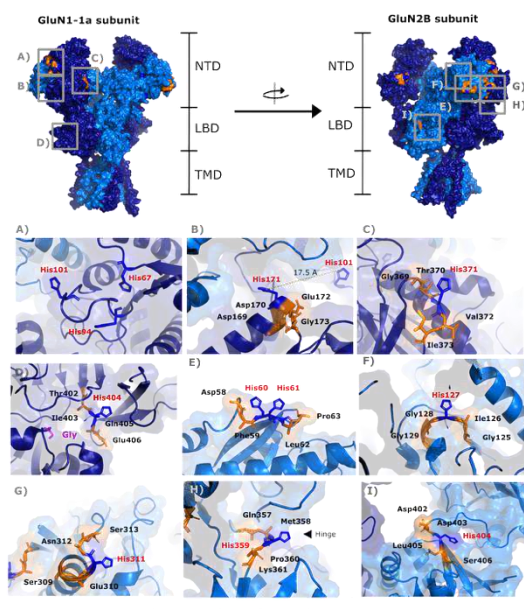


Copper-dependent modulation of the neuroreceptor NMDAR: Molecular insights from QM/MM simulations and correlation to spectroscopic studies

The N-Methyl-D-Aspartate (NMDA) receptors are neuroreceptors highly expressed in the Central Nervous System (CNS).¹ NMDAR forms a transmembrane pore permeable to calcium that is only gated by binding the neurotransmitter glutamate and its co-agonist glycine to the extracellular domains.¹ Calcium entry through NMDAR is highly regulated and plays a critical role in neurodevelopment and neurotransmission associated with sensory/motor integration, learning, and memory.^{1, 2} Altered expression or malfunction of these receptors is involved in a myriad of CNS diseases that include neurodevelopmental disorders such as autism, neurodegenerative disorders such as Alzheimer's disease, neurological conditions (such as epileptic seizures, traumatic brain injury, and neuropathic pain), and neuropsychiatric illnesses (such as depression, schizophrenia, and anxiety).²⁻⁴ Interestingly, accumulation of copper has been observed as a common factor in patients affected by most of these pathologies.⁵⁻⁷ More importantly, cell culture studies demonstrated that copper can modulate activity of NMDAR.⁸⁻¹³ Therefore, modulation of NMDAR receptors by copper could be a promising therapeutic target for neurological disorders, including Alzheimer's.

NMDAR has a tetrameric structure, typically composed of two NR1 subunits and two NR2 subunits, although in some cases, an NR3 subunit may also be present (Figure). This structure forms a central ion channel that is permeable to calcium (Ca^{2+}), sodium (Na^+), and potassium (K^+). Upon NMDAR activation by glutamate and glycine, Ca^{2+} entry into the cell promotes a signaling cascade that leads to Cu^{2+} ions release into the synapse, reaching concentrations up to 100 μM . At concentrations higher than 2 μM , Cu^{2+} ions are reported to modulate NMDAR activity,⁸⁻¹³ though the molecular basis of this copper-dependent modulation is not known. Being a d-block transition metal ion, Cu^{2+} displays chemical properties that are entirely different from alkaline-earth ions, such as calcium.¹⁴ Hence, the copper-dependent modulation of NMDAR might involve a direct interaction of Cu^{2+} ions with the active form of NMDAR, that likely occurs at a different site from the channel pore. Based on the coordination chemistry of copper, we have identified ten putative Cu^{2+} binding sites in the NMDAR involving several histidine and cysteine residues as putative metal ligands (Figure); the analysis was performed using Cryo-EM and crystal structures of the receptor.¹⁵⁻¹⁷

In this project, we propose to use computational tools, including quantum-mechanical/molecular mechanics (QM/MM) calculations to elucidate the nature of the interaction of Cu^{2+} ions with the active form of NMDAR, and to gain insights into the mechanism of copper-dependent modulation of NMDAR activity with atomic resolution. The specific aims of this project are: i) To obtain a molecular description of the active form of the NMDAR using exascale simulations; ii) To



CryoEM structure of NMDAR (9ARE) showing its GluN1-1a and GluN2B subunits (top) and six putative $\text{Cu}(\text{II})$ binding sites (A-I, bottom).

elucidate the nature of the binding sites for Cu²⁺ ions in the active form of NMDAR, identifying the amino acids involved in metal binding; iii) To evaluate the impact of Cu²⁺ ion binding in the conformation of the neuroreceptor. The results from the QM/MM simulations will be contrasted with spectroscopic data on the direct Cu(II) binding to the ligand binding domain of NMDAR, which is experimental work under progress at Cinvestav. This work will pave the road to design therapeutic strategies that target the role of copper and NMDAR in neurological disorders.

Project Participants at Cinvestav

Prof. Dr. Liliana Quintanar - Department of Chemistry and Center for Research in Aging, Cinvestav.

Betzabe Carbajal-Salazar, PhD student at the Department of Chemistry, Cinvestav.

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