Single-molecule structure determination

X-ray free-electron lasers may enable single-molecule structure determination.

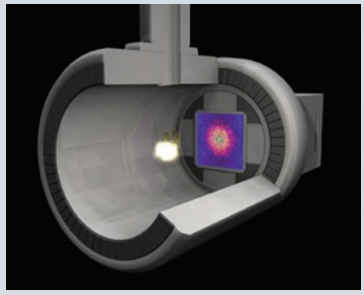
High-resolution, three-dimensional protein structure determination is a tedious process, involving protein overexpression, purification and crystallization. Many interesting biological structures, such as large complexes and membrane proteins, are all but intractable to this procedure. Moreover, information about the dynamic motions of proteins, crucial for understanding their functions, cannot be obtained from crystallography studies.

A technique to obtain snapshots of single molecules in motion would arguably revolutionize structural biology. With the construction of X-ray free-electron lasers (XFELs), such a methodological achievement seems within reach.

The world's first XFEL, the Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory in Menlo Park, California, USA, has been up and running since April 2009. This laser is a billion times brighter than previous hard X-ray sources (hard refers to the high-energy, short-wavelength end of the X-ray spectrum) and produces laser pulses with a wavelength of just 1.5 angstroms. These ultrabright, ultrafast pulses of light allow the LCLS to take a series of snapshots in rapid-fire succession with a shutter speed of about 100 femtoseconds. These unique properties have the structural biology community very excited.

But although building the LCLS was a true engineering feat, much methodological development is still needed before single-molecule structure determination will be possible. Reliable methods for injecting single molecules in the path of the XFEL beam and determining the best way to take snapshots such that a high-resolution, three-dimensional structure can be reconstructed from a diffraction pattern must be developed; these challenges will certainly be nontrivial. Analyzing the data that comes out will also be a huge hurdle and will necessitate algorithm and software development.

It may be several years or more before single-molecule structure determination becomes a reality, but progress so far is encouraging. Recently, a paper describing the LCLS's operation and performance was published (*Nat. Photonics* **4**, 641–647; 2010). The first application papers using the LCLS, including one reporting how the electrons in neon respond to XFEL radiation (*Nature* **466**, 56–61; 2010), are also beginning to appear in the literature. Two other XFELs are under construction: the Spring-8 XFEL in Japan is expected to be completed in 2011 and the European XFEL in Germany in 2014. We will certainly be watching for exciting further developments. **Allison Doerr**



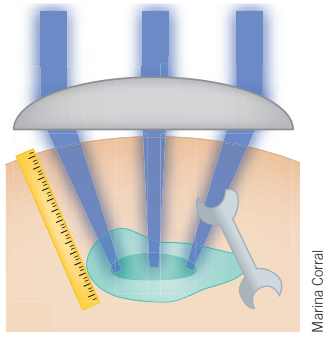
Taking a snapshot of a single molecule with an XFEL. Image courtesy of SLAC National Accelerator Laboratory.

Adaptive optics for biological imaging

The use of adaptive optics to correct light distortions promises to greatly improve the imaging quality of thick biological tissues.

Imaging technologies have greatly advanced our understanding of biological systems. However, biological specimens are far from optimal structures for imaging as they are rife with optical inhomogeneities that seriously degrade image quality. Both excitation and emission light need to travel across the tissue to and from the focus point, and any deviation from the ideal path causes optical distortions. The result is that imaging biological specimens, even with high-end research microscopes, achieves far from ideal results, with images being increasingly degraded as one goes deeper into the tissue.

Fortunately, astrophysicists have long encountered similar problems and devised ways to solve them. When imaging faraway galaxies using telescopes, light from remote stars enters the Earth's atmosphere, and atmospheric turbulence produces optical distortions that severely degrade the image. Telescopes can correct these distortions with adaptive optics, using a wavefront sensor that measures the distortions and a deformable mirror that is shaped to correct them.



Marina Corral

Adaptive optics can correct light distortions when imaging biological specimens.

So why can't these same principles be applied to improve the imaging of biological tissues? The application of adaptive optics in microscopy has lagged behind that in astronomy because of the difficulty in measuring the light's distortions in biological tissues (it is hardly possible to place a wavefront sensor within the specimen). Backscattered light from the sample can be used, but this method has only been successful when applied to relatively transparent samples such as the retina. To extend adaptive optics to many biological applications, especially *in vivo* tissue imaging, methods that enable measurement of the aberrations indirectly must be used.

One such method was recently described and applied to image cortical brain slices using two-photon microscopy (*Nat. Methods* **7**, 141–147; 2010). By illuminating the sample through different light subapertures and measuring the relative displacements in each group of rays as they travel through the specimen toward the focus, enough information can be obtained to then correct these distortions and produce a nearly perfect image.

Although the application of adaptive optics to biological imaging has yet to be widely taken up, we expect to see further substantial efforts at marrying adaptive optics and microscopy to enable researchers to see more deeply and clearly into tissue. **Erika Pastrana**

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