electrostatics free energy in guiding the functional cycle in other actin-based myosin motors.

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Different 3D Domain-Swapped Oligomeric Cyanovirin-N Structures Suggest Trapped Folding Intermediates
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Although it has long been established that the amino acid sequence encodes the fold of a protein, how individual proteins arrive at their final conformation is still difficult to predict, especially for oligomeric structures. Here, we present a comprehensive characterization of oligomeric species of cyanovirin-N that all are formed by a polypeptide chain with the identical amino acid sequence. Structures of the oligomers were determined by X-ray crystallography, and each one exhibits 3D domain swapping. One unique 3D domain-swapped structure is observed for the trimer, while for both dimer and tetramer, two different 3D domain-swapped structures were obtained. In addition to the previously identified hinge-loop region of the 3D domain-swapped dimer, which resides between strands β5 and β6 in the middle of the polypeptide sequence, another hinge-loop region is observed between strands β7 and β8 in the structures. Plasticity in these two regions allows for variability in dihedral angles and concomitant differences in chain conformation that results in the differently 3D domain-swapped multimers. Based on all of the different structures, we propose possible folding pathways for this protein. Altogether, our results illuminate the amazing ability of cyanovirin-N to proceed down different folding paths and provide general insights into oligomer formation via 3D domain swapping.

147-Plat
Structural Basis of Conformational Transitions Involved in Pseudopilus Assembly and Stability
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Type II secretion systems (T2SS), type IV pili and archaeal flagella use a conserved plasma membrane machinery to assemble helical filaments promoting macromolecule transport or motility. Using electron microscopy, we observed structural heterogeneity, predominantly twist-angle variations, in T2SS pili. Based on the measured twist-angles we generated 2500 pseudopilus structural models, recapitulating this conformational heterogeneity and twist-angle continuum, by an automated modeling procedure. We analyzed the ensemble of pilus models by making use of self-organizing maps, which allowed us to define a transition path between major conformational basins, leading from low to high twist-angles. We further characterized the free energy landscape of the pilus by performing short molecular dynamics calculations starting from each individual model combined with GBSA analysis. Experimental functional analysis based on predictions derived from our models showed that specific contacts at P-P^+3 and P-P^+4 interfaces determine fiber stability. Their disruption led to loss of surface pili but did not affect pseudopilus assembly and protein secretion. The results support the one-start assembly model for pseudopili and related fibers, and challenge the piston model of the type II protein secretion.

148-Plat
A Single Inter-Domain Salt Bridge within the Human Argonaute 2 Protein Crucially Affects Protein Folding and Consequently Enzymatic Activity
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RNA interference is a highly complex process involved in posttranscriptional gene regulation. Argonaute (Ago) proteins are the key component of the RNA-induced silencing complex (RISC) and mediate RNA interference (RNAi) in association with small RNAs. Ago2 proteins are composed of four domains: N, PAZ, Mid and PIWI, tethered by L1 and L2 linker regions [1, 2]. In the binary complex of Ago2 and single stranded RNA, the 3'-end of the RNA is bound to the PAZ domain and the 5'-phosphate is anchored within a binding pocket in the Mid domain. The PIWI domain harbors the active site, which is composed of a catalytic tetrad as observed in RNase H.
In the present study, we performed MD simulations on the hAgo2 protein in complex with a bound miRNA (pdb 4F3T). The protein undergoes prominent breathing motions dominated largely by movements of the PAZ and N domains. Most interestingly, we observed the transient formation a thitherto-undescribed inter-domain salt bridge. The introduction of a mutation preventing formation of this salt bridge drastically affected nucleic acid binding properties of recombinant hAgo2 [3] and abolished the enzymatic activity. In summary, the missing salt bridge not only affects overall protein conformation and stability but also seems to be crucial for proper positioning of the small RNA.
1. Elkayam, E., et al., (2012) The structure of human Argonaute-2 in complex with miR-20a. Cell 150(1), 100-110.
2. Schirle, N.T. and MacRae, I.J., (2012) The crystal structure of human Argonaute2. Science 336(6084), 1037-1040.
3. Deerberg, A., et al., (2013) Minimal mechanistic model of siRNA-dependent target RNA slicing by recombinant human Argonaute 2 protein. PNAS in press.

149-Plat
Mechanisms of Substrate Degradation by Energy-Dependent Proteases
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Energy-dependent proteases of the AAA^+ family catalyze the highly specific protein degradation involved in cellular protein quality control and the regulation of numerous vital processes, yet the detailed mechanisms coupling ATP hydrolysis with mechanical substrate unfolding and translocation remain poorly understood.
Our cryo-EM structural studies of the eukaryotic 26S proteasome show that its heterohexameric AAA^+ ATPase ring adopts a conformation with pronounced spiral-staircase arrangement of subunits in the absence of substrate, but transitions into a translocation-competent conformation upon substrate engagement. This substrate-engaged ring conformation is characterized by uniform interfaces between the six ATPase subunit, a widened central channel coaxially aligned with the peptidase, and a rearranged, more planar spiral orientation of ATPase subunits that suggests a highly coordinated rapid progression of ATP-hydrolysis events around the ring.
This coordinated ATP hydrolysis mechanism is further supported by our optical tweezers single-molecule studies of the related bacterial protease ClpXP and may be a general feature of AAA^+ translocases. ClpXP translocates substrate polypeptides in steps with constant frequency but variable length, depending on the number of ATP-hydrolyzing subunits.

150-Plat
Structure, Dynamics, Evolution and Function of a Major Scaffold Component in the Nuclear Pore Complex
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The Nuclear Pore Complex, composed of proteins termed Nucleoporins (Nups), is responsible for the nucleo-cytoplasmic transport in eukaryotes. NPCs form an annular structure composed of the nuclear ring, cytoplasmic ring, a membrane ring, and two inner rings.
Nup192 is a major component of the NPC's inner ring. We report the crystal structure of Saccharomyces cerevisiae Nup192 residues 2 to 960 [ScNup192(2-960)], which adopts an α-helical fold with three domains (i.e., D1, D2 and D3). SAXS and EM studies reveal that ScNup192(2-960) could undergo long-range transition between an ''open'' and ''closed'' conformations. We obtained a structural model of full-length ScNup192 based on EM, structure of ScNup192(2-960), and homology modeling.
Evolutionary analyses using ScNup192(2-960) structure suggest that NPCs and vesicle coating complexes are descended from a common membrane-coating ancestral complex.
We show that suppression of Nup192 expression leads to compromised nuclear transport and hypothesize a role for Nup192 in modulating the permeability of the NPC central channel.

151-Plat
Structural Gymnastics by Proteins make the Clock Mechanism go Round and Round
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Endogenous clocks regulate metabolism, physiology and behavior of most organisms in anticipation of daily swings in ambient light and temperature by