## Deep Learning Insights on $Na^+/I^-$ Symporter (NIS) transport cycle.

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We used cryoEM to determine the structures of apo-NIS and NIS with $I^-$ and an oxyanion ($ReO_4^-$) bound to it (Fig. 1a) [1]. All apo- and holo-structures showed a NIS molecule in an occluded conformation, poised to open up toward the intracellular medium. The previous lack of information about other conformations NIS adopts in the course of its transport cycle, particularly its outwardly open conformation (Fig. 1b), severely limited our understanding of the NIS transport cycle. To fill this gap in our knowledge, we carried out molecular dynamics simulations of NIS under close-to-physiological conditions (Fig. 1c). The goal of our computational study was to explore the conformational space of NIS and gain insights into the conformations it assumes over the course of its transport cycle (Fig. 1d).

We prepared a realistic simulation system that included the cryoEM structure of the NIS $Na^+ + I^-$ / $Na^+ + ReO_4^-$ complexes embedded in a central membrane with the mammalian lipid composition, with two half-membrane leaflets on either side (Fig. 1c). The external leaflets of this "sandwich" formed two separate chambers filled with ions at different concentrations, to mimic the physiological ion gradient. We simulated the membrane potential by applying a constant external electrical field to the system, thereby bringing about the equivalent of a membrane potential. The final molecular system comprised 320,999 atoms, including proteins, lipids, ions, and water. We are using this system to conduct unbiased and enhanced dynamics (metadynamics, Deep-Tica) MD simulations by means of the NPT statistical ensemble, using the program GROMACS (version 2023.3) with a modified CHARMM36 force field, to include non-bonded interactions between $I^-$ and the oxyanion $ReO_4^-$. In order to determine the effects of the potential on the outwardly open NIS events, simulations were conducted with three different membrane potentials—0, −70, and −140 mV—using the wild-type NIS with the anion and oxyanion bound to it, and using a double mutant with interesting transport characteristics.

The resulting molecular dynamics (MD) trajectories are currently being analyzed using a Python 3.11 script and extended to encompass rare events using a deep-learning approach. The Python script uses publicly available Python modules, including mdtraj (v1.9) for reading and manipulating trajectories, MDanalysis (v2.7) for preliminary analysis of trajectories, Pyemma (v2.5.12) for developing kinetics models, and mlcolvs and pytorch for deep-learning analysis, as well as several other modules (to a lesser extent). Metadynamics calculations of the transport of $I^-/ReO_4^-$ free energy along the transport path are currently being done for the different membrane potentials, using funnel bias metadynamics.

Topics; Keywords
Molecular dynamics software, Metadynamics, Deep learning, Lipids, Membrane potential, Proteins, Cryogenic electron microscopy, Statistical thermodynamics