5th ISMB Retreat
3 – 4 July 2013

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The Institute of Structural Molecular Biology (ISMB), set up to foster collaboration between researchers in University College London and Birkbeck who work in molecular and structural biology and allied disciplines, is now ten years old. It has established two series of biannual research meetings designed to grow these collaborations, inspire research excellence and encourage new ideas. The ISMB symposia, held in even years, feature lectures by world-class researchers in its constituent disciplines, and the residential retreats, held in odd years, are dominated by talks by postgraduate students and young postdoctoral researchers from its core departments. The retreat held on July 3 and 4 2013 was therefore the fifth in the series. It was also the third to be held in the quiet surroundings of Robinson College in Cambridge. About 120 delegates, almost all of whom are affiliated to Birkbeck or UCL, took part in a fascinating programme of science that included three keynote lectures by brilliant senior researchers and ten short talks by students and postdoctoral researchers. A poster session and an activity intriguingly titled "Professional Speed Dating", which was designed to expose the younger delegates to a wide variety of careers in science, completed the scientific programme.

The retreat was opened by Gabriel Waksman, head of both the Department of Biological Sciences at Birkbeck and UCL's Research Department of Structural and Molecular Biology and founder director of the ISMB. He welcomed delegates to Cambridge and to Robinson College and introduced the first keynote speaker, **Professor Chris Dobson** of the Department of Chemistry at the University of Cambridge and Master of St. John's College there. Dobson's research career has included periods at three of the world's leading research universities: first Harvard as a young Principal Investigator in the late 1970s, then Oxford and finally, since 2001, Cambridge. He has received numerous honours and awards and is a Fellow of the Royal Society and a Foreign Member of the National Academy of Sciences of the US.

Dobson's research has been particularly focused on the mechanisms through which proteins fold, or on some occasions misfold. This area of science has become increasingly important because of its link with human disorders and strategies through which they can be combatted. Dobson's recent work is summed up in the title of his talk: 'New Approaches to Understanding and Preventing Neurodegenerative Diseases'. He began with a discussion of this group of diseases, explaining how the prevalence of neurodegenerative disorders, and Alzheimer's disease in particular, is increasing dramatically as the population ages and how, unless there is a dramatic improvement in the therapies available, the yearly cost of caring for people with this condition will challenge the health care systems of the much of the world. For example, the estimated costs of Alzheimer's diseases in the USA will rise from $172 billion in 2010 to over a trillion dollars by mid-century.

Alzheimer's disease is a classic example of a "protein misfolding" or "amyloid" disorder. Most but not all proteins need to fold into a unique and often highly intricate structure in order to perform their functions, and this is a fascinating and complex process, particularly in the crowded environment of a living cell. Dobson drew attention in this context to the work of John Christodoulou at UCL who has made remarkable progress in defining in molecular detail the process through which a nascent protein chain folds as it leaves the ribosome. He also discussed the various ways in which proteins that mis-fold can cause disease. Alzheimer's disease is only one of over 50 different diseases in which the protein involved folds into an

aberrant structure known as an "amyloid fibril", and can become deposited in specific cells or tissues. Unlike correctly folded proteins, the amyloid "fold" is not encoded in the amino acid sequence. Many proteins, and even polymers of single amino acids, can under some circumstances form amyloid structures with very similar underlying characteristics.

A key detailed molecular model for a "cross-beta" amyloid structure, stabilised by main chain – main chain hydrogen bonding, was determined in conjunction with Birkbeck's Helen Saibil using cryo-electron microscopy more than a decade ago, and this collaboration has recently led to the first complete, atomic resolution structure of an amyloid fibril. Dobson believes that almost any protein may mis-fold into this structure, although some will do so much more readily than others. Understanding the detailed mechanism of this process provides key insights into the molecular origins of this class of diseases: Dobson and his colleagues in Cambridge and elsewhere are now developing strategies for the design of small molecules and other therapeutics that can interfere with the process of amyloid formation and that may therefore halt the progression of Alzheimer's and many other devastating diseases.

This lecture was followed by the first three of the short talks by student and postdoctoral members of the ISMB. **Natalie Dawson**, a PhD student in Christine Orengo's bioinformatics group in the Research Department of Structural and Molecular Biology at UCL had the challenging task of following immediately after Dobson. She described her work on the automatic annotation of protein sequences by function. Although it is becoming easier to annotate protein sequences structurally as the proportion of known folds grows, functional annotation of novel sequences is more challenging. The Orengo group has developed a tool for dividing proteins into classes based on their predicted functions, and has classified the proteins in each of CATH's structure-based super-families into these so-called 'FunFams'. Dawson described classifying enzymes of known structure using this method, showing that the functional sub-families in a given enzyme super-family may, or may not, share their chemistry or their catalytic residues, and that this precise relationship can relate to the evolutionary origin of the proteins. She is intending to apply the technique to proteins from the large, diverse bacterial populations that are found in environments such as the human gut.

Mun Peak (Peg) Nyon from the Department of Biological Sciences at Birkbeck gave the next talk. She returned to the theme of Dobson's keynote lecture, protein misfolding diseases, in describing her work on the molecular mechanisms of alpha-1 antitrypsin deficiency. In this disease, mutations in the protein alpha-1 antitrypsin cause the protein to mis-fold and accumulate in the endoplasmic reticulum within cells. She described the technique she used to study these deposits as a "bottom-up method", starting with an analysis of the proteome of the endoplasmic reticulum from healthy and diseased cells with and without doxycycline (which is known to induce alpha-1 antitrypsin expression). Over a thousand proteins were identified in one or more samples and 448 were found to be common between them all; the ER fraction from diseased tissue treated with doxycycline contained the highest concentration of alpha-1 antitrypsin. She is now examining the quantitative changes in protein expression between the different conditions with the aim of identifying novel response mechanisms in alpha-1 antitrypsin deficiency and other diseases involving the endoplasmic reticulum.

Paul Moody, a Wellcome Trust funded PhD student in the Department of Chemistry at UCL gave the next talk. He described the use of green fluorescent protein (GFP) as a model protein for the development of techniques to functionalise small, tractable protein scaffolds as biotherapeutics. Many proteins have been designed as therapeutic agents, but the largest class of these – antibodies – are large enough molecules to be difficult to express and study. Protein therapeutics are ideally homogeneous and can benefit from being modified chemically. Moody and his colleagues are developing a technique for the chemical modification of proteins that involve the incorporation of both dehydroalanine and sulphonium into the

protein chain in the place of cysteines. They have proved the principle of these techniques by incorporating functional groups into GFP, and are hoping to adapt them so that it is possible to incorporate more useful molecules, including drugs.

The afternoon's scientific presentations ended with the second keynote lecture, by **Leonid Sazanov** from the MRC Mitochondrial Biology Unit in Cambridge. Sazanov, originally from Belarus, had reached his present position via Minsk and Moscow, and then Birmingham University and Imperial College in the UK. His talk described some elegant structural biology carried out by his group in elucidating the structure of the largest of the protein complexes that carry out the crucial metabolic task of respiration.

Respiratory complex I is a huge – in molecular terms – "molecular machine" that is embedded in the membranes of both mitochondria and bacterial cells. In cellular terms, respiration involves the conversion of potential energy derived from food first into a proton gradient across the membrane, and then into chemical energy in the form of ATP. The reaction catalysed by respiratory complex I is, as the enzyme's name implies, the first in this process; it involves the transfer of two electrons from NADH to a quinone, coupled to the translocation of four out of a total of 10 protons across the membrane. Mutations in this complex have been associated with severe disease.

In humans, complex I comprises 44 subunits with a total molecular mass of about 1,000 kDa. The complex is much smaller in bacteria (although still very large for a protein, at about 550 kDa), and comprises about 14-16 subunits; however, these subunits form the "core" of the complex and each is homologous to one of the human subunits. It can therefore be taken as a model for the human complex. The structural biology of complex I has been pioneered by Sazanov and his group, and they have now finally published the first high-resolution structure of an intact complex. This structure, obtained using the enzyme from the thermophilic bacterium *Thermus thermophilus*, was the main topic of his fascinating lecture.

Sazanov described the overall L-shaped structure of the complex, with a hydrophobic, membrane-spanning base and a hydrophilic arm located in the cytoplasm that binds a string of nine iron-sulphur clusters. The base includes three similar antiporter-like subunits, each of which contains 14 conserved transmembrane helices, and each of which can translocate a single proton across the membrane. However, the proton channel in each of these subunits is seen to be "broken" into two half-channels that are separated in space.

The structure of one further transmembrane subunit, known as Nqo8/ND1, has proved to be the last remaining key of the puzzle to understand the mechanism of action of the whole complex. This, which is the most conserved of all the hydrophobic subunits in the complex, has a fold that is similar to half an antiporter protein. Further membrane-bound subunits located at the junction of the base and the arm of the complex combine with Nqo8 to form a fourth complete antiporter domain. The enzyme mechanism involves NADH binding to the top of the arm of the structure, and electrons passing down the chain of iron-sulphur clusters until they reach a quinone bound at the junction of the base and the arm. This leads to a series of conformational changes that are propagated along the base of the complex and that lead to the closing and opening of each of the four antiporter channels for proton transfer in turn. Knowing this mechanism, which can be assumed to be completely conserved in humans, is already providing Sazanov and his colleagues with insight into the molecular mechanisms of several important diseases. In thanking Sazanov for his talk, Waksman commented that the impact of this structure was only just being recognised, and speculated that he might possibly be in line for a future Nobel Prize.

Very few research students and post-docs, however, can have the talent and opportunity to forge a stellar research career like Dobson's or Sazanov's. The **Professional Speed Dating** event that followed the first day's scientific programme was set up to alert young scientists to the wide range of scientific career opportunities open to them both inside and outside the lab. All

participants were divided into four groups containing equal numbers of young scientists and more experienced "experts"; the experts sat at tables and the students and postdocs circulated with four minutes to discuss each expert's career. Professionals from outside the Institute from areas including publishing, patent law, drug discovery and scientific administration augmented the "home team" of lecturers and principal investigators. This fast-moving exercise was a rewarding, enjoyable and eye-opening experience for both students and experts, although with about forty people and twenty simultaneous conversations in each room many found it noisy and exhausting. The day ended with dinner and a well attended poster session.

The second day started early with two groups of student and post-doc presentations, separated by a welcome coffee break. **Geraldine Levy** from the Research Department of Structural and Molecular Biology at UCL gave the first talk. Her work is supervised by Bibek Gooptu and John Christodoulou, and, like Peg Nyon's, it concerns alpha-1 antitrypsin deficiency. She described using NMR and circular dichroism spectroscopy to study the mechanisms through which alpha-1 antitrypsin folds into normal (metastable, native) and pathogenic (hyperstable, polymeric) structures. Detailed structural studies show how conformational lability varies around the native fold and indicate that folding and unfolding of this protein in urea is a two-state process, the largest such process described to date. However, in physiological conditions, introduction of a disease mutation favours the population of an intermediate ensemble that is near-native in its structure and dynamics.

Marija Lesjak, originally from Serbia and now also at the Research Department of Structural and Molecular Biology at UCL, spoke next. She described her research into the role of quercetin in the process of iron homeostasis. Iron is an essential element in human metabolism, and its concentration in the body is determined by a complex series of processes. Iron deficiency, which is the most common of all nutritional deficiencies, and iron overload can both cause disease. Lejsak described an experiment to monitor iron homeostasis in rats that had been fed an iron-deficient diet and then dosed with quercetin. This showed that even a single dose of quercetin inhibits iron absorption through chelating the iron. These results suggest that this natural product might be useful in treating diseases of iron overload including haemochromatosis.

Mass spectroscopy is generally considered to be best suited for structural studies of small, soluble proteins. The next talk, by **Jun Yan** of the Research Department of Structural and Molecular Biology at UCL, highlighted the fact that this powerful technique is now being applied to the structures of membrane proteins and complexes. Mass spectrometers measure the mass-charge ratio of ionised molecules, and some modern ionisation techniques are 'soft' enough to keep complexes intact. Yan described applications of this technique to two membrane protein complexes from bacteria: the adhesin assembly complex from the Type 1 pilus or fibril that enables *E. coli* to attach to the bladder wall and cause urinary infections, and the core complex from the type IV secretion system. This secretion system is present in many species of bacteria and is responsible for the secretion of many substances, including toxins, from the bacterial cells.

The final speaker in the second session was also a student from UCL: **Leila Shariff** of the Department of Chemistry. She described some novel techniques for the synthesis of cyclic peptides. These are often more readily bioavailable than linear peptides, and can have important biological properties, but most current methods for their synthesis are complex and expensive. The method developed by Shariff and her colleagues in Derek Macmillan's group at UCL is an adaptation of a common method involving bonding a thioester at one end of the peptide to a free thiol at the other. Its unique feature is the exploitation of a novel method of forming a C-terminal thioester *in situ* from peptides that terminate in cysteine residues. She illustrated the technique with an example with potential medical applications: the synthesis of analogues of a naturally occurring cyclic peptide, sunflower trypsin inhibitor-1, that inhibit an enzyme involved in atopic dermatitis.

Anathe Patschull from the Department of Biological Sciences at Birkbeck started the final session of student and postdoc presentations with a talk about her research into the virus that causes Kaposi's sarcoma. This type of skin cancer mainly affects people with compromised immune systems and came to prominence in the 1980s in association with the AIDS epidemic. Its main causative agent is a double-stranded DNA virus in the herpes virus family, which undergoes both a lytic and a latent phase. Patschull described the role of a viral nuclease enzyme, SOX, in recognising and binding to specific target sequences and structures in host RNA molecules, which initiates the rapid degradation of the host mRNA. She has now discovered that the mechanism of RNA degradation involves an interaction between SOX and a host ribonuclease, Xrn1, and is beginning structural studies of SOX in complex with both Xrn1 and RNA.

Olena Myronova from the Research Department of Structural and Molecular Biology at UCL described her studies of cell signalling pathways that centre on a protein called mTOR (mammalian target of rapamycin). This protein is involved in many protein-protein interactions and has many functions in both normal and cancer cells.
"Myronova's work focuses in particular on studying the role of the S6K2 splicing isoform in the regulation of mTOR signalling. S6K1 and S6K2 have been implicated in cancer, diabetes and ageing, and mutant mice that lack S6K1 expression look normal but are small in size and live significantly longer than wild type mice. She has shown that a recently identified isoform of S6K2 lacking part of the kinase domain, S6K2-S1, can bind to mTOR and affect its signalling, particularly during starvation. Furthermore, S6K2-S1 is able to suppress the growth of tumours both *in vivo* and *in vitro*.

The last of the young scientists' presentations was given by **Isabella Tsai**, a post-doc in the Research Department of Structural and Molecular Biology at UCL. Working with her supervisor Snezana Djordjevic, she has solved the crystal structure of one domain of neuropilin NRP2, a cell surface receptor, bound to the C-terminus of the growth factor VEGFA. Understanding the direct interaction between these proteins sheds light on therapeutically important topics including the prevention of VEGF-signalling related diseases including cancers and metabolic conditions. She also used X-ray crystallography, bioinformatics and biophysical analysis to probe zinc binding sites on neuropilin 2 that had not been observed before.

The final keynote lecture was given by **Professor Sir Steve O'Rahilly** from the Institute of Metabolic Science in Cambridge. It proved to be a very engaging and entertaining one, even though it focused on some quite distressing medical conditions: obesity, metabolic syndrome and diabetes. O'Rahilly's theme was the interaction between genetics and these metabolic diseases; although we may think that our weight depends on our circumstances, a large part of whether an individual is obese or lean is determined genetically with estimates of the hereditability of body mass index (BMI) varying from 0.6 to 0.8.

O'Rahilly began by dividing studies of human genetics and its relationship with disease into two classes. The 'butterfly net' approach involves searching for rare, interesting variants, while the more pedestrian 'sweatshop' approach studies small effects in large groups of people. And O'Rahilly has applied both these approaches to obesity and diabetes. A small number of unfortunate individuals have rare genetic variants that predispose them to extreme obesity. O'Rahilly characterised these genes and, interestingly, found that they are all highly expressed in one region of the brain: the hypothalamus. Extreme obesity, therefore, appears to be mainly a neurological disorder of appetite control – people carrying 'obesogenic' variants are simply driven to eat. And in one case a very successful treatment has been found. The binding of a hormone called leptin to a particular neuropeptide produces the sensation of satiety (being full) and the rare individuals who lack functional leptin become severely obese in early childhood. Simply giving these people leptin as a drug will reduce their BMI to normal levels in a relatively short time.

Most obese individuals, however, cannot be associated with a single-gene condition. O'Rahilly has also applied the 'sweatshop' to the genetics of obesity, and identified a large number of genes that each have a small effect on BMI. About 60% of people have at least one copy of the 'obesogenic' variant of one gene, FTO, which has a moderate effect on BMI. These genes, however, all share one feature with those that have been associated with severe childhood obesity: they are expressed in the brain, and involved in the control of appetite and food choice.

Although obesity is a risk factor for diabetes, not everyone who is obese will become diabetic, and some who are merely overweight or even of normal weight do. The metabolic path through which excess adipose (fat) tissue can lead to problems with insulin signalling and to diabetes is a complex one that is affected by many genes. O'Rahilly ended his talk with an exploration of some of these. He described developing a strain of mice with an apparently infinite capacity to store fat: they become extremely obese but never develop diabetes.

After such a (non-obesogenic) feast of science, one very pleasant task remained: awarding the prizes for the best poster and the best student or post-doc presentation. The four young ISMB lab heads who judged the talks and posters were unanimous in praising the quality of both and highlighting the difficulty in choosing winners. They finally picked Jun Yan as the winner of the prize for the best talk. The poster prize was won by Ksenia Ryzhenkova from the Department of Biological Sciences at Birkbeck for a poster entitled "Structural study of the E1 helicase/DNA replication complex by negative stain electron microscopy". And Waksman ended the retreat by thanking the one person without whom the retreat would not have happened at all: the efficient, and invaluable, ISMB administrator Andrew Service.