PAN contains six identical ATPase subunits, we found that it exhibits three types of ATP binding sites: 2 high affinity conformations (ATP-binding sites), 2 with lower affinity (ADP-binding sites), and 2 with conformations that do not bind nucleotides. Correlation of ADP off rates with rates of ATP hydrolysis suggests that ADP leaving may be the rate limiting step in hydrolysis. ATP binding to the high and lower affinity sites has distinct functional consequences on the proteasome. With two ATP\(\gamma\)S molecules bound, PAN maximally stimulates opening of the gated channel for substrate entry into the 20S proteasome and has a high affinity for the 20S. However, the binding of 4 ATP\(\gamma\)S reduces PAN’s affinity for the 20S, which can be explained by steric hindrances in the PAN-20S interface. Because ATP binding drives the association of the C-termini of the ATPase with the 20S and only two ATPase subunits bind ATP for maximal function it’s likely that only two ATPases’ C-termini dock into the 20S at any time and in a predict-able pattern mirroring the cycllical pattern of ATP hydrolysis. This observation suggests how the symmetrically mismatched hexameric ATPase ring associates with the heptameric 20S proteasome to regulate substrate degradation.

104-MiniSymp
Eliminating ATP Binding in Specific ClpX Subunits Yields Functional ATP-Fueled Protein-Unfolding Machines
MIT, Cambridge, MA, USA.

AAA+ ring hexamers of ClpX utilize the energy of ATP binding and hydrolysis to unfold protein substrates and translocate the resulting denatured polypeptide into the degradation chamber of ClpP, an associated self-compartmentalized peptidase. Nucleotide-dependent conformational changes are necessary for ClpX binding to ClpP and to protein substrates as well as for allosteric function of ClpX. ClpX functions asymmetrically. In crystal structures, for example, four loadable subunits are competent for nucleotide binding, whereas two unloadable subunits are not. Moreover, ATP hydrolysis in one subunit can power conformational changes in the entire ring and protein degradation. Subunit-subunit interactions are crucial for ClpX function, but the communication mechanisms are poorly defined. For instance, it is not known whether the conformations of loadable and unloadable subunits remain fixed or interchange during the hundreds of cycles of ATP hydrolysis that are required for protein unfolding, translocation, and degradation. To probe subunit communication, we constructed covalently linked mutant hexamers in which the nucleotide affinity of specific subunits was dramatically reduced by mutations in the Walker A, sensor-II, or box II motifs and developed novel fluorescence assays to probe nucleotide-binding cooperativity as well as ATP binding to specific subunits. Strikingly, ClpX pseudo-hexamers bearing two opposed subunits with severe ATP-binding defects hydrolyze ATP at near normal rates and are able to unfold and translocate substrate molecules. For some variants, machine function was retained despite sensor-II-dependent abrogation of the positive cooperativity of ATP binding. Because hexamers with two ‘‘permanent’’ unloadable subunits retain basic ClpX machine functionality, subunit switching between unloadable and loadable conformations does not appear to be required for protein unfolding or translocation. Our results are most consistent with probabilistic models of ATP binding and hydrolysis rather than strictly sequential models.

105-MiniSymp
The Structure of the Dynein Motor Domain
Andrew P. Carter.
MRC Lab of Molecular Biology, Cambridge, United Kingdom.

Dyneins are microtubule-based motor proteins that power ciliary beating, transport intracellular cargos, and help to construct the mitotic spindle. Evolved from ring-shaped hexameric AAA-family deoxyribonuclease triphosphatases (ATPas), dynein’s large size and complexity have posed challenges for understanding its structure and mechanism. Here, we present a 6 angstrom crystal structure of a functional dimer of two ~300-kilodalton motor domains of yeast cytoplasmic dynein. The structure reveals an unusual asymmetric arrangement of ATPase domains in the ring-shaped motor domain, the manner in which the mechanical element interacts with the ATPase ring, and an unexpected interaction between two coiled coils that create a base for the microtubule binding domain. The arrangement of these elements provides clues as to how adenosine triphosphate-driven conformational changes might be transmitted across the motor domain.

106-MiniSymp
The Fellowship of the Ring
Francis T.F. Tsai, Amadeo B. Biter, Jungsun Lee, Nuri Sung, Sukyeong Lee.
Baylor College of Medicine, Houston, TX, USA.

AAA+ ATPases are a group of conserved, ring-forming, ATP-dependent molecular machines that harness the energy of ATP binding and hydrolysis to drive diverse biological activities, ranging from protein unfolding to DNA translocation. While common mechanisms are apparent, distinct structural features exist that confer specific functions. Yeast Hsp104 and its bacterial ortholog ClpB are ATP-dependent protein disaggregases, which, together with the cognate Hsp70 system, rescue stress-damaged proteins from previously aggregated state. The ability to do so is strictly dependent on the M-domain that forms an 85-A long coiled-coil and is a hallmark of the ClpB/Hsp104 family. While substrate translocation through the ClpB hexamer is essential for protein disaggregation, it remains unclear how ATP is coupled to the power stroke that drives protein unfolding and translocation. At this mini-symposium, I will present our latest, unpublished data on the structure, mechanism, and function of ATP-dependent protein disaggregases. This work was supported by grants from the National Institutes of Health (AR076239, GM067672, and RR002250) and the Robert A. Welch Foundation (Q-1530).

Platform: Computational Methods

107-Plat
Equilibrium Sampling using a Weighted Ensemble of Dynamical Trajectories
Carson Stringer, Matthew Zwier, Lillian Chong, Daniel Zuckerman.
Univ Pittsburgh, Pittsburgh, PA, USA.

The ‘‘weighted ensemble’’ (WE) method, originally designed for non-equilibrium path sampling, can also be applied to equilibrium sampling [J. Chem. Phys. 133: 014110 (2010)]. WE is a parallel method with multiple trajectories coupled periodically through configuration space in a statistically rigorous way. We demonstrate the first applications of equilibrium WE to molecular systems. Because ‘‘ordinary’’ dynamics trajectories are employed, the approach can simultaneously yield rate constants for transitions among arbitrary states.

108-Plat
Simple and Efficient Calculation of Scattering Intensities of Proteins in Solution from Atomistically Detailed Structures
Juergen Koefinger, Gerhard Hummer.
NIH/NIDDK, Bethesda, MD, USA.

Solution scattering experiments provide signatures of the atomistic structure of proteins, nucleic acids, and biomolecular assemblies under near physiological conditions. Coarse-grained structural properties like shape and volume can be inferred from an essentially model-free analysis using information in the small-angle regime. The wide-angle regime offers higher resolution information which can be interpreted using atomistic models. A combination of time-resolved scattering experiments, molecular simulations, and ensemble refinement methods helps reveal structural changes in proteins as they perform their biological functions. To address this challenge, we developed a mathematically simple and computationally efficient method to calculate the scattering intensity of atomistically detailed structures of proteins in solution. Compared to other methods, our method, which is based on Debye’s formula, has the advantage that there is no trade-off between computational efficiency and accuracy in the promising wide-angle regime. We present results for a variety of proteins and different water models and discuss some fundamental differences in interpretation of small- and wide-angle data.

109-Plat
The Simultaneous Determination of Diffusion Coefficients and PMFs through the OFR Method
Brandon, MB, Canada.

Progress in nonequilibrium, bidirectional work theorems have lead to the development of an important theory, known as the forward-reverse (FR)