How can protonation influence conformational switching: the curious mechanism of diphtheria toxin translocation (T-) domain

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Toxins and viruses excel at penetrating our membrane bilayers, often achieving this by pH-dependent conformational transitions [1,2]. One such case is found in the translocation domain (T-domain) of the diphtheria toxin, which uses to its benefit the acidification of the endosome as a translocation trigger for delivering its catalytic domain to the cell. Although the overall process is known, including the trigger role of His residues protonation [3], the molecular details of these translocation steps are not clear. Using constant-pH MD simulations, we intend to describe, at the molecular level, the effects of pH in key residues of the wt T-domain, the H223Q, H257Q, and E259Q single mutants, and the H223Q/H257Q double mutant. Combining pKa calculations and protonation data from our CphMD and NMR data from our collaborators it was possible to identify crucial pH-dependent features of the starting stages of the conformational transition of T-domain translocation. We confirmed the crucial role of the protonation in key histidines and how they impacted the activity of the mutated T-domains, while simultaneously revealed the presence of a strong latch-type mechanism between residues H223 and E259, which can modulate the trigger of the translocation domain.

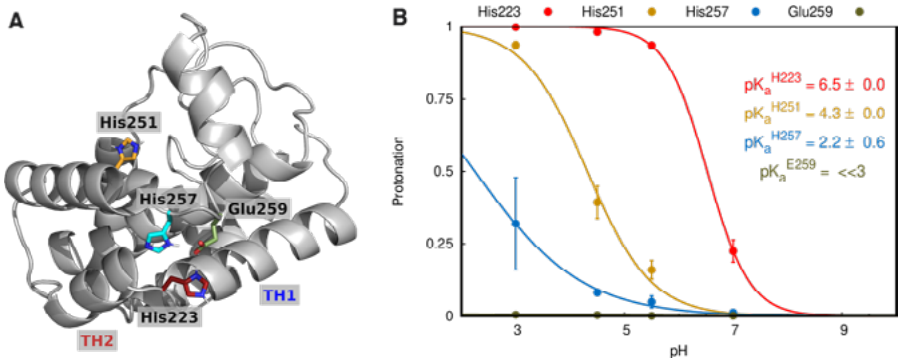

Figure1: Structural representation of the Wild type T-domain with the key Histidine residues highlighted (A) and pKa curves (B) of residues H223 (red), H251(yellow), and H257 (blue), and E259 (olive), obtained from CpHMD simulations.

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Extending the stochastic titration CpHMD method to AMBER14SB for acid-sensing ion channels modeling

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Acid-sensing ion channels (ASICs) are voltage-insensitive, proton-gated cation channels in membranes (Figure 1), widely expressed across the central and peripheral nervous systems, that are involved in physiological processes ranging from nociception to brain ischemia [1]. ASICs are activated by extracellular acidosis and ligands can act as antagonists or agonists for the channel's affinity for protons [2]. To discover ASIC activity modulators, one must understand the pH effects on the protein channel that result in altered cation membrane permeability. Constant-pH Molecular Dynamics (CpHMD) methods are pivotal to describe pH and its effects on the conformational space of biological systems [3]. The stochastic titration CpHMD (st-CpHMD) method has shown excellent performance over the years [3,4]. Until recently, our implementation of this method only supported the GROMOS 54A7 [3] and the CHARMM36m force fields [4], but we have now extended this method to also support AMBER 14SB, a force field particularly suited for studying disordered proteins and membrane channels. Using this method, we have started modulating ASICs activity at different pH values. We will present the preliminary results and some of the caveats that were surpassed to render the st-CpHMD method fully AMBER 14SB compatible.

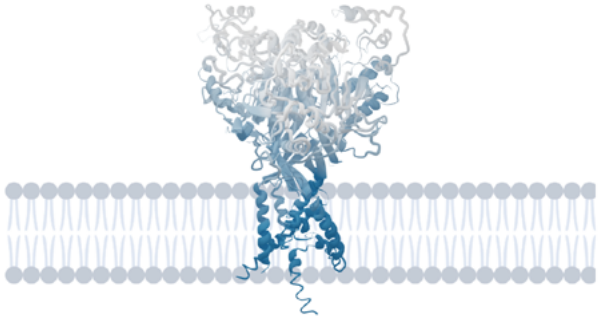

Figure 1. Cartoon representation of the ASIC protein.

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Optimizing the membrane protein system size for realistic MD simulations

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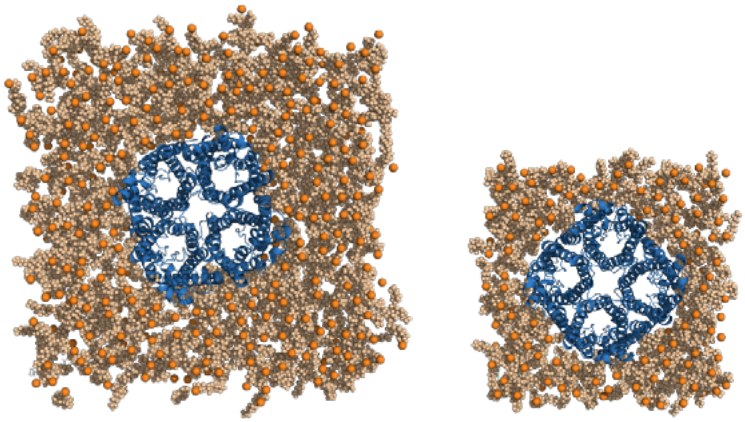
Aquaporins (AQPs) are responsible for permeating solutes across membranes. They can be divided into two subgroups: classical aquaporins, strictly selective for water, and aquaglyceroporins, those permeable to water and glycerol. The identification of AQP function modulators has been a difficult task due to the low target druggability and the unsuitability of the commonly used computational approaches. The crystallographic structures of AQPs often present inadequate conformations due to the crystal packing, which impairs the binding of the best modulator candidates. Despite attempts to use computational approaches to mitigate the mentioned problems [1], a fundamental issue remains: are we using the most adequate models, parameters, and force fields to simulate these membrane proteins?
In this work, we initially focused on studying the impact of different membrane sizes on AQPs' stability and function, followed by addressing the impact of different force fields. For this, we set up Molecular Dynamics (MD) simulations with the solved structure of the aquaglyceroporin hAQP7 (6QZI) [2] in a POPC lipid bilayer of different sizes (160, 200, 300, 400, and 500 lipids) as shown in Figure 1, with the Amber ff14SB and the CHARMM 36m forcefields. This protocol helped us understand the conformational behavior of the protein and identify the minimal lipid embedding environment required for a fully functional protein. These results will be useful for the identification of structural features that regulate the function of hAQP7, and later on of specific and efficient functional modulators that could be explored as therapeutic approaches for different diseases.



(a) (b)

Figure 1 (a) hAQP7 colored in blue embedded in a 500 POPC lipid bilayer, colored orange. (b) hAQP7 colored in blue embedded in a 200 POPC lipid bilayer, colored orange.

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