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.

Biological Importance

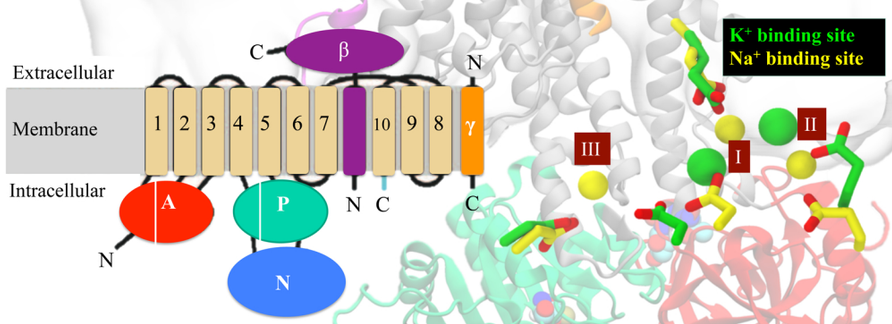
Primary Function

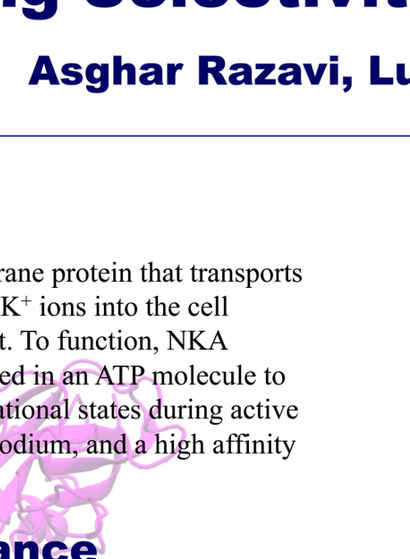
- Maintains Na^+/K^+ chemical gradient in cell

Related Disease

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

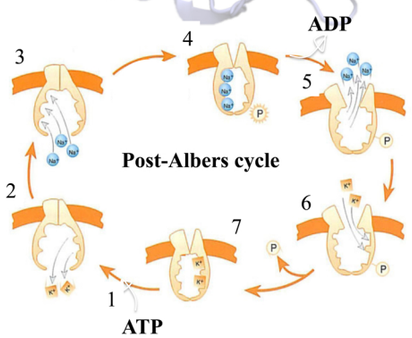
Structure





Mechanism

① ATP molecules binds to NKA.
② Intracellular gate open and K^+ release inside cell.
③ 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.



Objective

- Determine affinity of binding site for sodium and potassium.

Affinity depends crucially on protonation state of acidic binding residues. There are five acidic residues involved in binding, hence, total possible number of protonation states is 32 for each occluded state.

Methods and Simulations

Equilibrium and Metadynamics Simulations

Sodium state (PDBid:4HQJ) and potassium state (PDBid: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

Costume Asp and Glu residues 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.

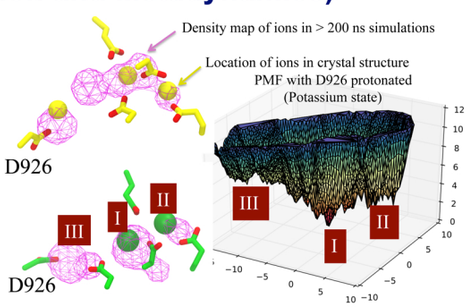
	20 window FEPs	40 window FEPs
4HQJ	12 runs (6 forward and 6 backward) each window 10 to 50 ns	12 runs (6 forward and 6 backward) each window 10 to 20 ns
3KDP	10 runs (5 forward and 5 backward) each window 10 to 50 ns	10 runs (5 forward and 5 backward) each window 10 to 20 ns

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

Results (Equilibration and Metadynamics)

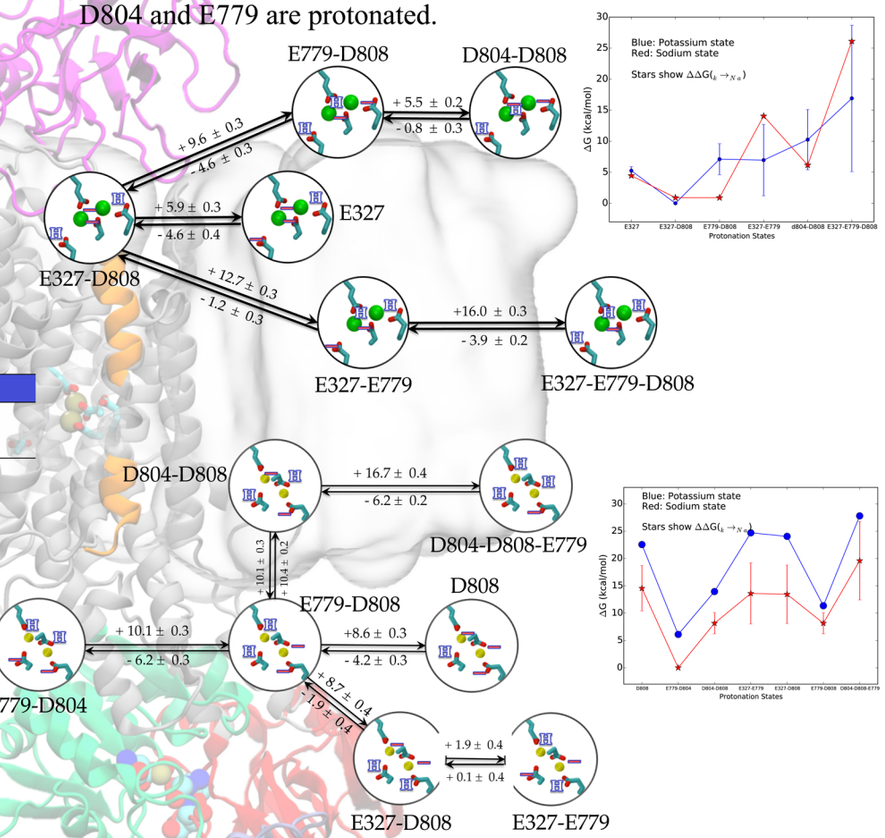
Equilibrium simulations rules out majority of the protonation states based on their deviation from crystal structure.

Metadynamics reveals that D926 has to be protonated in potassium state not in sodium state.



Results (FEP)

Equilibrium and metadynamic simulations rule out majority of the possible combinations. For the remaining, FEP simulations reveal the most probable protonation state: E327 and D808 are protonated in K^+ state, but for the Na^+ state, D804 and E779 are protonated.



Conclusions

We show 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.

Acknowledgments

- This research was supported in part by the National Science Foundation through major research instrumentation grant number CNS-09-58854.

References

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