CELL BIOLOGY

Pore puzzle

John D. Aitchison and Richard W. Wozniak

Where would you start in trying to work out the structure of a macromolecular machine consisting of 456 proteins? Taking a combined experimental and computational approach is one answer.

Consider a 1,000-piece jigsaw puzzle. There are millions of ways in which the pieces might fit together, yet there is only one solution. We solve the puzzle by considering each piece and ruling out those that don't fit physical restraints, such as colour patterns, the overall shape of the picture and the potential for interlocking. This is the basic premise for the multidisciplinary approach taken by Alber *et al.*[1,2] (pages 683 and 695 of this issue) to solve the structure of a large macromolecular machine, the yeast nuclear pore complex. But, in their case, the pieces were proteins, the various restraints were of a biochemical and morphological nature, and computers explored the placement of each protein into a single ensemble solution.

Nuclear pore complexes (NPCs) serve as regulated ports for transporting molecules into and out of the cell nucleus. Researchers have studied these complexes for decades. They have defined the overall morphology of the NPC, and have used various approaches to determine the inventory of its constituent proteins — the 30 different nucleoporins — and the abundance and rough localization of each nucleoporin. They have also begun to identify genetic and physical interactions between these proteins[3]. All NPCs — from those of yeast to human — have largely symmetrical, doughnut-shaped structures that lie within a pore that is formed by a membrane connecting the inner and outer layers of the nuclear envelope. Each NPC consists of eight morphologically similar units called spokes, which are arranged around a central channel through which transport occurs[3,4].

The previous studies were essential for characterizing NPC functions and for identifying subcomplexes within them. But because of the size and complexity of this structure, these earlier studies did not reveal the detailed architecture of the entire assembly. To tackle the NPC puzzle, Alber *et al.*[1,2] systematically generated comprehensive biochemical and morphological data of the sort collected in the past, and combined them with state-of-the-art computational integration strategies.

Using the known size and symmetry of the NPC, the team established more than 10,000 other restraints as input for their computational analysis. These included the stoichiometry (number) and localization of each NPC component, as well as interactions between different components. Their goal was to generate sufficient data so that, like the jigsaw puzzle, there could be only one possible solution.

But each data type had uncertainty associated with it. For example, because of the limitations of the imaging techniques, the localization of each nucleoporin could be narrowed down to only a relatively large volume within the NPC. Thus, each data type by itself was insufficient for determining the structure, because, by analogy, it was like a set of partly broken or faded puzzle pieces. The solution was data integration: even with uncertainty in every data type, the likelihood of satisfying all restraints with an incorrect structure becomes vanishingly small as more data are generated.

Once the experimental data had been translated into mathematical representations of restraints, the authors' procedure involved taking a random configuration of beads

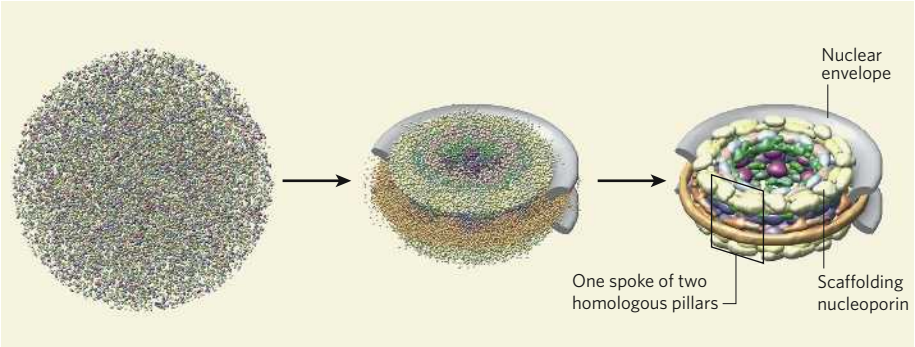

Figure 1 | Determining the structure of the nuclear pore complex. Alber *et al.*[1,2] started with 456 randomly distributed protein components of the complex (coloured beads), corresponding to several copies of each of the 30 different constituent nucleoporins. They moved the beads randomly, while computationally minimizing thousands of biochemical and morphological restraints. The final structure shows the distribution of proteins resulting from the best-scoring simulations. (Figure courtesy of F. Alber and A. Sali, University of California, San Francisco.)

50 & 100 YEARS AGO

50 YEARS AGO

The proposals of the South African Government to enforce racial segregation at the university-level and to exercise rigid control over projected colleges for non-whites has caused concern and indignation in university circles throughout the Commonwealth and beyond. Some of this concern found expression in a well-attended meeting held at the Caxton Hall in London ... The audience gave a polite but sceptical hearing to Prof. L. J. du Plessis, of the Potchefstroom University, who had come over from South Africa to present the case in favour of university apartheid ... Prof. du Plessis said :"If I were in England, I would be an integrationist too, because it is not a danger to English national character, and if I were in America I would be an integrationist too; the negroes in America are in no danger of destroying that great nation of America in its identity. But the Bantus and Indians and Coloureds of South Africa, if they are integrated more fully than they are now, would undoubtedly destroy our great South African nation".

From *Nature* 30 November 1957.

100 YEARS AGO

Since writing the notice of Mr. le Souef's book on Australian wild life ... I have been making inquiries as to the existence in collections of any examples of platypus egg definitely known to have been taken from the nest after extrusion ... In the central hall of the British Museum is shown an egg-shell of a platypus sent from Queensland by Mr. G. P. Hill in 1902, but this ... was doubtless found in its present broken condition. Such broken shells might, apparently, be extruded from the uterus with the foetus; and, so far as I can find, there still appears to be no definite evidence that the eggs are really laid entire.

From *Nature* 28 November 1907.

representing proteins in three-dimensional space. Then, again in a random process, the beads were iteratively moved to sample the potential space they could occupy, while simultaneously minimizing violations of restraints (Fig. 1). The configurations in each iteration were scored for their ability to satisfy the restraints. After thousands of trials, the best-scoring structures were superposed to produce a map of the localization of each component. As there was no gold-standard structure for validation, the final structure was assessed by criteria of self-consistency: all the restraints were satisfied; variability between possible solutions was small; similar structures were obtained when portions of the data were omitted; inclusion of incorrect data led to poorly resolved (frustrated) structures; and previously reported subcomplexes were rediscovered.

In biology, the motivation for structure determination involves an element of faith: that, once solved, the structure will tell you something fundamental. And this is the case here. For many years, subcomplexes of the NPC consisting of handfuls of nucleoporins have been identified and characterized in isolation. Alber and colleagues' determination of the molecular architecture of the NPC places these components in the context of the whole complex and provides researchers with a frame of reference for future studies, including devising more tightly directed approaches for testing existing and new hypotheses generated from the study. For example, the authors find that half of the nucleoporins in the yeast NPC form an interconnected network or scaffold that coats the pore membrane (Fig. 1). These proteins are composed of two types of structural domain with a similar configuration to that of the protein complexes that coat carrier vesicles involved in intracellular trafficking. This suggests that the two complexes — both of which are involved in the curvature of membranes — are evolutionarily related.

The authors also show that the core scaffold is built of repetitive structural pillars. Each of the eight symmetrical spokes is composed of paired groups of duplicated or structurally related nucleoporins, forming pairs of homologous columns (Fig. 1). This modularity has implications for ways in which the NPC structure might vary within a cell, and provides insight into how the structure could have evolved.

One class of nucleoporin is characterized by a repeated motif of phenylalanine–glycine (FG, in single-letter code) amino-acid residues, and is thought to control transport across the NPC. But how FG nucleoporins achieve this is a contentious issue[5]. As would be expected, Alber *et al.* find that these nucleoporins are exposed to the central pore, where they form a selective barrier to transport. But as the precise structure of this region of the NPC could not be resolved by this analysis, exactly how transport occurs remains unclear.

The combined experimental and computational approach that Alber and colleagues[1,2] took to determine a complex structure has yielded an excellent platform for further resolution of the NPC structure. Perhaps, more importantly, it serves as a paradigm for future data-integration strategies designed to understand the structure, dynamics and mechanistic properties of other large macromolecular machines. ■

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MICROSCOPY

Elementary resolution

Christian Colliex

The atoms and bonds that make up complex solids can be identified chemically — a feat made possible by cleverly combining spectroscopic and structural information conveyed by electrons scattered through a thin sample.

Spying out the atomic organization of solid structures has long been a dream of materials scientists. They have built ever more refined microscopes to realize that dream, using probes of electrons, or of local currents or forces, to build up increasingly detailed pictures of solids. But identifying the chemical nature of atoms in position has been a pricklier problem.

Two techniques have reached a stage where this is becoming possible. First, there is the three-dimensional topographic atom probe, which uses mass spectroscopy to identify atoms that have been made to evaporate gradually from the surface of a specimen solid[1]. Second, there is electron energy-loss spectroscopy (EELS), which measures the energy of electrons transmitted through a thin specimen in a scanning transmission electron microscope (STEM) (Fig. 1). On page 702 of this issue[2], Kimoto *et al.* report the use of EELS for element-selective imaging of columns of atoms in a crystal. This is a further stage in a healthily competitive race in which results are appearing at an accelerated pace.

The starting signal for this race was fired almost 15 years ago. In a News & Views article published at the time[3], L. M. Brown detailed the near-simultaneous publication of three papers[4–6], all of which described the measurement, with atomic-scale resolution, of compositional changes across an interface between two grains or components in a solid. In each case, a probing electron beam was directed down a column of atoms, and the resulting EELS spectrum was used to ascertain the atomic species as well as their valence states and local coordination. The question of precedence among these three papers gained renewed piquancy last year with the appearance of a corrigendum[7] to the first of the papers[4] in which the authors admitted errors in data processing. These errors did not, they said, modify their general claim of atomic resolution.

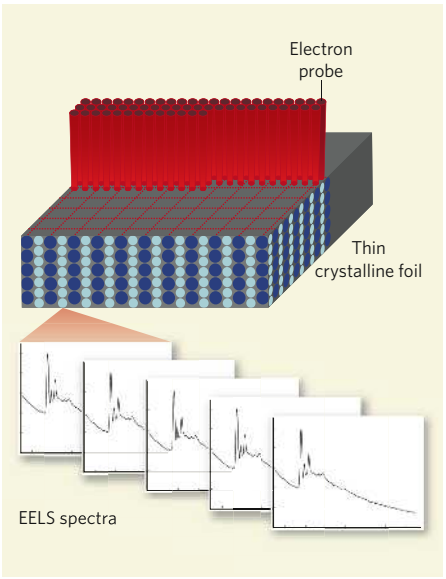

Figure 1 | EELS in action. In electron energy-loss spectroscopy (EELS), spectra are recorded successively by an ångström-size incident electron beam to build up an atomic-scale map of a thin crystalline foil. By comparing these signals with structural information from electrons scattered through large angles by interactions with the crystal atoms (the annular dark-field, or ADF, signal), Kimoto and colleagues[1] identified the chemical elements in a crystal structure.

Fortunately, advances in instrumentation and methods now allow the task to be tackled more reliably. First, the general improvement in all the electrical and mechanical components involved has reduced instability and performance degradation. Second, the so-called spectrum–image strategy[8] increases confidence in