Structures from tiny crystals

Protein structures can be determined from microcrystals using X-ray and electron diffraction.

Atomic structure determination by X-ray diffraction calls for large, well-formed protein crystals. Many proteins—especially membrane proteins—cannot be coaxed into forming large crystals but will often form nano- or micrometer-sized crystals. Such tiny crystals would have been considered useless just a few years ago, as they are extensively damaged before diffraction data can be recorded, but new technologies are enabling high-quality data to be collected from them.

One approach is femtosecond X-ray crystallography, in which tiny crystals are streamed into the path of femtosecond pulses from an extremely bright X-ray free-electron laser (XFEL). Because the X-ray pulses are so fast, diffraction snapshots can be collected from individual microcrystals before they are annihilated. Rapid developments since the introduction of the technique in 2011 have been expanding the range of applications. The year 2014 brought new structures and biological insights, as well as improved methods for introducing tiny crystals into the XFEL beam path and for data analysis. Last year also saw some of the first biological results from the XFEL facility in Japan. New facilities in Germany and Switzerland and upgrades to the first XFEL facility, in California, are expected in the next few years.

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New technologies allow protein structures to be solved from tiny crystals.

A newer technique may yet give these (extremely expensive) XFELs a run for their money. The MicroED method, introduced in late 2013 (*eLife* **2**, e01345, 2013), uses electron diffraction for atomic protein structure determination from three-dimensional microcrystals. Though electron crystallography has been around for decades, technical challenges have prevented the approach from becoming widespread. A recent generalized version of MicroED (*Nat. Methods* **11**, 927–930, 2014) exposes a microcrystal to a very low electron dose while it is continuously rotated about its axis, thereby allowing the data to be processed with software tools developed for X-ray crystallography.

It will be interesting to see whether some of the advantages attributed to XFELs can be ported to electron diffraction, which uses widely available electron microscopy instrumentation. Further developments of both X-ray and electron diffraction using tiny protein crystals are likely to have a lasting impact in structural biology. **Allison Doerr**

Super-resolution CLEM

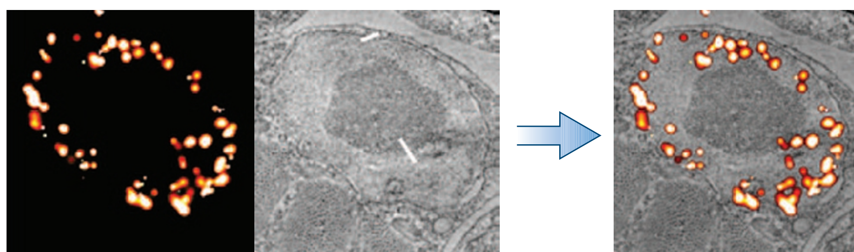
Correlated light and electron microscopy (CLEM) is particularly powerful when applied in super-resolution.

Super-resolution microscopy, which can be used to visualize objects as close together as a few tens of nanometers, has recently been much celebrated, with the 2014 Nobel Prize in Chemistry awarded for its development. Although it is exciting and useful to see a labeled protein in super-resolution, the resulting fluorescence data still lack cellular context. Where, in other words, is the labeled protein relative to the ultrastructure of the cell? Conversely, electron microscopy (EM) can give exquisite structural information, but identifying specific proteins with EM is relatively laborious and typically not quantitative. Correlation of electron micrographs with diffraction-limited fluorescence images, though informative, cannot be performed at the scale of a few tens of nanometers.

But super-resolution fluorescence imaging is now approaching the scale of an electron microscope (though there is still at least an order-of-magnitude difference in resolution between the two techniques). In the imaging of fixed samples in particular, correlating electron and super-resolution fluorescence microscopy offers exciting prospects.

Putting these two methods together is not necessarily a simple matter. The sample must be prepared in such a way that it can be imaged at high quality by both methods, with minimal distortion. Images obtained in both modes must be aligned precisely if they are to genuinely provide complementary information. For correlating transmission EM and interferometric photoactivated localization microscopy (PALM) images of cell surface structures, for instance, Taraska, Hess and colleagues recently developed sample preparation and alignment methods based on embedded gold nanorods, achieving correlation at 20-nanometer resolution (*Nat. Methods* **11**, 305–308, 2014). CLEM also requires fluorescent probes compatible with sample preparation for EM. To correlate PALM with cryo-electron tomography of bacterial cells, for example, Jensen and colleagues identified fluorescent proteins that could photoswitch in frozen conditions (*Nat. Methods* **11**, 737–739, 2014). They also needed to find ways to prevent ice-crystal formation due to warming of the sample during PALM.

Methods development for precise super-resolution CLEM is likely to continue and to result in an increased interest in correlative imaging more generally. **Natalie de Souza**



Correlated PALM and EM localize proteins within ultrastructure. Image adapted from Watanabe, S. *et al.* *Nat. Methods* **8**, 80–84 (2011), Nature Publishing Group.

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