

3rd ISMB Symposium

19-20 June 2008

Write-up of the event

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The Institute of Structural and Molecular Biology held its third symposium at one of its constituent institutions, University College London, on June 19 and 20, 2008. This biennial meeting brings together all researchers and postgraduate students of the five departments from UCL and Birkbeck College that make up the Institute together for a two-day lecture programme. This year, over 300 people packed into the lecture theatre of UCL's School of Chemistry to hear eleven excellent talks covering all four of the Institute's research areas: chemical biology, structural biology, biophysics (incorporating proteomics) and bioinformatics. Speakers were drawn almost equally from the Institute's five departments and from elsewhere, and they included promising newcomers as well as established "research stars".

The symposium was supported by both colleges at the highest level, with David Latchman, Master of Birkbeck, and Peter Mobbs, Executive Dean of Life Sciences at UCL, introducing sessions. Mobbs highlighted the depth and breadth of science within the ISMB, saying that its scientists had published over 200 papers in 2006, "the vast majority in upper quartile journals". Latchman stressed the strong collaboration between the two institutions and the resources being poured into infrastructure and research personnel, "keeping the future of science at Birkbeck secure".

The Director of the ISMB, **Gabriel Waksman**, opened the symposium. Waksman, who was recently elected as a Fellow of the prestigious Academy of Medical Sciences, also combines his role as director with heading both the School of Crystallography at Birkbeck and the Research Department of Structural and Molecular Biology at UCL. His welcome was followed by the first of the discipline-specific programmes, in chemical biology.

The first speaker in the chemical biology session was **Peter Seeberger** from **ETH Zurich, Switzerland**. Seeberger, a carbohydrate chemist, has attracted accolades from well beyond the rarefied world of chemical biology, being named among the hundred most important Swiss citizens in 2005. He described a novel process of glycan synthesis and how it is being applied to understanding the mechanisms of deadly diseases including malaria. The word "glycan" is applied to polymers of single sugar units (monosaccharides); if they are also linked covalently to other molecules, proteins or lipids, they are termed glycoconjugates. Monosaccharides are linked together and to other molecules by reactions catalysed by glycosyltransferases, and, as they can form branched chains, the number of possible combinations is enormous. Furthermore, there is no easy glycan equivalent of the PCR reaction for automatic protein synthesis.



Seeberger has developed a novel, solid-state method for fast and accurate glycan synthesis, reducing the timescale for the synthesis of complex molecules from years to days. His group has further developed this into a complete toolbox for glycobiology, including tools for, for example, identifying carbohydrate ligands and determining protein-carbohydrate binding constants. Using this technology, they have designed carbohydrate arrays and used them to the identification of molecules in human blood that act as markers for disease, and to the identification and synthesis of carbohydrate antigens as vaccine candidates. Seeberger is co-founder of a small biotechnology company, Ancora, based in Medford, USA, which is taking vaccine candidates through pre-clinical development. They hope to file an investigational new drug (IND) application for a vaccine against malaria in 2009.



Stephen Howorka, from the **Department of Chemistry at University College**, gave the Institute's contribution to the chemical biology programme. He, also, described a novel technology and the

development of a biochemical toolkit, in this case for sensing individual DNA molecules. This uses a technique called nanopore sensing, which, in turn, was developed about ten years ago from the Coulter counter, an invaluable tool for counting and sizing cells patented in 1953. The basic principle involves measuring the change in current when a particle passes through a pore, with the magnitude of current blockade proportional to the size of the particle. Remarkable progress has been made simply by refining the technology to measure smaller and smaller particles; Howorka uses single-protein pores with diameters appropriate for the measurement of single DNA molecules. For some time, these techniques have been theoretically sensitive enough to detect single base changes, but it has not been possible to translocate the DNA through the pores slowly enough to obtain this single-base resolution. Howorka has developed several strategies for slowing down DNA translocation, including one

in which the DNA is "tagged" with one or more peptides bonded to single bases to increase the diameter of the DNA and provide a steric barrier to translocation.

The structural biology programme was opened by another member of the "home team", **Neil McDonald** of the **School of Crystallography at Birkbeck and Cancer Research UK**. McDonald



described structural details of a human receptor tyrosine kinase, RET. Mutations of this protein have been implicated in several cancers and in a rare congenital disease of the colon called Hirschprung's disease. Tyrosine kinases are enzymes that catalyse the addition of phosphate groups to tyrosine residues in proteins, and are involved in the transmission of signals into and within cells. The extracellular portion of RET contains four cadherin-like domains, binding calcium between the second and third of these. McDonald's group have investigated the structure of this region of RET and presented a model for a ternary complex of RET bound to its ligand GDNF and its co-receptor, GFR-alpha based on available mutational and structural data.

McDonald and his colleagues have also mapped a large number of RET mutations involved in Hirschprung's disease onto the protein's structure. Some mutations cause a mild form of the disease, affecting only a small part of the colon, and others a more severe form. McDonald and his colleagues devised an assay investigating the export of RET from the endoplasmic reticulum (ER) and divided the mutations into two classes with different mechanisms of action, corresponding to mild and severe phenotypes. This has implications for the genetic counselling of affected individuals.

Stephen Harrison has been a faculty member at **Harvard Medical School, Boston, USA** since 1971 and has made a number of distinguished contributions to structural biology during those 37 years, working mainly on the structures of viruses and viral proteins. He now directs the **Center for Molecular and Cellular Dynamics** there. His presentation described the mechanisms involved in the reorganisation of cellular membranes as, for example, vesicles bud from the surface of some cells and fuse with others. Membrane reorganisation is involved in numerous physiological processes, including bacterial invasion, phagocytosis, and the re-ordering of neuron membranes after neurotransmitter release. Harrison and his co-workers have been studying the structures of vesicles and their clathrin coats using electron tomography. The protein clathrin has an extended structure that forms a trimeric structure which is known as a “triskelion” as it bears some resemblance to the symbol of the Isle of Man. These structures interact and assemble into lattices on the vesicle surfaces. Harrison has examined the detailed structure and the kinetics of triskelions and the interactions between them. He is now relating this to the mechanism by which the influenza surface protein, haemagglutinin, binds with a human cell membrane, forming a clathrin-coated pit, and hence invades the cell.



Marc Baldus, of the **Max-Planck Institute for Biophysical Chemistry in Göttingen, Germany**, also described structural studies of membranes, but using the technique of solid-state nuclear magnetic resonance (NMR). NMR is more often used to study the structures of molecules in solution. The strength of the inter-atomic interactions that it records is increased in the solid state, and novel technologies are needed to elucidate these. However, this technique enables a wide range of molecular structures and conformational changes to be studied. Knowledge of the high resolution solid state NMR structure of scorpion toxin, for example, has enabled the conformational change induced when it binds to potassium channels to be both elucidated and verified from electro-physiological measurements. A similar conformational change, in which the activation-gate within the pore is closed, occurs when potassium channels are inactivated by lowering pH. Baldus and his team are now using the same technique to explore the structure of a membrane-embedded histidine kinase, DcuS, in its active and inactive conformations.



Ruedi Aebersold, based in the new **Institute of Molecular Systems Biology (IMSB) at ETH Zurich**, opened the session on biophysics and proteomics. He was one of the pioneers of the development of mass spectrometry for the analysis of small quantities of large numbers of proteins, and has collaborated with Nobel Laureate Stanley Prusiner, discoverer of infective prions. In his talk, he focused on how proteomics techniques can be used to study the aggregation of proteins within cells to form functional complexes or modules. Humans and other vertebrates have a similar number of genes as some much simpler organisms; current theories explain the complexity of higher organisms in terms of networks of interacting proteins. Enormous efforts have already been made to map such networks, with mass spectrometry and the yeast two hybrid approach the most popular methods. However, the accuracy of some of these studies is questionable, and the vast

majority have used single-celled organisms, mostly yeasts. Aebersold and his colleagues have developed a mass spectrometry based pipeline for mapping and assessing the reproducibility of protein-protein interactions in mammalian cells. This technique was recently applied to a network centred on a protein phosphatase, PP2A, and suggested proportions of single proteins and assemblies that bind to it. Aebersold and his colleagues in the IMSB are partners in the Cytoscape project, developing an open source bioinformatics network for visualising protein-protein interactions¹.



Carlos Bustamante of the **University of California at Berkeley, USA**, presented a lecture on the packaging of DNA molecules in bacteriophages that he imaginatively titled “Grabbing the Cat by the Tail”.

The first manipulation of single molecules of DNA was published in *Science* in 1992. The motor at the base of the bacteriophage $\phi 29$ has to compact the phage DNA molecule 6000 times to fit it into its tiny 42nm x 54nm capsid. Bustamante and his colleagues have developed a novel method of measuring the tension in this single DNA molecule as it is packaged into the capsid using an “optical trap”. They have measured the mean force applied to the DNA to be about 55pN, over ten times stronger than the force generated by a molecule of the muscle protein myosin, and found that it generates a pressure of 60 atmospheres inside the capsid. This process requires the expenditure of ATP, which is provided, inadvertently, by the bacterium infected by the phage: the phage uses this enormous pressure and force to infect the bacterium. Applying Michaelis-Menten single molecule kinetics to the system, Bustamante and his colleagues discovered that the expenditure of one ATP molecule was needed to compact each two base pairs of DNA. Knowing that the motor predominantly tracks the 5'-3' DNA strand,

the group is now elucidating the atomic details of the interaction.

The “home team” contribution to the biophysics session was given by **Peter Rich** from the **Department of Biology at UCL**. He was formerly the director of the Glynn Research Institute in Cornwall, founded by Nobel Laureate Peter Mitchell, and moved the Institute to UCL in 1996. His research focuses on the application of vibrational infrared (IR) spectroscopy to the structures and mechanisms of large, complex proteins. IR spectroscopy is a classical analytical technique in chemistry that detects vibrations of molecules, but a typical protein will have many thousands of vibrational modes which are difficult if not impossible to resolve in absolute spectra. However, if a specific local change in a protein is induced, the resulting IR difference spectrum consisting of a small number of localised vibrational band changes can be interpreted in terms of structural and chemical changes arising only from the perturbed site. The spectra can give information on substrate or ligand binding, protein conformational changes, chemistry of intermediates and changes in protonation states of amino acids: Rich’s group have applied the methods to a range of protein reactions, in particular to ligand binding and heme-heme electron transfer in cytochrome c oxidase. In this protein, electron transfer between heme groups is coupled to proton translocation, and IR spectroscopy is an ideal technique for measuring both electron and proton movements at the same time. In one line of work, Rich and co-workers were able to distinguish changes in polypeptide backbone conformation and associated chemical and protonation changes on formation of one catalytic intermediate. The group is extending such studies to the time resolution of further electron, proton and chemical changes, with the eventual aim of reconstructing the kinetics and mechanism of the entire catalytic cycle.

The final session, on bioinformatics, began with a lecture from **Peer Bork**, from the **European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany**, who is currently Europe’s most cited researcher in genetics. His wide-ranging presentation on

¹ www.cytoscape.org

protein function prediction encompassed homology based and network based techniques, and the emerging discipline of metagenomics, which is concerned with the genomic analysis of mixed-species environmental samples. These techniques all depend on the availability of vast quantities of sequence information: we now have full sequences for well over 600 prokaryotic and over 40 metazoan genomes. Protein function prediction is aided by following the “shuffling” of domains within and between protein chains during evolution, and by exploring protein interactions with small molecules. Bork has developed the hypothesis that drugs that share side effect profiles may also share molecular targets into the database MATADOR: Manually Annotated Targets and Drugs Online Resource². Metagenomics involves harvesting organisms (mainly prokaryotes) from habitats such as sea water, farm soil or even a mammalian gut, and comparing their genomic content. The functional repertoire of the combined proteomes of the organisms harvested has been found to depend on their habitat: for example, active sodium and potassium transporters are found more often in bacteria from farm soil than in those from sea water.

The ISMB’s representative in bioinformatics was relative newcomer **Irilenia Nobeli**, who was appointed as a lecturer in the **School of Crystallography at Birkbeck** in 2007. She also tackled the topic of protein function identification, but, as a specialist in cheminformatics, she focused on the use of small molecule ligands in this process. Despite all recent advances in gene sequencing and protein identification, about 25% of predicted proteins still have no homolog of known function. Nobeli has developed a method for analysing proteins of known structure but unknown function by docking diverse small molecules into their predicted binding sites, on the assumption that the natural substrate or a similar compound will bind more tightly than any compound taken at random. Testing this approach with proteins with known ligands, she found that the substrate fell within the top 10% of binders about three-

quarters of the time, which she felt to be encouraging if not dramatically successful. This promising approach, however, is unlikely to predict successfully enzymes or substrates that are promiscuous binders, or substrates that are known to bind weakly to their known targets such as mannitol binding to mannitol dehydrogenase.

The last speaker in the bioinformatics session, and in the whole symposium, was **Mike Sternberg** from **Imperial College, London**. He provided an overview of current methods of predicting protein structure, function and interactions from sequence. Protein structure prediction can be divided into template-based (homology modelling and fold recognition) and template-free methods. Template-based methods, particularly homology modelling, are reliable and well understood; most progress is being made with the more experimental template-free methods, which are used for proteins with no known or expected structural analogs. In the last biennial blind trial of structure prediction methods, CASP7 in 2006, the most successful template-free method was that developed by David Baker’s group at the University of Washington; it involves assembling small fragments into complete structures. Sternberg is using a different approach, trying to simulate protein folding pathways using Langevin dynamics with a simple “lollipop” model of the protein backbone and side chains. This method is beginning to approach the accuracy of Baker’s prediction methods, but, significantly, uses far less computational time. Further projects in his lab are involved in predicting which proteins will change conformation when binding to other molecules, and predicting protein function from a combination of homology and ontology based methods.

In his introduction, Mobbs offered a debt of gratitude to Waksman in so ably promoting and facilitating the flourishing of molecular sciences in central London. This debt must be owed not least for his ability to attract such distinguished scientists, all also talented and engaging speakers, to present their work on the ISMB stage. In concluding the meeting, Waksman, in his turn, paid tribute to his ISMB colleagues and his able administrative team.

² <http://matador.embl.de>