A Free-Energy Landscape Approach Reveals the Global Conformational Dynamics of Proteasomal ATPases

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***Short Abstract* — A ring of heteroheximeric AAA+ ATPases on the 26S proteasome couples the chemical and mechanical cycles to achieve processive substrate unfolding and translocation during protein degradation. To understand the mechanism of how these ATPases coordinate, we developed a novel approach for delineating the nucleotide-dependent free-energy landscape (FEL) of the ATPases based on complementary structural and kinetic measurements. We used FEL to simulate the dynamics of the proteasome and found the predictions are consistent with diverse experimental observations. Our study reveals that the ATPase structure entails a unique free-energy minimum at each nucleotide state, dictating the cooperative movements of the six ATPases.**

***Keywords* — ATPase complex, proteasome, free-energy landscape (FEL), cooperativity**

I. Introduction

THE 26S proteasome, a 2.5-MDa protein degradation machine, consists of a barrel-shaped 20S core particle (CP) capped by 19S regulatory particles (RPs) on one or both ends. The RP harbors a heterohexameric ring of AAA+ ATPases that uses the energy from ATP hydrolysis to mechanically unfold substrates and translocate them into the CP for proteolysis. Recent advance in cryo-EM studies of the substrate-engaged proteasome reveals the molecular details of inter-domain and substrate-proteasome interactions. However, important questions pertaining to proteasome's intricate structural dynamics have not been approached, including how the chemical and mechanical cycles of an ATPase are coupled and how the six ATPases coordinate to achieve processive substrate unfolding and translocation.

To model the dynamics of the proteasome, a "sequential" model was proposed previously where the conformation and nucleotide-binding status of the ATPase hexamer may cycle consecutively through six different states with rotational equivalence. However, there are apparent contradictions in structural and functional studies. For example, only two out of the six predicted translocating conformational states were identified in cryo-EM studies; mutations on certain ATPases that impede their hydrolysis activity were well-tolerated in yeast. Therefore, we aimed to build an alternative model to better recapitulates the real dynamics of the proteasome as well as to resolve the problem of "symmetry-breaking".

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II. Results

We developed a novel approach to simulate the structural dynamics of the proteasome by constructing a physical model based on its nucleotide-dependent, free-energy landscape [1].

A. Structure-based construction of the FEL

To obtain the FEL, we first performed comparative analysis to identify the primary degrees of freedom (DOFs) of proteasome's conformational changes based on the published cryo-EM structures. This, in conjunction with the molecular details of the nucleotide-ATPase interactions, suggests a possible parameterization of the nucleotide-dependent FEL using nine parameters. We then determined these parameters by measuring the nucleotide-proteasome interaction kinetics in a single-molecule assay and from published results.

B. FEL predictions on the proteasome dynamics

We simulated the dynamics of the proteasome's ATPase complex as stochastic transitions on its FEL and subjected the predictions to extensive experimental examination using a sensitive fluorescent reporter for the translocation rate. We found that the FEL predictions were widely consistent with experimental measurements in this and previous studies.

The FEL model suggests that the coordinated movements of the six ATPases arise from the design of the ATPase hexamer entailing a unique energy-minimal conformation at each nucleotide-binding state, which can be triggered by the evolvement of energy-minimums on the FEL upon nucleotide hydrolysis and exchange. Interestingly, loss of the energy-minimal conformation at high ATP concentrations reduces the rate of translocation, as confirmed in a quantitative degradation assay. The FEL model also explains the "symmetry breaking" in cryo-EM state distributions and in functional mutations once we incorporate the Lid subcomplex-ATPase interactions into the model.

III. Conclusion

In summary, we demonstrate the practicality of simulating the dynamics of a large protein complex in a biologically-relevant time scale by determining its empirical FEL from structural and kinetic measurements. The results not only elucidate the mechanisms by which the proteasomal AAA+ hexamer operates in driving substrate translocation and, but also provide novel insights into the cooperative mechanism of ring-like ATPases.

References

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