Dynamical Coupling around a ring-shaped ATPase

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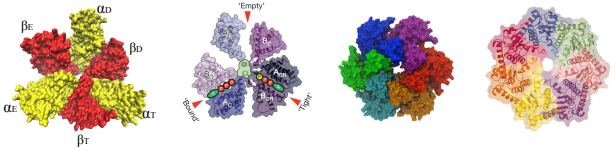
Introduction

Ring-shaped NTPases assemble multiple protein subunits into ring-like structures, with chemical sites for NTP binding and hydrolysis located at the interface of the neighboring subunits. To generate force or torque onto the nucleic acid or protein substrate threaded through the central channel of the ring, more or less coordination around the ring is needed. Currently we focus on studying the inter-subunit coordination in F_1-ATPase ring, where the three chemical sites work sequentially with or without the central gamma subunit. To probe how the sequential coordination arises even in the absence of the central subunit[1], we started simulating independent chemical site reactions and then gradually added a variety of neighbor-site couplings in the stochastic simulations. We notice that the sequential hydrolysis emerges under certain assumed couplings. Based on these clues, we perform atomistic molecular dynamics and coarse-grained simulations to enable targeted subunit conformational changes, and watch the neighbor-site responses dynamically or mechanistically. Comparative studies of a range of ring-shaped NTPases would be conducted to see how general and effective the inter-subunit coupling and coordination work.

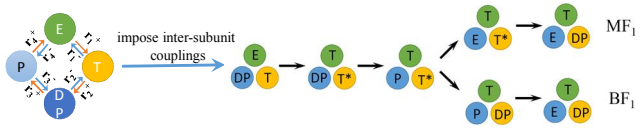


F_1-ATPase (no gamma) V_1-ATPase helicase ClpX

Methods

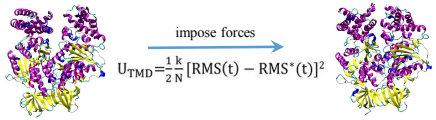
Stochastic simulation

Here we conduct Kinetic Monte Carlo simulation starting from unisite reactions, the rates of both mitochondrial F_1-ATPase (MF_1) and bacterial F_1-ATPase (BF_1) are obtained from experiments[2][3]. We impose indispensable inter-subunit couplings in order to generate sequential performance[4][5] around the ring and decide which couplings are the most important.



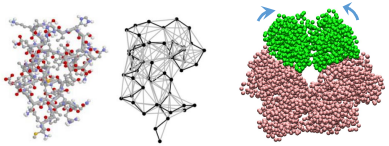
Targeted Molecular Dynamics simulation

Then we use Targeted Molecular Dynamics simulations to investigate the systems at an atomic level. We impose force on one of the three sites and monitor the responses from other sites.



Targeted Elastic Network Model

We also performed coarse-grained model (Targeted Elastic Network Model) to study the system from a long time scale.



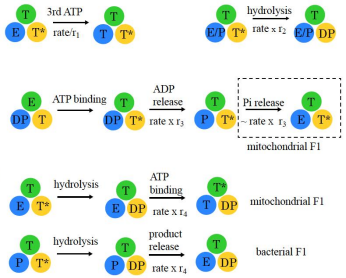
Stochastic simulation

Three independent subunits

For three independent subunits, our simulation results show independent and non-sequential patterns.

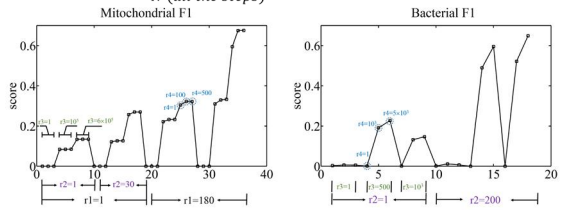
Simulation with imposed inter-subunit couplings

- At most two sites among the three can bind ATP.
- The site binds ATP earlier, hydrolyzes ATP earlier.
- Products release faster when the upstream site binds ATP.
- The site exchanges ATP/Pi faster when the downstream site hydrolyzes ATP.



results

Define score function: $s = \frac{n_{(sequential\ steps)}}{N\ (all\ the\ steps)}$



Some sequential patterns come out when we set the rates properly.

Targeted Molecular Dynamics

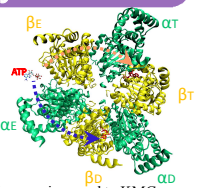
We think the sequential performance comes from the asymmetric structures and dynamical responses.

The transition path from current site to upstream site is:

pocket → current β → upstream α → upstream pocket

The transition path from current site to upstream site is:

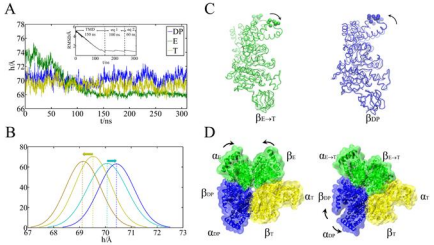
pocket → downstream α → downstream β → downstream pocket



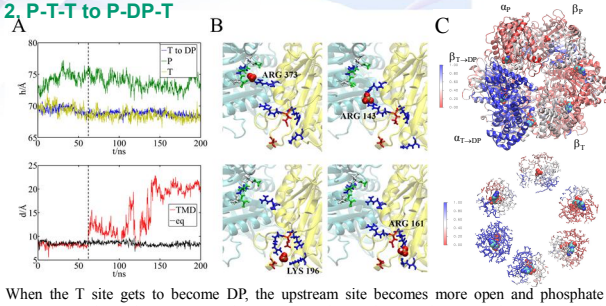
We conduct 4 Molecular Dynamics simulations to confirm the couplings we imposed to KMC simulation:

1. E-DP-T to T-DP-T

We measure the distance from DELSEED motif to the N-terminal plane to quantify the open degree of the structure. When the E site gets to be closed, its downstream DP site becomes more open.

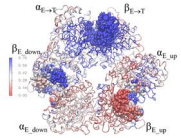


2. P-T-T to P-DP-T



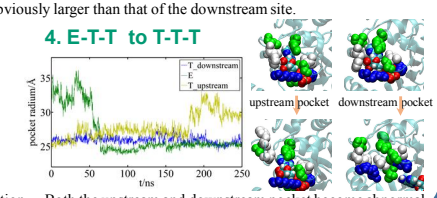
When the T site gets to become DP, the upstream site becomes more open and phosphate in the upstream pocket releases from P-loop. Fig C shows the correlation to the T→DP pocket, the correlation to the upstream site is obviously larger than that of the downstream site.

3. E-E-E to T-E-E



Downstream site shows larger correlation.

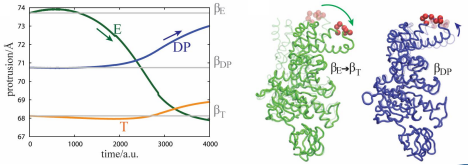
4. E-T-T to T-T-T



Both the upstream and downstream pocket become abnormal.

Coarse-grained ENM

We build an Elastic Network Model of the E-DP-T structure and mimic the close motion of the E site, the same as in the Molecular Dynamics simulation, the DP site becomes open .



Conclusion

We conduct several atomistic and coarse-grained simulations to confirm the couplings we imposed in the stochastic simulation.

I. E-DP-T to T-DP-T (ATP binding helps open the downstream site and ADP release)

II. P-T-T to P-DP-T (ATP hydrolysis help the Pi release in the upstream pocket)

III. E-E-E to T-E-E (asymmetric structures induce some bias)

IV. E-T-T to T-T-T (three ATP bound state is unstable)

References and Acknowledgements

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