger stiffnesses, as depicted in Fig.2. An off-center density maximum has indeed been found recently in experiments (T. Pfohl et al., University of Basel) using actin filaments.

**Structure Formation of Rotating Colloids in Microchannels** - Externally actuated and self-propelled micro- and nano-rotators show an intriguing variety of non-equilibrium structure formation and dynamics. Examples of such systems include super-paramagnetic colloidal particles in a rotating magnetic field, dipolar colloids in a rotating electric field, colloidal dimers rotated by laser tweezers and biological swimmers such as Volvox algae.

We consider suspensions of spinning colloids in linear and ring channels [4,5], see Fig.3. Symmetry implies that there cannot be any net fluid or colloid transport in a straight channel. However, we find that the particle distribution across the channel causes local directed motion: colloids spinning counter-clockwise move to the left/right near the lower/upper wall. For channels which are wider than two colloid diameters $\sigma$, this leads to lane formation. To quantify this effect, we calculate the average colloid velocity per half-channel. As shown in Fig.4, the colloid velocity in the half-channels increases with increasing colloid density $\Phi$ at low densities, because (i) when two colloids pass each other, they circle around each other, which implies an increased density near the walls where propulsion is most efficient, and (ii) the fluid volume each colloid has to move decreases as $1/\Phi$. At high $\Phi$, the average colloid velocity decreases again because lubrication forces between neighboring colloids and between colloids and the walls slow down the spinning motion, see Fig.4.

**FIG. 4:** Average colloid velocity per half-channel as a function of the colloid area fraction $\Phi$ for straight channels of width $D=2.5\sigma$ (square, blacksquare, red) and $D=3\sigma$ (circle, bullet, blue). Data are shown for three different torques, $L_{ext}0$ (blacksquare, bullet), $L_{ext}3$ (square, circle), and $L_{ext}9$ (inset).

In ring channels, the channel curvature breaks the translational symmetry and leads to a net fluid transport around the annulus with the same rotational direction as the colloidal spinning direction. The average tangential velocity $v_{tan}$ of all colloids in the channel is then a function of the median radius $R$ and the channel width $D$. For example, we find that $v_{tan} \sim 1/R$ for channel width $D>2\sigma$. Also, there is an interesting non-monotonic dependence of $v_{tan}$ on $D$.

Another promising method to generate net fluid and colloidal transport is to keep some colloids fixed near one of the two walls, for example by a laser tweezer [5]. We conclude that spinning colloids present a promising approach for flow generation and control in microfluidic devices.


Near-surface structure of micro-emulsions

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In the bicontinuous microemulsion phase of oil-water-surfactant mixtures, a planar hydrophilic wall induces lamellar order adjacent to the wall [1], while the bulk structure remains disordered. This near-wall structure is characterized on the basis of computer simulations, neutron-reflectivity and grazing-incidence small-angle neutron scattering [2]. Characteristic lengths are obtained for the lamellar decay and for the beginning of the bicontinuous structure. The simulations predict an intermediate structure formed by perforated lamellae, while is difficult to demonstrate experimentally.

Interfaces are ubiquitous in fluid systems. Liquid metals and simple liquids mostly tend towards surface freezing, also known as surface ordering; in a certain temperature range, the bulk material is liquid while the liquid-vapor interface has a higher degree of order, similar to a solid state. In contrast, many soft-matter systems tend toward surface melting; on top of the well-ordered bulk material a thin layer with a lower degree of order is often found, although surface ordering has been found as well, for instance for polymeric micelles or nanospheres.

On the application side, aqueous surfactant systems are often employed for the enhanced oil recovery for various reasons. The cracking fluid must be highly viscous to deposit the pressure energy in the sandstone. In contact with oil, the fluid forms low-viscosity microemulsions, which facilitates the oil production after the application. From this example it becomes clear, that surfactant systems adjacent to walls and in porous media are highly interesting. The influence of the wall on the microscopic structure of a microemulsion will help to understand the flow properties of the fluid in a porous medium. Lamellar structures parallel to the walls may lead to finite slip lengths and finally to a facilitated flow.

Our Monte-Carlo computer simulations are based on a Ginzburg-Landau theory [1,3] with extended terms for the selective hydrophilic surface [4]. A single scalar order parameter $\Phi(r)$ describes the three-dimensional local oil-water concentration difference. An isosurface analysis of the order parameter reveals the structure of the surfactant monolayer at the interfaces between the oil-rich and water-rich regions (see Fig.1). The induced lamellar structure can be clearly distinguished from the bicontinuous bulk structure. The perforated lamellar structure is found in between, and can be proven by cuts in parallel to the surface [2].

Reflectivity curves are obtained from the reflectometer TREFF/NOSPEC at the research reactor FRM II. This method probes the scattering-length density profile along the film normal [2]. Fig.2 shows typical reflectivity curves for D$_2$O and microemulsions with different surfactant concentrations γ. In the inset of Fig.2 the real-space scattering-length density profile of the sample with $\gamma$=0.18 is shown. As expected, alternating water and oil domains are found with decaying amplitudes into the bulk. The wave length of the lamellar order gives rise to the peak of the reflectivity curve at $q_0 = 0.025\text{Å}$. The real-space scattering-length density closely resembles the order parameter profile obtained from our computer simulations.

Grazing incidence small angle neutron scattering experiments (GISANS) were obtained from KWS-2 at the research reactor FRM II. The measurements are performed at incident angles close to the critical angle $\alpha_c$ of total internal reflection. In this regime, the evanescent (tunneling) wave propagates into amplitude of this evanescent wave decays the sample and gives rise to the scattering. The exponentially with
a penetration length $\Lambda_{\text{inc}} = \left[4\pi \Delta \rho \left(\frac{1 - \alpha_{\text{inc}}}{\alpha_{\text{inc}}}\right)^2\right]^{1/2}$. Here, $\alpha_{\text{inc}}$ is the angle of the incident neutron beam and $\Delta \rho$ is the difference of the scattering length densities of silicon and the overall microemulsion. The scattering depth $\Lambda = \left(\Lambda_{\text{inc}}^{-1} + \Lambda_{\text{out}}^{-1}\right)^{-1}$ arises from in- and outgoing waves (very large $\Lambda_{\text{out}}$ for $\alpha_{\text{out}} > \alpha_{\text{inc}}$). Thus, the length scale of the evanescent wave is sensitive to regions (lamellar/bicontinuous) while the scattering vector of the outgoing wave is comparable to the internal structure of the regions.

In GISANS, the preferred orientation of the lamellar phase gives rise to a peak in the scattering pattern (see Fig. 3). This pattern is described well by a Gaussian peak. The bicontinuous phase is locally isotropic, with a preferred domain size, so that the scattering from this phase leads to a ring-like structure. Both contributions can clearly be seen in different graphs of Fig. 3. The scattering pattern of the bicontinuous phase can be modeled by the formula of Teubner and Strey. While at high momentum transfers $Q$, the patterns are not influenced by damping, close to $Q_z = 0$ additional damping becomes visible. There, the outgoing wave becomes evanescent, too.

To obtain a characteristic depth from the GISANS experiments, the ratio between the bicontinuous ring and the lamellar peak was determined. A linear extrapolation finds a zero of this behavior which is connected to the beginning of the isotropic region. For smaller scattering depths the ratio is not exactly zero, but much smaller than the measured ratios. Experimentally, we have found the isotropic region for depths larger than 400Å. From computer simulations, the GISANS patterns were calculated in parallel, and the same intensity ratio has been evaluated. The ratio depends linearly as well, and the zero was found to be at a depth of 200Å. These perforations start to develop. So, from simulations it can be clearly stated that the proposed GISANS evaluation leads to the beginning of the perforated lamellae. This is plausible because the length scales of the perforations are similar to the domain spacing of the bicontinuous structure.

In summary, good agreement is found between experimental and simulation results for the near-wall structure of microemulsions. The laterally averaged structures agree very well with reflectivity experiments. The intermediate perforated-lamellar structure between the perfect lamellae and the bicontinuous structure is only clearly shown by the simulations. First spectroscopic experiments show considerably different dynamics of the adjacent lamellar and bicontinuous bulk structure [5].

Crumpling and collapse of thin elastic spherical shells

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Thin elastic shells deform when a sufficiently large load is applied. The shape of the deformed shells depends on the deformation rate, the reduced volume and on the elastic constants of the material that makes up the shell. For small deformation rates the ground state is a single indentation whereas for large deformation rates the shape is a heterogeneous network of indentations similar to a crumpled sheet of paper. For small indentations, the shape of the rim of the indentations is smooth, where stretching and bending energy are homogeneously distributed. However, for large indentations there is a transition to a polygonal shape which is driven by the condensation of the stretching and bending energy in so-called stretching ridges like in a crumpled elastic sheet.

Thin elastic shells are closed surfaces that can be characterized by a two-dimensional Young modulus $K_0$ and a bending rigidity $\kappa$. Biological examples of these shells range from very small entities like spherical viruses (the size is of order 10-50 nm) to much larger structures like red blood cells with a typical linear dimension on the order of several micrometers. In addition, special procedures have been developed to synthesize nano-sized shells which can for instance be used for drug delivery or even as artificial cells. It will be no surprise that the mechanical properties of native and artificial shells are of crucial importance for an optimal performance of these systems in their natural environment or in envisaged applications. For virus shells with icosahedral symmetry the mechanical properties have been investigated extensively using computer simulations [1].

When a sufficiently large external load is applied to an elastic spherical shell of radius $R_0$, it will deform into a state that is characterized by one or more indentations or by a hierarchical structure of wrinkles. The parameters that are expected to determine the shape of the deformed state are the initial shape, the elastic constants of the shell, expressed by the (dimensionless) Föppl-von-Kármán number $\gamma = K_0 R_0^2/\kappa$, the temperature (that is, thermal fluctuations) and deformation rate. In the investigations presented here Monte Carlo computer simulations were used to study the role of the aforementioned parameters on the deformation of elastic shells [1-4]. Elastic shells are modelled by permanent disordered networks that have no internal stresses in de undeformed spherical state. The deformations imposed in this study are achieved by reducing the volume of the shell using a volume constraint [2]. To set the scene two typical structures that are found in a computer simulation study using triangulated spherical shells are shown in Fig.1. It shows that by just increasing the Föppl-von-Kármán number a very different final state is observed.

In order to understand these observations the ground state for the indented elastic shells was investigated first. For a given reduced volume $V/V_0$ of a shell this turns out to be a single indentation, an example of which is shown in Fig.1A. This result is obtained numerically and in agreement with a simple scaling argument that originates from balancing bending and stretching energies for a rim of constant diameter $r$ and with a width $d$,

$$E \sim \kappa \gamma^{1/4} N \left( \frac{\Delta V}{N V_0} \right)^{3/4}$$

where $E$ is the elastic energy and $\Delta V=V-V_0$ the volume spread over the number of indentations $N$. Furthermore, this scaling relation predicts the energy of an indentation to scale like $\gamma^{1/4}$. Figure 2 shows the scaling of the energy of a single indentation with the Föppl-von-Kármán number. For small Föppl-von-Kármán numbers the $\gamma^{1/4}$ scaling of the energy predicted by Eqn.(1) is recovered. For very large Föppl-von-Kármán numbers ($\gamma \sim 10^7$) so-called ridge-scaling with $\gamma^{1/6}$ is expected [3,5]. The expected asymptotic ridge-scaling is difficult to recover numerically and preceded by a crossover region covering a very wide range of Föppl-von-Kármán
numbers. The change of the scaling characteristics originates from a change in the shape of the indentations. A first indication of this shape change is obtained by visual inspection of the indentations. In this way the transition between smooth and polygonal rims is localised by the purple line in Fig.2.

**FIG. 2:** Scaling of the total energy of an indentation with $\gamma$. The two regimes of smooth & polygonal indentations are separated by the purple line. The black lines indicate the limiting scaling regimes with exponent $1/4$ and $1/6$.

A more precise estimate of the location of this transition can be obtained from the stretching energy of the system as a function of $\gamma$ as indicated in Fig.3.

**FIG. 3:** Scaling of the stretching energy $E_s$ of an indentation with $\gamma$.

For small $\gamma$ the network is soft and the rim has a homogeneous smooth shape. When $\gamma$ is increased the local in plane compression becomes increasingly more unfavourable and the stretching energy rises until at a critical $\gamma_c$ the onset of the formation of stretching ridges leads to a strong reduction of the stretching energy. For larger values of $\gamma$ these ridges become more and more pronounced. The shape of the deflated shells was also studied as a function of deformation rate, Föppl-von-Kármán number and the reduced volume. An example for $\gamma=8 \times 10^4$ is shown in Fig. 4. For the fastest compression (top-row) the shells nucleate many indentations that deepen upon further compression. Since coarsening is in this case slower than further deformation the final state is disordered. However, for slow enough compression (bottom-row) the stresses that develop initially during compression have time to localise in a single spot that is the site at which the indentation forms when the shell buckles. A single indentation is formed. When the volume is kept constant at the final value, in this case $V/V_0=0.48$, and the system is left to relax, it slowly returns to the ground state. However, the relaxation rate depends strongly on the Föppl-von-Kármán number and for the largest Föppl-von-Kármán numbers studied the ground state is not reached within simulation time. The simulation results presented suggest that the irregular shapes that are found in experimental studies are non-equilibrium states.

**FIG. 4:** Snapshots for $\gamma=8 \times 10^4$, three different compression rates (from top to bottom) and 4 different reduced volumes (from left to right). Top: fast compression, $\tau_v=1.5 \times 10^{-6}$, middle: slower compression, $\tau_v=1.5 \times 10^{-7}$; bottom: slowest compression: $\tau_v=1.5 \times 10^{-8}$.

Simulations of thermophoretic colloids

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The motion of a colloid induced by a temperature gradient is investigated by means of mesoscopic simulations. The variation of the solvent colloid interaction from attractive to purely repulsive interestingly results to change from a thermophobic to a thermophilic behavior. We further consider a nanodimer with asymmetric thermal properties in solution. One monomer is globally heated while the thermophoretic properties of the second monomer produce a propulsion against or towards the heated particle, such that the nanodimer becomes a puller or pusher nanoswimmer.

The motion of a particle due to the presence of a temperature gradient is known as thermophoresis, thermodiffusion, or Soret effect [1]. Historically this effect has found a large number of very different applications and more recently, the establishment of experimental techniques that allow temperature control at small scales is originating the development of promising microfluidic applications. Thermodiffusion of colloids has been extensively investigated by means of various experimental techniques, different analytical approaches, and recently also by means of one type of atomistic simulations. Most colloids in solution show a thermophilic behavior, this is, they tend to migrate to cold areas, although examples of thermophilic colloids, that tend to migrate to warm areas, can also be found. The particularities of the interactions between the colloid and the surrounding solvent have shown to determine their thermophoretic behavior. Properties like the colloidal interfacial tension, solvent polarity, colloidal charge, average temperature, or particle size, strongly influence the form of the related thermophoretic forces. Moreover, systems with varying colloidal concentration have shown that also colloid-colloid interactions can contribute significantly to the thermodiffusive properties of the solution. So far no general theoretical explanation is found that can predict the direction or strength of the diffusive migration of the colloid along the temperature gradient. Simulations with well defined interactions and individually tunable parameters are therefore a promising approach to help in identifying the mechanisms of colloidal thermodiffusion.

We perform simulations with a hybrid algorithm. The solvent is provided by the mesoscale simulation method known as multiparticle collision dynamics (MPC) that has shown to properly include the effect of hydrodynamic interactions in colloidal and polymeric systems, and to be able of sustaining temperature inhomogeneities [2]. Colloidal interactions are considered with standard molecular dynamics (MD) such that the effect of varying colloid-solvent interactions can be straightforwardly investigated [3]. In order to determine the thermophoretic force, the colloid is attached to a harmonic spring, such that there is no average drift velocity and consequently no friction force. The thermophoretic force balances then the harmonic force that can be directly determined \( \mathbf{F}_T = k \nabla T \), where \( k \) is the harmonic constant and \( \nabla T \) the averaged deviation of the colloid from its neutral position.

Two examples of the time averaged \( \mathbf{F}_T \) as a function of time are displayed in Fig. 1 for colloids with repulsive and attractive interactions with the surrounding solvent. In the two perpendicular directions to the temperature gradient the force vanishes in all cases, while in the parallel direction to the gradient, the averaged force has a clear non-vanishing value which long time behaviour we account as the computed thermophoretic force \( \mathbf{F}_T \). The linear relation of \( \mathbf{F}_T \) and the temperature gradient

\[
\mathbf{F}_T = -\alpha T \nabla k B T \tag{1}
\]

determines the thermal diffusion factor of the particle \( \alpha T \), which is related to the Soret coefficient \( S_r \) by \( \alpha T = T \nabla S_r \). The positive value of \( \mathbf{F}_T \) in Fig. 1 for the repulsive colloid-solvent interaction indicates that the colloid goes on average towards the hot bath, i. e. it is thermophilic. Meanwhile, the negative \( \mathbf{F}_T \) value in the case of attractive colloid-solvent interaction show a thermophobic trend of the colloid. Experimentally, similar influence of the attraction interaction between solute and solvent on the thermodiffusive properties, has been observed in polymeric systems with different solvents. Although polymers are mostly thermophobic, in cases where the affinity between solute and solvent is higher the Soret coefficients show to be larger. Meanwhile in cases where the affinity is reduced, for example by diminishing the relevance of the hydrogen

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Fig1.pdf}
\caption{Thermophoretic force averaged as a time integral vs time for a colloid attached to a spring in a temperature gradient. Colloid with repulsive interaction with the solvent displays thermophilic behavior (left). Conversely, colloid with attractive interaction with the solvent displays thermophobic behavior (right).}
\end{figure}
bonds in water based solutions, the Soret coefficient can become eventually negative.

FIG. 2: (Left) Schematic diagram of the simulated nanodimer solution. The 'h' monomer can be heated to a fixed temperature and it is strongly linked to a second monomer 'p'. The system has then a radially symmetric temperature gradient. (Right) Swimming characteristic behaviors for a thermophobic p-monomer 'puller' and a thermophilic p-monomer 'pusher'.

Recent experiments with a Janus particle have shown to display self-propelled motion due to thermophoresis [4]. With a defocused laser, a half-metal coated colloidal sphere is heated. The higher heat absorption of the metal side produces a temperature gradient on the non-metal side, what translates into a self-propelled motion. We perform computer simulations [5] of two strongly bonded monomers which are immersed in a hydrodynamic solvent as depicted in Fig. 2. The heated bead 'h', can have a temperature higher than the surrounding fluid. If the average temperature $T_{av}$ of the system is kept constant, the surrounding solvent will sustain a steady temperature gradient with radial symmetry. The non-heated bead can generate a thrust due to thermophoresis, what will translate into a directed motion of the nanodimer along the bond direction. We will therefore refer to the non-heated bead as the propelling monomer 'p'. In case p-bead is thermophilic, it will tend to go to higher temperatures, and the nanodimer will behave as a pusher. Reciprocally, in case the p-bead is thermophobic, the nanodimer will behave as a puller.

During its motion the nanodimer can freely rotate, what implies that the directed motion will change orientation, which will effectively give rise to an enhanced diffusive behavior. In order to quantify and to explain the swimming behavior, we characterize the self-propelled velocity $v_p$ defined by projecting the nanodimer center of mass velocity $v_{cm}$ in the bond direction $n$. This is $v_p = <v_{cm} \cdot n>$. This directed velocity is related to the thermophoretic force with $v_p = \mu_p \nabla T$, where the mobility $\mu_p$ can be estimated by computing the mean squared displacement along the bond direction. Note that there is no thermophoretic force exerted on the h-bead since its surface is at constant temperature. By employing Eq. (2), the self-propelled velocity can then be expressed as,

$$v_p = -\mu_p \alpha_T \nabla k_B T.$$  \hspace{1cm} (2)

In Fig. 3, direct measurements of $v_p$ are presented for different temperature gradients and potential interactions (different $\alpha_T$). Simultaneously, the values of $v_p$ can be indirectly obtained by computing the right hand side of Eq. (2). The temperature gradient is locally estimated at the center of the p-bead, and $\alpha_T$ is obtained from single-particle simulations as shown in Fig. 1.

The agreement is satisfactory, though the indirect evaluated $v_p$ values are systematically 50% larger than the directly measured ones. The most relevant factor for this overestimation is surely due to the employed values for $\alpha_T$ of single particles. In the nanodimer environment, the existence of the h-bead, and the radial temperature gradient will have certain influence in the distribution of the solvent near the p-bead, and therefore in $\alpha_T$.

FIG. 3: Self-propelled velocity as a function of the temperature gradient in (a) and of the thermal diffusion factor in (b). Bullets correspond to direct simulation results, diamonds to the evaluation of Eq. (2), and lines to linear fits. (a) The p-bead has a repulsive colloid-solvent interaction. (b) $\nabla T = 0.02 k_B T$ is fixed and the colloid-solvent interaction varies.

In summary, we present a simulation model that is able to capture the thermophoretic properties of colloidal systems and that makes possible to study the behavior of thermophoretic nanowimmers. Therefore, this model is a promising tool to investigate and design synthetic nanomachines.

In the ICS-3 we investigate equilibrium and non-equilibrium phenomena in macromolecular systems. In equilibrium, various types of phases as well as the diffusive dynamics are of interest. An important aspect of our research is the response of macromolecular systems to external fields, like flow, electric fields, temperature gradients and high pressure. Patterns and structures can be induced by the external fields which do not exist in equilibrium, and which sometimes are of biological and technological relevance. To gain a fundamental understanding of these complicated processes we synthesize model systems with chemically tuned properties.

To study and characterize our systems of interest, the institute is equipped with a wide variety of standard and highly sophisticated, home-built, state-of-the art instruments. Amongst these are several specialized light-scattering set ups (including spatially resolved heterodyne light scattering, evanescent wave scattering, thermal diffusion forced Rayleigh scattering, and equipment specialized for studies in electric fields), optical techniques like Total Internal Reflection Microscopy (TIRM) and confocal microscopy, with a variety of dedicated sample environments for the application of external fields.
Research Highlights
Near wall dynamics and particle wall interaction in flowing suspensions

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We report on experiments, which were designed to elucidate the interplay of flow, rotational dynamics and particle-wall interactions. In particular we were investigating the particle velocity profile in streaming suspensions, the effect of flow on depletion interactions between a probe sphere and a planar wall, and the near wall rotational dynamics of spherical particles.

Near wall particle velocity in flowing suspensions:
Fluid flow near hard planar surfaces attracts attention because of its relevance in various fields, like tribology, micro-fluidics or rheology, reflecting the general need to control and understand flow close to surfaces. An important parameter to describe the near wall velocity profile this case is the slip length, which quantifies the deviation from the expectations for stick boundary conditions. Although a broad range of experimental techniques have been applied to measure velocity profiles close to surfaces the determination of the slip length suffered from a severe lack of resolution so far.

Recently we have developed a new optical technique, namely Near Field Laser Doppler Velocimetry (NFLDV) that provides a robust method for the measurement of slip lengths with a resolution of tens of nanometers for colloidal suspensions with high Reynolds numbers [1]. It is an extension to flowing samples of evanescent wave dynamic light scattering (EWDLS) which we used to evaluate diffusion near walls before [2].

The technique is based on the measurement of time auto-correlation functions (TACF) of the scattered light from a sample volume which is illuminated by an evanescent wave with penetration depth \( \kappa \). As shown in Figure 1, in the present case the TACFS consist of a superposition of a Lorentzian with a decay rate of \( \Gamma_1 \) and an oscillating contribution with a typical frequency \( \Gamma_2 \). The analysis of the penetration depth dependence of \( \Gamma_1 \) and \( \Gamma_2 \) allows the model free determination of the near wall shear rate and of the slip length with unsurpassed resolution.

Depletion interaction in flowing suspensions:
Dispersions of colloidal mixtures play a crucial role in a wide variety of technical applications and biological systems spanning drilling fluids and blood. Due to entropic excluded volume effects, depletion interactions between particles and confining walls are introduced, which have been widely studied for systems at rest.

Figure 1 top: measured TACFS \( g_m(t) \) at different penetration depths of the evanescent field (points) and best fits with a theoretical expression (lines). bottom: rates \( \Gamma_1 \) and \( \Gamma_2 \) as a function of the penetration depth.

However in technology or biology, systems at rest are rather the exception than the rule. It is therefore of crucial importance to understand how depletion interactions, induced by non-spherical colloids, are affected by shear flow.
character of rotational dynamics of the depletant changes with increasing concentration. It is therefore necessary to better understand the rotational dynamics of colloidal particles close to interfaces at rest and under shear. As a first step in this direction we investigated

Near wall rotational dynamics of colloidal spheres:
Theoretical predictions for the near wall rotational diffusion coefficients $D_{rot,p}$ and $D_{rot,n}$ (were the subscripts identify the orientation of the rotational axis with respect to the wall) have been available for about two decades, however to date they lack experimental verification. We have improved our EWDS setup to allow for the measurement of TACF in VH-geometry, which is a prerequisite to determine rotational diffusion coefficients. Further we have developed the necessary theoretical framework to relate the initial slope of the VH-TACF, $\Gamma$, to the rotational diffusion constants. As a final result, we found that rotational contribution to $\Gamma$ should depend linearly on the square of the normal component of the scattered light’s polarisation vector, $n^2_z$.

With this we could verify the theoretical predictions for the rotational diffusion coefficients by depolarized EWDS experiments, as it is shown in Figure 4. There we compare experimental data to parameter free theoretical predictions.

From the slopes and the intersects of linear fits to the data the rotational diffusion coefficients can be calculated, which agree with theoretical predictions within error bars.

1. B. Loppinet, P. R. Lang European patent application no. 10 162191.0 pending.
Probing the (hydro-) dynamic interactions of tunable colloidal rods

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The diffusive dynamics of rod-like colloids in the isotropic, nematic and smectic phase depends on the length, thickness, and stiffness of the colloidal particles. By chemical modification of virus particles, we systematically vary these parameters, and are able to infer the role of direct and hydrodynamic interactions. The role of hydrodynamic interactions on the diffusion rate in the nematic phase is tuned by using charged rod-like viruses and viruses coated with a brush of polymers in experiments, and with rods that mimic these systems in full hydrodynamic simulations. We show how the rod dynamics is influenced by the rod stiffness using viruses with different flexibility, which explains the shift in the nematic-smectic phase boundary. We also explored the two quite different "living systems", PB-PEO block-copolymer wormlike micelles and actin filaments in shear flow ("living" refers in this context to the scission and recombination of filaments).

The dynamical behaviour of colloidal particles in complex environments is of interest for a large variety of soft-matter and biological systems. It is set by the steric interactions between particles, i.e. the structure of the rods, the hydrodynamic interactions (HI) between the particles, i.e. the friction mediated by the solvent, and external fields like shear flow [1].

One of the most fundamental examples of dynamics in a structured environment is the diffusion of colloidal rods in the nematic phase, where the rods have an averaged alignment but no special ordering. We independently probe the effect of hydrodynamic interactions and steric interactions. Both experiments and simulations are performed [2]. In experiments wild-type fd-virus (fd-wt) is used, which are slender monodisperse particles with a contour length of 0.88 µm and a persistence length of 3 µm. The diffusion of the rods is monitored by labelling viruses with fluorescent molecules in a ratio of 1:10000. The fluorescent tracer viruses are tracked by fluorescence video microscopy. The relative importance of direct interactions and HI is tuned in experiments by varying the range of electrostatic interactions and grafting the rods with a hydrodynamically impenetrable polymer brush. In the simulations, HI is incorporated by embedding rods in a particle-based mesoscopic solvent, where HI can be also switched off. Repulsive rods are mimicked by long-range, repulsive Lennard-Jones spheres around the beads that constitute the rod, which are permeable for solvent and with a diameter of twice that of the beads. The principle idea is sketched on the right in Figure 1.

First, we show that the dynamics in the isotropic phase is governed by steric interactions, and that HI cause a slowing down of the diffusion by at most 30%. Second, in the nematic phase it can be clearly seen that repulsive rods, which cannot come into close contact, diffuse faster than rods that can come in close contact. HI are enhanced because they take place over the full length of the aligned rods. As a result, HI causes a slowing down of the diffusion by up to a factor of two. The range of the HI is about twice the thickness of the rods. This we can conclude from the fact that the diffusion rates as found from simulations without HI and with the permeable layer around the rods (green and red symbols in Figure 1) are almost the same.

Figure 1: The experiment (solid symbols) and simulated (open symbols) total diffusion rate constant as a function of volume fraction, where the grey stripe indicates the phase boundary between isotropic and nematic phase. The lines are guides to the eye. On the right: a schematic representation of the tuning of hydrodynamic and direct interactions for simulations (dashed lines) and experiments (solid lines).
From this dataset we also find that the anisotropy of the diffusion, i.e. the relative motion parallel and perpendicular to the director, strongly depends on the aspect ratio of the rods. For low aspect ratio the anisotropy is lower than for high-aspect ratio rods.

Another steric contribution to the diffusion and phase behaviour of rod-like colloids is due to the rod-stiffness. We accessed the effect of stiffness by comparing fd-wt with the much stiffer fd-Y21M (persistence length of 12 μm) [3]. This virus has a single point mutation in the amino-acid sequence of the major coat protein compared to fd-wt, which turns out to greatly enhance the stiffness. We found that fd-Y21M displays a broad concentration range where the smectic phase (one-dimensional ordering of layers of a liquid of rods) is stable. For the more flexible fd-wt the smectic phase is almost completely suppressed: there is a very small concentration region between nematic and columnar phase (two-dimensional ordering) where the smectic phase is found. Both types of particles display a hopping behaviour, where diffusion takes place via discrete jumps between the adjacent layers. Interestingly, however, the diffusion within the layer is almost completely suppressed in case of the flexible fd-wt, while the stiff fd-Y21M is also very mobile within the layer, see Figure 2. This is a clear dynamic signature for the suppression of the smectic phase, since the quenched dynamics within the smectic layers for the more flexible fd-wt hints to the proximity of the columnar phase, where the position of rods in the direction perpendicular to the long axis is fixed.

The morphology of the rod-like system also plays an important role in the flow behaviour of dispersions of colloidal rods. In the past we have studied the almost ideal fd-wt and PB-PEO block-copolymer wormlike micelles. For the latter system we combined rheological dynamical test with time-resolved small angle neutron scattering (SANS) experiments to find a link between phase behaviour and the collective rotational dynamics of the system, close to the isotropic-nematic phase transition [4]. For this polydisperse but relatively stiff system (persistence length of 500 nm, 29 nm diameter) we find, surprisingly, a very good match between theory for monodisperse hard-core rods and experiment, only using the location of the spinodal point as a fit parameter. In order to increase complexity in a controlled way, we tuned the PB-PEO system by addition of DMF. Using a combination of (dynamic) light scattering, SANS, and fluorescent microscopy we managed to have a full characterization of the system ranging from the contour-length distribution and persistence length to the aggregation number per unit length. As a function of increasing DMF concentration, we see that the worms become shorter, thinner and more flexible. This study allows us to assess the effect of these parameters on the phase and flow behaviour [5].

Due to the small value of the persistence length of PB-PEO as compared to the optical resolution of the optical microscope, it is not possible for the PB-PEO wormlike micelles to study the response of singles worms to shear flow. For this study we rely on very stiff actin filaments, which can also be considered as living polymers. We study these filaments in a counter-rotating cone-plate shear cell, mounted on top of an ultra-fast confocal microscope. Again, as with the fd-wt experiments, we use only a few labelled filaments in a background of many unlabelled filaments. Here we analyze the accessible microscopic parameters, like orientation of the filaments, contour lengths and filament curvature under the influence of shear deformation. These measurements will enable us to understand the viscoelastic properties of sheared actin solution on a microscopic level. For both F-actin and wormlike micelles we also plan to further exploit the ‘living’ character of the system.


Figure 2 : Displacement of the tracked particle, see inset, in the directions parallel (z, in red) and perpendicular (x, in black), to the normal to the smectic layers. The trace clearly shows the jumps between adjacent smectic layers and the self-diffusion occurring within the layers. Inset: Overlay of the DIC image and the trajectory of a virus tracked by fluorescence microscopy in the smectic phase ([fd-Y21M] = 96.7 mg/ml). The scale bar represents 2 μm.
Non-equilibrium states and dynamics in concentrated dispersions of charged rod-like colloids in electric fields

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ICS-3: Soft Matter

Experiments on concentrated suspensions of charged fibrous viruses (fd) in alternating external electric fields reveal various phases and dynamical states, and a “non-equilibrium critical point”. The observed phenomena are the result of field-induced rod-rod interactions, resulting from polarization charges and electro-osmotic flow. The phases and states are characterized with birefringence, vertically-aligned dynamic light scattering and polarization microscopy. The critical (and off-critical) divergence of a length- and time-scale have been determined by video-correlation spectroscopy. In addition we found non-diffusive behaviour at relative high frequencies of the external field within a field-induced uniform, homeotropic phase.

Very little is known about the response of concentrated dispersions of charged colloids to external electric fields at low frequencies, where the double layers and the layer of condensed ions are polarized, and where electro-osmotic flow is induced. We found that dispersions of rod-like colloids (fd viruses) which are in the isotropic-nematic two-phase region without the electric field exhibit a rich phase/state behaviour. The phase/state diagram is shown in Figure 1, together with the depolarized optical morphologies seen in the various phases/states [1]. We chose the fd-concentration such that there is an isotropic-nematic coexistence in the absence of the field (the N-phase). On increasing the field amplitude at low frequencies a chiral-nematic N*-phase is induced. On further increasing the amplitude a dynamical state (the D-state) is induced. In this state, the nematic domains melt and reform. At high frequencies the so-called H-phase is induced, which is a uniform homeotropically aligned nematic phase. The solid lines in Figure 1 indicate the sharp transitions between these phases/states, while the thick dotted lines indicate gradually changes in the overall morphology (which we will not discuss here). The almost horizontal lines at low frequencies are measured transition lines, while the corresponding lower lines are the transition lines that are corrected for electrode polarization (electrode polarization is important only for frequencies less than about 70 Hz).

With our specially designed small angle dynamic light scattering- and birefringence set up [2], we found that diffusion coefficients as well as the nematic orientational order parameter are independent on the electric-field amplitude and frequency throughout the entire H-phase [3,4]. This hints to the possibility that electro-osmotic flow stabilizes the H-phase. Orientational order in the H-phase depends, however, strongly on the fd-virus concentration [3].

There is a point in the phase/state diagram where various transition lines meet, which resembles that of a critical point in an equilibrium phase diagram. On approach of this point from the side of the H-phase, dynamic light scattering correlation functions develop a very slow mode, on top of the diffusive modes that also exist away from the critical point. Critical divergencies on approach from the H-phase are not yet systematically investigated. Surprisingly, this point also exhibits critical behaviour on approach from the side of the dynamical state (the D-state in Figure 1), in the sense that a length- and time-scale diverge [4]. The length scale is the nematic domain size, and the time scale is the time on which melting and forming of nematic domains occurs. The latter is measured by video correlation spectroscopy. The critical divergencies (also for off-critical approach of the N*-to-D transition line) have been fully characterized [5].
Performing dynamic light scattering experiments on approach of the N*-to-H transition line, we found an anomalous increase of the diffusion coefficient for motion perpendicular to the director (see Figure 2). Microscopy shows that this is due to the presence of large, blurry domains with a typical life time of minutes. These domains are present within the H-phase, about 100 Hz away from the transition line (the pink region in Figure 2). On crossing the N*-to-H transition line, just inside the N*-phase, very large “soliton-like structures” develop over a time of several tens of minutes (see the second image from the left in the upper panel in Figure 2). It is still unclear why these blurry domains exist, and why the soliton-like fibers are formed.

Within the H-phase we find “anomalous diffusion”, in the sense that relaxation rates of scattered intensity auto correlation functions do not vary like the wave vector q squared, depending on the fd-concentration and the ionic strength [4]. For larger fd-concentrations and lower ionic strengths, a linear q-dependence of relaxation rates is found (see Figure 3). It thus seems that when interactions are particularly strong, anomalous diffusion occurs. This is not yet understood.

Figure 2: The pink region indicates the region where very large, blurry transient domains exist within the otherwise uniform H-phase (such a domain can be seen in the third image from the left). The blurry domains give rise to an apparent increase of the measured diffusion coefficient as can be seen in the plot. The scale bar in the images is 200 micron.

As a first step toward the understanding of the origin of the electric field-induced phases and the dynamical state, the observed critical divergencies, as well as the anomalous diffusive behaviour, we theoretically analyzed the polarization charges of single rods in alternating electric fields [5]. The rod-rod interactions resulting from these polarization charges can now be calculated, and can be used as an input to kinetic equations which can be integrated to obtain equations of motion for the relevant macroscopic variables.

Thermal diffusion of stiff rod-like mutant Y21M fd-virus

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It turns out that a simple non-equilibrium environment created by a temperature gradient can be used to monitor the reaction kinetics of large proteins with small substrate molecules. This is probably caused by a change in the hydration layer of the protein which is influenced by subtle conformational changes induced by the binding substrate molecule. To obtain a better understanding of the shape and charge influence on the observed behaviour, we studied the thermal diffusion behaviour of a stiff rod-like mutant Y21M fd-virus at low and high salt content.

Recently, microscale thermophoresis has been used to analyse interactions of proteins or small molecules in biological liquids such as blood serum or cell lysate. This technique is based on thermal diffusion, which describes the mass flow induced by a temperature gradient. Due to its practical importance especially for biological molecules it is important to understand how the molecule size, shape, charge and hydration shell influence the thermophoretic motion. Therefore, a study of the thermal diffusion behaviour of biological relevant small [1] and large molecules [2] is essential.

Therefore, we investigated the thermal diffusion phenomena of a mutant filamentous bacteriophage fd-Y21M with a length of 910 nm and a diameter of 6.6 nm [2]. The wild-type fd-viruses have been widely used as a robust colloidal model system, which has the big to be rather monodisperse and having identical structures with the same physical parameters such as mass, length, and diameter. We investigated the virus in two different buffers of tris- (hydroxymethyl)aminomethane (TRIS), one with low salt content (2 mM TRIS-HCl buffer adjusted to pH 8.2) and another one with higher salt content (20 mM TRIS-HCl+100mM NaCl adjusted to pH 8.2).

To assure that all experiments relate to the isotropic phase, we characterized qualitatively the isotropic/nematic phase transition of fd-Y21M in both high salt (hs) and low salt (ls) buffers. In the experiment, we placed fd-Y21M solutions of different concentrations between two crossed polarizers (c.f. Figure 1). We started from a high-concentrated fd-Y21M solution and diluted the solution until we reached the isotropic phase. The object of this experiment is not to determine accurately the isotropic/nematic phase transition but to select the range of fd-Y21M concentrations that are in the isotropic phase. In Figure 1a, the results for the ls buffer are shown. As it can be observed, the isotropic/nematic phase transition is at ~3 mg/mL. Therefore, to be completely sure that we performed the experiments in the isotropic phase, we decided to study the system at concentrations up to 2 mg/mL. In Figure 1b, the results for the hs buffer are shown. In this case, the isotropic/nematic phase transition is ~13 mg/mL, which is in good agreement with the literature value of 13.9 mg/mL.

![Figure 1](image-url): (a) Isotropic/nematic phase transition for fd-Y21M at low salt content in a 2 mM Tris buffer and (b) at high salt content in a 20 mM Tris+100mM NaCl buffer at room temperature. At low salt content the fd-concentrations are (a) 6.5, (b) 5.2, (c) 3.7, (d) 3.0, and (e) 2.9 mg/mL and at high salt content (a) 19.8, (b) 15.0, (c) 13.5, (d) 13.1, and (e) 12.4 mg/mL. The images were taken between crossed polarizers.

All measurements have been performed with an improved infrared thermal-diffusion-forced Rayleigh scattering (IR-TDFRS) setup optimized for measurements of slowly diffusing systems such as the fd-Y21M. The usage of an IR-laser for heating the sample has the big advantage that no light absorbing dye is needed for aqueous mixtures.
In Figure 2, the thermal diffusion, collective diffusion, and Soret coefficients are shown as a function with increasing temperature. In ls solutions, $D_T$ is always positive in the studied range of temperatures, which means that fd-Y21M moves to the cold side. To some extent, the temperature dependence of $D_T$ is influenced by the viscosity change with temperature. Additionally, changes in the interactions with temperature influence the thermal diffusion behaviour. This becomes even more evident for the hs solutions, where $D_T$ changes its sign around 26 °C. At temperatures <26 °C, $D_T$ is negative, meaning that fd-Y21M moves to the warm side, whereas at temperatures >26 °C, $D_T$ is positive, which means that fd-Y21M moves to the cold region. The physical mechanism of this sign change with temperature is probably related to the fact that the system minimizes its free energy at low temperature by forming hydrogen bonds, while the system minimizes the free energy at high temperatures by maximizing the entropy.

The addition of salt has a similar effect as lowering the temperature. This induces a higher order in the inner and outer hydration cell around the ions and leads also to a lower $D_T$, so the reduction of the temperature as well as the addition of salt results in a higher structure of the water molecules, which leads to a more thermophilic behaviour of the fd-Y21M virus. The increasing salt content at constant fd-Y21M concentration as well as an increase in the temperature will decrease the structure between the rod like particles. Regarding these two aspects, we do not find a correlation with the thermal diffusion coefficient. Whereas $D_T$ increases with increasing temperature, it decreases with increasing salt content. Therefore, we conclude that the structural changes of the solvent surrounding the solute particles have a larger influence on the thermal diffusion behaviour than the interactions between the solute particles. This implies that the interparticle interactions, which are certainly present because all investigated concentrations are well above the overlap concentration, influence the dynamics of the solution but are less important for the thermal diffusion behaviour of the system. This finding confirms the importance of the solvent structure on the thermal diffusion.

Considering the collective diffusion coefficient, as one can expect, it increases with increasing temperature because of the decreasing viscosity with increasing temperature. Keeping the temperature constant, $D$ decreases by approximately 60-70% because of the added salt. As expected for this slow-diffusing charged rod-like particle, the determined Soret coefficients are almost three orders of magnitude larger than the values obtained for low-molecular-weight mixtures, but the values are comparable to those of other biopolymers such as DNA. Although the Soret coefficient is fairly large, the expected concentration separation in our setup, the IR-TDFRS, is only in the order of $\Delta c = 10^{-4}$mg/mL because of the small temperature difference in the order of $\Delta T = 100 \mu$K.


High pressure microscopy cell up to 100MPa

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Microscopy at high pressures is interesting for several reasons. For example it is a versatile tool for investigating the microbial inactivation in favor over thermal treatment as pressure does not produce so many unwanted chemical reactions. In situ observations of this process are indispensable. Other promising fields of application are in situ observations of cellular systems and dynamic studies of reacting systems by spectroscopic investigations, where especially modern fluorescence techniques play an important role.

In order to show the applicability of a high pressure microscopy cell that we recently designed and build, we have performed two kinds of investigations: Particle tracking from a colloidal suspension and fluorescence correlation spectroscopy. Using video microscopic imaging techniques, submicron spheres can be located typically to within tens of nanometres in the focal plane. Thus, particle tracking can give information on the mean square displacement of particles, provided the particles exact positions and trajectories can be safely obtained from the video sequences. Then we can relate the measured displacement \( r(t) - r(0) \) to the self-diffusion coefficient \( D \) via the Einstein–Smoluchowski equation

\[
\langle r(t) - r(0) \rangle^2 = 2dDt
\]

where \( d \) is the dimension of the system and \( D \) the self-diffusion coefficient. We observe by a particle tracking algorithm colloidal particles only in a confocal xy-plane, thus \( d=2 \).

Our test system to demonstrate the applicability of our set-up for high pressure experiments is a 0.02wt% dilute solution of stearyl coated silica spheres of about 0.1µm diameter in water/ethanol mixtures measured in the transmission mode. From previous measurements of the viscosity of these silica spheres in toluene as a function of pressure, we have shown that the silica sphere is a perfect test particle to map the solvent’s viscosity because its size is not pressure dependent [1] and can thus give access to the self-diffusion coefficient by applying the Stokes–Einstein relation:

\[
D(P) = \frac{kT}{6\pi\eta_r(P)r}
\]

Here \( r \) is the radius of the diffusing particle and \( \eta_r(P) \) is the pressure dependent viscosity. \( kT \) has the usual meaning. For a water/ethanol mixture it is known that \( D \) exhibits a large pressure effect depending on the water to ethanol ratio. This reflects, contrary to regular liquids, like toluene for example, the anomaly of the water viscosity and is explained in terms of the special water structure possessing hydrogen bonds. Specifically, within our observed pressure range the viscosity and hence the diffusion coefficient of water at ambient temperature does not depend on pressure. In fact, the viscosity may even exhibit a minimum depending on pressure and temperature and reflects thus the water structure anomaly. Adding more and more ethanol to water breaks the water structures based on hydrogen bonding and leads in the end to the viscosity/pressure behaviour of regular liquids, being intuitively characterized by a decrease of the viscosity with increasing pressure. For pure ethanol the decrease of \( D \) between 1 and 1000bar is about 40%. For a 50/50 mixture of water and ethanol it is still 20%.

We have chosen the 100% ethanol and 50/50 mixture of water and ethanol as examples to show the feasibility of our data evaluation and hence applicability of our high pressure microscopy device.

![Figure 1: The mean squared displacement as a function of time for various pressures, as indicated in the figure.](image)

In Figure 1 above our data are shown for the 100% ethanol sample. From initial slopes of the mean square displacement versus time, we obtain \( D \) using eq.1. We have disregarded the first point in all cases because there experimental artefacts can occur obscuring a sensible data analysis. The resulting diffusion coefficients versus pressure are plotted in Figure 2 for 50/50 ethanol/water. In comparison to our data, indicated by particle tracking, the expected behaviour of \( D(P) \) is shown using \( \eta(P) \) data from literature and using the value of \( D(P=1bar) \), which we have measured by dynamic light scattering.
We see that our method reproduces the correct trend and is capable to determine D within an accuracy of ±5%. Our method works sufficiently well and can give access to MSD and hence D under pressure, which is otherwise difficult to obtain. The other problem mentioned at the beginning is the evaluation of data obtained in the transmitted confocal image. This offers in principle the possibility to use our set-up for fluorescence correlation spectroscopy (FCS) measurements under high pressures. To obtain quantitative results with FCS the optics has to be well defined because otherwise the determination of the confocal volume is not possible and hence the determination of the self-diffusion coefficient is obscured. We have shown here that indeed the expected resolution, as can be calculated from the numerical aperture of the objective, is obtained despite of the diamond window, which is necessary to use for the high pressure cell. Clearly also a relatively small distance between the objective and the cell is set, however, with an air gap, possibly disturbing the conditions for FCS. To demonstrate that such a device allows nevertheless for FCS measurements we have performed an experiment using a Zeiss Confocor set-up. The free dye Rhodamine in a low concentration to assure single molecule detection was used. The result is shown in Figure 3, where we compare the correlation functions obtained from Rhodamine taken with the objective Olympus 40x, with NA=0.55 equipped with a correction ring for thick cover-glass, the same as used for video-microscopy. We find quantitative agreement between the data taken from a thin glass plate and the diamond, however, the count rate was different, as can be visualized by the different counting statistics shown in Figure 3. We have optimized each data set to the maximum in counts /molecule. The analytical expression for the fluorescence correlation function $G_D(\tau)$ using a most simple model i.e. free diffusion of small fluorescent dye-molecule is given by:

$$G_D(\tau) = \frac{1}{N} \left[ A \left( 1 + \frac{\tau}{\tau_D} \right)^{-1} \left( 1 + \frac{\tau}{\tau_K} \right)^{-1/2} \right]$$

Here the indices D refer to diffusion, A is the amplitude, $\tau_D$ the characteristic time and N is the number of fluorescing particles in the confocal volume. $\tau_K$ is the structure parameter. Fitting eq.3 to both experimental data sets as shown in Figure 3, we find a diffusion time $\tau_D \approx 84 \mu s$ and a structure parameter about 28, all parameters for both cases equal within the experimental error, except N of course. This is thus the adjustable parameter to match these two data sets shown in Figure 3. The important message here is that the shape of the correlation function is not affected, whether we measure thin plate or diamond with large cover glass correction.

A structure parameter of about 28 as obtained by fitting eq.3 to the experimental data means that the z component is enlarged; however, the definition of the xy plane still seems to hold, which is argued by Egner and Hell. They claim that this definition works best the closer is the distances from the surface. Deeper into the sample deviations in the experimental data, specifically in the single diffusion process, from eq.3 are visible, because then the correction would have caused severe distortions leading to an unphysical additional slow process (data not shown). The shape invariance shown in Figure 3 is the determining criterion for the suitability for FCS measurements. Thus we conclude here that the correction for thick cover glass up to 2.5mm compensates the thin diamond, which has a large index of refraction instead.

![Figure 2: The translational diffusion coefficient as a function of the applied pressure](image)

![Figure 3: FCS correlation functions for different cover-glasses.](image)

Dynamics and phase behaviour of dispersions of proteins and colloids

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Dispersions of charged particles are ubiquitously found in a broad range of particle sizes, from micron-sized colloids to nano-sized proteins. We report our recent work on the dynamics of crowded solutions of globular bovine serum albumin proteins, and dispersions of charged Gibbsite platelets. Scattering experiments and rheological measurements were compared to results obtained using our newly developed theoretical methods of great analytic simplicity. The measured static and dynamic properties are reproduced by these methods with an at least semi-quantitative accuracy. In addition, we have studied theoretically and experimentally the phase behaviour of aqueous lysozyme protein solutions in the presence of additives, and the liquid-crystal freezing lines in suspensions of charged colloidal silica spheres.

In a theoretical-experimental project, we have studied collective diffusion, and the steady-state and high-frequency shear viscosities in concentrated solutions of BSA proteins, as functions of protein and added salt concentrations [1]. Data obtained from dynamic light scattering and rheometry have been compared to theoretical calculations based on an analytically treatable spheroid model of BSA with isotropic screened Coulomb interactions. The only input to the dynamic calculations is the static structure factor which we have obtained from a consistent theoretical fit of small-angle x-ray scattering data, by invoking a newly developed analytic integral equation scheme [2]. This scheme combines high numerical efficiency with excellent accuracy. The experimentally determined dynamic properties are reproduced theoretically to good accuracy, including a peculiar maximum in the reduced viscosity as a function of the protein concentration. In addition, we have analysed the application range of a generalized Stokes-Einstein relation proposed by Kholodenko and Douglas which connects viscosity, collective diffusion and osmotic compressibility (see Figure 1).

In another project, polarized and depolarized dynamic light scattering experiments on suspensions of charged, flat Gibbsite platelets in the isotropic phase were analysed theoretically [3]. This analysis has allowed us to infer the (long-time) translational collective diffusion coefficient, and the long-time translational and rotational self-diffusion coefficients as functions of the volume fraction \( \Phi \) (see Figure 2).

![Figure 1: Experimental-theoretical test of a generalized Stokes-Einstein (GSE) relation connecting the high-frequency and steady-state solution viscosities \( \eta \) and \( \eta_\infty \) to the collective diffusion coefficient \( d_c \) and to the reduced osmotic compressibility \( S(q \to 0) \). In solutions of low salt content, the GSE relation is violated for non-zero BSA concentrations \( c_p \). From [1].](image1.png)

![Figure 2: Concentration dependence of the reduced collective diffusion coefficient \( d_c \) and the long-time translational self-diffusion coefficient \( d_\infty \). The depicted results have been obtained from a theoretical analysis of the dynamic light scattering data. For more details see [3].](image2.png)
While $d_C$ increases only moderately strongly, both self-diffusion coefficients decline extraordinarily strongly with increasing volume fraction, by two orders of magnitude, in the pre-transitional concentration region before the isotropic-liquid crystal phase transition is reached. This strong decline is explained by, for self-diffusion, pronounced translational-rotational diffusion coupling of the strongly anisometric particles.

In a combined theoretical and experimental effort, we have investigated the phase behaviour of aqueous lysozyme protein solutions in the presence of the additives glycerol and dimethyl sulfoxide (DMSO), and sodium chloride [4]. In biotechnology, additives are often used to modify the protein interactions, in order to stabilize the proteins against denaturation and freezing, or to inhibit protein aggregation. The experimentally observed changes in the metastable gas-liquid (gl) and stable fluid-solid (fs) coexistence lines caused by the additives are overall well captured by our theoretical predictions gained from thermodynamic perturbation theory in combination with the Derjaguin-Landau-Verwey-Overbeck (DLVO) pair potential model for the globular proteins (see Figure 3). Both glycerol and DMSO render the potential more repulsive, while sodium chloride reduces the potential strength.

In reference [5], freezing lines of charge-stabilized suspensions of colloidal spheres interacting by a Yukuwa-type pair potential have been constructed based on the accurate Rogers-Young integral equation scheme for the static structure factor, in conjunction with the empirical Hansen-Verlet rule for the onset of freezing (see Figure 4). The complete set of experimentally relevant two-parameter freezing lines has been obtained and discussed for a broad range of system parameters. The obtained freezing lines have been used to explain an experimentally observed fluid-bcc-fluid re-entrant transition of charged silica spheres suspended in dimethylformamide (DMF).

The selected results presented in this report also serve to demonstrate that theoretical methods developed originally for large globular colloids can be successfully applied, with rather simple modifications, to crowded solutions of small proteins and charged platelets.

The ICS-4 is dedicated to research in molecular and cellular neurobiology, signal transduction, information processing, and cellular biophysics.

In ICS-4, signalling processes are studied at different levels of complexity. We examine the molecular properties of individual signalling molecules, cellular pathways that mediate signalling and adaptation as well as information processing in small neuronal circuits, such as the retina or the olfactory bulb.

Two recent studies are representative for our work. First, we identified the molecular basis of a negative feedback mechanism acting on a G-protein coupled receptor that causes oscillations in the intracellular calcium concentration. Second, we unravelled a new function of HCN1 channels in the retina that is of utmost importance for regular vision. Under light conditions when both rods and cones are active, vision is only possible because HCN1 channels limit the response of rods and thereby save the retinal network from saturation.

In ICS-4, strength has always been the tight cooperation of biologists, chemists, and physicists. Our technical repertoire reaches from molecular biological, biochemical, and biophysical methods to physiological studies in vitro and in vivo in normal and in transgenic animals. Fluorescence-based optical methods and imaging play an important role in our research efforts. To monitor the concentration of intracellular messengers like calcium or cAMP, imaging techniques have been established, including two-photon fluorescence-lifetime imaging microscopy, single molecule fluorescence spectroscopy as well as camera-based ratiometric imaging. In addition to synthetic fluorescent dyes we employ genetically encoded sensors based on fluorescent proteins.

We envision that applying these different techniques and strategies to study cellular processes with high spatial and temporal resolution provides the basis for an in-depth understanding of the “physics of the cell”.
Research Highlights

HCN1 channels are essential for regular mesopic cone vision

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In retinal photoreception, the highly light-sensitive rods respond under dim light conditions (scotopic vision) but saturate under bright light conditions, whereas the less sensitive cones require stronger stimuli and still function under bright light (photopic) conditions. Both photoreceptor types contribute to vision under intermediate (mesopic) light conditions. Anatomically, the major rod and cone pathways in the retina are well established (Fig. 1A, B). Cones connect to ON- and OFF-cone bipolar cells, which in turn connect to ON- and OFF-ganglion cells. Rod signals feed into both ON- and OFF-channels in the inner retina via rod bipolar and All-amacrine cells (primary rod pathway, Fig. 1A), or via cone bipolar cells that rods access through gap junctional connections between rods and cones in the outer retina (secondary rod pathway, Fig. 1B). While the connections between rod and cone pathways have been verified using anatomical and electrophysiological approaches in vitro, the functional consequences for vision have not been fully elucidated. Here we show that proper cone vision under mesopic conditions requires rapid adaptational feedback modulation of rod output via hyper-polarization-activated and cyclic nucleotide-gated channels 1 (HCN1). When these channels are absent, rods display sustained responses that saturate the retinal network and lead to the entire loss of downstream rod and cone signalling [1].

Responses to mesopic flicker stimuli are strongly compromised in HCN1-/ mice

In rod photoreceptors, stimulation by light leads to the closure of cyclic nucleotide-gated channels and, subsequently, to hyperpolarization of the membrane. After a short delay hyperpolarization-activated and cyclic nucleotide-gated channels 1 (HCN1) located in the membrane of rod inner segments [2,3] conduct a sodium influx that drives the membrane potential back towards the dark state. The result is a substantial, but not complete, reduction of rod output (i.e. an increase in synaptic activity). We studied mesopic light responses in normal mice (wt) and in mice deficient in HCN1 channels (HCN1-/).
reduced the amplitude of the current deflection. At 20 Hz only the sustained outward current remained. The offset of the flicker stimulus elicited a pronounced inward current (OFF-response). In the OFF-GC of HCN1−/− retinae (Fig. 2A, middle panel), the transient outward current at stimulus onset was similar to that of wt OFF-GCs. However, already at 3 Hz, light-induced current fluctuations were strongly diminished and only the sustained outward current persisted. Moreover, we could not record an OFF-response at the offset of the flicker stimulus. Upon stimulus offset, it took roughly 3 s before the outward current had completely returned to the holding level (data not shown). ON-GCs of the HCN1−/− retina had a comparably reduced ability to follow flicker stimuli (data not shown). In current clamp recordings, wt cells responded with bursts of action potentials to each flash of a mesopic flicker stimulus (Fig. 2B, left panel). In contrast, in HCN1−/− retinae the same stimulus elicited tonic inhibition in OFF-GCs and unmodulated long lasting excitation in ON-GCs (Fig. 2B, right panel).

In summary, our experiments identify rods as the source of saturating responses in the HCN1−/− retina. We show that HCN1 channels in rod photoreceptors are essential for normal vision under mesopic conditions as they preclude saturation of the retinal network by prolonged rod responses.

Responses to mesopic flicker stimuli in HCN1−/− mice are restored upon ablation of rod signals or following disconnection of rod pathways

We hypothesized that in HCN1−/− retinae, rods exert exaggerated and sustained light responses that saturate the retinal network, obscuring all light responses. We tested this hypothesis by crossbreeding HCN1−/− mice with two other mutant strains. First, in HCN1−/− rhodopsin−/− mice, rod responses were ablated and, therefore, saturation could not occur. In agreement with our hypothesis, mesopic light responses were fully restored in these animals, despite the fact that HCN1 channels were lacking (Fig. 2A, right panel). Second, in HCN1−/− connexin36−/− mice, saturating input from the secondary rod pathway as well as from the primary rod pathway to the ON-channel was disconnected, as all gap junctions participating in these pathways rest on functional connexin36 (see Fig. 1). As a consequence, mesopic light responses in the ON-channel were fully restored in these animals. In contrast, mesopic light responses in the OFF-channel were still compromised, as the input from the primary rod pathway into the OFF-pathway is mediated by a chemical synapse, rather than a gap junction (see Fig. 1A). We, therefore, additionally blocked the primary rod pathway pharmacologically using L-APB (Fig. 3). Under these conditions, both primary and secondary rod pathways were disconnected and mesopic light responses in the OFF-channel were fully restored.

FIG. 2: Light responses to mesopic flicker stimuli of voltage-clamped OFF-ganglion cells (A) and current clamped ON-ganglion cells (B) in different mouse strains. In wt cells, mesopic flicker stimuli are well resolved. In HCN1−/− responses are saturated and unmodulated. Additional knock-out of rhodopsin restores light responses (A, right panel).

HCN channels are differentially expressed in the olfactory system

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ICS-4: Cellular Biophysics

In the olfactory bulb, input from olfactory receptor neurons is processed by neuronal networks before signals are relayed to higher brain regions. Information processing starts in the glomeruli, in which signals coming from receptor neurons are modified by input from a heterogeneous group of juxtaglomerular cells. In many neurons, hyperpolarisation-activated and cyclic nucleotide-gated (HCN) channels generate and control oscillations of the membrane potential. Oscillations also appear crucial for information processing in the olfactory bulb. Four channel isoforms exist (HCN1 - HCN4) that can form homo- or hetero-mers. Here, we describe the expression pattern of HCN isoforms in the olfactory bulb of mice using a set of antibodies against all four isoforms. HCN channels can be detected in most cell populations identified by marker antibodies. The combination of stainings with marker and HCN antibodies revealed at least 17 different staining patterns in juxtaglomerular cells. HCN isoforms give rise to an unexpected wealth of co-expression patterns. Our results suggest that HCN channels play an important role in olfactory information processing. The staining patterns are consistent with the possibility that both homomeric and hetero-meric HCN channels are involved in oscillations of the membrane potential of juxtaglomerular cells [1].

HCN isoforms are expressed in many different combinations

HCN channel isoforms are differentially distributed in the olfactory bulb

The distribution of HCN channels in the olfactory bulb was examined with 12 different antibodies against the four HCN channel isoforms. Each HCN isoform was recognized by three antibodies generated in different species. In addition, for each HCN isoform, two antibodies were generated against different epitopes. Each HCN isoform showed a characteristic expression pattern in the different layers of the olfactory bulb (Fig.1A-D). HCN1 was strongly expressed in the glomerular layer (GL), followed by some weaker expression in the internal plexiform layer (IPL) and the granule cell layer (Grl). Only weak staining was observed in the external plexiform layer (EPL). For HCN2, the most intense staining was observed in individual cell bodies distributed across all but the olfactory nerve layer (AL). HCN3 was strongly expressed in the IPL and the outer part of the EPL. Strong HCN4 staining was observed in all layers; in the AL, it appeared to be the only HCN isoform.

HCN isoforms are expressed in many different combinations

In the GL, antibodies against all HCN isoforms preferentially stained neurons because the gli-specific marker GFAP did not co-localize with HCN staining. Cells stained by HCN antibodies fell into two groups. The first group was characterized by relatively small somata and thin dendritic processes. This group comprised both periglomerular (PG) and short axon (SA) cells. The second group displayed large somata and usually possessed only one thick primary tuft which ramified within a single glomerulus; these features are characteristic for external tufted (ET) cells.

Triple and double labeling revealed subpopulations of PG- and SA-like cells that expressed either a single HCN isoform (HCN1, HCN2, or HCN4) or combinations of HCN isoforms (HCN1/3 or HCN1/4; Fig. 2). Coexpression of HCN isoforms was observed more often in ET-like cells than in PG- and SA-like cells. The following combinations were detected: HCN1/3, HCN3/4, and HCN1/3/4 (Fig. 3). These results demonstrate for the first time that up to three HCN channel isoforms are expressed within the same neuron. We used antibodies against eight different markers and 12 different antibodies against HCN channel isoforms in more than 140 combinations. We found seventeen distinct and highly reproducible co-localization patterns in different juxtaglomerular cell populations that in the following are referred to as immunohistochemical fingerprints.
An intriguing result of our study is the prominent co-
localization of HCN channel isoforms in
juxtaglomerular cells. Heteromerization could give rise
to functional diversity of HCN channels. HCN channels
may play a key role in neuronal activity e.g. in
rhythmic bursts of action potentials. The large variety
of expression patterns of HCN channels suggests a
functional diversity of juxtaglomerular cells. It will be
a challenge for future work to precisely examine in
quantitative terms the diverse functions of homomeric
and heteromeric HCN channels in the nervous system
and to establish a model of the sophisticated
information processing in the olfactory bulb.

**HCN channels are expressed in axons of olfactory
sensory neurons**

We detected HCN channel isoforms in axons of
olfactory sensory neurons (OSNs). Several lines of
evidence suggest that HCN channels may play a role in
the outgrowth of axons from the olfactory epithelium
[2]. Control of axon growth is of particular interest, as
OSNs have a limited lifetime and are continuously
replaced. Therefore, at each moment numerous axons
of OSNs have to find their way towards their target
glomeruli in the olfactory bulb.

Hyperpolarization-activated and cyclic nucleotide-
gated channels are differentially expressed in
juxtaglomerular cells in the olfactory bulb of mice. Cell

[2] Mobley, A.S., Miller, A.M., Araneda, R.C., Maurer, L.R.,
Müller, F., and Greer, C.A. (2010): Hyperpolarization-
activated cyclic nucleotide-gated channels in olfactory
sensory neurons regulate axon extension and
Control of cellular Ca\textsuperscript{2+}-signaling by a biogenic amine receptor

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Rhythmic activity of cells and cellular networks plays an important role in physiology. In the nervous system oscillations synchronize neuronal activity and are fundamental to cognitive functions in particular to learning and memory. Here we have unraveled the molecular basis of cellular Ca\textsuperscript{2+} oscillations controlled by a biogenic amine receptor (DmOct\textsubscript{α1Rb}) using a combination of pharmacological intervention, site directed mutagenesis, and functional cellular Ca\textsuperscript{2+}-imaging. Activation of the receptor by its ligand triggers Ca\textsuperscript{2+} release from intracellular stores. Phosphorylation of a single amino acid residue in the third intracellular loop of the GPCR by protein kinase C (PKC) is necessary and sufficient to desensitize DmOct\textsubscript{α1Rb}. The receptor is re-sensitized by dephosphorylation giving rise to a new Ca\textsuperscript{2+} signal. Our findings show that transient changes of the receptor’s surface profile have a strong impact on its physiological signaling properties.

Octopamine (OA) is a biogenic amine that binds to specific receptors belonging to the superfamily of GPCR proteins. Physiologically it has been shown that Oct1 receptors cause Ca\textsuperscript{2+} elevation whereas Oct2 receptors lead to cAMP production in cells (Roeder, 2005, Annu. Rev. Entomol. 50, 447‐477). We have previously shown that the DmOct\textsubscript{α1Rb} receptor causes Ca\textsuperscript{2+} oscillations in cells at OA concentrations ≤ 50 nM [1]. For various GPCRs it has been shown that termination of cellular signaling occurs by phosphorylation of the receptor. Re-entry of a desensitized GPCR into a signaling status can be achieved by dephosphorylation of the receptor. Here, we initiated a combined pharmacological and mutagenesis approach to unravel the molecular basis of DmOct\textsubscript{α1Rb} signaling characteristics [2].

Whether DmOct\textsubscript{α1Rb} activity is modulated by kinases was tested with a series of kinase-specific inhibitors. Simultaneous incubation of cells heterologously expressing the receptor with OA and PKC inhibitors led to reproducible and drastically changed Ca\textsuperscript{2+} responses. Blocking phosphorylation of the activated receptor resulted in a continuous Ca\textsuperscript{2+} signal instead of Ca\textsuperscript{2+} oscillations (Fig. 1A). Such responses were not observed with PKA or PKG specific inhibitors. These results suggested an important role for PKC in the desensitization process and thus shut down of DmOct\textsubscript{α1Rb}-mediated signaling. Since under control conditions the receptor could be re-activated in the continuous presence of OA, we examined whether the inhibition of cellular phosphatases had an effect on DmOct\textsubscript{α1Rb} signaling. Simultaneous incubation of DmOct\textsubscript{α1Rb}-expressing cells with OA and phosphatase inhibitors completely abolished Ca\textsuperscript{2+} oscillations (Fig. 1B). From these results we concluded that it was the interplay of receptor phosphorylation and dephosphorylation that was crucial for receptor evoked Ca\textsuperscript{2+} oscillations.

**FIG. 1:** Pharmacological intervention of octopamine induced cellular Ca\textsuperscript{2+} signaling in DmOct\textsubscript{α1Rb}-expressing cells. (A) Incubation of cells with 50 nM OA causes Ca\textsuperscript{2+} oscillations. Co-application of PKC inhibitors (Bis VIII) induces sustained increased levels of intracellular Ca\textsuperscript{2+}. (B) As in (A) incubation of DmOct\textsubscript{α1Rb}-expressing cells with OA causes Ca\textsuperscript{2+} oscillations which are completely abolished in the presence of phosphatase inhibitors (PPi IV).

In order to gain insight into the detailed molecular mechanism(s) governing these signaling events, we
inspected the primary structure of the receptor protein for potential phosphorylation sites. A total of 10 consensus motifs that could be phosphorylated by PKC were uncovered. Based on this bioinformatics approach, a mutagenesis strategy was initiated by which the phosphorylation prone residues were individually substituted for alanine residues. All mutants were transiently expressed in HEK293 cells and the \( \text{Ca}^{2+} \) signals evoked by 50 nM OA were registered. By this approach we identified a single threonine residue (T352) in the third intracellular loop of DmOctα1Rb (Fig. 2) as the target site for PKC-dependent phosphorylation mediating desensitization of the receptor.

**FIG. 2:** Position of amino acid residues in DmOctα1Rb that can be phosphorylated by PKC.

Since transient transfections of cells often suffer from rather inhomogeneous protein expression levels that might increase variability of cellular responses, we generated stably transfected cell lines with either the wildtype (WT) receptor gene or the T352A mutant gene. \( \text{Ca}^{2+} \)-imaging of these cell lines resulted in highly reproducible and homogeneous cellular responses to OA stimulation. Whereas WT receptors showed the typical \( \text{Ca}^{2+} \) oscillations (Fig. 3A) the T352A-mutant expressing cells showed sustained elevated \( \text{Ca}^{2+} \) (Fig. 3B).

**FIG. 3:** \( \text{Ca}^{2+} \)-imaging of cell lines transfected with either the WT (A) or the T352A mutant DmOctα1Rb receptor (B). Upon OA stimulation cells expressing the WT receptor show \( \text{Ca}^{2+} \) oscillations, whereas cells expressing the mutant show a sustained increased level of intracellular \( \text{Ca}^{2+} \).

In summary, we succeeded unraveling the molecular basis of a dynamic cellular signaling process, i.e. \( \text{Ca}^{2+} \) oscillation, down to the modification of a single amino acid residue that is necessary and sufficient to tune the GPCR’s activity profile. It would thus be interesting to examine whether this is a common principle at receptors sharing the same transduction pathway.

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Serotonin’s role in controlling the phototactic behavior of honeybees

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Serotonin (5-HT) is a biogenic amine that plays a key role in modulating physiological and behavioral processes in many species. The main route of 5-HT’s functional activity is exerted by its binding to different members of membrane receptors belonging to the GPCR superfamily. Here we cloned the first 5-HT receptor from the honeybee (Am5-HT1A) sharing high sequence similarity with the 5-HT1 receptor class. Activation of Am5-HT1A by 5-HT inhibited the production of cAMP in a dose-dependent manner. The receptor is highly expressed in brain regions participating in visual information processing. Using in vivo pharmacology, we could show that Am5-HT1A receptor ligands had a strong impact on the phototactic behavior of individual bees. These data mark the first comprehensive study of a 5-HT1A receptor in the honeybee which may allow identifying additional roles of this receptor subtype in the physiology and behavior of this social insect.

The biogenic amine 5-HT acts as a messenger substance in most animal phyla (Thamm and Blenau, 2011, Arthropod. Struct. Dev. 40, 381-394). Disruption of the serotonergic system has been linked to several human disease states, such as schizophrenia, migraine, depression, suicidal behavior, and obsessive-compulsive disorder (Jones and Blackburn, 2002, Pharmacol. Biochem. Behav. 71, 555-568). In the honey-bee, 5-HT is present in large quantities in the CNS. Some differences in 5-HT levels occur independently of age, but may be related to the specific task a bee performs. Current knowledge about 5-HT receptor subtype(s) in the honeybee is rather limited. Except for a 5-HT7 receptor no molecular data on other 5-HT receptors were available, yet. Here, we molecularly identified another member of the 5-HT receptor family, i.e. 5-HT1A. Using in vivo pharmacology and behavioral testing, we were able to show that 5-HT is involved in the regulation of honeybee phototactic behavior and that the Am5-HT1A receptor is a likely mediator of this effect [1].

The primary structure of Am5-HT1A was compared to a variety of 5-HT receptor sequences both of non-vertebrate and vertebrate origin (Fig. 1). From this analysis one can state that the honeybee receptor belongs to a clade containing members of the 5-HT1 subfamily. These receptors share the property to inhibit adenylyl cyclase activity [2], once bound to 5-HT.

In order to unravel whether the activated Am5-HT1A receptor caused a reduction of cAMP production, we generated a cell line constitutively expressing the receptor protein. Incubation of these and non-transfected control cells with an agonist of membrane bound adenylyl cyclases (NKH477) caused an increase in intracellular cAMP. Simultaneous incubation of the cell lines with NKH477 and 5-HT caused a reduction of cAMP production by 60% in Am5-HT1A expressing cells whereas cAMP production remained almost constant in non-transfected cells (Fig. 2A). The effect of 5-HT was dose dependent with an EC50 of ~16.9 nM (Fig. 2B). The effect of 5-HT could be mimicked by known 5-HT receptor agonists like 5-carboxamidotryptamine and 5-methoxytryptamine and was blocked by the antagonists methiothepin, prazosin, and WAY 100635.

FIG. 1: Phylogenetic analysis of Am5-HT1A (white box) and 5-HT receptors from various invertebrate species and human 5-HT receptors. Alignments were performed using the core amino acid sequences without the N- and C-terminus and the variable third cytoplasmic loop. The numbers at the nodes of the branches represent the percentage bootstrap support for each branch. The scale bar allows conversion of branch lengths in the dendrogram to genetic distance between clades.
The cellular distribution of Am5-HT1A was investigated by immunostaining of cryosections of the honeybee brain. Using specific antibodies, the highest labeling intensity was found in brain regions involved in visual information processing: the proximal lamina of the optic lobes and ocellar tracts. Immunoreactivity was also detected in the medulla and lobula of the optic lobes, the lip and basal ring of the mushroom body calyces, the pedunculus and α- and β-lobes of the mushroom bodies (Fig. 3A, B). The staining pattern of Am5-HT1A agrees well with results from radioligand-binding studies that have uncovered a relatively uniform distribution of [3H]5-HT binding sites in each of the three optic ganglia suggesting that 5-HT might modulate visual information processing and visually guided behaviors. Therefore, we have investigated the effects of 5-HT on the phototactic behavior of bees. 5-HT reduced the positive phototactic behavior toward a light source of low intensity (Fig. 3C). This effect could be prevented by the receptor antagonist prazosin. Although striking, the results do not rule out the possibility that additional 5-HT receptors contribute to the observed behavior. This interpretation is corroborated by the fact that the whole repertoire of honeybee 5-HT receptors and their distribution are not known, yet.

In summary, the detailed characterization of the Am5-HT1A receptor provides the basis to examine its functional contribution to honeybee behavior and physiology. So far, we have been able to establish a new role for 5-HT in the modulation of honeybee phototaxis and to provide evidence for Am5-HT1A being a likely mediator of this effect.


Physiological cellular processes rest on the interaction of cellular molecules. A prerequisite to understand such processes is to unravel the dynamics of the participating biomolecules. One challenging task is to determine a molecule’s position with high temporal and spatial resolution. In 2009 we started to implement single molecule localization based super-resolution methods in ICS-4. Here the progress in building an up-to-date super-resolution widefield fluorescence microscope as well as first applications and experimental results are summarized.

Fluorescence microscopy based methods have become indispensable in many areas of biology, especially in cell biology. These techniques are widely used to visualize and precisely localize proteins participating e.g. in cellular signal transduction and communication. Fluorescence images may be obtained from fixed cells or tissue but also from living cells. The ultimate goal of such approaches is to examine a proteins’ localization with high spatial and temporal resolution. However, a general problem of all experimental methods subsists in their limited observation capabilities, i.e., minimal and maximal distances, time periods and signal intensities that are detectable. Abbe’s limit of optical resolution constrains all optical methods thus preventing to spatially resolve most of the subcellular structures like organelles, membrane domains, protein complexes, or cytoskeletal components. Since about five years fluorescent methods subsumed as super-resolution microscopy or nanoscopy have been introduced that circumvent Abbe’s limit of resolution by different means e.g. (F)PALM, SIM, STED, (d)STORM) (Toomre and Bewersdorf, 2010, Annu. Rev. Cell Dev. Biol. 26, 285). These techniques reach unprecedented spatial resolution of biological structures between 10 to 100 nm. A subset of super-resolution microscopy techniques use single molecule detection of fluorescent proteins (FPs) or dye molecules that can be photoswitched or photoconverted (Patterson et al., 2010, Annu. Rev. Phys. Chem. 61, 345; van de Linde et al., 2011, Australian J. Chem. 64, 503).

We started PALM imaging on a home-built wide-field fluorescence microscope existing in our institute. Although images of subcellular structures below Abbe’s limit of resolution were recorded, the performance of the setup did not satisfy our expectations. Therefore we decided to build an improved wide-field fluorescence microscope to record super-resolution images. A schematic drawing of the microscope setup is shown in Fig. 1. The microscope allows applying several laser wavelengths, i.e., 405, 488, 514, 561, and 642 nm. These laser lines can be used individually or simultaneously. The microscope can also be used in ‘Total Internal Reflection Fluorescence’ (TIRF) mode. This allows to specifically excite fluorophores in a rather thin layer (~100-200 nm) of the sample adjacent to the glass-water interface, which significantly reduces background fluorescence originating from fluorophores localized outside of the focal plane.

**FIG. 1:** Super-resolution setup.
Additionally, we implemented a feedback mechanism that corrects for sample drift in the X, Y, and Z-direction. Such feedback correction is indispensable when determining molecule positions on a nm scale. The feedback mechanism is based on continuously monitoring non-fluorescent beads with IR-light. The correction itself is realized via movement of the piezo-stage that holds the sample. Another feature of the setup is the ability to choose the effective pixel size of the camera within the range of 64 to 130 nm (i.e., in combination with a 100x objective). Finally, it is also possible to use two cameras for simultaneous data acquisition to record two-colour images. As both cameras are mounted on a motorized translation stage, we can also apply an offset to one of the cameras, allowing retrieval of 3D information from the recorded data.

Our long-term goal is to determine the distribution of proteins that constitute elements of signalling cascades either in isolated cells or in tissue samples by super-resolution imaging. We decided to follow both approaches (1) dSTORM with secondary antibodies coupled to photoswitchable fluorescent dye molecules and (2) PALM with photoswitchable and photoconvertible FPs fused to the protein of interest. Research projects were initiated to optimize the parameters of dSTORM experiments by imaging tubulin fibres in different mammalian cell lines. In Fig. 2 individual tubulin fibres running in parallel can be seen. The width of an individual fibre has been determined to 40-50 nm, which is well below Abbe’s resolution limit. We also successfully imaged two different subcellular structures in the same cell, i.e., tubulin fibres and endocytotic vesicles, in two colour dSTORM measurements.

Furthermore, the distributions of integral membrane proteins, i.e., cyclic nucleotide-gated ion channels and adenyl cyclases, co-expressed in a genetically modified cell line have been determined. Both membrane proteins form punctuate and cluster-like patches of different sizes (Fig. 3). Whether both proteins are co-localized in these clusters is currently under investigation.

![FIG. 2: Normal wide-field (upper left), reconstructed dSTORM (upper right; scale bar 500 nm) images of tubulin fibres in COS-1 cell and cross-section through four parallel fibres (bottom).](image)

Collaboration with ICS-7: We visualized photoswitchable and photoconvertable FPs fused to proteins that are part of focal adhesions and cytoskeletal structures with PALM experiments. Different FPs were tested and their photophysics and photoconversion have been investigated as well.

Collaboration with ICS-6: One project of ICS-6 focusses on the development of diagnosis tools for Alzheimers’ disease. We labeled primary antibodies against different epitopes of the Aβ protein with various photoswitchable dyes. By applying super-resolution microscopy we started to characterize the number, shape, and size of Aβ oligomers and aggregates in body fluids (blood, CSF). In addition, we performed two-color dSTORM experiments with two antibodies against different epitopes to decipher the composition of Aβ aggregates.

![FIG. 3: Normal wide-field (upper left), reconstructed dSTORM (upper right and lower left; scale bars 10 μm and 500 nm) images of adenyl cyclases (AmAC3) heterologously expressed in HEK293 cells and (lower right; cluster analysis. The latter revealed the existence of small (75 nm) and large (700 nm clusters).](image)
FRET-based genetically encoded biosensors

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FRET-based biosensors employing fluorescent proteins for fluorescent read-out are important tools in life sciences. Various activities in our institute deal with the construction, characterization, optimization, and application of such sensors.

The importance of fluorescent methods in biology and medicine has grown tremendously in the past decades. Genetically encoded fluorescent molecules that became available first as the green fluorescent protein (GFP) opened the way for live cell imaging of single cells, tissues, organs, and whole organisms. Today, a huge number of fluorescent proteins (FPs) covering the whole visible spectrum are available. Also, different tags have been introduced enabling binding of organic dyes to proteins or nucleic acids with high specificity in living cells. Based on these possibilities far more than one hundred fluorescent biosensors have been constructed and applied to decipher components of signaling pathways and their interplay in physiological processes in living cells (Newman et al., 2011, Chem. Rev. 111, 3614). Generally, the sensors couple a sensing element, e.g. a binding domain for the molecule of interest, to one or more fluorophores. Upon binding of the ligand to the sensing element the properties of the fluorophore are altered (e.g. intensity, lifetime, emission or excitation spectrum). These changes are used as a read-out signal for the cellular process under investigation.

One of the first FP-based biosensors was cameleon, a calcium (Ca²⁺) sensor with two GFPs of different color, e.g. CFP and YFP, capable of undergoing Förster resonance energy transfer (FRET) (Miyawaki et al., 1997, Nature 388, 882). The two FPs are linked by a Ca²⁺ binding protein (calmodulin (CaM)) and a CaM binding peptide. The binding of Ca²⁺ ions to CaM causes a conformational change and leads to an altered FRET efficiency. This construction principle became a general motif for most of the FRET-based genetically encoded biosensors that make more than 50% of all biosensors (Newman et al., 2011, Chem. Rev. 111, 3614). Since the first publication in 1997 more than 20 sensors have been published with improved properties like Ca²⁺ binding affinity, dynamic range or response time. Nowadays, genetically encoded calcium sensors (GECIs) are applied in very sophisticated experiments, e.g. chronic imaging of neurons in the brain of living organisms or measurement of local calcium fluxes. The newest developments in this field have been recently reviewed [1].

Photophysical characterization of biosensors is mandatory: The biophysical properties of many FRET-based biosensors are very complex. This rests on the rich and sophisticated photophysics of the used fluorescent proteins and on the often unknown conformational changes that cause the FRET change. Furthermore, rather than to achieve a detailed understanding of the underlying photophysical processes or structural changes of a biosensor, most efforts have been put to obtain sensors with a better performance. The ill-defined knowledge of the origin of a biosensors’ signal can lead to serious misinterpretation of results originating from cellular measurements. We therefore put special emphasis on a detailed photophysical characterization including the fluorescence lifetime of biosensors and FPs used in our institute [2].

A well understood FRET-based biosensor: The fluorescence properties and the protein structure of the FRET-based GECI TN-XXL in the absence and presence of Ca²⁺ has been examined using small angle X-ray scattering, stationary and time-resolved FP and tyrosine fluorescence measurements as well as nuclear magnetic resonance spectroscopy. The results point towards a much closer distance between donor and acceptor in TN-XXL when Ca²⁺ is bound (Fig.1). This leads to a much more efficient FRET reflected in an increased (by > 300%) ratio of acceptor and donor fluorescence intensity and a reduced (by 50%) donor fluorescence lifetime (Fig.1). Such dynamic ranges are

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**FIG. 1:** Donor fluorescence decays (left panel: TN-XXL with non-absorbing acceptor (1), TN-XXL calcium free (2), TN-XXL calcium bound (3), instrument response function (4)), calcium titration of normalized average donor fluorescence lifetime and normalized ratio of acceptor and donor fluorescence intensity (middle panel) and low resolution shapes of TN-XXL obtained from small angle X-ray scattering (right panel).
especially useful for intracellular measurements. However, many FRET-based FP biosensors show much smaller fluorescence changes (only 10 – 20%), which may be caused by the alteration of the orientation of donor and acceptor FP rather than by a distance change. It is noteworthy, that TN-XXL’s change in donor fluorescence lifetime, which was quantified by Ca²⁺ titration (Fig.1), was that large that TN-XXL is recommended as the GECI of choice in fluorescence lifetime imaging applications.

A less well understood FRET-based biosensor:
Although Ca²⁺ plays a central role in cellular physiology, the investigation of other cellular molecules is of interest, too. In collaboration with IBG-1 we have determined the fluorescence properties of FRET-based FP biosensors that “sense” sugars. The fluorescence spectra of a glucose sensor (Deusche et al. 2005, Protein Science 14, 2304) seem to indicate an increase in FRET efficiency upon glucose binding. However, the donor fluorescence decay is almost unchanged. This is not consistent with a simple increase in FRET efficiency. Several lines of experimental approaches are currently followed to solve this obvious discrepancy, including ultrafast fluorescence spectroscopy.

New FPs in biosensors: We have shown by the two previous studies [4] that ECFP, the donor FP in those two but also in most FRET-based biosensors currently in use, should be replaced in the future by better suited FPs because of its complicated photophysics (e.g. an at least 4-exponential fluorescence decay [4]) and the existence of photoconversion reaction(s) upon intense illumination as used in laser-based fluorescence microscopies. There exist several optimized blue GFP-like FPs that will be tested in the future and also different FP classes derived from photoreceptor proteins like phytochromes or LOV-domains. The latter bind non-covalently a flavin chromophore. Certain mutants assemble to FPs with absorption in the near UV-blue and strong emission in the cyan-green part of the spectrum (e.g. FbFP FbFP (Drepper et al., 2007, Nature Biotechnol. 25, 443)). They show a very long and almost mono-exponential fluorescence decay [3]. In a first attempt, FluBO - a FRET-based biosensor (donor: FbFP, acceptor: YFP) - has been constructed, characterized and used to determine intracellular O₂ concentrations in bacteria [3]. Indeed, FluBO turned out to be advantageous for FLIM measurements compared to biosensors with ECFP as a donor.

**FIG. 2:** FRET-based FP biosensor for glucose: Decrease of donor and increase of acceptor fluorescence upon glucose binding indicate an increase of FRET efficiency (left panel). However, the donor fluorescence decay gets slightly slower upon glucose binding pointing towards no change or even a small FRET efficiency decrease (right panel; blue: glucose free; red: glucose bound).

**FIG. 3:** O₂ sensor FluBO [3]: Low concentrations of molecular oxygen prevent chromophore maturation of YFP in FluBO resulting in no FRET. In contrast, under aerobic conditions a strong FRET occurs (upper and lower left panel, the latter depicts the emission spectra of donor (FbFP), acceptor (YFP) and FluBO (λem=440nm)). Fluorescence lifetime images of E.coli expressing only the donor (FbFP, upper right) or the biosensor (FluBO, lower right) both under aerobic conditions pointing towards a non-invasive method to determine O₂ concentrations in individual bacteria and cells.

One ultimate goal in biophysics is to understand the functions of proteins and macromolecular complexes as well as whole cellular processes like signal transduction pathways in their cellular environment. For most biophysical techniques it is difficult or in many cases impossible to apply physical methods to living cells. ICS-5 follows several approaches to achieve this goal:

- Proteins or complexes are isolated, crystallized and high-resolution X-ray structures are determined from ground and intermediate states of their working cycles to obtain a nearly complete time-resolved image of their mechanisms.
- Protein unfolding and refolding as well as the interactions between proteins are studied in aqueous solution and in membranes.
- Cell-free transcription and translation systems in combination with fluorescence single molecule spectroscopy allow us to accompany protein synthesis and folding at the ribosomal machinery.
Research Highlights

Native and unfolded states of PGK studied by single-molecule FRET

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Single molecule Förster resonance energy transfer (FRET) measurements with phosphoglycerate kinase from yeast were performed at different concentrations of guanidine hydrochloride. From these steady-state measurements we obtained FRET efficiency histograms characterizing structural properties of individual proteins at different stages between the native and the fully unfolded state. At 0.5 M GdnHCl an unfolded state population shows up which exhibits a significantly expanded structure as compared to the native state. The unfolded state is characterized by a pronounced broadening of the efficiency distribution, which indicates a large structural and/or dynamical heterogeneity within the population. At high denaturant concentrations, well above the unfolding transition at C1/2 ~ 0.7 M, we observe a progressive expansion of the protein structure, a so-called globule-coil transition.

Since the unfolded state of proteins is rather dynamic and structurally very heterogeneous, the folding process itself exhibits a heterogeneity which is inherently difficult to study with classical ensemble techniques. In this respect single molecule techniques became of increasing importance in recent years because they allow for independent analysis of signals from different subpopulations. In particular single molecule Förster resonance energy transfer (smFRET) is a well suited method to study structural and dynamical properties of individual proteins. Förster resonance energy transfer between two fluorophores bound at different positions at one and the same protein can be used to measure the spatial extension of the protein and related changes thereof upon folding or unfolding. In particular studies on single molecule level offer the advantage to characterize unfolded state populations under conditions where the native state is dominating the ensemble. We performed smFRET studies on a large multi-domain protein, namely the native and unfolded states of phosphoglycerate kinase (PGK) from yeast (Mw: 44.7 kDa). In order to elucidate details of the folding or unfolding pathways, typically multiple intra-molecular distances have to be analyzed. For multi-domain proteins label positions can be chosen in a way that either distances within individual domains are measured or in another way that inter-domain distances are monitored. In the end both types of distances have to be measured to obtain a full picture of the complete folding process. Here we report a first series of smFRET measurements on PGK for which we analyzed inter-domain distances of the native state and evolving unfolded states at progressively increasing GdnHCl concentrations under equilibrium conditions.

Single molecule FRET studies were performed with a double cystein mutant of PGK which is displayed in Figure 1. At positions 135 (located in the N-terminal domain) and at position 290 (located in the C-terminal domain) maleimide functionalized dyes were bound to the protein. The distance between the C₅ positions of both cysteins is about 39 Å. The chosen FRET pair, Alexa 488 as donor and Alexa 647 as acceptor, is characterized by a Förster radius R₀ of 48.9 Å.

![Figure 1: Three dimensional structure of yPGK (PDB-code: 3PGK) and the fluorescent dyes (Alexa 488 and Alexa 647) displayed as attached to their respective positions in the N-domain (yellow color) and in the C-domain (cyan colored).](image)

The conformational characterization of native and unfolded states was performed by measuring FRET efficiencies for individual proteins [1]. Figure 2 shows FRET efficiencies histogrammed for PGK measured at different denaturant concentrations between 0 M and 6 M GdnHCl. Typically for each sample condition a few thousand efficiency values are measured which allow...
for the discrimination between folded and unfolded state populations.

At 0 M GdnHCl only the native population is visible, while at GdnHCl values above 0.5 M an unfolded state population shows up, which in addition increases progressively with increasing GdnHCl concentrations. Above 0.9 - 1 M GdnHCl the protein is fully unfolded, as shown in the corresponding histograms (rightmost column in Fig. 2).

In the transition regime between 0.5 and 0.9 M GdnHCl the native and the unfolded state populations are present at the same time, each of them represented by individual well separated peaks. In order to obtain relevant properties of the corresponding populations the histograms were fitted with Gaussian functions. The resulting three fit parameters are displayed as a function of GdnHCl concentration in Fig. 3. From the amplitude factors the relative fractions of native state and unfolded state populations were calculated (Fig. 3A). The dependence of these fractions on the GdnHCl concentrations enables us to determine the half transition concentration \( C_{1/2} \). The obtained value of 0.7 M for the labeled double mutant is somewhat smaller as compared to values obtained for unlabeled wild type PGK (\( C_{1/2} \sim 0.8 \)). The peak position of the Gaussian represents a mean FRET efficiency of the population and characterizes the structural dimension of PGK at the given environmental condition (Fig. 3B).

Under native state conditions (0 M GdnHCl) we observe a value of \( E \sim 0.80 \), which correspond to an averaged inter-dye distance of about 38 Å. In contrast to the native state, the unfolded state population exhibits efficiency mean values that decrease with increasing GdnHCl concentration.

We observe a strong decay of \( E \) values (from \( E \sim 0.35 - 0.2 \)) in the transition regime between 0.7 and 1 M. These values are related to a progressive structural expansion of unfolded PGK, leading to an increased inter-dye distance. Although the statistics of our data is not good enough to obtain reliable values for the unfolded state below 0.7 M, the data indicate that the unfolded state is even more compact at low GdnHCl concentrations. On the other hand the unfolded state exhibits a further structural expansion above 1 M and reaches the most expanded structure at 6 M (\( E \sim 0.1 \)); a phenomenon also known as globule-coil transition. In addition to peak positions, the width of the efficiency distributions is of interest (Fig. 3C). The smallest possible width which can be achieved in the absence of any heterogeneity from the sample is limited by the shot noise broadening (lines in Fig. 3C). While for the native state population the distribution width is almost constant over the entire range of denaturant concentrations (2\( \sigma \sim 0.24 \)), the unfolded state exhibits much broader distributions in the transition region (2\( \sigma \sim 0.37 - 0.3 \)). At higher denaturant concentrations (> 1.5 M GdnHCl) we observe the same distributions width as obtained for the native.

Membranes: Mechanisms of signal propagation and proton transport

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Rhodopsins, as well as GPCR-receptors, are seven transmembrane alpha-helical membrane proteins and are present in all kingdoms of life. Although structurally highly similar they are functionally quite diverse as they can act either as ion pumps, cation channels, or sensors. This broad diversity made microbial rhodopsins the target of an intensive research and of biotechnical applications. We study molecular mechanisms of signal propagation and vectorial proton transport using the complex of the receptor NpSRII with its cognate transducer NpHtrII and a proton pump – bacteriorhodopsin.

Signal transduction. Broad diversity of functions and applications of retinal proteins has been also confirmed in the new field of optogenetics which utilized channel rhodopsin and halorhodopsin, a chloride pump, as tools in neurophysiological research. Archaeabacterial photoreceptors mediate phototaxis by regulating cell motility through two-component signalling cascades. Homologs of this sensory pathway occur in all three kingdoms of life, most notably in enteric bacteria in which the chemotaxis has been extensively studied. The sensor complex of SRII and its cognate transducer HtrII has also been the subject of intensive research because of its importance as a model system for studying transmembrane signal transfer [1]. We solved crystal structures of the ground and active M-state of NpSRII/NpHtrII as well as the mutant NpSRII-D75N/NpHtrII complexes, in the space group I212121. It is shown that the relative orientation of symmetrical parts of the dimer of two proteins is "U"-shaped in contrary to the "V"-shaped form of the previously reported structures of the wild type NpSRII/NpHtrII complex in the space group P212121 [1] although the structure of the proteins themselves is nearly the same. Computer modelling of the HAMP domain in the obtained "V"- and "U"-shaped structures revealed that only the "U"-shaped conformation allows for tight interactions of the receptor with the HAMP domain (Fig.1). This is in line with the existing data and is in favor of biological relevance of the "U" shape in the ground state.

We performed additional investigations of signal transduction mechanisms [2,3], including a computer study, of the inter-HAMP regions of NpHtrII and other phototactic signal transducers by means of molecular dynamics [3]. Their structure is found to be a bistable asymmetric coiled coil, in which the protomers are longitudinally shifted for about 1.3 Å. Both flanking HAMP domains are mechanistically coupled with the inter-HAMP region, and are also asymmetric. The longitudinal shift in the inter-HAMP region is coupled with the in-plane displacement of the cytoplasmic part by 8.6 Å relative to the transmembrane part. The established properties suggest that 1) the signal may be transduced through the inter-HAMP domain switching; 2) the inter-HAMP region may enable cytoplasmic parts of the transducers to come close enough to form oligomers.

FIG. 1: The "U"-shaped conformation of the NpSRII/NpHtrII complex allows for tight interactions of the receptor with the HAMP domain. Electrostatic interactions between the receptor molecules of the dimer and the HAMP domain (at the top). Positive charges of the receptor interface are mapped in blue and negative of the HAMP domain are mapped in red.
Proton Transport. Bacteriorhodopsin (bR), a transmembrane protein of halophilic archa, is the most studied light activated proton pump. Bacteriorhodopsin (bR) provides light driven vectorial proton transport across a cell membrane. Creation of electrochemical potential at the membrane is a universal step in energy transformation in a cell. The published atomic crystallographic models of early intermediate states of bR show a significant difference between them and conclusions about pumping mechanisms have been contradictory. We grew highly ordered 3D crystals and clarified the origin of the lack of the consensus. We showed that merohedral twinning and radiation damage of the crystals corroborate the crystallographic data [4,5]. Here, we present a quantitative high resolution crystallographic study of conformational changes in bR induced by X-ray absorption. It is shown that X-ray doses usually accumulated during data collection for intermediate-state studies are sufficient to alter significantly the structure of the protein. X-ray induced changes occur primarily at the active site of bR. Structural modelling showed that X-ray absorption triggers retinal isomerisation accompanied by the disappearance of electron densities corresponding to the water molecule W402 bound to the Schiff base. It was demonstrated that these and other X-ray-induced changes may mimic functional conformational changes of bR leading to misinterpretation of the earlier obtained X-ray crystallographic structures of photo intermediates (Fig.2). The studies which have been done not only explain the origin of contradictions but provide all the necessary methodology to obtain true ultra high resolution structures of bR and finally elucidate the molecular mechanism of the universal first step in bioenergetic vectorial proton transport.

FIG. 2: Comparison of the X-ray-induced retinal conformation of ground state and those in the published models of the K-state and L-states. X-ray-induced retinal conformation of ground state is shown in red. Retinal conformations in K-state models with pdb-numbers: 1IXF – orange, 1MOK – green, 1QKP – violet - are shown in (a). Retinal conformations in L-state models with pdb-numbers: 100A – cyan, 2NTW – blue, 1VJM – magenta, 1EOP – dark green, 1UCQ – yellow - are shown in (b)


Membrane protein diffusion and interactions studied by 2f-FCS

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In order to monitor membrane protein binding in lipid bilayers at physiological protein concentrations we employed the recently developed dual-focus FCS technique. In a case study on a photoreceptor consisting of seven transmembrane helices and its cognate transducer (two transmembrane helices) the lateral diffusion for these integral membrane proteins was analyzed in giant unilamellar vesicles (GUV). The two-dimensional diffusion coefficients of both separately diffusing proteins differ significantly with \( D = 2.2 \times 10^{-8} \text{ cm}^2/\text{s} \) for the photoreceptor and with \( D = 4.1 \times 10^{-8} \text{ cm}^2/\text{s} \) for the transducer. In GUVs with both membrane proteins present together, we observed significant smaller diffusion coefficients for labeled transducer molecules indicative for larger diffusing units and therefore indicating inter-molecular protein binding. Based on the phenomenological dependence of diffusion coefficients on the molecule’s cylindrical radius we are able to estimate the degree of membrane protein binding on a quantitative level.

Membrane proteins have to cope with various specific tasks in the cell. They ensure the interaction and communication of the cell with its environment by forming example channels or by providing receptor molecules which transfer various kinds of information. In order to fulfill these requirements often larger oligomeric protein complexes need to be formed in the cell membrane. A well studied example in this respect is given by a photoreceptor (NpSRII) and its cognate transducer (NpHtrII), together forming a photo-signaling complex in *Natromonas pharaonis*. NpSRII consist of seven membrane spanning alpha-helices, while NpHtrII is made up of two membrane spanning helices and a cytoplasmic domain composed of a coiled-coiled four-helix bundle. EPR-spectroscopy and crystallographic studies on the Np(SRII/HtrII)-complex with a truncated version of the transducer (NpHtrII157, which is missing the cytoplasmic domain) indicated that NpSRII and NpHtrII form a 2:2 complex with a two-fold symmetry axis perpendicular to the lipid membrane surface. This complex formation is facilitated by the intermolecular binding of transmembrane transducer helices. According to these results the 2:2 complex is assumed to be the functional unit and both Np(SRII-HtrII) and Np(HtrII-HtrII) binding are essential to form the complex.

Recent Förster resonance energy transfer (FRET) measurements at protein concentrations (molar protein/lipid ratios: 1:200–8000) much lower than in EPR and crystallographic studies (typical protein/lipid ratio: 1:50) revealed a much stronger Np(SRII-HtrII) binding than the Np(HtrII-HtrII) binding [1]. Most probably the cytoplasmic transducer domain (which was missing in the above mentioned studies) is essential to ensure a stronger Np(HtrII-HtrII) binding which is required to obtain functional photo-signalling complexes in the cell. However, in the cell the concentration of photoreceptors is even lower, with approximately 400 molecules per cell which corresponds to a molar protein/lipid ratio in the order of 1:1,000,000. To achieve such low protein concentrations in a lipid bilayer system we incorporated the membrane proteins into giant unilamellar vesicles (GUV) for membrane protein binding studies. GUVs, with a typical size (diameter ~ 10–40 \( \mu \text{m} \)) very similar to that of cells, have often been used as well-defined cell membrane models. In contrast to supported lipid bilayers where the bilayer may interact with the solid support, GUVs provide a free standing (top) bilayer. This advantage is valuable for determining the precise lateral diffusion of integral membrane proteins and for analyzing membrane protein binding in an unperturbed environment (see Fig. 2). To measure how such protein complexes are
formed in lipid bilayers at physiological protein concentrations is still an experimental challenge. Advanced spectroscopic techniques such as fluorescence correlations spectroscopy (FCS), single particle tracking, as well as other kinds of single molecule techniques have been successfully applied to investigate proteins in artificial lipid bilayer systems and in cell membranes.

Here we applied the recently developed dual-focus FCS (2FCS) technique to measure the lateral diffusion of integral membrane proteins in GUVs [2]. The diffusion coefficients and the corresponding cylindrical radii from our measurements are given in the Table 1. The obtained value for lipid diffusion ($D_{2D} = 7.9 \cdot 10^{-8}$ cm$^2$/s) is in agreement with recently observed values, also measured with FCS in GUVs. We do not expect any dimerization and therefore assume monomeric diffusing molecules. Based on the above described size-dependent diffusion properties we investigated the NpSRII/NpHtrII binding by fusing non-labelled NpSRII molecules together with labelled NpHtrII into the GUVs. For this purpose, transducer molecules and photoreceptor molecules were reconstituted in different proteo-liposomes which were subsequently fused into GUVs. Upon Np(HtrII/SRII) binding we would expect a slower diffusion coefficient of the NpHtrII as compared to the diffusion of an unbound transducer. Indeed we clearly observe a significant slower diffusion in GUVs where the transducer and the photoreceptor are present together. The corresponding diffusion coefficient of NpHtrII ($3.1 \pm 0.46 \cdot 10^{-8}$ cm$^2$/s at 1.4 molecules per µm$^2$) is already smaller than that one for the single transducer ($4.1 \pm 0.39 \cdot 10^{-8}$ cm$^2$/s) and is therefore indicating that a fraction of all NpHtrII in the GUV is binding NpSRII.

In a second set of experiments we reconstituted labelled transducer and non-labelled photoreceptors together at the same molar stoichiometry into proteo-liposomes and thereafter fused them into GUVs. For this sample we observed an even smaller diffusion coefficient ($2.1 \pm 0.48 \cdot 10^{-8}$ cm$^2$/s at 1.2 molecules per µm$^2$) which is rather close to a value predicted from the $1/R$ dependence. The latter result indicates that at the given protein concentration (molar protein/lipid ratio $\sim 1/2,000,000$) nearly 80% of the transducer molecules form a Np(SRII/HtrII) complex with the related photoreceptor. Most probably, in the first experiment we do observe only a partial NpHtrII/NpSRII complex formation ($\sim 20\%$ complexes), because NpSRII loaded proteo-liposomes fused not to the same extend into GUV as compared with NpHtrII loaded liposomes. In the second approach the equal stoichiometry of NpHtrII and NpSRII is guaranteed intrinsically.

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We show that optical tweezers are a valuable tool to study the co-translational folding of a nascent polypeptide chain at the ribosome in real-time. The aim of this study was to demonstrate that a stable and intact population of ribosomes can be tethered to polystyrene beads and that specific hookups to the nascent polypeptide chain by dsDNA handles, immobilized on a second bead, can be detected. A rupture force of the nascent chain in the range of 10–50 pN was measured, which demonstrates that the system is anchored to the surface in a stable and specific way. This will allow innumerable future applications to follow protein folding using much lower forces.

Co-translational protein folding takes place in vivo during the production of the nascent chain by the ribosome and it varies significantly from in vitro refolding of proteins. Ensemble measurements can provide a mean value of such an asynchronous process taking place in the bulk. Single molecule studies, on the other hand, give valuable information about asynchronous processes and reaction intermediates by eliminating the temporal and population averaging characteristics of bulk ensembles.

In our attempt to understand co-translational protein folding in more detail, we performed in the past in vitro cell-free single molecule fluorescent studies, where translating ribosomes were immobilized on a surface [1]. In this study, we report the use of translating ribosomes immobilized on a polystyrene bead to measure the forces applied on the nascent polypeptide chain during co-translational protein synthesis with the use of optical tweezers [2].

L4 biotinylated ribosomes were specifically attached on micron sized polystyrene beads coated with streptavidin (Fig. 1A). The streptavidin on the bead that did not react with ribosomes was blocked with an excess of biotin. By using a fractionated cell free transcription/translation E.coli system, tethered ribosomes started to synthesize GFP. The GFP construct was extended at the C-terminal end by a sequence of 31 additional aminoacids in order to give the full length protein the possibility to fold correctly outside the ribosomal tunnel and protein release was suppressed to keep GFP bound on the ribosome after synthesis (Fig. 1B).

A biotin was inserted into the N-terminus of the nascent polypeptide chain according to the suppressor tRNA technique, followed by a linker sequence of 29 aminoacids plus a sequence of 6 histidines (6xHis) (Fig. 1B). In order to stop the synthesis at the desired position we used a histidine deficient transcription / translation system. Thus, the synthesis of GFP
proceeded up to the 6xHis and halted, at which point the biotinylated N-terminus was just appearing outside of the ribosomal tunnel. Following this pre-synthesis, ribosomes were incubated with streptavidin yielding a streptavidin–biotin N-terminus of GFP.

The beads, with streptavidin on the N-terminus appearing outside of the tethered ribosomes, were injected into a custom-made flow chamber. A single bead was trapped by the tweezers and was then held by a micropipette.

While holding on the micropipette a single bead with translating ribosomes halted at the 6xHis, a second set of polystyrene beads to which dsDNA was attached was injected into the chamber and one of them was trapped in the optical tweezers. The free end of dsDNA was biotinylated and could interact with the streptavidin of the nascent polypeptide chain.

By approaching the micropipette close enough to the bead in the optical trap a force was measured by the detector. In order to determine if the coupling was specific we pulled the micropipette slowly away and we monitored the applied force versus the extension until a rupture occurred. In most measurements the rupture was observed in the range of 10–50 pN (Fig. 2A and B), which is consistent with the force that is required to break the hydrogen bonding between the mRNA and the peptidyl-tRNA carrying the nascent chain. This indicates that the biotinylated end of the dsDNA interacted specifically with the streptavidin at the N-terminus of the paused nascent chain. In order to avoid the rupture in the coupling, we applied a constant force of 7 pN and we monitored the elongation versus time. It became clear that the N-terminus of the polypeptide chain remained coupled to the DNA handle through time (Fig. 2C).

In this study we described a method to immobilize fully active ribosomes on polystyrene beads and bind the N-terminus of a nascent polypeptide chain with a DNA handle attached on a second polystyrene bead trapped by optical tweezers. The described system provides the possibility to measure the forces applied on the nascent chain during its synthesis and potentially during its folding.

FIG. 2: Disruption of the nascent polypeptide chain from the ribosome and force measurements. (A) A characteristic diagram of force vs. elongation for a specific interaction between the N-terminus of the polypeptide chain and the DNA handle. Coupling ruptures at 10 pN. Arrow pointing upwards indicates extension of the elastic dsDNA linker, whereas the arrow pointing to the left indicates the return of the pipette bead back to its origin. (B) A characteristic diagram of force vs. elongation for a high force rupture of the streptavidin-dsDNA handle from the nascent polypeptide on the ribosome. (C) A diagram of elongation vs. time when a constant force of 7.0 pN is applied. No rupture is observed through a time of more than 40 seconds which should enable future polypeptide unfolding–refolding experiments while protein is translated.


Method and process development for membrane protein analysis

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The required amount of protein for CIMP crystallization has been reduced by a factor of 4. The applicability of this method has been proven conclusively by solving the structure of sensoryrhodopsin II from *H. salinarum*. Methods and protocols to apply CIMP crystallization to human membrane proteins were developed that allow obtaining several hundred micrograms of the target protein per liter culture by E. coli expression.

The number of non-redundant structures of membrane proteins is still below 400 deposits at the protein data bank. Progress that would remedy this situation requires new methodical approaches:

- Minimizing protein consumption in crystallization
- Maximizing success rate by optimized methods
- Increasing the yield of available protein

The development of CIMP, the the controlled in meso phase crystallization method, as an advancement of the conventional lipidic cubic phase crystallization allowed to reduce the protein consumption of membrane protein crystallization and decreased the amount of protein required to obtain the first crystal by incomplete factorial screening (TAB. 1).

<table>
<thead>
<tr>
<th>1st hit Plate number</th>
<th>Protein consumption Total (mg) per exp. (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR 1</td>
<td>0.8</td>
</tr>
<tr>
<td>HR 2</td>
<td>0.8</td>
</tr>
<tr>
<td>SR 8</td>
<td>12.8</td>
</tr>
</tbody>
</table>

**TAB. 1:** Protein consumption until 1st crystal was obtained. Plate with 96 different experiments using 450 nl protein solution Bacteriorhodopsin (BR), Halorhodopsin (HR), Sensoryrhodopsin II (SR) from *H. salinarum*

By downscaling the amount of monoolein, which forms with water the crystallization matrix, from 130 µg to 33 µg a further reduction of protein consumption by a factor of 4 was achieved. By design CIMP crystallization incorporates the membrane protein into the crystallization matrix, the meso phase, during the experiment. Thereby crystallization is steered from being an event in the bulk phase to being an event at the phase boundary between the meso and the aqueous phase. This allows further reducing protein consumption by employing films of meso phase (Fig.1).

![FIG. 1: Crystallization of BR using CIMP crystallization in a 'hanging drop' setup that promotes formation of films of meso phase.](image)

The investigation of bacteriorhodopsin has proved previously that high resolution data can be obtained by applying CIMP crystallization [1]. For the case of sensoryrhodopsin II from *H. salinarum*, a protein that resisted crystallization using other crystallization methods than CIMP crystallization (Fig.2), the structure has been obtained (Fig3). Thereby the potential of CIMP crystallization to overcome the bottle neck problem for crystallographic analysis of membrane protein structures is conclusively proven.

Current interest in membrane protein structures is strongly focused on human proteins because of the
evident benefit for the development of lead structures in the development of therapeutics.

The availability of human membrane proteins is restrained by (i) the yields obtainable with current expression methods and (ii) by the inherently higher instability of mammalian proteins as compared e.g. to archaean proteins like bacteriorhodopsin or sensoryrhodopsin.

A comprehensive protocol to obtain typically yields of several hundred micrograms of purified human membrane protein by heterologous expression in E. coli has been developed [2,3]:

- Use of codon-optimized constructs
- Screening for optimal E. coli strains
- Optimizing cell growth conditions
- Optimizing induction of protein synthesis
- Optimizing harvest condition
- Optimizing detergent for membrane extraction
- Use of affinity tags
- Optimizing detergent to insure protein stability

Employing the developed methods and protocols to obtain proteins and crystals of human membrane proteins led first positive results that are currently under investigation by X-ray crystallography.


Biophysical studies on the arrestin activation mechanism

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Arrestins are regulatory proteins that play a central role in the termination of G protein-coupled receptor (GPCR) mediated signal transduction pathways. In visual signalling, arrestin specifically binds to photoactivated and phosphorylated rhodopsin. In contrast, the p44 splice variant can terminate phototransduction by binding to nonphosphorylated light-activated rhodopsin. Here we report the crystal structure of bovine p44 at a resolution of 1.85 Å. Compared to native arrestin, the p44 structure reveals significant differences in the regions crucial for receptor binding, namely, flexible loop V-VI and polar core regions. Additionally, the electrostatic potential is remarkably positive on the N- and C- domains. The p44 structure represents an active conformation that serves as a model to explain the ‘constitutive activity’ found in arrestin variants.

Visual arrestin (arr-1) is one of the most abundant soluble proteins in vertebrate photoreceptors. A rod-specific splice variant of arr-1, termed p44, differs from arr-1 in three properties: the amino acid sequence is identical except for the C-terminus, where residues 371 to 404 are missing and Phe370 is replaced by Ala; it shows high affinity towards different rhodopsin species (Rh*, P-Rh*, P-Rh and C-terminally truncated rhodopsin that lacks the potential sites of phosphorylation; where * and P- denote light-activated and phosphorylated states, respectively); and is permanently localized in the rod outer segments (ROS).

Crystal structure of p44: The structure of p44 exhibits the typical arrestin fold containing two domains, each composed of a seven-stranded β-sandwich (Fig. 1). A single helix is present on the surface of the convex side of N-domain. The fulcrum of the domain interface harbors an intact polar core, composed of charged residues forming a hydrogen-bonding network including several salt bridges. It is noteworthy that the presented structure of p44 was solved at 1.85 Å, the highest amongst arrestin-1 structures reported so far. The lack of C-terminal residues (371-404) in p44 allows a tight crystal packing with one molecule in the asymmetric unit, improving the X-ray diffraction and the mosaic spread remarkably.

Structural differences between p44 and arr-1: The crystal structure of arr-1 reported previously shows two conformations amongst the 4 molecules present in the asymmetric unit, where two chains are in α-conformation and the other two in β-conformation. A comparison of the p44 model with the two arr-1 conformers reveals significant conformational differences in three loop regions (residues 68-78, 155-166 and 336-344, see Fig. 1); and in the polar core. Interestingly, these residues are located on the surface of the concave side of the molecule, which is involved in receptor binding.

Role of flexible nature of the loop V-VI (residues 68-79): Existing experimental data convincingly demonstrates that the conformational flexibility of loop V-VI is essential for arrestin to adopt a high-affinity binding state required for receptor binding. This loop has two different conformations in the α- and β- conformers of arr-1 (Fig. 1). Interestingly, the loop V-VI is disordered in our p44 structure. Considering the importance of flexibility of this loop in receptor binding and its predicted location on the arrestin-rhodopsin interface in the complex, we speculate that the disordered loop V-VI conformation in p44 is a property that is linked to its activation state.

FIG. 1: Superposition of the three dimensional structures of arrestin-1 and p44. Two conformers α and β of arrestin-1 are shown in color coral and cyan, respectively. The model of p44 is drawn in blue. The termini are indicated with capital letters. C* marks the C-terminus of p44. The loop regions with largest deviations are boxed, where the flanking residues are numbered.
Polar core region - consequences of the absence of C-tail: The polar core consists of several charged residues and has been identified as phosphorylation-sensitive region in arrestin. Although the phosphorylation-trigger residue Arg175 conformation is similar, a reorganization of the polar core is seen in p44 structure. The side chain of Arg29 points 'inside' the core, and interacts with Asp30 and Arg175, stabilizing the p44 polar core. Likewise, the side chain conformation of Asp303 forms an additional Asp303-N···ND1-His301 hydrogen bond.

Electrostatic potential on p44 protein: The N- and C-domain concave surfaces are highly positively charged, which is much more pronounced in case of p44 due to the absence of the negatively-charged C-terminal region (Fig. 2). This explains the energetically favorable interaction of p44 to Rh*. A strong positive potential in p44 will compensate for the lower negative charge in nonphosphorylated rhodopsin.

The molecular mechanism of arrestin activation: The crystal structures of visual arrestin determined so far are in basal or 'inactive' states and require activation by binding of the phosphorylated carboxy terminal segment of rhodopsin (1st trigger step) (Fig. 3). Binding of phospho-residues of rhodopsin causes the displacement of C-tail of arrestin, where the latter sterically blocks the polar core region. Lack of C-terminal segment (371-404) in p44 leaves the polar core region unmasked, allowing the binding of different rhodopsin species to the polar core. Our results are consistent with the current model of the arrestin activation mechanism [1] where the first phosphor-sensing step is likely to be bypassed in p44 which is already in an activated state [2].

Subsequently, in the 2nd activation step the presence of rhodopsin causes disruption of additional interactions in the polar core and in the three element interactions between the C-tail, N-terminal β-strand I and α-helix, activating the arrestin molecule (Fig. 3).

Fig. 2: Electrostatic potential maps around arrestin-1 (α conformation) and p44 showing the field which propagates into the solvent. The spreading of the positive electric field on the respective N-terminal domains indicates the relative ability to attract rhodopsin. Polar core region is indicated (white circles).

Fig. 3: A model of the interaction of rhodopsin with arrestin (arr-1). Arr-1 (cyan) in active state is defined by the α-conformer of loop V-VI (blue) and disruption of the polar core, which amongst others involves C-tail (residue range: 372 to 386; blue) to “swing-out”. The center of the polar core is indicated by residue R175 (cpk-mode of atom Ca). The model of light-activated and phosphorylated rhodopsin (orange) is based on the PDB codes 1U19 and 2X7Z. Upon light-activation, the retinal isomerizes and initiates changes in helices 5, 6 and the connecting loop (salmon). For an interaction with arr-1, the three serines are phosphorylated (cpk-mode of atom Ca for p-S334, p-S338, and p-S343; purple balls, marked by arrows), and the C-terminal end (salmon) is placed on top of the polar core of arr-1.


X-ray holography in biology

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X-ray Fourier Transform Holography was successfully applied to magnetic as well as biological objects. The phase problem is solved by the interference of the object wave with a reference wave. The phase information is encoded as intensity modulations within the hologram. A single Fourier transform yields the reconstruction of the object. In our approach, a small gold sphere close to the sample was used to create the reference wave. As a test object a single shell of a diatom was used [1].

The geometry of the X-ray holography experiment is shown in Figure 1: A single diatomic shell was prepared in the centre of a silicon nitride membrane carried by a silicon wafer. Diatoms are a type of green algae. A small gold sphere was placed close to sample. Both, diatom and gold sphere were positioned using micromanipulators. The gold sphere had a diameter of 250 nm and was placed in a distance of ~ 20 µm to the diatom. The achievable resolution depends on the covered scattering vector range and on the size of the gold reference particle. The experiment was performed at the synchrotron BESSY II in Berlin at a wavelength of 2.53 nm [1].

In Figure 2 a measured X-ray hologram of the sample and the corresponding 2D-reconstruction of the diatom at 0° tilt angle are shown. The object-reference modulations are clearly visible indicating sufficient coherence and adequate sampling. The reconstruction has a lateral resolution of ~ 130 nm which is limited by the size of the gold sphere.

In total 47 holograms were recorded from -60° to +55° in steps of 2.5°. Each hologram was illuminated from 80 s to 400 s depending on the angular position and the change of the incoming photon flux. The maximum angles are limited by the size of the silicone nitride membrane. High angular positions result in strong diffraction from the edges of the window, which disturbs the hologram. Before image reconstruction an inverse Gaussian filter was applied to the hologram to flatten the sharp edges of the beamstop. On the base of the 47 projections of the diatom the IMOD software package was used for tomographic image reconstruction and for visualization [1]. The pores of the shell were used for image alignment. No additional
introduction of fiducial markers into the sample was necessary.

In Figure 3 two slides of the tomographic back projection of the diatom, and a view of the 3D-rendered model are shown. The shell of the diatom has a height of 1.3 µm and a diameter of 9.5 µm. It is formed more like a soup bowl than a flat disk, which cannot be visualized by a single 2D projection only. The shape is typical for diatoms of genus *Stephanodiscus* and *Cyclostephanos*.

The resolution of the 3D tomogram was determined to be ~ 140 nm using Fourier shell correlation. We expect that a resolution of ~ 50 nm might be achievable using current synchrotron sources and smaller gold particles to generate the reference wave. The higher resolution would allow for example to image the three dimensional organisation and distribution of organelles in whole cells or to investigate the 3D structure of nano-sized complex materials. For that purpose a high photon flux is crucial to collect data with excellent signal to noise ratio up to the largest scattering vectors. To reduce the radiation damage of biological samples, which is unavoidable during the extended measurements with a very high incoming photon flux, the experimental setup will need to include a cryo-cooling stage.

It is also thinkable to connect a small gold sphere with only a few gold atoms to a protein complex via a linker. A Free Electron Laser could provide the extremely high flux, which would be needed to record a X-ray hologram of the protein. Thousands of shots must be recorded and analyzed to reconstruct the 3D electron map of the protein complex. It might also be feasible to combine this approach with pump-probe experiments and to image the dynamics of enzymatic reactions. The transparent mask concept using FTH is also a suitable method for conventional 2D-projection imaging of magnetic systems where it is necessary to rotate the sample. It could be used to image the out-of-plane magnetization at FELs where only linear polarized light is available. If the sample is rotated the magnetic moments will be moved out of the beam axis which makes linear polarized light sensitive to the out-of-plane magnetization. This can be used for imaging the dynamics of demagnetization and remagnetization processes in a pump-probe-experiment.

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**Figure 3.** Two slides from the tomogram of the diatom: one from top view (a) and another one from side view (b). The resolution of the tomogram was determined to 140 nm using Fourier shell correlation. On the base of the tomographic reconstruction a 3D rendering model (c) of the shell was reconstructed. Each scale bar corresponds to 2 µm.

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The ICS-6 contributes to the Helmholtz programmes “BioSoft: Macromolecular Systems and Biological Information Processing” within the research field Key Technologies and “Neuro: Function and Dysfunction of the Nervous System” within the research field Health. Research of the ICS-6 focuses on the development and application of methods to precisely investigate three-dimensional structures, dynamics, ligand interactions and molecular mechanisms of biologically and medically relevant macromolecules involved in basic cellular processes, like autophagy. Function and malfunction of most investigated proteins play decisive roles in the development and progression of infectious diseases, e.g. AIDS and Hepatitis C, as well as neurodegenerative diseases, e.g. prion diseases, Alzheimer’s and Parkinson’s disease. Thus, folding, misfolding and aggregation together with protein-protein and other protein-ligand interactions need to be investigated quantitatively and – if possible – in atomic detail by liquid and solid state NMR as well as X-ray crystallography and computational structural biology methods.

Because of their important role in the above mentioned processes, especially membrane associated and fibril forming proteins are explored, which are not readily amenable to standard methods for structural investigation. Therefore, a special research focus is on the development and application of methods that allow such kind of high resolution structural investigations.

In addition to basic research and methods development, applied research is carried out at ICS-6 as well. This includes the development of novel approaches for early diagnosis of neurodegenerative disorders. Furthermore, novel antiviral strategies are being developed as well as therapeutic and preventive approaches to Alzheimer’s disease.
Research Highlights

Structural basis of cyclic nucleotide-activated ion channel gating

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Cyclic nucleotide-sensitive ion channels play an important role in signal amplification pathways. They are activated by binding of cyclic nucleotides to a domain located on the cytoplasmic side of the channel. However, the mechanism by which the ligand binding opens these channels is not well understood. To elucidate the structural basis of the underlying gating mechanism, high resolution structures of the cyclic nucleotide-binding domain (CNBD) in the apo and holo state are required. In the present study, we describe the solution structures of the apo and holo CNBD of Mesorhizobium loti K1 channel (MloK1). A comparison of these structures reveals large conformational rearrangements upon ligand binding. These structures provide important insights into conformational events that accompany channel gating within the ligand-binding site.

Cyclic nucleotide-sensitive ion channels play crucial roles in excitability and signaling of sensory neurons. They belong to two subfamilies: Cyclic nucleotide-gated (CNG) channels, and hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels. Both channel types share a carboxy-terminal CNBD. HCN channels are activated by hyperpolarization and their activity is modulated by cyclic nucleotides. In contrast, CNG channels are voltage independent and require cyclic nucleotides to open. Binding of cyclic nucleotides to the CNBD promotes the opening of the channel in both cases.

Until recently, however, only crystal structures of CNBDs from HCN and the prokaryotic CNG channel MloK1 have been known. In the case of HCN, the apo and holo state did not reveal substantial differences (Zagotta et al., Nature, 2003; Taraska et al., Nat. Methods, 2009). For MloK1, structural information for the apo state has only been gained from mutant CNBDs (Clayton et al., Cell, 2004; Altieri et al., J. Mol. Biol., 2008). The MloK1 channel harbors six transmembrane domains (S1-S6), a “GYG” signature sequence for K+ selectivity, and a conserved CNBD is connected via a short C-linker to S6 (Fig. 1). The longer C-linker (~80 residues) of mammalian CNG channels is important for relaying the binding signal to the channel gate. Crystal structures of mammalian HCN channel CNBDs revealed that neighbouring C-linkers contribute virtually all contacts between subunits in the tetrameric protein (Zagotta et al., Nature, 2003; Flynn et al., Structure, 2007). The crystal structure of the isolated CNBD of MloK1 suggested that subunits are organized as dimers (Clayton et al., Cell, 2004). The dimer interface formed by the short linker has been proposed to be involved in channel gating. However, an electron microscopy study of the complete channel revealed a four-fold symmetry of subunit arrangement (Chiu et al., Structure, 2007). The CNBDs appear as independent domains separated by discrete gaps, suggesting that CNBDs are not interacting with each other. Furthermore, the MloK1 channel and the isolated CNBD bind cAMP with similar affinity (Kd ~ 107 nM) in a non-cooperative fashion (Cukkemane et al., EMBO Rep., 2007).

FIG. 1: Subunit topology and assembly of the MloK1 cyclic nucleotide-gated K+ channel. The channel consists of four subunits. Each subunit encompasses six transmembrane segments S1-S6 and an intracellular binding domain (shown in ribbon representation).
In the present study, we determined the solution structures of the holo [1,2] and apo [3, 4] wild-type CNBD of the MloK1 channel. The solution structures of both the cAMP-free and -bound CNBD feature a β-roll (consisting of eight antiparallel strands) with a short internal α-helix (known as the phosphate-binding cassette (PBC) which is responsible for interactions with the phosphate and ribose moieties of cAMP) that is topped by a helical portion of four α-helices (Fig. 2).

The potential dimerization of the CNBD upon ligand binding was addressed by relaxation measurements. Correlation times of 8.4 ns (cAMP-free state) and 8.5 ns (cAMP-bound state) for isotropic rotational diffusion of CNBD were derived from 15N average longitudinal (R1) and transverse relaxation (R2) rates. These data indicate that the CNBD is present as a monomer in solution.

Superposition of cAMP-free and -bound CNBD solution structures shows that the β-roll region is almost identical with an rms displacement of only 0.084 Å (Fig. 2C). In contrast, the helical parts show substantial rearrangements between the two states (rms displacement of 0.271 Å). Binding of cAMP causes a large reorientation of the helical part with respect to the β-roll of the CNBD. Ultimately, this leads to a sliding movement of the N-terminal helix by 8 Å.

The solution structures of the cAMP-free and –bound CNBD allow to reconstruct, unbiased by mutations or crystal contacts, the sequence of dynamical events that propagate through the binding fold on cAMP binding. Since the N-terminal helix connects directly to the transmembrane region of the channel it is conceivable, that the observed reorientation of secondary structure elements in the CNBD might induce reorientations of the transmembrane helices of MloK1, which may result in channel activation.

FIG. 2: Structure comparison of wild-type cAMP-free and -bound MloK1 CNBD. Solution structure of the cAMP-free (a) and cAMP-bound (b) CNBD. Superposition of cAMP-free and -bound CNBD structures (c). In contrast to the β-roll (blue), the helical portion (white and red) shows substantial rearrangements upon ligand binding (dashed arrows). The cAMP molecule is shown as a stick model, secondary structure elements are labelled.


Interaction between human CD4 and HIV-1 protein VpU

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Human cluster determinant 4 (CD4) is a type I transmembrane glycoprotein involved in T-cell signalling. Besides its cellular functions, CD4 serves as the main receptor for human immunodeficiency virus type I (HIV-1). The HIV-1 encoded virus protein U (VpU) is required for efficient viral release from human host cells and for induction of CD4 degradation in the endoplasmic reticulum (ER). The cytoplasmic domain of the membrane protein VpU (VpUcyt) and its interaction with the cytoplasmic region of CD4 is essential for the latter activity. We used solution NMR spectroscopy to determine the structures of both VpUcyt and the transmembrane and cytoplasmic domains of a cysteine-free variant of CD4 (CD4mut) in the presence of membrane-mimicking dodecylphosphocholine (DPC) micelles. Solution NMR was also utilized to study the interaction of CD4mut and VpUcyt in DPC micelles. Nanodiscs present a superior, detergent-free model membrane of relatively small size for biophysical studies of integral membrane proteins (IMP). We reconstituted CD4mut into nanodiscs and demonstrated the feasibility of solution NMR studies of IMPs in nanodiscs. Incorporation of IMPs in nanodiscs makes the membrane protein amenable to techniques, originally developed for interaction studies on soluble proteins. We reconstituted a CD4mut-based fusion protein into nanodiscs and used this IMP successfully as analyte in a binding study with surface plasmon resonance (SPR). Finally, we started solid state NMR experiments on CD4mut and full length VpU in liposomes.

Human CD4 is a 433 amino acid residue IMP and consists of an immunoglobulin-like extracellular region of 371 amino acids, a short transmembrane region, and a cytoplasmic domain of 40 amino acids. The 70-residue CD4mut resembles the C-terminal CD4(372–433) fragment, with its five cysteines being conservatively replaced by four serines and one histidine, plus an 8-residue tag at the N-terminus added for technical reasons [1]. We determined the three-dimensional (3D) structure of CD4mut in DPC micelles based on proton–proton distance constraints from nuclear Overhauser enhancement (NOE) data [1]. Two α-helices were found ranging from M372 to V395 and from A404 to L413, respectively. The positions of the two helices relative to the micelle were derived from paramagnetic relaxation enhancement (PRE) experiments. For this purpose, we doped the DPC micelles with 16-doxyl stearic acid or 5-doxyl stearic acid, respectively, which both place PRE probes into the hydrophobic core of the micelle, but at different positions. Paramagnetic probes reduce or even quench NMR signal intensity of nuclei located in the neighbourhood of the probe in a distance dependent manner and have a reach of up to 20 Å (Fig. 1). Information on protection of the two helical regions from water comes from H/D exchange data. The stability of the two helices was evaluated by secondary chemical shift analysis and heteronuclear 1H-15N-NOE measurements. The helix ranging from M372 to V395 is very stable and strongly protected from water. NMR signals of the amino acids in this helix are very sensitive to the presence of the PRE probes as expected for a micelle embedded helix. The amphipathic helix ranging from A404 to L413 is rather flexible and only partially protected from water, in agreement with a location on the micelle surface [1].

FIG. 1: Surface representation of CD4mut in DPC micelles. The N-terminal helix is depicted in a vertical orientation. The degree of NMR signal reduction due to presence of the PRE probe 16-doxyl stearic acid is indicated by different shades of green colour. Dark green indicates maximum quenching, white light green symbolizes intermediate effects. White indicates absence of signal reduction. Amino acid residues that could not be evaluated are coloured in magenta. (From Ref. [1])

The 81 amino acid sequence of VpU can be divided into three distinct domains. A short stretch of basic residues (Y27–K38, notation according to strain HIV1S1) connects the transmembrane part (I6–V26) and the extremely acidic cytoplasmic domain (I39–L81). We combined diverse solution NMR experiments to address the structure, dynamics and topology of VpUcyt comprising VpU-residues I39 to L81, in presence of membrane-mimicking DPC micelles [2]. Secondary chemical shift analysis of VpUcyt in aqueous solution at a physiological salt concentration revealed complete absence of secondary structure and the heteronuclear NOE data are consistent with a highly flexible protein. In contrast, addition of DPC micelles
induced two helices in VpUcyt covering residues I39–E48 and L64–R70 as well as a tight loop (L73–V78) close to the C-terminus (Fig. 2), and the conformational flexibility of VpUcyt was heavily reduced.

![Figure 2: Backbone line representation of the 20 lowest energy conformers of VpUcyt in 100 mM DPC solution calculated from NOD-based distance restraints (left). The ribbon diagram of VpUcyt (right) highlights the secondary structure elements. Side chains of SS3 and SS7 in the linker, forming a highly conserved phosphorylation motif, are visualized in ball-and-stick format (From Ref. [2]).](image)

The CD4 binding site on the surface of VpUcyt was derived from titration of 15N-labeled VpUcyt in DPC solution with increasing amounts of CD4mut in DPC micelles. First, chemical shift changes of VpUcyt resonances were monitored. Second, the PRE probe MTSLe was covalently linked to three single cysteine variants of CD4mut via a disulfate linkage (cf. Fig. 3). The replaced residues 397 and 422 are cysteines in the native CD4 sequence and are expected to be close to the VpU binding site of CD4 according to mutational studies. A third CD4mut variant contained a single cysteine in the artificial N-terminal tag and served as control.

![Figure 3: Cartoon illustrating the use of single PRE probes attached to CD4mut for mapping protein-protein contact sites on the surface of VpUcyt.](image)

Chemical shifts and intensities of VpUcyt (1H,15N) amide correlation signals were monitored in 1H,15N HSQC experiments. The data indicate that helix 3 and the C-terminal loop of VpUcyt make the closest contact to CD4 in the complex.

Nanodiscs are small lipid bilayer fragments of ~150 to 300 lipids encircled by two copies of an amphipathic, largely helical scaffold protein in a belt-like fashion. They provide a close to native environment for reconstitution of IMPS and can be formed by directed self-assembly (Fig. 4). We recorded aliphatic 1H,13C HSQC spectra of 13C-labeled CD4mut in nanodiscs [3]. The reasonably well resolved spectra highlight the great potential of solution NMR for studies of IMPS in nanodiscs.

![Figure 4: Reconstitution of CD4mut into nanodiscs by a self-assembly process (From Ref. [3]).](image)

We also demonstrated the applicability of SPR methodology to nanodisc-embedded IMPS on a fusion protein containing an N-terminal decahistidine tag followed by ubiquitin and finally the CD4mut sequence. Binding of the nanodisc-inserted histidine-tagged protein to a monoclonal anti-pentahistidine antibody immobilized on a sensor chip surface was quantified by SPR. The nanodisc-incorporated IMP showed nearly identical affinity toward the antibody as did the soluble decahistidine-tagged ubiquitin studied in a control experiment [4].

Finally, we started solid state NMR studies of liposome-reconstituted CD4mut and full-length VpU. High quality 2D double-quantum-single-quantum correlation and 13C,13C-correlation spectra of both proteins were recorded. For CD4mut, site-specific assignments for 70% of the residues in the transmembrane domain were obtained based on homonuclear interresidue correlations observed at 0 °C. The 13C chemical shifts closely match those of CD4mut in DPC micelles measured by liquid state NMR at 45 °C and are indicative of α-helical secondary structure.

GABARAP-like proteins – autophagy and beyond

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The GABA<sub>A</sub> receptor-associated protein (GABARAP) is part of the autophagic execution machinery, but also plays an important role in intracellular trafficking of several membrane proteins. Alterations in GABARAP expression or function have been associated with a number of human diseases like neurodegenerative disorders and cancer. In our previous work, we have demonstrated that GABARAP features two hydrophobic binding sites (hp1 and hp2), which are required for complex formation with protein ligands. Recent experimental data has not only extended the spectrum of known GABARAP interaction partners, but has also improved our understanding of its physiological functions.

Cellular integrity critically relies on the presence of degradation systems with the potential to dispose of defective biomolecules and organelles or to disintegrate cytoplasmic material for recycling of its constituents. One of these fundamental pathways is autophagy, which has been found to be deregulated in a series of human diseases. During autophagy, cytosolic components are engulfed by a crescent-shaped double membrane termed the phagophore, which is subsequently closed to form the autophagosome. Upon fusion of the autophago-some with lysosomes, its contents are decomposed by hydrolytic enzymes. In yeast, conjugation of the ubiquitin-like protein Atg8 with phosphatidyl-ethanolamine (PE) is a critical requirement for autophagosome formation. Atg8 has several homologues in mammals, including three variants of microtubule-associated protein light chain 3 (LC3A, LC3B, LC3C), GABA<sub>A</sub> receptor-associated protein (GABARAP), glandular epithelial cell protein 1 (GEC1) and Golgi-associated ATPase enhancer of 16 kDa (GATE-16). Similar to Atg8, these proteins are substrates of a lipid conjugation machinery, resulting in their membrane attachment.

GABARAP is a versatile protein with a wide range of interaction partners, which are involved in various aspects of vesicle transport and fusion as well as apoptosis. Structural studies using NMR spectroscopy as well as X-ray crystallography have revealed that most interactions of GABARAP are mediated by two hydrophobic pockets on its surface. Phage display screening for peptides interacting with GABARAP has led to the identification of the signature xxWxxΨ as a minimal binding motif. In this sequence, Ψ is hydrophobic, whereas x can be any residue, but at least one of these needs to be acidic. Based on this motif, we identified the Bcl-2 family member Nix as a direct interaction partner of GABARAP [1]. Nix, which is also known as Bnip3L, is a pro-apoptotic protein containing a single Bcl-2 homology (BH) 3 region, which can be sequestered and inactivated by Bcl-2. The interaction between Nix and GABARAP was verified by pull-down analysis, co-immunoprecipitation and immunofluorescence imaging. Structural information about this complex could be obtained by solution NMR spectroscopy. $^{1}$H-$^{15}$N heteronuclear single quantum coherence (HSQC) spectra of $^{15}$N-GABARAP recorded during titration with a soluble form of Nix clearly indicated a direct interaction of both proteins. The GABARAP residues most strongly affected upon Nix binding are located in a continuous region of its surface comprising the two hydrophobic pockets (Fig. 1). Since Nix binds to GABARAP via its N-terminal WVEL region, which conforms to the xxWxxΨ motif and is distinct from the Bcl-2 interaction surface, simultaneous binding of Nix to GABARAP and Bcl-2 may be feasible. Surprisingly, Bcl-2 turned out to also display significant affinity for the GABARAP molecule (P. Ma, M. Schwarten, J. Mohrlüder, M. Stoldt, B. Strodel, A. Methner, O. H. Weiergräber and D. Willbold, J. Biol. Chem., in revision).

**FIG. 1:** NMR structure of GABARAP (blue ribbon) with residues affected by Nix binding coloured yellow and orange, indicating large chemical shift differences and line broadening, respectively, in HSQC spectra.

As recombinant Bcl-2 is poorly soluble, we used a chimera in which the 35-91 loop of Bcl-2 was replaced...
found that GABARAP directly binds to Bcl-2 with higher affinity (Kd = 26 µM) than to Nix (Kd = 100 µM). The relevance of this interaction in vivo could be demonstrated by pull-down and co-immunoprecipitation experiments. As expected, immunofluorescence imaging revealed a partial colocalization of the two proteins.

In addition to these protein-protein interactions, we have used state-of-the-art methods to address important problems related to the GABARAP molecule itself. First of all, the structure of lipidated and membrane-associated GABARAP, which constitutes the active species promoting autophagy, has not been assessed experimentally. By using a thiol-reactive lipid compound, we have been able to anchor the GABARAP C-terminus to nanodisc particles, yielding a native-like model of the membrane-attached protein (Fig. 3). NMR studies revealed that this molecule largely retains its native fold, with its hydrophobic pockets accessible for interaction with target proteins [2]. Second, oligomerization of GABARAP has been demonstrated years ago, but its significance has remained unclear. Based on translational self-diffusion coefficients obtained from 1H-15N-DOSY-HSQC measurements, we have provided the first quantitative account of this process. Evaluation of the NMR data as a function of temperature revealed both concentration-independent and -dependent changes in the diffusion properties of GABARAP [3]. While the former are indicative of conformational changes, the latter reflect the oligomerization propensity of soluble GABARAP, with estimated dissociation constants in the low millimolar range.

FIG. 2: Molecular model of the complex formed by Bcl-2 (ribbon drawing) and GABARAP (surface representation). In the Bcl-2 molecule, the BH segments are highlighted by colour and labelled accordingly. See text for details.

Residues involved in the GABARAP-Bcl-2 interaction were identified using HSQC titrations. In Bcl-2, the most strongly affected resonances map to the 24-34 region, which overlaps with the end of the BH4 segment. Notably, the sequence does contain a tryptophan residue, but fails to fully match the xWxAΨ signature. In a complementary experiment, GABARAP residues involved in Bcl-2 binding were mainly found within a patch centered on the first hydrophobic pocket (hp1). Similar titrations using Bcl-2-derived peptides revealed that two acidic residues flanking W30 in Bcl-2 also play a crucial role for complex formation. Docking experiments guided by these results led to the model shown in Fig. 2. Note that the bulky tryptophan side chain (W30) of Bcl-2 is buried deeply in hp1 on the GABARAP surface whereas hp2 is not occupied. Furthermore, the complex is stabilized by several hydrogen bonds and salt bridges. The role of Bcl-2 family proteins in autophagy has only recently been appreciated. Specifically, Bcl-2 negatively regulates autophagy via its interaction with Beclin 1, an essential activator of the PI-3 kinase Vps34. Our data suggest that GABARAP might represent a novel target of Bcl-2 in autophagy. In support of this view, we could demonstrate that the expression level of Bcl-2 affects GABARAP lipidation and thus its subcellular distribution.

In addition to these protein-protein interactions, we have used state-of-the-art methods to address important problems related to the GABARAP molecule itself. First of all, the structure of lipidated and membrane-associated GABARAP, which constitutes

FIG. 3: GABARAP covalently attached to a phospholipid within a nanodisc. Residues of hp1 and hp2 are coloured blue and yellow, respectively, those displaying HSQC chemical shift changes upon coupling are shown in red. Blue cylinders indicate the membrane scaffold protein, which laterally shields the lipid bilayer (Figure provided by J. Glück).

The investigations outlined above have provided new insight into the function of GABARAP in the execution of autophagy. Current studies are focussing on the role of Bcl-2 homologues and their mode of regulation, as well as the structural characterisation of additional protein families involved in autophagosome biogenesis.

Protein structure determination at low resolution

G.F. Schröder

ICS: Structural Biochemistry

One of the grand challenges in structural biology is the determination of large biomacromolecules and in particular complexes thereof, which are often of key biological importance. For such systems, X-ray crystallography and single-particle cryo-electron microscopy (Cryo-EM) often yield only low-resolution data, which are inherently difficult to interpret. We have developed computational tools to improve the refinement of protein structures at low resolution and applied these tools to a variety of different protein systems, such as the chaperonin mm-Cpn and actin filaments. Our approaches are sufficiently general to be also useful to other types of experiments, as we have shown with applications to structural modeling using SAXS and single-molecule FRET data.

X-ray crystallography and single-particle cryo-electron microscopy (Cryo-EM) are particularly suited to determine the structure of large macromolecular systems such as protein assemblies. However, due to their inherent flexibility and inhomogeneity, high-resolution (<3.5 Å) structure determination of such large macromolecular systems is often impossible; experiments often yield only low-resolution data. The number of structures deposited to the PDB that are determined to resolutions lower than 4 Å is steadily increasing, which shows the increased interest.

Since the interpretation of low-resolution data is difficult, new algorithms and software tools are needed. We combine protein structure prediction approaches with model building and refinement of protein structures. We developed the Deformable Elastic Network (DEN) approach to improve crystallographic protein structure refinement at low resolution [1]. This new method adds specific information from known homologous structures but allows global and local deformations of these homology models. Our approach uses the observation that local protein structure tends to be conserved as sequence and function evolve. Cross-validation with Rfree (the free R-factor) determines the optimum deformation and influence of the homology model. For test cases at 3.5-5 Å resolution with known structures at high resolution, our method gives significant improvements over conventional refinement in the model as monitored by coordinate accuracy, the definition of secondary structure and the quality of electron density maps (see Fig. 1). For re-refinements of a representative set of 19 low-resolution crystal structures from the Protein Data Bank, we find similar improvements. Thus, a structure derived from low-resolution diffraction data can have quality similar to a high-resolution structure.

We are further developing the real-space refinement program DireX (http://www.simtk.org/home/direx) which is targeted at refining atomic models against density maps obtained from cryo-EM. We applied this software to several different systems in collaboration with experimental groups.

With the group of Wah Chiu (Baylor College, TX, USA), we determined four cryo-EM structures of Mm-cpn, an archaeal group II chaperonin, in the nucleotide-free (open) and nucleotide-induced (closed) states [2]. The 4.3 Å resolution of the closed conformation allowed building of the first ever atomic model directly from

FIG. 1: Electron density map improvement upon DEN refinement for three structures, PDB 3DMK, 1YE1 and 1XXI. The 1YE1 (c, d) and 1XXI (e, f) structures are among the cases that benefit most from DEN refinement, whereas the 3DMK (a, b) structure showed only moderate improvement of the Rfree value (Table 2). Nevertheless, in all three cases DEN refinement dramatically improves the electron density maps. The structures refined with DEN (DEN, in blue) and without DEN (noDEN, in orange) are superimposed, and the corresponding phase- combined SA-weighted 2Fo 2Fc electron density maps are shown in blue and red, respectively.
the cryo-EM density map, in which we were able to visualize the nucleotide and more than 70% of the side chains. The model of the open conformation was obtained by using the DEN modelling with the 8 Å resolution open-state cryo-EM density restraints. Together, the open and closed structures show how local conformational changes triggered by ATP hydrolysis lead to an alteration of intersubunit contacts within and across the rings, ultimately causing a rocking motion that closes the ring. Our analyses show that there is an intricate and unforeseen set of interactions controlling allosteric communication and inter-ring signalling, driving the conformational cycle of group II chaperonins. Beyond this, we anticipate that our methodology of combining single particle cryo-EM and computational modelling will become a powerful tool in the determination of atomic details involved in the dynamic processes of macromolecular machines in solution.

In collaboration with the group of Edward Egelman (University of Virginia, VA, USA), we have been studying the structure of actin filaments [3]. We showed that frozen-hydrated actin filaments contain a multiplicity of different structural states. We show (at ~10 Å resolution) that subdomain 2 can be disordered and can make multiple contacts with the C-terminus of a subunit above it. We link a number of disease-causing mutations in the human ACTA1 gene to the most structurally dynamic elements of actin. Because F-actin is structurally polymorphic, it cannot be described using only one atomic model and must be understood as an ensemble of different states.

More recently, we determined the structure of cofilin decorated actin filaments at a resolution of 9 Å, the highest resolution achieved for a complex of F-actin with an actin binding protein. Cofilin/ADF proteins play key roles in the dynamics of actin, one of the most abundant and highly conserved eukaryotic proteins. We show that the cofilin-induced change in the filament twist is due to a unique conformation of the actin molecule unrelated to any previously observed state. The changes between the actin protomer in naked F-actin and in the actin-cofilin filament are greater than the conformational changes between G- and F-actin. Our results show the structural plasticity of actin, suggest that other actin-binding proteins may also induce large but different conformational changes, and show that F-actin cannot be described by a single molecular model.

Our methods are not restricted to X-ray crystallography or Cryo-EM: we studied with our modeling techniques also the conformational transitions of phosphoglycerate kinase using small-angle X-ray scattering (SAXS) data [L. Zerrad, A. Merli, G. F. Schröder et al. JBC (2011) 286:14040-14048].

In addition, we have determined structures of branched DNA molecules with a combination of single-molecule FRET data and structural modeling [5]. Such branched DNA plays critical roles in DNA replication, repair, and recombination and are also key building blocks for DNA nanotechnology. Our study revealed an open, planar structure of a forked DNA molecule with three duplex arms and demonstrated an ion-induced conformational change.

The first high-resolution free electron laser (FEL) is now operational at the LCLS at Stanford University. We worked on one of the first data sets collected on this FEL: a 7 Å data set of the photosystem I. The methods that we have developed have proved to be very powerful in the refinement of this structure (manuscript submitted). With the new XFEL at DESY in Hamburg, it can be expected that more, comparable data sets will be obtained for other important biological structures in the near future and that the methods that we are developing will be valuable tools to exploit the full power of this new experimental technique.

Furthermore, we are developing physics-based structure prediction techniques to complement structural information that is missing from low-resolution data. With our prediction techniques, we participated successfully in the last CASP9 experiment (refinement category), where our group was ranked among the top 5 groups (out of 36).


Approaches for therapy of Alzheimer’s disease

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Alzheimer’s disease (AD) is a devastating neurodegenerative disorder. The clinical characteristics are difficulties with memory deficits, apathy, depression, impaired judgment, disorientation, confusion and others. In 2007 AD affected 27 million people world-wide with steadily increasing tendency. The greatest risk factor to develop AD is age. Up to now, no causative treatments are available. Presently available treatments cannot slow down disease progression. Specific neuro-pathological changes like neurofibrillary tangles and amyloid plaques define AD. Amyloid plaques are mainly composed of the amyloid-β peptide (Aβ). Several lines of evidence suggest that the formation and accumulation of Aβ plays fundamental roles for disease progression. Therefore, substances that target Aβ oligomers and reduce their toxicity are of great interest. In the past years, we have successfully developed agents that are capable to specifically address and abolish toxic Aβ oligomers.

Aβ is the major component of the amyloid plaques. It consists of 39 to 43 amino acid residues. Especially Aβ1-42 is prone to aggregation and forms oligomers, protofibrils, fibrils and plaques. As originally suggested by the amyloid cascade hypothesis, it appears likely that Aβ peptides and their aggregated forms initiate cellular events leading to the pathologic effects of AD. According to a previous version of the amyloid cascade hypothesis, fibrillar forms of Aβ, deposited in amyloid plaques, have been thought to be responsible for neuronal dysfunction. More recent studies support that small, diffusible Aβ oligomers are the major toxic species responsible for disease development and progression. We have developed substances which bind to toxic species of Aβ and influence their aggregation and cytotoxicity.

In our laboratory a phage displayed peptide library with more than 1 x 10⁹ different 12mer peptides was screened for peptides with binding affinity to the mirror image of aggregated Aβ(1-42). The basic idea was to identify ligands that interfere with the formation of toxic Aβ aggregates and their toxicity. Because we used a mirror image of the phage display procedure we obtained D-enantiomeric peptides, which are known to be very resistant to degradation in vivo and are therefore applicable for therapeutic approaches in animals and humans. One of the identified D-peptide ligands, denominated D3, was shown to have a dramatic potential to modulate Aβ aggregation and cytotoxicity in vitro and in vivo [1]. In vitro data suggest that D3 precipitates toxic Aβ oligomer species and converts them into non-amyloidogenic, non-fibrillar and non-toxic amorphous aggregates without increasing the concentration of monomeric Aβ.

The APP-PS mouse model expressing human APPswe and PS1-ΔE9 develops elevated levels of Aβ42 at the age of about four months. At around five months of age they show typical Aβ plaques. Young, four-months old female mice were orally treated with D3 by adding D3 to the drinking water for eight weeks. After seven weeks of D3 treatment, all groups of mice were tested in the Morris water maze. The D3 treated mice showed a significantly improved learning behaviour during the week of testing, but the untreated mice did not (Fig. 1).

After completion of the behavioural testing, the animals were sacrificed and the brains assessed for AD pathology. In the sections that were stained for Aβ it becomes obvious that brain tissue sections of D3 treated mice had a significantly lower Aβ load as compared to the untreated mice (Fig. 2).

Next to extracellular Aβ, intraneuronal Aβ has important pathological functions in AD. Agents that specifically interfere with the oligomerization processes either outside or inside of neurons are highly desired for the elucidation of the pathologic mechanisms of AD and might even pave the way for
new AD gene therapeutic approaches. In parallel to D3, we selected an L-enantiomeric Aβ binding peptide L3 and characterized its influence on Aβ oligomerization in vitro. Preliminary studies in cell culture demonstrated that stably expressed L3 reduced cell toxicity of externally added Aβ in neuroblastoma cells (Funke SA, et al Rejuvenation Research (2012) in press). Very similar to D3, L3 might interfere with Aβ oligomers. In contrast to D3, however, L3 may be intra- and/or extracellularly produced by neurons or glia cells upon transfection with suitable DNA constructs. Such an approach might help to investigate potential gene therapeutic approaches to AD.

In collaboration with T. Schrader (Organic chemistry department of the University Duisburg-Essen) we were involved in the development of a drug directed against the pathological self-association of Aβ based on a small organic molecule designed as a β-sheet breaker. In our study we established a number of complementary assays for the characterization of the in vitro aggregation behaviour of the Aβ peptide in the presence of the test compounds. By sedimentation velocity centrifugation analysis of Aβ peptide solutions it is possible to determine quantitatively as well as qualitatively aggregate size- and shape-distributions delivering a far more detailed picture of the aggregation process than standard procedures. With this method we could show that a rationally designed small organic inhibitor, that is build out of a trimeric aminopyrazole moiety with a 5′-nitro group on one end and a 3′-carboxyl group on the other end lead to the formation of smaller and more extended aggregates as compared to the control solutions without the inhibitor [2]. Based on these results further research followed in two directions. First, continuing our collaboration with T. Schrader we investigated derivatives from the trimeric aminopyrazole synthesized in order to elucidate the mechanistic details for the interaction with Aβ as well as to generate new compounds with enhanced inhibitory effects. The evolving picture for the aggregation inhibiting mechanism by these newly designed ligands implies transformation of well-ordered fibrils into less structured aggregates with a high molecular weight [3].

Second, in cooperation with the Swiss company AC-Immune SA we assisted in the pharmaco-kinetical optimization of a lead compound derived from the aminopyrazole scaffold by providing a screening assay based on fluorescence fluctuation correlation spectroscopy. This assay gives a measure for aggregate formation in a sample independent of the amyloid nature of the aggregates, complementing thus the results from ThT-fluorescence assays. These studies resulted in the identification of an Aβ aggregation inhibitor fulfilling the requirements for further testing in animal models and in clinical trials. Within this collaboration we also participated in the biophysical characterization of a liposomal vaccine against AD [4].

In a third cooperation project with T. Schrader which also involved C. Korth (Heinrich-Heine-University Düsseldorf) and H. Sticht (University of Erlangen) we developed the hybrid compound [M169], which covalently binds the D-enantiomeric peptide D3 with a β-sheet breaking compound via a linker substance. We demonstrated that the hybrid compound was more efficient in vitro than the sum of its components and had novel properties concerning Aβ aggregation inhibition [5]. This compound is planned to be thoroughly investigated in mouse models soon.

Alzheimer’s disease (AD) is a neuro-degenerative disorder associated with synaptic failure and extracellular accumulation of senile plaques composed of the neurotoxic amyloid-β peptide (Aβ). The major alloforms of Aβ are Aβ40 and Aβ42, which differ by the presence of two amino acids, Ile41 and Ala42 at the C-terminus of the latter. The more hydrophobic Aβ42 is the prevalent alloform seen in amyloid plaques and has a greater tendency to aggregate into fibrils and plaques. There is acceptable evidence suggesting that Aβ exerts its cytotoxic effect by interacting with membranes of neurons and other cerebral cells. While there are numerous studies showing such effects, information about structural transformations of Aβ at and within membranes is still lacking. We aim to provide insights into the neurotoxic effect of Aβ at the molecular level by performing large-scale simulations of Aβ in water and in interaction with membranes.

**Influence of pH on the Aβ structure.** Using explicit solvent molecular dynamics (MD) simulations on the microsecond time scale we investigated different influences affecting the secondary structure of the Aβ peptide. We have acquired extensive knowledge about the importance of sufficient conformational sampling of proteins as a prerequisite to obtain reliable information about the dynamics and kinetics of their structural transitions [1,2]. We have applied this knowledge to study the dynamics and aggregation of various peptides including Aβ [3,4]. Our comparative study of Aβ40 and Aβ42 revealed that Aβ42 has a higher tendency to form intramolecular β-sheets, which may serve as seed for Aβ42 aggregation [4]. We further studied the effect of protonation of the three histidine residues in Aβ42, which reduces the total peptide net charge from -3 to zero and induces interactions of the otherwise hydrophilic N-terminal domain with the hydrophobic C-terminal domain. We observed that the protonation of the three histidine residues significantly increased the β-sheet content in Aβ42. We suggest this effect as an important driving force for the fast aggregation kinetics reported for the amyloid peptide at acidic pH via a reduction in intramolecular electrostatic repulsion. Given the slightly acidic nature of accompanying inflammatory response in AD, acidic pH-facilitated aggregation is likely to be of importance in disease development. It has been shown that brains from patients who die from AD are more acidic than brains from patients who die suddenly with no brain disease.

Aβ interacting with a D-enantiomeric peptide. The molecular events surrounding its causative role in the onset of AD make Aβ a viable target for the treatment of the disease. Various therapeutic strategies try to prevent the aggregation of Aβ into toxic oligomers, which is probably the key pathogenic event. A plethora of organic molecules have been shown to inhibit Aβ aggregation and toxicity. Many inhibitors are peptides or peptidomimetics, which, however, suffer from a significant drawback, that is the fast rate at which they are cleared from circulation by endogenous peptidases. Approaches employed in circumventing this disadvantage include the use of D-enantiomeric peptides. Recently, a 12-residue arginine-rich D-peptide called D3 was identified by mirror-image phage display in the group of Willbold (ICS-6) and was shown to reduce Aβ plaque load and cognitive deficits in transgenic mice in vivo. In addition, it also specifically precipitates toxic Aβ oligomers into nontoxic non-amyloidogenic amorphous aggregates in vitro. We used molecular simulations to unravel how D3 binds to Aβ42 in isolation [4]. Using a global-optimization approach we generated 6000 optimized Aβ42/D3 complexes of which the one hundred structures lowest in energy were further investigated using MD simulations. The interaction energies between Aβ42 and D3 in the resulting complexes were decomposed into residual contributions from both peptides revealing that their interaction is mediated by strong electrostatic attraction between the arginine residues of D3 and the negatively charged aspartic and glutamic residues in the N-terminal part of Aβ42. Our findings are supported by dot blot experiments of various Aβ fragments with fluoresceinisothiocyanat labeled D3 in the group of Willbold (ICS-6), which showed that D3 binds only to the N-terminal fragments containing the negatively charged residues Asp and Glu. The electrostatically driven association between Aβ42 and D3 thus brings D3 to the local vicinity of the N-terminal half of the amyloid peptide, which includes the central hydrophobic core 17LVFFA21. This suggests that D3 prevents the aggregation of Aβ42 into toxic β-sheets by shielding this amyloid-aggregation nuketiding sequence. From our simulation study we can confirm that D3 binding leads to a significant decrease in β-sheet contents in Aβ42.
**Aβ interacting with lipid membranes.** The amphipathic nature of the Aβ peptide has been suggested to contribute to its capacity to coat or lie on the surface of the membranes, or penetrate and insert into membranes. A potential pathway for Aβ toxicity lies in its ability to alter biophysical membrane properties causing membrane disruption and increased permeability, which enables excessive leakage of ions, particularly calcium ions. However, there is conflicting experimental evidence as to the secondary structure and oligomerization state of membrane-bound Aβ. Molecular simulations provide the possibility to gain atomic information on the dynamics and structure of Aβ interacting with lipid bilayers. We performed simulations with the aim to identify the most stable structures of the transmembrane Aβ42 monomer and oligomers (up to the octamer). To this end we employed a global optimization approach based on Monte Carlo with minimization to investigate Aβ42 in an implicit membrane and found β-sheets as most stable membrane-spanning structures [4]. The oligomeric β-sheets, especially tetramers and hexamers, are stabilized by peptide-peptide interactions. Based on these results we confirmed the experimental result that Aβ pores as observed in atomic force microscopy (AFM) images may consist of tetrameric and hexameric β-sheet subunits (FIG. 1).

**FIG. 1:** Aβ channel-like structures. (A) Individual channel-like structures at high resolution from an AFM image of Aβ40 reconstituted in membrane bilayers. This picture was reproduced from Quist, A. et al. Proc. Natl. Acad. Sci. USA 102, 10427-10432 (2005). (B) Tetrmeric and hexameric β-sheets obtained from a global optimization simulation of transmembrane Aβ42, which are likely structures for the subunits in the Aβ channel-like structures observed in AFM experiments A.

To study the effects of these structures on membrane integrity we performed atomistic MD simulations of the β-sheet monomer and tetramer at the submicrosecond time scale considering lipids with different headgroups and different tails (C. Poojari, A. Kukol & B. Strodel, submitted). Due to conflicting experimental results as to whether membrane-bound Aβ is helical or in a β-sheet conformation we also performed MD simulations of a helical structure obtained from NMR experiments for Aβ42 in a membrane-mimicking solvent. Our MD simulations, in which Aβ42 always remained embedded in the bilayer, revealed a relatively high structural stability irrespective of the Aβ42 starting structure and despite negative hydrophobic mismatch. We found that the surface charge and the type of lipid tails are determinants for transmembrane stability of Aβ42 with zwitterionic surfaces and unsaturated lipid tails as in palmitoyloleoyl phosphatidyl-choline (POPC) membranes promoting stability. The MD simulations further confirmed that from the considered structures the β-sheet tetramer is the most stable structure as a result of interpeptide interactions and that it is the only structure, which facilitates increased water permeability compared to peptide-free lipid bilayers. This finding allows us to conclude that permeabilization of lipid bilayers as observed experimentally must be due to transmembrane Aβ oligomers and not monomers as some studies conjectured. In a further study we investigated the effects of mutations on the stability of transmembrane Aβ42 (C. Poojari & B. Strodel, submitted).

Another main conclusion from our atomistic MD simulations of membrane-bound Aβ42 at the submicrosecond time scale is that this time scale is still not sufficient to follow the assembly of Aβ42 into oligomers on the membrane surface or when inserted in membranes, let alone the insertion of Aβ42 into membranes. To study the dynamics of these membrane-induced processes coarse-grained peptide and lipid models are required in order to extend the length and time scale of our MD simulations. To this end we started to employ the coarse-grained MARTINI force field to simulate lipid bilayers (G. Sansanwal, C. Poojari & B. Strodel, unpublished results). We followed the self-assembly of pure POPC bilayers and POPC bilayers with sphingomyelin and/or cholesterol added to it. The resulting model raft and non-raft membranes will be used in future studies of Aβ42 self-assembly on the membrane surfaces and inside the membranes.

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**References:**


Surface-FIDA: single particle detection as diagnostic tool

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Alzheimer’s disease, Prion diseases and Parkinson’s disease are neurodegenerative diseases that are characterized by the formation of protein aggregates during the progression of the disease. Whether these aggregates are causative for, or symptoms of, the disease is still under intensive research. However many studies show that aggregates or even oligomers of the according proteins are neurotoxic and lead to neurodegeneration. To understand disease-associated or causative mechanisms in respect to protein aggregation, as well as new diagnostic approach an ultrasensitive method to quantify these disease-related aggregates is required. In this project we introduce a novel diagnostic tool to count, specify and characterize even single protein aggregates.

Alzheimer’s disease (AD) is a fatal neurodegenerative and progressive disorder. AD is the most prevalent dementia, affecting nearly 2% of the population in the western world, where the risk of AD increases dramatically in individuals beyond the age of 70. The major component of the amyloid plaques is the Amyloid-β (1-42) peptide. Currently, no reliable biomarker for pre-symptomatic diagnosis or therapy monitoring is available. Recent studies indicate that especially soluble Aβ oligomers are the major toxic species during development and progression of AD. Therefore, we suggest that the number of Aβ oligomers in body fluids can be used as the most direct biomarker for AD.

Parkinson’s disease (PD) is one of the most common age-related neurodegenerative diseases in humans associated with motor deficiencies. The symptoms are caused by the death of dopaminergic neurons in the brain, which are accompanied by the misfolding and aggregation of the protein α-synuclein. Diagnosis is based on the incidence of clinical symptoms, although they only appear as a result of the irreversible damage of neurons during the disease. Identification of a suitable biomarker would allow predilential diagnosis.

Prions are the causing agent of transmissible spongiform encephalopathies (TSEs) such as Creutzfeldt-Jakob disease (CJD) in man, bovine spongiform encephalopathy (BSE) in cattle and Scrapie in sheep. These diseases are characterised by an abnormally folded form of the host-encoded prion protein (PrP). The cellular, i.e. non-pathological isoform of PrP (PrPC) is present in most tissues, especially the central nervous system. Following infection, PrPSc undergoes a conformational change, leading to altered physicochemical properties such as aggregation, insolubility and β-sheet rich secondary structure. This pathological isoform is designated PrPSc.

Within this project we focussed on the detection of Aβ, α-synuclein and PrP aggregates. In all three diseases protein aggregates occur during disease progression. Therefore we established a highly sensitive and specific tool to detect even single protein aggregates [1, 2, 3] called surface-FIDA (sFIDA) (fig. 1).

Our new test system is based on fluorescence microscopy using fluorescence intensity distribution analysis (FIDA). It is quantifying the number and size of aggregates simultaneously labelled by two different antibodies for dual colour FIDA. Only aggregates and oligomers but not monomeric proteins are counted. To increase the sensitivity, particles were concentrated on a chip surface immobilized by capture antibodies. Laser beams are scanning the surface systematically, so even single particles are detected.

We report on the successful use of sFIDA as diagnostic tool for prion diseases. Therefore we were able to detect PrP-aggregates in brain tissue of cattle afflicted with BSE, hamsters afflicted with Scrapie, sheep afflicted with Scrapie and humans afflicted with CJD. In body fluids we detected PrP-aggregates in the cerebrospinal fluid (CSF) of BSE afflicted cattle and even blood plasma of Scrapie afflicted sheep (fig 2). Furthermore during the pathogenesis of BSE infected cattle, we could detect first PrP-aggregates in the brain down to 16-24 month after infection, which is 12 month before clinical signs occur.
We introduced an approach to quantify single α-synuclein aggregates as a possible biomarker for PD [1]. Therefore, we used *in vitro* generated α-synuclein aggregates (fibrils) and showed the proof of principle, that these aggregates could be detected and counted as single aggregates by sFIDA (FIG. 3).

![FIG. 3: sFIDA: visualization of single alpha-synuclein aggregates (0.001 µg and 0.01 µg of *in vitro* generated alpha-synuclein fibrils) in colocalized fluorescent images (ImageG), 165x193mm (300 x 300 DPI). Buffer and 0.1 µg alpha-synuclein monomers serve as control samples (figure according [1]).](image)

We challenged the assay with CSF samples from 14 AD patients and 12 age-matched control subjects. The Aβ oligomer count revealed a surprisingly clear distinction between both groups. All samples of the control group showed homogeneously low numbers of Aβ oligomers, while the samples of the AD group had comparably high levels of Aβ oligomers and displayed high variability (FIG. 5). The Aβ oligomer levels clearly correlated with the patients’ mini-mental state examination (MMSE) scores.

![FIG. 2: Detection of PrP particles in blood plasma of scrapie-infected sheep in a blinded study. PrP aggregates from 15 plasma samples were prepared, applied to sFIDA. Image processing e.g. background reduction was done using ImageJ. The total number of crosscorrelated pixels of the processed images is given for each sample. After decoding samples were assigned as scrapie-positive (red) or uninfected controls (green).](image)

During the next steps we will improve the sFIDA-assy in respect of sensitivity and specificity e.g. using high resolution techniques like STROM (stochastic optical reconstruction microscopy), using high throughput imaging techniques like TIRFM (total internal reflection fluorescence microscopy) and adapt the highly sensitive test system for diagnosis of human diseases like AD, CJD, PD using body fluids like CSF and blood.

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ICS-7: Biomechanics

Within the organism many processes of vital importance depend crucially on mechanical properties and processes of living cells. Prominent examples are connective tissue tension caused by continuous force generation of embedded cells, heart contractions produced by the regular beat of heart muscle cells and cell locomotion during embryogenesis. Moreover, living cells react to mechanical stimuli from their environment like mechanical stiffness or oscillatory strain. The gamut of these reactions ranges from subtle changes of the cellular cytoskeleton to initiation of differentiation processes changing the whole cell. In the broad field of biomechanical processes of living animal cells our work is centered on the force producing actin cytoskeleton, force transmission to the external environment via cellular adhesion structures bridging the plasma membrane, and cellular mechanosensing. We employ modern methods of cell biology and cell biophysics. When necessary we also develop tailored methods, e.g., to apply carefully calibrated mechanical stimuli to cells under nature-like conditions or to incorporate tracer molecules into the biomembrane and map their interactions with adhesions. Many of our present experimental approaches are based on micromanipulation, microfabrication, replica molding of soft silicone elastomers, physical chemistry of lipids, quantitative light microscopy of living cells, mathematical analyses, and model formulation. In our quest for quantitative understanding we complement work on living cells with tailored in vitro model systems of the cytoskeleton and the biomembrane.
Research Highlights

Cyclic Stress at mHz frequencies aligns cells in direction of zero strain

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Recognition of external mechanical signals is vital for mammalian cells. Cyclic stretch, e.g. around blood vessels, is one such signal that induces cell reorientation from parallel to almost perpendicular to the direction of stretch. Here, we present quantitative analyses of both, cell and cytoskeletal reorientation of umbilical cord fibroblasts. Cyclic strain of preset amplitudes was applied at mHz frequencies. Elastomeric chambers were specifically designed and characterized to distinguish between zero strain and minimal stress directions. For all stretch amplitudes, the angular distributions of reoriented cells are in very good agreement with a theory modelling of stretched cells as active force dipoles.

Cell adhesion and the corresponding mechanical coupling to the environment are prerequisites for the survival and functionality of numerous cell types. The direct interaction between the cell and its extracellular matrix (ECM) enables cells to actively sense and respond to varying environmental conditions and thereby to maintain mechanical homeostasis. This is a fundamental feature, since adherent cells not only exert active cellular forces on the ECM but are also constantly exposed to various externally generated mechanical forces such as shear flow or uniaxial strain. The latter is the focus of this work.

When cells are exposed to strain, their actin bundles reorient and thereby change the transmission of stress through the filaments to maintain optimum mechanical conditions. A major open question in this respect is if stress (force per area) or strain (material deformation) is the relevant control parameter. It is well accepted that the reaction of cells to cyclic stress is an “escape mechanism” to avoid stretch [4], where the degree of reorientation perpendicular to the strain direction seems to correlate with the stretch amplitude.

To explain mechanically induced cell reorientation, De and Safran (Biophys. J. 94: L29) developed a coarse-grained theoretical model. This model considers forces induced both actively by cells due to cytoskeleton rearrangement as well as passively by substrate stretch. Focusing on cells with bipolar morphologies, the model idealizes stationary adhering cells as force dipoles composed of two oppositely directed forces exerted at the cell extremities and their separation distance.

The theory assumes ideal material behavior under uniaxial stress. However, calibrations of the cell culture chambers (cf. Fig. 1) revealed additional stress perpendicular to the direction of external stretch [1]. To rectify this idealization of our experimental situation we used the measured perpendicular shrinkage ratio \( \kappa \) instead of the Poisson’s ratio \( \nu \). Only this choice ensures that the effective free energy \( F \) is minimal in the direction of zero strain \( \alpha_0 \) which is the only distinct direction. Here \( u_4 \) is proportional to the amplitude of the applied cyclic strain. It is multiplied by a parameter describing the cell’s tendency to follow such orientation cues multiplied by the set point stress.

In vivo analyzed cells do not perfectly align along this direction. Instead a substantial scatter is observed (Fig. 2). This scatter arises from noise in the system which can be modelled as an effective temperature and a Boltzmann distribution function of angles. This yields the distributions of cytoskeletal angles. The only unknown parameter in these distributions is the effective temperature which we determine by a simultaneous fit to all measured distributions. The resulting theoretical distributions are displayed as dotted lines in Fig. 2.

By analyzing human umbilical cord fibroblasts we present here quantitative experiments on large cell populations that answer the question of whether cells reorient at mHz frequencies in direction of zero strain or low stress. For this purpose we designed two different stretching chambers. These chambers were specifically designed to define and to vary directions of zero strain upon cyclic stretch.
The direction of zero strain, $\alpha_0$, is given by:

$$\alpha_0 = \arcsin \left( \frac{1}{1 + \kappa} \right)$$

were $\kappa$ is the transversal shrinkage ratio $\kappa$ ($\kappa = -\Delta y/\Delta x$). By modifying the wall thickness of our elastomeric chambers we are able to tune $\alpha_0$. For box-shaped setup with $\kappa=0.15$ resulting in $\alpha_0=69°$, whereas $\alpha_0$ for ribbon-like chambers was $62°$.

The interplay of elongation and contraction in stretching chambers is shown in Fig. 1.

Cellular response was evaluated at the level of reorientation of cytoskeletal stress fibers as well as of the entire fibroblast cell (cf. [1] for data). Automated microscopy and image data processing allowed the evaluation of hundreds of cells per experiment. The predominant cytoskeletal orientations were determined for cells stretched for 16 hours at, amongst others [1], amplitude $a_5$ (32%, 772 cells) in box-shaped chambers ($\kappa = 0.15$) and at amplitude $b_5$ (31.7%, 588 cells) on ribbon-like substrates ($\kappa = 0.29$). Control measurements showed random distributions indicated as a dashed line. The dotted lines express the theoretical description of the measured actin angle distributions. (B) Cytoskeletal reorientation after cyclic stretch as described before. Cells were fixed and stained for actin. Scale bars, 50 µm.

Actin angle distributions of cells stretched under these conditions in box-shaped chambers ($a_5$) were reduced by 81% for the angle range of $0°$ to $50°$ and increased by as much as 401% for angles above the angle of zero strain ($70°$ to $90°$).

In contrast, actin distributions at $b_5$ show a clearly peaked distribution with its maximum shifted towards direction of zero strain (Fig. 2). Cells with orientations of low stress and therefore high contractile strain are significantly reduced in number compared to $a_5$ although stretch frequency and amplitude were unchanged. Here, angles in the range of $70°$ to $90°$ were reduced by 10% and even by 28% for the minimal stress range of $80°$ to $90°$.

At all analyzed stretch parameters, the distribution of actin stress-fiber orientations in our cell population were well described by the theoretical model [1].

Shape normalization as a tool to quantify typical cell behaviour

C. Möhl, N. Kirchgeßner, C. Schäfer, B. Hoffmann, R. Merkel

Spatially ordered formation and disassembly of focal adhesions is a basic requirement for effective cell locomotion. Since focal adhesions couple the contractile actin-myosin network to the substrate, their distribution determines the pattern of traction forces propelling the cell in a certain direction. Here we mapped averaged focal adhesion growth dynamics to a standardized cell coordinate system. These maps revealed distinct zones of focal adhesion assembly, disassembly and stability and were strongly interrelated with corresponding actin flow and traction force patterns.

Adhesion of animal cells to substrates occurs at specific sites, so-called focal adhesions (FA). These micron-sized structures connect the intracellular actin cytoskeleton to the extracellular matrix (ECM). Therefore they are the spots where mechanical forces are transmitted between the cell and its environment. In locomoting cells adhesions undergo a dynamic turnover. They are generated at the cell front. Subsequently they grow in size, mature in protein composition and activity, and, finally, dissolve again. With respect to the substrates, adhesions are almost but not entirely stationary.

All events during adhesion lifetime and the molecular players involved have been extensively studied by cell and molecular biology. However, no two cells are identical and even the state of a given cell undergoes permanent changes. For these reasons, comparison between cells is difficult and knowledge of what comprises "typical" versus "atypical" cell behaviour is mostly based on experience. Thus many of the aforementioned processes have been understood to some extent on a very intuitive basis. Obviously, only dramatic effects can be tackled by such an approach.

To overcome this barrier we devised a method to map micrographs of living cells onto a standardized geometry [1]. For this we employed life cell microscopy on cells transfected with either GFP-actin or GFP-vinculin. This enabled real time imaging of cytoskeleton (GFP-actin) or focal adhesions (GFP-vinculin). As cell system we used human keratinozytes cultivated under standard conditions whose locomotion activity was initiated by EGF (epidermal growth factor).

Transforming an object to a normalized shape involves: (1) shifting, (2) rotating, and (3) mapping the shape. For these steps we made the following choices: (1) The origin of the coordinate system is the cell center. (2) Images were rotated to keep the direction of locomotion constant. Cells appeared slightly...
elliptical (median ratio of long to short half axis 1.24). Yet, the direction of locomotion didn’t correlate with the orientation of the ellipse. Therefore the resulting average cell shape was almost circular. Thus we decided (3) to map micrographs of cells onto the unit circle. This procedure is exemplarily shown in Fig. 1.

Using this mapping method we created scaled and averaged maps of actin density, actin flow velocity (determined by the algorithm of Danuser, J. Microscopy 220: 150), focal adhesion density, area, shape, sliding velocity, growth rate and applied forces [2]. Some examples are shown in Fig. 2.

This procedure enables averages over long movies of many cells. Moreover, maps of diverse cell properties can be compared. Thus the resulting data are of high statistical significance. For the example in Fig. 2 we conclude that actin flow velocity is highest where vinculin (i.e. anchoring strength) is lowest. At the same location FA sliding is most pronounced whereas traction forces are lowest. By analysing many more parameters we arrived at the cell locomotion scheme displayed in Fig. 3.

In conclusion, we have presented a first cell shape normalization scheme and exploited its power to study adhesion and locomotion of keratinocytes. Results with high statistical significance have been achieved.

**FIG. 2:** Top: Average brightness of GFP-vinculin (false colors) overlayed with actin flow velocity (length of arrow 10 \(\mu m/min\)). Middle: Sliding velocity of focal adhesions (arrow 0.15 \(\mu m/min\)). Bottom: Brightness of vinculin overlayed with traction forces (length of arrow 5 nN).

**FIG. 3:** Scheme of processes during cell locomotion.


Migration of cells is one of the most essential prerequisites to form higher organisms and depends on a strongly coordinated sequence of processes. Early migratory events include substrate sensing, adhesion formation, actin bundle assembly and force generation. While substrate sensing was ascribed to filopodia, all other processes were believed to depend mainly on lamellipodia of migrating cells. We show for motile keratinocytes that all processes from substrate sensing to force generation strongly depend on filopodial focal complexes. In a coordinated step by step process, filopodial focal complexes have to be tightly adhered to the substrate and to actin filaments to enlarge upon lamellipodial contact forming classical focal adhesions. Upon cell progression, the incorporation of filopodial actin bundles into the lamellipodium goes along with a complete change in actin cross-linker composition from filopodial fascin to lamellipodial α-actinin and leads to elevated force levels applied at filopodial adhesions. Furthermore, even within filopodia, fascin concentration does not stay stable but becomes downregulated by actin retrograde flow after stable filopodial adhesion. Consequently, adhesion sites formed independently of filopodia are not connected to detectable actin bundles, transmit weak forces to the substrate and disassemble within a few minutes without having been increased in size.

Recent experiments have proven that many lamellipodial focal adhesions (FAs) derive from stably attached filopodial focal complexes (FXs). Unfortunately, structural as well as functional analyses over the whole lifetime of lamellipodial structures of filopodial origin are rare. For this reason we first analyzed stably adhered filopodial FXs in locomoting keratinocytes over time [1,2,3]. We could show that filopodial FX remained in place when overgrown by the lamellipodial leading edge to form classical FAs just by enlargement (Figure 1A, white arrows). Statistical analyses revealed that 65% of all analyzed FXs (120/184) grew from FXs directly behind filopodia. These FAs displayed a constant increase in size and persisted over several minutes. In contrast, 35% of lamellipodial FAs were formed without filopodial influence. These adhesions developed within the lamellipodium but remained small and disassembled already after a few minutes (Figure 1B, white arrows).

Given data argue for the presence of two different types of lamellipodial adhesion sites. We therefore analyzed all lamellipodial adhesions in more detail by measuring their whole lifespan. Interestingly, FAs with lifetimes of not more than 6 min were almost constant in mean area as well as aspect ratio. Taking this lifetime as threshold to classify the two groups of FAs as stable and unstable ones, we found that 86% (111/129) of stable focal adhesions were originated from filopodial FXs. In contrast, 84% (46/55) of lamellipodial FAs with lifetimes below 6 min were formed without filopodial attendance. These results demonstrate the high value of FA lifetime to discriminate between the two groups of lamellipodial FAs. Sorted like this, stable adhesions (n = 129) exhibited a mean lifetime of about 13.9 min (s.e. = 0.5 min), a mean area of 0.68 μm² (s.e. = 0.03 μm) and a mean aspect ratio of 2.38 (s.e. = 0.07). In contrast, unstable FAs (n = 55) revealed a mean lifetime of about 2.9 minutes (s.e. = 0.2 min), a mean area of 0.20 μm² (s.e. = 0.01 μm) and a mean aspect ratio of 1.77 (s.e. = 0.05). All mean values for the two groups of FAs were significantly different (p-value < 0.001).

Based on the two types of lamellipodial adhesions traction force measurements on single adhesion sites were performed. Stable FAs experienced an increasing force whereas force transmission for unstable FAs levelled off (Figure 2). This is shown exemplarily for stable (top) and unstable (middle) FAs in a time series.
from FA assembly (left) to disassembly (right). Mean force values of stable FAs increased constantly with time while force values of unstable FAs remained on a low level (Figure 2, bottom, \( n_{\text{stable, } t=0} = 38, n_{\text{unstable, } t=0} = 124 \)).

**Figure 2.** FA stability depends on force transmission. Motile keratinocytes were seeded onto elastic silicone rubber substrates and transfected with GFP-vinculin. Substrate displacements were analyzed by tracking fluorescent beads (not shown). Force application on every newly formed FA was calculated over time (top and middle). FA lifetime was additionally used to classify all FAs as stable (top) or unstable (middle, lifetime below 6 min) ones. Red arrows denote measured forces at these adhesions. Last images of the time series show the time point of FA disassembly. Force scale bar (red arrow) = 3 nN. Time points are given in seconds. Scale bars = 5 μm. Cell edges are indicated by white lines. Bottom: mean force transmission of stable (light gray) and unstable (dark gray) FAs for the first 8 min. Error bars display the standard error (s.e.).

Since formation and elongation of filopodia strongly depend on actin cross-linkers, here mainly fascin, we analyzed fascin localization and relative fluorescence intensity during filopodial elongation cycles by live cell imaging of migrating, GFP-fascin transfected keratinocytes. We found a homogeneous fascin signal along the complete length of filopodia during elongation (Figure 3, see 0°). This fascin signal remained visible over time, as long as filopodia extended in length. Interestingly, a strong decrease in fascin intensity was found in stably adhered, not growing filopodia. Here, fascin intensity did not decrease homogeneously within filopodia. Instead, the intensity decreased continuously as a sharp border moving retrogradely from the filopodial tip to the lamellipodial leading edge (Figure 3, 40°–120°).

In order to analyze whether filopodial actin bundles additionally change in protein composition upon contact with the lamellipodium, keratinocytes were transfected with GFP-α-actinin. Subsequently cells were fixed and stained for fascin. For every filopodial actin bundle extending into the lamellipodium (basically all) we found a separated localization of both proteins. While fascin was present only in filopodial extensions of the actin bundles, α-actinin could be localized exclusively in the lamellipodial part of the bundle (Fig. 4). A small overlap region of both proteins at the shafts of filopodia argues for a transition zone where fascin becomes exchanged by α-actinin within the same actin bundle.

Taking together, given results clearly proof a strong dependency of lamellipodial adhesion site formation and cell force generation at the leading edge of migrating keratinocytes on filopodial adhesion complexes and their interaction with actin bundles.

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Presenilin affects focal adhesion site formation via c-Src

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Presenilin 1 (PS) is a critical component of the γ-secretase complex that cleaves transmembrane proteins. This process leads to the release of products from cellular membranes and plays an essential role in signal transduction or vital functions as cell adhesion. Here, we found for PS1−/− cells an altered morphology with significantly reduced sizes of focal adhesion sites compared to wild-type. Furthermore, cell forces were reduced by 50%. On the regulatory level, PS1 deficiency was associated with decreased phospho-tyrosine levels of focal adhesion site specific proteins. The reduced tyrosine phospho-rylation was caused by a transcriptional down-regulation of c-Src kinase. The direct regulatory connection between PS1 and c-Src could be identified with ephrinB2, a receptor mediating cell-cell adhesion. EphrinB2 becomes cleaved by PS1 with a subsequent translocation of the ephrinB2 intracellular domain (ICD) into the nucleus. Here ephrinB2-ICD functions as transcriptional coactivator for c-Src transcription leading to a full suppression of the PS1−/− phenotype in terms of focal adhesion formation, function and regulation.

PS1 is an aspartyl protease forming the active components of the γ-secretase complex. This complex cleaves transmembrane proteins within their transmembrane domains leading to the release of the two cleavage products from the membrane. The freed products can play important roles in different signalling pathways by translocating into the nucleus and acting as transcriptional coactivator. Due to the wide range of substrates many mechanisms are affected by presenilin. One of them is the ephrinB/Eph receptor mediated cell-cell adhesion. Since ephrins additionally bind to cellular sarcoma protein kinase (c-Src), a protein vitally involved in focal adhesion (FA) formation, it is speculated that PS might be an important regulator for switching cell function from a sessile to a dynamic, moving phenotype.

All cell analyses described here, were performed using wild-type mouse embryonic fibroblasts as well as PS1−/− mutant strains. Cell biological procedures were performed according to standard protocols. Cells were either analyzed in phase contrast or transfected with GFP-fusion proteins and analyzed by fluorescence microscopy. Alternatively, cells were fixed and proteins were stained by immunolabelling. Northern blot, western blot as well as qRT-PCR experiments were performed according to standard protocols. Elastomeric substrates were prepared and calibrated (Young’s modulus = 13 kPa, Poisson’s number = 0.5) as described earlier [1].

To characterize the exact function of PS in cell-matrix adhesion processes wild-type (WT) and presenilin 1 knock out mouse embryonic fibroblasts were cultured and used for immunofluorescence analyses against actin and vinculin as marker of focal adhesion sites (FAs, Fig. 1). WT cells were characterized by well visible FAs. These sites with an average size of 0.9 µm² were mainly located at the cortex of cells and had an elongated shape. To every FA thick F-actin bundles, called stress fibers, were connected. Very different results were found for PS1−/− cells. Here, the number of FAs was reduced by 40%. The average size of FAs was diminished by 30 to 40% and reached 0.65 µm² on average. Their spatial distribution changed from a cortical to a disperse localization. Prominent actin stress fibers were absent.

Fig. 1 (A) WT as well as PS1−/− cells were stained for actin and vinculin in immunolabelling experiments. (B) Cell types as in (A) were grown on elastomeric substrates and substrate deformations (yellow) were determined. Underlying forces applied at every FA (red) as well as the generalized first moment (blue) were calculated as described in Cesa et al. [3].
For a quantitative measurement of cell forces WT as well as the PS1/− mutant strain were seeded on micropatterned, soft PDMS-substrates. Displacements of the regular micropattern were visualized by RICM and the generating forces were calculated from these data. For single FAs of WT cells, analyses revealed forces in the range of 13 nN (Fig 1B) with a generalized first moment of about 8.5 pNm (σ=4.7 pNmn; n=86). PS1/− cells instead applied forces in the range of just 7 nN per focal adhesion with a generalized first moment of 3.0 pNmn (σ=3.1pNmn; n=36).

γ-secretase regulates expression of c-Src. Since earlier experiments indicated an influence of PS on ephrinB1 putatively affecting c-Src activity, we analyzed the phospho-tyrosine levels of FAs in WT and PS1/−. These analyses were performed in fixed cells using a phospho-tyrosine specific antibody and revealed high levels of phosphorylation in FAs of WT cells while phosphorylation of PS1/− FAs was almost absent (data not shown).

![Fig. 2](image.png)  
**Fig. 2** Crude protein (A) as well as total RNA (B) were isolated from WT and PS1/− and analyzed for indicated proteins/mRNAs. α-tubulin and 28S rRNA, respectively, were used as internal standards.

Since phosphorylation in FAs is mainly performed by activated c-Src kinase we analyzed the activation status of this kinase at tyr 418 as well as its expression level in WT and PS1/− cells. As given in Figure 2A, c-Src phosphorylated at tyr 418 was decreased by 90% in the absence of PS1 compared to WT. As such result might have been caused by either regulation of autophosphorylation or regulation of expression, northern as well as western analyses for c-Src were performed. Protein levels of c-Src were reduced by 60% in PS1/− cells (Fig 2A). Northern analyses identified an almost identical reduction of c-Src transcripts. Here, levels of c-Src mRNA were reduced by 50% in PS1/− cells (Fig 2B). Reduced levels of c-Src protein were therefore likely caused by transcriptional downregulation rather than increased protein degradation.

EphrinB2 cytoplasmatic domain is translocated in a PS1 dependent manner into the nucleus. To complete the signal transduction pathway from PS1 function to c-Src activity, we checked γ-secretase targets for a putative influence on c-Src. Since two of them, ephrinB1 and ephrinB2, were also known binding partners of c-Src, we analyzed ephrinB2 in more detail. Experiments given above revealed c-Src regulation mainly at the transcriptional level. We therefore tested ephrinB2 intracellular domain (EB2-ICD) for transcriptional co-activator function. An EB2ICD-GFP construct was expressed in WT as well as PS1/− cells and its localization was compared to GFP only. Life cell imaging revealed intense nuclear localization of EB2ICD-GFP (Fig. 3A). At the same time, FAs of transfected PS1/− cells were restored in localization, tyrosin phosphorylation intensity and size (1.0 µm; σ=0.4 µm; n=100 FAs) (Fig. 3A). Furthermore, qRT-PCR experiments revealed a strong increase of c-Src mRNA levels in these cells compared to untransfected PS1/− cells. A similar increase was observed for c-Src in western analyses (data not shown).

![Fig. 3](image.png)  
**Fig. 3** (A) PS1/− cells were transfected with an EB2ICD-GFP construct and analyzed in fixed cells for GFP (green) as well as tyrosine phosphorylation of FAs. (B) WT and PS1/− cells were transfected with full length EB2-GFP and analyzed for nuclear translocation of an EB2-GFP fragment. Note that translocation was only detected in the presence of functional PS1.

Although toxic at high concentrations or enhanced incubation times, a full length EB2-GFP construct, expressed in WT and PS1/− cells, identified the dependency of EB2-ICD translocation on PS1 function. While nuclear localization could be observed for EB2-GFP in WT cells no such signal was present in PS1/− (Fig 3B). These data proof EB2 to be cleaved by γ-secretase and that an EB2 cleavage product is transduced into the nucleus were it functions as transcriptional coactivator for c-Src [2].


Probing bio-membrane dynamics with microinterferometry

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Membrane fluctuations are of central importance during the initial steps of cell contact formation. Yet, due to the complex interplay of active as well as passive processes in a living organism, quantitative studies on the specific role of fluctuations are difficult. Therefore, we used giant unilamellar vesicles (GUVs) as extremely simplified and well-defined biomimetic model system enabling quantification of the effects of individual system parameters. Here, GUVs adhered via specific ligand-receptor interactions to patterned receptor sites. Dual-Wavelength Reflection Interference Contrast Microscopy (DW-RICM) was employed to monitor membrane topography and fluctuations with 4 nm axial and 20 Hz temporal resolution. Steady state analysis and the systems’ dynamic adaptation to osmotic pressure changes gave insight into the physical forces involved [1].

Membrane fluctuations are highly relevant for course and final state of adhesion, as they are responsible for both, retardation and acceleration of adhesion, at the same time: while constant collisions between membranes seem to hamper stable bond formation, receptors embedded in the membrane can probe a larger area on the opposite surface, thereby increasing the probability of binding. Whether one or the other process dominates depends on other influencing factors such as membrane bending modulus, lateral mobility and concentration of molecules, etc. Much is known about the membrane composition, its molecular properties as well as the principle interactions governing adhesion. In this context membrane fluctuations have been treated as an inevitable implication to cell and vesicle adhesion before. However, comprehensive understanding of their specific role and a systematic study of influencing factors such as membrane tension or confining geometries are still missing. This is mostly due to a sparseness of techniques providing sufficient resolution to monitor membrane fluctuations as well as to make 2D maps across the entire membrane.

Use of interferometry at the micron scale makes it possible to measure nano-metric distances in the vertical direction and thus to reconstruct a three dimensional image of the membrane [1-4]. Therefore, we used dual-wavelength reflection interference contrast microscopy, a micro-interferometric technique, for measuring absolute membranes heights up to several 100 nm above the substrate. We developed an improved analysis based on a recently established theory to account for partial coherent light and implemented algorithms for an automated, time-resolved reconstruction of membrane conformations. FIG.1 illustrates the principle of DW-RICM.

FIG. 1: Dual wavelength-reflection interference contrast microscopy (DW-RICM): (A) Polarized light is reflected at different interfaces within the sample and interferes. The amount of reflected light is calculated from the refractive indices $n_0$ and thickness $d_2$, yielding the theoretical curves shown in (B). (B) Normalized intensity plotted as a function of height $h = d_1$ for the wavelengths $\lambda_g = 546$ nm and $\lambda_b = 436$ nm. Simultaneous recording of two interferograms allows for assigning an unambiguous height, as indicated by measured data (black and grey dots). (C) Interferogram of a vesicle adhering to microcontact printed molecules. Scale bar 7 µm. The white frame marks the fluctuation area whose intensities are plotted in (B).
Vesicles adhering to microstructured adhesive substrates exhibited regions of bound and fluctuating membrane, in accordance with the underlying pattern (FIG. 1C). Within the fluctuating zone, the membrane presented itself as a flat-topped hill, saturating in a plateau at 79±9 nm. FIG. 2 shows an example of reconstructed topography within a fluctuation region. In this plateau the fluctuation amplitude was found to be 10±3 nm [1].

In addition, we investigated the system while administering osmotic shocks, as this effectively alters the vesicle tension. We found that the plateau height could be tuned from 0 to several 100 nm. FIG. 3 illustrates the time course of bio-membrane topography upon osmotic deflation: Within one minute the membrane rises up to several 150 nm in height. Moreover, an increase in fluctuation amplitudes upon equilibration was observed.

These measurements have two important consequences: First, macroscopic membrane flow is possible even through tightly adhered area. Thus the membrane is able to reach an equilibrium state. Hence, similar average heights measured in steady state indicate the position of a potential minimum. Second, upon osmotic deflation and corresponding membrane tension decrease, average membrane heights within fluctuation regions rise. Apparently, the system changes to an energetically favorable state, which is highly influenced by the Helfrich entropic repulsion – a membrane tension dependent interaction, which arises from thermally induced fluctuations.

Our results show that model membranes are a perfect means for systematic studies of membrane fluctuations affected by different adhesion geometries or membrane tension. Applying DW-RICM, static and dynamic membrane properties are analyzed with high precision. At present we are exploiting this powerful technique for a systematic investigation of fluctuation effects in biomembrane adhesion to microstructures of defined geometries.

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**FIG. 2:** Reconstructed membrane topography within one fluctuation area. The membrane rises from the bound edge toward the middle and saturates in a plateau.

**FIG. 3:** Osmotic pressure changes drive the membrane upwards. The change is dynamically monitored with DW-RICM. Micrographs (left) represent snapshots of time points after osmotic pressure change, false colour images (right) the reconstructed heights. Scale bar 7 μm.

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In polymer solutions, entanglements between neighboring filaments impose topological constraints on the Brownian motion of individual polymers. The most successful phenomenological approach to describe the polymer motion is the tube model introduced by Edwards. It reduces the complex many-filament problem to a tractable single-filament problem, where the topological constraints imposed by the surrounding polymers are condensed to the concept of an impenetrable tube. Theoretical approaches so far assumed that this tube is homogeneous along the filament contour. Our results on filamentous actin, an important class of semi-flexible biopolymers, indicate, however, substantial heterogeneities which can be described in a segment fluid model.

Filamentous actin (F-actin) form by self-aggregation of the protein actin and exhibit lengths of some tens of microns. In solution, these filaments undergo thermal shape fluctuations but, due to their bending stiffness, maintain an average direction and are therefore referred to as semi-flexible; their persistence length is ~20 µm for phalloidin stabilized filaments.

For semiflexible polymers, a typical confinement tube is pictured as thin and relatively straight. A thermally fluctuating filament will encounter collisions with the tube wall, whose average distance is given by Odijk’s deflection length. Expanding this concept Semenov derived a scaling law for the dependence of tube width on monomer concentration \( c \) and predicted the width of the confinement tube to be homogeneous along the filament contour and proportional to \( c^{-3/5} \) (measurements in [1]).

In our work we show that in entangled F-actin solutions, however, the confinement tube exhibits substantial heterogeneities, which we quantify in terms of the tube radius distribution \( P(R) \). As a main result, \( P(R) \) is found to be described by a universal master function with a broad tail. We developed a systematic theory of tube fluctuations that explains this observation and provides the basis for a characterization of the structural and elastic properties of semi-flexible polymer solutions [2].

F-actin solutions were prepared at various monomer concentrations \( c = 0.2, 0.4, 0.6, 0.8 \) and 1.0 mg/ml. Rhodamine-phalloidin labeled solutions were mixed with unlabeled solutions at a ratio of 1:1000. Time series of typically 150 pictures of individual labeled filaments were recorded using an inverse confocal microscope (LSM510, Carl Zeiss Jena, Germany). The pictures were then superimposed to obtain a time-averaged image of the fluctuating test filament, where intensity reflects the residence time of the filament (Fig. 1(a)).

FIG. 1: (a) Superimposed confocal microscopy images of an actin filament (the red spline represents the tube backbone); scale bar: 5 µm; (b) tube radius profile \( R(s) \); (c) normalized tube radius distribution for different actin concentrations; (d) rescaled distribution: experimental data for the concentrations as in (c) (symbols), and self-consistent theory described in the main text for \( \gamma = 2 \) (solid line).

Smooth tube contours connecting the intensity maxima were constructed using standard algorithms and the local tube radius \( R \) was identified with the standard deviation of Gaussian fits to the intensity profiles transverse to the backbone of the tube (Fig. 1(a)). Along a single test filament, \( R \) exhibits pronounced undulations (Fig. 1(b)), from which the tube radius distribution \( P(R) \) was inferred (Fig. 1(d)). The peak position and width, corresponding to the typical value and the fluctuations of \( R \), respectively, decrease with increasing \( c \).
In order to describe the skewed shape of the experimentally observed tube radius distribution \( P(R) \), we developed a theoretical approach along the lines of the binary collision approximation (BCA). It replaces the many-body interactions in an entangled polymer solution by an individual test polymer of length \( L \) in a tube-like harmonic confinement \( \beta/\sigma \) per unit length, where \( h(s) \) parameterizes the transverse contour undulations. Here, the tube stiffness represents the cumulative contribution from collisions in all possible tube configurations. The latter is subtle, since for semi-flexible polymers the topology is not uniquely determined by the center-of-mass positions and orientations (Fig. 2), rather the distance between filaments may be denoted as “above” and “below” and need to be distinguished.

The wormlike chain model in the weakly-bending rod limit and equipartition then yields the mean-field (projected) tube radius

\[
R^2(\phi) = \int \frac{d^2}{2L} \langle h^2 \rangle_\phi = 2^{-3/2} I_p^{-1/4} \phi^{-3/4}
\]

where natural units \( k_BT = 1 \) are used and \( I_p \) is the bending rigidity.

Similarly, one gets the coarse-grained interaction potential (BCA potential) between two tubes of radii \( R_0 \) and \( R_1 \) at separation \( x \) along \( \phi = \phi_x \),

\[
h(x) = -\ln\Phi(x) = \frac{\sigma x}{R_0 + R_1} + \Phi(x) = \frac{\sigma x}{\sqrt{2}R_0 + \sqrt{2}R_1} \phi_x + \Phi(x)
\]

the partition function for the contour fluctuations \( h(s) \). Considering that transverse displacements of the filament tube at an arbitrary angle with respect to the direction \( \phi_x \) of closest approach (Fig. 2) do not change topology and averaging over all possible configurations yields the mean-field tube radius \( R^* \propto \rho^{-3/5} I_p^{-1/5} \). Here, \( \rho \) is the line concentration (polymer length per volume) of the filaments. The conventional BCA, as a self-consistent mean-field theory, is exclusively concerned with the average values \( \phi \) and \( R^* \). To get hold of the spatial tube width fluctuations as obtained from experimental data, an ensemble of \( N + 1 \) independent entanglement segments of length \( L \) are introduced which are characterized by their individual fields and corresponding tube radii \( R_0 \) before averaging over the segment ensemble. In a formal generalization of the BCA that is called segment fluid model, any overlapping pair in the ensemble interacts with the BCA pair potential. Here, the collision, respectively, overlapping region of two segments is denoted as the shaded region of two test tubes with orientation \( u \) and \( u' \) as depicted in Fig. 2. The confinement potential for a test segment is then computed as the cumulative effect from the collisions with all overlapping segments.

Using this fluid segment approach one finally obtains the tube radius distribution \( P(R) \) in entangled solutions of semi-flexible polymers:

\[
P(R) \propto \frac{R_0/\gamma}{R^*^{1/5}} \exp \left[ -1.41 \gamma \left( \frac{R}{R^*} - \frac{1}{2} \right)^2 \right]
\]

with \( \gamma \) a numerical coefficient of the order 1.

The predictions of this equation are plotted in Fig. 1(d) as solid lines and compare favorably with the measured tube radius distribution. In this comparison, \( \gamma \) is set and the ratio \( \rho \gamma \) is used as a single global fit parameter \( (\rho \gamma = 5.5 \mu m^2 \text{ [mg/ml]}) \). It turns out to be about an order of magnitude smaller than the value expected if all monomers were bound in strongly entangled polymers, probably reflecting the effects of the critical polymerization concentration and of polydispersity. Also note that the solution for \( P(R) \) implies that the concentration dependence enters the tube radius distribution only through the average tube radius \( R \), such that \( \rho \gamma \) defines a concentration-independent master function of \( R/R^* \).

Fig. 1(d) (symbols) demonstrates that the data indeed scale satisfactorily.

A consistency check for our model is the spatial autocorrelation of the tube radius profile in Fig. 1(b). It should decay over a characteristic length comparable to the entanglement length, \( \lambda \propto \rho^{-2/5} \) (Fig. 3(a)). Indeed, the magnitudes and in particular the concentration scaling (Fig. 3(b)) of \( \lambda \) compare favorably with the expectation.

The fact that heterogeneities give rise to pronounced tails in \( P(R) \) underscores the importance of shifting the attention from characteristic parameters such as tube width, entanglement length etc. to their skewed leptokurtic distributions. As demonstrated here for the example of the tube width \( R \), these distributions and other higher-order correlation functions, which can provide a comprehensive characterization of the micro-structure of semi-flexible polymer solutions, are readily accessible in our BCA-based segment fluid model.


Novel fusogenic liposomes for \textit{in vivo} fluorescent cell labelling

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Efficient delivery of biomolecules into membranes of living cells as well as cell surface modifications are major biotechnology-cal challenges. Here, novel liposome systems based on neutral and cationic lipids in combination with lipids modified by aromatic groups are introduced for such applications. Fusogenic liposomes enabled highly efficient incorporation of molecules into mammalian cell membranes within 1 to 30 min at fully unchanged cell growth conditions and did not affect cell behavior. Selected applications ranging from basic research to biotechnology are envisaged here.

Classical liposomes used for DNA delivery are based on a composition of neutral and positively charged lipid molecules, e.g. DOPE and DOTAP. The cellular uptake processes of these vesicles have been investigated earlier and have shown a strong preference of endocytotic pathways with a limited efficiency. Here, the addition of a third lipid component converted these liposomes to a nearly universal fusogenic system allowing complete lipid merging between liposomal and cellular membranes [1]. The third lipid component needed to meet the criteria of 1) amphiphilic molecular character to be easily incorporated into biological or biomimetic membranes and 2) a delocalized conjugated \( \pi \) electron system located in the polar or apolar molecular range. Interestingly, these criteria were fulfilled by almost all fluorescent labels when coupled to phospholipids.

Fig. 1 shows fusion processes between liposomal membranes composed as described above and plasma membranes of HEK293 cells in culture. As fluorescent lipid a head labeled phospholipid, BODIPY FL-DHPE, was applied and its green fluorescent signal was detected over time by laser scanning microscopy. As can be seen in Fig. 1a, immediately after vesicle addition (Fig. 1a: 0 s), small green dots, i.e. liposomes, were observed in the fluorescence BODIPY FL channel. After 100 seconds the first fluorescently labeled cell membranes appeared and 200 seconds later most of the plasma membranes were homogenously labeled by BODIPY FL without liposome dumps on the surface.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Vesicle fusion with plasma membranes. (a) HEK293 cells were incubated with vesicles made from DOPE/DOTAP/BODIPY FL-DHPE (1/1/0.1 w/w) and analyzed over 300 seconds in fluorescence (left) and phase contrast (right). Within this time period, efficient membrane fusion was detected with unchanged cell behavior. Scale bars = 50 \( \mu \)m. (b) Fluorescence recovery curve of LR-DOPE in HEK293 plasma membrane after membrane fusion. The diffusion parameters of LR-DOPE calculated from the FRAP curve confirm the incorporation of fluorescently labeled lipid into the cell membrane.}
\end{figure}
Unchanged cell behavior and cell growth were detected in the phase contrast channel simultaneously with the appearance of the fluorescent signal in the cell membranes. After lipid merging, cellular mechanisms need distinct time periods to recognize the new components and to sort them according to their biological function and chemical properties into intracellular organelle membranes. We observed that 24 h after administration most of the analyzed lipids were digested by cells.

Successful incorporation of aromatic lipid molecules was furthermore controlled by detecting their lateral diffusion constants using fluorescence recovery after photobleaching technique (FRAP). A typical FRAP recovery curve is shown in Fig.1b. The resulting diffusion constants of all investigated dye lipids were found to be relatively similar with values around 0.18 ± 0.06 µm²/s. Fluorescence recovery, frequently called mobile fraction, was also determined and the values also appeared statistically similar to each other (around 78 ± 6%) independent of the dye.

The ubiquitous ability of our system to induce fusion was successfully proven by labeling many other cell types as human keratinocytes, human macrophages, rat embryonic cortical neurons, bronchial smooth muscle cells and rat myofibroblasts. For all of these cell types, cell labeling efficiency reached an almost complete labeling of all cells. Only in the case of myocytes, an increased resistance against fusogenic liposomes was observed. To prove the efficiency of cellular tissue labeling rat embryonic pericardium, a multi-cell layer of defined thickness around the contractile heart apparatus was chosen. In this case confocal imaging revealed an almost complete staining of all pericardial surface cells.

Our results open a large number of possible medical and biotechnological application fields as schematically summarized in Fig. 2. Fluorescent membrane labeling is only one of the numerous cell membrane modifications (Fig. 2 I.). This membrane modification can be completed in an additional step by transmembrane protein incorporation into the cellular plasma membrane as well (Fig. 2 II.). With addition of other functionalized molecules like chelator lipids, biotinylated or antigenic lipids surface functionalization of living cells can be achieved by protein coupling to the cell surface (Fig. 2 III.). Nevertheless, if the membrane mixing is accompanied by content mixing, highly efficient delivery of soluble molecules to living cells can be performed (Fig. 2 IV.).

FIG. 2: Overview of the potential fields of application for fusogenic liposomes: (I.) plasma membrane visualisation (II.) Transmembrane protein incorporation into plasma membranes, (III.) cell surface functionalization, and (IV.) content delivery.

In our research we focus on the functional connection of biological and electronic systems where we in particular examine the molecular, cellular and electronic and electro-chemical processes at this interface. This enables fabrication of sensors that detect very small amounts of pollutants or biochemical substances in the environment or in body fluids or even exchange signals with cells. These developments may lead to new approaches for the development of sensitive implants to replace destroyed sensory cells.

We operate onsite facilities for method developments, prototyping and testing bioelectronic systems, fabricating and measuring the performance of devices, and testing the interaction with biological materials. The Process Technology (PT) Group maintains micro- and nanoelectronics equipment, supports internal and external users of the cleanroom, and offer services for external clients.

This work is embedded in the research field of key technologies of the Helmholtz Association, in which our Institute is embedded within the programs FIT: Fundamentals for future information technologies and BioSoft: Macromolecular and Biological Systems Information Processing.
Research Highlights

Axon guidance of rat cortical neurons by microcontact printed protein gradients

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In this study, we constructed a discontinuous substrate-bound gradient to control neuronal cell position, pathway of neurite growth and axon directionality. Gradient patterns were fabricated by microcontact printing using laminin/ poly-L-lysine (PLL) or PLL alone. The gradients were tested on neurite growth and their impact on axon guidance effects of embryonic rat cortical neurons. The neurite length was determined and the axon was evaluated by Tau-1 immunostaining. We found that the microgradients of laminin/ PLL and PLL directed neurons adhesion, differentially controlled the neurite growth and guided up to 84% of the axons. Our approach proved to be very successful in guiding axons of single multipolar neurons with very high efficiency. It could thereby be useful to engineer defined neural networks for analyzing signal processing of functional circuits as well as to unravel fundamental questions of axon guidance mechanism.

All of the sophisticated and complex functions of the brain are based on highly ordered cell architecture and precisely wired neural circuits. This process is regulated by extrinsic guiding cues which are expressed as numerous spatio-temporal gradient patterns. Even though numerous techniques were already established [2] to construct substrate-bound gradients which affect axon guidance those procedures still fail to guide the axons of multipolar neurons. Thereby, the aim of this study was controlling 1) neuronal position, 2) pathway of neurite growth, 3) directionality of axon growth. Based on the study of Philipsborn et al. [2006] we fabricated a modified gradient and produced substrate-bound discontinuous patterns of laminin/ poly-L-lysine (PLL) and PLL alone. The gradient was tested for rat cortical neurons in respect to neurite outgrowth and axon guidance. We used laminin since it is the most commonly used ECM protein to foster neurite growth. Our results provide a simple and reproducible microstructuring method to guide axons and control neurite growth in neural networks with defined architecture. For the production of substrate-bound discontinuous gradients, a variety of micropatterns were designed with slight changes in slope, width, and length (Fig. 1). Microstamps were produced by photolithographically generating a master and then casting Poly-(dimethylsiloxane) (PDMS) microstamps. The stamps were used to print discontinuous gradient patterns made of laminin/-PLL or PLL alone.

For all experiments, the fluorescein isothiocyanate (FITC)-conjugated poly-L-lysine was used to visualize the printed protein. Dissociated cortical neurons were seeded in a low density ensuring localization of one single neuron per gradient structure and minimizing interference from trophic factors produced by neighboring neurons on neurite outgrowth. After three days in culture, cells were fixed and immunostained for MAP2- and TAU-1 to evaluate neurite growth and axon guidance effects. Fluorescence images were analyzed from structures where only one single neuron adhered with the soma directly on the node. Different gradients were analyzed for their ability to control the neurite growth and in more detail to differentially promote the growth of neurites towards one specific side of the guiding structure. The neurite growth direction along the increasing concentration was defined as the positive direction, while neurites growing along the decreasing concentration were defined as growing in the negative direction. Next to the effect on neurite growth, the gradients were also

Fig. 1. Basic features of designs for the discontinuous gradient structures: Example of structures with different scopes. Measurements are given in µm.
analyzed for their potential to guide the axon. After evaluating the axon by TAU-1 immunostaining (Fig. 2), the axon growth in the positive and negative direction was quantified. Comparing all gradients tested for their effects on axon-guiding pointed out that all patterns, independent of the different geometric parameters, evoked an effect on axon growth by directing axons (>50%) in the positive direction along the gradient. The highest effect could be seen on PLL-S4-W4 (84%) and the lowest effect on the PLL-S1-W2 pattern, which did not reach significance. On the S1-W2 pattern where PLL induced nearly no effect on axon guidance, laminin/PLL mediated a high and significant effect. This clearly shows that laminin mediates an additional effect on axon guidance in comparison to PLL alone, but the additional effect was restricted to the low slope and 2 µm wide structures. On the 4 µm and 8 µm width structures, the axon guiding effect of laminin was reversed. Whereas laminin/PLL mediated only a slight response, PLL triggered high and significant effects (8 µm width). Thereby, it could be shown for the first time that a discontinuous gradient of polycationic polymer is sufficient to induce axon guidance.

Discontinuous microgradients of laminin/PLL, and PLL alone, direct neuron adhesion, controlling both neurite growth and axon guidance of single primary neurons. The potency of the structure to guide axons depends on the proteins used and geometric parameters (width and slope). The best results of axon guidance could be obtained by the structure with a slope of 0.04, a width of 4 µm and made of PLL. Similar effects could be obtained on structures with a shallower slope if they were made of laminin/PLL [1]. The data have shown that the growth cones can respond on gradients made of mixtures of extracellular guiding cues combined with positive charges, as well as on gradients of positive charges alone. This method proved to be very successful in obtaining discontinuous protein gradients, by which a model of in vivo protein gradient conditions was achieved in vitro.

![Fig. 2: Immunolabeling of neurons at DIV 3 with MAP2 and Tau-1 antibody. Neurons adhered onto the nodes and neurites grew out mostly along the pattern, forming bipolar neurons. The MAP2-TAU-1 immunostained neurons were used to determine the length of the neurites grown on the positive side of the gradient. Tau-1 positive neurites are the axons. (A-D): Laminin/PLL (E-H): PLL pattern Tau-1 positive neurites grew in positive direction of the gradient. Scale bars 10 µm.](image)

![Fig. 3: Quantification of the axon guidance effect of the different patterns made of laminin/PLL (A) and PLL (B). For statistical analysis, individual neurons were analyzed. Data represent the relative frequency of axons grown on the positive side of the gradients, error bars confidence intervals; *p < 0.05; p*** <0.001.](image)

The fabricated guiding structure is therefore useful to guide axons of single multipolar neurons with high efficiency in the design of defined neuronal networks in culture. Electrophysiological measurements may be used to confirm that these guiding structures could further be used to direct signal propagation.


Light induced stimulation and delay of cardiac activity

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A model system was developed in which light can be used to control the activity of cardiac tissue. By introducing the ion channel Channelrhodopsin 2 (ChR2), cells can be depolarized with blue light. The behavior of the HL-1 cardiomyocyte-like cell line during manipulation with light was monitored using a custom built microelectrode array (MEA) measuring setup. The components of light induced current and action potentials (APs) could be distinguished in the MEA recordings, and kinetic characteristics of the light sensitive channel extracted. Using this system, the position of the cardiac pacemaker could be induced and furthermore, the exposure of spontaneously beating cell sheets to flashes of blue light could be used to delay beat rate.

The coupling of electrogenic cells to electronic devices is used to both measure and stimulate cells. Such electrically coupled extracellular systems allow good spatial resolution in combination with high temporal resolution. However, it is desirable to separate stimulation and recording modes to improve the quality of signal detection. Electrical stimulation from recording electrodes suffers from the drawbacks of scar formation, cell permeabilization, and blind time as the applied pulse dissipates from the electrode. Combining light based stimulation with MEA recordings maintains high accuracy in the recorded signal while increasing the available stimulation points and providing fast, non-invasive cell manipulation.

Channelrhodopsin 2 is a small protein from C. reinhardtii, which can be transected into electrogenic cells. The protein binds trans-retinal to form ChR2, a directly gated cation channel activated by blue light [G. Nagel et al., Proc. Natl. Acad. Sci. 100, 13940-5 (2003)]. The flow of cations during illumination depolarizes electrogenic cells, and if depolarization exceeds threshold, the cell fires an AP. The cardiomyocyte-like cell line HL-1 [W. Claycomb et al., Proc. Natl. Acad. Sci. 95, 2979-84 (1998)] was transfected with this protein to allow us to manipulate the location of the pacemaker cell and beat rate using light.

HL-1 cells were transfected with a Channel-rhodopsin 2-YFP construct with roughly 50% efficiency, creating a mixed network of light sensitive and light insensitive cells. Cells were cultured on MEAs for 2-4 days before measuring. The HL-1 cells divide until a confluent layer is formed, then build gap junctions between the cells, and finally become spontaneously active, sending waves of APs over the whole cell sheet. Fluorescence intensity was used to select presumed highly expressing cells for light based manipulation.

Our stimulation and recording setup is shown schematically in Fig. 1. A targetable laser spot, ~10 µm in diameter, couples into the microscope optics to apply flashes of 473 nm light from above with a pre-fiber intensity of 0.40 mW. From below, the cells are recorded by an 8x8 gold MEA.

![Schematic of the laser stimulation & MEA recording setup.](image)

Comparison of the light induced AP and spontaneous APs on the same channel allows extraction of the light induced component of the recording. The isolated light induced component was fit with single exponentials for ChR2 opening and ChR2 closing. From this the time constants for the kinetics of ChR2 opening and closing were determined to be $11.3 \pm 0.2$ ms and $34.5 \pm 0.2$ ms respectively.

In addition to being able to induce AP firing in a single cell, network effects were visible. Light induced APs propagated across the network at propagation speeds equivalent to spontaneously generated APs. The location of a light generated pacemaker cell could be seen in the change in AP propagation direction across the MEA array. For example, in a culture with a
naturally occurring pacemaker to the right of the array, a light induced pacemaker was generated near channel 55. The propagation of the AP was then observed to propagate in concentric rings from near channel 55 instead of from right to left, most clearly visible in the much later arrival of the AP in the top right corner of the array (see Fig. 2)[1].

FIG. 2: APs arrival times are color coded on the array, white electrodes did not register an AP. All APs propagate regularly across the array, but the origin can be moved using light stimulation. a) Naturally occurring pacemaker to the right of the array. b) Light induced pacemaker near channel 55. Propagation lines are a guide to the eye. c) Propagating APs register on all functional channels. f) Propagation speed of light stimulated APs is normal at .61 cm/s, as determined by linear fit of the distance traveled vs. the time elapsed.

In cultures that were not mature, and showed no spontaneous APs, the frequency of AP firing did not always match the frequency of illumination. This failure to trigger an AP may occur when the subsequent flash arrives during the absolute refractory period of the cell. The ChR2 induced depolarization generates a light elongated refractory period (LERP) by extending the plateau phase of the cardiac action potential instead of triggering a new AP. Further evidence for this mode of action is provided by AP suppression when ChR2 depolarization is triggered immediately after a spontaneously occurring AP wave in mature cultures. In these mature cultures, extension of the plateau phase by light induced depolarization prevents spontaneous beating and slows beat rate. LERP may be a localized effect in the illumination area. However, when the cell captured by LERP is the naturally occurring pacemaker cell, the effect of suppression is global. If illumination is halted, the culture returns to its native spontaneous activity without any observable persistent effects of light induced modulation, see Fig. 3.

The generation of LERP in a non-pacemaker region of the culture would only locally suppress APs and provides a means of generating cardiac cultures with inhomogeneities in conduction. Such systems have become important models for cardiac disease, and currently rely on mixed cell type cultures or physical barriers within the tissue.

A further application of light-controlled cardiac activity is the temporally precise determination of contraction for imaging studies. Since LERP prevents APs, and therefore prevents cell contractions, precise periods can be defined when cultures can be imaged without movement. This prevents use of chemical blocking agents that may alter other cell behaviors while blocking contraction. The optical systems of confocal microscopes would be sufficient for both the control of LERP and imaging during the non-contractile period.

The use of ChR2 to generate a light sensitive cardiomyocyte-like culture has been shown, without detectable alterations in AP firing or AP propagation in the dark. MEA recordings of APs occurring spontaneously in the dark and APs triggered by illumination with blue light propagate similarly through the tissue. Differences in signal shape allow the extraction of the light induced component of the recorded signal, and subsequent determination of channel kinetics of the ChR2. The light induced depolarization can be used to generate a pacemaker cell in immature cultures or to re-locate the pacemaker in mature cultures. It was furthermore shown that ChR2 activation by light not only allows the triggering of APs but also AP suppression by LERP in spontaneously beating cultures.

Nanoelectronic devices for direct communication with cells

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Si-based nanowire field-effect transistors (SiNW-FETs) were used to record the extracellular potential of the spontaneous activity of cardiac muscle HL-1 cells. Based on their sensitivity and interface with cell membranes large signals can be recorded. On the other side we used graphene as a nanoelectronic device. Here we used arrays of Graphene-fieldeffect transistors (G-FETs) for the detection of the electrical activity of electrogenic cells. The propagation of signals from cardiac myocytes across the layer was successfully tracked. Analysis of the recorded cell signals and the associated electronic noise of the transistors in the arrays suggest that both SiNW as well as G-FETs could open up unique opportunities in the field of bioelectronics.

Investigation of neuronal cell activity through interfacing with electronic devices is of great interest for many applications such as monitoring cell signal processing within neuronal networks, neuroprosthetic devices, and the use as a biosensor to monitor pharmaceutical agents, pollutants, etc. This requires the advancement of novel solid-state sensors and actuators with a further improvement in the signal detection capability, a superior stability in biological environments, and a more suitable compatibility with living tissue.

Silicon nanowire (NW) transistors had been used in recent times as biosensors for the detection of molecules such as DNA or proteins, antigens and viruses. We have fabricated Si-nanowires using nanoimprint lithography followed by anisotropic wet chemical etching of silicon-on-insulator substrates with tetramethylammonium hydroxide. The combination of these methods was highly reproducible, yielded very smooth surfaces (Fig. 1) and is suitable for a high integration density [1, 2].

These devices were used to record the extracellular potential of the spontaneous activity of cardiac muscle HL-1 cells. Their signals were measured by direct dc sampling of the drain current. An improved signal-to-noise ratio compared to planar field-effect devices was observed [3]. Furthermore, the signal shape was evaluated and could be attributed to different membrane currents. With these experiments, a qualitative description of the properties of the cell-NW contact was obtained and the suitability of these sensors for electrophysiological measurements in vitro was demonstrated.

SiNW-FETs were used for the detection of the extracellular potential of electrogenic cells. The arrays were fabricated at the Walter-Schoottky-Institute in Munich using large-area graphene films grown by CVD on copper foil. Cardiomyocyte-like HL-1 cells were cultured on these arrays and exhibited a healthy growth. After characterizing the transistors in an electrolyte, the action potentials of these cells could be detected and resolved by the G-FETs under the cells (Fig. 3a). Using the complete transistor array, the
propagation of the cell signals across the layer was successfully tracked. Analysis of the recorded cell signals and the associated electronic noise of the transistors in the arrays confirms that graphene FETs surpass the performance of state-of-the-art commercial devices for bioelectronic applications.

The HL-1 cells seeded on the transistor arrays exhibited healthy growth and formed a densely packed layer covering the entire sample surface including the open transistor areas after several days. Figure 4a depicts a differential interference contrast (DIC) microscopy image of an array with the HL-1 cells, combined with a fluorescence image of the same region, where the confluent layer of healthy cells is revealed by calcein staining. Figure 4b shows the current response of eight graphene transistors measured simultaneously on one sample. The transistor bias was chosen such that the transconductance was maximized for all of the transistors. Repeated spikes can be seen for all working transistors at similar times, which can be attributed to the propagation of action potentials across the cells. By using the transistor transconductance, the current spikes can be converted to gate voltage spikes and reach values up to 900 μV with a root mean square (RMS) noise level of 50 μV (Figure 4d).

Signals recorded from cardiomyocytes with SiNW and with G-FET indicate that both devices are suitable for extracellular recordings and that a stable interface between cells and electronic device is formed. In addition, comparisons of peak-to-peak recorded signals with G-FET devices versus NW-FETs will allow in future an assessment of relative resolution in extracellular recording. The complementary of G- and NW-FETs could open up interesting opportunities in the field of bioelectronics in the future.

FIG. 3: a) Schematic view of a G-FET with a cell on the gate area. The graphene is shown between the drain and source metal contacts, which are protected by a chemically resistant layer. b) Optical microscopy image showing eight transistors in the central area of a G-FET array. The transistor active area between the drain and source contacts is 10 × 20 μm². The scale bar is 100 μm.[4]

FIG. 4: a) Combination of an optical microscopy image of a transistor array and a fluorescence image of the calcein-stained cell layer on the same array. The scale bar is 100 μm. b) Simultaneous current recordings of eight transistors in one FET array over tens of seconds. d) Exemplary single spikes. The current response has been converted to an extracellular voltage signal. The upper spike resembles a capacitive coupling followed by the opening of voltage-gated sodium channels whereas in the bottom one the ion channels dominate over the capacitive coupling.[4]

Development and characterization of carbon nanotube sensors

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The main aim of the project “Development and characterization of carbon nanotube sensors” is developing low-noise high-speed test device structures on the basis of single-wall carbon nanotubes (SWNTs) for biosensor applications. The noise spectroscopy results allow us to analyze the mechanisms of conductivity in fabricated carbon nanotube structures. Furthermore, we showed that functionalization of the electrolyte-insulator-semiconductor (EIS) structure with multilayers of polyamidoamine dendrimer and single-walled carbon nanotubes leads to an essential reduction of the 1/f noise compared with noise in a bare EIS device. Thus the control of molecular architecture is important to obtain the enhanced performance of multilayer structures interfacing with electrolyte.

Carbon nanotubes (CNTs) are unique materials due to the high degree of ordering of carbon atoms on the surface of the tube, which represents a very uniform channel for high-speed electron transport. Therefore CNTs are very promising for various applications, including extremely sensitive biosensors. Noise spectroscopy is a powerful method for studying the transport properties, performance, and reliability of material structures, especially scaled down to the nanoscale [1]. The fluctuation phenomena contain important information on the material performance and may be utilized as a valuable method for the characterization of nanoscaled materials and devices.

We studied the phenomena of charge transport in structures consisting of bundles of carbon nanotubes [2] prepared by applying uniaxial pressure. The Raman spectra data demonstrate that the bundles mainly consist of single-walled nanotubes. Low-frequency noise and transport properties of the structures were studied for samples fabricated in the form of dumbbells, containing a constriction with a characteristic size of 100×100 μm² in cross-sectional area and a length of about 200 μm. In the temperature range from 20K to 200K, the measured data demonstrate the characteristic for Luttinger liquid temperature dependence of resistance $R \sim T^{-\alpha}$ with exponent of $\alpha = 0.4$. At the temperatures higher than 200K, the measured dependence deviates from this simple function. At lower temperatures, $T = (4.2 - 20)$ K, the resistance follows an exponential function $R \sim \exp(T_0/T)^{25}$. Exponential dependence of the resistance versus the sample temperature with an exponent of 0.25 indicates the hopping mechanism of conductivity with variable hopping length. The noise spectra of the sample have 1/f dependence, which is a characteristic function of flicker noise. To analyze the nature of the noise current in the nanotube bundles, we measured the spectral density of current noise, as a function of the current, $S_I \sim I^\beta$, at different temperatures in the range from 4.2K to 300K. Based on these results, we found the dependence of the coefficient $\beta$ on the temperature (Fig.1).

![Figure 1: The current noise exponent $\beta$ of power function $S_I \sim I^\beta$ determined from measured noise spectra as a function of temperature, corresponding to: (1) hopping mechanism of conductivity; (2) transition region; (3) Luttinger liquid conductivity; (4) diffusion conductivity.](image)

A transition region is recorded in the temperature range $T = (8 - 25)$ K, which indicates the transition from hopping conductivity to Luttinger liquid conductivity. Our experiment showed that the conductivity of Luttinger liquid is less "noisy" than the conductivity of the Fermi liquid. A sharp increase of noise in this latter region can be explained by increased phonon scattering and, as a result, a decrease in the mean free path of carriers. At the same time, the conductivity of Luttinger liquid up to the temperature $T \approx 200K$, is of a ballistic nature and is characterized by a low noise level, weakly dependent on temperature.

On the other hand, noise spectroscopy is a very sensitive method for the analysis of the semiconductor/insulator interface quality as well as for characterization of the quality of functionalization/passivation layers in liquid environments. We studied the low-frequency noise properties of a capacitive field-effect EIS structure functionalized with...
polyamidoamine (PAMAM) dendrimer/SWNT multilayers (Fig. 2) and compared with noise of a bare EIS device [3].

In contrast to transistor structures, capacitive EIS sensors are simple in layout and cost-effective in fabrication (usually, no photolithographic process steps or complicated encapsulation procedures are required). Capacitive Al-p-SiO2-Ta2O5 structures with a 30 nm thermally grown SiO2 and a 55 nm Ta2O5 layer were fabricated. The Ta2O5 layer has been prepared via electron-beam evaporation of 30 nm Ta followed by thermal oxidation at 515 °C for about 30 min. As the contact layer, a 300 nm Al film was deposited on the rear side of the Si wafer and then, the wafer was cut into single chips of 10 mm x 10 mm size. The EIS sensor was mounted into a home-made measuring cell and sealed by an O-ring to protect the side walls and backside contact of the chip from the electrolyte solution. The contact area of the EIS sensor with the solution was about 0.5 cm².

The LF noise spectra were measured in buffer solutions of pH 3, pH 7, and pH 11 in accumulation, depletion, and inversion regions of the EIS structure by applying different gate voltages. We have observed that the pH value of the buffer solution did not significantly influence the noise spectral density. This is in agreement with results obtained on pH ion sensitive field-effect transistors (ISFETs) previously. It has been reported that the gate-referred 1/f noise in a channel current of pH ISFETs is also independent of the pH, and the origin of LF noise in these ISFET devices is the trapping/detrapping of carriers at the Si-SiO2 interface. Moreover, it has been discussed that the ionic strength of the solution does not affect the noise magnitude of liquid-gated SWNT transistors. These results may suggest that the interface between the solution and the gate insulator does not or not significantly contribute to the 1/f noise. It has been observed that the gate current noise in EIS structures with thin gate oxides is increased with increasing the gate voltage or leakage current. This has been attributed to the more slow-trap states available for a trap-assisted tunneling with increasing gate voltage. The noise spectral density exhibits an 1/f dependence with the power factor γ of 0.8 and γ(0.8 – 1.8) for the bare and functionalized EIS sensor, respectively.

It has been revealed that functionalization of the EIS structure with multilayers of polyamidoamine dendrimer and single-walled carbon nanotubes leads to considerable reduction of the 1/f noise.

The gate-current noise behavior in bare and functionalized EIS devices has been modelled. The experimentally observed gate-voltage dependence of the noise in capacitive EIS structures is explained by the gate-voltage-dependent changes in the occupancy of the oxide trap levels resulting in a modulation of the conductivity of current paths or charge carriers passing through the EIS structure. Physical processes in the semiconductor, insulator, and electrolyte medium responsible for low-frequency charge fluctuations are discussed based on an electrical equivalent scheme for the capacitive EIS structure [4].

Thus, CNTs offer new perspectives for development of biosensors. We demonstrated that noise can be used to investigate transport phenomena and factors determining the performance of electrolyte-insulator-semiconductor structures. Biomolecules and biological objects contain a backbone carbon atoms. Therefore, interfacing living systems with nanocarbon materials is promising direction for biosensor applications.


The project on “nanotechnology tools for cell-chip communication” deals with the investigation of functional nanostructures for detecting cellular signals in vitro. This includes the development of new chip-based devices for electrical and electrochemical recording. Our main focus has been directed at studying techniques based on nanocavity redox cycling. This approach allows one to detect minute quantities of redox-active molecules down to the single-molecule level by boosting the electrochemical signals exploiting fast diffusion on the nanoscale. We applied the nanocavity redox cycling technique in a sensor array design and demonstrated that concentration gradients of the neurotransmitter dopamine can be mapped on the chip. Furthermore, we showed that the nanocavity approach can be used for highly localized recording of electrical activity inside a cellular network.

Redox cycling amplification is a powerful tool for increasing the sensitivity of electrochemical detection. Molecules that can undergo reversible redox reactions are repetitively oxidized and reduced at independently biased electrodes which are located in close proximity to each other (see sketch in Fig. 1). Since the oxidation state is “recycled” after exchanging electrons with the electrodes, this approach allows multiple reactions of a single molecule at the same electrode, resulting in an amplified electrochemical signal.

Very efficient redox cycling can be performed in confined geometries where the electrode distance is well below 100 nm. Nanofluidic channels with integrated electrodes have especially demonstrated an increase in molecular sensitivity by several orders of magnitude compared to conventional voltammetry. The distinct advantages of confined redox cycling make this technique an interesting candidate for biosensing applications. One application is the on-chip spatiotemporal detection of redox-active neurotransmitters with the prospect to investigate localized release of neurotransmitters from neuronal networks with high sensitivity. In the last years, we have developed arrays of circular nanocavities [1] as well as dual-electrode nanopore sensor platforms [2], which were structured by means of optical and template-based lithography, respectively. We have demonstrated the redox cycling efficiency of these sensors and their applicability for the detection of redox active compounds such as hexacyanoferrate and the neurotransmitter dopamine. Furthermore, we have shown that chemical gradients and changes in the local concentration can be spatiotemporally characterized on-chip using our array-based nanocavity approach [1]. This feature is a crucial requirement for future applications such as mapping neurotransmitter release from chemical synapses in a cellular network.

**FIG. 1:** Sketch of the redox cycling principle at a nanocavity device. Molecules are repetitively oxidized and reduced at two closely spaced electrodes, leading to an effective amplification of the electrochemical current.

The efficiency of this recycling method depends on the average number of cycles a molecule performs before it escapes and the time it takes to undergo a complete redox cycle. Thus, it is strongly influenced by the geometry and size of the device.

**FIG. 2:** Electrochemical current of a nanocavity sensor in response to different concentrations of hexacyanoferrate [1]. During the measurement, one electrode is kept constant at -200 mV vs. Ag/AgCl while the second electrode is swept from -200 mV to 600 mV. The anticorrelation of anodic and cathodic currents reveals a redox cycling efficiency close to 100%.
Another interesting feature of nanocavity redox cycling sensors is the anticorrelation of the anodic and cathodic current, which is useful for the discrimination of interfering signals (see Fig. 2). The high amplification gain in combination with the anticorrelation signatures make nanocavity redox cycling devices powerful tools for a variety of electrochemical investigations. Applications range from investigations of rapid electron transfer kinetics [3] to single-molecule studies [4].

Furthermore, we could show that nanocavity array devices can be used for highly localized on-chip detection of electrophysiological activity from individual cells in a network (see Fig. 3). We have demonstrated this by monitoring action potential propagation in a cardiomyocyte-like cell line (HL-1) at µm-sized spots [5]. There are obvious advantages of this new concept for extracellular microelectrode array recordings. As the overall impedance is dominated by the size of the electrode-electrolyte interface, the liquid filled nanocavity effectively reduces the impedance of the sensor. In contrast, the spatial resolution of the sensor is not affected and is only determined by the aperture connecting to the nanocavity. The high resolution provided by small apertures allows the exact assignment of individual cells to the respective recording electrodes. Furthermore, single cells are able to completely cover the apertures leading to an increased seal resistance and consequently a better cell-electrode coupling.

The strong coupling in combination with a low electrode impedance now enables the analysis of individual cells, that are integrated into dense clusters. Our results demonstrate the versatility of the nanocavity approach. The next goal is to advance cell-chip communication employing simultaneous stimulation and recording techniques at high temporal and spatial resolution.

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**FIG. 3:** Nanocavity device for recording electrophysiological activity of individual cells in a network. A) Schematics of cell-chip coupling. B) Action potential recordings from HL-1 cells. C) SEM image of a FIB cross section at the cell sensor interface. The scale bars in the main and enlarged image are 2 µm and 1 µm, respectively. See details in [5].
Electrochemically transduced logic gate on molecular level

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Organic molecules that are able to generate a detectable response to an external stimulus are of great scientific interest since they can be used for data processing, as for instance information storage, transformation, and communication. Logic gates such as AND, OR, INHIBIT, XOR, XNOR, and NOR Boolean functions have been realized on the molecular level and are able to process information and to perform arithmetic operations. In the scope of the sub topic “molecular bioelectronics” we have realized electronic functions typically performed by solid state devices like current rectification and logic operations by electrochemical means. A surface confined redox mediator is tethered to the electrode surface which controls the charge transfer between soluble redox probes and electrode.

Although numerous molecular systems performing different computing operations have been developed so far, this research is still in a very early experimental and theoretical stage. In order to achieve practical applications, the future of molecular logic elements is strongly related to the successful linkage of molecules onto a conductive or semi-conductive support. However, logic gate functions performed by molecule functionalized electrodes are relatively rare. Here, we make use of a reaction scheme that is widely used in nature to regulate biological charge transfer and to realize a unidirectional current. Therefore, a number of redox species is arranged such that the electrons pass through these locally separated redox compounds in a defined order. The driving force of these processes is the release of energy when electrons are transferred from redox species with low redox potentials to species with higher anodic potentials. Based on this concept, we demonstrated the realization of different functions with electrical readout by immobilizing robust, redox-active (bio-) molecules on a solid-state surface and applying different redox probes as input signals. The different functional units presented here have in common that they are all based on electrochemical rectifiers (ECR). An electrochemical rectification is characterized by a unidirectional redox current which is transferred between redox probes in solution and a metal electrode functionalized with the molecular redox mediator. Therefore, the electrode is modified by an insulating layer (typically alkanethiols) inhibiting the direct electron transfer between redox probe and electrode [1,2].

Furthermore, an enzymatic redoxmediator (microperoxidase, MP-11) is attached to the insulating surface layer. The unidirectional current develops due to selective electron transport from the functionalized gold electrode to the redox probe (ferri/ferrocyanide) or vice versa such that the redoxmediator controls the read-out of the coupled redox system. A high forward current can be observed if electrons are continuously transferred from the source to the drain via states of lower energy, Fig. 1 top right. If one state has a higher energy than the source state then only marginal
leakage currents can be observed (reverse direction, Fig. 1 top left). The relative position of donor, mediator, and acceptor redox potentials defines the charge transport and hence the potential-dependent electrochemical current rectification.

Therefore, the redox probes are considered as inputs information contained in electrical signals is required. To perform the XOR operation, binary encoding of the mediator, and acceptor redox potentials defines the potential-dependent electrochemical current rectification.

Electrochemical rectifiers do not only have electrical / electrochemical characteristics similar to conventional diode rectifiers, they also can be combined to perform higher logic operations. Therefore, we linked a cathodic and anodic ECR together to realize a XOR logic gate [3]. Redox active ferrocenylthiol is attached to a gold electrode acting as redox mediator for two different redox inputs. If the redox potentials of the redox probes are clearly separated (ferrocyanide 0.23 V, hexachloroiridate 0.71 V) and the redox potential of the mediator is located in between (0.34 V) then one obtains two linked ECRs of opposite sign, Fig. 2 b, c. To perform the XOR operation, binary encoding of information contained in electrical signals is required. Therefore, the redox probes are considered as inputs and the absolute electrochemical outputs which are assigned to 0 and 1.

As can be seen in Fig. 2, a strong current signal at 0.50 V was present (output: 1) when either ferrocyanide or iridate[IV] ions were added (input: b:1,0 or c:0,1). A pronounced current signal was absent (output: 0) when neither or both of the inputs were applied (input: a:0,0 or d:1,1). Figure 3 shows the current responses of the modified electrode with respect to different input signals. By summarizing input and output signals in a truth table, it becomes obvious that an XOR logic gate function was realized with high switching ratio between output signals “1” and “0”. Additionally, the modified electrode can be used to successively perform XOR logic gate functions (Fig. 3) without renewing the system (electrode and solution) after finishing the first operation of the XOR logic gate function. The total level of the output signal encodes additional information of the input signal history. This property will allow application of several input cycles, thus underlining the reproducibility of the system and is a prerequisite for many electronic applications. It is worth noting that an XOR logic gate can also be operated as a comparator, which distinguishes whether the input signals have the same value or not.

The strategy to link different surface bound and unbound redox centers to electrified interfaces can be further expanded to the development of other molecular logic gates with electrical readout, which might pave the way to integrate logic and sensing operations for advanced sensor performance. The opportunity to use different sensory inputs being processed by a logic gate will improve the reliability of sensor responses by suppressing cross sensitivity and linking the signal of several (bio)-markers together. Furthermore, it can be envisioned that this XOR logic gate is operated as switch triggered by redox probe inputs.

**FIG. 2:** Cyclic voltammograms of a HDT / ferrocenylthiol functionalized gold electrode in a) PBS (input 0,0), b) 0.5 mM ferrocyanide (input 1,0), c) 0.5 mM iridate[IV] (input 0,1), and d) a mixture of 0.5 mM ferrocyanide and 0.5 mM iridate[IV] ions (input 1,1). b) and c) are representing an anodic and cathodic electrochemical rectifier response.

**FIG. 3:** Left: Current responses (output signals) of a modified electrode corresponding to various input signals at the potential of 0.50 V. Here, the current is shown as absolute value independent of current direction during first and subsequent operations. Right: schematic of a molecular XOR logic gate based on two linked electrochemical rectifiers.

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Intermolecular electron transport investigated by break junctions

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Theoretical and experimental investigations of charge transport through (bio) molecules have attracted considerable attention driven by the interest in fundamental aspects of charge transport and the vision of future applications in molecular electronics and biosensors. Within the sub-topic “molecular bioelectronics” we address open mechanistical questions of electron transport in model systems which are relevant for protein charge transport. We investigated the relation between inter- and intramolecular electron transport, considered the role of metal ions in metal-organic complexes, and showed that electrostatically bridged molecules have a lower conductance than the same molecules bridged by covalent bonds.

Within this project period, we established a mechanically controllable break junction setup (MCBJ) which facilitates electrical addressing of individual molecules. A micro-fabricated metal wire is bent in a mechanical bending apparatus until it breaks, resulting in two closely spaced nanoelectrodes (Fig. 1). The molecule of interest is anchored between the electrodes and subsequently electrically characterized by recording I/s and I/V responses. The current-voltage characteristics of alkanes typically exhibit a transition from direct tunneling to field emission as the applied bias exceeds a threshold voltage. This voltage is proportional to the effective tunneling barrier and shifts to lower values as the distance between the nanoelectrodes decreases. Previously, this shift was assigned to an enhanced intermolecular electron transport between parallel molecules. With MCBJ experiments we demonstrated that also for single molecules an enhanced field emission can be observed as the molecular junction was compressed [1]. I/V measurements with various numbers of molecules in the junction at a given gap size revealed that intermolecular electron transport between adjacent molecules was of minor importance and alkanedithiols molecules can be considered as individual transport channels.

Furthermore, we have used MCBJ for the investigation of the influence of metal ions on conductivity of metal complexes. Metal complexes play an important role for many biochemical redox processes and their charge transport depends on the nature of anchored metal ions. We have investigated metal-molecule-metal junctions which were built from aminocarboxylic acids (C2-NTA), a general sequestering agent. Breaking a gold junction modified by (C2-NTA) generated a clear peak in the conductance histogram (Fig. 2). After addition of metal ions, a second set of peaks at lower conductance values was observable in the histogram. The position of these peaks shifts depending on the kind of entrapped metal ion. A statistical analysis of the recorded conductance traces revealed that the complex conductance depends strongly on the type of coupling ion with the order: Ca2+>>Zn2+>Ni2+ [2]. The conductance was found to be reciprocally dependent on the complex stability. The observed relation between type of metal ion and conductivity of molecular junctions helps us to understand in detail the role of metal ions in biological charge transport. Furthermore, it may pave the way for novel design strategies of molecule based devices.

![Image of working principle of the mechanically controllable break junction (MCBJ)](image_url)

**FIG. 1:** Working principle of the mechanically controllable break junction (MCBJ). a) Scanning electron microscopy images of micro-fabricated MCBJ consisting of a freestanding metal bridge with a central constriction. Left) before and right) after breaking of the constriction. b) Schematic of the MCBJ mounted into a three-point bending configuration. Bottom: I/V curves of single octane dithiol junctions with three different gap sizes. Curves B and C were recorded after the gap size was decreased by approximately 0.2 and 0.4 nm compared to the gap of curve A, respectively. The insert shows corresponding ln(I/V2) versus 1/V characteristics. An inflection point can be observed, which shifts to lower biases as the gap size decreases.
To investigate charge transport processes on a single molecule level in aqueous solution, an electrochemical break junction setup was established (Fig. 3). Pairs of facing nanoelectrodes were fabricated and encapsulated on a silicon chip. The two electrodes were initially separated by a submicron gap and subsequently narrowed by electrochemical deposition until a gold junction was formed. We tuned the electrode surface morphology by the deposition potential to generate a dendritic surface, which results in high junction stability and small contact area between the electrodes [3]. The electrochemical break junction setup was used to electrostatically bind ferrocene dicarboxylic acid (FDA) to cysteamine modified electrodes. This coupling is possible only within a small pH range, where the two molecules are oppositely charged. However, within this pH range, it was possible to bridge two opposing nanoelectrodes by electrostatically linked cysteamine(+)/(−)FDA(-)/(+)cysteamine junctions in aqueous solution and to determine corresponding conductance histograms. The obtained single molecule conductance of the electrostatically linked junction was one order of magnitude smaller than the conductance of covalently linked cysteamine–FDA–cysteamine, which indicates that the intermolecular electron transport through electrostatic interfaces has lower efficiency compared to intramolecular electron transport [4].

This finding is of relevance to re-evaluate the role of electrostatically linked complexes for interprotein charge transport.

In summary, the obtained results show that biologically relevant questions of charge transport can be investigated by means of MCBJ on the level of individual molecules. The synthetic model molecules used so far will be substituted in the next step by more complex oligopeptides, the building blocks of proteins [5].

Strained SrTiO$_3$ films on sapphire

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JARA-FIT: Jülich-Aachen Research Alliance

Owing to the strong connection between strain and ferroelectricity, large shifts of the Curie temperature and polarization are observed in strained ferroelectric material. In this work, the effects of lattice-mismatch induced stress upon the crystallographic structure, strain, and generation of different types of defects in epitaxial SrTiO$_3$ films on CeO$_2$ buffered sapphire are examined and discussed in context with the resulting impact of strain on the polarization of the ferroelectric layers. Depending on the thickness of the SrTiO$_3$ layer, characteristic changes in their structural perfection and crystallographic orientation with respect to the substrate system are observed. For thin films, misfit dislocations partially compensate the stress in the SrTiO$_3$ layer, whereas cracks develop in thicker SrTiO$_3$ films. The structural modifications and the formation of defects can be explained in a model based on lattice misfit induced stress and energy considerations. It is demonstrated that intrinsic mismatch and thermal mismatch must be considered to explain strain dependent effects such as induced ferroelectricity and modifications of the permittivity of these complex heteroepitaxial layer systems.

Pseudomorphic strained-layer epitaxial growth has quickly turned from a mere curiosity into a major technology for advanced semiconductor devices and structures for solid state research. It has been demonstrated that mechanical strain can have dramatic effects on the electromagnetic properties of thin films. The most significant effect has been observed in the incipient ferroelectric oxide SrTiO$_3$ (STO) where a ferroelectric transition can be induced and an enhancement of the transition temperature T$_c$ up to room temperature has been reported [1,2]. For epitaxially grown thin films, the strain is naturally induced by the underlying substrate (clamping effect). The strain is caused by the lattice mismatch and the differences in thermal expansion coefficients between the substrate and the STO film. Therefore, the development of strained epitaxial STO films on suitable substrates and the analysis of their ferroelectric and structural properties are of large interest for various applications in the semiconductor technology, ranging from high-frequency devices to specific sensor applications.

Here we report on induced ferroelectricity of STO films grown on different substrates. We will focus on epitaxially grown films on the microwave suitable substrate sapphire. For this system, ferroelectricity is observed up to T$_s$ $\approx$ 200K which cannot be explained by standard structural analysis obtained from room temperature XRD measurements.

A series of STO films is grown on NdGaO$_3$ (110) (NGO), DyScO$_3$ (110) (DSO), and CeO$_2$ buffered r-cut sapphire ($\alpha$-Al$_2$O$_3$) via pulsed laser deposition and magnetron sputter technology. Due to the lattice mismatch, STO films on DSO substrates are expected to be exposed to tensile strain, whereas NGO would lead to compressive strain parallel to the film surface (in-plane). As demonstrated in fig. 1b, the STO films on NGO and DSO behave as expected. The in-plane lattice parameter a$_{\parallel}$ is elongated or distorted for STO on DSO or on NGO, respectively, and compensated by the out-of-plane parameter a$_{\perp}$. As a consequence and in agreement with the theory [3], in-plane ferroelectricity is induced in STO on DSO, whereas no ferroelectricity is measured in STO on NGO (see fig. 1a).

![Fig. 1: Ferroelectric transition temperature $T_c$ (a) and out-of-plane lattice parameter (b) for STO on different substrates as function of in-plane lattice parameter. The dashed lines represent the theoretical predictions for the regime of the phase transition from ferroelectric to dielectric behavior according to [3] in (a) and the nominal lattice parameter for the different substrate systems in (b).](image)

The STO films on CeO$_2$ buffered sapphire behave quite differently. The lattice mismatch between the CeO$_2$ and STO does not seem to affect the in-plane lattice parameter of the STO in the same way observed for the other systems (STO on NGO and DSO). STO films on CeO$_2$/sapphire seem to be nearly unstrained at room temperature (Fig. 1b). However, the ferroelectric properties of the films are affected. In contrast to the theoretical predictions [3], in-plane ferroelectricity is induced in STO on sapphire, and $T_c$ values up to 200K
are observed. In order to understand this unexpected behavior, we analyzed the structural and mechanical properties of STO films on sapphire in more detail.

A first indication that stress is also imposed on the films on sapphire and results in a modification of structural and dielectric properties is provided by a comparison of XRD and permittivity data for films of different thicknesses (Fig. 2). A clear indication is given by the observation of crack formation in thick STO films on sapphire (Fig. 3).

All samples with a thin STO layer ($d_{STO} \leq 350\text{nm}$) show a smooth surface and no cracks. In contrast, all thick STO films ($d_{STO} \geq 525\text{nm}$) reveal cracks. As shown in Fig. 3b ($d_{STO} = 525\text{nm}$), first a few cracks with finite lateral extension (typically < 200$\mu\text{m}$) are present. All cracks point in the same crystallographic direction, i.e. the [010] direction of STO which is identical to the [1210] direction of r-cut sapphire. With increasing thickness, a ‘network of cracks’ develops (see fig. 3c). The cracks are oriented along two crystallographic directions, i.e. along the [1210] and the [010] direction of the r-cut sapphire. Since there is no distinguished crystallographic orientation of the STO and CeO$_2$ layers equivalent to the [1010] direction of the r-cut sapphire, this indicates that the cracks are generated by the difference in the expansion coefficients of sapphire and STO, and that the (in-plane) expansion coefficient of sapphire is slightly anisotropic.

Heteroepitaxial strained-layer growth signifies a major technology for use in advanced semiconductor and correlated devices, as well as for other structures in solid state research. The most fundamental questions in strained-layer growth are, (i) up to what thicknesses are heteroepitaxial layers stable, (ii) which type of misfit defects will develop and (iii) what happens upon modifications to the misfit (for instance due to cooling of the film)? It is generally believed that below a critical thickness the strained layer is the thermodynamic equilibrium state, and above a critical thickness a strained layer may be metastable or it may relax [4]. Different critical thicknesses might be associated to different types of misfit defects, e.g., dislocations, misalignments, or even cracks [5]. Stress in heteroepitaxially grown films results from both an intrinsic and a temperature-dependent component. The main reason for the development of intrinsic stress in heteroepitaxially grown films is given by the nominal lattice mismatch $\varepsilon = (a_{film} - a_{substrate})/a_{film}$ at growth conditions [4,5], where $a$ represents the lattice parameter of the film and substrate. The thermal contribution to the stress arises from the difference in thermal expansion coefficients of the film and the underlying substrate. These two (intrinsic and extrinsic (here temperature-dependent)) contributions generate defects (ranging from misfit dislocations to cracks) in our heteroepitaxial STO films. A detailed description of the model is given in ref. [6].

The importance of intrinsic and thermal induced stresses for the understanding of structural modifications, the formation of defects, and the resulting ferroelectric properties of heteroepitaxially grown STO films is demonstrated. A model (details in ref. [6]) is developed to describe (i) the generation of misfit dislocations during growth and (ii) the crack formation during cooling of the sample. In general, the misfit in lattice constant and thermal expansion can be a very powerful tool to engineer the properties of heteroepitaxial films and, in particular, the ferroelectric properties of STO, suitable for various applications in semiconductor technology ranging from tunable high-frequency devices to microwave sensor concepts.

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Low field magnetic resonance imaging with tuned high-\(T_c\) SQUID

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In order to improve low-field (LF) Magnetic Resonance Imaging (MRI) measurements with a tuned high-\(T_c\) radio-frequency (rf) Superconducting Quantum Interference Device (SQUID) as a signal detector, we use a permanent magnet pair for sample pre-polarization. MRI images are acquired by using filtered back projection reconstruction. The projections are obtained by recording free induction decay (FID) or spin echo (SE) signals with the gradient field applied at different angles. For every projection, the sample is first pre-polarized in the gap of the PM pair and then mechanically transported to the measuring position underneath the tuned SQUID. Taking 12 projections, two-dimensional LF-MRI images of two water phantoms are obtained without averaging, exhibiting a spatial resolution of about 0.2 mm.

Recently, LF-MRI using a SQUID as a signal detector was demonstrated [1] at low measurement field \(B_m\) in the \(\mu\)T range. The magnetization \(M\) of the sample is proportional to the pre-polarization field \(B_p\) while \(B_m\) determines the Larmor frequency \(f_e\). The precession of signals scale only with \(B_p\) when a SQUID is employed as the detector because of its frequency-independent sensitivity. However, the technical relevance of MRI at low field is usually limited by the relatively low signal-to-noise ratio.

It is very difficult to generate a polarization field \(B_p\) larger than 50 mT using copper-wire-wound coils at room temperature [2] because of the dissipated heat due to the large coil current, the difficulty to switch off \(B_p\) quickly because of the large coil inductance, and flux trapping in the SQUID. In order to overcome this, we use sample pre-polarization by a permanent magnet (PM). Two NdFeB disk magnets are integrated with a soft iron yoke, generating a \(B_p\) field of about 1 T. A tuned high-\(T_c\) (HTS) rf SQUID is used as the signal detector. The MRI images are acquired by using filtered back projection reconstruction. Each measurement is performed by first pre-polarizing the sample in the gap of the PM pair and then mechanically transporting it to the measuring position. Because of the strong polarizing field and the high sensitivity of the tuned SQUID, the FID and SE signals can be recorded without averaging.

Our LF-MRI measurement setup, schematically shown in Fig. 1, is designed for small-size samples. It consists of four parts: a coil system, a tuned HTS rf SQUID, a PM pair with yoke, and a mechanical transport system.

![Figure 1: Schematic of the LF-MRI setup. Labels are defined in the text.](image)

The 2-D MRI coil system includes a Helmholtz coil pair for the generation of the measurement field \(B_m\), three coil pairs generating the gradient field components \(G_{zx}\), \(G_{zy}\), and \(G_{zz}\), and an excitation field \(B_{ac}\) coil pair to generate the \(\pi/2\) or \(\pi\) pulse.

A Helmholtz coil pair of 22 cm diameter provides a \(B_m\) of 212 \(\mu\)T along the z-direction. An excitation field \(B_{ac}\) in the y-direction is produced by a coil pair (not shown in Fig. 1). The gradient field \(G_{zx} = dB_x/dz\) is generated by a Maxwell coil, whereas the other two gradient fields, \(G_{zy} = dB_y/dx\) and \(G_{zz} = dB_z/dy\) are produced by planar multi-loop gradient coils. When the filtered back projection reconstruction is utilized to create 2-D MRI images, the direction of the gradient field in the y-z plane can be rotated stepwise by adjusting the strength of \(G_{zx}\) and \(G_{zy}\).

The tuned HTS rf SQUID combines an rf SQUID magnetometer and a LC resonant circuit, which is inductively coupled to the SQUID [3]. The tuned rf SQUID not only improves the sensitivity to 6 ~ 7 \(fT/\text{Hz}\) at \(f_c\approx 9\, \text{kHz}\) but also provides the large detection area of the \(L\) coil which is 40 mm in diameter [3].
Once the sample arrives at the measuring position, a micro-switch produces a TTL pulse to start the measurement sequence shown in Fig. 2. First, a \( \pi/2 \) pulse of \( B_{ac} \) is applied to tilt the sample magnetization \( M \) perpendicular to \( B_0 \). A Q-switch circuit damps the ringing in the LC circuit induced by the ac pulse [4] before the FID signal is recorded. Subsequently, a \( \pi \) pulse of \( B_{ac} \) is applied to obtain a SE signal. After the recordings, the sample is transported back to the gap of the FM pair for pre-polarization and the gradient field is rotated to acquire the next projection.

The filtered back projection reconstruction was utilized for 2-D MRI measurements [5]. In the \( y-z \) plane, each projection corresponded to a gradient field \( G(y,z) \) of about 20 Hz/cm, with its direction rotated step by step to cover 180°. For all projections, both FID and SE signals were recorded without averaging.

We performed LF-MRI measurements with water phantoms [6]. Image quality generally increases with increasing number of projections. To illustrate this effect, images were reconstructed from 6 and from 12 projections, as shown in Fig. 3 (a). Here, sample #1 with five isolated cylinder-shaped bores filled with tap water (1 × 0.2 ml with diameter of 6 mm in the center and 4 × 0.35 ml with diameter of 8 mm around it) was utilized. The MRI image reconstructed from 6 projections was distorted because of the low number of projections. When the number of projections was increased to 12, the image quality was clearly improved. Usually, the echo signal is utilized to reconstruct the image. Here we make a comparison between the images of sample #2 reconstructed from FID and Echo signals. The sample #2 is a plexiglass slab with two cylinder-shaped bores, 7.5 mm in diameter, filled with tap water (0.35 ml each), and separated by a distance of 13 mm. The photo of the sample #2 and its reconstructed 2-D MRI images with 12 projections are shown in Fig. 3 (b). Geometrical shapes of the images reconstructed from both FID and SE signals are in good agreement with the dimensions of the sample.

The spatial resolution \( \Delta x \) of MRI in one dimension can be expressed as \( \Delta x = 2\pi\delta l / \gamma G \), where \( \delta l \) is the linewidth of the NMR spectrum, \( \gamma \) the gyromagnetic ratio of proton \( (\gamma/2\pi = 42.58 \text{ MHz/T}) \), and \( G \) the applied gradient field strength \( (47 \mu \text{T/m}) \). For the measured 0.4 Hz linewidth of sample #2, a spatial resolution of about 0.2 mm was reached.

The quality of the images demonstrates that it is feasible to image small-sized samples with 1 T pre-polarization, sample movement and a tuned SQUID detector [C. Liu, Y. Zhang, LQ. Qiu, H. Dong, H.-J. Krause, X. Xie, A. Offenhäusser, submitted to SUST (2012)]. The performance was significantly improved over pre-polarization by a copper coil. MRI images were reconstructed from 12 projections without averaging. In our present scheme, a relatively long relaxation time \( T_2 \) of the sample on the order of seconds is required. In the future, not only the homogeneity of \( B_0 \) should be improved to reduce the distortions of the reconstructed MRI image, but also the sample transport time should be shortened to lower the requirement on the sample’s \( T_2 \).

![FIG. 2: Pulse sequence used in the measurements.](image)

![FIG. 3: Sample photos and their 2-D LF-MRI images. The tap water in the sample photos was pink-colored and the sample substrates were made from plexiglass. (a) Photo of sample #1 and its 2-D MRI images reconstructed from FID signals for different numbers of projections. (b) Photo of sample #2 and its 2-D MRI images reconstructed from FID and SE signals.](image)

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Education, Dissemination, Workshops and Conferences

The directors of ICS are jointly appointed by Forschungszentrum Jülich and one of the universities of Aachen, Bonn, Düsseldorf, Cologne, and Münster. Together with further senior scientists from the ICS, they contribute to the curricula of these universities. This way, students from the universities gain access to the research capabilities of the ICS and can participate in the graduate and postgraduate training. Besides the individually arranged postgraduate training, the ICS initiated as a joint activity, the first structured postgraduate training in Jülich and one of the first international research schools within the Helmholtz Association: The International Helmholtz Research School on Biophysics and Soft Matter.
International Helmholtz Research School on Biophysics and Soft Matter

The International Helmholtz Research School on Biophysics and Soft Matter (IHRS BioSoft) provides a course programme for PhD students at the interfaces of biology, chemistry, and physics. In addition to this educational role, the aim of the school is to promote interdisciplinary research projects.

In 2011, more than 30 PhD students were fellows of the IHRS BioSoft. Faculty members of the school have jointly selected all new students. So far, the fellows of the school have been very successful: Out of the fourteen alumni, five have achieved the highest grade "summa cum laude" for their PhD project. The selection and the education of graduate students is a central common activity within the PoF programme BioSoft that significantly strengthens the interactions between the institutes and that provides links to the universities in Cologne and Düsseldorf, and to caesar Bonn on the basis of collaboration agreements.

Since its foundation in 2006, the IHRS BioSoft has hosted foreign fellows from China, the USA, France, Italy, Greece, Zimbabwe, Great Britain, India, Iran, Kazakhstan, and Ukraine. Currently, half of the highly qualified PhD fellows are non-German. Being fellows of the school, the students benefit not only from lectures, seminars, and lab courses given by experts in the field, but also from courses in transferable skills that are jointly conducted by tutors from the Imperial College London and by German tutors from Helmholtz centres. These courses, as well as regular retreats, provide opportunities to network with PhD students from other fields.

The last three-year course programme consisted of a lecture series on complex fluids by Prof. Strey, an introductory course on optical microscopy jointly offered by Prof. Merkel, Prof. Egelhaaf, and Prof. Seidel, a course on macromolecular physics and chemistry by Prof. Richter together with colleagues from the ICS-1, a lecture series "Molecules of Life" coordinated by PD Dr. Fitter, lectures and exercises on statistical mechanics and its applications in soft matter and biophysics by Prof. Gompper and Prof. Dhont, courses on computer simulations by Prof. Winkler and Dr. Strodel, a lecture series on biological macromolecules by Prof. Büldt and colleagues from the ICS-5, as well as a lecture series on cell biology by Prof. Müller and Prof. Baumann. In addition, Prof. Vermant from KU Leuven presented a guest lecture series on rheology. These main courses were complemented by lab courses on fluorescence spectroscopy, electron microscopy, NMR, optical microscopy, neutron scattering, protein purification, MD simulations, plant phenotyping, and an in-house course on scientific writing. At the 2011 retreat, that took place at caesar in Bonn, lectures on scientific ethics were given by Prof. Löwer and Prof. Kaupp, and a collaboration workshop was organized by Dr. Auth.
Individually assembled PhD committees with scientists from different institutes advise the fellows on their projects and point out opportunities for interdisciplinary research. This is reflected by the topics of the theses that focus on the research interests of the individual groups:

- Annukka Aho: Analyse der Heteromerisierung von hyperpolarisationsaktivierten und zyklisch nukleotid-gesteuerten Ionenkanälen
- Christoph Brenker: Die Rolle von Ionenkanälen in menschlichen Spermien
- Christine Gerstl: Kettenkonformation und Dynamik verschiedener Poly(alkylenoxid)e
- Normann Goodwin: Untersuchung der nicht-genomischen Wirkung von Progesteron auf menschliche Spermien
- Sudipta Gupta: Dynamic phase diagram of soft colloids
- Marco Heinen: Charged colloids and proteins: Structure, diffusion, and rheology
- Richard Poire: Effect of light and temperature on plant canopy growth
- Tobias Rosenkranz: Time-resolved single molecule FRET studies on folding, unfolding transitions and on functional conformational changes of phosphoglycerate kinase
- Sabine Schetzberg: Progress and first milestones in formulating food-grade microemulsions
- Ivana Fehr: Vesicles in simple binary water
- Michael Klostermann: Quantitative characterisation of supercritical microemulsions and their use for preparing highly porous sugar foams
- Justin Lecher: NMR studies on the isolated C39 peptidase-like domain of ABC transporter Haemolysin B from E. coli: Investigation of the solution structure and the binding interface with HlyA
- Kristian Marx: Self-propelled rod-like swimmers near surfaces
- Vanessa Maybeck: Tools for non-invasive communication with electrogenic cells: Optogenetic stimulation and diamond recording devices

Besides the PhD education, the IHRS BioSoft provides a framework for common activities that cannot be offered by single groups and that help to increase the visibility within the scientific community. An example is the guest student programme that took place for the first time in 2011: On August 1st, eleven master level guest students started to work with PhD fellows and postdocs in IHRS BioSoft institutes. The students, selected for academic excellence, from Sweden, Switzerland, Croatia, Iran, and China have worked on 10-week research projects, participated in lab courses offered by other IHRS-BioSoft groups, and presented their projects in short talks. For the fellow PhD students of the IHRS BioSoft, the guest student programme provided the possibility to gain experience in advising younger students.
Centre for Structural Biology and Helmholtz Nanoelectronic Facility II

Two major investment plans of the ICS are part of the Roadmap “Research Infrastructure” of the Helmholtz Association. The Roadmap outlines a concerted schedule of outstanding research infrastructures which are strategically relevant for the Helmholtz Association and for the implementation of the scientific portfolios in the respective research fields for the next 10 years. Based on these considerations, the research infrastructures of the Helmholtz Roadmap are regarded as essential. The ICS contributes to the Helmholtz Roadmap with the Centre for Structural Biology and the Helmholtz Nanoelectronic Facility II.

The Centre for Structural Biology is a common proposal of Forschungszentrum Jülich and KIT (Karlsruhe Institute of Technology) to establish a new state of the art research platform for biomolecular ultra high field liquid and solid state NMR spectroscopy. It will be embedded in the ICS-6 and Karlsruhe Institute of Research on Oriented Systems (KAIROS) at KIT. The core of the proposed research infrastructure are four NMR spectrometers: For the Jülich site a 1.2 GHz NMR spectrometer dedicated to liquid and solid state NMR and a solid state 800 MHz widebore NMR spectrometer equipped with DNP as well as a building with space for laboratories and offices and a hall to host the two NMR spectrometers is requested. The Karlsruhe site plans to host a 950 MHz NMR spectrometer and a solid state 800 MHz widebore NMR device dedicated for use with oriented samples and for micro-imaging spectroscopy.

The proposal received enthusiastic support from an international, external review. One of the reviewers stated that “the 1.2 Gigahertz machine will slingshot the structural biology at FZJ to the very frontier of international science. The concerted action of such a machine together with the 950 MHz NMR spectrometer dedicated to liquid and solid state NMR and a solid state 800 MHz widebore NMR device equipped with DNP as well as a building with space for laboratories and offices and a hall to host the two NMR spectrometers is requested. The Karlsruhe site plans to host a 950 MHz NMR spectrometer and a solid state 800 MHz widebore NMR device dedicated for use with oriented samples and for micro-imaging spectroscopy.

The Helmholtz Nanoelectronic Facility (HNF) at the Forschungszentrum Jülich is the central technology platform for nanoelectronics within the Helmholtz Association. In the HNF several institutes have joined forces to provide technologies for the exploration, production, and characterisation of nanoscale and atomic structures for the research field Key Technologies. The HNF-user facility enables a broad access to these technologies for universities, research institutions, and industry as well as the direct transfer of knowledge to the community. The recently constructed 3-story building will accommodate a 1000 m² clean room on the second floor and basement space for further experiments and equipment. The facility will be followed up by
Education, Dissemination, Workshops and Conferences

a second building as office and laboratory combination for most advanced characterisation and analysis methods for procedure and process control (HNF II). Modern use concepts for virtual access to the infrastructure and integration of external users will be implemented. A centre for "Bottom-up" technologies is located at the decoupled ground floor. Nano-technology and -electronics are enabling technologies with diverse application fields: They are the key to research and advancements in automation, the automotive industry, entertainment and consumer electronics, security technology, life sciences, and medicine. Technology drivers are mass-market applications (automotive, entertainment, and consumer electronics), but also new special applications in life sciences and medicine. In particular, the combination of nano-technology and -electronics with biological systems is considered as an area with great future potential.

The ICS and related partners take an active role in the discussion and dissemination of the relevant scientific issues in the field of biophysics and soft matter, e.g. by organizing workshops, spring schools, and conferences:

- International Conference on Natural and Biomimetic Mechansensing, Dresden (2009)
42nd IFF Spring School 2011: Macromolecular systems in soft and living matter

The IFF spring school has a long tradition. Organized for more than 40 years, this two-week school presents an intensive course of lectures, lab visits and courses about varying topics of high current interest. The topic of the 42nd IFF spring school was concerned with the interdisciplinary field of macromolecular sciences, which is equally important for biologists, chemists, and physicists. It was attended by more than 200 participants from more than 25 countries.

The spring school 2011 intended to give an introduction and an overview of current research topics of macromolecular systems in soft matter and cellular biology. Macromolecules such as synthetic polymers, polyelectrolytes, amphiphiles, and colloids are the key building blocks of many modern materials. The understanding of their structural and dynamic properties is challenging due to the large number of relevant degrees of freedom, the weak interactions between the components which imply an important role of thermal fluctuations, and the large structural length scales in the range of nanometers to micrometers. Macromolecules such as DNA, proteins, and lipids, are the key building blocks of living systems. In addition to the complexity of synthetic systems, the enormous variety of different molecules and their very specific interactions pose many new challenges, which range from the understanding of the tertiary (spatial) structure of proteins from their amino-acid sequence to the complex interplay of many components in the cell. Therefore, many aspects of macromolecules and composite systems are important for soft and living matter alike.

The goal of this spring school was not only to teach soft matter and biophysics to students and postdocs in physics, chemistry, and biology, but also to establish the interdisciplinary connection between these fields. This includes, in particular, to introduce biologists and chemists to physical experimental methods and theoretical modelling, and to introduce physicists to the large variety of fascinating biological phenomena.

Introductory lectures presented the basics of soft matter science and biophysics. These lectures were intended to establish a common level of basic interdisciplinary knowledge. Subsequent lectures then treated more advanced topics within both disciplines and emphasized interdisciplinary aspects. In addition, experimental and computer simulation techniques were introduced, explained, and examples of applications were given.

Programme:
The school provided about 50 hours of lectures, including discussions, and offered the opportunity to visit the participating Institutes at Forschungszentrum Jülich. All lectures were given in English. All registered participants received a book of lecture notes, which contains all the material presented during school. The following topics were presented within the lectures:

Techniques and Methods:
Many modern experimental and simulation techniques are used in soft-matter and biophysics research. The experimental methods include NMR, scattering methods (light, X-ray and neutron scattering), imaging, microscopy and single-molecule techniques, dielectric spectroscopy and rheology are grouped together in three sections, ordered according to increasing length scales and complexity, and a fourth section dealing with techniques and methods.
Cells and Viruses:

An introductory lecture reminded physicists about the structure of the eucaryotic cell, and the function of its main components. Advanced lectures covered several topics related to cellular biophysics: Motor proteins connect to actin filaments in the cytoskeleton and give this active polymer network unique properties, red blood cells deform in capillary flows, and sperm cells bacteria are microswimmers in a highly viscous environment. Cells and viruses have interesting mechanical properties. Cells react actively to external mechanical and electrical stimuli. Finally, bioelectronics explores the interface between microelectronic and the living world. Widely used numerical techniques that were discussed are molecular dynamics, Monte Carlo, and mesoscale hydrodynamics simulations.

Lecturers:

In addition to members of the organizing institutes (ICS-1, ICS-2, ICS-3 and ICS-6) lectures were given by A. Baumann (ICS-4), J. Fitter (ICS-5), O. Weiergräber (ICS-5), B. Hoffmann (ICS-7), R. Merkel (ICS-7), and A. Offenhäusser (ICS-8). In addition, external speakers contributed their special expertise; we are grateful to B. Byrne (Imperial College London), S. Egelhaaf (Düsseldorf), K. Gerwert (Bochum), U.B. Kaupp (caesar Bonn), C. Ligoure (Montpellier), R.R. Netz (TU München), J.O. Rädler (LMU München), and G. Schoehn (EMBL Grenoble) for their contributions.
The Jülich Soft Matter Days

G. Gompper, J.K.G. Dhont, D. Richter

We organize a yearly workshop, the “Jülich Soft Matter Days”, to bring together scientists from the different soft-matter disciplines (colloids, polymers, and surfactants), as well as scientists from biophysics and biology. This workshop series was started in 2001 and attracts typically about 200 scientists and students every year.

It has been recognized over the last decades that colloids, polymers, and surfactants are by far not as distinct materials as previously assumed. Indeed, there is essentially a continuum of molecules and systems, which fills the triangle of materials illustrated in the figure below. The two main axes of this triangle are, roughly speaking, amphiphilicity as abscissa and elongation or flexibility as ordinate. Let us illustrate this by following the left-hand side of the triangle from colloids to flexible polymers. Traditionally, colloids are hard, spherical particles. However, there are also rod-like colloids.

As the aspect ratio, the ratio of rod length to rod diameter, becomes larger, rods typically become more flexible. An example is the fd-virus which has been used as a model colloidal particle to study the behaviour of anisotropic colloids. For even larger aspect ratios, the length exceeds the persistence length; this is the regime of semi-flexible polymers, for which DNA is an example of enormous importance. In the limit of very small persistence lengths, we arrive at the classical, flexible synthetic polymers.

Mixtures of several components of colloidal, polymeric or amphiphilic character are becoming increasingly important, because they open up the possibility to tune and control material properties. Well-known examples are the depletion interaction between colloidal particles induced by polymers in solution, the intriguing mesophases in mixtures of spherical and rod-like colloids, the tuning of membrane properties by anchored polymers, and amphiphilic block copolymers, or the modification of the properties of polymers melts by addition of colloidal particles to form nano-composites.

Biological and biomimetic systems share many macromolecules and properties with soft matter.
systems. Indeed, the application of physical concepts and ideas to biological systems has become one of the most intense activities in soft condensed matter in recent years.

The 2011 meeting, consisted of four sessions devoted to:

- Biosystems
- Colloids
- Polymers
- Self Assembly.

The invited speakers came from the USA (10 speakers), Germany (8), France (4), The Netherlands (2), England (2), Japan, Greece, Israel, Poland and Spain (1 each). There were 130 posters distributed over two sessions.

The Jülich Soft Matter Days have been organized since 2001 on a yearly basis. In 2007, we initiated and co-organized a large International Soft Matter Conference in Aachen; such a conference is now organized every three years (2010 in Granada [Spain], 2013 in Rome [Italy]). The Jülich Soft Matter Days are attended typically by about 200 scientists and students from Europe, Asia, and the Americas.

As in previous years, the Jülich Soft Matter Days 2011 took place at the Gustav-Streeesemann-Institute in Bonn. This is a very convenient conference centre, because lecture halls, poster rooms, accommodation, and catering are all in the same place, which enables and fosters intense discussions.

Poster session during the Jülich Soft Matter Days


A cartoon of a colloid-polymer nano-composite
1st BioScience Workshop 2010: Expanding the frontiers of biomolecular science

M. Bachmann¹, G. Gompper¹, G. Schröder², B. Strodel², D. Willbold²

¹ICS-2: Theoretical Soft Matter and Biophysics
²ICS-6: Structural Biochemistry

The BioScience 2010 workshop was the beginning of a biennial workshop series on biomolecular sciences. This first workshop on “Expanding the Frontiers of Biomolecular Science”, which was held from 15 to 17 November at Forschungszentrum Jülich, attracted 95 researchers from 14 countries and was remarkably well received. The workshop was aimed at experimentalists and theoreticians alike, and focused on current and future techniques for characterizing structure, function, and dynamics of biomolecules. It was organized by the ICS-2 and ICS-6.

This workshop brought together both experimentalists and theorists with the goal of identifying and discussing the state-of-the-art and emerging techniques for biomolecular structure, dynamics, and function characterization. The function of a specific biomolecule is closely coupled to a multitude of other biomolecules and the ultimate goal is to understand how interactions between biomolecules shape the complex organization, behaviour, and evaluation of biomolecules. In this workshop, different biomolecular interactions were considered, including protein-protein interactions, interactions between proteins and DNA, as well as protein-lipid interactions. In addition to the variety of biomolecular systems, the advancement of existing, and the development of new, experimental and simulation techniques were discussed. Newer experimental techniques enable higher position and time resolution, integral to understanding biomolecular structure and dynamics. Experimental techniques discussed in this workshop included solution and solid-state NMR, infrared spectroscopy, and single molecule fluorescence spectroscopy. Simulation approaches, such as coarse-graining, large-scale molecular dynamics, and Monte-Carlo techniques were discussed in terms of their ability to extend the length and time scales of biomolecular simulations. By inviting experimentalists and theorists to the workshop we aimed to bridge the gap between theory and experiment.

The workshop comprised 28 presentations given by invited speakers with about half of the speakers coming from Germany and the others from around the world. The other participants were given the possibility to present their work during a poster session on the first evening, which stimulated lively discussions in a sociable atmosphere. The workshop dinner on the second evening, in addition to coffee breaks between talks, was a good opportunity for the workshop participants to get to know each other, discuss their work and exchange scientific ideas.

Following the success of this workshop, the next BioScience workshop will be held in November 2012 for which 25 internationally renowned speakers are already confirmed.

In 2010, the following presentations were given during the workshop:

- B. Brutscher, Université Joseph Fourier, France: Exploring the free-energy landscape of proteins by multidimensional NMR spectroscopy
- P. Neudecker, University of Toronto, Canada: High-resolution structure determination of an amyloidogenic low-populated folding intermediate from NMR relaxation dispersion experiments
- H. Heise, Heinrich Heine University Düsseldorf/Forschungszentrum Jülich, Germany: MAS NMR-spectroscopy for the study of membrane-protein interactions and paramagnetic protein assemblies
- B. Groot, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany: The molecular dynamics of molecular recognition
- M. S. Li, Polish Academy of Science, Poland: Factors governing fibrillogenesis of polypeptide chains
- Irbäck, Lund University, Sweden: All-atom Monte Carlo study of Abeta42 monomers and dimmers
- D. Teplow, David Geffen School of Medicine at UCLA, USA: “What makes Aβ42 ‘Aβ42’?”—Computational and experimental studies of
amyloid β-protein monomer folding and assembly

- F. Chiti, Università di Firenze, Italy: Investigation of the structure of toxic protein oligomers associated with protein deposition diseases
- Clementi, Rice University, USA: A multiscale approach to characterize macromolecular dynamics and functions
- G. Stock, University of Freiburg, Germany: How complex is the dynamics of protein folding?
- J. Heberle, Freie Universität Berlin, Germany: Surface-enhanced infrared absorption spectroscopy (SEIRAS) of membrane proteins
- K. Hauser, University of Konstanz, Germany: Site-specific folding dynamics of peptides studied by IR-spectroscopy combined with different isotopic substitution methods
- E. Pebay-Peyroula, University of Grenoble, France: Mitochondrial carriers: a molecular dissection of the ADP/ATP carrier
- V. Dötsch, Goethe University, Frankfurt am Main Germany: Structural investigation of the C-terminal fragment of presenilin-1
- F. X. Schmid, Universität Bayreuth, Germany: Prolyl isomerization in protein folding and protein function
- Anselmetti, Universität Bielefeld, Germany: Single molecule force mechanics
- P. Carloni, German Research School for Simulation Sciences, Aachen and Jülich, Germany: Molecular simulations of proteins undergoing fibrillation in neurodegenerative diseases
- B. Schuler, University of Zurich, Switzerland: Structure and dynamics of unfolded proteins from single molecule fluorescence spectroscopy
- Y. Okamoto, Nagoya University, Japan: Generalized-ensemble algorithms for multiscale biomolecular simulations
- Lindahl, Royal Institute of Technology Stockholm, Sweden: Combined cluster & distributed computing for quantitative simulation of million-atom systems
- V. Helms, Universität des Saarlandes, Germany: Adhesive water networks facilitate binding of protein interfaces
- Gräter, Molecular Biomechanics, HITS GmbH, Germany: Protein function from force distribution analysis – answers to gene expression and blood coagulation
- P. Varnai, School of Life Sciences, University of Sussex, UK: Switching mechanism of bistable RNA studied by a coupled structure-based potential
- W. Wenzel, Karlsruhe Institute of Technology, Germany: Protein structure prediction and folding with free energy models: From algorithms to treatment options
- C. Dellago, University of Vienna, Austria: Water in narrow pores: How orientational defects and protons move along 1d water wires
- D. Wales, University of Cambridge, UK: Energy Landscapes: From biomolecules to mesoscopic systems
- S. J. Marrink, University of Groningen, Netherlands: Lipid-mediated protein clustering and sorting.
## Publications

**ICS-1/JCNS-1**

### Peer-reviewed Publications

#### 2009

<table>
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<th>Author(s)</th>
<th>Title</th>
<th>Journal/Volume</th>
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<tr>
<td>Kirstein, O.; Prager, M.; Schneider, G. J.</td>
<td>Rotational dynamics and coupling of methyl group rotations in methyl fluoride studied by high resolution inelastic neutron scattering</td>
<td>Journal of Chemical Physics, 130 (2009) 21, 214508</td>
<td>2009</td>
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#### 2010

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<tr>
<td>Brodeck, M.; Alvarez, A.; Moreno, A.J.; Colmenero, J.; Richter, D.</td>
<td>Chain motion in non-engulfed dynamically asymmetric polymer blends: Comparison between atomistic simulations of PEO/PMMA and a...</td>
<td></td>
<td>2010</td>
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Correlation of mass fractal dimension and cluster size of silica in styrene butadiene rubber composites
Schneider, G.J.; Vollnhals, V.; Brandt, K.; Roth, S.V.; Göritz, D.
Journal of Chemical Physics, 133 (2010), 024903

Strain induced anisotropies in silica polydimethylsiloxane composites
d’Errico, G.; Silipo, A.; Mangiapia, G.; Vitiello, W.; Georgi, R.; Su, Y.; Doster, W.
Using polarization analysis to separate the coherent and incoherent scattering from protein samples

Generic bead-spring model

Publications

Characterization of Lipopolysaccharides from Burkholderia Cenocepacia, Burkholderia Multivorans and Agrobacterium tumefaciens: from the Molecular Structure to the Aggregate Architecture
Physical Chemistry Chemical Physics, 12 (2010) 41, 13574 – 13585

Doster, W.; Busch, S.; Gaspar, A.M.; Appavou, M.-S.; Wuttke, J.; Scheer, H.
Dynamical Transition of Protein-Hydration Water
Physical Review Letters, 104 (2010) 9, 098101

Friedrich, T.; Tieke, B.; Meyer, M.; Pyckhout-Hintzen, W.; Pipich, V.
Thermoresponsive copolymer hydrogels based on n-isopropylacrylamide and cationic surfactant monomers prepared from micellar solution and microemulsion in a one-step reaction

Frielingshaus, X.; Brodeck, M.; Holderer, O.; Frielingshaus, H.
Confined Polymer Dynamics on Clay Platelets

Gaspar, A.M.; Busch, S.; Appavou, M.-S.; Haeussler, W.; Georgi, R.; Su, Y.; Doster, W.
Using polarization analysis to separate the coherent and incoherent scattering from protein samples

Biochimica et Biophysica Acta, 1804 (2010) 1, 76 – 82

Gerstl, C.; Schneider, G.J.; Pyckhout-Hintzen, W.; Allgaier, J.; Richter, D.; Huber, P.
Segmental and normal mode relaxation of poly(alkylene oxide) melts studied by dielectric spectroscopy and rheology
Macromolecules, 43 (2010), 4966 – 4977

Generic bead-spring model

Structural and Dynamic Properties of Decorated Lamellar Microemulsions in the Brush Regime
Hegger, E.; Allgaier, J.; Meinke, M.; Stellbrink, J.; Pyckhout-Hintzen, W.; Richter, D.
Synthesis of polymer/silica hybrid nanoparticles using anionic polymerization techniques
Macromolecules, 43 (2010) 2, 856 - 867

Harms, S.; Rützke, K.; Faupel, F.; Schneider, G.J.; Willner, L.; Richter, D.
Free Volume of Interphases in Model Nanocomposites Studied by Positron Annihilation Lifetime Spectroscopy

Heiss, A.; Pipich, V.; Jahnen-Dechent, W.; Schwahn, D.
Fetuin-A is a mineral carrier protein: Some angle neutron scattering provides new insight on Fetuin-A controlled calcification inhibition

Holderer, O.; Frielingshaus, H.; Byelov, D.; Monkenbusch, M.; Allgaier, J.; Richter, D.
Dynamic Properties of Decorated Lamellar Microemulsions in the Brush Regime
Zeitschrift für physikalische Chemie, 224 (2010) 1/2, 243 – 251

Hübner, E.; Allgaier, J.; Meyer, M.; Stellbrink, J.; Pyckhout-Hintzen, W.; Richter, D.
Synthesis of polymer/silica hybrid nanoparticles using anionic polymerization techniques
Biochemistry, 49 (2010) 34, 7332 – 7343

Inoue, R.; Biehl, R.; Rosenkranz, T.; Fletter, J.; Monkenbusch, M.; Radulescu, A.; Farago, B.; Richter, D.
Large Domain Fluctuations on 50-ns Timescale Enable Catalytic Activity in Phosphoglycerate Kinase

Jain, A.; Kulkarni, A.; Bligou Koubma, A.M.; Wang, W.; Busch, P.; Laschewsky, A.; Müller-Buschbaum, P.; Papadakis, C.M.
Micellar Solutions of a Symmetrical Amphiphilic ABA Triblock Copolymer with a Temperature-Responsive Shell
Macromolecular Symposia, 291-292 (2010) 1, 221 - 229

Polymer dynamics in nanochannels of porous silicon: A neutron spin echo study
Macromolecules, 43 (2010) 19, 8162 - 8169


Le Coeur, C.; Teixeira, J.; Busch, P.; Longeville, S.
Compression of random coils due to macromolecular crowding: Scaling effects
Physical Review E, 81 (2010) 6, 061914

Lund, R.; Willner, L.; Stellbrink, J.; Lindner, P.; Richter, D.
Logarithmic chain-exchange kinetics of diblock copolymer micelles

Martin, J.; Krutyeva, M.; Monkenbusch, M.; Arbe, A.; Allgaier, J.; Radulescu, A.; Falus, P.; Maiz, J.; Mijangos, C.; Colmenero, J.; Richter, D.
Direct Observation of confined Single Chain Dynamics by Neutron Scattering
Physical Review Letters, 104 (2010) 19, 197801

Matern, N.; Geuring, T.; Thomas, J.; Goerigk, G.; Franz, H.; Eckert, J.
Phase separation in Ni-Nb-Y metallic glasses

Monkenbusch, M.; Richter, D.; Biehl, R.
Observation of protein domain motions by neutron spectroscopy
Chemical Physics, 11 (2010) 6, 1187 - 1194

Conformations of silica-poly(ethylene-propylene) nanocomposites
Macromolecules, 43 (2010) 23, 8537 - 8547

Papagiannopoulos, A.; Karayianni, M., Mountrichas, G.; Pispas, S.; Radulescu, A.
Self-Assembled Nanoparticles from a Block Polyelectrolyte in Aqueous Media: Structural Characterization by SANS

Microscopic structures and dynamics of high- and low-density liquid trans-1,2-dichloroethylene

Schneider, G.J.; Göritz, D.
Strain induced anisotropies in silica polydienylsloxane composites
Journal of Chemical Physics, 133 (2010), 024903

Schneider, G.J.; Vollnhals, V.; Brandt, K.; Roth, S.V.; Göritz, D.
Correlation of mass fractal dimension and cluster size of silica in styrene butadiene rubber composites

146
Publications


Nusser, K.; Mosbauer, T.; Schneider, G.J.; Brandt, K.; Weidemann, G.; Goebbels, J.; Riesemeier, H.; Göritz, D. Silica Dispersion in Styrene Butadiene Rubber Composites studied by Synchrotron Tomography Journal of Non-Crystalline Solids, 1 (2011) 1, 1


Oberdisse, J.; Genix, A.C.; Cauty, M.; Schneider, G.J.; Pyckhout-Hintzen, W. 3rd European Workshop on Nanocomposites and Polymer Dynamics Applied Rheology, 21 (2011), 299 - 300

Press, W.; Zamponi, M.; Prager, M. Rotational tunneling in CH4 II: Disorder Effects Journal of Chemical Physics, 135 (2011), 224509


Pyckhout-Hintzen, W.; Allgaier, J.; Richter, D. Recent developments in polymer dynamics investigations of architecturally complex systems European Polymer Journal, 47 (2011) 4, 474 - 485


Zorn, R. The boson peak demystified Physics, 4 (2011), 44

Books/Book contributions


2011 148
Publications

Brückel, T.; Heger, G.; Richter, D.; Roth, G.; Zorn, R. (Eds.)
Laboratory Course Neutron Scattering (Experimental Manuals)
Jülich, Forschungszentrum Jülich, Zentralbibliothek, 2011
Schriften des Forschungszentrums Jülich: Reine Schlüsseltechnologien / Key Technologies

Patents


Publications

ICS-2/IAS-2

Peer-reviewed Publications

2009

Auth, T.; Gompper, G.
Budding and vesiculation induced by conical membrane inclusions
Physical Review E, 80 (2009), 031901 [1-10]

Auth, T.; Gov, N. S.
Diffusion in a fluid membrane with a flexible cortical cytoskeleton
Biophysical Journal, 96 (2009), 818 – 830

Baumgaertner, A.
Fast-ion transport in peptide nanochannels
Materials Science and Engineering B, 165 (2009), 261 - 265

Belushkin, M.; Gompper, G.
Twist grain boundaries in cubic surfactant phases
Journal of Chemical Physics, 130 (2009), 134712

Chatterjee, S.; Schütz, G. M.
Diffusion of hydrocarbon mixture in one-dimensional zeolite channel: an exclusion model approach
Microporous and Mesoporous Materials, 125 (2009), 143 - 148

Chelakot, R.; Lipowsky, R.; Gruhn, T.
Self-assembling network and bundle structures in systems of rods and crosslinkers - A Monte Carlo study
Soft Matter, 5 (2009), 1504 - 1513

Cherstvy, A.
Positively Charged Residues in DNA-Binding Domains of Structural Proteins Follow Sequence-specific Positions in DNA Phosphate Groups
Journal of Physical Chemistry B, 113 (2009), 4242 - 4247

Cherstvy, A.
Probing DNA-DNA Electrostatic Friction in Tight Superhelical DNA Piles
Journal of Physical Chemistry B, 113 (2009), 5350 - 5355

Comets, F.; Popov, S.; Schütz, G. M.; Vachkovskaia, M.
Billiards in a General Domain with Random Reflections
Archive for Rational Mechanics and Analysis, 191 (2009), 497 - 537

Dhont, J. K. G.; Gompper, G.; Richter, D.
Julich Soft Matter Days 2008 - Conference Report II
Applied Rheology, 19 (2009), 176 - 178

Eisenriegler, E.
Density profiles around nanoparticles and distant perturbations
Journal of Chemical Physics, 130 (2009), 134902 [1-13]

Elgeti, J.; Gompper, G.
Self-Propelled Rods near Surfaces
Europhysics Letters, 85 (2009), 38002

Fontleitner, J.; Lo Verso, F.; Kahl, G.; Likos, C.N.
Ordering in Two-Dimensional Dipolar Mixtures
Langmuir, 25 (2009), 7836 - 7846

Frank, S.; Winkler, R. G.
Mesoscale hydrodynamic simulation of short polyelectrolytes in electric fields
Journal of Chemical Physics, 131 (2009), 234905

Multi-Particle Collision Dynamics: A Particle-Based Mesoscale Simulation Approach to the Hydrodynamics of Complex Fluids
Advances in Polymer Science, 221 (2009), 1 - 87

Gupta, S.; Mukamel, D.; Schütz, G. M.
The robustness of spontaneous symmetry breaking in a bridge model
Journal of Physics A - Mathematical and Theoretical, 42 (2009), 405002

Haan, M.; Gwan, J.F.; Baumgaertner, A.
Correlated movements of ions and water in a nanochannel
Molecular Simulations, 35 (2009), 13 - 23

Head, D.A.
Critical Scaling and Aging in Cooling Systems Near the Jamming Transition
Physical Review Letters, 102 (2009), 138001

Hirschberg, O.; Mukamel, D.; Schütz, G. M.
Condensation in Temporally Correlated Zero-Range Dynamics
Physical Review Letters, 103 (2009), 090602

Huang, C.C.; Ryckaert, J.-P.; Yu, H.
Structure and dynamics of cylindrical micelles at equilibrium and under shear flow
Physical Review E, 79 (2009), 041501

Junghans, C.; Bachmann, M.; Janke, W.
Statistical Mechanics of Aggregation and Crystallization for Semiflexible Polymers
Europhysics Letters, 87 (2009), 40002

McWhirter, J. L.; Noguchi, H.; Gompper, G.
Flow-induced clustering and alignment of vesicles and red blood cells in microcapillaries
Proceedings of the National Academy of Sciences of the United States of America, 106 (2009), 6039 - 6043

Messinger, S.; Schmidt, B.; Noguchi, H.; Gompper, G.
Dynamical regimes and hydrodynamic lift of viscous vesicles under shear
Physical Review E, 80 (2009), 011901

Noguchi, H.
Swinging and synchronized rotations of red blood cells in simple shear flow
Physical Review E, 80 (2009), 021902

Schnebel, S.; Bachmann, M.; Janke, W.
Elastic Lennard-Jones Polymers Meet Clusters: Differences and Similarities
Journal of Chemical Physics, 131 (2009), 124904

Schütz, G. M.; Pigearde Almeida, Prado; Harris, R.J.; Belitsky, V.
Publications

Short-time behaviour of demand and price viewed through an exactly solvable model for heterogeneous interacting market agents
Physica A, 388 (2009), 4126 - 4144
Tripathi, T.; Schütz, G. M.; Chowdhury, D.

RNA polymerase motors: dwell time distribution, velocity and dynamical phases
Verberck, B.; Vliegenthart, G.A.; Gompper, G.

Orientational ordering in solid C60 fullerene-cubane
Journal of Chemical Physics, 130 (2009), 154510 [1-14]
Vogel, T.; Neuhaus, T.; Bachmann, M.; Janke, W.

Ground-state Properties of Tubelike Flexible Polymers
European Physical Journal E, 30 (2009), 7 - 18
Vogel, T.; Neuhaus, T.; Bachmann, M.; Janke, W.

Thermodynamics of tubelike flexible polymers
Physical Review E, 80 (2009), 011802
Vogel, T.; Neuhaus, T.; Bachmann, M.; Janke, W.

Thicknes-Dependent Secondary Structure Formation of Tubelike Polymers
Europhysics Letters, 85 (2009), 10003 [1-5]
Winkler, R. G.; Huang, C.C.

Stress Tensors of Multiparticle Collision Dynamics Fluids
Journal of Chemical Physics, 130 (2009), 074907
Wysocki, A.; Royall, C.P.; Winkler, R. G.; Gompper, G.; Tanaka, H.; van Blaaderen, A.; Löwen, H.

Direct observation of hydrodynamic instabilities in a driven non-uniform colloidal dispersion
Soft Matter, 5 (2009), 1340 – 1344

2010
Bachmann, M.
Contact-Density Analysis of Lattice Polymer Adsorption Transitions
Bachmann, M.

Statistical Analysis of Structural Transitions in Small Systems
Physica Procedia, 3 (2010), 1387 - 1395

Microscopic Mechanism of Specific Peptide Adhesion to Semiconductor Substrates
Bereau, T.; Bachmann, M.; Deserno, M.

Interplay between Secondary and Tertiary Structure Formation in Protein Folding Cooperativity
Journal of the American Chemical Society, 132 (2010), 13129 - 13131
Burkhardt, T.W.; Yang, Y.; Gompper, G.

Fluctuations of a long, semiflexible polymer in a narrow channel
Physical Review E, 82 (2010), 041801
Chatterjee, S.; Schütz, G. M.

Importance of boundary effects in diffusion of hydrocarbon molecules in a one-dimensional zeolite channel
Chatterjee, S.; Schütz, G.M.

Determinant representation for some transition probabilities in the TASEP with second class particles
Journal of Statistical Physics, 140 (2010), 900 – 916
Chelakkot, R.; Winkler, R. G.; Gompper, G.

Migration of semiflexible polymers in microcapillary flow
Europhysics Letters, 91 (2010), 14001
Cherstvy, A.

Collapse of Highly Charged Polyelectrolytes Triggered by Attractive Dipole-Dipole and Correlation-Induced Electrostatic Interactions
Journal of Physical Chemistry B, 114 (2010), 5241-5249
Comets, F.; Popov, S.; Schütz, G.M.; Vachkovskaia, M.

Knudsen Gas in a Finite Random Tube: Transport Diffusion and First Passage Properties
Journal of Statistical Physics, 140 (2010), 948-984
Comets, F.; Popov, S.; Schütz, G.M.; Vachkovskaia, M.

Quenched invariance principle for the Knudsen stochastic billiard in a random tube
Annals of Probability, 38 (2010), 1019-1061
Ellegi, J.; Kaupp, U. B.; Gompper, G.

Hydrodynamics of Sperm Cells near Surfaces
Biophysical Journal, 99 (2010), 1018 - 1026

Blood flow and cell-free layer in microvessels
Microcirculation, 18 (2010), 615 - 628
Fornleitner, J.; Kahl, G.

Pattern formation in two-dimensional square-shoulder systems
Journal of Physics: Condensed Matter, 22 (2010), 104118
Fornleitner, J.; Kahl, G.; Likos, C.N.

Tailoring the Phonon Band Structure in Binary Colloidal Mixtures
Physical Review E, 81 (2010), 060401
Götzke, I.; Gompper, G.

Mesoscale Simulations of Hydrodynamic Squirmer Interactions
Physical Review E, 82 (2010), 041921
Götzke, I.O.; Gompper, G.

Flow Generation by Rotating Colloids in Planar Microchannels
Europhysics Letters, 92 (2010), 64003
Head, D.A.; Mizuno, D.

Non-local fluctuation correlations in active gels
Physical Review E, 81 (2010), 041910
Head, D.A.; Tanaka, H.

Superdiffusive mass transport as a causal mechanism for large-scale structure formation
Europhysics Letters, 91 (2010), 40008
Huang, C.C.; Chatterji, A.; Sutmann, G.; Gompper, G.; Winkler, R. G.

Cell-level canonical sampling by velocity scaling for multiparticle collision dynamics simulations
Publications

Biophysical Journal, 100 (2011), 2321 - 2324
Fedosov, D.A.; Caswell, B.; Karmiadakis, G.E.
Wall Shear Stress-Based Model for Adhesive Dynamics of Red Blood Cells in Malaria

Biophysical Journal, 100 (2011), 2084 - 2093
Fedosov, D.A.; Caswell, B.; Suresh, S.; Karmiadakis, G.E.
Quantifying the biophysical characteristics of Plasmodium-falciparum-parasitized red blood cells in microcirculation

Fedosov, D.A.; Lei, H.; Caswell, B.; Suresh, S.; Karmiadakis, G.E.
Multiscale Modeling of Red Blood Cell Mechanics and Blood Flow in Malaria
PLoS Computational Biology, 7 (2011), 1002270,
Fedosov, D.A.; Pan, W.; Caswell, B.; Gompper, G.; Karmiadakis, G.E.
Predicting human blood viscosity in silico

Götze, I.O.; Gompper, G.
Dynamic self-assembly and directed flow of rotating colloids in microchannels
Physical Review E, 84 (2011), 031404

Greenall, M.J.; Gompper, G.
Bilayers Connected by Threadlike Micelles in Amphiphilic Mixtures: A Self-Consistent Field Theory Study
Langmuir, 27 (2011), 3416 - 3423

Grisi, R.; Schütz, G.M.
Current symmetries for particle systems with several conservation laws
Journal of Statistical Physics, 143 (2011), 1499 - 1512

Groß, J.; Janke, W.; Bachmann, M.
Massively parallelized replica-exchange simulations of polymers in GPUs
Computer Physics Communications, 182 (2011), 1638 - 1644

Head, D.A.; Briels, W.J.; Gompper, G.
Spindles and active vortices in a model of confined filament-motor mixtures
BMC Biophysics, 4 (2011), 18

Hirschberg, O.; Mukamel, D.; Schütz, G.M.
Approach to equilibrium of diffusion in a logarithmic potential
Physical Review E, 84 (2011), 041111

Huang, C.-C.; Saltaim, G.; Gompper, G.; Winkler, R.G.
Tumbling of polymers in semidilute solution under shear flow
Europhysics Letters, 93 (2011), 54004

Hur, K.; Jeong, C.; Winkler, R.G.; Lasevic, N.; Gee, R.H.; Yoon, D.Y.
Chain Dynamics of Ring and Linear Polyethylene Melts from Molecular Dynamics Simulations
Macromolecules, 44 (2011), 2311 - 2315

Ji, S.; Jiang, R.; Winkler, R.G.; Gompper, G.
Mesoscale hydrodynamic modeling of a colloid in shear-thinning viscoelastic fluids under shear flow
Journal of Chemical Physics, 135 (2011), 134116

Junghans, C.; Janke, W.; Bachmann, M.
Hierarchies in nucleation transitions
Computer Physics Communications, 182 (2011), 1937 - 1940

Karalus, S.; Janke, W.; Bachmann, M.
Thermodynamics of polymer adsorption to a flexible membrane
Physical Review E, 84 (2011), 031803

Kerscher, M.; Busch, P.; Mattauch, S.; Frielingshaus, H.; Richter, D.; Belushkin, M.; Gompper, G.
Near-surface structure of a bicontinuous microemulsion with a transition region
Physical Review E, 83 (2011), R030401

Lei, H.; Fedosov, D.A.; Karmiadakis, G.E.
Time-dependent and outflow boundary conditions for Dissipative Particle Dynamics
Journal of Computational Physics, 230 (2011), 3765 - 3779

McWhirter, J.L.; Noguchi, H.; Gompper, G.
Deformation and clustering of red blood cells in microcapillary flows
Soft Matter, 7 (2011), 10967 – 10977

Möddel, M.; Janke, W.; Bachmann, M.
Adsorption of finite polymers in different thermodynamic ensembles

Murthy, K.P.N.; Schütz, G.M.
Mean bubble formation time in DNA denaturation
Europhysics Letters, 96 (2011), 68003

Pan, W.; Fedosov, D.A.; Caswell, B.; Karmiadakis, G.E.
Predicting dynamics and rheology of blood flow: A comparative study of multiscale and low-dimensional models of red blood cells
Microvascular Research, 82 (2011), 163 - 170

Pigeard de Almeida Prado, F.; Schütz, G. M.
Loss of ergodicity in the transition from quenched to annealed disorder in a finite kinetic Ising model
Journal of Statistical Physics, 142 (2011), 984 - 999

Popkov, V.; Schütz, G.M.
Transition probabilities and dynamic structure factor in the ASEP conditioned on strong flux
Journal of Statistical Physics, 142 (2011), 627 - 639

Popkov, V.; Schütz, G.M.
Large deviation functions in a system of diffusing particles with creation and annihilation
Physical Review E, 84 (2011), 021131

Pavlovskiy, A.M.; Priezzhev, V.B.; Schütz, G. M.
Generalized Green Functions and current correlations in the TASEP
Journal of Statistical Physics, 142 (2011), 754 - 791

Ramachandran, S.; Komura, S. Seki, K.; Gompper, G.
Dynamics of a polymer chain confined in a membrane

Ripoll, M.
Helicopter rotation and smectic-isotropic coexistence of strongly attractive rods
Publications

Physical Review E, 83 (2011), 040701
Schnabel, S.; Janke, W.; Bachmann, M.
Advanced multicanonical Monte Carlo methods for efficient simulations of nucleation processes of polymers
Journal of Computational Physics, 230 (2011), 4454 - 4466
Singh, S.P.; Winkler, R.G.; Gompper, G.
Nonequilibrium Forces between Dragged Ultrasoft Colloids
Physical Review Letters, 107 (2011), 158301
Toyota, T.; Head, D.A.; Schmidt, C.F.; Mizuno, D.
Non-Gaussian athermal fluctuations in active gels
Soft Matter, 7 (2011), 3234 - 3239
Verberck, B.; Cambedouzou, J.; Vliegenthart, G.A.; Gompper, G.; Launois, P.
A Monte Carlo study of C(70) molecular motion in C(70)@SWCNT peapods
Carbon, 49 (2011), 2007 - 2021
Vliegenthart, G.A.; Gompper, G.
Compression, crumpling and collapse of spherical shells and capsules
New Journal of Physics, 13 (2011), 045020
Vogel, T.; Bachmann, M.
Adsorption of polymers at nanowires
Yang, M.; Ripoll, M.
Simulations of thermophoretic nanoswimmers
Physical Review E, 84 (2011), 061401

Books/Book contributions

2009
Bachmann, M.
Statistical Analysis of Structural Transitions in Small Systems
Proceedings of the 22nd Workshop on Recent Developments in Computer Simulation Studies in Condensed Matter Physics, 23 - 27. Feb. 2009. - Athens, Georgia, USA
Chatterjee, S.; Schütz, G. M.
Diffusion in a one-dimensional zeolite channel: an analytical and numerical study

2010
Schütz, G.M.
Asymmetric exclusion process with long-range interaction
Oberwolfach Reports 7 (2010), 2951-2954, Hrsg. G. Landim, S. Olla, H. Spohn
Sutmann, G.; Huang, C.C.; Winkler, R. G.; Gompper, G.
Semidilute Polymer Systems under Shear Flow

2011
Auth, T.
Mesoscopic Membrane Systems
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Dhont, J.K.G.; Gompper, G.
Introduction: Soft and Living Matter
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Fedosov, D.A.
Blood Cells and Blood Flow
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Fedosov, D.A.; Pivkin, I.V.; Pan, W.; Dao, M.; Caswell, B.; Kaniadakis, G.E.
Multiscale modeling of hematologic disorders
Gompper, G.
Microswimmers
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Priezzhev, V.B.; Schütz, G.M.
Bethe ansatz solution in the finite Bernoulli matching model of sequence alignment
Ripoll, M.
Mesoscale Hydrodynamics
Ripoll, M.
Molecular Dynamics
Schütz, G.
Motion of Molecular Motors
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Schütz, G.M.
How stochastic dynamics far from equilibrium can create non-random patterns
Vliegenthart, G.
Viruses
Publications

Winkler, R.G.
Polymers in Flow, Molecular Properties
42nd IFF Springschool 2011, Macromolecular Systems in Soft and Living Matter, organized by the Institute of Complex Systems of the Forschungszentrum Jülich, Schriften des Forschungszentrums Jülich. - 978-3-89336-688-0
Publications

ICS-3

Peer-reviewed Publications

2009
Banachowicz, E.; Kozak, M.; Patkowski, A.; Meier, G.; Kohlbrecher, J.
High-pressure small-angle neutron scattering studies of glucose isomerase conformation in solution
Journal of Applied Crystallography, 42 (2009), 461 - 466

Delgado, J.; Kriegs, H.; Castillo, R.
Flow velocity profiles and shear banding onset in a semidilute wormlike micellar system under couette flow

Gapinski, J.; Patkowski, A.; Banchio, A.J.; Buitenhuis, J.; Holmøvist, P.; Lettinga, M.P.; Meier, G.; Nägele, G.
Structure and Short-Time Dynamics in Suspensions of Charged Silica Spheres in the Entire Fluid Regime
Journal of Chemical Physics, 130 (2009), 084503

Gögelein, C.; Nägele, G.; Buitenhuis, J.; Turnier, R.; Dhont, J.K.G.
Polymer depletion-driven cluster aggregation and initial phase separation in charged nanosized colloids
Journal of Chemical Physics, 130 (2009) 20, 204905

Heinen, M.; Kull, H.-J.
Radiation boundary conditions for the numerical solution of the three-dimensional time-dependent Schrödinger equation with a localized interaction

Holmøvist, P.; Ratajczyk, M.; Meier, G.; Wensink, H.H.; Lettinga, M.P.
Supersaturated dispersions of rodlike viruses with added attraction
Physical Review E, 80 (2009) 3, 031402

Kang, K.; Dhont, J.K.G.
Criticality in a non-equilibrium, driven system: Charged colloidal rods (fd-viruses) in electric fields

Lettinga, M.P.; Manneville, S.
Competition between shear banding and wall slip in wormlike micelles
Physical Review Letters, 103 (2009) 24, 248302

Nygaard, K.; Satapathy, D.K.; Buitenhuis, J.; Perret, E.; Bunk, O.; David, C.; van der Veen, J.F.
Confinement-induced orientational alignment of quasi-2D fluids
EuroPhysics Letters, epl, 86 (2009), 66001

Nygaard, K.; Satapathy, D.K.; Bunk, O.; Perret, E.; Buitenhuis, J.; David, C.; van der Veen, J.F.
Grating-based holographic X-ray diffraction: theory and application to confined fluids
Journal of Applied Crystallography, 42 (2009) 6, 1129 - 1138

Polyakov, P.; Rossinsky, E.; Wiegand, S.
Study of the Soret Effect in Hydrocarbon Chain/Aromatic Compound Mixtures
Journal of Physical Chemistry B, 113 (2009) 40, 13308 - 13312

Polyakov, P.; Wiegand, S.
Investigation of the soret effect in aqueous and non-aqueous mixtures by the thermal lens technique
Physical Chemistry Chemical Physics, 11 (2009), 864 - 871

Vavrin, R.; Kohlbrecher, J.; Wilk, A.; Ratajczyk, M.; Lettinga, M.P.; Buitenhuis, J.; Meier, G.
Structure and phase diagram of an adhesive colloidal dispersion under high pressure: A small angle neutron scattering, diffusing wave spectroscopy, and light scattering study
Journal of Chemical Physics, 130 (2009), 154903

Zhang, Z.; Krishna, N.; Lettinga, M.P.; Vermant, J.; Grelet, E.
Reversible gelation of rod-like viruses grafted with thermoresponsive polymers

2010
Abade, G.C.; Cichocki, B.; Ekiel-Jezewska, M.; Nägele, G.; Wajnryb, E.
Short-time dynamics of permeable particles in concentrated suspensions
Journal of Chemical Physics, 132 (2010) 1, 014503

Abade, G.C.; Cichocki, B.; Ekiel-Jezewska, M.L.; Nägele, G.; Wajnryb, E.
Dynamics of permeable particles in concentrated suspensions
Physical Review E, 81 (2010) 2, 020404

Abade, G.C.; Cichocki, B.; Ekiel-Jezewska, M.L.; Nägele, G.; Wajnryb, E.
High-frequency viscosity and generalized Stokes-Einstein relations in dense suspensions of porous particles
IOP Select article

Abade, G.C.; Cichocki, B.; Ekiel-Jezewska, M.L.; Nägele, G.; Wajnryb, E.
High-frequency viscosity of concentrated porous particles suspensions
Journal of Chemical Physics, 133 (2010), 084906

Artl, B.; Datta, S.; Softmann, T.; Wiegand, S.
Soret Effect of n-Octyl beta-D-Glucopyranoside (C8G1) in Water around the Critical Micelle Concentration
Blanco, P.; Kriegs, H.; Artl, B.; Wiegand, S.
Thermal Diffusion of Oligosaccharide Solutions: The Role of Chain Length and Structure

Blanco, P.; Wiegand, S.
Study of the Soret Effect in Monosaccharide Solutions
Journal of Physical Chemistry B, 114 (2010) 8, 2807 - 2813

Cichocki, B.; Wajnryb, E.; Blawzdziewicz, J.; Dhont, J.K.G.; Lang, P. R.
The intensity correlation function in evanescent wave scattering
Journal of Chemical Physics, 132 (2010) 7, 074704

Dhont, J.K.G.; Kang, K.
Electric-field-induced polarization and interactions of uncharged colloids in salt solutions
European Physical Journal E, 33 (2010) 1, 51 - 68

Dhont, J.K.G.; Kang, K.; Lettinga, M.P.; Briels, W.J.
Shear-banding instabilities
Gapinski, J.; Patkowski, A.; Nägele, G.
Generic behavior of the hydrodynamic function of charged colloidal suspensions

Heinen, M.; Holmqvist, P.; Banchio, A.J.; Nägele, G.
Short-time diffusion of charge-stabilized colloidal particles: generic features
Journal of Applied Crystallography, 43 (2010), 970 - 980

Heinen, M.; Kull, H.-J.
Numerical calculation of strong-field laser-atom interaction: An approach with perfect reflection-free radiation boundary conditions

Holmqvist, P.; Nägele, G.
Long-Time Dynamics of Concentrated Charge-Stabilized Colloids

July, C.; Lang, P.R.
Depletion interactions effected by different variants of fd virus

Kang, K.
Diffusivity in an electric-field-induced homeotropic phase of charged colloidal rods
EPL: A Letters Journal Exploring the Frontiers of Physics, 92 (2010) 1, 18002

Kang, K.
Mesoscopic relaxation time of dynamic image correlation spectroscopy
Journal of Biomedical Science and Engineering, 3 (2010) 625 - 632

Kang, K.; Dhoht, J.K.G.
Charged fibrous viruses (fd) in external electric fields: dynamics and orientational order
New Journal of Physics, 12 (2010) 063017

Kang, K.; Dhoht, J.K.G.
Electric-field induced transitions in suspensions of charged colloidal rods
Soft Matter, 6 (2010), 273 - 286

Kishikawa, Y.; Wiegand, S.; Kita, R.

Lettinga, M. P.; Dhoht, J.K.G.; Zhang, Z.; Messlinger, S.; Gompper, G.
Hydrodynamic interactions in rod suspensions with orientational ordering
Soft Matter, 6 (2010) 18, 4556 - 4562

Stiakakis, E.; Wilk, A.; Kohlbrecher, J.; Vlassopoulos, D.; Petekidis, G.
Slow dynamics, aging, and crystallization of multiarm star glasses

Vad, T.; Sager, W.F.C.; Zhang, J.; Buitenhuis, J.; Radulescu, A.
Experimental determination of resolution function parameters from small-angle neutron scattering data of a colloidal SiO2 dispersion

Osmotic shrinkage in star-linear polymer mixtures

Zhang, Z.; Buitenhuis, J.; Cukkemane, A.; Brocker, M.; Bott, M.; Dhoht, J.K.G.
Charge reversal of the rodlike colloidal fd virus through surface chemical modification

2011

Abade, G.C.; Cichocki, B.; Ekiel-Jezewska, M.L.; Nägele, G.; Wajnryb, E.
Rotational and translational self-diffusion in concentrated suspensions of permeable particles
Journal of Chemical Physics, 134 (2011) 24, 244903

Abade, G.C.; Cichocki, M.L.; Ekiel-Jezewska, M.L.; Nägele, G.; Wajnryb, E.
First-order viral expansion of short-time diffusion and sedimentation coefficients of permeable particle suspensions
Physics of Fluids, 23 (2011) 8, 083303

Ballesta, P.; Lettinga, M.P.; Manneville, S.
Interplay between a hydrodynamic instability and a phase transition: the Faraday instability in dispersions of rodlike colloids
Soft Matter, 7 (2011) 24, 11440 - 11446

Blanco, P.; Kriegs, H.; Lettinga, M.P.; Holmqvist, P.; Wiegand, S.
Thermal Diffusion of a Stiff Rod-Like Mutant Y21M fd-Virus
Biomacromolecules, 12 (2011), 1602 - 1609

Briels, W.J.; Vlassopoulos, D.; Kang, K.; Dhoht, J.K.G.
Constitutive equations for the flow behavior of entangled polymeric systems: Application to star polymers
Journal of Chemical Physics, 134 (2011) 12, 124901

Dhoht, J.K.G.; Kang, K.
Electric-field-induced polarization of the layer of condensed ions on cylindrical colloids
European Physical Journal E, 34 (2011) 4, 40

Heinen, M.; Banchio, A.J.; Nägele, G.
Short-time rheology and diffusion in suspensions of Yukawa-type colloidal particles
Journal of Chemical Physics, 135 (2011) 15, 154504

Heinen, M.; Holmqvist, P.; Banchio, A.J.; Nägele, G.
Pair structure of the hard-sphere Yukawa fluid: An improved analytic method versus simulations, Rogers-Young scheme, and experiment
Journal of Chemical Physics, 134 (2011) 4, 044532

Heinen, M.; Holmqvist, P.; Banchio, A.J.; Nägele, G.
Pair structure of the hard-sphere Yukawa fluid: An improved analytic method versus simulations, Rogers-Young scheme, and experiment
Journal of Chemical Physics, 134 (2011) 12, 129901

July, C.; Kleshchanok, D.; Lang, P.R.
Depletion interactions caused by polydisperse platelets
Soft Matter, 7 (2011) 14, 6444 - 6450

Kang, K.
Image time-correlation, dynamic light scattering, and birefringence for the study of the response of anisometric colloids to external fields
Review of Scientific Instruments, 82 (2011) 5, 053903

Klein, M.; Wiegand, S.
The Soret effect of mono-, di- and tri-glycols in ethanol
Physical Chemistry Chemical Physics, 13 (2011) 13, 7090 - 7094
Publications

Lonetti, B.; Tsigkri, A.; Lang, P.R.; Stellbrink, J.; Willner, L.; Kohlbrecher, J.; Lettinga, M.P.
Full Characterization of PB-PEO Wormlike Micelles at Varying Solvent Selectivity
Macromolecules, 44 (2011) 9, 3583 - 3593

Pouget, E.; Grelet, E.; Lettinga, M.P.
Dynamics in the smectic phase of stiff viral rods
Physical Review E, 84 (2011) 4, 041704

Reddy, N.K.; Pérez-Juste, J.; Pastoriza-Santos, I.; Lang, P.R.; Dhont, J.K.G.; Liz-Marzan, L.; Vermant, J.
Flow dichroism as a reliable method to measure the hydrodynamic aspect ratio of gold nanoparticles
ACS Nano, 5 (2011) 6, 4935 - 4944

Probing glassy states in binary mixtures of soft interpenetrable colloids

Vad, T.; Sager, W.F.C.
Comparison of iterative desmearing procedures for one-dimensional small-angle scattering data
Journal of Applied Crystallography, 44 (2011) 1, 32 - 42

Viscosity and diffusion: crowding and salt effects in protein solutions
Soft Matter, accepted for publication; to appear in 2012.

Kleshchanok, D.; Heinen, M.; Nägeli, G.; Holmqvist, P.
Dynamics of charged gibbsite platelets in the isotropic phase
Soft Matter, accepted for publication; to appear in 2012.


Reviews
Dhont, J.K.G.; Kang, K.; Lettinga, M.P.; Briels, W.J.
Shear-banding instabilities

Patents
Druckzelle zur optischen Beobachtung unter Druck
Meier, Gerhard, Lettinga, Pavlik
Patentnr.: 102005061984, März 2011

Verfahren zur Ermittlung der Geschwindigkeit von in einer strömenden Lösung despersierten Partikeln
Lang, Peter, Loppinet, Benoit
Patentnr.: 102008023679, November 2008

Verfahren zur Ermittlung der Geschwindigkeit von in einer strömenden Lösung despersierten Partikeln
Europäische Patentanmeldung
Lang, Peter, Loppinet, Benoit
Patentnr.: 10 162 191.0, August 2010
Publications

ICS-4

Peer-reviewed Publications

2009

An atypical CNG channel activated by a single molecule controls sperm chemotaxis  
Sciencesignaling 2 (2009) ra68

Kanyshkova, T.; Pawlowski, M.; Meuth, P.; Dube, C.; Bender, R. A.; Brewster, A. L.; Baumann, A.; Baram, T. Z.; Pape, H.-C.; Budde, T.  
Postnatal expression pattern of HCN channel isoforms in thalamic neurons: Relationship to maturation of thalamocortical oscillations  
Journal of Neuroscience, 29 (2009), 8857 - 8847

Caged progesterone: A new tool for studying rapid nongenomic actions of progesterone  
Journal of the American Chemical Society, 131 (2009), 4027 - 4030

Kimmreck, D.; Daiber, P.C.; Bruähl, A.; Baumann, A.; Moehrlen, F.; Frings, S.  
Bestrophin 2: An anion channel associated with neurogenesis in chemosensory systems  
Journal of Comparative Neurology, 515 (2009) 5, 585 - 599

Rotte, C.; Krach, C.; Balfanz, S.; Baumann, A.; Blenau, W.  
Molecular characterization and localization of the first tyramine receptor of the American cockroach (Periplaneta americana)  
Neuroscience, 162 (2009), 1120 - 1133

Solution structure of the Mesorhizobium loti K1 channel cyclic nucleotide-binding domain in complex with cAMP  
EMBO Reports, 10 (2009) 7, 729 - 735

Wässle, H.; Puller, C.; Müller, F.; Haverkamp, S.  
Cone contacts, mosaics, and territories of bipolar cells in the mouse retina  
Journal of Neuroscience, 29 (2009) 1, 106 – 117

2010

Fried, H.-U.; Kaupp, U. B.; Müller, F.  
Hyperpolarization-activated and cyclic nucleotide-gated channels are differentially expressed in juxtaglomerular cells in the olfactory bulb of mice  
Cell and Tissue Research, 339 (2010) 3, 463 - 479

Fuss, N.; Mujačić, S.; Wachtten, S.; Erber, J.; Baumann, A.  
Biochemical properties of heterologously expressed and native adenylyl cyclases from the honeybee brain (Apis mellifera L.)  
Insect Biochemistry and Molecular Biology, 40 (2010) 8, 573 - 580

Mobley, A.S.; Miller, A.M.; Araneda, R.C.; Maurer, L.R.; Müller, F.; Greer, C.A.  
Hyperpolarization-activated cyclic nucleotide-gated channels in olfactory sensory neurons regulate axon extension and glomerular formation  
Journal of Neuroscience, 30 (2010) 49, 16498 - 16508

Schröper, F.; Baumann, A.; Offenhäusser, A.; Mayer, D.  
Bidirectional immobilization of affinity-tagged cytochrome c on electrode surfaces  
Chemical Communications, 46 (2010), 5295 - 5297

Sommerhage, F.; Baumann, A.; Wroble, G.; Ingebrandt, S.; Offenhäusser, A.  
Extracellular recording of glycine receptor chloride channel activity as a prototype for biohybrid sensors  
Biosensors & Bioelectroctics, 26 (2010) 1, 155 - 161

Thamm, M.; Balfanz, S.; Scheiner, R.; Baumann, A.; Blenau, W.  
Characterization of the 5-HT1A receptor of the honeybee (Apis mellifera) and involvement of serotonin in phototactic behavior  
Cellular and Molecular Life Sciences, 67 (2010) , 2467 - 2479

Troppmann, B.; Balfanz, S.; Baumann, A.; Blenau, W.  
Inverse agonist and neutral antagonist actions of synthetic compounds at an insect 5-HT1 receptor  

2011

Blenau, W.; Rademacher, E.; Baumann, A.  
Plant essential oils and formamidines as insecticides/acaricides: what are the molecular targets?  
Apidologie, Published Online: 17. November 2011 Doi: 10.1007/s13592-011-0108-7

High glucose enhances thrombin responses via protease-activated receptor-4 in human vascular smooth muscle cells  
Arteriosclerosis, Thrombosis, and Vascular Biology, 31 (2011) 3, 624 - 633

Hoff, M.; Balfanz, S.; Ehling, P.; Gensch, T.; Baumann, A.  
A single amino acid residue controls Ca2+ signaling by an octopamine receptor from Drosophila melanogaster  

Seeleger, M.W.; Bromback, A.; Weier, R.; Humphries; P.; Knop, G.; Tanimoto, N.; Müller, F.  
Modulation of rod photoreceptor output by HCN1 channels is essential for regular mesopic cone vision  
Nature Communications – online published 8.11.11 DOI: 10.1038/ncomms1540

The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm  

Books/Book contributions

2009

Baumann, A.; Blenau, W.; Erber, J.  
Biogenic Amines  

Gensch, T.  
Voltage sensitive dyes used for FLIM studied by two-photon-excitation  

Müller, F.; Kaupp, U.B.
Publications

Nervensystem

2010
Fringes, S.; Müller, F.
Auditorisches System, Stimme und Sprache
Fringes, S.; Müller, F.
Gustatorisches und olfaktorisches System
Fringes, S.; Müller, F.
Vestibuläres System
Fringes, S.; Müller, F.
Visuelles System - Auge und Sehen

2011
Baumann, A.
Cell Biology
Gensch, T.; Kaschuba, D.
Fluorescent genetically encoded calcium indicators and their in vivo application
Gensch, T.; Wirth, M.
Determination of calcium concentrations in cells and tissue with fluorescence lifetime imaging (FLIM)
Proc. SPIE 7903, 790322 (2011); doi:10.1117/12.974507
Fluorescence lifetime of fluorescent proteins

Patents
Adenylat-Zyklase, für die Adenylat-Zyklase kodierende Gensequenz, Vektoren und Zellen, sowie deren Verwendung
Baumann, A.; Wachten, S.
Europäisches Patent 1971683, PT 1.2259 PCT/EP
2009

Journal of the American Chemical Society, 131 (2009) 47, 17194 - 17205

Hemoglobin senses body temperature.
European Biophysics Journal : with Biophysics Letters, 38 (2009) 9, 589 - 600

Balem, F.; Yanamala, N.; Klein-Seetharaman, J.
Additive Effects of Chlorin E6 and Metal Ion Binding on the Thermal Stability of Rhodopsin in Vitro.
Photochemistry and Photobiology, 85 (2009), 2, 471 - 478

Heterolytic reduction of fatty acid hydroperoxides by cytochrome c/cardiolipin complexes: antioxidant function in mitochondria.
Journal of the American Chemical Society, 131 (2009) 32, 11288 - 11299

Block, H.; Kubicek, J.; Labahn, J.; Roth, U.; Schauer, F.
Production and comprehensive quality control of recombinant human interleukin-1 beta: A case study for a process development strategy.
Protein Expression and Purification, 57 (2009) 2, 244 - 254

Block, H.; Maertens, B.; Spirestersbach, A.; Brinker, N.; Kubicek, J.; Fabis, R.; Labahn, J.; Schäffer, F.
Immobiled-metal affinity chromatography (IMAC) - a review.

Fabiani, E.; Stadler, A.M.; Madern, D.; Hirai, M.; Zaccai, G.
Dynamics of apo-myoglobin in the alpha-to-beta transition and of partially unfolded aggregated protein.
European Biophysics Journal : with Biophysics Letters, 38 (2009) 2, 237 - 244

From Shell to Cell: Neutron Scattering Studies of Biological Water Dynamics and Coupling to Activity.
Faraday Discussions, 141 (2009), 171-180

Unravel Twins: Comparison of two enantiocomplementary hydroyxynitrile lases with a/s-hydrolase fold.

Mitochondrial targeting of electron scavenging antioxidants: Regulation of selective oxidation vs random chain reactions.
Advanced Drug Delivery Reviews, 61 (2009) 14, 1375 - 1385

Fast biosynthesis of GFP molecules - a single molecule fluorescence study.

Kriegsmann, J.; Brehs, M.; Klare, J.P.; Engelhard, M.; Fitter, J.
Structural rhodopsin/Transducer complex formation in detergent and in lipid blayers studied with FRET.
BBA - Biomembranes, 1788 (2009) 2, 522 - 531

Kriegsmann, J.; Gregor, I.; von der Hocht, I.; Klare, J.; Engelhard, M.; Enderlein, J.; Fitter, J.
Translational Diffusion and Interaction of a Phototransducer and Its Cognate Transducer Observed in Giant Unilamellar Vesicles by Using Dual-Focus FCS.

Moiseeva, E.S.; Reshetnyak, A.B.; Borshchevskiy, V.I.; Baeken, C.; Bült, G.; Gordielj, V. I.
Comparative Analysis of Quality of Membrane Bacteriothorodopsin Crystals Obtained in Oxyglucoside and Ocythylglucoside.
Journal of Surface Investigation : X-ray, Synchrotron and Neutron Techniques, 3 (2009) 1, 29 - 32

Moiseeva, E.S.; Reshetnyak, A.B.; Borshchevskiy, V.I.; Baeken, C.; Bült, G.; Gordielj, V. I.
Comparative analysis of the quality of membrane protein bacteriothorodopsin crystals during crystallization in oxyglucoside and ocythylglucoside.
Journal of Surface Investigation : X-ray, Synchrotron and Neutron Techniques, 3 (2009) 1, 29 - 32

Journal of the American Chemical Society, 131 (2009) 47, 17194 - 17205

Rosenkranz, T.; Katranidis, A.; Atta, D.; Enderlein, J.; Gregor, I.; Grzelakowski, M.; Gregor, I.; Meier, W.; Fitter, J.
Obseving proteins as single molecules encapsulated in surface-tethered Polymeric Nanocontainers.

Tanizawa, H.; Tanizawa, M.; Ghimire, G.D.; Mitaku, S.
Prediction of fragile points of coiled coils.
Chem-bio Informatics Journal, 9 (2009), 12 – 29

Thielmann, Y.; Wiegergraber, O.H.; Ma, P.; Schwarten, M.; Mohrlüder, J.; Willbold, D.
Comparative modeling of human NSF reveals a possible binding mode of GABARAP and GATE-16.
Proteins: Structure, function and Bioinformatics, 77 (2009) 3, 637 - 646

Thielmann, Y.; Wiegergraber, O.H.; Mohrlüder, J.; Willbold, D.
Structural characterization of GABARAP-ligand interactions.
Molecular BioSystems, 5 (2009), 575 - 579

Thielmann, Y.; Wiegergraber, O.H.; Mohrlüder, J.; Willbold, D.
Structural framework of the GABARAP-calreticulin interface - implications for substrate binding to endoplasmic reticulum chaperones.
FEBS Journal, 276 (2009) 4, 1140 - 1152

Tirupula, K.C.; Balem, F.; Yanamala, N.; Klein-Seetharaman, J.
PH-dependent Interaction of Rhodopsin with Cyanidin-3-glucoside. 2. Functional Aspects.
Photochemistry and Photobiology, 85 (2009), 2, 463 - 470

Yanamala, N.; Tirupula, K.C.; Balem, F.; Klein-Seetharaman, J.
PH-dependent Interaction of Rhodopsin with Cyanidin-3-glucoside. 1. Structural Aspects.
Photochemistry and Photobiology, 85 (2009), 2, 454 - 462
Publications

Borshchevskiy, V.I.; Round, E.S.; Popov, A.N.; Budt, G; Gordeliy, V.I.
X-ray-radiation-induced changes in bacteriorhodopsin structure
Journal of Molecular Biology, 409 (2011) 4, 813-825

Dimitrova, N.; Klein-Seetharaman, J.; Beyerlein, P.; Tewfik, A.H.
Signal processing in genomics and proteomics: defining its role
IEEE Signal Processing Magazine, 29 (2011) 1, 19 - 21

Discovering Pathways by Orienting Edges in Protein Interaction Networks

Gushchin, I.; Reshehnyak, A.; Borshchevskiy, V.; Ishchenko, A.; Round, E.; Grudinin, G.; Engelhard, M.; Bueldt, G; Gordeliy, V.
Active State of Sensory Rhodopsin II: Structural Determinants for Signal Transfer and Proton Pumping
Journal of Molecular Biology 4 (2011) 574

Gushchin, I.Y.; Gordeliy, V.I.; Grudinin, S.
Role of the HAMP domain region of sensory rhodopsin transducers in signal transduction
Biochemistry, 50 (2011) 4, 574-580

Topography of tyrosin residues and their involvement in peroxidation of polyunsaturated cardiolipin in cytochrome C cardiolipin peroxidase complexes
Biochim Biophys Acta, 1808 (2011) 9, 2147-2155

Force measurements of the disruption of the nascent polypeptide chain from the ribosome by optical tweezers

Conformational dynamics of helix 8 in the GPCR rhodopsin controls arrestin activation in the desensitization process

The enzymatic oxidation of graphene oxide
ACS Nano 5 (2011) 3, 2098-2108

Do the size effects exist?

Analysis of neutron spectra and fluxes obtained with cold and thermal moderators at IBR-2 reactor: Experimental and computer-modeling studies
Physics of Particles and Nuclei Letters, 8 (2011) 2, 119-128

New opportunities provided by modernized small-angle neutron scattering two-detector system instrument (YuMO)

Kumari, A.; Rosenkranz, T.; Kayastha, A.M.
Structural stability of soybean (Glycine max) α-amylase: Properties of the unfolding transition studied with fluorescence and CD spectroscopy
Protein and Peptide Letter 18 (2011) , 253-260

Murugova, T.N.; Solodovnikova, I.M.; Yurkov, V.I.; Gordeliy, V.I.; Kuklin, A.I.; Ivanov, O.I.; Kovalyv, Yu.S.; Popov, V.I.; Teplova, V.V.; Yagudinsky, L.S.
Potentials of small-angle neutron scattering for studies of the Structure of "Live" Mitochondria
Neutron News, 22 (2011) , 11-14

Rosenkranz, T.; Schlesinger, R.; Gabba, M.; Filter, J.
Native and unfolded states of phosphoglycerate kinase studied by single molecule FRET
ChemPhysChem. 12 (2011) 3, 740-710

X-ray scattering and volumetric P-V-T studies of the dimyristoylphosphatidylcholine-water system
Journal of Surface Investigation: X-ray, Synchrotron and Neutron Techniques 5 (2011) 1, 7-10

Stadler, A.M.; van Eijck, L.; Demmel, F.; Artmann, G.
Macromolecular dynamics in red blood cells investigated using neutron spectroscopy
Journal of the Royal Society Interface 8 (2011), S50-S60

Uguroglu, S.; Tastan, O.; Klein-Seetharaman, J.; Leuba, S.H.
Editorial: Identification of Potentially Relevant Citeable Articles using Association Rule Mining
Medicinal Chemistry, 1 (2011), 101

Zhao, Z.; Xia, J.; Tastan, O.; Singh, I.; Kehrirasarag, M.; Carbonell, J.; Klein-Seetharaman, J.
Virus interactions with human signal transduction pathways
International Journal of Computational Biology and Drug Design 4 (2011) 1, 83-105

Reviews

2009
Fitter, J.
The perspective of studying multi-domain protein folding
Cellular and Molecular Life Sciences, 66 (2009), 1672 – 1681

2010
Stadler, A.M.
Dynamics in Biological Systems as seen by QENS
Zeitschrift für physikalische Chemie, 224 (2010), 201 - 214

2011
Fitter, J.; Katranidis, A.; Rosenkranz, T.; Atta, D.; Schlesinger, R.; Bültdt, G.
Single molecule fluorescence spectroscopy: A tool for protein studies approaching cellular environmental conditions
SoftMatter 7 (2011), 1294-1295

Books/Book contributions
Publications

2009
Gordeliy, V. I.; Moiseeva, E.A.
In meso Approaches to Membrane Protein Crystallization

2010
Deniaud, A.; Moiseeva, E.; Gordeliy, V. I.; Pebay-Peyroula, E.
Crystallography of Membrane Proteins: From Crystalization to Structure

Jasnin, J.; Stadler, A.M.; Zaccai, G.
Water in Membranes, Water in Cells: Neutrons Reveal Dynamics and Interactions

2011
Fitter, J.
Lecture Notes of the 42nd IFF Spring School “Macromolecules in Soft and Living Matter”
Chapter: Protein Folding, B.8.1-B8.15, Forschungszentrum Jülich, 2011

The status and perspectives of small-angle neutron scattering YUMO spectrometer
Book of Abstracts of SANS-YUMO USER MEETING at the start-up of scientific experiments on IBR-2M devoted to the 75-th anniversary of Yu.M. Ostanovich Birth, Dubna, 2011

Kuklin, A.I.; Rogov, A.D.; Erhan, R.V.; Manoshin, S.; Ivankov, O.I.; Gordeliy, V.I.
New configuration of YuMO spectrometer and moderator at modernized IBR-2 reactor
Book of Abstracts of SANS-YUMO USER MEETING at the start-up of scientific experiments on IBR-2M devoted to the 75-th anniversary of Yu.M. Ostanovich Birth, Dubna, 2011

inz, U.
Physical and Biological Rationale for Using Ions in Therapy
Ion Beam Therapy, Biological and Medical Physics, Biomedical Engineering, Springer Heidelberg, Berlin, 2011

Patents

Kristallisation von Membranproteinen in kubischer Lipidphase
Kubicek,J., Labahn,J., Buldf, G., Schafer,F.; Europäische Patentanmeldung 08017180.4 mit der Fa. Qiagen
2009

Demeler, B.; Brookes, E.; Nagel-Steger, L.
Analysis of heterogeneity in molecular weight and shape by analytical ultracentrifugation using parallel distributed computing.

Helix formation in Arrestin accompanies recognition of photoactivated Rhodopsin.
Biochemistry, 40, 10733-42 (2009)

Integral membrane proteins in nanodiscs can be studied by solution NMR spectroscopy.
Journal of the American Chemical Society, 131 (2009) 34, 12060 - 12061

Görz, P.; Opatz, J.; Siebler, M.; Funke, S. A.; Willbold, D.; Lange-Asschenfeldt, C.
Transient reduction of spontaneous neuronal network activity by sublethal amyloid S (1-42) peptide concentrations.

Oligomer assembly of the C-terminal DISC1 domain (640-854) is controlled by self-association motifs and disease-associated polymorphism 5704C.
Biochemistry, 48, 7746-7755 (2009)

Pyroglutamate formation influences solubility and amyloidogenicity of amyloid peptides. A driving force in different neurodegenerative disorders?
Biochemistry, 48 (2009) 29, 7072 - 7078

Solution structure of the Mesorhizobium loti K1 channel cyclic nucleotide-binding domain in complex with cAMP.
EMBO Reports, 10 (2009) 7, 729 - 735

NIX directly binds to GABARAP: A possible crosstalk between apoptosis and autophagy.
Autophagy, 5 (2009) 5, 690 -696

Schwarten, M.; Stoldt, M.; Mohrüder, J.; Willbold, D.
Sequence-specific 1H, 13C and 15N resonance assignment of the autophagy-related protein Atg 8.
Biomolecular NMR Assignments, 3 (2009), 137 - 139

Thielmann, Y.; Weiéggräber, O.H.; Ma, P.; Schwarten, M.; Mohrüder, J.; Willbold, D.
Comparative modeling of the human NSF reveals a possible binding mode of GABARAP and GATE-16.
Proteins - Structure Function and Bioinformatics, 77 (2009) 3, 637 - 546

Thielmann, Y.; Weiéggräber, O.H.; Mohrüder, J.; Willbold, D.
Structural characterization of GABARAP-ligand interactions.
Molecular BioSystems, 5 (2009), 575 - 579

Thielmann, Y.; Weiéggräber, O.H.; Mohrüder, J.; Willbold, D.
Structural framework of the GABARAP–calcineurin interface – implications for substrate binding to endoplasmic reticulum chaperones.
FEBS Journal, 276 (2009) 4, 1140 - 1152

van Groen, T.; Kadish, I.; Wiesehan, K.; Funke, S. A.; Willbold, D.
In vitro and in vivo staining characteristics of small, fluorescent, Aβ42 binding D-enantiomeric peptides in transgenic AD mouse models.
ChemMedChem, 4 (2009) 2, 276 - 282

Wittlich, M.; Koenig, B. W.; Stoldt, M.; Schmidt, H.; Willbold, D.
NMR structural characterization of HIV-1 virus protein U cytoplasmic domain in the presence of dodecylophosphatidylcholine micelles.
FEBS Journal, 276 (2009) 22, 6560 - 6575

2010

Differently Selected D-Enantiomeric Peptides Act on Different A beta Species.
Rejuvenation Research, 13 (2010) 2-3, 202 - 205

Batra-Safferling, R.; Granzin, J.; Moedder, S.; Hoffmann, S.; Willbold, D.
Structural studies of PISK SH3 domain in complex with a peptide ligand: role of anchor residue in ligand binding.
Biological Chemistry, 391 (2010) 1, 33 - 42

Bauer, M. S.; Strodel, B.; Fejer, S. N.; Koslover, E. F.; Wales, D. J.
Interpolation Schemes for Peptide Rearrangements.

Helix Formation in Arrestin Accompanies Recognition of Photoactivated Rhodopsin.
Biochemistry, 48 (2009) 45, 10733-10742

Oral Treatment with the D-Enantiomeric Peptide D3 Improves Pathology and Behavior of Alzheimer Disease Transgenic Mice.
ACS Chemical Neuroscience, 1 (2010) 9, 639 - 648
[Highlighted in: Chemical & Engineering News, 88(33), August 16, 2010]

Funke, S. A.; Wang, L.; Birkmann, E.; Willbold, D.
Rejuvenation Research, 13 (2010) 2/3, 206 - 209

Galkin, V.E.; Orfova, A.; Schröder, G.F.; Egelmann, E.H.
Structural polymorphism in F-actin.
Nature Structural and Molecular Biology, 17 (2010) 11, 1318 - 1323

Glück, J.M.; Hoffmann, S.; Koenig, B. W.; Willbold, D.
Single vector system of efficient N-myristoylation of recombinant proteins in E.coli.
PLOS One, 9 (2010), e10081
Books/Book contributions

Analytical Biochemistry, 408 (2011) 1, 46 – 52
Sequence-independent control of peptide conformation in liposomal vaccines for targeting protein misfolding diseases

Rational Design of G-ßSheet Ligands Against Aß(42)-Induced Toxicity
Journal of the American Chemical Society, 133 (2011) 12, 4348 – 4358

Jänicke, E.; Büchler, K.; Decke, H.; Markl, J.; Barends, T.; Schröder, G.F.
The Refined Structure of Functional Unit II of Keyhole Limpet
Hemocyanin (KLH-I) Reveals Disulphide Bridges
IUBMB Life, 63 (2011) 3, 183 - 187

Nuclear Medicine and Biology, in press (2011)

Conformational dynamics of helix 8 in the GPCR rhodopsin controls arrestin activation in the desensitization process

Klein, K.; Strodel, B.; Wales, D.J.; Wenzel, W.
Modelling Proteins: Conformational Sampling and Reconstruction of Folding Kinetics
BBA - Proteins and Proteomics, 1814 (2011) 8, 977 - 1000

Kubo, T.; Block, H.; Maertens, T.; Spiessensbach, A.; Labahn, J.
Expression and Purification of Membrane Proteins, Methods Navigator, Elsevier
Methods Navigator, (2011), online Publication

Lecher, J.; Stoldt, M.; Schwarz, C.K.W.; Smits, S.H.J.; Schmitz, L.; Willbold, D.
1H, 15N and 13C resonance assignment of the N-terminal C39 peptide-like domain of the ABC transporter Haemolysin B (HybB)
Biomolecular NMR Assignments, 53 (2011) 2, 199 – 201

C-Src is required for complex formation between the hepatitis C virus-encoded proteins NS5A and NS5B: A prerequisite for replication of hepatitis B

Sarib, T.; Schröder, G.F.; Toulinin, A.; McGlynn, P.; Magennis, S.W.
Global Structure of ForKed DNA in Solution Revealed by High-Resolution Single-Molecule FRET
Journal of the American Chemical Society, 133 (2011) 5, 1189 – 1191

Structural characterization of polyglutamine fibrils by solid-state NMR spectroscopy
Journal of Molecular Biology, 412 (2011), 121 - 136

Schünke, S.; Stoldt, M.; Lecher, J.; Kaupp, U.B.; Willbold, D.
Structural insights into conformational changes of a cyclic nucleotide-binding domain in solution from mesorhizobium lot K1 channel

Crystallographic and preliminary X-ray crystallographic studies of an oligomeric species of a refolded C39-peptide-like domain of the Escherichia coli ABC transporter Haemolysin B
Acta Crystallographica Section F: Structural Biology and Crystallographic Communications, 57 (2011) 5, 630 - 633

Seebach, A.; Dinkel, H.; Mohrler, J.; Hartmann, R.; Vogel, N.; Becker, C.M.; Sticht, H.; Enz, R.
Structural characterization of intracellular C-terminal domains of group III metabotropic glutamate receptors
FEBS Letters, 585 (2011) 3, 511 - 516

Stöhr, J.; Ehrlnk, K.; Weinmann, N.; Wille, H.; Willbold, D.; Birkmann, E.; Riesner, D.
In vitro conversion and seeded fibrillation of posttranslationally modified prion protein
Biochemistry, 402 (2011) 5, 415 - 421

Suresh, P.S.; Olubiyi, O.; Thirunavukkarasu, C.; Strodel, B.; Kumar, M.S.
Molecular modeling of human alkaline sphingomyelinase
Bioinformatics, 0 (2011) 2, 78 – 82

Wang, W.; Dong, H.; Pacheco V.; Willbold D.; Zhang Y.; Offenhauser A.; Hartmann R.; Weirich T.; Ma P.; Krause H.; Gu Z.
Relaxation behavior study of ultra-small superparamagnetic iron oxide nanoparticles at ultra-low and ultra-high magnetic fields.

A Spring Loaded Release Mechanism Regulates Domain Movement and Catalysis in Phosphoglycerate Kinase.

Reviews

2009
Funke, S. A.; Birkmann, E.; Willbold, D.
Detection of Amyloid-ß aggregates in body fluids: A suitable method for early diagnosis of Alzheimer's disease?

Funke, S. A.; Willbold, D.
Mirror image phase display - a method to generate D-peptide ligands for use in diagnostic or therapeutical applications?
Molecular BioSystems, 5 (2009) 8, 783 – 786

Mohrler, J.; Schwarten, M.; Willbold, D.
Structure and potential functions of GABARAP
FEBS Journal, 276 (2009) 18, 4089 – 5005

2011
Funke S.A.
Detection of Soluble Amyloid-ß Oligomers and Insoluble High-Molecular-Weight Particles in CSF: Development of Methods with Potential for Diagnostic and Therapy Monitoring of Alzheimer’s Disease

Klein, K.; Strodel, B.; Wales, D.J.; Wenzel, W.
Modelling Proteins: Conformational Sampling and Reconstruction of Folding Kinetics
Biochimica et Biophysica Acta – Proteins and Proteomics, 1814 (2011) 8, 977-1000
Publications

2009
Wales, D. J., Carr, J. M., Khalili, M., de Souza, V., Strodel, B. & Whittleston, C. S.
Pathways and rates for structural transformations of peptides and proteins.

2011
Funke, S.A.; Willbold, D.
Quantitation of Amyloid-ß Oligomers in Human Body Fluids for Alzheimer's Disease Early Diagnosis or Therapy Monitoring?
Koenig B.W., Schünke S., Stoldt M., Willbold D.
NMR methods for the determination of protein-ligand interactions.
Protein-Ligand Interactions (Editor: H. Gohlke) (series "Methods and Principles in Medicinal Chemistry" by Wiley-VCH, in press (2011)
van Groen T., Kadish I., Funke S.A. and Willbold D.
Staining of Amyloid Beta (Abeta) Using (Immuno) Histochemical Techniques and Abeta42 Specific Peptides

Patents
An die Lck-SH3- und Hck-SH3-Domäne bindende Peptide
Willbold, D.; Hoffmann, S.; Wiesehan, K.; Tran, T.T.
06009706, status issued

An die Lck-SH3- und Hck-SH3-Domäne bindende Peptide
Willbold, D.; Hoffmann, S.; Wiesehan, K.; Tran, T.T.
08009541, status issued

Peptide for diagnosis and therapy of Alzheimer's disease
Willbold, D.; Wiesehan, K.
PCT/EP2002/02737926, status issued

Verfahren zum Auffinden von spezifisch an einen Köder bindende Moleküle, sowie spezifisch an einen Köder bindende Moleküle und deren Verwendung
07012129.8-2404, status pending

Zusammensetzung zur Herstellung von anti-Amyloid beta-Peptid-Antikörpern
Willbold, D.; Korth, C.; Müller-Schiffmann, A.; Funke, S.A.
PCT/EP2009/065308, status pending

Mittel zur Behandlung der Alzheimerschen Demenz
10 2010 019 338.4-41, status pending

Hybrid-Verbindung, deren Verwendung und Verfahren zu deren Herstellung
10 2010 017 130.1, status pending
Publications

ICS-7

Peer-reviewed Publications

2009
Direct observation of the tube model in F-actin solutions: Tube dimensions and curvatures

Beltram, G. L.; Giesen, M.; Ibach, H.
Anomalous Helmholtz-Capacitance on Stepped Surfaces of Silver and Gold
Electrochimica Acta, 54 (2009) 18, 4305 - 4311

Fenz, S.F.; Merkel, R.; Sengupta, K.
Diffusion and Intermembrane Distance: Case Study of Avidin and E-Cadherin Mediated Adhesion

Regulation of NK Cell Trafficking by CD81
European Journal of Immunology, 39 (2009) 12, 3447 - 3458

Becoming Stable and Strong: The Interplay between Vinculin Exchange Dynamics and Adhesion Strength During Adhesion Site Maturation
Cell Motility and the Cytoskeleton, 66 (2009) 6, 350 - 364

Moiseeva, M.; Pichardo-Pedrero, E.; Beltram, G. L.; Ibach, H.; Giesen, M.
Reconstruction on Au(001) Vicinal Surfaces in UHV and in Sulfuric Acid Solution
Surface Science, 603 (2009) 4, 670 - 675

Monzel, C.; Fenz, S.; Merkel, R.; Sengupta, K.
Probing Bio-Membrane Dynamics by Dual-Wavelength Reflection Interference Contrast Microscopy

2010
Novel Fusogenic Liposomes for Fluorescent Cell labeling and Membrane Modification
Bioconjugate Chemistry, 21 (2010) 3, 537 - 543

Dieluweit, S.; Csiszar, A.; Rubner, W.; Fleischhauer, J.; Houben, S.; Merkel, R.
Mechanical Properties of Bare and Protein-Coated Giant Unilamellar Phospholipid Vesicles. A Comparative Study of Micropipet Aspiration and Atomic Force Microscopy

Tube Width Fluctuations in F-actin Solutions

Eekhoff, A.; Bonakdar, N.; Alonso, J.L.; Hoffmann, B.; Goldmann, W.H.
Glomerular podocytes: A study of mechanical properties and mechano-chemical signaling
Biochemical and Biophysical Research Communications, 406 (2011) 2, 229 - 233
Publications

Switching from Ultraweak to Strong Adhesion  
Advanced Materials, 23 (2011) 22/23, 2622 - 2626

Fenz, S.; Smith, A.S.; Merkel, R.; Sengupta, K.  
Inter-membrane adhesion mediated by mobile linkers: Effect of receptor shortage  
Soft Matter, 7 (2011) 3, 952 - 962

Ibach, H.; Beltramo, G. L.; Giesen, M.  
Interface capacitance of nano-patterned electrodes  
Surface Science, 605 (2011) 1/2, 240 - 247

Ligezowska, A.; Boye, J.; Eble, B.; Hoffmann, B.; Klösgen, B.; Merkel, R.  
Mechanically enforced bond dissociation reports synergetic influence of Mn2+ and Mg2+ on the interaction between integrin alpha7beta1 and invasin  
Journal of Molecular Recognition, 24 (2011) 4, 715 - 723

Loritz, H.M.; Kirchgeßner, N.; Born, S.; Hoffmann, B.; Merkel, R.  
Mechanical Strength of Specific Bonds Acting Isolated or in Pairs: A Case Study on Engineered Proteins  

Viale-Bouroncle, S.; Völlner, F.; Möhl, C.; Köpper, K.; Brockhoff, G.; Reichert, T.E.; Schmalz, G.; Morsczeck, C.  
Soft matrix supports osteogenic differentiation of human dental follicle cells  
Biochemical and Biophysical Research Communications, 410 (2011) 3, 587 - 592

Diatom frustules show increased mechanical strength and altered valve morphology under iron limitation  
Limnology and Oceanography, 56 (2011) 4, 1399 - 1410

Lorenz, B.; Persson, B.N.J.; Dieluweit, S.; Tada, T.  
Rubber friction: Comparison of theory with experiment  

Schäfer, C.; Faust, U.; Kirchgeßner, N.; Merkel, R.; Hoffmann, B.  
The filopodium: A stable structure with highly regulated repetitive cycles of elongation and persistence depending on the actin cross-linker fascin  
Cell Adh. Migr. 5 (2011) 431-438

Sackmann, E.; Merkel, R.  
Lehrbuch der Biophysik  

Books/Book contributions

2010  
Alt, W.; Bock, M.; Möhl, C.  
Coupling of cytoplasm and adhesion dynamics determines cell polarization and locomotions In: Cell Mechanics: From Single Scale-Based Models To Multiscale Modeling / ed.: Chauviere, A.; Preziosi, L.; Verdiere, C.  
Boca Raton, FL, USA, CRC Press Inc., 2010. 86-125

2011  
Giesen, M.; Beltramo, G.  
Dynamics and Stability of Surface Structures  

Patents

2009  
Patentanmeldung PT 1.2444 vom 19.06.2009  
Autofokusverfahren und Vorrichtung zur Durchführung des Verfahrens  
Kirchgeßner, N.; Merkel, R.

Patentanmeldung PT 1.2448 vom 09.07.2009  
Mischung amphipathischer Moleküle und Verfahren zur Zellmembranmodifikation durch Fusion  
Hoffmann, B.; Császar, A.; Hersch, N.; Merkel, R.

Patentanmeldung PT 1.2461 vom 14.10.2009  
Internationale Patentanmeldung PCT/DE2010/001139  
Vorrichtung zur Untersuchung von Zellen mit einem Elastomer sowie Verwendung der Vorrichtung  
Kirchgeßner, N.; Hoffmann, B.; Houben, S.; Merkel, R.

Deutsche Patentanmeldung 102006029051.8-41  
Europäische Patentanmeldung 07764371.8  
Japanische Patentanmeldung 2009-519897  
US-amerikanische Patentanmeldung 12/308.789  
Verfahren zur Herstellung von Zellkulturverfahren  
Hoffmann, B.; Born, S.; Merk, R.

Internationale Patentanmeldung PCT/DE2010/0005.28  
Zuleitungssystem  
Füllerer, C.; Fried, H.-U. Brombas, A.

2010  
Deutsche Patentanmeldung 102005005121.9-43  
Europäische Patentanmeldung 06705874.3-2115  
Japanische Patentanmeldung 2007-553553  
US-amerikanische Patentanmeldung 11/683,893  
Verfahren zur Herstellung eines Elastomers und Elastomer  
Hoffmann, B.; Cesa, C.; Merkel, R.; Hersch, N.

Internationale Patentanmeldung PCT/DE2010/00081  
Mischung amphipathischer Moleküle und Verfahren zur Zellmembranmodifikation durch Fusion  
Hoffmann, B.; Császar, A.; Hersch, N.; Merkel, R.
Patentanmeldung PT 1.2533 vom 06.10.2011
Molekülmischung, umfassend eine amphiphatische Molekülsorte A, welche im hydrophilen Bereich eine positive Gesamtladung aufweist und eine amphiphatische Molekülsorte B sowie ein Polyphenol C, Verfahren zur Herstellung der Molekülmischung und deren Verwendung
Csiszar, A.; Kleusch, C.; Hoffmann, B.; Merkel, R.
2009
Abouzar, M. H.; Poghossian, A.; Razavi, A.; Williams, O. A.; Bijnens, N.; Wagner, P.; Schöning, M. J.
Characterization of capacitive field-effect sensors with a nano-crystalline diamond film as transducer material for multi-parameter sensing
Biosensors and Bioelectronics, 24 (2009) 5, 1298 - 1304

2009
Arida, H.; Mohns, Q.; Schöning, M. J.
Microfabrication, characterization and analytical application of a new thin-film silver microsensor

2009
Bäcker, M.; Beging, S.; Biselli, M.; Poghossian, A.; Wang, J.; Zang, W.; Wagner, P.; Schöning, M. J.
Concept for a solid-state multi-parameter sensor system for cell-culture monitoring

2009
Analysis of Some Nondestructive Evaluation Experiments Using Eddy Currents

2009
Caban, K.; Offenhäusser, A.; Mayer, D.
Electrochemical Characterization of the Effect of Gold Nanoparticles on the Electron Transfer of Cytochrome c

2009
Diamond Transistor Array for Extracellular Recording From Electrogenic Cells
Advanced Functional Materials, 19 (2009) 18, 2915 - 2923

2009
Dong, H.; Zhang, Y.; Krause, H.-J.; Xie, X.; Braginski, A. I.; Offenhäusser, A.
Suppression of ringing in the tuned input circuit of a SQUID detector used in low-field NMR measurements
Superconductor Science and Technology, 22 (2009), 125022

2009
Irradiation oxide microelectrode arrays for in vitro stimulation of individual rat neurons from dissociated cultures
Frontiers in Neuroengineering, 2 (2009), 16

2009
Action Potentials of HL-1 Cells Recorded With Silicon Nanowire Transistors
Applied Physics Letters, 95 (2009) 8, 083703

2009
Fardmanesh, M.; Kokabi, H.; Pourhashemi, A.; Moftakhari, A.; Khorasani, S.; Banzet, M.; Schubert, J.
2D Analysis of the Effects of Geometry on the Response of High-critical-current Superconducting Bolometric Detectors

2009
Fardmanesh, M.; Sarreesh, F.; Pourhashemi, A.; Ansari, E.; Vesaghi, M.A.; Schubert, J.
Optimization of NOE Characterization Parameters for a RF-SQUID Based System Using FEM Analysis

2009
Impedimetric detection of covalently attached biomolecules on field-effect transistors

2009
Gilles, S.; Meier, M.; Prömpers, M.; van der Hart, A.; Kügeler, C.; Offenhäusser, A.; Mayer, D.
UV nanoimprint lithography with rigid polymer molds
Microelectronic Engineering, 86 (2009) 4/6, 661 - 664

2009
Goluch, E.D.; Wolfum, B.; Singh, P.S.; Zevenbergen, M.A.G.; Lemay, S.G.
Redox cycling in nanofluidic channels using interdigitated electrodes
Analytical and Bioanalytical Chemistry, 394 (2009) 2, 447 - 456

2009
Gun, J.; Rizkov, D.; Lev, O.; Abouzar, M. H.; Poghossian, A.; Schöning, M. J.
Oxygen plasma-treated gold nanoparticle-based field-effect devices as transducer structures for bio-chemical sensing
Microchimica Acta, 164 (2009) 395 - 404

2009
Holtmann, E.; Schubert, J.; Kutzner, R.; Wöderweber, R.
Stress generated modifications of epitaxial ferroelectric SrTiO3 films on sapphire
Journal of Applied Physics, 105 (2009), 114104

2009
High tunability of the soft mode in strained SrTiO3/DyScO3 multilayers
Journal of Physics: Condensed Matter, 21 (2009), 115902-1 - 115908-8

2009
Kadlec, C.; Skoromets, V.; Kadlec, F.; Nemec, H.; Schubert, J.; Panaitov, G.; Ku el, P.
Temperature and electric field tuning of the ferroelectric soft mode in a strained SrTiO3/DyScO3 heterostructure
Physical Review B, 80 (2009) 17, 174116

2009
Kianer, A.; Lenk, S.; Mayer, D.; Mourzina, Y.; Offenhäusser, A.
Determination of the Stability Constant of the Intermediate Complex during the Synthesis of Au Nanoparticles Using Arous Halide

2009
Kladko, V.P.; Kolomys, A.F.; Slodbodan, M.V.; Streichuk, V.V.; Raycheva, V.G.; Belyaev, A.E.; Bukalov, S.S.; Hardtdegen, H.; Sydoruk, V. A.; Klein, N.; Vitusevich, S. A.
Internal strains and crystal structure of the layers in AlGaN/GaN heterostructures grown on sapphire substrate
Journal of Applied Physics, 105 (2009) 6, 063515-1-9

2009
Kladko, V.P.; Kuchuk, A.V.; Saffryuk, N.V.; Machulin, V.F.; Belyaev, A.E.; Hardtdegen, H.; Vitusevich, S. A.
Mechanism of strain relaxation by twisted nanocolumns revealed in AlGaN/GaN heterostructures
Applied Physics Letters, 95 (2009), 031907

2009
Krämer, M.; Pita, M.; Zhou, J.; Ortnakta, M.; Poghossian, A.; Schöning, M. J.; Katz, E.
Coupling of biocomputing systems with electronic chips: Electronic interface for transduction of biochemical information

2009
Kügeler, C.; Meier, M.; Rosszin, R.; Gilles, S.; Waser, R.
High density 3D memory architecture based on the restatic switching effect
Solid-State Electronics, 53 (2009) 12, 1287 - 1292

2009
Quantum confinement effect on the effective mass in two-dimensional electron gas of AlGaN/GaN heterostructures
Journal of Applied Physics, 105 (2009) 7, 073703

2009
The use of microelectrode array (MEA) to study the protective effects of potassium channel openers on metabolically compromised HL-1
cardiomyocytes

Physiological Measurement, 30 (2009), 155 - 167

Optical band gap and magnetic properties of unstrained EuTiO3 films

Applied Physics Letters, 94 (2009), 212509

 Resistively switching Pt/SnO2 nanocells for non-volatile memories fabricated with UV nanoimprint lithography

Microrheologic Engineering, 86 (2009) 46, 1060 - 1062

Meier, M.; Schindler, C.; Gilles, S.; Rosezin, R.; Rüdiger, A.; Kügeler, C.; Waser, R.
 A nonvolatile memory with resistively switching molybdenum-silicotine oxide

IEEE Electron Device Letters, 30 (2009), 8 - 10

 Non-invasive Determination of Plant Biomass with Microwave Resonators

Plant, Cell and Environment, 32 (2009), 368 - 379

 Ofchemical image scanner based on FDM-LAPS

Sensors and Actuators B, 137 (2009) 2, 533 - 538

 A micron-sized nanoporous multifunction sensor device


Näther, N.; Henkel, H.; Schneider, A.; Schöning, M. J.
 Investigation of different catalytically active and passive materials for realizing a hydrogen peroxide gas sensor


Poghossian, A.; Abouzar, M. H.; Razavi, A.; Bäcker, M.; Bijvens, N.; Williams, O. A.; Haenen, K.; Moritz, W.; Wagner, P.; Schöning, M. J.
 Nanocrystalline diamond thin films with high pH and penicillin sensitivity prepared on a capacitive Si–SiO2 structure

Elecrotechnica Acta, 54 (2009) 25, 5981 - 5985

Poghossian, A.; Ingelbrandt, S.; Offenhausser, A.; Schöning, M. J.
 Field-effect devices for detecting cellular signals

Seminars in Cell & Developmental Biology, 20 (2009) 1, 41 - 48

Pretzel, A.; Krause, H.-J.; Zhang, Y.; Offenhausser, A.
 HTS rf SQUID System for Magnetic Nanoparticle Detection


Qu, L. Q.; Krause, H.-J.; Zhang, Y.; Dong, H.; Braginski, A. I.; Offenhausser, A.
 The Effect of Low Frequency Disturbance to SQUID Based Low Field NMR


Qu, L. Q.; Zhang, Y.; Krause, H.-J.; Braginski, A. I.; Tandoka, S.; Offenhausser, A.
 High-Performance Low-Field NMR Utilizing a High-Tc rf SQUID


Qu, L.; Zhang, Y.; Krause, H.-J.; Braginski, A. I.; Offenhausser, A.
 Low-field NMR measurement procedure when SQUID detection is used

Journal of Magnetic Resonance, 196 (2009), 101 - 104

Rosezin, R.; Nauenheim, C.; Trenfellkamp, S.; Kügeler, C.; Waser, R.
 Electrical properties of Pt interconnects for passive crossbar memory arrays

Microelectronic Engineering, 86 (2009) 11, 2275 - 2278

 Time-dependent observation of individual cellular binding events to field-effect transistors

Biosensors and Bioelectronics, 24 (2009), 1201 - 1208

Schöning, M. J.; Abouzar, M. H.; Poghossian, A.
 pH and ion sensitivity of a field-effect EIS (electrolyte-insulator-semiconductor) sensor covered with polyelectrolyte layers

Journal of Solid State Electrochemistry, 13 (2009) 1, 115 - 122

Shafroost, E. N.; Klein, N.; Vitusevich, S. A.; Barannik, A.A.; Cherpak, N.T.
 High sensitivity microwave characterisation of organic molecule solutions of nanoliter volume


Siqueira, J. R. Jr.; Abouzar, M. H.; Zucolotto, V.; Poghossian, A.; Oliveira, O. N. Jr.; Schöning, M. J.
 Carbon nanotubes in nanostructured films: Potential application as amperometric and potentiometric field-effect (bio-)chemical sensors


Siqueira, J. R. Jr.; Abouzar, M. H.; Poghossian, A.; Zucolotto, V.; Oliveira, O. N. Jr.; Schöning, M. J.
 Penicillin biosensor based on a capacitive field-effect structure functionalized with a dendrimer/carbon nanotube multilayer

Biosensors and Bioelectronics, 25 (2009) 2, 497 - 501

Siqueira, J. R. Jr.; Werner, C. F.; Bäcker, M.; Poghossian, A.; Zucolotto, V.; Oliveira, O. N. Jr.; Schöning, M. J.
 Layer-by-layer assembly of carbon nanotubes incorporated in light-addressable potentiometric sensors


Song, F.; Muller, F.; Behr, R.; Klushin, A. M.
 Coherent emission from large arrays of discrete Josephson junctions


Spelthahn, H.; Poghossian, A.; Schöning, M. J.
 Self-aligned nanogaps and nanochannels via conventional photolithography and pattern-size reduction technique


 Artificial intelligence/huzy logic method for analysis of combined signals from heavy metal chemical sensors

Elecrotechnica Acta, 54 (2009) 25, 6082 - 6088

 Noise spectroscopy of AlGaN/GaN HEMT structures with long channels

Journal of Statistical Mechanics : Theory and Experiment, (2009), 01046-1-10

 Low frequency noise in 2DEG channel of AlGaN/GaN heterostructures

AIP Conference Proceedings, 1129 (2009), 487-490

 Top-down processed silicon nanowire transistor arrays for biosensing


 Gate-controlled Quantum collimation in nanocolumn resonant tunneling transistors

Nanotechnology, 20 (2009), 465402
Publications


2010

Abouzar, M. H.; Pedrazza, A.; Schöning, M. J.; Poghosssian, A. Label-free DNA hybridization and denaturation detection by means of field-effect nanoplate SOI capacitors functionalized with gold nanoparticles Procedia Engineering, 5 (2010), 918 -921


Belyaev, A.E.; Boltovets, N.S.; Vitusevich, S. A.; Ivanov, V.N.; Konakova, R.V.; Kudryk, Y.Ya.; Lebedev, A.A.; Milien, V.V.; Svechnikov, Yu.N.; Sheremet, V.N. Effect of microwave treatment on current flow mechanisms in Au-Ta/TiN (++n++)+GaN/AZO GaN ohmic contacts Semiconductors, 44 (2010) 6, 742 - 751


Bohm, U.; Stütz, E.; Fleischer, M.; Schöning, M. J.; Wagner, P. Real-time detection of CO by Eukaryotic cells Procedia Engineering, 5 (2010), 17 - 20


Kätelhön, E.; Hoffmann, B.; Banzet, M.; Offenhäusser, M.; Wolfrum, B. Time-resolved mapping of neurotransmitter fluctuations by arrays of nanocavity redox-cycling sensors Procedia Engineering, 5 (2010), 950 - 956


Kirchner, P.; Oberländer, J.; Friedrich, P.; Berger, J.; Ryssdag, G.; Keusgen, M.; Schöning, M. J. Realization of a calorimetric gas sensor on polycrystalline foil for applications in asptic food industry Procedia Engineering, 5 (2010), 264 - 267

Kiselov, V.S.; Lytvyn, P.M.; Yukhymchuk, V.O.; Belyaev, A.E.; Vitusevich, S. A. Synthesis and properties of porous SiC ceramics Analytical Letters, 107 (2010) 9, 093510


Liu, Y.; Offenhäusser, A.; Mayer, D.
An Electrochemically Transduced XOR Logic Gate at the Molecular Level

Liu, Y.; Offenhäusser, A.; Mayer, D.
Electrochemical current rectification at bio-functionalized electrodes
Bioelectrochemistry, 77 (2010) 2, 89 - 93

Liu, Y.; Offenhäusser, A.; Mayer, D.
Molecular rectification in metal-bridge molecule-metal junctions

Liu, Y.; Offenhäusser, A.; Mayer, D.
Rectified tunneling current response of bio-functionalized metal-bridge-metal junctions
 Biosensors and Bioelectronics, 25 (2010) 5, 1173 - 1178

Elastin strain and dopant activation in ion implanted strained Si nanowires

Miniaturized chemical imaging sensor system using an OLED display panel
Procedia Engineering, 5 (2010), 516 - 519

Moers, J.; Gerharz, J.; Rinke, G.; Musser, G.; Trellekmamp, S.; Grützmacher, D.
Influence of the epitaxial growth and device processing on the overlay accuracy during processing of the d-DotFET

Moshchalkov, V.; Wördenweber, R.; Lang, W.
Nanoscience and Engineering in Superconductivity

Rosezin, R.; Meier, M.; Trellekmamp, S.; Kügeler, C.; Waser, R.
Observation of unipolar resistance switching in silver doped methyl-silsesquioxane

Sarreshtedari, F.; Jahed, NMS.; Hosseni, N.; Pourhashemi, A.; Banzet, J.; Fardmanesh, M.
FEM enhanced signal processing approach for pattern recognition in the SQUID based NDE system

Sarreshtedari, F.; Pourhashemi, A.; Asad, N.; Schubert, J.; Banzet, J.; Fardmanesh, M.
An Efficient Finite-Element Approach for the Modeling of Planar Double-D Excitation Coils and Flaws in SQUID NDE Systems

Sawade, G.; Krause, H.-J.
Prüfung von Spannbetonbauteilen mit magnetischen Methoden
Beton- und Stahlbetonbau, 105 (2010) 3, 154 - 164

Schöning, M. J.
Development of an electrolys-sulator-semiconductor (EIS) based capacitive heavy metal sensor for the detection of Pb2+ and Cd2+ ions

Spelthahn, H.; Schaffarth, S.; Coppe, T.; Rufi, F.; Schöning, M. J.
Miniaturized chemical imaging sensor system using an OLED display panel
Procedia Engineering, 5 (2010), 520 - 523

Vu, X. T.; Stockmann, R.; Wolfrum, B.; Offenhäusser, A.; Ingebrandt, S.
Transport properties of single-walled carbon nanotube transistors after gamma radiation treatment
Journal of Applied Physics, 107 (2010), 063701
Publications


Wolff, S.; Lägel, B.; Trelleken, S. Incident angle dependent damage of PMMA during Ar+-ion beam etching Microelectronic Engineering, 87 (2010), 1444 - 1446


Yi, Z.; Banzet, M.; Offenhäuser, A.; Mayer, D. Fabrication of nanogaps with modified morphology by potential-controlled gold deposition Physica Status Solidi (RRL), 4 (2010), 73 - 75


2011

Abouzar, M.H.; Moritz, W.; Schönöing, M.J.; Poghossian, A. Capacitance-voltage and impedance-spectroscopy characteristics of nanoplate EISOI capacitors Physica Status Solidi A, 208 (2011) 6, 1327 - 1332


Barannik, A.A.; Cherpark, N.T.; Ni, N.; Tanatar, M.A.; Vitusevich, S.A.; Skesanov, V.N.; Canfield, P.C.; Prozorov, R.; Glamazdin, V.V.; Tokorhiti, K.I. Millimeter-wave study of London penetration depth temperature dependence in Ba (Fe0.926Co0.074)2As2 single crystal Low Temperature Physics, 38 (2011) 8, 912 - 915


Hong, H.-B.; Krause, H.-J.; Song, K.-B.; Choi, C.-J.; Chung, M.-A.; Son, S.-w.; Offenhäusser, A.
Detection of two different influenza A viruses using a nitrocellulose membrane and a magnetic biosensor
Journal of Immunological Methods, 365 (2011) 1/2, 95 - 100

Hüské, M.; Wollfrum, B.
Fabrication of a nanoporous dual-electrode system for electrochemical redox cycling
Physica Status Solidi A, 208 (2011) 6, 1265 - 1269

Jungk, F.; Schillberg, S.; Krause, H.-J.; Schröper, F.
Der mobile Pflanzenidoktor - Magnetische Immundetektion von Pflanzenpathogenen
GfT Labor-Fachzeitschrift, 95 (2011) 12, 870 - 871

Kampeis, P.; Lieblang, M.; Krause, H.-J.
Eine Nutzung von Magnetfiltern in der Bioverfahrenstechnik - Teil 3: Neues Messverfahren zur Quantifizierung von Magnetbeads in strömenden Suspensionen
Chemie Ingenieur Technik, 83 (2011) 6, 851 - 857

Kirchner, P.; Li, B.; Spelthahn, H.; Henkel, H.; Schneider, A.; Friedrich, P.; Kolstad, J.; Keusgen, M.; Schöning, M.J.
Thin-film calorimetric H(2)O(2) gas sensor for the validation of germicidal effectivity in aseptic filling processes
Sensors and Actuators B, 154 (2011) 2, 257 - 263

Kirk, P.; Oberländer, J.; Friederich, P.; Berger, J.; Suso, H.-P.; Kupyna, A.; Keusgen, M.; Schöning, M.J.
Optimisation and fabrication of a calorimetric gas sensor built up of a polyimide substrate for H(2)O(2) monitoring
Physica Status Solidi A, 208 (2011) 6, 1235 - 1240

The Role of Oxidative Etching in the Synthesis of Ultrathin Single-Crystalline Au Nanowires
Chemistry-a European Journal, 17 (2011) 34, 9503 - 9507

Klocke, D.; Schmitz, A.; Solterr, H.; Bousack, H.; Schmitz, H.
Infrared receptors in pyrophilous fly loving insects as model for new un-cooled infrared sensors
Beilstein Journal of Nanotechnology, 2 (2011) 186 - 197

Liu, B.; Zhang, Y.; Mayer, D.; Krause, H.-J.; Jin, Q.; Zhao J.; Offenhäusser, A.
A simplified poly(dimethylsiloxane) capillary electrophoresis microchip integrated with a low-noise contactless conductivity detector
Electrophoresis, 32 (2011) 6/7, 697 - 704

Panaitov, G.; Thiery, S.; Hofmann, B.; Offenhäusser, A.
Fabrication of gold micro-spike structures for improvement of cell/device adhesion
Microelectronic Engineering, 88 (2011) 8, 1840 - 1844

Sanetra, N.; Feig, V.; Wollfrum, B.; Offenhäusser, A.; Mayer, D.
Low-impedance surface coatings via nanopillars and conductive polymers

Analytical Model for the Extraction of Flux-Induced Current Interactions for SQUID NDE Applications on Applied Superconductivity, 21 (2011) 1, 3442 - 3446

Terres, B.; Dauber, J.; Volk, C.; Trellenkamp, S.; Wichmann, U.; Stamper, C.
Disorder induced Coulomb gaps in graphene constructions with different aspect ratios
Applied Physics Letters, 98 (2011) 142506

Wagner, T.; Werner, C. F.; Miyamoto, K.; Schöning, M. J.; Yoshinobu, T.
A high-density multi-point LAPS set-up using a VCSEL array and FPGA control
Sensors and Actuators B, Chemical, 154 (2011) 2, 124 - 128

Weber, D.; Mourzina, Y.; Brüggemann, D.; Offenhäusser, A.

Xiang, D.; Pyatkov, F.; Schröper, F.; Offenhäusser, A.; Zhang, Y.; Mayer, D.
Molecular Junctions Bridged by Metal Ion Complexes
Chemistry-a European Journal, 17 (2011) 47, 13166 - 13169

Xiang, D.; Zhang, Y.; Pyatkov, F.; Offenhäusser, A.; Mayer, D.
Gap size dependent transition from direct tunneling to field emission in single molecule junctions
Chemical Communications, 47 (2011) 1, 4760-4762

Yukhmitschuk, V.O.; Kiselov, V.S.; Belyaev, A.E.; Valakh, M.Ya.; Chrusanova, M.V.; Danailov, M.; Vitsusevich, S.A.
Raman Spectroscopy of bio-SiC ceramics

Zevenbergen, M.A.G.; Singh, P.S.; Goluch, E.D.; Wollfrum, B.L.; Lemay, S.G.
Stochastic Sensing of Single Molecules in a Nanofluidic Electrochemical Device
Nano Letters, 11 (2011) 7, 2831 - 2838
Publications

Zhang, G.; Zhang, Y.; Dong, H.; Krause, H.-J.; Xie, X.; Braginski, A.I.; Offenhäusser, A.; Jiang, M.
An approach to optimization of the superconducting quantum interference bootstrap circuit
Superconductor Science and Technology, 24 (2011) 6, 065023

Comparison of noise performance of the dc SQUID bootstrap circuit with that of the standard flux modulation dc SQUID readout scheme

Reviews

2009
Krause, H.-J.; Rath, E.; Sawade, G.; Dumat, F.
Bestimmung der Feuchte und des Chloridgehaltes von Beton mit der "Radar-Magnet-Betontest"-Methode

2010
Capacitive field-effect (bio-)chemical sensors based on nanocrystalline diamond films

Books/Book contributions

2009
Ingebrandt, S.; Offenhäusser, A.
Label-Free, Fully Electronic Detection of DNA with a Field-Effect Transistor Array

Offenhäusser, A.; Ingebrandt, S.; Pabst, M.; Wrobel, G.
Interfacing Neurons and Silicon-Based Devices

Schöning, M. J.; Schütz, S.
Der Käfer/Chip-Sensor als bioelektronischer "Schnüffler"

2010
Sawade, G.; Krause, H.-J.
Magnetic flux leakage (MFL) for the nondestructive evaluation of pre-stressed concrete structures

2011
Vitusevich, S.; Gasparany, F.
Low-frequency noise spectroscopy at nanoscale: carbon nanotube materials and devices.

Wördenweber, R.
Fundamentals of high-temperature superconductors

Wördenweber, R.
Ferroelectric Thin Layers

Patents

filed patents:
Anordnung aus Träger und supraleitendem Film
Wördenweber, R.
DE 19964555.9-09 (2009)

Transistorsiabasierter Sensor mit besonders ausgestalteter Gateelektrode zur hochempfindlichen Detektion von Analyten
Goryll, M.; Moens, J.; Lüth, H.; Odenthal, M.; Offenhäusser, A.

Vorrichtung zur Frequenzabstimmung eines Hohlraumresonators oder dielektrischen Resonators
Klein, N.; Krishnananda, D. S.
EP 03025954.3-2215 (2009)

Waferhalter
Müller, J.
DE 102 58 368.4-09 (2009)

Vorrichtung und Verfahren zum Nachweis von geladenen Makromolekülen
Cherstvy, A.; Ingebrandt, S.; Keusgen, M.; Poghossian, A.; Schöning, M. J.
EU 05774381.7-1240 (2009)

Nahfeldsonde
Klein, N.; Kuzel, P.; Kadlec, F.

Anordnung aus Träger und supraleitendem Film, Vortexdiode, umfassend eine derartige Anordnung sowie Verwendung von Vortexdioden
für Filter
Wördenweber, R.

Implantat zum Einführen in Hohlkörper
Zhang, Y.; Otto, R.; Offenhäusser, A.; Bousack, H.
EP 06147778.2-2320 (2009)

A method of patterning molecules on a substrate using a micro-contact printing process

A method of applying a material on a substrate
US 7,964,054 (2011)

Identification of a probe in anBehälter, z. B. im Check-In Bereich bei der Reisegastabfertigung, durch Bestimmung der Resonanzfrequenz und der Güte eines dielektrischen Resonators, an dem der Behälter angeordnet wird
Klein, N.; Krause, H. J.
US 12311,236 (2011)
Publications

Verfahren zur Herstellung von Durchkontaktierungen und Leiterbahnen
Bousack, H.; Viehweger, K.
EP 07846377.5-1235 (2011)

A method of preparing a substrate having a layer or pattern of metal on it

Resonatoranordnung und Verfahren zur Untersuchung einer Probe mit der Resonatoranordnung
Klein, N.; Vitusevich, S.; Danylyuk, S.

granted patents:

Sensoranordnung
Offenhäusser, A.; Bousack, H.; Schmitz, H.
EP 09729347.6 (2009)

SQUID with a coil inductively coupled to the SQUID via a mutual inductance

Vorrichtung zur Ableitung elektrophysiologischer Signale von Zellen
Banzet, M.; Offenhäusser, A.; Wolftrum, B.; Hofmann, B.; Kätehön, F. E.
DE 102010012840.6-62 (2010)

Verfahren zur elektrischen Messung der Bandlücke in amorphen Materialien sowie Verstärker und Vorrichtung zur Durchführung
Offenhäusser, A.; Sydronik, V.; Vitusevich, S.; Petrychuk, M.

Verfahren zur Herstellung einer Hydrogel-Mikrostruktur, Hydrogelstruktur, sowie Verwendung der Hydrogelstruktur
Füllerer, C.; Meffert, S.; Fricke, R.; Offenhäusser, A.; Merkel, R.
DE 102010022675.9-54 (2010)

Sensoranordnung
Banzet, M.; Mayer, D.; Soltner, H.; Bousack, H.
DE 102010027346.5-52 (2010)

Method for applying a first metal onto a second metal, an isolator or semiconductor substrate, and the respective binding units
EP 10008481.3 (2010)

Vorrichtung und Verfahren zur Abscheidung von Magnetpartikeln
Soltner, H.; Pretzell, A.
DE 102010048551.9-44 (2010)

Verfahren zur Herstellung einer Vorrichtung zum Nachweis eines Analyten sowie Vorrichtung und deren Verwendung
DE 1020111010767.3-52 (2011)

Elektrodenanordnung und Verfahren zum Betreiben der Elektrodenanordnung
Mayer, D.; Offenhäusser, A.; Liu, Y.
DE 102011108885.0 (2011)

Method for identifying a sample in a container, e.g. when conducting a traveler survey in the check-in area, by determining the resonance frequency and the quality of a dielectric resonator to which the container is arranged
Klein, H.-J.; Krause, W.; Zander

Method and device for selectively detecting ferromagnetic or superparamagnetic particles
P. Miethe, H.-J. Krause, Y. Zhang, N. Wollens, D. Plaksin