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Meeting report

Watching proteins in motion

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Abstract

A report of the 23rd Protein Symposium 'Proteins in Motion', Boston, USA, 23-27 July 2009.

This year, the annual symposium of the Protein Society highlighted the dynamic motions that enable almost all protein function. The ability of polypeptide protein chains to adopt intricate and highly-defined shapes, as elucidated in crystal structures, has long been appreciated for enabling the multitude of functions that proteins carry out. More recently, as the field of protein science has progressed, it has become clear that all proteins are in constant motion and that protein motions have a tremendous influence on function. The meeting covered a broad array of protein functions, experimental approaches, and scales from single molecules to signaling networks and complex cellular organizations. In this report, we describe a small fraction of these presentations that are representative of both influential contributions and new directions of the field.

Dynamic proteins and complexes

Beyond the beautiful static structures that we see using crystallography, proteins are inherently dynamic, and many sample a plethora of different conformations. While dynamics are vital to the function of virtually all proteins, a major goal is to understand the dynamics of two types of proteins that have critical roles in many human diseases: intrinsically disordered proteins, and proteins that aggregate to form amyloid-like fibrils. These proteins are not easily studied by classic crystallographic techniques, but instead highlight the importance of nuclear magnetic resonance (NMR) and other biophysical methods to study conformational changes and dynamics. Intrinsically disordered proteins often have short amphipathic regions with no regular structure until complexed with specific binding partners. As a family, these proteins are predicted to comprise a significant proportion of the eukaryotic proteome, where many of them function as 'hubs' in protein-interaction networks, interacting with numerous partner proteins.

Peter Wright (Scripps Research Institute, La Jolla, USA) focused on NMR studies of intrinsically disordered

proteins, such as the transcriptional coactivators CREB-binding protein (CBP) and p300, and the tumor suppressor p53, to gain structural insights into their transformation from disordered to structured on binding their partners. The free-energy cost associated with folding of the disordered regions results in weaker interactions with high specificity that can readily dissociate to terminate a signaling event. Taking disordered proteins to the next level, Julie Forman-Kay (Hospital for Sick Children, Toronto, Canada) described how the functions of highly dynamic protein complexes can be modulated by post-translational modifications. NMR experiments with the cystic fibrosis transmembrane regulator (CFTR) indicate that the regulatory region of this protein is prone to intrinsic disorder; the balance between assuming structure and binding to the nucleotide-binding region and disorder is modulated by phosphorylation of the regulatory region. Raymond Norton (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) presented structural studies on an intrinsically disordered protein, MSP2, that is a major surface protein on *Plasmodium falciparum* and an important candidate for an anti-malarial vaccine. In solution, MSP2 is largely unstructured, but appears to form fibrils, which are predicted to be biologically relevant when present on the surface of the parasite.

Many different proteins (some of which are also intrinsically disordered) have been shown to aggregate into amyloid-like fibrils, which are the hallmarks of several neurodegenerative diseases. Jean Baum (Rutgers University, Piscataway, USA) presented the results of NMR experiments along with molecular simulations that indicate the rate of aggregation of α -synuclein (in Parkinson's disease) is dependent on the pH and aspects of protein sequence, including charge distribution. Using complementary approaches, Yuji Goto (Osaka University, Japan) described how ultrasonication can create monodispersed minimal fibrils of β_2 -microglobulin (implicated in Alzheimer's disease) that can be readily studied *in vitro*. Fluorescence microscopy and hydrogen/deuterium exchange NMR experiments were then used to follow the kinetics and mechanism of subsequent fibril nucleation and growth at high resolution. A major goal of these studies is a detailed understanding of how normal proteins can aggregate into

higher-order structures, and how to prevent such aggregation, with the hope of developing successful therapeutic approaches to treat amyloid-based human diseases.

Multivalent association and higher-order organization

The combination of multiple contact points between associated molecules leads to tighter binding than for individual contact points in isolation. This intuitively simple concept is vital to the function of many important protein systems and was widely discussed at the meeting. A number of speakers presented data indicating that multivalent interfaces in repeat-containing proteins or homo-oligomers lead to stabilization of protein structure. This reservoir of stability is thought to serve as a capacitor for functional evolution of these proteins by maintaining structure in the face of destabilizing mutations that increase the potential for altered or novel function.

Experimental approaches demonstrated the influential role of multivalent association on protein stability. Doug Barrick (Johns Hopkins University, Baltimore, USA) presented stability measurements on engineered proteins consisting of a variable number of consensus ankyrin repeats. Structurally, each ankyrin repeat is composed of two α -helices and each repeat packs against itself as well as neighboring repeats. By using consensus repeats of identical sequence, Barrick distinguished the energy of folding of each repeat from the energy of association with neighboring repeats. This analysis revealed that association of each subunit with its neighbors provides the energy to drive structure formation, and that the folding of individual subunits is on its own unfavorable.

Sarah Teichman (MRC Laboratory of Molecular Biology, Cambridge, UK) presented bioinformatic analyses indicating that protein structures have a preference for homo-oligomerization. Of the structures deposited in the Protein Data Bank (PDB), 95% contain a single polypeptide sequence with one to eight subunits in the unit cell. Teichman described how counting the number of copies in the unit cell provides an estimation of the prevalence of different oligomeric forms. Utilizing this approach it was revealed that a distinct preference exists for homo-oligomers in general and for homo-oligomers with an even number of subunits in particular. These findings suggest that homo-oligomers have an evolutionary advantage. The advantage was hypothesized to derive from the stability benefit of homo-oligomerization, enabling the sampling of increased mutational diversity without compromising folding.

Tight-binding inhibitors are highly sought after as potential drugs and Michael Kay (University of Utah, Salt Lake City, USA) gave an impressive presentation on the design of a class of multivalent tight-binding HIV fusion inhibitors. These inhibitors bind to a groove present in

triplicate on the homotrimeric gp41 surface protein of HIV and hinder fusion of the virus with host cells. Kay described how, by utilizing flexible polyethylene glycol linkers to connect three binding motifs in one inhibitor molecule, the apparent binding affinity was lowered from a micromolar level to sub-nanomolar. As with most multivalent interactions, the off-rates of the trivalent inhibitors were slowed because dissociation requires all three binding sites to dissociate at the same time. For the tightest-binding inhibitors, the off-rate was so slow that, once bound, the inhibitor-gp41 complex did not dissociate on biologically relevant timescales. With binding of this strength, infectivity was observed to be controlled by the on-rate kinetics and not by thermodynamic binding affinity. Because resistance mutations usually affect off-rates and not on-rates, these inhibitors were refractory to the development of drug resistance in multi-pass infectivity experiments.

Proteins that control membrane dynamics

Although many of the eukaryotic proteins involved in the dynamic behavior of membranes - in cell movement, endocytosis, exocytosis, and cell division - have been identified, many of the mechanisms by which they curve and pinch membranes into different configurations have yet to be elucidated. Pietro De Camilli (Yale University, New Haven, USA) showed, using fluorescence and electron microscopy combined with high-resolution structures, how the dimeric, banana-shaped BAR and F-BAR families of proteins interact with membranes to create and sense membrane curvature, which is necessary to form transport vesicles. Using synthetic giant unilamellar vesicles and fluorescence microscopy, Randy Schekman (University of California, Berkeley, USA), similarly demonstrated that the Sar1 GTPase, required for COP II vesicle trafficking of cargo between the endoplasmic reticulum and the Golgi complex, plays a direct role in creating membrane curvature for vesicle formation and scission. This reaction was highly dependent upon GTP and the amino-terminal helix of Sar1. Jenny Hinshaw (National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, USA) studies the large GTPase dynamin, and a related protein Dnm1, using cryo-electron microscopy. She presented remarkable images showing how the proteins self-associate on lipid tubules in a helical fashion. Upon GTP hydrolysis, they wrap tightly around each other to squeeze the membranes together, resulting in membrane constriction and scission.

In contrast to proteins that cause vesicle curvature and scission from the outside of the vesicle neck, the endosomal sorting complex required for transport III (ESCRT III) complex is used both for formation of internal endosome vesicles (the opposite orientation from budding of transport vesicles) and for the budding of HIV particles from the outside of the cell. Chris Hill (University of Utah, Salt Lake City, USA) described how the ESCRT III subunits

assemble into spirals that pinch off newly forming vesicles from the inside. Currently, the field awaits even higher-resolution information, especially that of specific protein-lipid and protein-protein interactions, in order to fully understand these mechanisms of vesicle scission.

The nuclear pore complex (NPC) was seemingly unrelated to vesicle budding, but turns out to have some striking similarities. Stephen Brohawn (Massachusetts Institute of Technology, Cambridge, USA) presented high-resolution structural data showing that several of the NPC subunits are homologous to the coat proteins (such as COP II) that shape newly forming vesicles, indicating a common ancestor for proteins that bend membranes. Andrej Sali (University of California, San Francisco, USA) presented a *tour de force* of computational, electron microscopic, biochemical and mass spectrometric approaches that has succeeded in modeling the entire NPC, which contains multiple copies of up to 30 different proteins. Several of these proteins line the pore and are responsible for bending the membranes to form the pore, while others on the inside of the complex regulate traffic in and out of the pore.

Future challenges

Drug resistance is an unmistakable current and future challenge for the treatment of all human illnesses that involve rapidly multiplying genomes, which includes most viral and bacterial infections and cancer. Despite the diversity of illnesses affected by drug resistance, the field has just begun to appreciate that common molecular and physical principles form the foundation of resistance in seemingly unrelated illnesses. Simply stated, rapidly dividing organisms have the opportunity to sample many different mutations in the search for protein sequences that retain biological function while weakening binding to drug. These concepts were aptly presented by many speakers. Michael Eck (Harvard Medical School, Boston, USA) presented data showing that a drug-resistance mutation in the epidermal growth factor receptor caused increased affinity for its endogenous substrate ATP, which enabled ATP to out-compete the binding of the inhibitory drug. Madhavi Kolli (University of Massachusetts Medical

School, Worcester, USA) provided evidence that in patients treated with HIV protease inhibitors, mutations in the protease and its substrates coevolve. For example, the multi-drug-resistant protease mutation I84V correlates with compensatory changes at the P1 cleavage site in the nucleocapsid substrate. In this case, the compensatory change in the nucleocapsid substrate site causes increased drug resistance. The field is beginning to appreciate the commonalities in drug-resistance mechanisms and the future hope is that this understanding will lead to the development of improved therapies.

One of the most pressing challenges in protein design is the ability to accurately predict charge-charge interactions. Electrostatic charge-charge interactions contribute greatly to the stable formation of protein structure and association of protein complexes, as well as to the binding of substrates and small-molecule effectors. Predicting the magnitude of charge-charge interactions is challenging because the dielectric constant and polarizability inside proteins is difficult to predict accurately. Bertrand Garcia-Moreno (Johns Hopkins University, Baltimore, USA) presented an impressive experimental characterization of the polarizability at multiple positions within staphylococcal nuclease. Polarizability was determined by introducing ionizable amino acids and measuring their pK_a shift. The data obtained from testing a family of 100 variants revealed that contrary to most computational models, the interior of this model protein was highly variable in its polarizability. This experimental analysis should enable improved future computational predictions of charge-charge interactions.

Overall, the meeting highlighted a number of exciting protein structures, functions and dynamics, and the wide variety of techniques and strategies used to understand them. We look forward to seeing many more at the next meeting, to be held in San Diego on 1-5 August 2010.

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