Computer Simulations of Remodeling of the Weak Mechanical Resistance Substrate Protein Mediated by Clp ATPase Nanomachine

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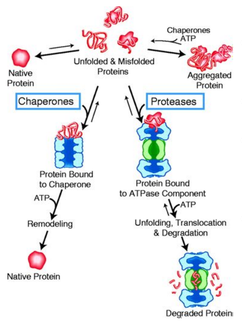
Abstract: Bacterial caseinolytic proteases (Clp) nanomachines support protein quality control through the degradation of misfolded or excess proteins which helps to maintain protein homeostasis. These hexameric nanomachines utilize cyclical allosteric motions to apply mechanical force onto the Substrate Protein(SP) that enables SP unfolding and translocation through the central pore of the machine. Haloalkane Dehydrogenase (Halo Tag) is a good candidate to study the unfolding and translocation mechanisms due to its biological and experimental importance. Topologically, HaloTag is an α-helix rich bulk protein that consists of a central buried-sheet registry. To study the mechanisms of the unfolding of HaloTag, we performed Langevin molecular dynamics coupled with targeted molecular dynamics (TMD). Here we used the Effective Energy Function (EEF1) implicit solvation model to represent the proteins. Our simulation setup consists of restrained or unrestrained geometries that mimic the experimental (Laser Optical Tweezers/ Atomic Force Microscopy) and in vivo degradation mechanisms respectively. We observe that, in the unrestrained geometry, ClpY nanomachine assists the HaloTag to orient in a direction that favors the remodeling of the SP but this process is stalled upon formation of a large number of non-native contacts from the α helices, specifically at α4 and α8. In the restrained geometry, SP rotation about the axial direction enables an efficient mechanism of removal of exposed α helices that overcomes the large number of non-native contacts.

Protein Quality Control



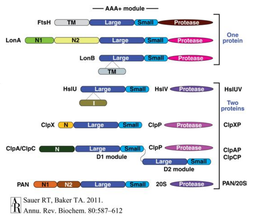
- Many native cellular proteins possibly do not interact with chaperones and are repelled to the degradative
- About 20% of newly synthesized polypeptides are degraded
- While recycling amino acids, in order to prevent potential fatal aggregation in the intracellular environment, misfolded or impared proteins are proteolytically degraded
- Degradation is also applied to eliminate completely functional proteins for regulatory purposes or when they are no longer required

Adrian O. Olivares, Tania A. Baker and Robert T. Sauer | Nature reviews Microbiology (2015)

Sue Wickner, Michael R. Maurizi, Susan Gottesman | Science (1999)

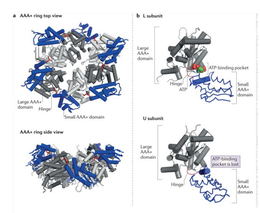
AAA+ (ATPase Associated with diverse cellular activities) proteases

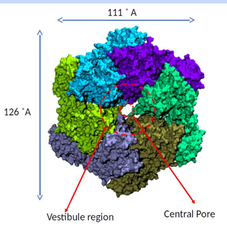
- AAA+ proteases are existed in all kingdoms of life.
- Families of AAA+ are in accordance to the sequences of their ATPase, protease, and auxiliary domains.
- AAA+ proteases exist in bacteria, mitochondria, and chloroplasts involve ClpXP, ClpAP, ClpCP, HslUV, Lon and FtsH2

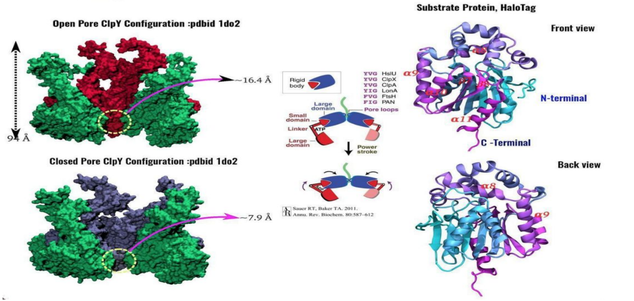


Sauer RT, Baker TA. 2011. Annu. Rev. Biochem. 80:587–612

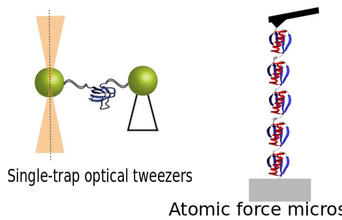
General Structure of Bacterial Caseinolytic Protease (Clp)

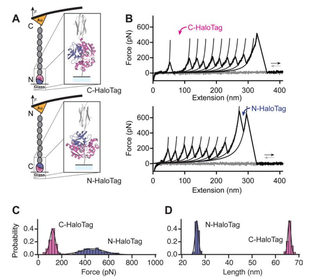






Protein Degradation probed by Single Molecular Experiments





Open Question

The Dependence of HaloTag unfolding and translocation pathways on the direction of force applied by ClpY

Implicit Model of ClpYΔI - HaloTag system

Bonded Interactions

$$V_{\text{bonded}} = \sum_{\text{bonds}} k_b(b - b_0)^2$$

$$V_{\text{angle}} = \sum_{\text{angles}} k_\theta(\theta - \theta_0)^2$$

$$V_{\text{improper}} = \sum_{\text{impropers}} k_\omega(\omega - \omega_0)^2$$

$$V_{\text{dihedrals}} = \sum_{\text{dihedrals}} k_\phi[1 + \cos(n\phi - \delta)]$$

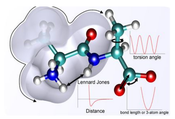
Langevin Dynamics

$$m\frac{d^2x}{dt^2} = -\nabla V_{\text{total}} - \gamma\frac{dx}{dt} + R_{(t)} \qquad F = -\nabla V_{\text{total}(r)} \qquad V_{\text{total}} = V_{\text{bonded}} + V_{\text{non-bonded}}$$

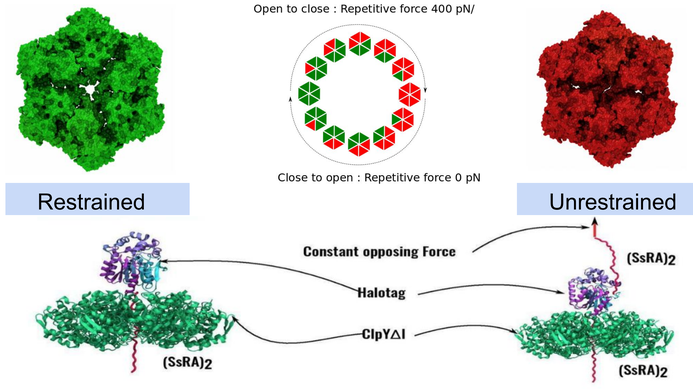
Non-Bonded Interactions

$$V_{\text{non-bonded}} = \sum_{\text{non-bonded}} \left\{ 4\epsilon \left[\left(\frac{\sigma_{i,j}}{r_{i,j}}\right)^{12} - \left(\frac{\sigma_{i,j}}{r_{i,j}}\right)^{6} \right] + \frac{q_i q_j}{\epsilon_1 r_{i,j}} \right\}$$

EEF1.1 Implicit solvent Model
T. Lazaridis and M. Karplus | proteins (1999)



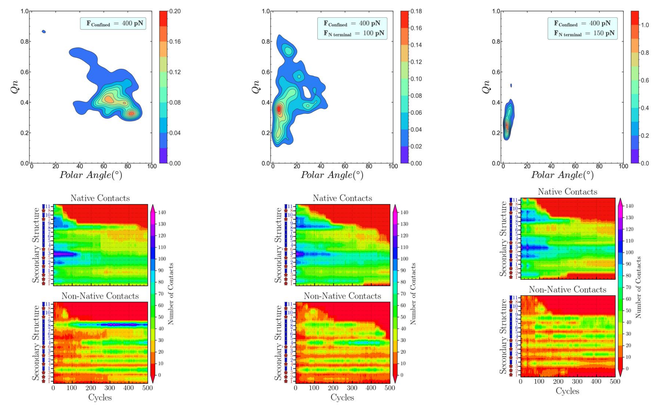
Targeted Molecular Dynamics Coupled with Repetitive force



The fraction of Native Contact (Qn) drops different rates with different orientation observed in unrestrained and restrained geometry

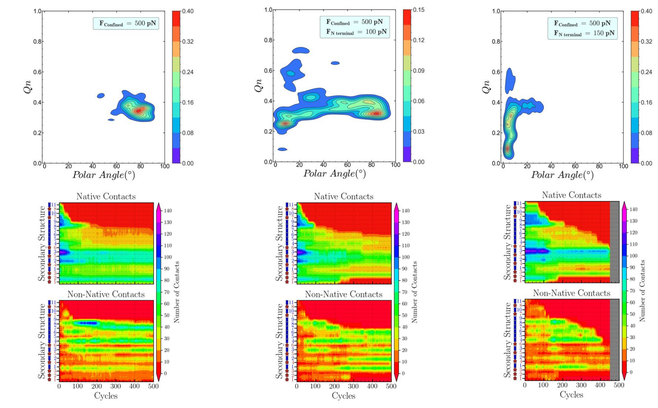
Unrestrained Geometry with F(mean) 400

Restrained Geometry with F(mean) 400 and Fnter 100/150



Unrestrained Geometry with F(mean) 500

Restrained Geometry with F(mean) 500 and Fnter 100/150



Conclusion

- Unfolding and Translocation of SP relate with local stability model
- Both Restrained and Unrestrained geometries, β registry unfolds utilizing zipping mechanism while force nearly applying perpendicular to β registry surface
- SP with Unrestrained Geometry efficiently unfolds and translocate w.r.t restrained geometry

Acknowledgment

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