5th ISMB Symposium
20 & 21 June 2012

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This year, 2012, is the tenth since the Institute for Structural and Molecular Biology (ISMB) was founded to promote closer links between individuals and departments engaged in these areas of research in Birkbeck and University College, London. Every other year since 2004, the ISMB has held a summer symposium at one of its constituent colleges, featuring presentations from some of the most innovative researchers in the disciplines it covers. The fifth such symposium was held in Birkbeck College on 20 and 21 June, 2012. The auditorium of Birkbeck's Clore Management Centre was packed with researchers and postgraduate students who were treated to thirteen excellent talks from UK and overseas researchers, all working at the very top of their respective disciplines.

The symposium was introduced by the ISMB director, Professor **Gabriel Waksman**, who had been elected as one of forty-four new Fellows of the Royal Society in April 2012. After welcoming delegates, he highlighted the inclusion of a fifth featured research discipline: biochemistry and cell biology, joining structural biology, biophysics, computational biology and chemical biology to form a symposium programme that was as broad as it was deep. Each of the five programmes included one talk by one of the ISMB's core researchers.

The first set of talks in the symposium featured structural biology, essentially the determination of the structures of biological molecules at or near atomic-level resolution. The three speakers, chosen, as always, to complement each other, included all the main techniques that form the backbone of structural biology: X-ray crystallography, nuclear magnetic resonance (or NMR) and electron microscopy.

First to present was NMR spectroscopist **Lewis Kay**, from the Department of Biochemistry at the University of Toronto, Canada. He described techniques developed in his group for examining protein conformations that are higher in energy than the most stable ground states of those proteins and are therefore rarely observed. He used an analogy involving stick boys walking and running to illustrate the way that the presence of higher-energy protein conformations can be observed with short NMR pulses in an accessible way.

Kay then described how the technique is being applied to the biologically important problems of protein folding and mis-folding. Intermediate or "half folded" states, such as the ones observed using his NMR technique, can be "decision points" on a pathway after which a protein may either fold into its functional form or mis-fold to form a potentially toxic aggregate. His group is probing this mechanism using proteins that have been mutated into forms that favour the intermediate form; this may have important applications in the study of the many diseases that involve protein mis-folding.

Birkbeck's **Carolyn Moores**, an electron microscopist who is becoming as acclaimed for scientific communication as for her excellent research, gave the "home" contribution to the structural biology programme. Her research interests focus on the structures and dynamics of the cytoskeleton, a protein scaffold that is present in all cells and that helps to determine their shape and movement. In her talk at the ISMB symposium, she focused on one component of the cytoskeleton – the microtubules – and the proteins that bind to microtubules and determine how they form and grow.

Microtubules are hollow tubes made from a protein called tubulin. Molecules of this protein first associate together to form dimers of alpha- and beta-tubulin; the dimers associate into thin proto-filaments; and these then associate to form the hollow microtubules. Most microtubules formed *in vivo* contain thirteen micro-filaments. A range of proteins generically termed microtubule-associated proteins, or MAPs, are known to bind to microtubules, to stabilise them and to help to regulate their growth and decay. Moores' group has been using electron microscopy to examine the structures of several of these MAPs and explore how they bind to microtubules. One of these is a protein called doublecortin, which is essential for normal brain development; its loss causes a devastating neurological disease called lissencephaly. Electron microscopy revealed that this protein "decorated" the whole of the outside of the microtubule structure except at a discontinuity running along it known as the seam, with one doublecortin domain binding at the corner of four tubulin dimers. Moores has now turned her attention to a second type of MAP, a protein that binds to the microtubule ends and seems to control how tubulin dimers are added to the growing structure. Elucidating the structure of this "end-binding protein" is allowing her and her team to explore the mechanism through which these proteins control microtubule dynamics; furthermore, it has thrown up some unexpected similarities between their binding and that of doublecortin.

David Barford of the Institute of Cancer Research in London gave the concluding talk in the structural biology programme.
His research uses both electron microscopy and the third of the "big three" structural biology techniques, X-ray crystallography, to explore the structure of a large protein complex found in eukaryotic cells, the anaphase promoting complex (or APC). This is part of the machinery that controls how cells progress through the cell cycle of growth and division; it is consequently important in cancer, which is above all a disease of abnormal cell division. It acts to "tag" a number of signalling proteins for destruction by proteases through the addition of a small protein called ubiquitin. Removing proteins that characterise one phase of the cell cycle allows others to move in and so triggers progression through the cycle. In particular, the APC triggers the transition into and out of mitosis by binding to the cell-division proteins cdc20 and cdh1.

The APC is a large complex comprised of many subunits. Barford's group has been probing its structure using a two-pronged approach, using electron microscopy to obtain a low-resolution picture of the whole complex and X-ray diffraction to obtain atomic resolution structures of the individual components, and then docking the component structures into the outline of the complete complex to gradually build up the complete all-atom structure. Most, but not all, of the subunit structures are now known. Overall, the complex has a "triangular" structure with a central cavity into which its substrates are bound. Barford and his colleagues are using this structure to probe how the enzyme recognises and binds

to a specific sequence motif termed the "D-box" in its substrates. A further complex, termed the mitotic checkpoint complex or MCC, is known to inhibit APC activity by blocking substrate binding. Barford's elegant structural studies of all these proteins are already providing important new insights into cell cycle control that may inform the design and development of novel anti-cancer drugs.

The second session, on the topic of biochemistry and cell biology – new to the ISMB symposium for 2012 – also featured three speakers. First to present was **Fiona Watt**, Director of the Centre for Stem Cells and Regenerative Medicine at King's College London, whose research into the properties and interactions of stem cells also has important implications for the understanding of cancer development. Stem cells are non-differentiated cells that have the ability either to continue dividing or to differentiate into a variety of cell types. There is a layer of stem cells attached to the basement membrane of mammalian epidermis; cells in this layer detach from the basement membrane, differentiate and move up through the epidermal layers until they reach the surface and are lost.

Watt's group has been using preparations of artificial skin to study the process through which these epidermal stem cells divide and differentiate for many years. She has discovered that the behaviour of these cells is crucially determined by their immediate environment. All stem cells pick up cues from this "micro-environment" to determine whether they divide or differentiate. Watt found that differentiation of epidermal stem cells is controlled through several environmental factors. Cells exert a mechanical force on substrate-bound extracellular matrix proteins and gauge the feedback to make cell fate decisions.

Jeremy Brockes from the Research Department of Structural and Molecular Biology at UCL represented the ISMB in the cell biology programme. His work, which also has links with cancer, concerns the mechanism through which amphibian limbs can re-generate following amputation. This process is observed in salamanders and a few other amphibian species uniquely among vertebrates. It begins with cell proliferation in the limb stub to form a structure termed a blastema, which grows and differentiates to form limb structures that can scarcely be distinguished from the original ones. Regenerated limbs can themselves regenerate, throughout the normal lifespan of the animal.

There are clear analogies between blastema and tumour formation. These prompted Brockes' group to study the expression and function of the tumour suppressor protein p53 during limb regeneration. As expected, they found p53 to be down-regulated during blastema formation and to return to normal expression levels as the new limb differentiates. The expression levels of several genes known to be targets of p53, including *Gadd45*, were found to follow similar patterns. Brockes also discovered that giving compounds that regulate p53 activity during regeneration can control that process. Nutlin-3a, which stabilises p53 levels, was found to prevent blastema formation, whereas adding the p53 inhibitor pifithrin to an already formed blastema prevented limb differentiation. Experiments in cell culture models further underlined the importance of p53 in controlling the process of limb regeneration, and Brockes' group is now moving on to examine the role of p73 and other members of the p53 protein family in this process.

The final talk in the cell biology session also concluded the first day's lectures. It was given by microbiologist **David Holden** from Imperial College, London, UK who quipped that the take-home message of his talk was "Salmonella – not salamanders". Bacteria in the genus *Salmonella* are responsible for two dramatically different forms of intestinal disease. One is a normally self-limiting gut infection that, nevertheless, causes over three million deaths a year: the other, the much more frequently fatal typhoid fever, is caused by the strain *Salmonella enterica typhi*. All Salmonella species look very similar to benign Gram negative enterobacteria such as *E. coli*, but unlike these their cell walls are packed with type III secretion systems through which virulence factors enter the host cells. These are proteins that help the bacteria invade those cells and survive and replicate inside them.

The cells invaded by Salmonella are macrophages, which are adapted to trigger immune responses and to remove foreign material such as bacteria. Holden and his group have been investigating the mechanisms that enable the bacteria to evade this host response and survive inside these hostile cells by monitoring the presence of the bacteria inside the cells in real time using fluorescence proteins. Interestingly, they found an enormous variation in the behaviour of cells inside different macrophages. Some macrophages harbour populations of replicating bacteria, others bacteria that respond to signals but do not replicate, and still others bacteria that appear dormant. The bacteria that are replicating are in some ways the most vulnerable because they are the only ones that respond to beta-lactam antibiotics. In contrast, dormant bacteria, which are unresponsive to antibiotics, form reservoirs of infection that may be responsible for chronic or recurrent infection.

A lively session of 'Any Questions?' followed the first day's talks. All postgraduate students at the ISMB had been asked to submit questions on any aspect of science or research. The most interesting of these were chosen for submission to a panel of experts made up of all the day's speakers plus **Hagan Bayley** of Oxford University from the second day's programme.

Topics covered ranged from institutional research and publication strategies to the ethics of genome sequencing; one of the questions under the last topic was triggered by a media report of the whole-genome sequencing of a fetus before birth. The panellists all answered the questions frankly, in detail and with generosity to their young questioners.

The second day of the symposium began with the biophysics programme. The first of two speakers under this programme was **Taekjip Ha**, from the physics department at the University of Illinois, Urbana-Champaign, USA. His research involves the use of a form of fluorescence resonance energy transfer (FRET) to measure precise distances within single DNA and protein molecules and thence to explore changes in their conformation. One of the examples he chose was a system with single-stranded DNA bound to a protein just called "single-stranded DNA binding protein" or SSB. Waksman had been the first to publish the structure of this protein-DNA complex, in 2001. By binding fluorophores to two different sites on the DNA molecule and

monitoring the distance between them, Ha and his team were able to show how the DNA molecule slides along the protein in a motion that is slightly reminiscent of the way that a snake glides.

Ha also described a novel method for assaying the nature of protein-protein interactions within cells that combined this single-molecule fluorescence with more traditional "pull-down" techniques. Termed SiMPull (for single-molecule pull-down), this technique enables researchers to discover the number, as well as the type, of proteins forming complexes within cell extracts. He gave a simple example of the technique using protein kinase A, which forms a "dimer of dimers" and explained that it was possible to use it for sample preparation as well as as an analytical tool.

The biophysics of amyloid formation was described by **Dan Raleigh** from the Department of Structural and Molecular Biology at UCL. Raleigh was the most recently appointed ISMB researcher to speak at the symposium, having arrived there from Stony Brook University, New York, USA only earlier this year. The semi-ordered protein aggregates known as amyloid have been implicated in at least 25 human diseases. In his talk, Raleigh focused on islet amyloid polypeptide (IAPP), a protein that forms aggregate in patients with type 2 diabetes that exacerbate their symptoms.

Raleigh described the complex kinetics through which this protein first forms oligomers, then protofibrils and finally mature amyloid fibres. The intermediate forms are known to be important in pathogenesis, with some results suggesting that they, and not the mature fibres, are the most toxic forms of the protein. Although amyloid fibres are filaments of beta strands, they are now believed to pass through an alpha-helical stage as they are formed. Raleigh illustrated how his group was using point mutations to accelerate the formation of mature IAPP fibrils, potentially reducing the concentration of the most toxic particles. This work may lead to the development of drugs to control the process of amyloid formation enough to retard disease.

Sarah Teichmann from the MRC Laboratory of Molecular Biology, Cambridge, UK was the first of two speakers in the computational biology programme. Her research has always focused on protein interactions; she started her career at UCL in the 1990s under Janet Thornton who was deservedly made a Dame in the 2012 Queen's Birthday Honours. She started her talk on the evolution and assembly of protein complexes by explaining that the interior of the cell is crowded with proteins, and the processes required to ensure that the correct proteins come together to form multimers must be very complex.

Teichmann's group has set up a database, 3dcomplex.org, to hold a hierarchical classification of protein complexes. The most basic division is into homomers, containing multiple copies of the same protein, and heteromers which involve more than one protein type. The most common type of homomer is a simple dimer of two identical molecules; HIV protease is a well-known example of this type of structure. She illustrated some functional advantages of homomer formation and described how the way in which these complexes form as shown by the sizes and residues involved in the interfaces between the chains appear to have been conserved during evolution.

The ISMB speaker in the computational biology programme was **Maya Topf** from the Department of Biological Sciences at Birkbeck. She introduced her talk by describing the range of techniques within "structural biology" that are used to elucidate structures at different resolutions, from 0.1nm for an atomic radius to 100nm for some structures within cells. It is often necessary to combine information from several techniques in order to gain a complete picture of a biological structure. This can be illustrated by, for example, the use of X-ray crystallography with cryo-electron microscopy to build up a high

resolution structure of a large protein complex such as those described by Barford in the first symposium session.

This combination of crystallography with electron microscopy involves fitting atomic-resolution models of part structures into a lower resolution map of the intact complex. Topf described several informatics methods developed in her group for improving this fitting technique. Firstly, she described several methods used to score the "goodness of fit" of the structures and explained how she compares and validates these using examples in which with electron density maps of the intact complexes had been obtained to different resolution. She also discussed problems that can be caused when the complex is flexible and electron microscopy observes significant variation between structures. It is important to avoid "over-fitting" to an "expected" structure in these cases. Finally, she emphasised the importance of allowing all available information, including computational simulations and structural information from low-resolution experimental techniques such as pull-down and FRET to feed into such structural studies.

Chris Schofield of the Department of Chemistry at the University of Oxford, UK is no stranger to the ISMB; he gave one of the keynote lectures at the ISMB retreat held in Cambridge in 2009. This year, his talk was the first of three in the final symposium programme, on chemical biology. His research into the molecular mechanisms by which mammals sense and respond to the presence of oxygen has won many awards. He described how the long-term response to low oxygen levels (a situation known as hypoxia) is regulated by a transcription factor known as Hypoxia Inducible Factor 1 (HIF-1). The transcription levels of over 1% of human genes are believed to be regulated by HIF-1; one of its most important functions is the regulation of a protein, erythropoietin, which is involved in the production of red blood cells.

The activity of HIF-1 is regulated through two post-translational hydroxylation reactions. One of these involves the hydroxylation of an asparagine residue that directly blocks the interaction between HIF and its co-activator proteins, so this modification acts as a "switch" to turn off HIF-related transcription. The other mechanism involves a protein called HIF prolyl hydrolase (PHD) which oxidises proline residues in HIF, targeting it for degradation by the proteasome. Schofield described X-ray crystal structures of the complex between PHD amd HIF, showing that the interaction between these two proteins involves a significant conformational change. The protein that catalyses asparagine hydroxylation, FIH (Factor Inhibiting HIF) is less specific than PHD but more active at low oxygen concentrations. Compounds that interfere with the complex interplay between these proteins may form useful treatments for anaemia and related conditions.

Jamie Baker from the Department of Chemistry at UCL then described his research in developing synthetic methods for the chemical modification of proteins. His group has been using molecules with a core structure based on a five-membered ring system known as maleimide to add known chemical groups specifically to the side chains of free cysteine residues in proteins. Adding a group containing a halogen atom, for example, creates a reactive centre on the protein that can be manipulated in further reactions. Similar reactions can be used to add fluorescent labels and to tag the protein with groups that make it accessible to pull-down reactions.

Many protein therapeutics contain accessible disulphide bonds that can be

cleaved into their constituent sulfhydryl groups and then cross-linked with a maleimide group to add functional groups. Baker and his colleagues are applying this technology to insulin and to antibodies; the latter application can be used to produce antibody-drug conjugates. A start-up biotech company, ThioLogics, has now been created to exploit this technology in novel antibody-based therapeutics.

The final symposium speaker was **Hagan Bayley**, like Schofield from the Department of Chemistry at Oxford University. His research focuses on the properties of membrane proteins, particularly ion channels and pores; he chose to describe a relatively new project building networks of water droplets in oil, each surrounded by a membrane-like bilayer into structures referred to as "minimal tissues". Functional membrane proteins can be inserted into these bilayers. One of the simplest examples of these structures is a simple assembly of two droplets with an electrode in each one, forming a "patch clamp amplifier".

Slightly larger networks of droplets can be built with the properties of capacitors and resisters and combined into nano-scale circuits. Simply embedding molecules of rhodopsin into the bilayer of a four-droplet network can form a circuit that may act as a light sensor or as a miniscule source of electric power. Other potential applications of this technology include ultra-precise drug delivery; conducting electric current through minimal tissues as nerves do through real ones; and detecting the assembly and dis-assembly of molecules. This last application allows the sensing of individual DNA bases and can be used for ultra-rapid, single molecule DNA sequencing, and was the basis of the spin-out, in 2005, of the biotech company Oxford Nanopore.

The symposium was concluded with a few words from Waksman and from **Nicholas Keep**, Dean of the Faculty of Science at Birkbeck. In wrapping up another very successful symposium, Waksman thanked the able ISMB administrator, Andrew Service; Keep paid tribute to Waksman's vision and leadership; and both encouraged their colleagues and students to go back to their laboratories and emulate the "inspiring" scientists they had heard speak during two very full days.

The ISMB Symposium will next take place in the summer of 2014.