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In Silico Model for NaAtm1 type ATP Binding Cassette Exporter Conformational transitions

Yinling Liu
Clemson University

Brian N. Dominy
Clemson University

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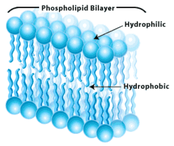
Yinling Liu, Brian N. Dominy. Department of Chemistry, Clemson University, Clemson, SC 29634.

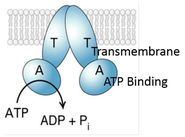
Introduction

ABC Transporter is Membrane Protein



- Membrane proteins are targets of over 50% of all modern medicinal drugs.
- 20–30% of human genes encode membrane proteins.

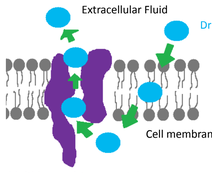
ATP Binding Cassette Transporters



- One of the largest membrane protein superfamilies.
- Active pumps: Use the energy provided by ATP hydrolysis for directional transport of diverse substrates.

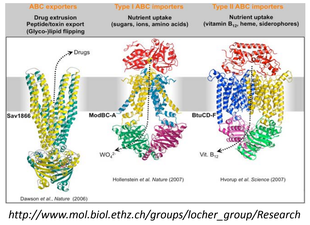
$$ATP + H_2O \rightarrow ADP + P_i$$
$$\Delta G^\circ = -30.5\ kJ/mol$$

Significance of ABC Transporters: Multi-drug Resistance



- Extrusion of cytotoxic compounds used for cancer therapy.

Importers & Exporters



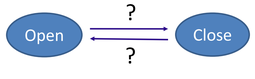
http://www.mol.biol.ethz.ch/groups/locher_group/Research

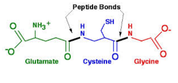
*Na*Atm1 ABC Exporters

- Glutathione derivatives serves as substrates and its overexpression in *E.coli* confers protection against Ag, Hg toxicity.
- *Na*Atm1 ABC exporter is homodimer: each subunit has 6 Transmembrane (TM) helices fused to nucleotide binding domain (NBD).

Open Ended Questions & Motivation

How dynamics and structural changes in the inward-open conformation state in wild-type?



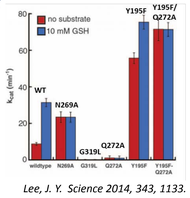


Reduced glutathione (GSH)

How ligand binding affect conformation changes?

How do mutations affect transport activity?

- What are the actual catalytic amino acids in transportation?
- How is binding affinity of apo protein different from its mutants?
- How do mutations cause conformational changes related to function?



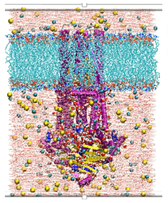
Lee, J. Y. Science 2014, 343, 1133.

Methods

Modeling Systems

- Apo, GSH-binding and their mutants.

1198 amino acids, 301 lipid molecules, 34860 solvent molecules, 115 Na^+ and 119 Cl^-, box size:110*110*146Å^3, total:163907 atoms.



Molecular Dynamics

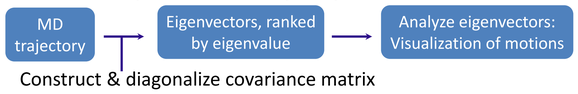
- Model atomic motion by solving Newton's equation.

- Powerful research tool in studying membrane proteins.

Principal Component Analysis

- A mathematical technique applied to MD simulation trajectories to detect the global, correlated motions of the system.

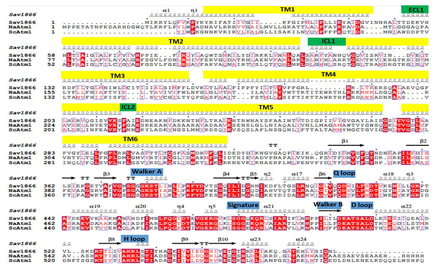
$$C_{ij} = \langle (x_i - \langle x_i \rangle)(x_j - \langle x_j \rangle) \rangle$$



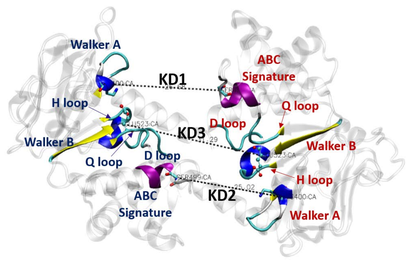
Results & Discussion

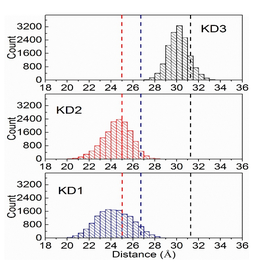
Conformation Changes During Transport

- The "alternating access" model:
  - ✓ Open, inward-facing conformation;
  - ✓ Closed, outward-facing conformation.
- Open, inward-facing conformation of *Na*Atm1 ABC exporters were observed in crystal structures.
- Sequence identity:
  - ✓ 45% with *Sc*Atm1; 32% with *Sav1866.*



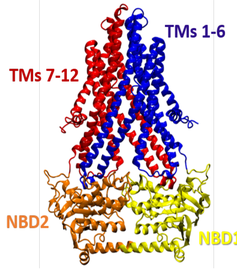
Movements of NBDs in Apo Protein

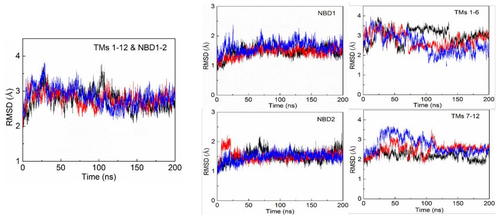




- Key distances (KD1~3) were used to monitor large conformational changes of NBDs.
- A 'semi-closed' geometry observed in MD that is closer than starting crystal structure suggested that two NBDs approached each other such that ATP molecules could be sandwiched between the walker A and the signature motifs.
- Asymmetric movement (ΔKD1 > ΔKD2) showed that there is allosteric communication between TMDs and NBDs.

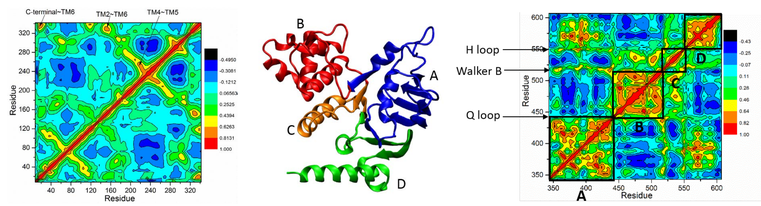
Cα-RMSDs for Individual Domains





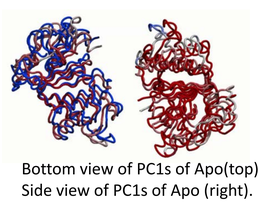
- All RMSDs converged well to around 2-4 Å and NBDs have relatively smaller RMSDs than that of TMDs implied that the ATP molecules acts as a molecular glue on the NBD-NBD interface to stabilize their dimer structure.

PCA Analysis of Domain Dynamics

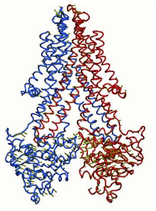


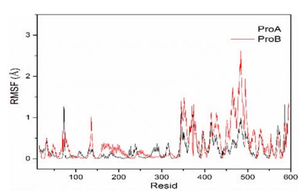
Covariance maps from snapshots of 200 ns cMD simulations of the apo protein TMD (left) and NBD (right)

- Key residue groups with high covariance in motion that brings the two NBDs close to each other are identified, which provides an important insight into the design of drugs.



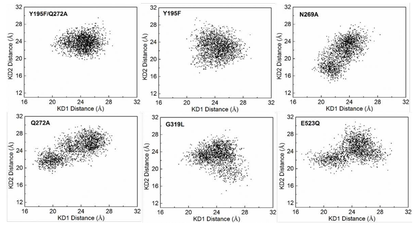
Bottom view of PC1s of Apo(top).
Side view of PC1s of Apo (right).





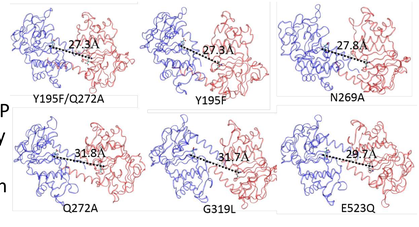
- Bottom view of NBD from PCA showed that two NBDs rotate in opposite directions, which resulted in the occurring of the asymmetric fashion of the NBD dimerization.

Conformation Changes of Mutants



- Differences in conformational ensembles were found between mutants and apo through statistical analyses of KD1 and KD2.
- Some mutants may contain conformations not competent to hydrolyze ATP.

- Strong interactions of two C-terminal α-helices prevent wider opening.
- MD simulations of apo mutants involving several residues in both the substrate and ATP binding sites indicate that those mutants may work by keeping the NBDs apart thus presumably inhibit ATP hydrolysis, resulting in a reduced basal ATPase activity.



Future Work

Investigate the export pathway between inward and outward close conformations using homology model. Is there any intermediate could be identified from each pathway? Are the intermediates different for different substrates GSH and GSSG? Study the binding affinity of GSH-bound protein as well as its mutants and thus whether be able to predict the catalytic activities of mutant variants.

Acknowledgement

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