STRUCTURAL BIOLOGY

Molecular machinery in action

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Nuclear magnetic resonance is the best way to study motion in proteins, but it could be applied only to small systems. This limitation has been overcome to reveal the dynamics of a large protein complex.

If biology were a car, structural biologists would be looking under the bonnet to find out how the engine works. Put more prosaically, structural biology aims to understand how biology works at the molecular level. Much information is gleaned by studying at atomic resolution the three-dimensional structures of molecules that make up living organisms, and the interactions of these molecules with one another.

But, just as a photograph of a car engine doesn't tell us how that engine actually works, static biological structures alone may not suffice for us to work out the functional mechanisms of biomolecules. Instead, a video of the structural dynamics — analogous to observations of a running car engine made from inside and out — would be much more revealing. Nuclear magnetic resonance (NMR) spectroscopy provides just such an observational tool, allowing precise measurement of both the range of molecular motion and the speed at which elements within biological structures move. On page 618 of this issue, Sprangers and Kay[1] report their use of an original NMR technique to garner details of atomic motions in a molecular 'machine': the 20S proteasome core particle (CP)*. With a molecular mass of 670 kilodaltons (kDa), this protein complex is much larger and more complicated than anything previously studied at this level of detail with NMR. The study provides fresh insight into how the proteasome performs its function of removing damaged and misfolded proteins from cells.

So far, the most common use of NMR in structural biology has been the determination of three-dimensional structures in solution. For this purpose, NMR has the advantage over X-ray crystallography in that it can be applied to systems that cannot be crystallized, of which there are many. However, X-ray crystallography is applicable to large molecular assemblies — such as intact viruses or the ribosome complexes responsible for protein assembly — that NMR has been unable to tackle.

The NMR frequency of any given atomic nucleus depends on the local dipolar magnetic fields from other nearby nuclei, and the orientation of its surrounding electron cloud relative to the magnetic field that is applied during an NMR experiment. To obtain a sharp spectrum, solution NMR spectroscopy relies on the rapid brownian tumbling of molecules to average the orientation-dependent interactions to zero over time. But the larger the molecule, the slower it tumbles, and the poorer the resolution in the NMR spectrum. In practice, this limits detailed structural NMR studies to systems smaller than about 50 kDa. The motion-dependent fluctuations of the local magnetic fields cause nuclear-spin magnetization to return to its natural equilibrium state, in a process known as relaxation. For molecules with internal flexibility, relaxation rates are governed not only by the overall tumbling, but also by the magnitude and rate of motions within the macromolecule.

Almost 10 years ago it was demonstrated that, although relaxation-causing magnetic interactions are generally additive, they can also interfere with one another[2]. This interference can lead to greatly enhanced resolution for 'backbone' amide signals — that is, for the NMR signals from the amide bonds that connect the amino acids in a protein — using a procedure known as transverse relaxation optimized spectroscopy (TROSY). These amides are not only the cornerstones for determining protein structures by NMR, they also provide information about protein molecular motion.

However, it is the side chains of proteins that interact most frequently with other molecules, so how can information be obtained about these peripheral groups? During the past few years, Kay and co-workers have developed an analogous TROSY technology that focuses on methyl (CH_3) groups[3,4]. This technique is even more powerful than the original amide TROSY method, and results in sharp NMR signals for molecules exceeding 1 MDa in size. Methyl-TROSY also takes advantage of the fact that methyl groups, such as those found in certain amino-acid side chains, spin very rapidly about their axes of three-fold symmetry, so scaling down their effective magnetic interactions before overall tumbling.

Sprangers and Kay[1] now use this technique to examine the 20S proteasome CP. The three-dimensional structure of this complex, which had previously been elucidated by X-ray crystallography, is constructed from two types of protein building block, referred to as α and β. Each type of building block forms two heptameric rings (α_7 and β_7), which stack together in an α_7–β_7–β_7–α_7 manner to form the barrel-shaped CP (see Fig. 3c of ref. 1). The two outer α_7 rings form the entrance channels for substrates and the binding sites for other multimeric protein complexes, including that of the heptameric 11S activator, which modulates proteasomal activity. The interfaces between the α_7 and β_7 rings enclose two antechambers where protein substrates are stored before degradation, and the two inner β_7 rings enclose the catalytic chamber where the active sites for degradation are sequestered within the lumen of the barrel.

The authors use methyl-TROSY to study the motions of almost 100 methyl groups that are found in isoleucine, leucine and valine amino-acid residues in the α-subunits. They do this for the α-subunit in isolation, for assembled α_7 rings and for the full CP, and also examine the effect of binding of the 11S activator to the CP. In this way, they identify rapid, large-amplitude motions for a region of the α-protein implicated in transport of the proteasome to cell nuclei in mammalian systems. These movements occur on a time scale that is fast, compared with the nearly 200 nanoseconds it takes the CP to change its orientation by one radian as it tumbles. Much slower motions (of the order of a millisecond) are seen for methyl groups lining the inside of the β-barrel. These all occur at the same rate, which strongly suggests that they are correlated with one another and that they form part of a unique mechanistic process — presumably the transport of substrate to the catalytic chamber.

The remarkable success of Sprangers and Kay in obtaining high-quality NMR data for such a large system derives in part from their careful choice of a highly symmetrical complex, in which all of the subunits of the same type (either α or β) are magnetically equivalent. The CP complex can be dissected into stable components, which can then be studied in isolation and as reassembled particles. This feature was essential to assigning each of the almost 100 simultaneously detected NMR signals to its specific methyl group. However, the need for high symmetry will be reduced by the substantial increase in sensitivity afforded by the next generation of NMR machines. The prospects are therefore excellent that NMR will provide information about the internal dynamics and transient interactions of a wide variety of molecular machines. This information, when combined with structural data from X-ray diffraction and electron microscopy, will guide our understanding of how biomolecules work at a molecular level. ■

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