Physics inside: solving protein structures without crystals

Cryo-EM gets atom-level resolution:

The development of cryo-EM into a mainstream structural biology technique
Nogales, E. Nat. Meth. **13**, 2427 (2016). DOI: 10.1038/nmeth.3694
Single-particle cryo-EM at crystallographic resolution Cheng, Y. Cell **161**, 450457 (2015). DOI: 10.1016/j.cell.2015.03.049

The Physics behind it:

Cluster imaging with a direct detection CMOS pixel sensor in transmission electron microscopy
Battaglia, M., Contarato, D., Denes, P. & Giubilato, P. Nucl. Instrum. Meth. A **608**, 363365 (2009). DOI: 10.1016/j.nima.2009.07.017
Comparison of optimal performance at 300 keV of three direct electron detectors for use in low dose electron microscopy McMullan, G., Faruqi, A. R., Clare, D. & Henderson, R. Ultramicroscopy **147**, 156163 (2014). DOI: 10.1016/j.ultramic.2014.08.002

Recommended with a Commentary by Simon J. L. Billinge,
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In the early 1990's Intel had a problem. Their 386 and 486 microprocessor chips were dominant and in the vast majority of all new personal computers that were flooding into people's homes, revolutionizing our lives. Yet, no one had heard of Intel. People would buy IBM and Hewlett-Packard computers, powered by Intel, but who knew? This prompted the now famous "Intel Inside" campaign, where every computer containing an Intel processor inside, had on the outside a little sticker stating this fact. The campaign was enormously successful and these stickers survive to this day; in fact I am looking at one now as I type this. Physics in general, and condensed matter physics in particular, shares this same problem to a greater or lesser extent. We are all aware that physics is behind everything in the physical world around us, but the general public; not so much. And more specifically, the massive impacts that CMP research has on their lives remains a closed book. This of course becomes

a problem when we, increasingly, have to fight to maintain funding for physics research.

There is a beautiful recent example that is not yet impacting Jane Public, but will have a major impact in due course, and right now is fueling a veritable revolution in protein structure determination. The ability to solve macromolecular structures has been key to understanding fundamental aspects of biological function, and is a central pillar in modern drug discovery efforts. There are by now more than one hundred thousand protein structures solved and deposited in the protein database (PDB). Proteins are large molecules, but in their functional form in organisms they fold into a complex but (generally) unique blob with diameters of a few nanometers, and the fold is absolutely key to giving the proteins their functional properties. The folded proteins are actually *nanoparticles*! Proteins are macromolecules, but the *key* to understanding protein function is to solve the structure of a nanoparticle: the folded protein. This presents a massive problem because of the nanostructure inverse problem (NIP) [1]. Whilst (thanks in very large part to physics research) we have been able to solve the structure of crystals, there are not robust and reliable methods for solving the structure of nanoparticles. One workaround would be to take your nanoparticles and form them into a translationally and orientationally ordered array, i.e., to crystallize them, and to use powerful crystallography to solve the structure. This is protein crystallography, and it accounts for a vast majority of protein structures in the PDB, but only a tiny minority of proteins have successfully been coaxed into crystals. On the other hand there are multiple efforts to solve nanostructure directly, without crystallization. This article is not intended as a review of this, but I want to focus on one, cryo-electron-microscopy (cryo-EM).

Electrons interact strongly with matter which makes them a good candidate for getting a signal from a very small object like a nanoparticle. The downside that comes along with the strong interaction is a high probability of multiple scattering making the scattering inverse problem (given the scattering pattern what was the atomic arrangement that gave rise to it) difficult to solve. But nanoparticles are themselves small which lowers the probability of multiple scattering. Unlike x-rays, it is possible to make electron lenses, allowing an image to be directly reconstructed on the detector rather than a diffraction pattern that requires further processing. High energy electrons have very short wavelengths, shorter than interatomic spacings making the biggest limitation to imaging atoms in nanoparticles to be aberrations of the electron lenses themselves. High resolution (sub angstrom) images

are now available thanks to aberration corrected microscopes. But these images are just projections of the particle, not 3D reconstructions of atomic arrangements. In parallel with improvements in image resolution came novel data acquisition protocols and algorithms for reconstructing tomographically a 3D electron density from thousands of noisy, low-dose (so as not to destroy the object), low-resolution, randomly oriented copies of the same object. All these things led to great progress in Cryo-EM and profoundly increased its scientific impact, with physicists making multiple contributions along the way.

But the final step that can make single-particle reconstruction cryo-EM, and its close relatives such as atomic electron tomography (TEM), truly transformative in nature, will be to bring the resolution for 3D reconstructions down to the sub-angstrom level. The final piece of the puzzle is now in place and we are likely on the verge of a revolution in structure science almost as profound as crystallography 100 years ago. The final puzzle piece is an elegant piece of applied physics: direct electron detection detectors. For many years, film was the detection medium of choice, which in later years was digitized using densitometers. Image plates (IP) followed, which are like film but they don't need to be chemically processed to digitize the image. Next came charge coupled device (CCD) cameras, but the CCD chips were made to be sensitive to visible light. The electron detecting layer is a phosphorescent material that absorbs electrons and emits light that is captured and directed to the CCD. These have faster readout but have issues with noise, cross-talk between pixels, quantum efficiency and so on. Optimally, one would have a detector where the electron detection device and the readout chip were one and the same, and this is the case for CMOS based direct electron detection devices These can integrate VLSI electronics directly onto the chip, so that that timing, control, the analog to digital conversion and signal processing all takes place on the detector chip, potentially allowing for very fast readout It is this fast readout, together with very high quantum efficiency and low noise of the latest detectors described in the two physics papers highlighted, that is having the transformative effect on Cryo-EM (the two bio papers).

To be fair, there is a lot of "electrical engineering" inside too, but the physics contributions, and the physics research driving many of the developments including detectors for high energy and space physics has been central to, as a recent Nature news item called it, "the revolution that will not be crystallized" [2]. In a final twist to the tale, the developments are being turned back onto problems of direct interest to condensed matter physics:

the structure of inorganic nanoparticles [3] with very exciting prospects in store moving forward.

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[1] S. J. L. Billinge and I. Levin, Science **316**, 561 (2007), URL http://www.sciencemag.org/content/316/5824/561.abstract.

[2] E. Callaway, Nature **525**, 172 (2015), ISSN 0028-0836, 1476-4687, URL http://www.nature.com/doifinder/10.1038/525172a.

[3] J. Miao, P. Ercius, and S. J. L. Billinge, Science **353**, aaf2157 (2016), URL http://science.sciencemag.org/content/353/6306/aaf2157.