Single-particle electron cryomicroscopy

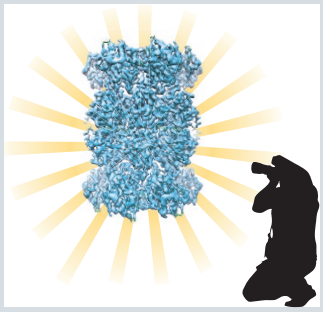
Single-particle electron cryomicroscopy reaches for atomic resolution.

Though first described several decades ago, single-particle electron cryomicroscopy (cryo-EM) has been a relatively specialized technique, straddling the shadowy area of structural biology between light microscopy and X-ray crystallography. Very recent technology advances that substantially improve the resolution of cryo-EM are currently reenergizing the field.

In a single-particle cryo-EM experiment, macromolecular assemblies are frozen in a thin layer of ice and imaged with an electron microscope. Thousands to millions of images of individual assemblies must be computationally aligned and merged to arrive at a three-dimensional structure.

A clear advantage of cryo-EM over X-ray crystallography is that crystallization is not required, allowing for more native-like reconstructions. But though it has long been recognized that cryo-EM has the potential to reach atomic resolution, severe technical limitations have gotten in the way. These have included difficulties in producing sufficient amounts of sample, structural heterogeneity, radiation damage, electron beam–induced sample motion and poor camera efficiency.

Until very recently, cryo-EM users have had two options for capturing electron microscope images: an inefficient digital charge-coupled device (CCD) camera or inconvenient photographic film. New cameras that detect electrons directly allow much faster and more efficient image collection than either previous option, addressing several of the above limitations. These direct electron-sensing cameras allow a movie of a sample to be recorded over the course of its exposure to the electron beam, permitting beam-induced motion to be computationally corrected by aligning the frames. Two papers published in 2013 using this strategy (*eLife* **2**, e00461; 2013 and *Nat. Methods* **10**, 584–590; 2013) powerfully demonstrated the ability to obtain near–atomic resolution structures for relatively small, asymmetrical assemblies, the ribosome and proteasome. Notably, the resolution in these studies was so good that substantially fewer images than typical were needed for structure determination.



New cameras for cryo-EM promise to generate atomic-resolution macromolecular structures.

Combined with rapidly improving sample-preparation methods, increasing automation of tedious tasks and new algorithms for data analysis, single particle cryo-EM is well-poised to enable new insights about macromolecular assemblies that are currently challenging to structurally characterize with any technique. **Allison Doerr**

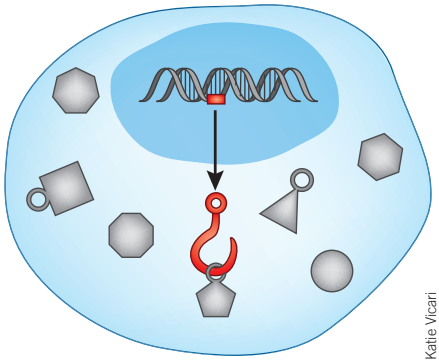
Intracellular mini-binders

Small, genetically encoded probes for real-time detection and perturbation of cellular events are gaining attention.

Tracking single molecules and interfering with their activity can be instrumental to understanding how living cells work. With the improvement in the resolution of microscopy methods, more of the cell is now accessible to the eye. In this context, choosing the right probe for imaging or manipulation experiments in living cells is more critical than ever.

Probes that label intracellular proteins should be specific for their target and not interfere with the protein's function, localization or expression. They should also be small enough so that they can access proteins in tiny corners of the cell. Genetically encoded probes are preferable, so they can be easily introduced in specific cells of interest or targeted to organelles.

To meet these challenges, researchers are increasingly turning to old friends such as intrabodies and aptamers. These probes have already received much attention for their putative pharmaceutical value, but they are now being used to develop biosensors and probes for basic biological studies.



Small, genetically encoded probes that bind to specific proteins are gaining users.

Intrabodies are small, recombinant, antibody-like proteins that bind to specific antigens. They can be obtained from animals that naturally produce very small single-domain antibodies, such as camels or sharks, or they can be engineered from larger mammalian antibodies or other proteins such as fibronectin. Aptamers, even smaller probes, are either oligonucleotide or peptide based, and are created through engineering and selection.

The genes encoding for these 'mini-binders' can be fused to other genetically encoded elements or proteins to monitor and perturb cellular components and processes. In recent work, intrabodies have been tagged with fluorescent proteins to track proteins in small subcellular structures (*Neuron* **78**, 971–985, 2013). They have also been fused to protein-degradation machinery to target the protein of interest for degradation under controlled conditions (*Nat. Struct. Mol. Biol.* **19**, 117–121, 2011) or used as a scaffold to bring different molecular components together and drive gene expression (*Cell* **154**, 928–939, 2013). Intrabodies that detect specific conformational states of the target protein can be used as biosensors to probe dynamic conformational changes with high spatiotemporal resolution in living cells (*Nature* **495**, 534–538, 2013).

Further optimization of these probes for cellular applications will make their use easier and more wide-spread. But given their unique properties, these small binders are no doubt promising tools for biological experiments. **Erika Pastrana**