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SAXS and the Working Protein

In this issue of *Structure*, Davies et al., 2005 present shape reconstructions for the molecular motor p97 using small angle X-ray scattering (SAXS) and offer insights into how ATP consumption is coupled to cyclical domain motions. This work emphasizes the emerging potential of SAXS for visualizing the workings of biological machines in solution.

AAA proteins are oligomeric ATPases that take part in a remarkably wide range of cellular processes, including protein unfolding and degradation, cell motility, transcriptional regulation, and DNA replication (Patel and Latterich, 1998). Despite this wide ranging functionality, a common mechanistic theme unites these proteins—they all use the coordinated binding and hydrolysis of ATP to generate mechanical forces for executing tasks such as pulling proteins apart or feeding them through pores (Neuwald et al., 1999). AAA proteins are identified by the presence of one or more copies of a highly conserved ~220-residue module that includes an ATP binding domain. Additional domains present in particular AAA proteins result in the coupling of ATP-generated conformational changes to a wide variety of functional outputs.

Valosin-containing protein (VCP)/p97 is an AAA protein that is most notably involved in extracting misfolded proteins from the endoplasmic reticulum (Wang et al., 2004). P97 forms a three-tiered homohexameric ring, each subunit of which consists of a unique N-terminal effector domain (N), thought to be responsible for interactions with target proteins, followed by two AAA modules, denoted D1 and D2. One of the mysteries surrounding p97 function, and indeed that of other AAA proteins, is how ATP consumption is linked to force generation. High-resolution crystal structures are available for the hexameric rings formed by the N-D1 domains (Zhang et al., 2000) and lower resolution views are available for full-length p97 in the nucleotide free and ADP-AlF_x states (DeLaBarre and Brunger, 2003; Huyton et al., 2003). CryoEM reconstructions in various nucleotide bound states have suggested that relatively small domain motions occur during ATP binding and hydrolysis (Rouiller et al., 2002). These studies were unable to resolve the position of the N-domain in states other than that in which p97 is bound to the transition state analog, ADP-AlF_x. This inability to visualize the N-domain was attributed to flexibility. The fit between the cryoEM maps and the crystal structure for the ADP-AlF_x state, in which the N-domains were resolved, was not exact, perhaps because of packing effects that might alter the structure in the crystals (Brunger and DeLaBarre, 2003).

In this issue, Davies et al. (2005) use SAXS to reveal structural changes that occur in p97 during the ATP hydrolysis cycle. We are in the midst of a revolution in the application of SAXS to the analysis of large molecular complexes. Although this classical technique has been in use for years, most structural biologists have assumed that the method is limited to providing a restricted set of information, such as the radius of gyration of the assembly, or at best, highly smoothened approximations to the rough and corrugated shapes of protein and nucleic acid complexes. The breakthrough in the application of SAXS to biomolecules has come from the utilization of a simulated annealing approach in which the shape of the complex is modeled by a large number of close-packed beads, which are moved around so as to match the observed scattering profile as best as possible (Svergun, 1999; Svergun and Koch, 2002). The remarkable result is that in cases where we know what to expect for the shape of the molecular complex in solution, the SAXS results have provided a stunning correspondence in molecular envelopes. While these early correspondences were met with skepticism by many structural biologists, the steady accumulation of convincing results for many different systems is now leading to a sense of optimism that SAXS might provide a reliable window into macromolecular conformation in solution. The latest results from Weis and coworkers (Davies et al., 2005) should help with this ongoing revival of SAXS as a premier biophysical technique.

Skepticism regarding SAXS arises from the fact that shape reconstruction by this method is an inherently underdetermined problem, that is, more than one bead arrangement will account for the observed scattering adequately. To minimize this problem, it is common practice in SAXS reconstructions to run multiple trials and average them to produce a model that may be more likely to correctly represent the molecular shape. Ultimately however, the most satisfying validation for a SAXS shape reconstruction is to have at hand a high-resolution crystal structure in the same state (e.g., a particular nucleotide state) for comparison. Relative changes that occur in different states can then be ascertained with greater confidence. In this case, a crystal structure of p97 bound to the transition state analog, ADP-AlF_x, was available (DeLaBarre and Brunger, 2003). Comparison with the corresponding SAXS reconstruction of p97 bound to ADP-AlF_x reveals that it recapitulates the overall shape of the crystal structure remarkably well (Figure 1).

With this established the authors go on to carry out shape reconstructions using SAXS data for p97 in the presence of AMP-PNP (ATP mimic) and ADP, and also without nucleotide bound. What they find are significant changes in the shape. Initially, in the absence of bound nucleotide, p97 appears as a relaxed and relatively flexible hexameric ring with the N-domains coplanar with D1. The bulk of the change in shape appears to take place on binding and hydrolysis of ATP, which results in a progressive tightening of the structure concomitant with a sliding of the N-domain ring in and out of the plane of the D1 ring. Additionally, there are changes in the pore size and rotation of the D2 ring relative to the D1 ring. Repeating this analysis using a

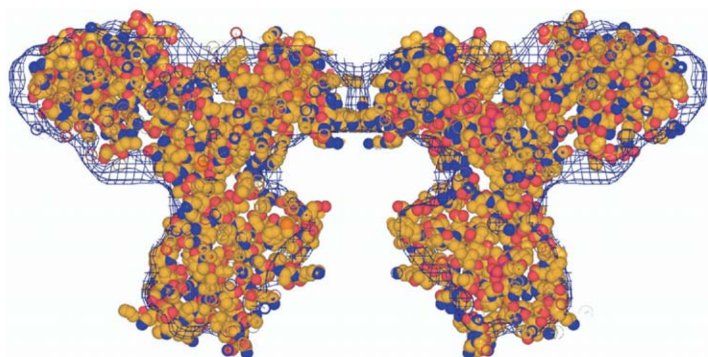

Figure 1. Overlay of the p97:ADP-AlF$_x$ Crystal Structure (PDB code 1OZ4; Colored Spheres) onto the Corresponding SAXS Shape Restoration (Blue Mesh)

shortened construct in which the D2 domain was deleted, the authors show that the N-domain does not move during ATP hydrolysis by D1. Thus, the energy of ATP hydrolysis is proposed to be transmitted from the D2 domain to the N-domain via the inert D1 domain. This is in agreement with the nucleotide exchange assays, showing that only the D2 domain is capable of ATP hydrolysis in full-length p97. The active site for ATP hydrolysis is generally found at the interface of successive subunits in most AAA oligomers. ATP hydrolysis usually results in motions that can lead to weakening of the interfaces. Thus, the function of the D1 domain is likely to maintain p97 hexamerization (Wang et al., 2003) during catalysis and to act as a rigid platform through which conformational changes can be transmitted. The authors postulate that following binding of effector proteins by the N-domains, this concerted up-down shifting of the N-domain ring could be responsible for performing useful work. Interestingly, in the related protein NSF, the positions of the D1 and D2 rings are swapped where the force-producing ring is closest to the N-domains in NSF (Brunger and DeLaBarre, 2003). This different organization is likely a reflection of their distinct cellular functions.

Determining the precise molecular correlates for ATP-coupled motions will ultimately require high-resolution crystal structures in all nucleotide states. Nonetheless, this study highlights the value of the SAXS method as a probe for large conformational transitions in solution. We look forward to the continuing development of synchrotron beamlines that are optimized for macromolecular SAXS and the improvement of the analysis programs so that they are more robust and efficient. Particularly exciting is the feasibility of carrying out time-resolved experiments using SAXS, which might allow shape reconstructions for evolving molecular changes after a chemical transformation is triggered.

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