

Structural rules

- The staircase architecture of PL1s
- 2~3 open interfaces in each ATPase complex
- At least 1 disengaged ATPase per complex; disengaged ATPases move to the top of the staircase
- ATPase interfaces adopt approximate binary states
- Translocation by ATPase power stroke

Kinetic measurements

- Nucleotide-proteasome interaction
- Degradation kinetics at different ADP concentrations
- ATP hydrolysis rate (published) and etc.

The FEL Model

Conformational coordinates
The FEL model parameters

ATPase mechanisms

- The cooperativity mechanism of the ATPases (section III)
- ATP-hydrolysis model for the ATPase complex (section IV)
- How are conformational changes coupled to the ATP cycle? (section IV)
- The mechanism of translocation directionality (section IV)
- And etc...

Global ATPase dynamics

Predictions

Kinetics-related validations

- Degradation kinetics at different ATP and ATP-γS concentrations: Quantitatively consistent
- Degradation kinetics of difficult-to-unfold (DHFR) substrate: Quantitatively consistent
- Kinetics of backward translocation: Quantitatively consistent
- Activities of Walker-B mutant proteasomes: Qualitatively consistent

Structure-related validations

- Steady-state conformational occupancies of wild-type and Walker-B mutant proteasomes: Qualitatively consistent
- Correlation between ATPase conformation and nucleotide status: Consistent

Approximations & assumptions

- The six ATPases share identical parameters
- The detailed process of conformational change is treated “adiabatic”; the rate is described by the Arrhenius equation
- The resistive force from substrate during translocation is approximated by two constants
- The effect of Lid-ATPase interaction is simplified as stabilizing S_A -like conformations.
- Phosphate release is assumed fast enough so that ADP-Pi state can be ignored.

Figure 4-figure supplement 1. The workflow of this study, including the observations used for model construction, experimental validations of the simulated ATPase dynamics, and the major insights into the ATPase mechanism.