7th ISMB Retreat
29–30 June 2017

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The Institute of Structural Molecular Biology was set up in 2003 to foster collaborations between researchers in these disciplines in Birkbeck and University College London. Each summer, the staff, postdocs and students based in its constituent departments gather for a research meeting. In even years this takes the form of a symposium with talks from ISMB core members and world-leading scientists in its constituent disciplines; in odd years the focus turns to the student and post-doc members with an ISMB Retreat.

The 2017 retreat, held on June 29 and 30, was the seventh in the series, and the fifth to be held in Robinson College, Cambridge. Well over a hundred delegates from the ISMB's constituent departments – Biological Sciences at Birkbeck, and Chemistry and Structural & Molecular Biology at UCL – took part in a fascinating scientific programme that included three splendid keynote lectures; nine talks from talented young scientists based at the ISMB, many of which contained unpublished work; and a lively poster session. The programme was, as usual, complemented by an activity designed to expose the younger ISMB delegates to scientific careers outside academic research. This year's, a 'Dragon's Den' style competition for biotech business ideas, was generally considered to have been one of the most original and enjoyable so far.

The ISMB's director, Gabriel Waksman, opened the retreat with a few words of welcome before handing over to Birkbeck's Carolyn Moores to chair the first session. Moores introduced a fellow electron microscopist, **Lori Passmore** of the MRC Laboratory of Molecular Biology (LMB), Cambridge, as the first keynote speaker. Passmore grew up in Canada and moved to the UK in 1999 to study for her PhD at the Institute of Cancer Research in London. She has been based at the LMB since completing her doctorate, first as a career development fellow and then as a group leader. She has already received several awards and is part of the prestigious EMBO Young Investigator programme. Her talk was divided into two sections, one describing some of her group's largely unpublished research into the process through which messenger RNA molecules – the 'templates' from which proteins are synthesised – are completed by the addition of a 'tail' of adenosine residues, and the other focusing on the advances in electron microscopy techniques that have made these and similar structural studies feasible.

Experimental structural biologists rely on three main techniques: X-ray crystallography, nuclear magnetic resonance and electron microscopy. Until very recently, however, only the first two of these could visualise structures in enough detail to show the positions of individual atoms. The 'resolution revolution' in electron microscopy over the last few years, which has enabled structures of some complex 'molecular machines' to be seen in detail for the first time, has arisen from simultaneous technical improvements in the microscopes, the detectors for the electrons forming the images, and the software used for image analysis. Nevertheless, some important limitations still remain. One of these arises from the fact that protein molecules move in the electron microscope beam, even when frozen, and this causes the images to distort and blur.

Conventionally, protein specimens in electron microscopes are mounted on amorphous carbon substrates that bend and deform in an electron beam. This motion can be reduced but not eliminated by the addition of materials such as graphene. A physicist, Chris Russo, who joined Passmore's group at about the same time that the latest

generation of electron detectors arrived at the LMB, has worked with her to design a novel, ultra-stable substrate that can almost eliminate motion during electron irradiation. This substrate is made from a single material: poly-crystalline gold. Using one material eliminates differences in thermal contraction as the specimen cools; the choice of gold came about because of its stability and biocompatibility. Passmore presented some unpublished structures of a multi-protein complex known as the cleavage and polyadenylation factor (CPF) that her group has obtained using these substrates. These are beginning to shed light on the detailed process through which this 'molecular machine' processes newly-synthesised messenger RNA molecules by adding poly-adenosine tails and thus regulate protein synthesis.

Moores then introduced the first two short research talks from young scientists based at the ISMB. **Lisa Redlingshoefer**, a final-year PhD student in Frances Brodsky's lab at UCL was the first to speak. She described structural studies of a protein, clathrin, which forms lattices that coat and shape the membranes surrounding vesicles within cells. Each clathrin molecule consists of three heavy and three light chains that interact to form an elegant spiral shape known as a triskelion, and these triskelions intertwine to form the lattice coat. Light chains come in two types known simply as A and B; Redlingshoefer's work centres on the distribution of these types in individual triskelions and in lattices, and how this affects the stability, size, budding efficiency and eventual disassembly of the vesicles that they surround.

The second talk in this session was given by **Alex Yon**, a PhD student in the London Centre for Nanotechnology at UCL. Yon's PhD is co-sponsored by MedImmune, a subsidiary company of the pharma giant AstraZeneca that develops antibodies and other immune-based therapeutics. His research involves atomic-force microscopy (AFM), which is a type of high-resolution scanning probe microscopy that can examine the structures of single molecules directly with no need for the sample to be crystallised or frozen. It focuses on the structures of ion channels, which are membrane proteins that – as their name suggests – control how charged ions pass into and out of cells. Yon described how he has been able to use AFM at the very limit of its resolution to study one such ion channel. This work suggests how therapeutics might be directed against these proteins, which have been implicated in many different diseases but are under-exploited as drug targets.

The next session included three more talks by young scientists at the ISMB. **Jennifer Booker**, a PhD student in Bonnie Wallace's lab at Birkbeck, continued the ion channels theme. Research in the Wallace lab focuses on one class of channel: voltage-gated sodium channels, which are found in the membranes of several types of mammalian cell. They have been implicated in many diseases and drugs that selectively block specific channel isoforms are being developed for conditions as diverse as pain, epilepsy and heart arrhythmias. Mammalian channels of this type are notoriously difficult to purify and crystallise, so most structural studies have used related bacterial channels that share the same mechanism but are easier to work with. Booker presented a recently-published structure of such a bacterial sodium channel in its open conformation that illustrates the mechanisms through which the channels sense change in voltage and open to allow sodium ions to cross the membrane. One tryptophan residue that is conserved in all known members in this family, from humans and other mammals as well as from bacteria, was found to lie in a 'hinge' region close to the voltage-sensing domain; changing this residue specifically into any other amino acid reduced or removed the channel activity.

Camilla (Millie) Pang, a PhD student in Christine Orengo's bioinformatics group at UCL, gave an accessible talk about her use of protein structure analysis to identify so-called 'driver mutations' in cancer. Comparing the genomes of tumour and normal cells will reveal large numbers of mutations in the coded proteins, but only a few of the changes will have been necessary for tumour development. Even proteins that have been widely implicated in cancer, such as the fibroblast growth factor receptor (FGFR) kinase, will contain many harmless 'passenger' mutations. Driver mutations,

however, will frequently cluster together in 'hotspots' on structurally and functionally important regions of the protein structure. Pang and her co-workers compared FGFR kinase sequences and structures from many different tumours, locating clusters of likely driver mutations in the molecular brake, active site and activation loop. No FGFR kinase inhibitors have yet been approved as anticancer drugs and this analysis may suggest where on the structure such inhibitors might most usefully be targeted.

The final talk on the first day was given by **Alistair Jagger**, a PhD student in John Christodoulou's group at UCL. Research in the Christodoulou group focuses on the dynamic structures of proteins as they fold, often using nuclear magnetic resonance (NMR). Human alpha-1-antitrypsin is a serine protease inhibitor that circulates in a 'metastable' state, binding to and inhibiting proteases when needed. As it is intrinsically unstable it is prone to forming misfolded variants, particularly when mutated, and this can cause serious disease. The most common disease-forming mutation, E342K, causes the protein to aggregate into polymers and collect in liver cells, but it is the loss of soluble protein from the circulation that causes the most serious disease. X-ray crystallography revealed very little difference between the structure of the wild type alpha-1-antitrypsin monomer and that of this mutant (which is known as the Z form). Jagger is using NMR to compare the structures of the wild type and mutant in solution, identifying structural instabilities that may contribute to misfolding and aggregation. This model may be applicable to other diseases that arise from misfolded proteins.

The student and postdoctoral delegates spent the time between this session and the conference dinner in a productive and enjoyable group activity entitled '**From Idea to Business: Fostering an Entrepreneurial Mindset**'. This was designed by ISMB principal investigators Kostas Thalassinos and Alan Lowe. "The activity aimed to highlight the broader context of basic scientific research in society by exploring industrial-academic connections, commercialisation and the way entrepreneurs think", explained Lowe. Birkbeck's Renos Savva, who co-founded a drug discovery company, Domainex, in 2001 and now directs the college's Bio-Business MSc, helped refine the idea and also invited the industry and sector specialists who ably put the teams through their paces.

Twelve teams of six or seven young scientists were each assigned a senior scientist with industrial experience as a mentor and given a task: to create a fictional life sciences company to a specific brief and present their idea in a brief 'elevator pitch' to a panel of mock investors. The brief was either to design a drug or other therapeutic; to develop a new computational tool; or to produce a new scientific instrument or piece of lab equipment. One team with each brief was chosen to go forward to a final round, where they were asked to present their idea to a panel of 'dragons' and be quizzed on it in front of all other delegates.

All the teams settled to their tasks well and there was soon a lively buzz in all three rooms. Even in the short time available, each 'company' was able to produce an idea that held water to at least some extent.

The delegates who assembled for the final were treated to a set of excellent pitches from the winning companies I-protein,

TerraNova and C-Three. The inventions they presented were, respectively, a program for selecting sets of crystallisation conditions from a protein's sequence; an antibody-drug conjugate for primary progressive multiple sclerosis; and a dishwasher for fragile, sterile laboratory glassware that could not otherwise be re-used.

Each company was quizzed by four 'dragons' who had not been involved in their heat. The feedback on all three presentations was very positive but there could only be one winner, and that was the drug discovery company TerraNova for developing 'a plausible concept that addresses a huge medical need'. The C-Three presentation, however, was highly commended for accessibility and humour. A splendid dinner and well-attended poster session rounded off the first day.

The second day began with a keynote lecture by **Bill Rutherford** from Imperial College London, introduced by Chris Kay from UCL. Kay explained that Rutherford had studied for his PhD at UCL with Professor Michael Evans in the late 1970s, done postdoctoral work in the US and Japan and spent most of his independent research career in France before returning to London in 2011. He has received many honours including membership of EMBO (2001) and fellowship of the Royal Society (2014).

Rutherford's engaging talk, which was accompanied by colourful hand-written slides, was an overview of the research area he has made his own: the mechanism of the reaction centre photosystem II. The lecture started with a few basic principles and ended with some very recent unpublished results. Photosynthesis is well-known to high school biology students as the process through which carbon dioxide and water are converted, with the addition of solar energy, into sugar (reduced carbon) and oxygen.

Its emergence on Earth about 2.6 billion years ago led to the rapid increase in the concentration of oxygen in the atmosphere that was necessary for the development of multicellular life. Much reduced carbon was eventually deposited in the ground as fossil fuels, only for humans to release it into the atmosphere again 'in the blink of a geological eye'.

Photosystems, or photosynthetic reaction centres, are membrane-bound multi-protein complexes that absorb light and convert it into chemical energy. This is done by light-triggered charge separation occurring between chlorophyll pigments. A series of stabilising electron transfer steps then occurs along a chain of co-factors. A small loss of energy occurs on each step, resulting

in high yields of charge separation per photon. Photosystem II, found in the membranes of plants, algae and cyanobacteria, absorbs visible light most efficiently at a wavelength of about 680 nm. Electron transfer along the co-factor chain requires bound bicarbonate and metal clusters, and the co-factors must be 'tuned' to specific energy levels. In fact, the whole photosynthesis process is not particularly efficient, and Rutherford suggested that this was in part because its evolution began before the rapid increase in atmospheric oxygen. The mechanism had to adapt to a high oxygen environment, but these adaptations reduced its overall efficiency. He suggested that this sacrifice of efficiency for survival in oxygen was common to all biological electron transfer in aerobic environments and ended his talk with some interesting comments taken from unpublished work about the prospect of artificial photosynthesis.

Another four talks by PhD students from the ISMB followed Rutherford's lecture. The first of these, by **Sapir Ofer** from Finn Werner's group at UCL, concerned the structural and molecular biology of histones – proteins that bind to and pack DNA – in single-celled organisms known as archaea. These share many characteristics with bacteria, including the lack of a cell nucleus, but are evolutionarily closer to eukaryotes. The different ways that eukaryotes and bacteria package long DNA molecules into their cells are very well known, but much less is known about the process in archaea. Ofer and her colleagues have studied the structures of DNA-packing proteins in archaea, which have the same fold as eukaryotic histones, showing that they shorten and compact long DNA chains in a similar way. Ofer's talk was to have been followed by one from Tom Peskett, a PhD student who is jointly supervised by Alan Lowe and Birkbeck's Helen Saibil, but he had to drop out due to illness.

Klaudia Cybulska from Erik Arstad's group at UCL gave a very practical talk about the synthesis of novel radioactive probes for positron emission tomography (PET). This medical imaging technique can be used to observe and monitor metabolic activity in living tissue in real time. Radioactive isotopes with relatively short half-lives are incorporated into chemicals that replace naturally-occurring ones, and the functionality of those molecules is monitored as the isotopes decay. One of the most common probes is an isotope of fluorine (^{18}F) but this is not always easy to incorporate into bio-active molecules. Cybulska discussed methods for incorporating this isotope into the aromatic rings that are found in many drug-like molecules, and showed how some of the synthesised molecules can be used to monitor the function of the adrenal glands in primary hyperaldosteronism, a disease that causes extremely high blood pressure.

Anaïs Cassaignau described research for her recent successfully-defended PhD within John Christodoulou's group. Much of the group's work uses NMR to study the dynamics and developing structure of polypeptide chains as they are translated on their parent ribosomes (at which point they are known as nascent chains). Anaïs described some examples of nascent chain structures. The first elucidated the co-translational folding of a sequential pair of immunoglobulin domains, where interactions between the nascent chain and the ribosome surface suggest that native folding only occurs when the entire N-domain has emerged well beyond the exit tunnel, so folding appears to be delayed. The second nascent chain was of a protein that is natively disordered: alpha-synuclein, which forms aggregates in Parkinson's disease brains. The determinants of nascent chain interactions in this protein were described and compared to those of the immunoglobulin nascent chains.

The final student talk was another NMR one: **Ruth Dingle** from Flemming Hansen's group at UCL, who presented some elegant unpublished work analysing the conformations of the flexible, positively charged side chain of the amino acid arginine on the surface of protein structures.

A keynote lecture by **Bart Vanhaesebroeck**, professor of cell signalling at UCL's Cancer Institute, completed two days of excellent science. A native of Belgium, Vanhaesebroeck moved to London in 1995 for postdoctoral research with Mike Waterfield at UCL and has stayed in the city ever since. His research throughout his career has focused on the structure, mechanism and

role in disease of a family of proteins known as phosphoinositide 3-kinases (PI3K). He has always maintained an interest in protein structure and has come full circle, using the now well-known structures of these enzymes as a guide to ongoing drug development.

Proteins in the PI3K family have been implicated in a wide range of diseases: most often cancer, but also heart disease, metabolic syndromes and even 'overgrowth syndromes' that cause patients' tissues to keep growing out of all proportion to the rest of their bodies. Eight separate PI3K isoforms are known, most of which could be targeted in different disease contexts. Most of Vanhaesebroeck's talk focused on two isoforms, PI3Kα and PI3Kδ, and their role in solid tumours and blood cancers respectively. His group used PI3Kα kinase-dead mice, in which the active site of the kinase is mutated to remove enzyme activity, to model the action of small molecule inhibitors and to uncover the precise function and mechanism of the isoform. PI3K is part of a pathway that is hyperactive in most cancers, but small-molecule inhibitors of the kinase have little intrinsic cytotoxic activity against tumour cells, at best PI3K inhibitors are cytostatic. Cancer cells can live and even proliferate with little PI3Kα activity, and if inhibitors of this enzyme are to be useful against cancer it will have to be in combination with other therapies, such as hormonal therapy in breast cancer.

The delta isoform (PI3Kδ) is expressed mainly in white blood cells. Drugs that target it have been approved for the treatment of certain B-cell specific blood cancers such as chronic lymphocytic leukaemia (CLL), and are also being trialled in some inflammatory diseases.

These PI3Kδ drugs appear to be ineffective against other blood cancers, and even in CLL there is no evidence for direct cytotoxicity.

The drugs most likely act by interfering with the interaction of the malignant cells with their surrounding stroma. Here, again, it is likely that PI3Kδ inhibitors will be most effective in combination with other drugs. Vanhaesebroeck ended his talk by providing evidence that inhibitors of PI3Kδ and other isoforms might be effective in stimulating an anti-tumour immune response. The idea that PI3K inhibitors could be used in cancer immunotherapy is very exciting, and is currently being tested in clinical trials.

All that remained for Waksman to do in closing another successful retreat was to present prizes for the best talk and poster and to thank all who had been involved in making it go so well. This included all speakers and chairs; the organising committee; the leaders and 'dragons' involved in the entrepreneurship exercise; and the ISMB's unflappable administrator, Andrew Service. The poster prize went to Stefan Schwenk, who works for Kristine Arnvig at UCL, for a poster on the role of small RNA molecules in the pathogenicity of *Mycobacterium tuberculosis*; the judges were unable to decide on a single best talk and finally awarded two equal prizes to Jennifer Booker and Sapir Ofer. Waksman closed with a date for everyone's diaries: the next ISMB symposium, on 18 and 19 June 2018. I, for one, will be there.