Meeting report

Harry Potter and the structural biologist's (Key)stone

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A report on the first European Keystone symposium 'Multi-protein complexes involved in cell regulation', Cambridge, UK, 18-23 August 2006.

After the first Keystone symposium held outside America, which took place in October 2005 in Singapore, the first in Europe was held at St John's College, Cambridge, UK. As stated by one of the speakers and clearly felt by many others, the venue of St. John's College gave a real 'Harry Potter' feeling to the conference, which brought together a multidisciplinary group of scientists interested in structures of large protein complexes and in how structural insights can aid understanding of cell regulation.

Cells are giant, highly dynamic molecular assemblies. They contain thousands of protein complexes, the molecular machines that carry out most of the textbook biological processes, from DNA replication to metabolism. These machines are themselves highly regulated and dynamic, and this regulation is carried out by a host of signaling processes mediated, in turn, by a great variety of protein interactions. The conference saw contributions covering all aspects of cell structure and regulation, from the atomic to the cellular level, and with subjects ranging from methods for solving structures to applications of hybrid approaches for the elucidation of structural aspects of biological processes.

Structures of complexes and structural biology

Wolfgang Baumeister (Max Planck Institute for Biochemistry, Martinsried, Germany) presented a global vision of the cell derived from a combination of proteomics and electron tomography. Tomograms of cells at molecular resolution are essentially three-dimensional images of the cell's entire proteome and reveal the spatial relationships of macromolecules directly. Approaching 3 nm in resolution, they provide a fascinating insight into the principles of supramolecular organization and a basis for studying higher cellular functions. These tomograms have a fundamental problem, however: very often one does not know what one is looking at. To get around this, Baumeister and colleagues are assembling a molecular atlas of large complexes determined by X-ray or electron microscopy (EM) methods, in which each complex is represented as a three-dimensional template that can be used as a probe to find possible candidates inside each tomogram, and subsequently to study aspects of 'molecular sociology', or the real networks of molecules in living systems.

Purification methods, together with proteomics based on mass spectrometry (MS), have identified hundreds of protein complexes. Standard proteomics techniques cannot, however, provide the stoichiometry, subunit interactions and organization of assemblies. Moreover, because they are heterogeneous and often present at relatively low abundances large complexes can be very difficult to isolate in quantities suitable for structural studies. New developments are already addressing these limitations, however. The composition of complexes can be determined on the large-scale by techniques such as tandem affinity purification (TAP). Bertrand Seraphin (CNRS, Gif-sur-Yvette, France) reviewed the structural and functional analysis of protein complexes, starting with TAP coupled to MS (TAP/MS) for determination of stoichiometry, and described how sufficient material for structural studies can now be obtained for low-abundance complexes by coupling overexpression with TAP. He discussed applications of his approaches, in concert with structural techniques such as small-angle scattering and X-ray to study various assemblies, including the exon-junction complex.

On a related theme, Carol Robinson (Cambridge University, Cambridge, UK) explored the interplay between MS and electron microscopy to uncover the composition, stoichio-

metry and structure of complexes. The overall shape of complexes can be determined by MS by measuring the traveling time through the device, in a manner similar to gel filtration. But the shape describes the external envelope and not details of what is inside. She also demonstrated an innovative way to deduce subunit organization by the analysis of subcomplexes derived from a larger assembly. She also presented a fascinating possibility: coupling MS to electron microscopy by placing the microscopy grid on the collision detection device of the spectrometer in order to visualize complexes directly. In this way TAP and MS with electron microscopy can be combined to compensate for the individual challenges of each technique: TAP can be used to isolate sufficient quantities of highly pure native complexes, and MS of the intact assemblies and subcomplexes can be used to determine their structural organization.

There is still a large gap between the number of complexes thought to exist on the basis of data from two-hybrid or affinity-purification screens and those for which three-dimensional structures are available. Moreover, there are many lower-resolution structures now produced for large complexes by electron microscopy, and models for protein complexes can often help to interpret them. This has defined the next generation of structure prediction - the techniques that must now tackle whole complexes or systems if they are to have the most impact in biology.

Andrej Sali (University of California, San Francisco, USA) presented an approach for determining low-resolution structures of complexes by the satisfaction of restraints derived from a plethora of experimental and theoretical data, and its application to the yeast nuclear pore complex, which is approximately 50 MDa in size and contains about 480 proteins. The spatial restraints on the symmetry, protein positions and protein relationships were determined using affinity chromatography, electron microscopy and ultracentrifugation measurements by the groups of Michael Rout and Brian Chait at Rockefeller University (New York, USA). The final nuclear pore complex structure resolves the approximate position of each protein and has already provided a number of insights into the function and evolution of this complex.

One of us (R.R.) discussed some 500 complexes deduced from a full genome screen using TAP/MS and described how complex structures that are already known can be used as templates to model others inside the interactome. This talk highlighted the growing number of interactions known to be mediated by short peptide stretches and described methods to find short recurring peptides that bind particular domains, possibly providing new target sites for allosteric drug-discovery approaches, such as that of Jim Wells (see below).

Reversing the paradigm: interactions as drug targets

Protein interfaces were a hot topic this year, with many presentations devoted to their study and to new ways of modulating them for applications in disease. The principles of protein interaction were reviewed by Tom Blundell (Cambridge University, Cambridge, UK), who opened the meeting. He focused specifically on comparing the interfaces of signaling complexes with those in other complexes. He discussed how a multitude of weak binary interactions can lead to stable multiprotein complexes in a 'velcro-like' manner. He also summarized the traditional pharmaceutical company criteria for 'druggability' of surfaces (their suitability for targeting by drugs), which largely dismiss flat, shallow, flexible surfaces. He went on to suggest that proteins that form interactions with ligands comprising a continuous region of flexible peptide could be more druggable than preformed complexes of globular protein structures.

Jim Wells (University of California, San Francisco, USA) presented the technique of disulfide tethering for identifying binding or allosteric sites in protein-protein interaction. Allosteric inhibitors are of growing interest for drug discovery, particularly when traditional active-site inhibition fails to deliver good candidate molecules. In the approach presented, some residues on a protein surface near the targeted site are mutated to cysteines, which lock in thio-labeled chemical fragments whose affinity is, at best, in the low micromolar range. Interlinking these cysteines, followed by some optimization by synthetic chemistry, can quickly lead to molecules of sub-nanomolar affinity; for example, inhibitors have been found in this way for caspases, for which active-site inhibitors have a poor clinical history. Perhaps the most impressive display of the technique was the targeting of the surface of interleukin-2 near to the known receptor-binding site. Although the site did not seem druggable, Wells and colleagues managed to synthesize a compound that clearly mimics receptor binding and binds with sub-nanomolar affinity.

Similarly, Steve Fesik (Abbott Laboratories, Abbott Park, USA) has applied nuclear magnetic resonance (NMR) and many other structural approaches to derive inhibitors for the anti-apoptotic protein Bcl-2 with a view to developing new cancer drugs. After developing compounds targeting the interaction between Bcl-2 and the pro-apoptotic protein Bak, and facing off difficult challenges such as eliminating binding to serum albumin, they obtained a specific nanomolar inhibitor that mimics the helical conformation of Bak.

Nadia Milech (University of Western Australia, West Perth, Australia) showed that it is sometimes sensible to abandon direct approaches to designing molecules that target molecular interactions, and instead to see if there is a suitable candidate already occurring in nature. She has searched for random fragments of bacterial genomes (phylomers) that act as inhibitors of protein-protein interactions, and discussed

fascinating potential applications of candidate peptides to wound healing.

Hybrid approaches in development and in practice

"At structural conferences of ten years ago", Chris Dobson (Cambridge University, Cambridge, UK) commented, "you might have heard a little bit about electron microscopy, and something about mass spectrometry, but today nearly every structural problem has been studied using at least three techniques, or more." This captured one of the themes of the meeting, that hybrid approaches are the order of the day, and indeed, when dealing with large molecular assemblies, they are a must.

There are still new hybrid approaches to be explored, including such seemingly unlikely bedfellows as NMR and small-angle scattering (SAXS). Determination of the three-dimensional structures of multidomain proteins by solution NMR methods presents unique challenges related to the fact that these proteins are normally much larger than structures typically solved by NMR, and the usual scarcity of constraints at the interdomain interface, which often results in a decrease in structural accuracy. Alexander Grishaev (National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA) demonstrated that in this respect, experimental information from SAXS can be used as a complement to NMR, as it provides an independent constraint on the overall shape a molecule can have. SAXS is not affected by isotopic labeling and measurements can be done very quickly, in small sample volumes, and in conditions that match the NMR experiment. Moreover, SAXS data can be incorporated naturally into NMR structure calculations. Whatever the combination of methods used, the power of hybrid approaches is best illustrated by applications to particular systems, of which plenty were presented at the meeting. A variety of multidisciplinary approaches were applied to a multitude of complexes, and interdisciplinarity and system-level analysis were mentioned by most speakers.

Much of the complexity of signaling was nicely put together in a provocative talk by Yosef Yarden (Weizmann Institute, Rehovot, Israel), who presented a model of a signaling network that was based on an analogy with electrical circuits and other human-built networks. Specifically, he argued that it is useful to envisage signaling by the epidermal growth factor receptor ErbB as a bow-tie-shaped evolvable network, which shares modularity, redundancy and control circuits with robust biological and engineered systems. Because network fragility is an inevitable trade-off of robustness, a systems-level understanding would be expected to generate therapeutic opportunities to avoid aberrant network activation. The fragility of the ErbB network provides opportunities for cancer therapy; it predicts better efficacy for drugs targeting multiple aspects of the same pathway, such as phosphorylation and binding of Hsp90 to the same kinase, as has been found for some inhibitors.

Amyloids everywhere

Amyloids are insoluble fibrous aggregations, sharing a common β-cross structure, formed by many different proteins. Some of the biggest players in the world of amyloids were present at the meeting, and this provided for a fascinating session on this subject. David Eisenberg (University of California, Los Angeles, USA) first reviewed the principles of amyloid fibril formation and then discussed his work studying the structures of amyloid fibrils using X-ray crystallography. The structures revealed very tight, close-packed interfaces, and certain common patterns of formation, in particular self-complementarity, which allows tight interdigitation. This group extended this work with David Baker (University of Washington, Seattle, USA) to find new sequences that fit onto the close-packed structure, which led to several surprising predictions of amyloid formation (such as by myoglobin and lysozyme). Context does have a role in the ability of a protein segment to form amyloids, however, because ribonuclease, which seems to contain a suitable segment, has never formed amyloids in more than ten years of harsh laboratory treatment.

Dobson explained that there was little in common among the 60 proteins that have so far been converted to form amyloids, and that perhaps amyloid formation is a generic feature of proteins and that proteins differ only in terms of the propensity to form these structures. He then presented applications of nanotechnology (such as nanoscale cantilevers) to uncover the strength and structure of amyloid fibers, and ended with his group's attempts to treat amyloid formation in flies by redesigning amyloid fibers. He also presented fascinating early work studying folding and misfolding on the ribosome by NMR, which promises to revolutionize our understanding of nascent chain folding.

Sheena Radford (University of Leeds, Leeds, UK) followed by discussing the 'knife-edge' in folding landscapes, meaning the delicate balance between folding, aggregation and amyloid formation. She studied β_2-microglobulin, almost all of which can form amyloid, and found mutations that isolated an amyloid-forming folding intermediate (at an edge strand in the structure). The electron microscopy pictures of these fibers reveals some surprises: they do not seem to form a generic β-cross structure.

Predicting function and interaction from structure(s)

More than half of the genes in most genomes are still of unknown function, and the output from structural genomics

initiatives has now provided structures for thousands of these that (alone) say little about protein function. Computational procedures are still needed to make sense of a bewildering array of data. Janet Thornton (EMBL European Bioinformatics Institute, Hinxton, UK) opened a computational section of the meeting by discussing her work on predicting function from structure. Her group's Catalytic Site Atlas [http://www.ebi.ac.uk/thornton-srv/databases/CSA/] describes the residues involved in catalysis, as identified by structural and biochemical experiments, for nearly 500 proteins. They have also developed approaches to compare these sites, and the ligands that bind to proteins of known structure, in order to predict new potential catalytic or binding sites on protein structures of unknown function. All these tools are being used to help predict function from structure in European and US structural genomics projects.

As was so often demonstrated at the meeting, proteins rarely act alone. Thus, the many thousands of structures now known are likely to interact with others, but determining complex structures experimentally remains difficult. This makes methods to predict how two protein structures might interact - docking methods - ever more relevant in structural biology. To develop effective methods one needs first, however, to understand principles of interaction. Joel Janin (CNRS, Orsay, France) described an approach to understanding what it is about interaction interfaces that makes them distinct from crystal packing. In addition, the approach also revealed interesting properties of the true biological interfaces.

Janin also introduced the Critical Assessment of the Prediction of Interactions (CAPRI) experiment, in which docking approaches are subjected to regular double-blind trials. Progress has been clear, with at least one group now providing a near correct structure for nearly every target submitted. Juan Fernandez-Recio (Institute for Research in Biomedicine, Barcelona, Spain) then presented his work on one of the most successful approaches, showing how recent improvements in docking methods, particularly information from binding-site predictions or evolutionary conservation, can improve performance dramatically.

In conclusion, hybrid experimental and computational approaches have put us well on the way towards determining structures for many thousands of complex structures, and to placing them in the context of the whole cell. This will not only reveal the real molecular organization of a cell but will also allow systems biology to move from abstract representations to the physical world. The time when we can wave Harry Potter's magic wand and zoom in on any part of a cell at atomic level detail is surely just around the corner.

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