Supplement material

Computer modelling reveals new conformers of the ATP binding loop of Na+/K+-ATPase involved in the transphosphorylation process of the sodium pump

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# Methods

***Comparative modeling of the open conformation***

For modeling, known structures of Na+/K+-ATPase deposited at the RCSB Protein Data Bank (http://www.pdb.org/) were used. In order to create the model based on the above mentioned sequence, the solved crystal structures of Na+/K+-ATPase with RCSB Protein Data Bank (http://www.pdb.org/) accession codes 3B8E ([Morth, Pedersen et al. 2007](#_ENREF_4)) and 3KDP([Morth, Pedersen et al. 2007](#_ENREF_4)) were used as the templates for our modeling. The multialignment of the chosen target (P50993, AT1A2\_HUMAN) sequence and the two templates (3B8E, 3KDP) for open conformation was prepared by MODELLER program (salign module) – see Figure S-1.

***Comparative modeling of the closed conformation***

As in the previous comparative modeling procedure, we have used the sequence P50993 (AT1A2\_HUMAN) for modeling of Na+/K+-ATPase in the closed conformation. However, the solved crystal structures of the RCSB Protein Data Bank (http://www.pdb.org/ - accession codes 3WGU, 3WGV and 4HQJ([Kanai, Ogawa et al. 2013](#_ENREF_3), [Nyblom, Poulsen et al. 2013](#_ENREF_5))) were used as the templates for our modeling – see Figure S-2.

***Molecular dynamics***

The PME method ([Darden, York et al. 1993](#_ENREF_2)) with a length parameter of 1 nm was used to describe Coulomb type electrostatic interactions and the cut-off method with a length parameter of 1 nm for the calculation of van der Waals interactions. As the first step of the MD simulation, the system of protein and water was energetically optimized using the method of steepest descents, followed by a conjugate gradient minimization algorithm with maximum 2.5x104 steps (see supplement material). The Berendsen coupling method ([Berendsen, Postma et al. 1984](#_ENREF_1)) was employed for the temperature and pressure coupling of a system to reflect the reference temperature of 300K and the pressure of 1 bar. The leap-frog integration with 104 steps was used for stabilization, with integration step of 1 fs, corresponding to 10 ps simulation time to reach the equilibrium of the rectangular box. This stabilized rectangular box was used for the main thirty simulations with 5x106 steps (2 fs single step), corresponding to 10 ns for each stabilization using the same simulation parameters as for the box stabilization (see Figure S-3).

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