Proposition de thèse à l'Ecole Doctorale INTERFACES

Simulations of the ion transport controlled by NMDAR membrane neurotransmitter

Simulation du transport des ions contrôlé par le neurotransmetteur membranaire NMDAR

INTRODUCTION

Functions: NMDA receptors (NMDAR) are neurotransmitter receptors located in the post-synaptic membrane of a neuron that receive signals across the synapse from a previous nerve cell. NMDARs are involved in signal transduction and control the opening and closing of ion channels. They play an important role in learning and memory formation. NMDAR dysfunction has been found to be involved in various neurodegenerative and psychiatric disorders, such as Alzheimer's, Parkinson's and Huntington's diseases, and in stroke, in which NMDAR subunits undergo transcriptional and/or posttranslational modifications [1]. NMDAR has also been suggested to play a pathological role in chronic peripheral disorders, such as type 2 diabetes mellitus [2] and cancer [3]. Consequently, NMDAR is a crucial therapeutic target intensively studied in context of different maladies.

The NMDAR consists of two obligatory GluN1 chains, and two GluN2 chains (splice variants GluN2A, GluN2B, GluN2C and GluN2D) modulating channel properties, or a GluN2/GluN3 subunit combination. It is activated by glutamate (Glu) and glycine (Gly) or D-serine (the ligand-dependent activation), in membrane depolarization conditions (the voltage-dependent activation) [4]. *Once* synaptic membrane has been depolarized and the Glu and Gly are attached, the channels open. Despite having distinct sequence composition and length, the NMDAR chains share structurally and functionally conserved structural domains consisting of the extracellular region composed of the amino-terminal domain (ATD) and the ligand-binding domain (LBD), the transmembrane domain (TMD) and the intracellular C-terminal domain (CTD) (**Fig. 1**).

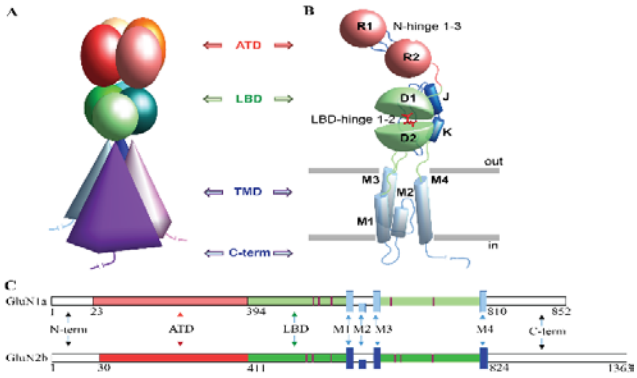

Fig. 1. Architectural organisation and topology of NMDA receptors. (**A**) A tetramer modular complex is formed with two GluN1 and two GluN2 chains schematized in (**C**). (**A**-**C**) Each chain is composed of the amino-terminal domain (ATD), the ligand-binding domain (LBD), the transmembrane domain (TMD) and the intracellular C-terminal domain (CTD). The ATD consists of two lobes, R1 and R2, connected by N-hinge 1-3, which is linked by a loop with the two lobes (D1 and D2) of LBD that is in turn coupled with the TMD formed by four helices, M1-M4. Structural domains in **A**-**C** are distinguished by colors that vary within the same spectral region for each domain denoting different chains. Ligand (G) in the LBD binding cleft is shown using red sticks.

Each domain of this modular receptor performs its specific functional roles and contributes to a well-coordinated complex system, accomplishing the regulation of cation transport between extracellular and cytoplasmic matrices of a cell (a flux of Ca^{2+} and Na^{+} into the cell and K^{+} out of the cell throughout the TMD) [5]. The other role of NMDAR relates to performing the modifications (by CTD) of a variety of proteins with the kinase or phosphatase functions [6].

Structure: The crystallographic structures of NMDAR for *Rattus norvegicus* (4PE5) [7] and *Xenopus laevis* (4TLL) [8], have revealed important insights into the receptor architecture and structure in the inhibited state with a closed channel. Furthermore, the structure of a limited resolution (cryo-EM) of *ratus* NMDAR in the inhibited and activated states was reported [9,10]. The coarse-grained modelling of *ratus* NMDAR has provided interesting information on the receptor dynamics during activation [11]. Combination of targeted molecular dynamics with application of the pore-lining helix repacking approach has generated a model of the open state of *Xenopus laevis* NMDAR [12]. However, despite the knowledge accumulated regarding NMDARs, the structure

of human NMDARs has not yet been characterized. Moreover, the structural movements leading to the ion channel gating in NMDARs have not been fully described, and the receptor activation mechanisms are still unclear.

Our preliminary results: We developed a 3D model of a human NMDA receptor (hNMDAR) and studied its molecular dynamics (MD) before and after the binding of functional agonist ligands, Glu and Gly. Using a limited in the time-scale MD data (300 ns) for this very large molecular system (~1 million simulated atoms), we evidenced the ligand-induced global reduction in molecular flexibility and a higher cooperative regularity of moving. We postulated that the ligand-induced synchronization of motion, identified on all structural levels of the modular hNMDAR, is apparently a fundamental factor in channel gating. We proposed a mechanistic dynamic model of the ligand-dependent gating mechanism in the hNMDAR: at the binding of the ligands, the differently twisted conformations of the highly flexible receptor are stabilized in a unique conformation with a linear molecular axis, which is a condition that is optimal for pore development. By searching the receptor surface, we have identified three new pockets, which are different from the pockets described in the literature as the potential and known positive allosteric modulator binding sites. This study, performed by Z. Palmai (PostDoc), was summarized in [13].

PROJECT

In this PhD project, we plan (i) to introduce the physical conditions essential for NMDAR activation, (ii) to apply alternative methods, which can lead to the channel opening, (iii) to develop new methods for analysis of simulations data, and (optionally) (iv) to extend the research topics focusing on the NMDAR properties.

Channel gating: As the 300 ns MD simulations was not sufficient to observe the channel gating, we will first continue these simulations with the longest extent in time (at the level of μs). Second, we will use the *accelerated molecular dynamics* that is an enhanced-sampling method that improves the conformational space sampling by reducing energy barriers separating different states of a system [14]. We will also apply the *parallel tempering*, known as '*replica exchange MCMC sampling*' and '*transition path sampling'* [15]**.** In simulations, together with '*atomistic*' model we will use '*coarse-grained*' model. This combination of different technics will be used for complete sampling of conformational spaces. The simulated system − NMDAR inserted into membrane and solvated with water − will be enriched with Ca^{2+} ions to model cation flow through the channel by *classical* MD simulation and by *steered* MD (SMD) simulation [16]. The parameters for the SMD simulation are mostly same as the classical MD, except for the application of an external force to pull a calcium ion from the intracellular environment to the extracellular region through the membrane channel.

Data analysis: MD data will be analyzed by the widely-used routines (RMSDs, RMSFs, PCA, clustering, …) and the more rigorous statistical techniques '*in-house-made*' [17-19] or adapted from literature. In particular, the conformational space explored by NMDAR during MD simulations will be analyzed with *ConfigScan* [17] that use a population estimate (density) of conformations in reduced space. The energy landscape will be presented as a 3D surface that display the evolution of dynamics as a random walk in this landscape. The energy landscape is useful for the tracing of visited main and secondary basins and for calculating the locations of energy minima. The channel characterization will performed with *Functional Mode Analysis* [20].

Next to channel gating, other questions remain open for the NMDAR functions, the deepest of which relates to the real gating mechanism. How is allosteric communication transmitted from the binding sites of ligands to the other regions, engaged in channel opening ? Which pockets are localized at proximity to communication pathways? The communication pathways will be analyzed with MONETA [18], the surface pockets search will be performed with FPockets [21] and TRAPP [22]. This study will open the door to different applications – structure-based drug design or a search for NMDAR interactions using presynaptic terminals.

Methodological development: While for the achieving of channel gating we will use different simulation techniques, the generated data will be multi-scale in a time, in a level of protein representation/description (all-atom, coarse-grain) and will feature both stationary properties and transition process (conformational

dynamics of NMDAR, ions flux through the channel). To compare these heterogeneous data and to extract the biologically relevant information, a development of the new method will be required.

Equipment: The PhD student will generate the MD simulation data on HPCs of GENCI (TGCC-Curie, IDRIS) and FUSION (ENS P-S & CentraleSupelec). The data processing and analysis will be performed using the local CMLA cluster TopDyn and performant personal working station. The immersive visualization platform SHIVA of ENS Paris-Saclay (Cachan), a unit of DIGISCOPE (http://www.digiscope.fr/en/platforms/shiva), will be used for a modelling at conception level and for the results representation.

This interdisciplinary project – a modelling of the biological process working as a physical engine represented by mathematical models with using bioinformatics technics – will be supervised by L. Tchertanov (Directeur de Recherche au CNRS, CMLA ENS Paris-Saclay) and A. Trouvé (Professeur ENS Paris-Saclay, CMLA).

References: (our papers are denoted in blue, with our names in bold)

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