Understanding Selectivity of Na^+/K^+-ATPase by Computational Approach

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Introduction

Na^+/K^+-ATPase (NKA) is a membrane protein that transports Na^+ ions out of the cell and brings K^+ ions into the cell against their concentration gradient. To function, NKA harnesses the chemical energy stored in an ATP molecule to cycle between two major conformational states during active pumping: a high affinity state for sodium, and a high affinity state for potassium. Recent crystal structures of both sodium- and potassium-bound conformations, now makes it possible to computationally investigate determinants of selectivity for the NKA.

Biological Importance

Primary Function

- Maintains Na+/K+ chemical gradient in cell

Related Disease

- Familial hemiplegic migraine 2 (FHM2)
- Rapid-onset dystonia Parkinsonism
- Heart failure

Objective

- *Determine protonation state in sodium-bound E1P and potassium-bound E2P conformations*

Extracellular ion transport by NKA is sixteen times more selective for sodium ions than potassium ions, while intracellular transport is 3000 times more selective for potassium than sodium.

Considering that the binding sites for sodium and potassium ions are the same, what is the origin of ion selectivity?

Recently it is shown affinity of the binding site depends crucially on protonation state of acidic binding residues, which might explain selectivity. The firs step, however, is to determine and compare the protonation state in both sodium- and potassium-bound conformations.

Methods and Simulations

Equilibrium and Metadynamics Simulations

Sodium state (PDB ID:4HQJ) and potassium state (PDB ID:3KDP) are inserted in POPC lipid bilayer and solvated with TIP3P water in 0.15 M Na^+/K^+ concentration using charmm-gui web interface. For metadynamics simulations, *distancez* collective variable implemented in NAMD software is used to confine one Na^+ or K^+ in a 30×20 $Å^2$ box in the binding site. Converged results obtained after 70 ns simulations.

PDB id	# atoms	# ions	# lipids	# water	Equilibrations	Metadynamics
4HQJ	233660	Na^+:185 , Cl^- : 164	314	56953	16 runs, each ~ 100 ns	1 run for 70 ns
3KDP	261581	K^+: 207 , Cl^- : 185	336	65179	16 runs, each ~ 100 ns	1 run for 70 ns

Protonation Combinations
E327, E779, D804, D808
E327-D804, E327-D808, E327-E229, E779-D804, E779-D808, E327-E779
E327-D804-D808, E327-E779-D804, E327-E779-D808, E779-D804-D808

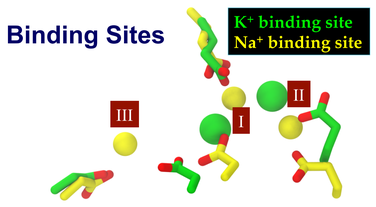
Free Energy Perturbation (FEP) simulations

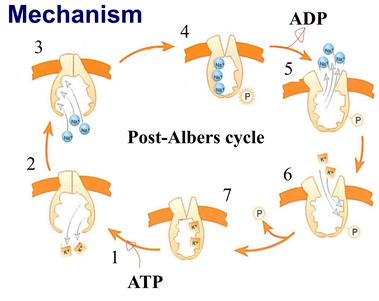
Custom Asp and Glu residue topologies are prepared that contain both protonated and un-protonated versions of Asp and Glu. Harmonic restrains are applied between appearing and disappearing residues to keep them close to each other during FEP. Initially, twenty FEP windows are used in chronological order each with 10-60 ns simulation time. Then, where possible, 20 windows are split in half to perform FEP with 80 windows.

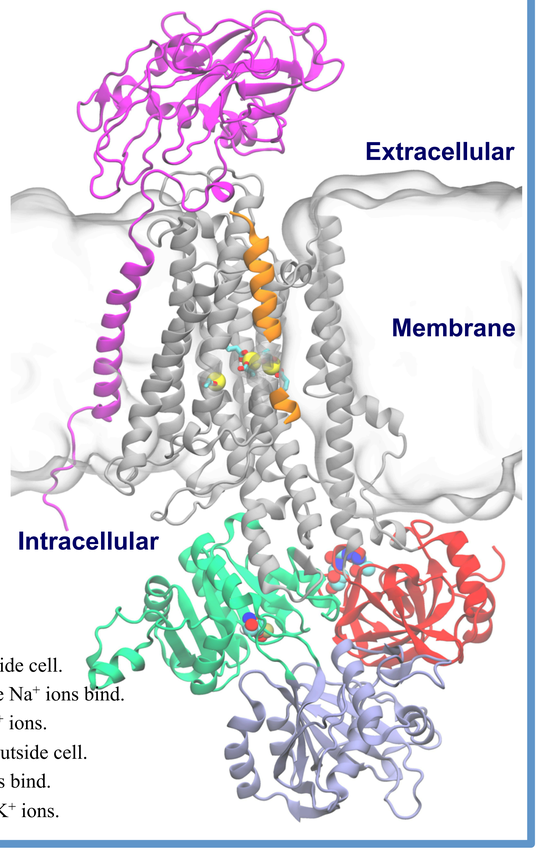
FEP Simulations	
Sodium State	D808 and E779-D808 | E779-D808 and E327-D808 | E779-D808 and E779-D804 | E779-D808 and E327-E779 | E327-D808 and E327-E779 | D804-D808 and D804-D808-E779
Potassium State	E327 and E327-D808 | E327-D808 and E779-D808 | E327-D808 and E327-E779 | E779-D808 and D804-D808 | E327-E779 and E327-E779-D808

	20 window FEPs	40 window FEPs
4HQJ	12 runs (6 forward and 6 backward) each window 10 to 50 ns	12 runs (6 forward and 6 backward) each window 10 to 20 ns
3KDP	10 runs (5 forward and 5 backward) each window 10 to 50 ns	10 runs (5 forward and 5 backward) each window 10 to 20 ns

Structure, Binding Sites, and Mechanism







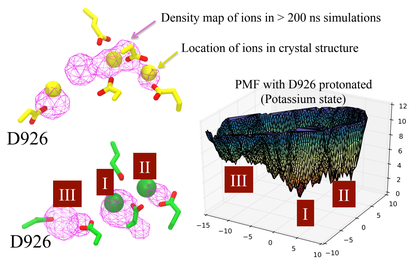
① ATP molecules binds to NKA.
② Intracellular gate opens and K^+ ions release inside cell.
③ Now NKA has high affinity for Na^+ ions. Three Na^+ ions bind.
④ Phosphorylation follows upon occlusion of Na^+ ions.
⑤ Extracellular gate opens and Na^+ ions release outside cell.
⑥ NKA has high affinity for K^+ ions. Two K^+ ions bind.
⑦ Dephosphorelation follows upon occlusion of K^+ ions.

Results and Discussion

Equilibration and Metadynamics results

Equilibrium simulations rule out majority of the protonation states based on their deviation from crystal structures.

Metadynamics reveal that D926 has to be protonated in potassium state.

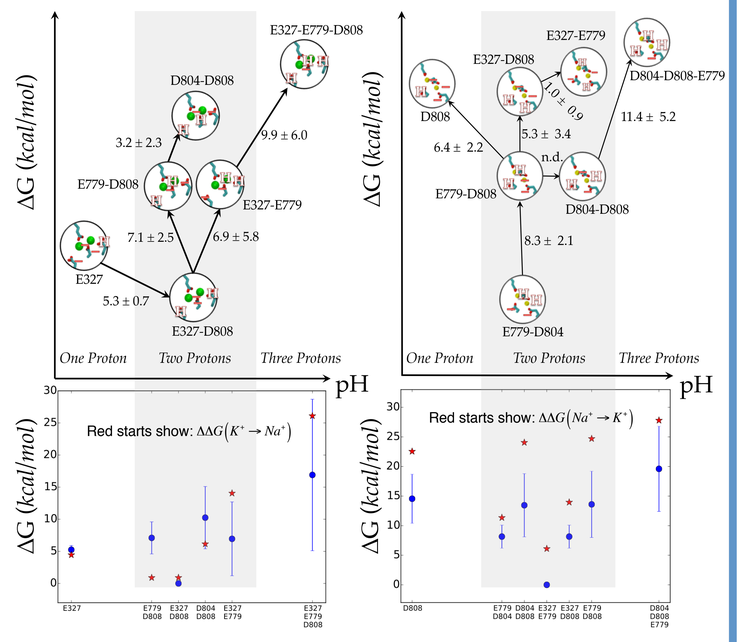


FEP results

FEP simulations reveal the most probable protonation states: E327 and D808 are protonated in K^+ state, but D804 and E779 are protonated in the Na^+ state.

Discussion

We showed that Na^+ and K^+ states have fundamentally different protonation states and switching between these protonation states seems to be necessary for proper ion binding. These findings suggest the possibility of a gate-opening conformational transition followed by ion occlusions that are coupled to changes in the protonation state.



Acknowledgements

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