A novel US-CpHMD protocol to study the protonation-dependent mechanism of the ATP/ADP carrier.

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Electrostatic interactions are key participants in biomolecular processes, being the main driving force of molecular interactions [1]. However, describing these forces accurately is quite challenging using both experimental and computational methods. We propose a new computational protocol combining Umbrella Sampling with Constant-pH Molecular Dynamics [2] to overcome the time-scale limitations of conventional MD simulations and to allow protonation changes of all molecules in our simulations. Such protocol was employed to study the transport of two highly negatively charged molecules (ATP and ADP) through the ATP/ADP carrier (AAC), where electrostatic interactions have previously been shown to be very important [3]. Until now, these complete transport processes have not been studied thoroughly and with the correct description of pH. Therefore, our US-CpHMD simulations can bring an unprecedented realism to these complex processes by capturing both conformational and protonation changes occurring during transport.

In our work, the potential of mean force (PMF) profiles of our US-CpHMD simulations at pH 7 show a clear selectivity in the import of ADP, compared to ATP, while in the export, no selectivity was observed. We also observed that, in the import process, AAC was able to sequester both substrates at longer distances and transiently protonate them while crossing the cavity. These features were not observed in the export process and may be an important advantage to counteract the unfavorable mitochondrial membrane potential. Finally, we observed a substrate-induced disruption of the matrix salt-bridge network, which can promote the conformational transition (from the C- to the M-state) required to complete the import process. This work unraveled several important structural features where the complex electrostatic interactions were pivotal to interpret the protein function and illustrated the potentiality of applying the US-CpHMD protocol to other transport processes involving membrane proteins.

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