4th ISMB Retreat
21-22 June 2011

Report by Dr. Clare Sansom, Department of Biological Sciences, Birkbeck

The Institute of Structural and Molecular Biology holds a residential retreat every two years. These are designed to foster collaboration between researchers in the Birkbeck and UCL departments that comprise the Institute, in particular by giving PhD students and postdoctoral researchers the chance to present their work. In 2011 researchers from Birkbeck and UCL were joined by some in the MRC National Institute for Medical Research (NIMR) in Mill Hill, north London. The majority of the talks were given by young scientists; there was an extensive poster session; and students and post-docs also competed for two fictitious research jobs before an audience of their colleagues in a fun and fast-moving session named "Scientific Apprentice" after the well-known TV series.

Robinson College, Cambridge

The 2011 retreat was the fourth in the series, and the second in succession to be held in Robinson College, founded in 1981 and still the newest of Cambridge's thirty-one colleges. Besides the talks from student and post-doc members of the ISMB, the programme featured three excellent keynote lectures, all given by group leaders at the MRC Laboratory of Molecular Biology (LMB). The LMB has a research track record that must be almost unique, certainly in Europe: thirteen of its distinguished scientists have shared nine Nobel Prizes between them since it was founded in 1947.

The meeting opened with a short address by the director of the ISMB, Professor Gabriel Waksman, to welcome all delegates, and this was followed by the first of the three keynote lectures.

Jason Chin, head of the MRC Centre for Synthetic Biology at the Laboratory for Molecular Biology was the first to speak. His lecture was titled, intriguingly, "Reprogramming the Genetic Code"; this summed up concisely the ambition of his research programme to exploit the "central dogma of molecular biology" – the process through which the information in DNA passes through RNA to make functional proteins – to construct molecules with novel functions. Protein translation is a complex process, and changing it to produce a completely new molecule requires synthetic changes to the synthesis machinery, the ribosome, and to the transfer RNA (tRNA) molecules that form a physical link between triplets of RNA bases and amino acids. All sixty-four possible codons are matched with amino acids in the standard genetic code apart from two that encode "stop signals", and the simplest feasible synthetic changes to the genetic code involve making changes to the tRNA that recognises one of these, the so-called "amber stop" codon.

A system for incorporating a non-standard amino acid into a protein therefore involves mutating an aminoacyl-tRNA synthetase so it will synthesise a tRNA molecule that covalently links the amino acid in question, and mutating a ribosome to recognise that

tRNA via the "amber stop" codon. Working mainly with the model bacterium *E. coli*, Chin and his co-workers mutated a synthetase from the archaeobacter *Methanosarcina mazei* to create a tRNA to recognise and incorporate an acetylated lysine residue into bacterial proteins. They have now begun investigating means of incorporating more than one non-standard amino acid into a protein. This, necessarily, involves mutating ribosomes to recognise patterns other than the standard 64-codon (base triplet) set; these, also necessarily, must evolve in parallel with "non-evolvable" wild type ribosomes. Chin has now developed a series of synthetic ribosomes that recognise four RNA bases, rather than three, and incorporate non-standard amino acids into proteins in response using the same archaeal synthetase-tRNA pairs. It should, eventually, be possible to synthesise protein-like molecules incorporating over 200 different unnatural amino acids using this approach.

Dr Max Yun of the Research Department of Structural and Molecular Biology, UCL gave the first of the student and post-doc presentations. Her research, within the group of Professor Jeremy Brockes, concerns the mechanisms through which amphibians, and particularly salamanders, are able to regenerate amputated limbs. This process involves two basic stages: first the proliferation of a mass of cells known as a blastema and then the differentiation and morphogenesis of the blastema into a functional limb. Blastema cells have similar properties to tumour cells although, interestingly, regenerated limbs are remarkably resistant to carcinogenesis. If a limb is amputated over the site of a tumour, that tumour will regress.

Dr Yun therefore investigated the involvement of tumour suppressor genes such as p53 in salamander limb re-growth, finding that the activity of this important gene was down-regulated during blastema formation but returned to normal levels during differentiation. A similar pattern was observed in some of the principal p53 target genes, and inhibition of the p53 protein during the differentiation phase prevented differentiation from taking place. Further work by Yun and her colleagues will focus on investigating the molecular mechanism of this tight regulation of p53 during limb re-growth, and why p53 downregulation during blastema formation does not increase the propensity for the limb to form tumours.

The second presentation was given by **Asif Tamuri**, a research student in Richard Goldstein's group in the Department of Mathematical Biology at NIMR. It described his statistical analysis, derived from phylogenetic data, of the relative advantage or disadvantage conferred by mutations. The advantage conferred by a mutation can be represented by a selection coefficient, S; negative values of S imply that the mutation is deleterious to the organism, while positive values indicate a fitness advantage. The distribution of selection coefficients in a sequence family over time, which peaks close to S=0, is crucial to, for example, the analysis of the genetic basis of complex diseases. This can be predicted theoretically or derived in practice from "wet lab" experiments or phylogeny. Several people have shown that mutation numbers decline exponentially as S increases. Adaptations during rapid evolution, where many mutations are accepted, can be picked up rapidly from S distributions, and Tamuri and his colleagues are using this to study the genetic shifts that occur when, for example, a strain of the influenza virus shifts from an avian to a human host or *vice versa*.

The first talk in the second section was given by **Dr Arefeh Seyedarabi** from the Research Department of Structural and Molecular Biology at UCL. She described an analysis of the structure and function of neuropilins and their interactions with the growth factor VEGF and with heparin. Neuropilins are cell surface receptors essential for normal development of both the neural and the vascular systems. They are transmembrane, multi-domain proteins, with several domains of the extra-cellular region involved in growth factor binding, and the sulphated polysaccharide heparin is known to enhance that binding. Seyedarabi, who is supervised by Snezana Djordjevic and supported by the British Heart Foundation, has over-produced and purified the neuropilin and VEGF domains involved in complex formation; she now intends to analyse the complexes using X-ray

crystallography, NMR and thermodynamics techniques.

The first day's final two presentations were given by research students from the Department of Chemistry at UCL. **Felix Schumacher**, who is supervised by James Baker, gave a talk entitled "Functionalisation of Disulfide Bonds with Maleimides". Baker's group, not unlike Chin's, is studying methods of inserting functional groups into proteins. He, however, is targeting the reactive side chains of cysteine residues with a reaction that cleaves disulphide bonds – the only covalent bonds to form between side chains in unmodified proteins – and immediately inserts a small aromatic compound called a maleimide between the sulphur atoms. This essentially broadens the covalent link between the two cysteine residues and adds a functional group attached to the maleimide. Schumacher has modified disulphide bonds in a small peptide hormone, somatostatin, and an antibody fragment in this way and proved that the altered molecules are still active. He is now exploring whether modifying disulphide bonds in an intact antibody will maintain its activity. If so, he will have introduced a "handle" for bio-conjugation into a functional antibody.

Schumacher was followed by **Mikiembo Kukwikila**, a PhD student with Stefan Howorka. Howarka's group is developing a very sensitive and precise method of sensing the activity of proteases – enzymes that catalyse the cleavage of proteins or peptides – using nanopores. These are tiny holes in lipid or synthetic bilayers; when a voltage is applied across the pores, ions flow through them giving rise to a measurable current. A molecule inside the pore will block the passage of ions and thus cause a downward "blip" in the current. When a protease substrate is immobilised above such a nanopore, protease activity will free a peptide to pass through the pore, enabling protease activity to be measured at the single molecule level. Kukwikila and her colleagues have shown this to work in principle using trypsin, and measured the frequency of peptide release and the time it takes the peptide to pass through the pore. She is now applying the technique to renin, a protease involved in the control of mammalian blood pressure.

The gap between the first day's talks and the splendid conference dinner and poster session was filled by the **Scientific Apprentice** session. Before the retreat, each participating student and post-doc had completed an application for one or two fictitious jobs, a research position in virology in a small biotech company, ISEMBA, and a lectureship at the ISMB; each was interviewed during the retreat by one member of staff. Interestingly, applicants for the industrial position outnumbered those for the academic position by five to two. The interview panels then chose four finalists for each position, and these candidates were grilled by the respective panels in front of the other delegates in a plenary session. Just as in the TV Apprentice, it was the audience who judged who was appointed to each position. Zakiyya Ahmed, a PhD student in Biological Sciences at Birkbeck with several years' experience in the pharmaceutical industry won the industry section, and the "lectureship" was awarded to Dr Nicolas Werbeck, a post-doc from UCL.

When questioned afterwards, most delegates said they found the Apprentice session enjoyable, and participants generally found it useful. All delegates, however – except, possibly, those already at the top of their careers – could have benefited from advice given after the exercise by the heads of the two fictitious interview panels. Professor Peter Goodfellow, a former vice-president of SmithKlineBeecham who chaired the industry panel, praised all candidates as "articulate" before advising job candidates to give interview panels an idea of themselves as people, not just scientists. He also advised those wishing to work in industry to get some "good, solid postdoctoral training" behind them first.

Gabriel Waksman, as "real life" head of the ISMB, was the obvious choice as chair of the academic panel. He was unequivocal about what he looked for in appointments to new academic positions: "the best researchers from the very best institutes", and he advised postgraduates with ambitions to work in academic research to choose the

laboratories and supervisors for their postdoctoral studies very carefully indeed.

Evening poster session, 21 June 2011

The second day began with a fascinating keynote lecture by a young MRC-LMB group leader **Dr M. Madan Babu**. As recently as 2004, Madan Babu was awarded a prize at the LMB as an outstanding graduate student; he then spent two postdoctoral years in the US before returning to Cambridge to set up his own group there. His research is concerned with the mechanisms of regulation in cellular systems; his talk at the retreat dealt with one part of that work, the regulation and control of so-called intrinsically unstructured proteins. About 40% of any eukaryotic proteome consists of proteins that lack well-defined three-dimensional structures, either wholly or for a significant part of their length. These proteins are thought to be particularly important in protein-protein interaction networks as they are able to interact with many binding partners, and their expression patterns are altered in cancer and neurodegenerative diseases.

Madan Babu's research addresses the question of how, given the "crowded" nature of the intracellular environment, unstructured proteins avoid "toxic" aggregation, suggesting that the availability of these proteins must be tightly and specifically regulated. He and his colleagues used programs including DisoPred2 from David Jones' research group at the ISMB to predict disordered regions in about a third of all proteins in the yeast proteome, and then investigated the abundance of the mRNA transcripts that encode these proteins. He found that although transcripts encoding unstructured proteins are synthesised at the same rate as other mRNAs, they are degraded more quickly, perhaps because they are recognised by a specific set of RNA-binding proteins and have short poly-A tails. Similarly, he found that these proteins are themselves degraded more quickly than proteins with defined structures, and linked this to the abundance of so-called PEST sequences in them. Taken together, these results imply that the abundance of unstructured proteins in cells is tightly regulated via the fast degradation rates of both these proteins and the mRNA molecules that encode them. This leads to the rapid clearance of unstructured proteins from cells, allowing them little time to aggregate.

The third section of presentations from postgraduate and postdoctoral researchers began with a talk by **John Hales**, a student in the Research Department of Structural and Molecular Biology at UCL. Hales works in Professor John Ward's group and with the London Centre for Nanotechnology, and he uses biological components to make materials and devices at the "nano" scale (1nm = 10^{-9}m, or a millionth of a millimetre: the same scale as biological macromolecules). Hales and his colleagues have developed a method of engineering bacteriophages – virues that infect bacteria – to produce cylinders of different lengths, structures and chemical compositions by mutating the sequence of their DNA. These are easier to make than the carbon nanotubes that they resemble, and their surface properties can be controlled more exactly.

Next, **Dr Adeline Goulet**, a postdoc working with Carolyn Moores in the Department of Biological Sciences at Birkbeck, presented her studies of the structure and function of the motor domain of the human protein kinesin-5. The movement of kinesins along the surface of microtubule filaments is essential for cellular functions including mitosis. This movement, which requires ATP hydrolysis, has been modelled as a "walk" in which separated, ATP-binding head or motor domains of an intertwined kinesin dimer advance in turn along the microtubule, very much like right and left feet. The structure of an isolated motor domain with an ATP

analogue bound has been solved using X-ray crystallography; Goulet and her colleagues used cryo-electron microscopy to determine the structure of this domain bound to a microtubule. They have identified features of the domain structure, including one very flexible loop, which are involved in the conformational change that is necessary for the consecutive binding and unbinding events of the kinesin walk.

The next two talks concerned the structures and mechanisms of bacterial proteins. **Irene Farabella**, a student in Maya Topf's group in Biological Sciences at Birkbeck, described her studies of the structure and function of an outer membrane protein from *E. coli*, PapC, in collaboration with Waksman's group. This plays an important role in the formation of a structure known as the P pilus, a filament on the bacterial surface that adheres to the surface of human kidney cells, causing the disease pyelonephritis. Waksman and his co-workers recently published a landmark paper describing the structure and mechanism of the complete molecular machine for pilus biosynthesis[1]. Farabella's role in this was to investigate the "gating" mechanism of the pore-like PapC protein, which controls when the proteins that assemble to form the filament are able to pass out of the membrane. She found that mutant proteins lacking several domains retain some of this gating activity, suggesting that the barrel is highly flexible, and used molecular dynamics and analysis of sequence conservation to identify the most flexible regions. This identified a region of four beta strands as likely to be involved in the barrel's characteristic conformational change.

Liisa Chisty, a PhD student in the Department of Physical Biochemistry at NIMR, used a very different, fluorescence-based technique to study the mechanism of a helicase protein, PcrA, which is involved in the replication of plasmid DNA in bacteria. Crystal structures of this protein have been solved both with and without bound double-stranded DNA, and these show a substantial conformational change. Observing the fluorescence of functional mutant PrcA proteins in which residues close to functional regions have been mutated to cysteine can throw light – almost literally – on the mechanism of this conformational change and thus the mechanism of action of the protein. Chisty's results indicated the interaction site of PcrA with the plasmid unwinding complex, and gave a signal and measurements for its translocation along the DNA strand.

The last ISMB speaker was **Paul Ashford**, a Ph.D. student with Irilenia Nobeli in the Department of Biological Sciences at Birkbeck. Small molecules generally bind to definitive "pockets" on the surface of protein structures, and there is much industrial interest in predicting the locations of these binding sites. Methods based on single structures, however, may miss features arising from conformational variability. Ashford has developed a method for visualising pocket locations based on an ensemble of similar structures, for example homologous members of a protein family or "snapshots" of a structure taken from a molecular dynamics run, and validated it using sets of kinases and interleukin-2 homologues. This method works with large datasets and can be used to answer complex questions about the variability of binding sites on protein surfaces.

Dr Harvey McMahon from the LMB completed the scientific proceedings with a final keynote lecture. His research focuses on the dynamics of cell membranes and how cells and their organelles and trafficking intermediates are shaped. Exocytosis, the process through which membrane vesicles inside cells fuse with the limiting membrane of cells secreting their contents into the environment, and endocytosis, the reverse process through which cells engulf molecules by enveloping them in a membrane, both involve extreme changes in the local membrane shape.

McMahon studies proteins that modify membrane curvature. He described proteins that act, either as "wedges" inserted into the membrane from the outside or as scaffolds that form a lattice on the membrane surface. The latter category includes protein domains in the BAR super-

[1] Phan *et al.* (2011). Crystal structure of the FimD usher bound to its cognate FimC–FimH substrate. *Nature* **474**, 49–53

family, which bind to curved membranes but can also reinforce the curvature, and clathrins, which form polyhedral lattices around membrane surfaces. Both these families of proteins are involved in endocytic vesicle formation. A different set of proteins is involved in exocytosis; some of these contain calcium-binding C2 domains, and it is thought that these domains bind to the outer membrane surface in the form of wedges and so increase membrane curvature. McMahon introduced mutations into C2 domains and found that some abolish and others enhance membrane fusion. He explained that all the processes he had described donate energy to membranes by introducing "curvature stress" which is necessary but not sufficient for exocytosis.

Before closing the meeting, Waksman had one final and very pleasant task, presenting prizes to the author of the best poster and the presenter of the best student or postdoc talk. The poster prize was awarded to Anna Adams, a Ph.D. student in the Department of Chemistry at UCL, whose poster was entitled "Thioester formation via *N* → *Se* acyl shift"; Felix Schumacher from the same department won the talk prize for his presentation on the chemical modification of disulphide bonds. Finally, Waksman thanked all who had helped organise the retreat, particularly the invaluable ISMB administrators Andrew Service and Anne-Cecile Maffat, and invited all delegates to join him at the ISMB symposium to be held next June.

Pictures: Robinson College