STRUCTURAL BIOLOGY

# Protein holography

**Low-energy holography enables imaging single proteins and protein complexes.**

State-of-the-art protein structure determination requires averaging; X-ray crystallography averages the diffracted light from many proteins within a crystal, while cryogenic electron microscopy averages diffraction patterns acquired from many individually observed proteins. The challenge to measuring single molecules is simple—proteins are susceptible to radiation damage caused by the imaging beam and are destroyed before imaging is completed. Now, a team led by Hans-Werner Fink and Jean-Nicolas Longchamp from the University of Zurich and Stephan Rauschenbach and Klaus Kern from the Max Plank Institute for Solid State Research in Stuttgart have imaged individual proteins with subnanometer resolution, revealing distinct protein conformations.

Inspired by the resistance of biomolecules to damage from low-energy electrons, the team used low-energy electron holography for protein imaging. By placing an electron point source as little as 100 nm above a sparsely distributed protein sample, a diverging beam of electrons illuminates a single protein. Some electrons are transmitted through the protein, forming the reference beam, while others are elastically scattered by the atoms along their trajectory. The reference and scattered electrons overlap in space and interfere on a detector, forming an electron hologram that contains the full 3D positional information of each atom in the protein.

For this to work, the proteins must be supported by a substrate that is both physically strong and transparent to low-energy electrons—graphene fits the bill. Longchamp and Rauschenbach gently deposited native proteins onto a graphene membrane by soft-landing electrospray ion-beam deposition and then transferred the sample into the electron microscope. A beam of low-energy electrons illuminated the sample, allowing the repeated acquisition of holographic snapshots, each with an electron signal sufficient for reconstructing the structure of the protein.

The team imaged cytochrome C, BSA and hemoglobin with subnanometer resolution, successfully obtaining conformations in agreement with existing structures. By reducing mechanical vibrations and protein diffusion, the hope for achieving atomic resolution still remains. "We will get much better resolution, maybe two or three angstroms," says Longchamp. He notes that by observing conformational dynamics, "you can start to understand how proteins actually work."

**Zachary J Lapin**

RESEARCH PAPERS

Longchamp, J.-N. *et al.* Imaging proteins at the single-molecule level. *Proc. Natl. Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1614519114 (2017).