# 9th ISMB Retreat, 22-23 July 2021

Shortly after the ISMB was set up in 2003, its founder director, Gabriel Waksman, initiated a series of annual events to foster collaborations between the students, postdocs and established researchers of its constituent departments at Birkbeck and UCL and to showcase the research conducted there. There are two series of these: the symposia, in even-numbered years, focus on the work of established researchers at the ISMB and elsewhere, and the retreats, in odd-numbered years, focus on that of the ISMB's students and postdocs. The retreat held on July 22 and 23, 2021 was thus the ninth in the series.

Two things distinguished the 2021 retreat from its predecessors. First, and most obviously, it was subject to COVID restrictions and thus held online in Microsoft Teams. All present missed the well-appointed lecture hall and meeting rooms of our usual venue, Robinson College in Cambridge, and the superb food we had become used to, but the online format held several important advantages. There was no cap on attendance, and no need for the trouble and expense of travel: three of the five keynote speakers – two more than at the 2019 retreat – were based at (and gave their talks from) the USA. The second difference was that it was the first to be held since Waksman retired as ISMB director to concentrate on leading his research goup. This retreat was held under the able leadership of **Finn Werner**, who combines the role of interim director with leading a large RNA polymerase research group at UCL.

## Thursday 22 July

The retreat began with the first of those five keynote lectures. **Buzz Baum** left UCL in 2020 to set up his lab at the MRC's prestigious Laboratory of Molecular Biology (LMB) at the University of Cambridge. His research focuses on the evolution of cell division, trying to answer the intriguing question of how primeval prokaryotes evolved into the more complex eukaryotes, particularly multicellular ones. Baum began by explaining the complexity of cell division: in order to divide, a single cell must duplicate its contents, identify the 'middle', separate everything (including its genome) into the halves, and then split. We know that eukaryotes evolved from archaea, so understanding the process of 'eukaryogenesis' will begin with archaeal cell division.

The cell cycle in higher eukaryotes is controlled by a complex patten of cyclin-dependent kinases (CDKs) and cyclins, which explains why CDKs are useful targets for anti-cancer drugs. We now know that this process is quite similar in archaea, but they lack cyclins. Instead, cell division is driven by the proteolysis of cell cycle proteins including CSKs by proteasomes. These include the cytosolic protein complex ESCRT-III, which is found in eukaryotic cells as

well as archaea and is involved in membrane scission. Baum described a physical model of this protein from the archaeon *Sulfolobus*, obtained during cell division. This is an extremophile that flourishes in volcanic springs at 75$^{o}$C, and he explained the challenges involved in maintaining it at these temperatures during imaging. *Sulfobus* forms stable communities of cells and can be thought of as a near relative of the simplest eukaryotes.

The first of the 17 young scientists to speak was **Tom Foran**, a PhD student in Carolyn Moores' lab at Birkbeck who is studying the dynamics of microtubules using cryo-electron microscopy. Microtubules are long, hollow tubes that form by the polymerisation of a dimer of alpha- and beta-tubulin. They are dynamic at the ends and bind GTP in solution. They are regulated and stabilised by the binding of doublecortin, which is encoded by a gene on the X-chromosome. Lack of doublecortin expression in males with mutations in this gene causes a devastating developmental disorder known as lissencephaly (the name means 'smooth brain'). Foran's project involves investigating the structures of microtubules bound by a different doublecortin family protein, doublecortin-like kinase, and under different nucleotide states using Birkbeck's Krios electron microscope. He has already generated images that, if published, would rival the best EM structures of microtubules in the PDB.

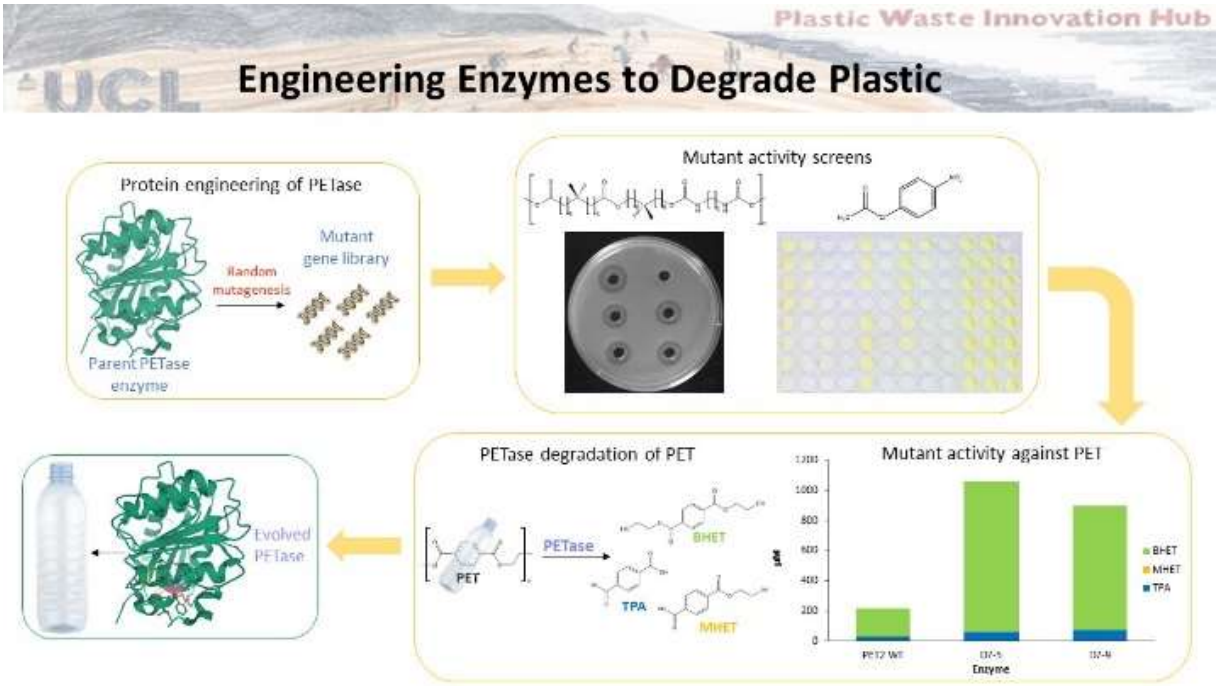
**Gorjan Stojanovski** from John Ward's synthetic biology group at UCL described how his research into aminoglycosides might help address the growing global health crisis of antibiotic resistance. These compounds are inhibitors of bacterial protein synthesis, but they are not ideal antibiotics because they are challenging to synthesise and have toxicity issues. Stojanovski's research addresses the first of these issues by exploring enzymatic pathways to aminoglycoside synthesis. He described identifying seven diverse transaminases and selecting two of them as particularly promising biocatalysts. The most promising of all was coupled into a pathway that could be used to produce novel, unnatural aminoglycosides as potential antibiotics.

The final talk in the first session also focused on antibiotic development. Many drugs are available to treat tuberculosis, the main focus of Sanjib Bhakta's research at Birkbeck, but resistance is gradually making current treatments less effective. **Mousumi Shyam**, a Newton Babba PhD Placement Fellow in Bhakta's group, is investigating some novel compounds – inhibitors of mycobactin biosynthesis, a metabolic process found only in mycobacteria – as potential efflux pump inhibitors. These drugs prevent the ejection of co-prescribed antibiotics from bacterial cells. So far, the programme has yielded two candidate efflux pump inhibitors that are also effective against non-tubercular species of mycobacteria that infect the lungs of immunocompromised patients.

The focus on infectious disease continued after the first break with a talk by **Gwenny Cackett**, a final year PhD student in Finn Werner's lab at UCL, on the transcription mechanism of the virus that causes African swine fever (ASF). This rapidly spreading virus is a major threat to world agriculture, affecting over 10% of pigs in some Asian countries. There is no vaccine or cure and affected pigs must be slaughtered. The ASF virus is large and, unusually, can replicate independently of the machinery of the host cell; many of its genes encode proteins involved in transcription. Cackett described the pattern of gene expression after infection occurs, identifying and sequencing those genes that are expressed 'early' (5 hours after infection) and 'late' (16 hours after), and identifying an early promoter motif that is similar to one found in the *Vaccinia* virus that is the source of the modern smallpox vaccine.

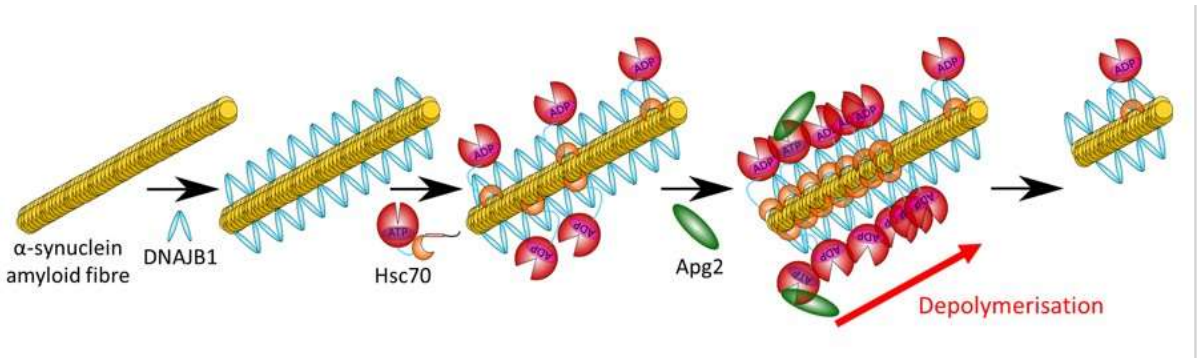
**Gabi Heller** joined Flemming Hanssen's lab at UCL in 2020 after a PhD in molecular dynamics at the University of Cambridge. Her talk focused on her doctoral studies of a group of proteins that have, until recently, been ignored as drug targets: those that are intrinsically disordered. Most of the structural biology and modelling techniques that have been developed over the last half-century are of very little use to study proteins with no intrinsic, stable structure. The two techniques that can be used are molecular dynamics and NMR spectroscopy. Heller aims to answer questions about how small, drug-like molecules bind to disordered proteins using these techniques. She cited examples of molecules that can prevent their disordered target proteins from aggregating or from binding DNA: both of these functions might prove therapeutically useful.

Then, **Maria Bawn**, a postdoc in the Department of Chemistry at UCL, described her mainly unpublished research, engineering enzymes so that their substrates change and they can digest, and so degrade, plastics. She explained that the main environmental problem is not plastics as such but plastic waste, and particularly waste derived from single-use plastics. Bawn is part of the Plastic Waste Innovation Hub at UCL, a multi-disciplinary team working on all issues to do with plastic waste. She is introducing mutations into enzymes in the so-called 'PETase' family, which break down the common plastic polyethylene terephthalate (PET) by hydrolysis, so that the digestion process is made faster and more efficient.




*Maria Bawn: Engineering enzymes to degrade plastic*

Many diseases, particularly neurological ones, arise when proteins aggregate to form amyloid fibres. **Jim Monistrol**, a final-year PhD student in Helen Saibil's electron microscopy group at Birkbeck, is studying the disaggregation mechanism of one of these, alpha-synuclein. Aggregates of this protein are observed in the post-mortem brains of patients with Parkinson's disease. Monistrol explained the mechanism through which, in healthy brains, the chaperone Hsc70 binds to fibrous aggregates of this protein with two other proteins – a heat shock protein DNAJB1 and an autophagy-related protein, Apg2 – causing hydrolysis and depolymerisation of aggregates. He is now using cryo-electron tomography to elucidate the details of this process.




*Jim Monistrol: Disaggregation of alpha-synuclein amyloid fibres by the Hsc70 system*

The afternoon session opened with a keynote lecture by **Michael Jewett** from Northwestern University, Evanston, Illinois, USA. He used his talk to highlight the importance of synthetic biology in addressing many of today's environmental and health challenges. Synthetic biology gives us the opportunity to make things that are beyond the reach of ordinary synthetic chemistry, which has many potential applications: not least in medicine.

The WHO estimates that about 30% of the world's population has no or very limited access to essential medicines. Some of these are protein-based therapeutics which are particularly expensive and challenging to manufacture, transport and store in resource-poor regions. This category includes conjugate vaccines, which are very effective at preventing bacterial infections. Jewett and his group have developed a method for expressing the proteins that form these vaccines in cell-free, freeze-dried *E. coli* lysates. Their platform, known as iVAX, is fast, modular (and so easily adaptable for manufacturing different vaccines), portable, safe and effective. Jewett described a proof of the principle of this platform using a synthetic vaccine against the bacterium *Francisella tularensis*. Mice injected with the vaccine, composed of an O-antigen polysaccharide from the bacterium linked to an immunostimulatory protein carrier, were protected against challenge with a lethal strain of the bacterium. Jewett described further novel techniques using bacteria for the synthesis of carbon-negative chemicals and acetone as a substitute for jet fuel. He ended his talk by calling for a significant increase in synthetic biology capacity worldwide to respond to these challenges.

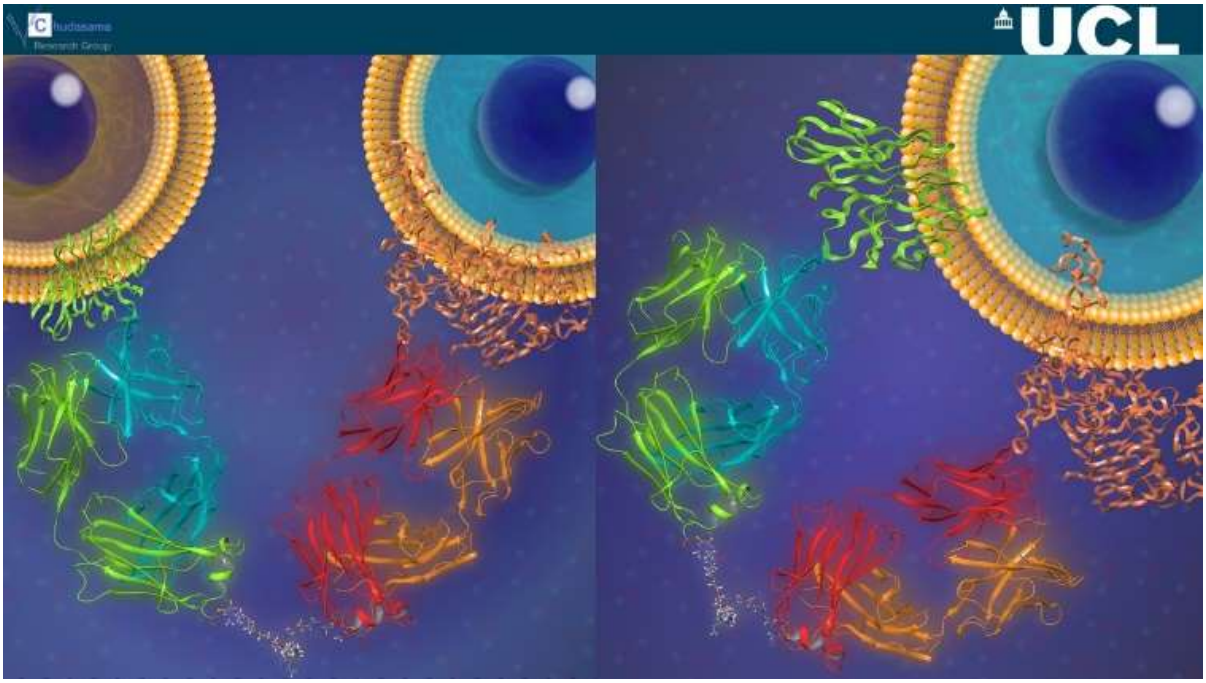

*Michael Jewett: The iVAX platform for vaccine synthesis*

Jewett's talk was followed by three more presentations by young scientists, starting with **Kamila Kamuda**, a PhD student at the UCL Centre for Respiratory Biology. She is using

structural cell biology techniques, including cryo-focused electron beam scanning electron microscopy (cryo-FEB) to study another protein that is prone to aggregation, alpha-1-antitrypsin. Any dysfunction of this protein, which is a serpin (a serine protease inhibitor), can lead to lung tissue breakdown. Her structural studies have shown that cells expressing alpha-1-antitrypsin that has been mutated to make it prone to aggregation contain elongated mitochondria that merge together to form single objects. She is now investigating how this change in the morphology of the mitochondria affects their, and the cells', function.

**Ivana Bukvin**, a PhD student on the UCL-Birkbeck MRC-DTP and based in the Ribosome Laboratory at the ISMB presented her structural investigations of nascent polypeptides folding during their biosynthesis while on their parent ribosome - a process known as co-translational protein folding. Her research aims to determine the molecular details and mechanism of the emerging role of the ribosomal surface as a chaperone that guides the protein folding as the nascent chain is being made. The highly dynamic polypeptide can realistically only be structurally examined by NMR as it rapidly becomes too flexible for cryo-EM. But even for NMR this is a major undertaking and Ivana and colleagues have had to develop the methodology to determine a set of distances between residues within the nascent chain itself and also to the ribosome surface. She has established the use of paramagnetic relaxation enhancement (PRE) NMR on stalled snapshots at various stages during the elongation of a nascent chain. Introducing single cysteine residues at chosen points in both the nascent chain and into gene-edited ribosomes has allowed Ivana to chemically introduce a nitroxide spin-label which enables distance measurements through observing the resonance broadening in the resulting NMR spectra of the ribosome-bound nascent chains. The resulting data can be used (together with her colleagues) to determine structural ensembles that define the progressive emergence of the nascent polypeptide.

Finaly in this session, **Peter Szijj**, a PhD student with Vijay Chudasama in the Department of Chemistry at UCL, described his research on bispecific antibodies as therapies for cancer and type 2 diabetes. Bispecific antibodies, as their name implies, bind to two different targets. They may be designed to bind to cells that express two different antigens, or to two different cells, each expressing one of the antigens. Szijj described the process of designing these proteins and their combinations as a form of 'antibody Lego'. He then outlined interesting applications of each of the two types of design: firstly, selectively targeting a subset of T cells that expresses two different receptors, and secondly, binding to both an immune cell and a cancer cell, so the two cells approach each other closely enough for the immune cell to kill the cancer cell.

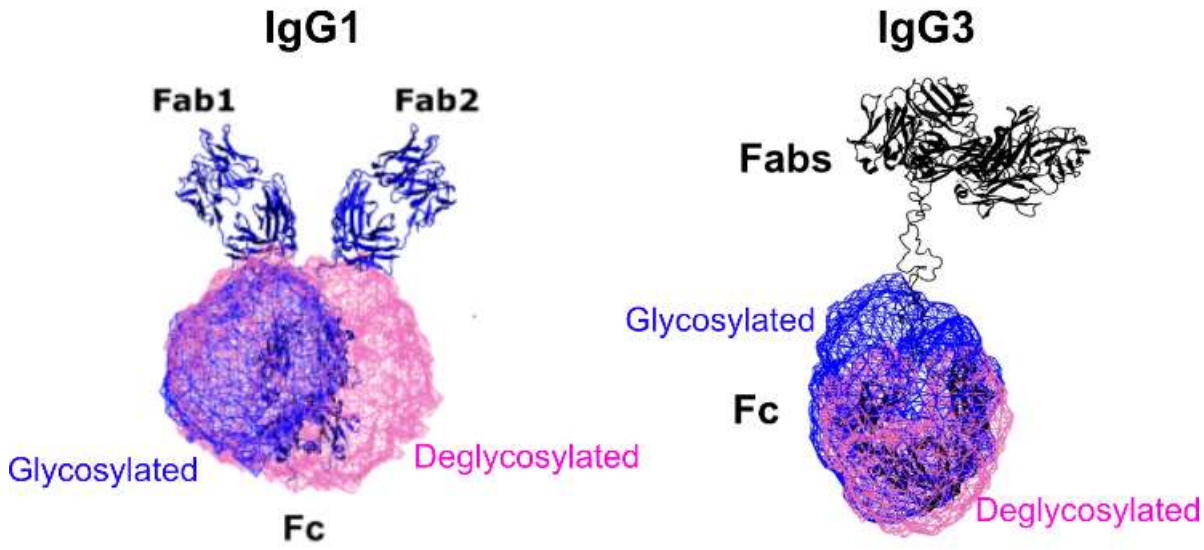



*Peter Szijj: Designing bispecific antibodies (antibody Lego)*

The last scientific session of the day began with another keynote lecture, given by **Debora Marks**, a computational biologist from Harvard Medical School, Boston, Massachusetts, USA; her talk on machine learning in biomedicine was billed as confidential. It was followed by a further three talks by young scientists. Firstly, **Sammy Chan** gave the second presentation from John Christodoulou's NMR lab: he completed his PhD in that lab in 2017 and stayed on as a postdoc. His presentation of unpublished research described the development of 19F NMR spectroscopy to probe the conformations of nascent proteins during their biosynthesis. His studies enabled the first observations of protein folding intermediates on the ribosome, and reveal how the ribosome strongly stabilise their formation to guide co-translational protein folding.

People who inherit mutated forms of the gene for alpha-1 antitrypsin may develop alpha-1 antitrypsin deficiency, which often leads to lung disease by early middle age. **James Irving**, a senior research associate in the Centre for Respiratory Biology at UCL, described a small molecule discovered in the group that binds to and prevents the aggregation of the commonest form of this protein, known as the 'Z' form. This mutation of glutamic acid to lysine replaces a negatively charged residue with a positively charged one. It is located at the end of a beta sheet, and the change in charge leads to a conformational change that in turn leads to aggregation. The molecule discovered by Irving and his co-workers binds to this site, stabilising the monomer.

The structure and properties of different forms of the human immunoglobulin IgG was the topic of **Valentina Spiteri's** presentation, which was the last of the day. She has just finished her PhD in Steve Perkins' lab at UCL. This is the commonest form of human immunoglobulin; it has four highly conserved subtypes, of which IgG1 is the most abundant in serum. These subtypes differ mostly in the hinge region and the adjoining constant domain. The subtype IgG3 is the largest, and its hinge region is the longest, so it is the most flexible; it is this flexibility that makes Spiteri's chosen analytical technique of small angle scattering a particularly appropriate one. The long hinge contributes to a heightened affinity between IgG3 and its receptor because this interaction is not blocked by the Fab region.




*Valentina Spiteri: Human antibodies with different hinges*

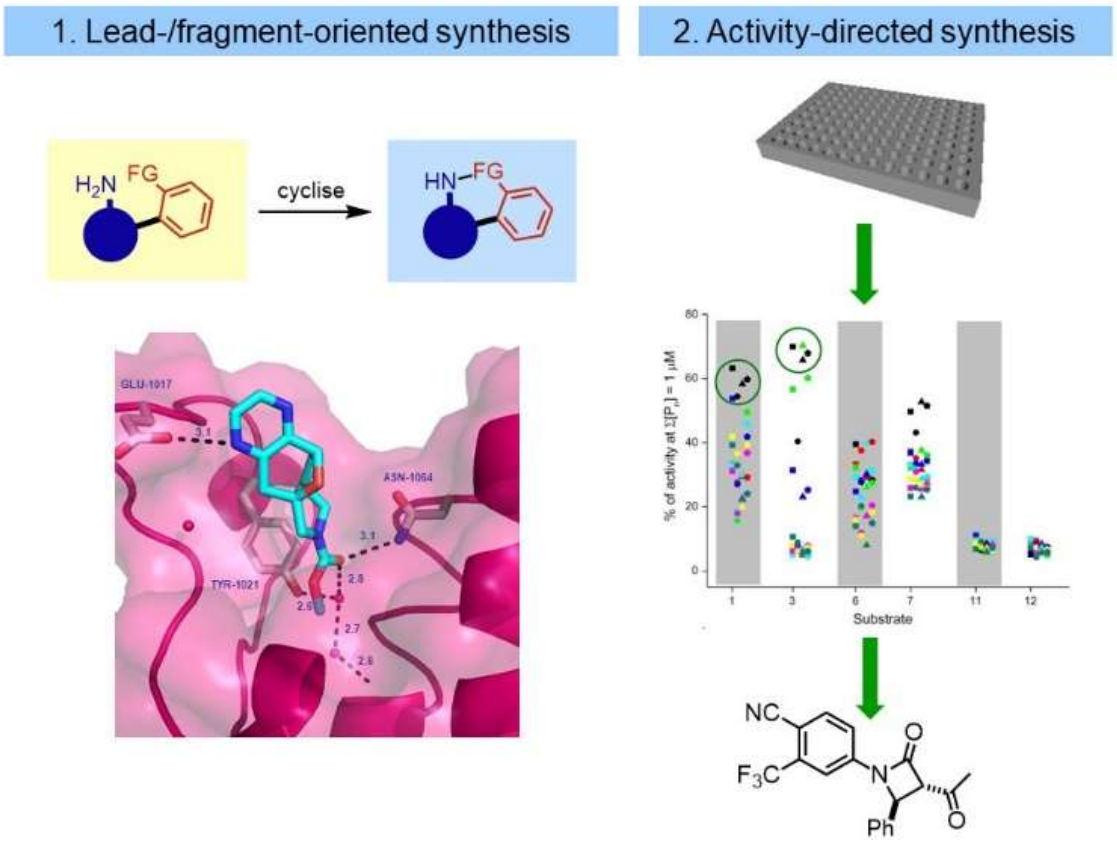
The paucity of social events is self-evidently a disadvantage of online conferences: or is it? The first day of the retreat ended with an online 'escape room' activity, a Jewel Heist, which all who took part in found rather fun. We were randomly assigned into teams, thrown together into a simulation of a locked room and invited to find stolen jewels – and the key to the room – by solving puzzles. These were challenging without being too frustrating, and most groups completed the whole puzzle in the time allowed.

## Friday 23 July

The retreat's second day began with a keynote lecture by **Adam Nelson**, Professor of Chemical Biology at the University of Leeds. His talk focused on novel methods for increasing the chemical diversity of the small molecules that are being discovered, synthesised and tested

to provide starting points for drug discovery. Several estimates exist for the number of possible compounds with appropriate physical properties, and they are all huge: Nelson quoted the figure of $10^{35}$. However, the part of so-called 'chemical space' that is actually being explored is very narrow. Half all the active compounds known come from only 0.25% of the available chemical families. Medicinal chemists focus on a few synthetic pathways and are, for example, 'very good at making amides'. Nelson described two novel approaches for increasing the diversity of compounds synthesised: lead-oriented and activity-directed synthesis. The first of these emphasises structure, and the second function.

Lead-oriented synthesis involves a stepwise approach to synthesis that harnesses chirality and cyclisation to produce diverse and novel molecular scaffolds. He showed fragments based on these scaffolds can provide useful starting points for molecular discovery by high-throughput crystallographic screening against exemplar protein targets. In contrast, activity-directed synthesis is a structure-blind and function-driven discovery approach. Here, crude reaction mixtures are assayed against a specific enzyme or receptor target at increasingly lower concentrations. Reactions that yield active products then inform further rounds of synthesis. This flexible strategy generates multiple series of lead molecules in parallel and can be applied to many different protein targets.

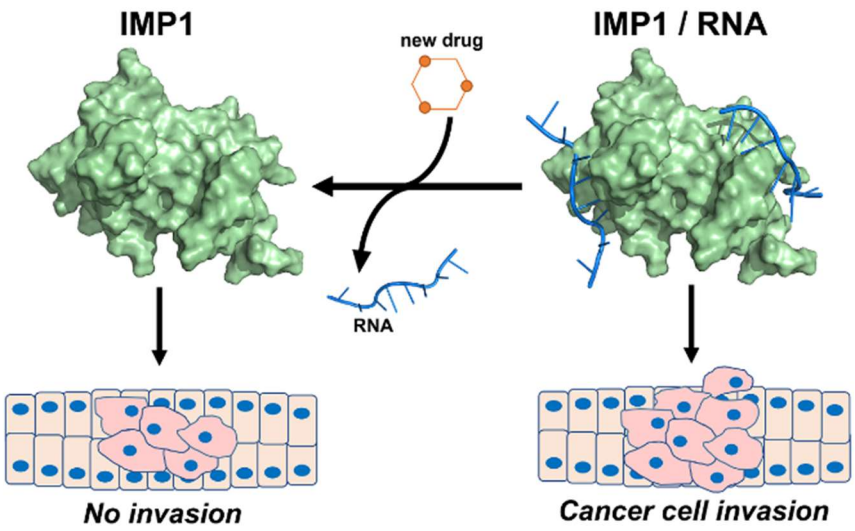



*Adam Nelson: Exploring chemical space for bioactive molecule discovery*

Over half the seafood consumed today is produced by aquaculture ('fish farming'). Farmed fish, like livestock, need to be protected against disease, but delivering therapeutics to fish stocks is challenging. The next presentation, by **Luyao Yang**, a first-year PhD student in Saul Purton's algal biotechnology group at UCL, described a novel method for delivering vaccines and growth hormones to fish by genetic modification of the algae in their diet. This is a stepwise process, beginning with the *in silico* design of the DNA sequence to be expressed. This is assembled into a cassette and inserted into a plasmid that is be incorporated in an algal chloroplast for expression; the presence and sequence of the expressed gene can then be confirmed by Western blotting and PCR.

**Sioned Jones**, a final-year PhD student with Stefan Howorka at UCL, summarised her research into how DNA can be made to interact with both biological and synthetic membranes. DNA is a hydrophilic molecule with a charged backbone, and membranes are largely hydrophobic, so they are not natural interaction partners. However, hydrophobic tags can be added to the end or centre of DNA duplexes so they can embed into membranes; the exact nature of this interaction depends on the nature and length of the tag. Jones has explored these interactions using fluorescence microscopy and simulated them using molecular dynamics, showing that a central hydrophobic belt generates a stronger interaction with the membrane than an end tag.

Next, **Giancarlo Abis**, a postdoc working in Andres Ramos' RNA regulation lab, described their recent advances on the RNA-binding protein IMP1. The function of this protein is to regulate the metabolism of several messenger RNAs during embryogenesis. When re-expressed in differentiated tissues, IMP1 leads to cancer cell phenotype. IMP1 has six RNA-binding domains organised in three pairs called "di-domains", which work as structural and functional independent units. Abis described their approach to identify a new drug able to target IMP1 in lung cancer cells where the protein is over-expressed. This new small molecule affects one of the three "di-domains" of IMP1, leading to the release of the RNA targets and reducing the replication growth and cell invasion ability of the lung cancer cells. This work paves the way to the synthesis of a new class of therapeutics for potential treatment of lung cancer.




*Giancarlo Abis: The mechanism of IMP1 binding to RNA*

The final talk in the morning session was given by **Hannah Britt**, a postdoc working with Konstantinos Thalassinos at UCL. She explained how mass spectrometry (MS) is now being used in structural biology, and, particularly, how she is using structural mass spectrometry to explore the dynamics of glycoprotein complexes. About half the proteins in the human proteome have one or more glycan chains covalently bonded to amino acid side chains and are therefore classed as glycoproteins. However, the glycan moieties are difficult to observe using most structural biology techniques because of their flexibility and their heterogeneity. Structural MS is able to deal with both these difficulties. As a case study, Britt described her studies of beta-2-glycoprotein 1, a heavily glycosylated protein that has been linked to the symptoms of the autoimmune disease APS. She has shown that this protein takes up a more extended conformation in samples taken from APS patients than in those from healthy controls.

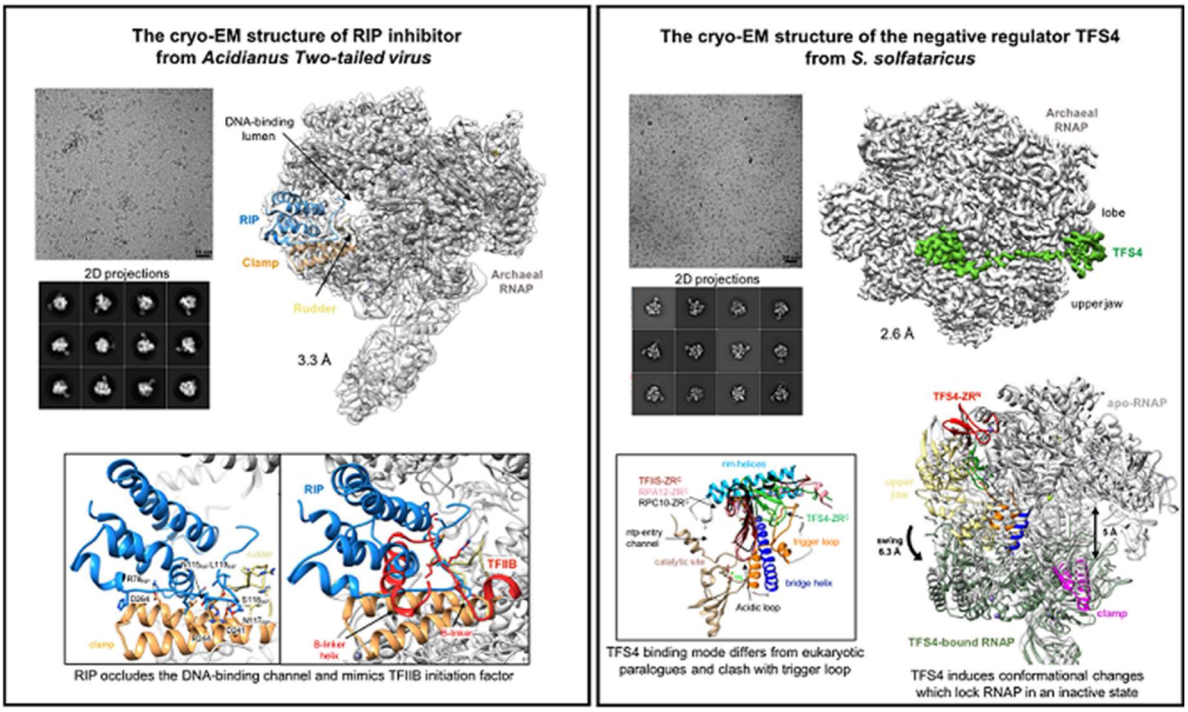
The first session after the lunch break was an engaging and useful workshop on pitching research ideas to funding panels, led by **Simon Cain** from Westbourne Training and Consulting, UK. Cain explained that panel members were unlikely to be experts in a candidate's own research field and that they may well have only read the lay summary. He introduced his audience to the '3 P's' of pitching – Project, Person and Place – and gave tips on slide production and presentation skills before going through the process using interim ISMB director **Finn Werner** as his 'guinea pig', with a project on the structure of RNA polymerase from African swine fever virus.

The final session began immediately after the workshop with a keynote lecture from **Richard Ebright**, Professor of Chemistry and Chemical Biology at Rutgers University, New Jersey. He

has published over 160 papers, and the work he described on the structural basis of transcription-translation coupling in bacteria was published in *Science* in 2020. Coupling means that in bacteria, RNA transcription and protein translation occur in the same compartment at the same time, with the movement of RNA polymerase along the DNA molecule coordinating with the movement of the first, or 'lead' ribosome along the mRNA. In fact, the lead ribosome on an mRNA makes physical contact with the RNA polymerase via proteins that act as coupling factors.

Ebright described the nature and structure of the coupling factors *NusG* and *NusA*. *NusG* has two domains, separated by a flexible linker; the N-terminal domain interacts with the RNA polymerase and the C-terminal domain with the ribosomal proteins. His group has obtained structures of the transcription-translation complexes (TTC) including both *NusG* and *NusA* using cryo-electron microscopy, and showed that these structures depend on the length, in codons, of the 'spacer' between the transcription elongation complex and the P-site of the ribosome. If this spacer contained seven codons or fewer, *NusG* in the resulting structure was unable to form a bridge between the RNA polymerase and the ribosome, and NusA was unable to bind. In contrast, if the spacer contained at least eight (but, in these experiments, no more than 10) codons, a structure formed that could bind both domains of *NusG* to form the bridge. Furthermore, this structure enabled *NusA* to bind. This structure, which has been termed TTC-B, is now considered to mediate transcription-translation coupling in bacteria.

The next talk, by **Simona Pilotto**, a postdoc in Finn Werner's lab at UCL, continued the theme of RNA polymerase structures. Pilotto presented two new structures of RNA polymerase inhibitors, solved using cryo-electron microscopy. One of these is found in the genome of a virus that infects archaea, and the other, the negative regulator TFS4, is a protein from the archaeal host. The former inhibitor, RIP (for RNAP Inhibiting Protein) from the *Acidianus* two-tailed virus, binds inside the DNA channel of the archaeal polymerase, preventing the nucleic acid and initiation factors from accessing this site. In contrast, TFS4 expression is induced in the host by viral infection, and this binds inside the secondary channel, inducing a conformational change to an inactive state that is similar to inactive states of RNA polymerase that have been observed in eukaryotes. Both these inhibitors contribute to an 'arms race' between virus and host.




*Simona Pilotto: Structures of virus and host RNA polymerase inhibitors in archaea*

**Stephanie Webb**, a PhD student in Anthony Roberts' lab at Birkbeck, gave the final scientific talk of the retreat, on the structure and mechanism of the motor protein kinesin-2. Kinesins are found predominantly in cilia, which are thread-like structures found on the surface of most human cells, and their function is to transport cargo along microtubules towards their tips (and, therefore, away from the cell body). Kinesin-2 is a heterotrimer comprising the related subunits Kif3A and Kif3B and an accessory protein; each of Kif3A and Kif3B has three domains, an N-terminal motor domain, a coiled-coil domain and a tail. These two proteins can exist in two states, either elongated and active or compact and autoinhibited. Webb explained that serial deletions of residues in the tail have shown that the function of this region is to stabilise the inactive state.

**Finn Werner** concluded the two days of fascinating talks by announcing two winners of prizes for the best talks by young scientists. The first prize was awarded to **Peter Szijj** for his talk on bispecific antibodies, and the second to **Gwenny Cackett** for hers on African swine fever virus transcription. He then thanked the indefatigable ISMB administrator, Andrew Service, for working so hard to lay on such a successful virtual ISMB retreat. The next retreat will be held in the summer of 2023, by which time the next ISMB director should be well established. Hopefully, too, we will be able to hold it in person, perhaps back in our old venue of Robinson College.

- *Dr Clare Sansom, Birkbeck, September 2021*