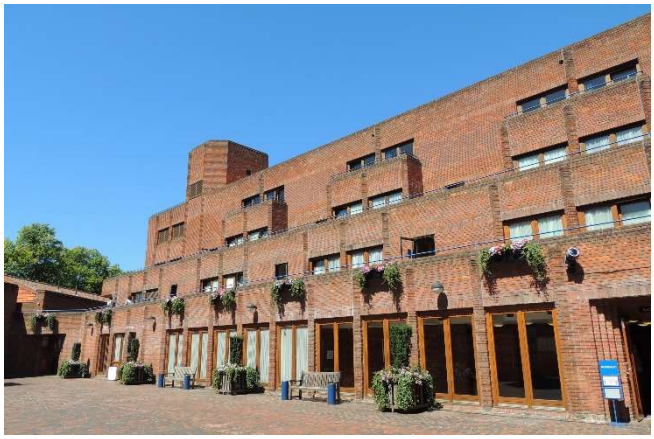
8th ISMB Retreat
11-12 July 2019

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

Soon after the Institute of Structural Biology was founded in 2003 its director, Gabriel Waksman, instituted a programme of events to bring together the students, postdocs and established research staff of its constituent departments in Birkbeck and University College London. The most important of these are the intense research meetings held each summer. In even-numbered years these take the form of symposia with talks from world-class researchers; in odd-numbered ones, students and postdocs based at the ISMB are given the chance to showcase their research at the ISMB retreat.



Robinson College, Cambridge

The 2019 retreat was thus the eighth in this particular series, and, like all since 2009, it was held in Robinson College, Cambridge. It took place a little later in the summer than usual, on July 11 and 12, and was well attended with the main lecture theatre packed with attentive delegates. This made it a very encouraging 'swan song' for Waksman as not only ISMB director but also Head of the Biological Sciences Department at Birkbeck and of the Research Department of Structural and Molecular Biology at UCL. He retired from these prestigious positions on 31 July, although he will continue to run his large, successful research group here for some years.

Each ISMB retreat includes three keynote lectures from scientists at the top of their research fields. This one began with a talk by **John Briggs**, who leads a research group at the MRC Laboratory of Molecular Biology (LMB), Cambridge. The chair of the first session, Birkbeck's Carolyn Moores, described his research area of cryo-electron microscopy as both a 'hot topic' in structural molecular biology and, self-evidently (since it uses samples frozen to temperatures close to that of liquid nitrogen) a very cold one.

Following degrees at Cambridge and Oxford and postdoctoral work in Munich Germany, Briggs established his research group at the European Molecular Biology Laboratory (EMBL) in Heidelberg, in 2006; he moved his group to the LMB in 2017. He started his talk by describing how he 'vitrifies' biological samples at a low temperature and places them on the grid of a transmission electron microscope for analysis by electron tomography. This is a fast-progressing technique for obtaining structures of macromolecular assemblies on the nano-scale *in situ,* and his group applies it to the structures of coated vesicles and enveloped viruses, including HIV. This virus synthesises its proteins in long chains, separated by flexible linkers that are cleaved by the virus' protease into individual proteins that then assemble to form the mature virions.

The talk focused on the GAG polyprotein, which is cleaved in five places to form the structural proteins that self-assemble to form the HIV capsid. This unusual, cone-shaped structure, which encases the viral genome, is constructed from a lattice of hexamers and pentamers of capsid protein. The lattice is tightly curved in some places and almost flat in others. Briggs described high-resolution electron micrographs of the hexamer and pentamer components and explained how the protein's flexibility allows the complex cone to assemble. Compounds that can inhibit this process could be useful as anti-AIDS drugs, and some of these 'maturation inhibitors' are already in clinical trials. Later in the talk, Briggs described the

structure of a retrotransposon that is encoded by the yeast genome and that is thought to shed light on how retroviruses evolved from these segments of eukaryotic genomes.

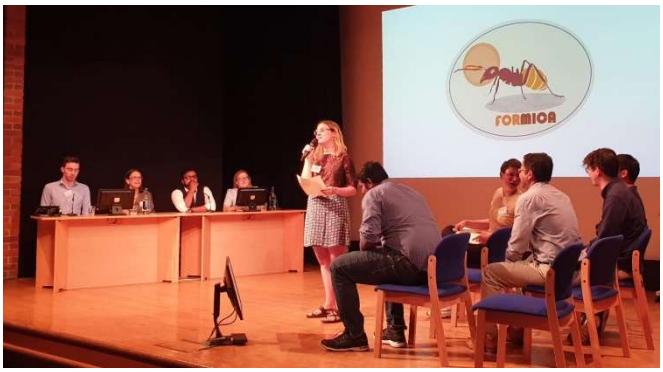
The first of eight students and postdocs from the ISMB to speak was **Josh Hutchings**, a fourth-year PhD student working in Giulia Zanetti's electron microscopy group at Birkbeck. Zanetti's group studies the trafficking of proteins between membrane-bound compartments in eukaryotic cells, and in particular the structures of the coat proteins that assemble on the membrane surfaces to promote the formation of vesicles. One of these, known simply as coat protein II (COPII) is responsible for trafficking proteins between the endoplasmic reticulum and the Golgi apparatus. It polymerises to form a double-layered protein coat around the vesicle as it forms, allowing the membrane to curve. Each vesicle is about 60-100 nm in diameter. Hutchings and his colleagues used cryo-electron tomography to show how COPII polymers are organised to form these layers, and how this further dictates the shape of the vesicles' membranes. He started by describing the detailed structure of the inner coat, which includes several subunits arranged in a tight lattice; lattice formation induces the insertion of long, amphipathic helices that allow the vesicles to curve. The outer coat is recruited to the vesicle through binding to a motif of three consecutive proline residues; similar motifs are found in other proteins that bind COPII. Hutchings ended his talk with a brief description of unpublished work on the structure of the outer coat.

Stephen McCarthy joined the Department of Chemistry at UCL as a PhD student from the pharma industry. His research project concerns the structure and function of a peptide toxin derived from tarantula venom. This peptide, protoxin II, is a selective inhibitor of a human voltage-gated sodium channel, $Na_v1.7$, that is involved in the pain response, Solid-state methods quite easily produce a reduced peptide that must be oxidised to form the three disulphide bonds that enable it to fold into its characteristic 'cysteine knot' structure. Oxidation in water produced an inactive mixture of products with different numbers of disulphides, whereas oxidation in a redox buffer produced a single, active folded product with the correct ones. The fold of this synthetic peptide has been confirmed by X-ray crystallography and separately, by a different group, using NMR; Stephen and his colleagues are now using cross-linking mass spectrometry to investigate the mechanism by which it inhibits the sodium channel.

The second session featured two talks about proteins from important human and animal pathogens. Malaria still causes well over half a million deaths each year, and the molecular details of its complex life cycle are not yet wholly understood. **Trishant Umrekar**, a PhD student in Helen Saibil's group at Birkbeck, is using electron microscopy to study the structure of a protein found on the surface of malaria parasites during the 'blood stage' in which they infect human red blood cells (erythrocytes). The parasites reproduce inside erythrocytes, and the progeny are released from the cells in order to infect others in a poorly understood process known as egress. Merozite surface protein 1 (MSP1) is tethered to the parasite surface via a GPI anchor. This protein is known to play a major role in this process, and egress is severely limited in parasites in which MSP1 is truncated and the GPI anchor missing. Umrekar has shown that parasites with truncated MSP1 cannot readily break the erythrocyte membranes and are trapped in a membrane 'bag'. Completely knocking out the MSP1 gene produces a similar phenotype.

African swine fever is a highly infectious, lethal haemorrhagic fever widespread in many tropical and sub-tropical countries that recently hit the headlines through a devastating outbreak in Asia. The virus that causes it, ASFV, is one of the largest known with a diameter of about 200 nm and a double-stranded DNA genome encoding over 150 proteins. **Gwenny Cackett**, a PhD student in Finn Werner's lab in UCL, is investigating the processes of gene transcription in this virus. It encodes an RNA polymerase that, surprisingly, has strong similarities to the eukaryotic RNA polymerase II. Many of its subunits have been modelled based on their equivalents in this eukaryotic protein, and Cackett is aiming to crystallise a major part of the complex. This work is still ongoing; she is also mapping the viral transcriptome to determine the pattern of RNA expression in the hours following infection. She has found that about two-thirds of the genes are differentially expressed at time points separated by 11 hours, with almost equal numbers up-regulated and down-regulated at the later point. Some of the most highly expressed proteins might be useful as drug targets for this important veterinary disease.

The first day of an ISMB retreat is incomplete without a training activity for students and postdocs. The 'Dragon's Den' style business planning activity at the last retreat had proved so popular that it was repeated this year.



Before the retreat the participants had been divided into teams, and each was asked to come up with a name and logo for their companies. In the first part of the activity, the teams worked with visiting experts, or dragons, to produce a business plan for a new drug or other therapeutic product; a piece of software; or a scientific instrument. They then pitched their ideas to the team of dragons associated with that area and one drug, one piece of code and one piece of kit was chosen to go through to the final. New for 2019 was a separate competition for the best company logo, run through the ISMB's Twitter account (@ISMBLondon).

The final took place in the main auditorium, where the three winning teams got to pitch their business plans to, and be grilled by, a group of dragons from the other areas. The chosen inventions – an aptamer as a drug for late-stage breast cancer, a software tool for diagnosing liver disease, and a bench-sized cryo-electron microscope – were all well thought through, and the team presentations were lively and engaging. The dragons' decision was a hard one, but they finally selected the company that had pitched the electron microscope – named FORMICA – as the overall winner.

The second day started with a keynote lecture by **Mariann Bienz** from the MRC Laboratory of Molecular Biology in Cambridge. She initially set up her group in Zurich in 1986, moved it to Cambridge in 1991 and has been based there ever since, studying Wnt/beta-catenin signalling, a cell communication pathway that controls numerous cell fates during animal development and stem cells in adult tissues, and that is also a major cancer pathway. Most notably, hyperactivation of Wnt/beta-catenin signalling in the intestinal epithelium initiates virtually all cases of colorectal cancer. Typically, this occurs through mutational inactivation of the tumour suppressor protein APC, which binds and downregulates beta-catenin.

Dr. Mariann Bienz

Bienz has been studying the molecular mechanisms underlying this signalling pathway for most of her long career. In her talk, she focussed on a component of this pathway that was given its intriguing name – Dishevelled – because this protein was first discovered in flies where its mutation causes disordered body and wing hairs. One of her earliest papers on Dishevelled, published in 2005, showed that it forms punctate structures in cells that are dynamic protein assemblies. These require the DIX domain of Dishevelled, which undergoes dynamic head-to-tail polymerisation. This enables Dishevelled to bind to low-affinity ligands, including a key effector called Axin to which it binds via a DIX-DIX interaction, thereby blocking the activity of Axin in downregulating beta-catenin. However, assembly of functional 'Wnt signalosomes' not only requires DIX polymerisation, but also cross-linking of DIX polymers into three-dimensional meshes (corresponding to the punctate structures seen in cells) via another domain of Dishevelled called DEP. This led to the concept of the 'Dishevelled paradigm' – namely the cross-linking of dynamic polymers – which, according to Bienz, could also underlie other signalling systems that depend on transient low-affinity interactions between signalling components. High-resolution crystal structures of the DIX and DEP domains were crucial for elucidating the molecular mechanisms underlying Wnt signalosome assembly.

Naturally occurring antibodies can be engineered through mutation and grafting to bind proteins very specifically; these have many therapeutic applications, but the engineered antibodies often lack stability. **Gil Ferreira Hoben**, who is studying for a PhD in bioinformatics at UCL while working at the pharma company UCB in Slough, is using machine learning to investigate determinants of antibody specificity. Machine learning is an umbrella term for any program that extracts patterns from data and then uses those patterns to predict results from unseen data; Hoben's work involves natural language processing, which is one

of a class of 'deep learning' approaches to this type of problem. He created a large number of artificial antibodies by mixing light and heavy chains from a UCB database, measured their biophysical features and combined this experimental data with protein sequence-derived data into a dataset for 'training' neural networks to predict antibody stability from sequences. So far, the best algorithms can predict the threshold temperature at which an antibody is no longer stable with an accuracy of about 80%.

With the next talk, by **Alex Cook** from Carolyn Moores' group at Birkbeck, the retreat returned to the topic of malaria and the technique of cryo-electron microscopy. Kinesins are stalk-like motor proteins that 'walk' along microtubules, powered by ATP; they are found throughout eukaryotes, including in single-celled parasitic protozoa, and they have many different functions. Kinesin 5 is an essential component of the mitotic spindle that forms in cell division, and the human version is a very attractive target for anti-cancer drugs. It is thought that the equivalent protein in the malaria parasite *Plasmodium falciparum* might be an equally good target for antimalarials. Cook is studying the structure of this protein bound to the microtubule using electron microscopy. So far, he has obtained structures of the microtubule to 4.5 Å resolution and the more flexible kinesin at 7.5 Å resolution. Despite this low resolution, which partly derives from the high proportion of unoccupied kinesin binding sites, these structures suggest that there are enough differences in kinesin-microtubule binding between parasite and human cells to class this protein, also, as a good drug target.

The next talk was given by the ISMB's newest group leader, **Dr Graeme King**, who has recently been appointed as a lecturer in single-molecule biophysics at UCL. He moved to London from Amsterdam, where he had been developing methods for studying the structures of single molecules of DNA *in vitro*. In living cells, DNA comes under constant mechanical strain; stretching during mitosis, bending around histone proteins, and twisting as it unwinds for replication and transcription. He uses fluorescence to visualise DNA-protein binding and dynamics, and 'optical tweezers' to measure the piconewton forces involved.

Dr. Graeme King

Stretching DNA causes the standard B-form double helix to break down, forming different types of micro-structure that can be detected with fluorescent labelling. Torsional (twisting) forces are much more important during transcription and DNA replication, however; measuring these is more difficult, although possible using some of King's techniques. All the changes in DNA structure that can be caused by torque stresses are collectively known as supercoiling. Mitochondrial DNA is particularly frequently supercoiled, and the amount of supercoiling affects the dynamics of transcription factor binding and thus RNA transcription. King is currently also investigating the role of supercoiling in topoisomerase binding to DNA.

The only NMR-based talk at the retreat was given by **Lucas Siemons**, a PhD student in the Hansen lab at UCL. He is using this technique to study the conformations of the amino acid side chains in proteins. The chemical groups making up the side chains – charged, polar, aliphatic or aromatic – must lie in particular positions to bind ligands, to take part in chemical reactions, and even for the proteins to fold. All side chains occupy some conformations, known as rotamers, much more frequently than others. Siemons' research uses ^{13}C chemical shifts measured by NMR to determine what side chains are doing, starting with isoleucine: a branched, hydrophobic amino acid in which the side chain conformation can be described using two dihedral angles, $\chi 1$ and $\chi 2$. Each of these will take up one of three possible orientations, *gauche+*, *trans* and *gauche-*; this gives a total of nine rotamers for the amino acid, four or five of which are frequently seen. He has measured these in four small, very well characterised proteins, and in a GIG peptide to simulate random coil. So far, he has determined the rotamer population of isoleucine in its ground and excited states and shown that, in isoleucine at least, 'random coil' is not wholly random.

The final two talks concerned the structures of chaperones, a large class of proteins that assist protein folding and protect cells in three different ways: preventing misfolding and aggregation, reducing the toxicity of misfolded proteins, and disassembling aggregates. Firstly, **Joseph Beton**, a PhD student in Helen Saibil's group at Birkbeck, described the structure and function of the human Hsp70 chaperones. These are important components of the system that disaggregates

'pathogenic' amyloid fibrils. He used alpha-synuclein fibres, which have been implicated in Parkinson's disease, as a model system to study the disaggregation process, asking questions about the underlying mechanism and the effect of mutations. Using atomic force microscopy, he has been able to watch the fibres shortening and fragmenting from one end when mixed with the chaperones. Cryo-electron microscopy of the system has yielded two novel folds for the alpha-synuclein fibres and a low-resolution structure of the fibres with a co-chaperone known as a J-protein bound.

Joseph Beton

The final keynote lecture, by **Justin Benesch** of the Department of Chemistry at the University of Oxford, concerned another class of chaperones: the small heat shock proteins (sHSPs) that protect cells against stress. All living cells experience three types of stress: acute stress from injury or extreme heat; chronic stress from ageing; and cyclical stresses. The rhythm of the heartbeat and the circadian rhythm are examples of cyclical stressors on different timescales. Small heat shock proteins protect proteins in cells under stress from forming deleterious associations such as amyloid, and promote protective ones, most often through assembling into complexes.

Benesch and his group study the structure, kinetics and thermodynamics of small heat shock proteins using two related quantitative methods: mass spectroscopy and mass photometry. The latter technique is much the less well known. It involves imaging the mass of single molecules in solution – 'weighing' them – through analysing their interaction with scattered and reflected light. It is now used routinely in his lab for distinguishing between oligomeric states and counting the number of monomers in protein oligomers and assemblies.

He uses mass spectrometry to probe how evolutionarily related sHSP monomers with very similar structures assemble into the exact oligomers that are required for each function. Taking two closely related plant sHSPs that function as homo-dodecamers, he constructed a set of chimeras by combining three regions of each protein together in all possible combinations, titrated them together and obtained their mass spectra. This showed that, although many different combinations formed dimers, only the native proteins spontaneously self-assembled into the 12-mers, preserving the inter-subunit interfaces. Finally, he discussed an interaction between a cardiac actin-binding protein, filamin C, and a chaperone, HSPB1, that binds to it under stress. This chaperone is activated by phosphorylation, and he has shown that this system is disrupted by mutations that have been implicated in cardiomyopathies.

Waksman ended the retreat by presenting prizes, starting with those for the entrepreneurship activity. Besides the overall winning company, FORMICA, the team behind a company named aDhOC won a small prize for the best logo.

aDhOC

The final speaker, Joseph Beton, won the prize for the best talk by a young scientist, and the poster prize went to Sioned Fôn Jones, a PhD student in Stefan Howorka's lab at UCL, for a poster entitled 'DNA-based Molecular Rulers to measure Membrane Thickness in Live Cells'.

After Waksman had thanked all the internal speakers, the three excellent keynote lecturers, and Renos Savva and his team of dragons, Frances Brodsky, head of the Division of Bioscience at UCL, gave a special vote of thanks to Waksman himself for over fifteen years' inspirational leadership of the ISMB. Although Waksman's successor will not be in place until late next year, the Institute will be in three pairs of good hands in the interim: Carolyn Moores at Birkbeck, Snezana Djordjevic at UCL and Finn Werner taking overall charge of the ISMB. Besides continuing to run his research group, Waksman intends to set up a charity to plant trees to offset the carbon emissions associated with scientists' conference travel, so it was fitting that Werner presented him with a gift of a tree.

The next ISMB symposium, which will take place on June 28 and 29 2020, will provide an opportunity to discuss Waksman's achievements as a scientist. The next ISMB director should be well established in post by the time the next retreat is held, in the summer of 2021.