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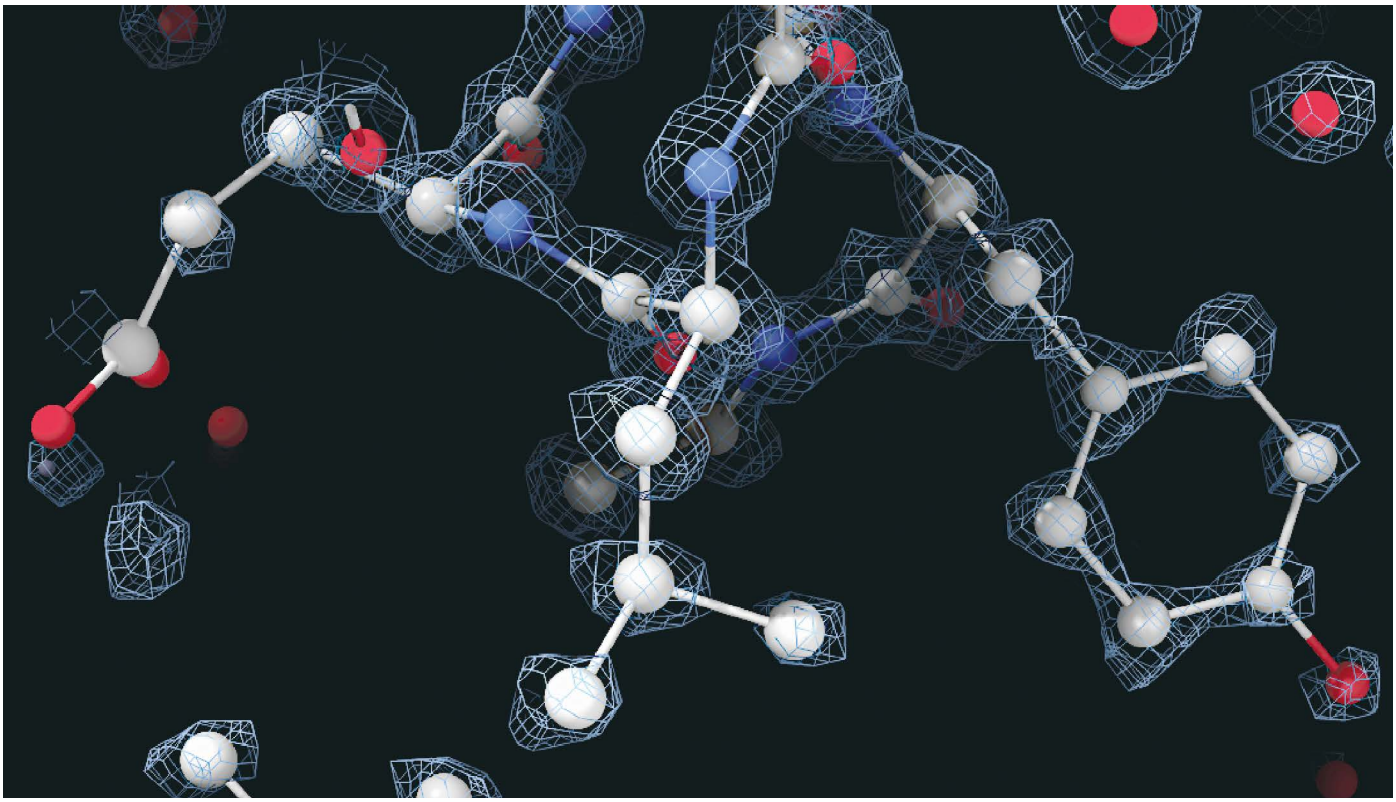

MARK HERZIK

Figure 1 | Individual atoms mapped using cryo-electron microscopy (cryo-EM). Yip *et al.*[1] and Nakane *et al.*[2] report that they have crossed the atomic-imaging threshold for this key structural-biology technique, presenting protein structures at a resolution of approximately 1.2 ångströms. A region of the apoferritin protein is shown, generated using results reported by Nakane and colleagues. The blue mesh represents the cryo-EM density data, superimposed on an atomic model of the molecular structure of the protein in which individual atoms are shown as spheres, and bonds as sticks. Carbon, nitrogen and oxygen atoms are grey, red and blue, respectively.

Structural biology

Cryo-electron microscopy reaches atomic resolution

Mark A. Herzik Jr

A structural-biology technique called cryo-electron microscopy has attained the ability to locate individual atoms within a protein. What are the implications of this advance? **See p.152 & p.157**

A founding principle of structural biology is that, once researchers can directly observe macromolecules in enough detail, it should be possible to understand how their 3D structures confer their biological functions. Indeed, many scientific advances have relied on directly observing the world around us in as much detail as possible, and efforts are increasingly being dedicated to visualizing the atomic structures of biological components that have a key role in human disease. Nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography and cryo-electron microscopy (cryo-EM) are the three main structural-biology techniques in use. Of the three, cryo-EM has emerged as the current 'go to' method for determining the structures of large and dynamic complexes that have proved difficult to obtain by the other approaches.

Writing in *Nature*, Yip *et al.*[1] (page 157) and Nakane *et al.*[2] (page 152) report the sharpest images yet obtained by using a method termed single-particle cryo-EM, enabling the location of individual atoms in a protein to be determined for the first time. Breakthroughs reported by other groups have also produced notable improvements in the resolution of cryo-EM images[3,4]. Ultimately, these developments will help researchers gain a better understanding, at unprecedented resolution, of how proteins work in health and disease, with the potential to aid the design of better therapeutics.

Although cryo-EM is a decades-old technique, it has garnered increasing interest since around 2013 due to a series of technological and algorithmic advances that together drove a striking improvement in the resolution obtainable by this technique (described as the 'resolution revolution')[5].

Collecting single-particle cryo-EM data

begins with a protein sample that has been applied to a special sample grid. Plunging it into liquid ethane flash-freezes and traps the protein particles in a thin film of amorphous ice. Two-dimensional images of the individual particles in the sample grid, obtained by applying a beam of electrons, are averaged computationally to yield a 3D structure. The 2D images are incredibly 'noisy' because a low dose of electrons must be used to avoid damaging the radiation-sensitive biological sample. As such, these images have historically been unsuitable for determining structures at an atomic level of detail. However, the advances reported since 2013 have allowed single-particle cryo-EM data to be collected that rival those obtained using X-ray crystallography.

The resolution revolution of cryo-EM has continued to advance[6]. Yip *et al.* and Nakane *et al.* harnessed technological improvements to determine the structures of a stable iron-storing protein called ferritin (termed apoferritin in the absence of metals) to a resolution of approximately 1.2 ångströms. These structures are the highest-resolution single-particle cryo-EM reconstructions so far determined, and the data are of sufficiently high quality to resolve the individual atoms in apoferritin (Fig. 1). This unprecedented feat would not have been thought feasible merely a decade ago.

Yip and colleagues' success relied on hardware advances, including components such as a spherical-aberration corrector plus a monochromator device that applies a series of filters to ensure that only electrons with a narrow spread of energies interact with the specimen, thereby enhancing the resolution of the final image. Nakane and co-workers applied a different technology, a cold field-emission gun that also generates electrons with a narrow energy spread, together with a technology that reduces noise in each image by filtering out those electrons that interact non-productively with the specimen. Moreover, Nakane *et al.* captured data with a next-generation, highly sensitive electron-detecting camera.

In addition to analysing apoferritin, Nakane and colleagues obtained a structure at 1.7 Å resolution of a form of the receptor for γ-aminobutyric acid type-A ($GABA_A$) that was engineered to be more stable than the common form found in humans. This receptor is a protein complex that resides in the cell membrane of neurons and is a target for numerous therapeutics. Obtaining such a high resolution by single-particle cryo-EM had been deemed near impossible for a biological specimen such as this, one that exhibits a high level of flexibility in terms of its structural mobility compared with structurally rigid molecules such as apoferritin. The structure reveals details of the $GABA_A$ receptor that have never been seen before, providing insights, for example, into the binding of a molecule called histamine in the core of the protein.

The developments in cryo-EM hardware described by Yip, Nakane and their respective colleagues have driven a major advance in the resolution of single-particle cryo-EM. Each team used hardware that tackled distinct aspects of cryo-EM imaging that had previously limited the resolution attainable. With these technologies, the increased signal-to-noise ratio of cryo-EM images will expand the technique's applicability. For example, this might include using the technique to determine high-resolution structures of heterogeneous samples such as those formed of membrane proteins, or macromolecular complexes that vary in conformation or composition. Perhaps the melding of these technologies will enable the determination of cryo-EM structures at a resolution beyond even 1 Å. This once might have seemed a near impossible quest to embark upon.

However, these technologies represent the elite echelon of cryo-EM instrumentation and are currently out of reach for most institutes because of the cost of purchase and operation. Moving forward, these types of advance will help us learn more about what is limiting the attainable resolution and might therefore enable the design of better instrumentation. Although such high-resolution structures are not necessary to answer every biological question, the extra detail such hardware can provide would limit inaccuracies in 3D structures and provide a better platform for understanding biological functions. Nevertheless, for most macromolecules, the inherent structural flexibility and structural heterogeneity will instead probably be the resolution-limiting factor, regardless of the capabilities of the instrumentation available. For such less-stable specimens, the application of new sample-preparation technologies, together with improvements in data-collection throughput and algorithm advances, will offer fresh ways to probe the conformational landscapes of these complexes. Thus, although cryo-EM's resolution revolution might be nearing its end, more revolutions await in the years to come that will make this technique even more powerful and applicable to the investigation of diverse biological questions.

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Nuclear physics

Why neutrons drip off nuclei

Calvin W. Johnson

The neutron drip line refers to the maximum number of neutrons that can be packed into the atomic nuclei of each chemical element. A mechanism has been proposed that could explain the long-debated origin of this drip line. **See p.66**

Whereas some people play extreme sports, many nuclear physicists seek the thrill of extreme isotopes, by finding, for each chemical element, the largest possible number of neutrons that can be held by an atom. This boundary of nuclear existence, called the neutron drip line, has not been fully mapped – although the construction of rare-isotope facilities[1] will bring the goal closer. Moreover, even the theoretical location of the drip line is uncertain[2,3]. On page 66, Tsunoda *et al.*[4] argue that the mechanism responsible for the drip line is more subtle than previously understood and is related to deformation, a hallmark of much of ordinary nuclear physics.

The strong nuclear force that binds protons and neutrons together favours equal numbers of each particle. By contrast, weaker but longer-range electrostatic repulsion discourages the accumulation of protons in atoms. Competition between these two forces produces the valley of stability – the V-shaped