fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

ISSUE 21 · MICHAELMAS 2024

UNIVERSITY OF OXFORD

An interview with Maria Leptin

The scale of the problem: Combating AMR

MatchBio: the latest Dunn School spinout

Building a more equitable, diverse and inclusive community

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Welcome...

It's always a pleasure to write the welcome to a new issue of Fusion. Not only do I enjoy the articles written by Dunn School colleagues and friends, and reminding myself of the highlights of the last year, but it also gives me a chance to reflect on the Dunn School diaspora, the alumni with whom we maintain such warm relationships, sometimes over decades. It is wonderful that such a strong bond of collegiality lasts over long distances of time and space, and it is one of the things that makes the Dunn School special. Please do continue to keep in touch, and pass the message on to any alumni who have fallen off our radar.

I make no apology about again mentioning the upcoming centenary, as I did last year, and no doubt will next. 2027 is approaching fast and there is a consequent acceleration of our planning activity. Details remain to be resolved but we are moving from outline concepts to more concrete specifics, which we'll be rolling out over the year. As described elsewhere in this issue, we are actively seeking ideas and input from you, our friends and alumni. You can always contact us through alumni@path.ox.ac.uk, but there will also be an online planning event this November, to which anyone interested is warmly invited. And don't forget to mark 7th July 2027 as the date of our primary celebration: we very much look forward to welcoming back as many old friends as possible.

The centenary is also an opportunity to raise funds for the next 100 years of the Dunn School (the best is yet to come, after all!). As I announced last year, we have made people our strategic priority. The specific goals are to endow up to twelve new graduate studentships, ensuring that we can accept as many as possible of the brilliant student applicants we get from all over the world; and to endow the faculty positions that support our individual group leaders, who are ultimately responsible for our research leadership. More details in future issues, but I can now report that we have already made good progress in both challenges, and we are extremely grateful to the people who have helped.

The annual cycle also provides an opportunity to welcome new members of the Department. We get many new students, postdocs, research and support staff every year so I hope you'll forgive me if here I only mention the new group leaders. It's been another bumper year, with the appointment and arrival of Anjali Hinch, Katerina Toropova and Kevin Foster, each of them either starting their first group or moving an already-thriving group to the Dunn School. More details elsewhere in this issue but the point I want to emphasise is that recruiting new group leaders is both the most important and most enjoyable aspect of my job. They are the future of the department, and of biomedical science, and it is an immense privilege that we get such extraordinary applicants, allowing us to make such exceptional appointments. The future looks very bright indeed.

Inevitably, the other side of the coin is departures and farewells. Again, there is always turnover at all levels and, while I can't realistically mention everyone here, rest assured that every single person, whatever their position, is encouraged to remain part of the extended Dunn School family.

I hope you enjoy this issue. It has been edited by Cat Vicente, who has the rather cryptic title of Head of Scientific Strategy and Projects, but who in practice is at the centre of nearly everything! As always, Fusion aims to provide a mix of news, insight into exciting current research, and reflections about the past. It is one of the vehicles by which we hope to communicate with the whole Dunn School community, past and present. But staying in touch is a two-way process so let me finish by repeating how much we like to hear from you. Please stay in touch by any means including, best of all, in person. We are always pleased to meet former colleagues, to hear their stories, and to show them how the department has changed. If you are thinking of visiting, just drop a note to alumni@path.ox.ac.uk.

And if not before, I look forward to seeing many of you in 2027!

Matthew Freeman

Centenary Celebrations Open House

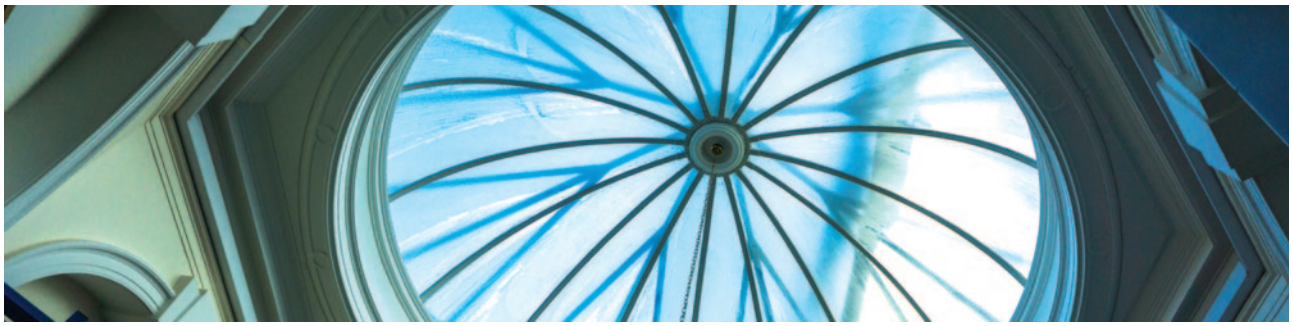
The Dunn School will be celebrating its centenary in 2027. Do you want to know what we are planning? Do you have ideas that you would like to share? Would you like to connect with other former members?

Join us this November for an online gathering to discuss all things centenary!

22nd of November at 2.30pm GMT (9.30 ET, 22.30 CST)

Sign up via this form:

Contents



Photograph Tatjana Terentjeva

Some arrivals and departures

Arrivals

The last year has seen the arrival of three new research groups to the Dunn School.

Anjali Hinch relocated her group from the Wellcome Trust Centre of Human Genetics to the Dunn School. Anjali has a computational background and in fact started her career in finance, but moved into computational genomics, completing a doctorate in population genetics. Her group is supported by a Wellcome Trust Henry Dale Fellowship, and aims to understand the mechanisms underlying the induction and repair of germline DNA breaks and their impacts on human disease.

Meanwhile **Kevin Foster** was appointed to the Statutory Professorship of Microbiology. Kevin Foster combines his unique background in maths, ecology, evolution and microbiology to study microbial communities. He is particularly interested in bacterial competition, and has applied the logic of ecology and evolution to microbiology. Previous holders of the Chair of Microbiology include Professor Susan Lea, Professor Stephen Bell and Professor Jeff Errington. It is held in conjunction with a Fellowship at Wadham College.

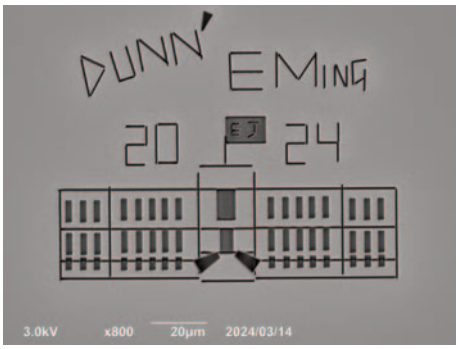
We also welcome **Kat Toropova** as a new group leader. Supported by an MRC Career Development Award, her group will aim to understand the three-dimensional structure of molecular machines within our cells, in order to understand how they normally function and how they go awry in disease. The Toropova groups solves atomic resolution structures, and places them in their cellular context, using multiscale cryo-electron microscopy (cryo-EM) and in situ cryo-electron tomography (cryo-ET) techniques. They combine this with *in vitro* reconstitution, cell biology, and live fluorescence imaging to dissect molecular mechanisms underlying function.

In the support teams, Raquel Perez Marina joined us as Finance Assistant, while Mervat Zen Aldin is the new Grants Administrator. Louise Bryant is a new apprentice in the services team. Meanwhile Raman Dhaliwal has returned as our EM Support Scientist following a stint as a teacher in South Korea.

Departures

Joan Monks retired this year, after 40 years as a research assistant and lab manager in Nick Proudfoot's group. Perhaps not technically a Dunn School record, that is nevertheless an amazing stint. We wish Joan a happy and fulfilling retirement!

Meanwhile, **Errin Johnson**, our Electron Microscopy Facility Manager, moved back to her native Australia to take on the position of Biological Electron Microscopy Manager at Sydney Microscopy & Microanalysis, at the University of Sydney. Errin will be remembered not just by her technical skill and fantastic efforts to continually push the boundaries of EM at the Dunn School, but also for cake-making, Father Christmas videos and all other contributions to the Dunn School community. One of her leaving gifts was a very (very) small etching of the Dunn School in a resin heart, by the equally skilled new manager of the EM facility, Charlotte Melia.



Also moving to sunnier climates, **Alberto Baena-Lopez** relocated his group to the elite Centro de Biología Molecular Severo Ochoa (CBM) in Madrid, Spain. Alberto was a group leader in the Dunn School for about 10 years, and a leading force in *Drosophila* research in Oxford, alongside developing an international reputation as a leader in the field of non-apoptotic caspases.

News

A round-up of news from the last year. You can read these and other stories on our website www.path.ox.ac.uk

Honours for Dunn School researchers: some of the highlights

Liz Robertson was awarded the title of Commander of the Order of the British Empire (CBE) in the 2024 New Year's Honours list, in recognition of her world-leading role in mammalian developmental genetics.

Meanwhile **Chris Tang** was elected to the Fellowship of the American Society of Microbiology, one of the largest and oldest professional societies This honour recognises his ground-breaking work on the fundamental biology of bacterial pathogens, as well as the opportunities his discoveries provide for improving human health.

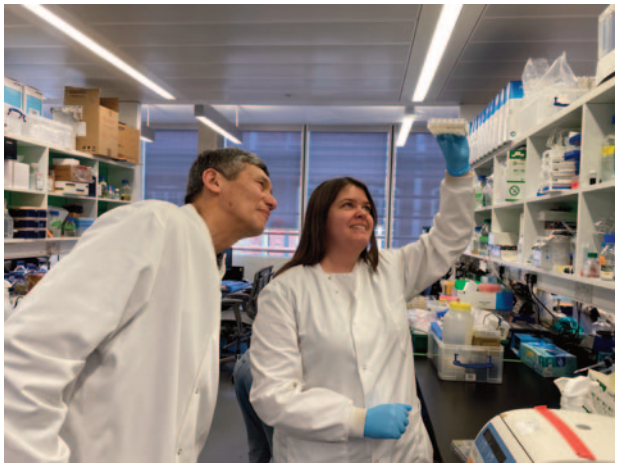
Ivan Ahel was elected as Fellow of the Academy of Medical Sciences, recognising his leading contribution to the field of ADP-ribosylation and genome stability.

Our congratulations also go to **Sumana Sanyal**, who was awarded the title of Professor of Molecular Pathology by the University of Oxford, recognising her research on the cell biology of viral infection, as well as her teaching contributions.

Finally, **Yiqi Zhao** was awarded the 2024 Peter Medawar Prize for Immunology. This prestigious prize, supported by the Medawar family and awarded by the Oxford Immunology Network, honours an Oxford researcher who has made exceptional contributions to the field of immunology, both through their scientific excellence and through their broader contributions to the academic community. Yiqi is a postdoc in the Smith group, and her work focuses on how the immune system interacts with viruses. Her recent paper published in *Nature* particularly focused on the protein TRIM5α.

In other news...

New meningitis-B vaccine developed by the Tang lab
The collaboration between the Serum Institute of India Pvt. Ltd and the University of Oxford will provide lifesaving protection against Men-B through the production of a chimeric protein-based vaccine.



Meningitis is the inflammation of the tissues surrounding the brain and spinal cord and is usually caused by infection. It can be fatal and requires immediate medical care. Meningitis can be caused by several species of bacteria, viruses, fungi and parasites. Bacterial meningitis is the most dangerous form of meningitis and can be fatal within 24 hours. Although meningitis affects all ages, young children are most at risk, and millions are affected worldwide.

Invasive meningococcal disease (IMD) is caused by six serogroups (Men-A, -B, -C, -W, -Y, and -X) of the bacterium. After five years of extensive work in collaboration with the Serum Institute of India, the team at the Sir William Dunn School of Pathology formulated a quadrivalent vaccine consisting of four chimeric proteins to tackle Men-B. Preliminary results indicate that the new protein-based vaccine advances several aspects including improved safety, efficacy, and coverage compared to present-day licensed vaccines.

dCas9 roadblocks paving new ways for CRISPRi use
In a study published in Nature Structural & Molecular Biology, the Proudfoot lab demonstrates how the use of dCas9 might affect transcriptional elongation and RNA processing, and how it can be harnessed to manipulate Pol II progression along the gene.

Our approach to genome editing changed drastically in 2012, with the discovery and implementation of the CRISPR-Cas9 system. CRISPR, short for Clustered Regularly Interspaced Short Palindromic Repeats, refers to unique sequences in the bacterial genome, which act as its immune system. Cas9 is an endonuclease protein that associates with CRISPR-derived RNAs, that in effect target formation of DNA breaks at very precise locations. Although primarily known as a tool for gene editing, it is becoming abundantly clear that the CRISPR-Cas9 mechanism can be used for a variety of other research applications – from the detection of specific nucleic acid sequences, to the control of gene expression.

The latter involves the use of enzymatically "dead" Cas9, dCas9, which continues to bind specific DNA sequences with the use of CRISPR guide RNAs (gRNAs), but now lacks its nuclease activity. It can be linked to effector domains such as in CRISPRa and CRISPRi – systems for, respectively, activating or inhibiting transcription. However, despite the plethora of available gRNAs for targeting distinct points within the genome, the full picture of the effects of dCas9 binding remains somewhat blurry. Previous investigations have shown that dCas9 bound to the gene transcription start site blocks its expression, but little is known about the impact of targeting dCas9 across downstream positions on the gene.

In their new work, Inna Zukher *et al*. employed the dCas9-KRAB CRISPRi suppression system to corroborate that binding dCas9 to the 5' end of a gene significantly reduces its expression. Correspondingly, targeting it downstream of the polyadenylation signal (PAS) causes a blockade for transcriptional elongation, as judged by a decrease in RNA readthrough. Natural anti-termination effects, for instance stress-induced, can still counteract early termination caused by CRISPRi. Pol II pausing is seen to occur just upstream of the DNA-bound dCas9, and is further associated with increased Thr4 phosphorylation in the polymerase's C-terminal domain, which eventually triggers termination. Although the KRAB domain recruits histone methyltransferases and increases the local H3K9me3 levels, the Pol II stalling effect seen in CRISPRi-induced early termination was found not to be dependent on repressive chromatin marks, but rather on the dCas9 roadblock itself.

Importantly, the data obtained in the investigation has underlined that the CRISPRi roadblock is orientation specific. Although dCas9 can target both DNA strands equally well, it does not have any pronounced transcriptional effects when bound to the template strand outside of the transcription start site region. These results could be of critical use when designing dCas9 experiments where minimal transcriptional disturbance is required without compromising on binding efficacy. The exact consequences of dCas9 roadblocks on early termination were further analysed by targeting the protein to different sites around the PAS. It was shown that when paired with an antisense guide, dCas9 acts as a sturdy roadblock to the elongation complex, but only transiently stops Pol II progression. The resulting collision between the polymerase and dCas9 culminates in earlier termination of transcription, but with no change to overall gene expression if there is an active PAS upstream of the block. However, if the PAS is nowhere close by, the transcript fails to get polyadenylated and gets degraded, which in turn suppresses gene expression. The site of dCas9 binding is also important when the gene has two active PAS, in which case alternative polyadenylation events are employed. Finally, the transient nature of the block is further verified by its lack of effect on alternative splicing, which is usually influenced by Pol II kinetics.

Taken together, the findings collected by Zukher and colleagues shed a new light on the elusive nature of CRISPRi interactions with Pol II and the elongation complex, and so help us better understand how the dCas9 roadblock might affect gene expression based on the gene site it targets. Ultimately, gaining better knowledge of this elegant system will help achieve greater robustness in the ever-expanding catalogue of CRISPR experiments.

Written by Aleksandra Pluta (Murphy lab)

New portrait of Alan Williams unveiled
A pioneer in the field of leukocyte membrane proteins, Alan Williams was elected Head of Department in 1991.

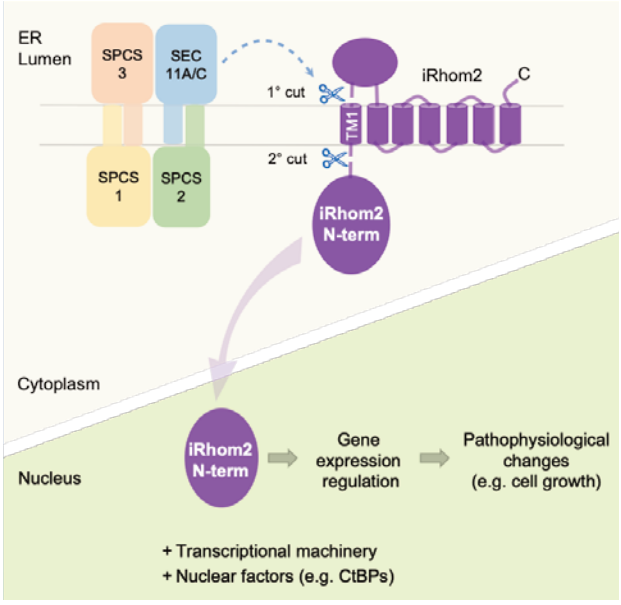
The unveiling of the new photographic portrait was hosted by the current Head of Department Professor Matthew Freeman earlier this year, in the company of friends and family. The new portrait is located in the main staircase of the Dunn School Old Building.

Alan Williams made leading contributions to the development of methods for the characterisation and subsequent isolation of cell surface proteins. His analysis of Thy-1 antigen established approaches used for a variety of other molecules, and he was also a pioneer in the use of monoclonal antibodies for these purposes. His discovery that the brain Thy-1 protein is evolutionarily related to immunoglobulins suggested that this type of domain would have a role beyond that in immunology, developing the concept of the immunoglobulin superfamily. He also showed that Thy-1 was anchored by a novel lipid method and many seminal findings. Alan Williams was appointed Director of the MRC Cellular Immunology Unit at the Dunn School in 1977, and elected Professor of Pathology and Head of Department in 1991. His untimely death at the age of 46 in 1992 deprived the Dunn School community of a trusted colleague. Professor Emma Slack, the inaugural Barclay-Williams Professor of Molecular Immunology, said "*Alan was one of the giants of Immunology, whose work underpins everything that I do and teach in Immunology today. It a huge honour for me to hold the Barclay-Williams chair, that Alan's family, together with Neil Barclay, have established. When I arrive at the lab each morning, I will look up at this new picture and be instantly reminded of my commitment to continue grow and develop the Dunn School's tradition as a world-leading centre for highly innovative, paradigm-shifting*"

iRhom2's Pseudoprotease Cleavage Unveils a Novel ER-to-Nucleus Signalling Pathway
New research led by Dr Iqbal Dulloo, a senior postdoctoral researcher in Matthew Freeman's lab at the Dunn School of Pathology, has uncovered a previously unknown signalling pathway between the endoplasmic reticulum (ER) and the nucleus, involving the pseudoprotease iRhom2.

Key findings of this study were published online on 5 January 2024 in *Molecular Cell*, titled 'Cleavage of the pseudoprotease iRhom2 by the signal peptidase complex reveals an ER-to-nucleus signaling pathway'.

The research demonstrates that the polytopic iRhom2 protein, known for its role in regulating growth factor and inflammatory signalling pathways, is a non-canonical substrate of the signal peptidase complex (SPC), and acquires an unexpected function in the nucleus following proteolytic cleavage. The cleaved N-terminal domain of iRhom2 translocates to the



Source: Dulloo *et al*, (2024) Cleavage of the pseudoprotease iRhom2 by the signal peptidase complex reveals an ER-to-nucleus signaling pathway, Molecular Cell, 84(2) 277-292

nucleus, leading to changes in the cellular transcriptome. Elevated levels of nuclear iRhom2 were observed in various human skin pathologies such as lesional psoriasis, suggesting potential disease association.

Dr Dulloo believes the research is significant as it sheds light on a new mechanism of action for iRhom pseudoproteases. Unlike their previous functions, which are associated with the recognition of transmembrane domains of their client proteins, this research uncovers an unexpected feature of iRhoms as substrates of the SPC, contributing to a more comprehensive understanding of their role in intracellular signalling.
The study also highlights the importance of pseudoenzymes, present in many enzyme families, but yet often neglected relative to their active counterparts. This new role of iRhom pseudoproteases further exemplify these proteins as essential regulators of intracellular signalling pathways.
The conventional belief regarding the primary function of the signal peptidase complex is also questioned. Traditionally associated with removing signal peptides, SPC is shown here to have a more diverse and complex role in cellular signalling.

Dr Dulloo commented: "*This project has been a labour of love to say the least, highly risky and getting binned a few times along the way, but I'm delighted that in the end, the findings turned out to be quite unexpected, exciting and potentially significant for several fields of research.*"

Written by Jo Peel (HoD office)

The scale of the problem: Combating AMR

Georgia Isom and Emma Slack

Dr Georgia Isom (MRC Career Development Fellow) and Professor Emma Slack (Barclay Williams Professor of Molecular Immunology) are part of a new community of microbiology and immunology groups at the Dunn School. In this issue of Fusion, they look at how their two groups (and others in the Dunn School) are approaching this problem at different scales.

It is perhaps surprising that *E. coli*, probably the best-studied organism on planet earth, is also at the heart of the antibiotic resistance crisis. There are still major gaps in our knowledge, including how *E. coli* maintains and grows its cell membranes to protect itself against antibiotics, and how our own immune system has evolved to resist *E. coli* infections. With antibiotic resistant invasive *E. coli* driving morbidity and mortality worldwide, the race is on to find new ways to prevent and treat *E. coli* infections.

Probing new drug targets at the nano scale: The bacterial outer membrane as a key contributor to AMR

A significant proportion of the AMR in *E. coli* can be attributed to the impermeable nature of its cell envelope, which provides the bacterium with several layers of protection. This cell envelope consists of an inner and outer membrane, separated by an aqueous periplasm that houses the cell wall (Figure 1). Some bacteria have an additional slime layer outside the cell envelope called the capsule. However, in bacteria that lack a capsule, the outer membrane is the first line of defence against the external environment and is very impermeable to a huge range of antibiotics. It is therefore essential that we understand the composition of this outer membrane, and the mechanisms by which *E. coli* and other Gram-negative bacteria build it.

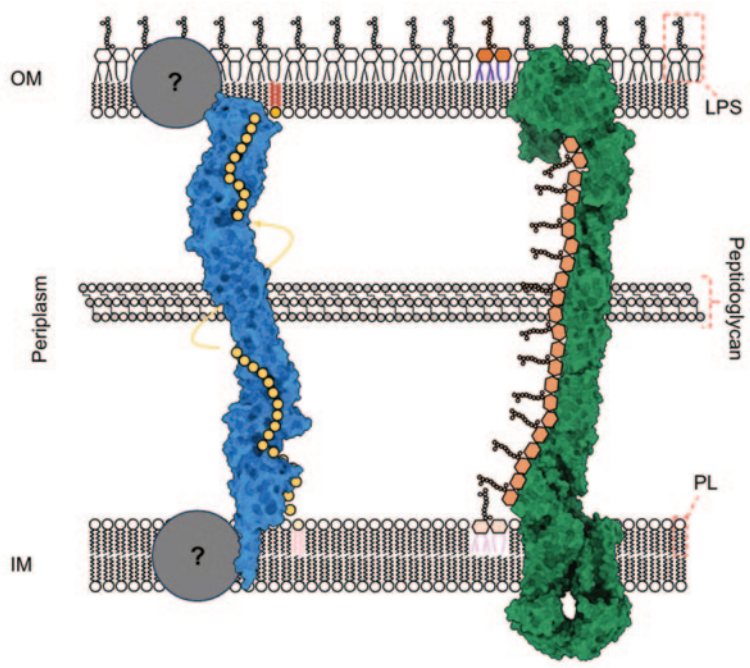


Figure 1: The *E. coli* cell envelope and machineries that build the outer membrane. The Gram-negative bacterial cell envelope consists of an inner membrane (IM) phospholipid (PL) bilayer, and an asymmetric outer membrane (OM) consisting of the PL inner leaflet and the lipopolysaccharide (LPS) outer leaflet. The two membranes are separated by the periplasm, housing the peptidoglycan cell wall. On the left, YhdP, the major PL transporter, is depicted as an envelope spanning groove through which PLs can travel. On the right is the well-characterised Lpt LPS transporter, which forms an envelope spanning bridge to allow transport of LPS from the inner to the outer membranes (Botos *et al.*, 2016; Owens *et al.*, 2019; Suits *et al.*, 2008).

The outer membrane (OM) is an asymmetric lipid bilayer made of phospholipid in the inner leaflet and lipopolysaccharide

(LPS) in the outer leaflet. The asymmetry of this leaflet is required to maintain outer membrane homeostasis. The OM is also scattered with a variety of proteins that do important functions for the cell, from building the OM to allowing import/export of molecules. The components of this OM are primarily synthesised in the cytoplasm, and thus require transport across the cell envelope to reach their destination. Over the past few years, it is emerging that many of these transporter proteins directly span the periplasm, allowing protected transport of OM components directly from the inner to the outer membranes.

The best characterised of these transporters is the Lpt system, responsible for transport of LPS to the OM. The Lpt transporter forms an envelope spanning bridge, which shields the hydrophobic acyl chains of LPS, while exposing the hydrophilic sugar moieties to the aqueous periplasm (Figure 1, right) (Chng *et al.*, 2010). This transport process is driven by ATP hydrolysis at the inner membrane, which pushes one LPS molecule at a time into the bottom of the bridge. The length of the bridge is lined with LPS molecules, such that insertion at the bottom of the bridge will push out an LPS molecule at the top of the bridge, which is subsequently incorporated into the outer membrane. This mechanism of transport is frequently described as analogous to a PEZ sweet dispenser (Okuda *et al.*, 2016).

Comparatively, research into phospholipid transport to the OM lies decades behind. The Isom lab is focussed on contributing to this major gap in our knowledge. In the past 5 years, the leading candidates for fulfilling this transport role belong to the AsmA protein family (Kumar & Ruiz, 2023). These proteins are conserved across life and are always found in locations where lipid transport between adjacent membranes is required. An AsmA protein in *E. coli*, YhdP, is anchored to the inner membrane and is emerging as the key player in phospholipid transport to the OM (Douglass *et al.*, 2022; Grimm *et al.*, 2020; Ruiz *et al.*, 2021). The release of AlphaFold has allowed us to view a predicted high-resolution structure of YhdP, revealing this rod-type structure forms an elongated groove with a hydrophobic interior (Figure 1, left) (Cooper *et al.*, 2023). The Isom lab has captured phospholipids inside this groove, leading to a model where the hydrophobic acyl chains are shielded inside, with the hydrophilic head groups exposed to the periplasm (Cooper *et al.*, 2023). The length of the protein would be large enough to span from the inner to the outer membrane, resulting in a continuous conduit for phospholipids across the periplasm. At a glance, this mechanism of transport is remarkably similar to the Lpt transporter, even though the two systems have no detectable sequence homology. Importantly, how YhdP is energised and how phospholipids would be inserted into the inner leaflet of the outer membrane, remains entirely unknown. Nevertheless, it appears that Gram-negative bacteria such as *E. coli* have evolved bridge-type mechanisms for transport of a range of lipid molecules in building the outer membrane.

These models largely stem from high resolution protein structures or predictions, allowing us to understand the molecular mechanism and, importantly, potentially design small molecules to inhibit their function. Indeed, two recent studies revealed a novel antibiotic that targets the Lpt transporter and is specifically effective against the Gram-negative pathogen Acinetobacter baumannii (Pahil *et al.*, 2024; Zampaloni *et al.*, 2024), which is listed at the top of the WHO list of priority pathogens. High resolution structures reveal that the antibiotics function by binding both the Lpt transporter and LPS in tandem inside the bridge, preventing LPS transport. This new antibiotic demonstrates the importance of studying transport systems that build the OM. This Isom lab's work paves the way for designing similar inhibitors for transporters such as YhdP, allowing targeting of *E. coli*.

From nano- to micro-scale: Using host immunity and microbe-microbe interactions

In labs, we often study *E. coli* in isolation. However, in the real world, bacteria are continuously interacting with other microbial species, as well as with dynamically changing ecosystems such as the biology of their mammalian hosts. The *E. coli* OM is its interface to the environment, including the host immune system and other microbes.

Interestingly, almost all bacteria cover the outer surface of their outer membrane with glycans - long polymers of sugars. In *E. coli*, these are synthesized as glycolipids in the cytoplasm or periplasm and transported out to the cell surface via dedicated transport systems (Whitfield *et al.*, 2020), similar to those described above. The glycolipids can be separated into two categories depending on their anchoring lipid. Lipopolysaccharides consist of a glycan, called the "O-antigen" linked to a highly conserved hexa-acyl lipid, while capsular polysaccharides, known as "K-antigens" are linked to a diacyl lipid and are often highly charged (Figure 2).

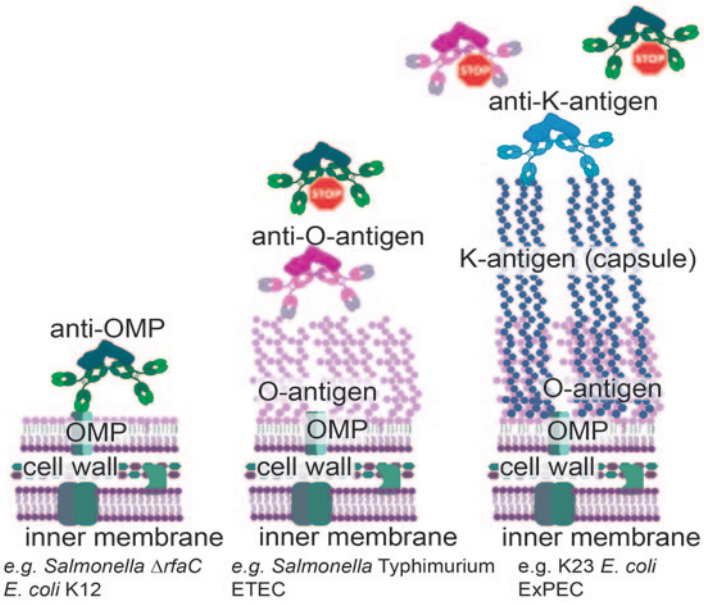


Figure 2: *E. coli* O- and K- antigens mask underlying structures such as outer membrane porins (OMP) from antibody-mediated recognition.

Both O-antigens and K-antigens are considered critical for *E. coli* virulence, providing protection for the cells from host immunity, bile and detergents. It should be noted that while diagrams often depict these glycolipids as sparce antennae-like structures, in reality they form a dense carpet over the cell surface and contribute significantly to the strength of the bacterium's outermost layer (Figure 3). These glycans are also common targets of both bacteriophages (viruses that infect bacteria) and the host immune response. Therefore, understanding the biochemistry, cell biology and structural biology of the *E. coli* cell surface not only generates new targets for drug therapies, it is also key to developing alternatives to antibiotics: namely phage therapies and vaccines.

Targeting surface glycolipids with *E. coli* vaccines?

There are no currently licensed *E. coli* vaccines for human use, although some vaccines based on *E. coli* surface proteins exist for use in farm animals (Pokharel *et al.*, 2023). One of the major challenges in this field is the diversity of potentially pathogenic *E. coli*. There are more than 180 known O-antigen types and more than 80 known capsular polysaccharides that can be produced by *E. coli*. While some of these structures are common in pathogenic *E. coli*, others are more common in gut commensal (non-pathogenic) strains, or can be found in both. Additionally, *E. coli* cause a range of infections including superficial infections such as self-limiting gastroenteritis and lower urinary tract infections, but also pyelonephritis, sepsis and neonatal meningitis (Poolman & Wacker, 2016). The immune mechanisms targeting blood stream infections are well understood, but these infections are rare, compared to urinary tract infections that will affect one in three women over their lifetime. Advances in bacterial genomics now indicate that urinary tract infections, as well as invasive *E. coli* diseases, originate from *E. coli* that colonize the patient's gut lumen. Logically, targeting *E. coli* in the gut lumen, i.e. before spread to problematic locations occurs, requires secretory antibody responses that are not reliably induced by standard intramuscular injected vaccines, but should provide robust protection.

The Molecular Mucosal Immunology lab (Emma Slack's group) is interested in understanding how protective immunity against *E. coli* can be induced and can act in the gut. For *E. coli* and *Salmonella* strains that do not produce a capsule, secretory antibodies targeting the O-antigen are necessary and sufficient to generate protection (Moor *et al.*, 2017). Anti-O-antigen antibodies are induced in the gut either by prior exposure to the live bacterium or by swallowing high doses of killed bacteria. However, if a capsule is present, anti-O-antigen antibodies become obsolete, as the capsule forms a larger slimy layer over the O-antigen, blocking antibody binding (Figure 2). Interestingly, we completely fail to produce protective capsular polysaccharide-specific antibodies when we encounter whole bacteria. This problem is also known from *Streptococcus pneumoniae, Haemophilus influenzeae*, and *Neisseria meningitidis* (Berti & Adamo, 2013). Licensed vaccines against these species are "glycoconjugates". The capsular polysaccharides are purified and chemically linked to a carrier protein - typically an inactivated version of the tetanus or diphtheria toxin, which overcomes the poor antigenicity of capsular glycolipids. This permits induction of high-affinity antibodies. However, there is a catch. Neither tetanus toxoid nor diphtheria toxin are useful carrier proteins in oral vaccines due to rapid digestion. A challenge is to develop glycoconjugate carrier proteins that function in the gut lumen, for example based on highly protease-resistant virus-like particles.

From micro-scale to ecosystem biology

Antibodies in blood, for example IgG, can activate systems that kill bacteria, such as the complement system and neutrophils. However, the mechanisms of secretory antibodies in the gut are more subtle (Hockenberry *et al.*, 2023).

To understand this, it is important to realise that the human gut houses an incredibly dense and diverse ecosystem: the gut microbiota. Moreover, the human gut handles in the order of nine litres of fluid per day, such that there is continuous flow of fluid from mouth to sigmoid colon. Any microorganism that should colonise this environment long-term must grow at least fast enough to replace the bacteria lost in fluid flow, i.e. needs to compete for nutrients. In fact, the microbiota itself is a major part of defence against infection. This is referred to as "colonisation resistance". Several groups, including the Foster lab (recruited to the Dunn School in 2024), have shown that colonisation resistance can be strengthened by supplementing the microbiota with bacterial strains that strongly metabolically overlap with the targeted pathogen (Spragge *et al.*, 2023). This "nutrient blocking" approach can suppress the gut load of *Klebsiella* or *Salmonella* more than 100'000-fold.

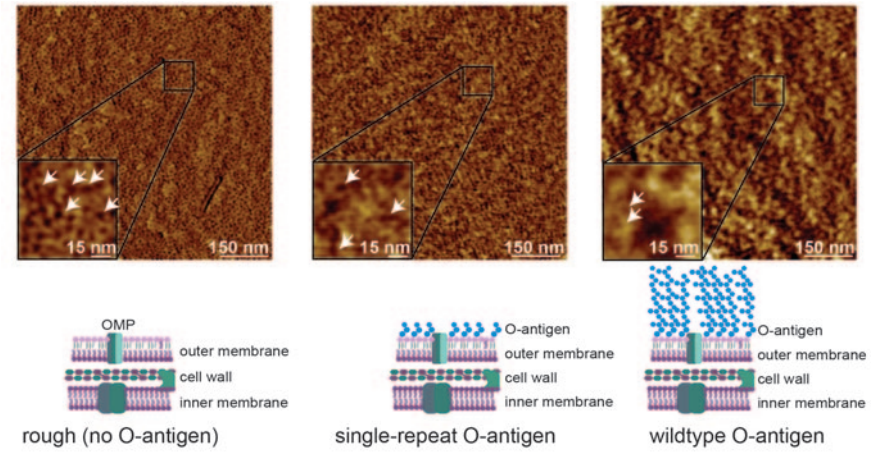


Figure 3: O-antigens form a dense carpet over the surface of most *Enterobacteriaceae*. Atomic force microscopy imaging, and diagrams, of the surface of *Salmonella enterica subspecies enterica* serovar Typhimurium with no O-antigen (left), a very short O-antigen (center) or a wildtype O-antigen (right). White arrows indicate visible outer membrane porins. Wildtype O-antigen forms a dense carpet that largely masks outer membrane porins. Figure modified from Diard *et al*, *Nature Microbiology* 2017.

Secretory antibodies in the gut have evolved in the continuous presence of both fluid flow and microbiota-driven colonisation resistance. These are dimeric antibodies, i.e. they have four identical binding sites. This multivalency makes secretory antibodies ideal for sticking things together, including targeted intestinal bacteria. This often happens as gut bacteria grow and divide, where even for low-abundance species, there will be two identical adjacent bacteria at the point of septation, allowing the antibodies to crosslink the two daughter cells (Moor *et al.*, 2017). Bacterial clumps, crosslinked by secretory antibodies, are then more efficiently cleared in the flow of gut content than planktonic cells. To maintain population size, a bacterium being cleared faster, would need to grow faster. There is therefore strong overlap between microbiota-driven colonisation resistance, which prevents growth of a pathogen and secretory-antibodies, which increase the pathogen's clearance rate. If we combine nutrient blocking with an oral vaccine, then we can decrease the abundance of a bacterium in the gut lumen from more than 1 billion bacteria per gram to effectively zero (Lentsch *et al.*, 2022). We coined this process "Vaccine-enhanced competition".

Vaccine-enhanced competition can therefore generate sterilising immunity in the gut lumen, eliminating a potentially pathogenic gut bacterium before it can invade tissues and cause disease. This also generates herd-immunity: i.e. overall reducing exposure of vulnerable individuals to potential pathogens, as well as decreasing the spread of antibiotic resistance genes to other pathogens via horizontal gene transfer (Diard *et al.*, 2017; Moor *et al.*, 2017).

Working across scales matters

If you want to fix something, it is extremely helpful to know how it really works. Between the groups of Georgia Isom, Emma Slack, and Kevin Foster, we can bridge from nano- to macro-scale in understanding how we can reverse the antibiotic resistance crisis. This requires diverse skills sets and expertise, as well as the ability to communicate efficiently and effectively between the fields of structural biology, immunology and microbial ecology. Embedding these activities within the strong translational environment of the wider Medical Sciences Division at Oxford puts us in a strong position to both advance our fundamental understanding of *E. coli* biology, and to translate our findings into the clinic.

References

Berti, F., & Adamo, R. (2013). Recent mechanistic insights on glycoconjugate vaccines and future perspectives. *ACS Chemical Biology*, 8(8), 1653–1663.

Botos, I., Majdalani, N., Mayclin, S. J., McCarthy, J. G., Lundquist, K., Wojtowicz, D., Barnard, T. J., Gumbart, J. C., & Buchanan, S. K. (2016). Structural and Functional Characterization of the LPS Transporter LptDE from Gram-Negative Pathogens. *Structure* , 24(6), 965–976.

Chng, S.-S., Gronenberg, L. S., & Kahne, D. (2010). Proteins required for lipopolysaccharide assembly in Escherichia coli form a transenvelope complex. *Biochemistry*, 49(22), 4565–4567.

Cooper, B.F., Clark, R., Kudhail, A., Bhabha, G., Ekiert, D. C., Khalid, S., & Isom, G. (2023).Phospholipid transport to the bacterial outer membrane through an envelope-spanning bridge. *Biorxiv*

Diard, M., Bakkeren, E., Cornuault, J. K., Moor, K., Hausmann, A., Sellin, M. E., Loverdo, C., Aertsen, A., Ackermann, M., De Paepe, M., Slack, E., & Hardt, W.-D. (2017). Inflammation boosts bacteriophage transfer between spp. *Science*, 355(6330), 1211–1215.

Douglass, M. V., McLean, A. B., & Trent, M. S. (2022). Absence of YhdP, TamB, and YdbH leads to defects in glycerophospholipid transport and cell morphology in Gram-negative bacteria. *PLoS Genetics*, 18(2), e1010096.

Grimm, J., Shi, H., Wang, W., Mitchell, A. M., Wingreen, N. S., Huang, K. C., & Silhavy, T. J. (2020). The inner membrane protein YhdP modulates the rate of anterograde phospholipid flow in. *Proceedings of the National Academy of Sciences of the United States of America*, 117(43), 26907–26914.

Hockenberry, A., Slack, E., & Stadtmueller, B. M. (2023). License to Clump: Secretory IgA Structure-Function Relationships Across Scales. *Annual Review of Microbiology*, 77, 645–668.

Kumar, S., & Ruiz, N. (2023). Bacterial AsmA-Like Proteins: Bridging the Gap in Intermembrane Phospholipid Transport. *Contact*, 6, 25152564231185931.

Lentsch, V., Woller, A., Moresi, C., Fattinger, S.A., Aslani, S., Hardt, W. D., Loverdo, C., Diard, M. & Slack, E. (2022). Combined oral vaccination with niche competition can generate sterilizing immunity against entero-pathogenic bacteria. *Biorxiv*

Moor, K., Diard, M., Sellin, M. E., Felmy, B., Wotzka, S. Y., Toska, A., Bakkeren, E., Arnoldini, M., Bansept, F., Co, A. D., Völler, T., Minola, A., Fernandez-Rodriguez, B., Agatic, G., Barbieri, S., Piccoli, L., Casiraghi, C., Corti, D., Lanzavecchia, A., ... Slack, E. (2017). High-avidity IgA protects the intestine by enchaining growing bacteria. *Nature*, 544(7651), 498–502.

Okuda, S., Sherman, D. J., Silhavy, T. J., Ruiz, N., & Kahne, D. (2016). Lipopolysaccharide transport and assembly at the outer membrane: the PEZ model. *Nature Reviews. Microbiology*, 14(6), 337–345.

Owens, T. W., Taylor, R. J., Pahil, K. S., Bertani, B. R., Ruiz, N., Kruse, A. C., & Kahne, D. (2019). Structural basis of unidirectional export of lipopolysaccharide to the cell surface. *Nature*, 567(7749), 550–553.

Pahil, K. S., Gilman, M. S. A., Baidin, V., Clairfeuille, T., Mattei, P., Bieniossek, C., Dey, F., Muri, D., Baettig, R., Lobritz, M., Bradley, K., Kruse, A. C., & Kahne, D. (2024). A new antibiotic traps lipopolysaccharide in its intermembrane transporter. *Nature*, 625(7995), 572–577.

Pokharel, P., Dhakal, S., & Dozois, C. M. (2023). The Diversity of Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *Microorganisms*, 11(2). https://doi.org/10.3390/microorganisms11020344

Poolman, J. T., & Wacker, M. (2016). Extraintestinal Pathogenic Escherichia coli, a Common Human Pathogen: Challenges for Vaccine Development and Progress in the Field. *The Journal of Infectious Diseases*, 213(1), 6–13.

Ruiz, N., Davis, R. M., & Kumar, S. (2021). YhdP, TamB, and YdbH Are Redundant but Essential for Growth and Lipid Homeostasis of the Gram-Negative Outer Membrane. *mBio*, 12(6), e0271421.

Spragge, F., Bakkeren, E., Jahn, M. T., B N Araujo, E., Pearson, C. F., Wang, X., Pankhurst, L., Cunrath, O., & Foster, K. R. (2023). Microbiome diversity protects against pathogens by nutrient blocking. *Science*, 382(6676), eadj3502.

Suits, M. D. L., Sperandeo, P., Dehò, G., Polissi, A., & Jia, Z. (2008). Novel structure of the conserved gram-negative lipopolysaccharide transport protein A and mutagenesis analysis. *Journal of Molecular Biology*, 380(3), 476–488.

Whitfield, C., Wear, S. S., & Sande, C. (2020). Assembly of Bacterial Capsular Polysaccharides and Exopolysaccharides. *Annual Review of Microbiology*, 74, 521–543.

Zampaloni, C., Mattei, P., Bleicher, K., Winther, L., Thäte, C., Bucher, C., Adam, J.-M., Alanine, A., Amrein, K. E., Baidin, V., Bieniossek, C., Bissantz, C., Boess, F., Cantrill, C., Clairfeuille, T., Dey, F., Di Giorgio, P., du Castel, P., Dylus, D., ... Bradley, K. A. (2024). A novel antibiotic class targeting the lipopolysaccharide transporter. *Nature*, 625(7995), 566–571.

An interview with Maria Leptin

Aleksandra Pluta

In this edition of Fusion, we talk to Prof Maria Leptin – the current president of the European Research Council, and Dunn School's Visiting Professor. Outside of her academic career as a developmental biologist and the head of two research groups at the University of Cologne and EMBL, Prof Leptin's experience ranges from working on journal editorial boards to multiple science leadership roles, such as being the head of EMBO in the years 2010-21. She is a Foreign Member of the Royal Society, an elected member of EMBO, of the Academia Europaea and the German National Academy of Sciences, an Honorary Fellow of the Academy of Medical Sciences, and an International Member of the National Academy of Sciences. In our interview, Prof Leptin offers some insights on current science topics, her perspective shaped by a diverse professional background spanning multiple countries and continents.

Source: EMBL Photolab

How did you find the move from being a group leader to doing science policy? What have been the highs and lows of your current position?

First of all, unless you really protect that, you're a pure group leader only at the very beginning of your research career. That changes as soon as you're in a university or a research institution. You get involved in the governance of the institute, faculty meetings, committee meetings, etc. – you get in touch with policy fairly soon. It distracts you from your work (that's the bad bit), but it also lets you do things, move your institution in different directions. I changed the graduate program in biology in Cologne, which was later picked up by other faculties, it clearly had an impact. It was fun too, because you get to do with a graduate school what you think is right and get to interact with students who also think it's right.

The first time that policy became a major part of my job was when I went to EMBO, which I knew from my previous work on committees and on the council. It was an interesting job and I also kept my lab. I said I wanted 30% protected time for research, and I got it. Most university professors will not receive much more than that. Now, unfortunately, I had to give up the lab. I still see people and we have several papers to write up, so I still get to talk and think about science. Science keeps you grounded – it's about the truth. There's nothing political there. There might be two ways of interpreting anything you see, but eventually the truth is only one way. Eventually, nature will tell you how things are and you can't see it any other way. It's the connection to this absolute truth that I miss.

Then, of course, there are the interactions with people in the lab, and the simple joy of seeing results and thinking about them. Recently I had a meeting with one of my postdocs, who still works on a project we started together ages ago. A year and a half ago she got results, which at the time seemed impossible. We just said: "It can't be. It doesn't work. There's no explanation for it..." It just didn't make any sense. She's continued working on it and just recently got some new stuff that makes it clear – what we saw was not a fluke. It was not something she'd done wrong nor the experiment behaving strangely. We found the explanation. Nothing else replaces that feeling.

Your job involves trying to convince European leaders to increase their science spending. What do you find can sway their opinion?

It does depend on who you talk to, but most of them agree and know that science is important. They realize that we need science to solve the problems that we are currently facing. And Europe, of course, is not a region packed with natural resources. Difficulty arises when you look at the roads that need to be built, food that needs to be grown, climate change that needs to be addressed very directly, immigration problems, political crises, etc. There are a lot of issues that have to be firmly dealt with. Additionally, European politicians have to bring direct financial benefit to their own countries. Science doesn't always sit in there very easily. Instead of going back to your constituency and saying "We're going to get money to build a hospital", you can say: "We're going to have more science." But there's no immediate reward for that.

At the same time, everybody sees that without the science we would not have had, for example, the Covid vaccine. And the only way we can deal with climate change is by using our knowledge of the natural world and the climate. I was talking to a scientist in Madrid, who was working on natural fluorine compounds that protect the climate by attacking methane. This was from long, long years of work. Importantly, it hadn't started

as a project connected to the climate – he was only excited about the science, he cared about the knowledge. People see and understand that. You can use these things to remind politicians that if we don't do the research that may not be of interest to them today, then we have no way of dealing with problems that may arise tomorrow.

Do you think scientists trying to get funding have to justify their research by tying it to current global issues?
I personally don't think it is a good idea. If you're interested in the science, you shouldn't say, for example: "I'm interested in cell shape changes in the Drosophila embryo. Understanding that might allow us to comprehend cancer cell migration." Maybe it will – but that's not why I'm doing it! I don't think we should lie. We can just say "it may or may not", but knowledge in itself is important. I've always told my lab: "if you write an abstract and end it with a sentence like that, I'll cross it out." And I do.

Why do you think certain journals are not affiliated with Review Commons? What could be the decisive factor in changing their mind?
At the beginning, Review Commons was very experimental. It's handled mostly by the editors of the EMBO journals who, of course, have a huge breadth, but they can't cover everything. For example, they're not as extensive in neuroscience as some of the neurobiology journals. It is important to note that some journals don't join Review Commons simply because so far it covers only some fields in the life sciences. So, reason number one: not sufficient expertise on the side of EMBO to cover in depth all the fields that some journals would like. To address this, Review Commons has recently teamed up with editors from Development and Journal of Cell Science, precisely to expand its editorial bandwidth. More journals may join forces according to this model, thus quickly expanding the editorial expertise to other fields – neuroscience, plant sciences, ecology, etc...

Additionally, journals have their own sets of editors and referees they like to use. If I'm an editor at a journal, and I know that I can trust Jackie S completely with her reviews, but Bill M is always really, really negative, I will know how to take their feedback. If I get feedback from reviewers that other editors have selected, I may not be able to read them as accurately. I find that some journals like to work with their own stable set of reviewers.
However, more and more journals are changing their minds – there are 28 which have joined Review Commons so far. I'm no longer very directly involved, but I am still on the board and I'm very curious to hear which other journals they've been recruiting. I think it's going well and seems to be well received by many in the community. I am hoping it'll expand further – but it doesn't need to be the only kid on the block.

Although the situation for women in senior science positions is slowly getting better, it is still far from ideal. What are your thoughts on making science more inclusive for working mothers?
Thirty years ago, many women in leadership positions in science didn't really have children, but if you look around, this is much less the case now. Further, men are also parents, and many of them take a lot of time to look after their children. I know relationships where the man is a stay-at-home father. It all exists, and it is not "women-only". Of course, the woman in the end does more, but we mustn't forget that it is hard for both parents. Ultimately, you can define your own way. I have a colleague who simply says "no" to many requests, and agrees to being available only at certain times. She says: "No, I have children. I do my work, I'm happy to be on committees, but I need to protect my time." I know many mothers – and fathers – who do that, and it has become generally acceptable.

Last week I travelled to and gave speeches in four different places. Science minister in Germany – female. Science minister in France – female. Science minister in Italy – female. Science minister in Spain – female. Today I had a delegation of Tuscan University rectors, everybody in the room was female. Head of UKRI, Head of the NAS, President of ERC, President of EMBO – all female. How many more role models do we need? There are so many examples... So if you want a career and a family, just press on. If you want to do it, it'll work. Yes, women may have it harder. Yes, they can't have it all. It might mean ten years with no cinema, no hairdresser, no new clothes, but I think it's manageable. It's not easy, but there are many things that aren't easy.

The hardest thing for women, isn't doing whatever is necessary to pursue a tough path – they can accomplish all that. What's truly difficult is hearing the pushback from their environment. In some cultures, combining work and motherhood is still not broadly accepted – that includes Germany, and especially West Germany. In the East, it was just far more normal for women to put their kids in day-care at the age of three months and go back to work. Going against the environment is not the easiest thing to do.

You have travelled around the world and worked in many institutions. During your journeys, was there a country (or an institute) that struck you as doing something visibly better for its science community or faculty members?
Between institutes, there are many differences. For example, some do more for women than others. Individual institutions can help their researchers to prepare better grants, or provide better infrastructure. Usually, these things are not distributed equally. There are huge disparities, and I can't think of a specific place where one could say "here's the shining example of an institute that does everything right." Institutes differ and they may not know, but they'll see the effects of that. You get what you set yourself up for.

Some of the differences are not institute-specific but rather country-specific. For example, career structures are very different in different countries. In some countries, institutions will have a big professor with a huge "empire". Everyone works under that professor, and even the junior independent groups aren't really that independent because they rely on the space

and the funds that the professor gives them. In other countries, if you're hired as an independent young group leader, you are just that – independent and a leader. Nobody else can tell you anything except the top-level management at the institution. These are obviously more attractive for pursuing an independent career. At the end of the day: young people vote with their feet.

You said "The value of the ERC is higher if the UK is in because the level of competition is higher – for both sides." How can we ensure that research institutions across Europe get equal funding opportunities? How can institutes from less-developed countries compete for funding with, e.g. the UK?

It does tie in with the previous question. First of all, these countries need good scientists and researchers. And in order to get them, they have to provide an environment that is attractive for those people to do their work. Imagine you get an offer from a university that gives you your own space, that helps you with administration and housing, where there is a clear career structure and good infrastructure... On the other hand, you can get an offer where someone says: "Here's a small room for when you're here, we'll see if we can find you a microscope." You know where you will go.

How can one help? The institutions themselves have to make themselves attractive. The countries have to give their institutions the means – and that's usually money – to be attractive, but ultimately, it's the universities or research institutions that make these changes. Then people will come and when the institutions have those really good people, they should help them. They should get them coaching, give them freedom, allow them to take time off teaching in order to prepare grants, and provide rewards for people who were ranked as excellent but could not be funded. And some institutions and countries do that. The positive outcomes might not be immediate, but with time one sees the benefits of that approach.

Would you agree that there is a problem with science not having a clear career progression system?

There is no one specific career path for scientists. For example, in some disciplines, there are temporary positions for five, nine years for starting group leaders. They often come with huge benefits, like very good salaries, very good infrastructure, all the perks I mentioned above. That's why they're there – to allow people to really just get on with their work with maximum help and minimum fuss about anything else. That's why this system is possible. There might be a feeling of unfairness that one has to leave after nine years, but in return, there is the freedom and the support. Then there are the slightly stonier paths at less well funded institutions, but you may get security sooner there. There is also a limit to how many senior people a system can accommodate. We cannot train people as PhDs while pretending they can or should all become PIs. It has to be clear that there is a relatively shallow pyramid leading up. I do think it's wonderful to train people in how to do research. But we, and they, have to be okay with them doing something other with their degree than become a PI. I think many are in fact seeing their PhD training in this way now – a majority of PhDs, even in top institutions, say that they're doing a PhD with no aim of becoming a PI, and have other plans for their future.

Of course there is precarity, but unfortunately, I don't see a good solution. Giving everyone a permanent position early on would be a quick fix, but then you'd have to have a much tougher competition early on. If people want permanent positions at the postdoc level, then there will be very few postdocs that can be funded. You have to balance these two against each other. It's a simple fact – if everybody becomes permanent, then that "everybody" has to be very few people. It just doesn't work otherwise.

What advice would you give someone looking to venture into science policy?

While working with institutions like the Wellcome Trust, here at the ERC or at EMBO, I met some great people – the editors, the programme managers, analysts, policy advisers, etc. The fact that they have a science background is unbelievably positive. Having been trained with a research background translates into an open way of communicating, understanding each other, appreciating where we're all coming from. Importantly, I think they enjoy their work outside academia a lot. There are problems with all jobs, but anybody who wants to try science policy should go for it. It's worth doing, and you can apply for internships in these places. I know many who did and were very happy.

Optimising immune cell recognition of cancer cells

Omer Dushek and P. Anton van der Merwe

MatchBio is a new immunotherapy spinout from the Dunn School

White blood cells such as T cells continuously patrol the body in search of abnormal cells, such as infected or cancerous cells. T cells detect molecules called 'antigens' on the surface of abnormal cells using their T cell antigen receptors (TCRs). T cells are remarkably sensitive: they can become activated by the presence of a single antigen on a cell[1]. This sensitivity is important because infectious organisms and cancer cells are very good at hiding from T cells by lowering the amount of antigen on the cell surface[2].

An exciting new treatment for cancer is to redirect T cells to target a patient's cancer cells[3]. This is done by using genetic engineering to express chimeric antigen receptors (CARs) on T cells (Figure 1). CARs have an extracellular part that binds a target antigen on the cancer cell and an intracellular part derived from the TCR that send an activating signal into the T cell. The antigens that CARs target on cancer cells cannot normally be recognised by TCRs, which is why CARs effectively re-direct and boost a patient's T cells to kill cancer cells. This therapy is very effective for targeting antigens expressed at high levels on leukaemias and lymphomas. However, many patients relapse when cancer cells emerge that have lower levels of antigen on their surface[4]. One reason why this escape is possible is that CARs are not very sensitive and so are unable to 'see' these new cancer cells. There is an urgent need to increase the sensitivity of CARs to prevent these relapses. More sensitive CARs would also allow CAR T cells to be used in treating a wider variety of cancer antigens that are expressed at low levels.

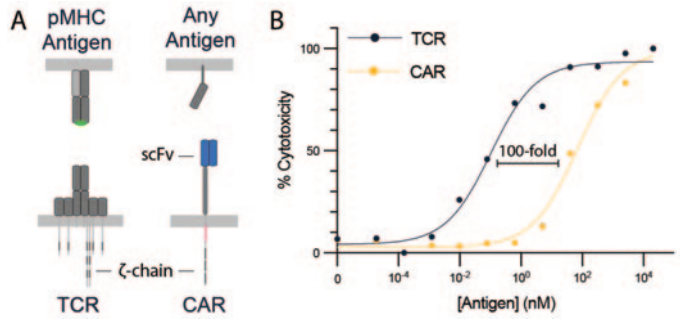


Figure 1: CAR-T cells have a major defect in antigen sensitivity.
A) The native T cell receptor (TCR) recognises peptide antigens presented on major-histocompatibility-complexes (pMHC). CARs are synthetic antigen receptors that can potentially recognise any surface antigen. This is achieved by fusing a single-chain variable fragments (scFv) of an antibody to an intracellular signalling chain from the TCR.
B) T cells require >100-fold more antigen to kill a target cell through a synthetic CAR compared to their native TCR. Data adapted from Burton *et al* [5].

T cells achieve high sensitivity partly by using 'accessory' receptors on their cell surface, which bind to ligands on the surface of cells. We therefore investigated whether CARs can exploit these accessory receptors to improve sensitivity and specificity of antigen recognition[5]. We showed that engagement of the T cell accessory receptors CD2 and LFA-1 with their ligands contributes to the ability of T cells to recognise low levels of antigen using the native TCR. In contrast, these accessory receptors are unable to enhance the sensitivity of CARs to antigens, providing an explanation for the low sensitivity of CARs (Figure 2). Based on our understanding of the molecular mechanism by which accessory receptors modulate antigen recognition, we have engineered new synthetic accessory receptors that are more effective at enhancing the sensitivity of CARs.

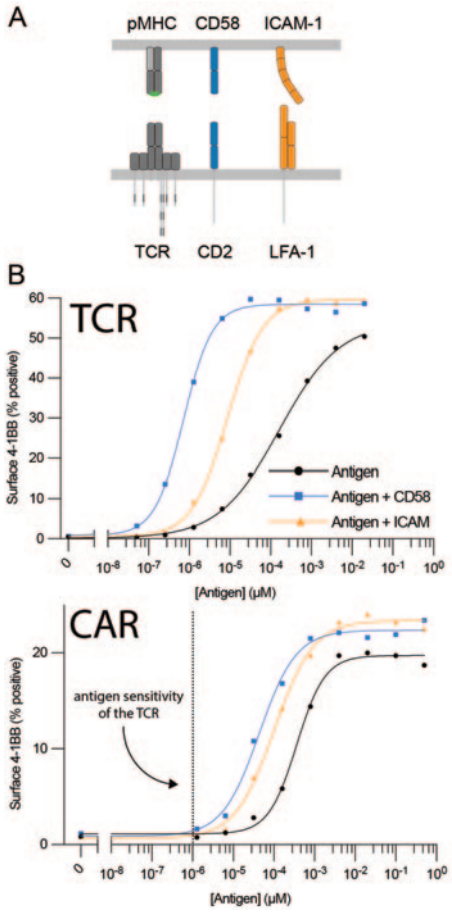


Figure 2: Inefficient exploitation of accessory receptors reduces the sensitivity of CAR-T cells.
A) Schematic of the T cell surface in contact with an antigen presenting cell showing the accessory receptor CD2 and LFA-1 with their ligands CD58 and ICAM-1, respectively.
B) T cell activation at increasing concentrations of antigen when T cells recognise antigen by their native TCR (top) or a synthetic CAR (bottom). Data adapted from Patel *et al* [6].

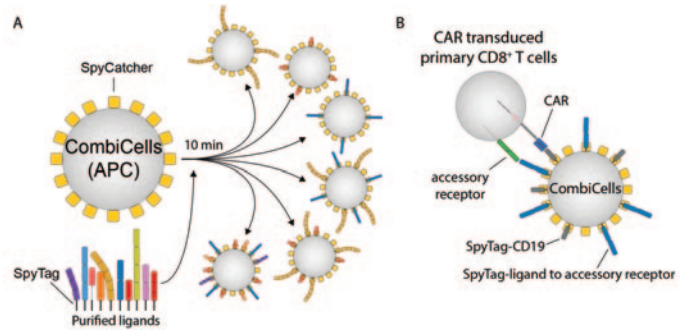


Figure 3: A platform for the combinatorial display and titrations of ligands directly on the cell surface (CombiCells).
A) Expression of Spycatcher on the cell surface enables the covalent coupling of purified extracellular domain of proteins fused to Spytag. Cells expressing any combination and concentration of ligands can be generated within minutes.
B) CAR-T cell antigen sensitivity, and its dependence on accessory receptor ligands, can readily be performed by titrating ligands and antigens, such as CD19, directly on the target cell surface. Adapted from Patel *et al*[6].

Performing the experiments described above required a method to precisely manipulate accessory receptor ligands and antigens on cell surfaces, something which is notoriously difficult to do. To solve this problem, we exploited an existing split-protein technology developed in Oxford by Mark Howarth, called SpyTag/SpyCatcher (Figure 3). We first expressed a chimeric protein which presents SpyCatcher on the cell surface, producing what we call CombiCells. SpyCatcher forms a spontaneous covalent bond with the SpyTag peptide. By fusing SpyTag to the extracellular domain of any protein of interest, we can now use CombiCells to easily produce large panels of cells presenting any antigens and accessory receptor ligands at different combinations and surface densities[6]

We are also developing methods to improve the specificity of CAR T cells, taking advantage of our insights into signal integration between the TCR and other receptors. To exploit these technologies for optimising CAR-T cell sensitivity and specificity, we have founded a spinout company, MatchBio Ltd. While improved CARs will transform the treatment of cancer, they may also prove useful for treating intractable infections and autoimmune disease.

CAR T cells are likely to be the first of many examples where immune cells are modified to benefit human health. Our ongoing research programme on immune recognition will help us improve immune cell function.

The work has been funded by the Wellcome Trust, the Medical Research Council, and through a Guy Newton Translational Grant. The authors thank past and present members of their laboratories.

Conflict of interest: Omer Dushek and P. Anton van der Merwe are founders and consultants to MatchBio Ltd. Omer Dushek is a director of MatchBio Ltd. Omer Dushek and P. Anton van der Merwe have financial interest in patents describing CombiCells and methods for improving CAR function.

References

[1] Huang J, Brameshuber M, Zeng X, Xie J, Li QJ, Chien YH, Valitutti S, Davis MM. A single peptide-major histocompatibility complex ligand triggers digital cytokine secretion in CD4(+) T cells. Immunity. 2013 Nov 14;39(5):846-57. doi: 10.1016/j.immuni.2013.08.036. Epub 2013 Oct 10. PMID: 24120362; PMCID: PMC3846396.
[2] Siller-Farfán JA, Dushek O. Molecular mechanisms of T cell sensitivity to antigen. Immunol Rev. 2018 Sep;285(1):194-205. doi: 10.1111/imr.12690. PMID: 30129204.
[3] Labanieh L, Mackall CL. CAR immune cells: design principles, resistance and the next generation. Nature. 2023 Feb;614(7949):635-648. doi: 10.1038/s41586-023-05707-3. Epub 2023 Feb 22. Erratum in: Nature. 2023 Jul;619(7968):E26. PMID: 36813894.
[4] Majzner RG, Mackall CL. Tumor Antigen Escape from CAR T-cell Therapy. Cancer Discov. 2018 Oct;8(10):1219-1226. doi: 10.1158/2159-8290.CD-18-0442. Epub 2018 Aug 22. PMID: 30135176.
[5] Burton J, Siller-Farfán JA, Pettmann J, Salzer B, Kutuzov M, van der Merwe PA, Dushek O. Inefficient exploitation of accessory receptors reduces the sensitivity of chimeric antigen receptors. Proc Natl Acad Sci U S A. 2023 Jan 10;120(2):e2216352120. doi: 10.1073/pnas.2216352120. Epub 2023 Jan 4. PMID: 36598945; PMCID: PMC9926289.
[6] Patel A, Andre V, Eguiguren SB, Barton MI, Burton J, Denham EM, Pettmann J, Mørch AM, Kutuzov MA, Siller-Farfán JA, Dustin ML, van der Merwe PA, Dushek O. Using CombiCells, a platform for titration and combinatorial display of cell surface ligands, to study T-cell antigen sensitivity modulation by accessory receptors. EMBO J. 2024 Jan;43(1):132-150. doi: 10.1038/s44318-023-00012-1. Epub 2023 Dec 18. PMID: 38177315; PMCID: PMC10897201.

Building a more equitable, diverse and inclusive Dunn School community

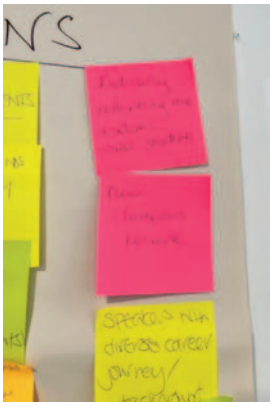
Emma Slack and Jordan Raff

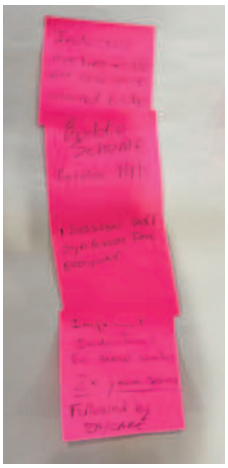
Since October 2023 Professors Emma Slack and Jordan Raff have co-chaired the Dunn School's EDI Committee. In this article, they discuss why Equality, Diversity and Inclusion (EDI) is important, and describe recent efforts to develop a new departmental EDI strategy that reflects everyone's views and contributions.

Like many national and international organisations, the University of Oxford has started to take issues of Equality, Diversity and Inclusion (EDI) much more seriously over the past few years, with a plethora of new committees at University, Divisional and Departmental levels tasked with developing and implementing EDI-relevant strategies. Sadly, such EDI initiatives have recently become something of a politically charged "culture wars" issue, with some claiming that organisations are not taking these issues seriously enough (and that any attempts to do so are merely window-dressing or box-ticking), and others believing that all such efforts are a waste of time and money (at the time of writing, the UK Government's "Common Sense" minister, Esther McVey had just announced a ban on jobs dedicated to EDI in the Civil Service[1]).

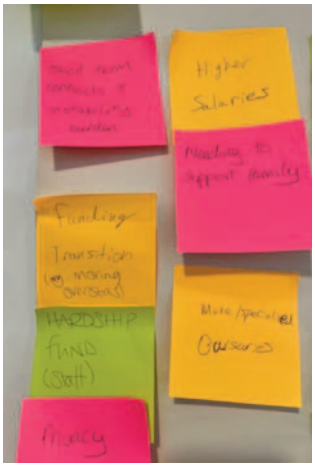
Whatever your views on this subject, there is a substantial body of evidence that having a diverse and inclusive culture is beneficial to any organisation, leading to increased creativity and innovation[2], improved problem solving[3], and many other tangible benefits. If we take a look around, it is pretty clear that the Dunn School, and the University of Oxford more generally, does not currently have such a culture. While there are no easy-fixes to this problem, The Dunn School has decided that it is worth establishing an EDI Committee (EDIC) to explore ways in which we can try to improve this situation. As the current Co-Chairs of the EDIC, we are writing this piece to explain how the EDIC was set up, how it currently operates, and how it has developed a strategic plan that can be viewed by all staff and students on the Dunn School Intranet.

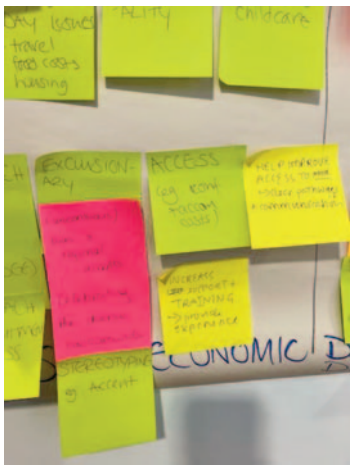
An EDI working group was established in September 2022 under the joint-leadership of Fumiko Esashi and Quentin Sattentau to identify EDI issues that were relevant to the Dunn School, and to formulate a plan for dealing with them. The working group concluded that an EDIC should be convened with overall responsibility for anything EDI-related. This meant that several existing committees (e.g. Athena SWAN, bullying and harassment) would become incorporated as sub-committees of the EDIC. Unusually, it was agreed that although the EDIC would have two Co-Chairs, *all Dunn School staff and students* would be welcome to attend any EDIC meetings to hear what is being discussed and to directly raise any issues of concern. The full minutes of all meetings are available to all staff and students. The EDIC was established in the summer of 2023, and October 2023, we took over as Co-Chairs (although both Fumiko and Quentin remain heavily involved). The committee's work is very effectively supported by the EDI specialist Louise Cotterell, as well as by representatives of the Graduate Student Association, Postdoc and Research Staff association, Professional and Support Staff (PSS) and many volunteers from across the department.





It was important that, when deciding what to focus on going forwards, we maintained this inclusive and open approach. The EDIC organised an open "Strategy Consultation" that took place in the Combination Room in early March 2024. The aim of this meeting was to clarify the priorities that were important to all staff and students, and to discuss effective strategies that the EDIC might employ to tackle these issues. The results from the University-wide Staff





Experience Survey (SES) were used to identify many of the key areas for discussion, which fell into six broad categories: (1) Childcare and Caring Responsibilities; (2) Communication of EDI issues; (3) EDI in Funding, Hiring and Management; (4) Mentoring and Sponsorship; (5) Culture of Inclusivity; (5) Addressing Socioeconomic Discrimination. The meeting exceeded all our expectations. It was incredibly impressive that more than 50 Dunn School staff and students (from all parts of the department) were willing to give up an afternoon to discuss these issues, and the quality of the suggestions that emerged provided a fantastic resource that has ultimately led to the formulation of several concrete aims, which span from increasing inclusivity in social events through to establishing a regular departmental induction day for all staff. A big thank-you to all who took part and, for anyone interested, we have retained a master document that captures all the major points that emerged from these discussions. This is also available to all staff and students on the Intranet, and will serve as an important future reference; even if a particular point did not make it into the final top priorities, it will not be forgotten.

From the outset, the committee decided to focus on priority areas where there was a realistic chance that we (and of course ultimately the Dunn School/University more widely) could make a difference, and we will try to identify specific ways to measure progress (often a very difficult task in this area). The staff survey remains a major benchmark, but for areas not covered (currently, for example, there is no in-depth, regular survey of graduate students, and very little data for some historically underrepresented groups), we will aim to carry out our own internal data collection. We encourage all Dunn School staff and students to have a look at the strategic plan, and to feel free to point out anything you think is wrong, or any issues you think it neglects. We certainly view the plan as a "living document" that will constantly be reviewed, amended and updated over time.

We also note here that some of our activities are, to some extent, experiments. For example, we will pilot a "get to know your Dunn School" lunch event, where we aim to get people talking across the traditional divides of research groups, seniority levels, academic/support staff etc. We hope that this sharing of ideas and experiences across barriers will further foster the collegiality that is at the core of what makes the Dunn School a special place to work and learn. We also want to explore ways to better reward "beyond the call of duty" work, for example in outreach or in fostering department culture. In trying these things, we recognise the possibility that not everything will work out, and that these kinds of interventions, no matter how well intentioned, may be less effective than hoped. By keeping our activities under review, we will aim to keep our energies focused on the most effective actions. As Co-Chairs of the EDIC, we passionately believe that the best research and teaching will happen when there are no barriers, when everyone feels that their contributions are valued, and where we operate within a culture of mutual respect. We believe that the strategies outlined have a good chance of contributing to achieve this atmosphere. We hope you will join us as we collectively attempt to make the Dunn School an ever more equitable, diverse and inclusive working environment.

References

[1] **Wastefull Whitehall diversity and inclusion spending will end**. Esther Mcvey, *Daily Telegraph*, 11th May 2024.

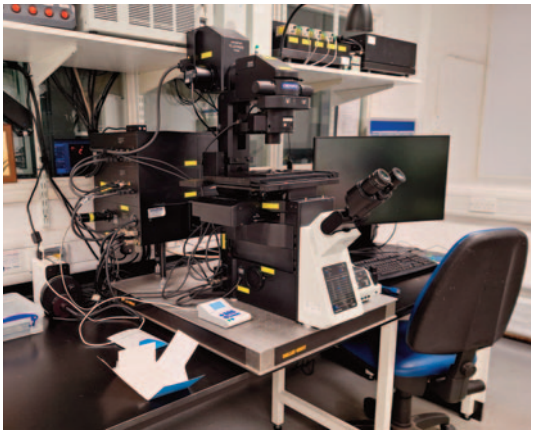
[2] **Diversity in Innovation**. Paul Gompers and Sophie Wang, *Harvard Business School Publications*, 2017.

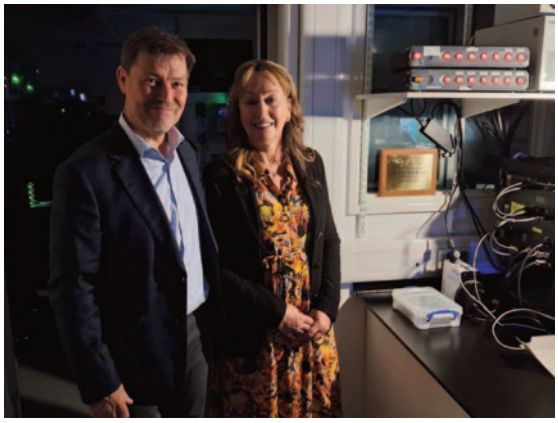
[3] **Groups of diverse problem solvers can outperform groups of high-ability problem solvers**. Lu Hong, and Scott Page, *PNAS*, 2004.

Generous gift allows upgrading of Dunn School microscope

Catarina Vicente

Dunn School research relies on state of the art scientific equipment, which is provided via our several scientific facilities. The Dunn School Biomaging Facility is unique in Oxford in offering both light and electron microscopy, which allows our researchers to image across a wide range of scales, from the whole organism, down to individual cells and even proteins. We are extremely thankful to Sarah and Nigel McLean, whose kind gift allowed the upgrading of our Olympus FV1200 confocal microscope. This is one of the most popular microscopes in our facility, but was nearly 10 years old, with outdated technology no longer supported by the manufacturer. There was a real risk that ongoing research would be disrupted if a fault arose. The McLeans' generous gift allowed us to acquire a new state of the art microscope system that is already in use in the facility, catalyzing a wide range of research projects.





We were very honoured to host a recent visit by Sarah and Nigel, who had the opportunity to see their microscope in action. They also met our Head of Department, as well as chatting with Prof Jordan Raff (Academic lead of the bioimaging facility) and with PhD student Annie Shaw about the impact of their gift.

Making a gift to the Dunn School

The Dunn School owes its existence to a philanthropic gift, and over the years has been the beneficiary of many acts of philanthropy, not least from those who have worked and studied here. Any gift made to the Dunn School helps to further research here, whether it is made to support a specific initiative, such as our centenary goal to endow graduate studentships, or at the discretion of the Head of Department. If you would like to make a gift to the Department this year, please get in touch with us at alumni@path.ox.ac.uk.

You will have the option to select gift aid so that we can reclaim tax on your gift, and if you are a higher rate tax-payer, you can also set your gift against your tax liability for the year. All gifts made to the Dunn School from the USA are also fully tax-deductible, when made through the University's 'giving vehicle' there, the Americans for Oxford, Inc organization.

Leaving a legacy

If you are considering leaving a gift to the Dunn School in your will, we will be pleased to have an informal chat with you, providing any guidance you may need and the wording to share with your solicitor or family members. A will is very personal, and we quite understand if you prefer to keep your intentions private. However, if you do wish to let us know about a gift in your will to the Dunn School, we will be delighted to be able to thank you personally.

From Dunn School student to High Court judge

Jo Peel

Reflections on the inspiring journey of Omphemetse Mooki

In a career journey that transcends boundaries, Omphemetse Mooki's story stands as a testament to the limitless possibilities that determination and a pursuit of excellence can achieve. An alumnus of William James' lab at the Dunn School of Pathology his remarkable academic and professional trajectory to High Court Judge in South Africa, is truly inspiring.

Born in South Africa on 17 August 1968, Omphemetse's academic journey began at Stanford University, where he was awarded a University Scholarship to pursue a Bachelor of Science degree in Immunology and Microbiology. This solid foundation in the sciences provided him with the springboard to venture into more challenging domains, setting the stage for the transformative path he would later tread. His tenure at Stanford was further enriched when he was awarded the prestigious Rhodes Scholarship, an acknowledgment of his intellectual prowess and commitment to his chosen field of study.

As part of his academic odyssey, Omphemetse joined the Dunn School as an MSc student in William James' lab in Michaelmas 1993. Here, he engaged in ground-breaking research focusing on blocks to HIV infection in murine cells.

The transition from the scientific world to the realm of law is a remarkable facet of Omphemetse's journey. His passion for the legal profession led him to the University of Cape Town, where he obtained a Bachelor of Laws degree. This paved the way for a career as a legal professional, culminating in his call to the Bar in 2003 and the subsequent award of Letters Patent in 2018, formally recognising him as Senior Counsel.

Before stepping into the courtroom as a barrister, Omphemetse honed his skills as a commercial attorney with the prestigious international law firm of White & Case LLP. His extensive experience also includes a role as a Senior Legal Advisor for one of South Africa's major local banks.

Today, Omphemetse's legal expertise spans a diverse range of practice areas, from commercial law and competition law to public law, medical negligence disputes, and labour law. He appears in various courts in South Africa, including the Competition Tribunal, the Competition Appeal Court, the High Court, Labour Court, Labour Appeal Court, Supreme Court of Appeal, and the Constitutional Court. Omphemetse reached the pinnacle of his legal career by being formally appointed a High Court Judge in November 2023.

Furthermore, Omphemetse's contributions to legal scholarship are substantial. Several of his articles and scholarly works have been cited in publications.

Commenting on the transferable skills from his scientific to legal career, Omphemetse said:

"*I am often asked about the transition from being a scientist to practising law, which some consider "radical." This has puzzled me. The sciences, fundamentally, teach a habit of mind: how to go about making sense of information. This includes the ability to anticipate issues that have not been addressed to-date. I apply the same mode in the practise of the law.*"

Omphemetse Mooki's journey serves as a shining example of what dedication and a pursuit of excellence can accomplish, and it is a source of inspiration for all of us at the Dunn School of Pathology and beyond.

A launch pad for success: the Dunn School postdoc programme

Catarina Vicente and Matthew Freeman

At the Dunn School, we are very proud of our postdocs and research staff. They lead much of our research output, and many are future stars. Despite their importance to science, elsewhere postdocs are often a silent and rather overlooked majority. Not in the Dunn School, where they have an active and central voice in the life of the department. Indeed, we were one of the first departments in Oxford to introduce a postdoc association, and we benefit enormously from having an engaged, enthusiastic and driven postdoc community (now called the Postdoc and Research Staff Association, PRSA, to encompass research staff more widely).

It is worthwhile comparing the situation for postdocs with that for graduate students. There are obviously parallels between the two communities, both of which are integral to the Dunn School's research mission, but there are also significant differences. Unlike with students there is a formal employment relationship between postdocs and the University. In addition, postdocs represent a much more heterogeneous group, ranging from recent PhDs who are aiming to do a relatively short postdoctoral fellowship as a stepping stone to an independent research career, to highly experienced scientists with other career plans. They also range very widely in age and life stage. Finally, postdocs are recruited continually and do not form an annual cohort, of the kind that provides an automatic peer community to graduate students.

Regardless of these complexities, we are committed to providing a best-in-Oxford experience for our postdocs, both while they are here and in supporting their future career goals. In this context, we decided that, in addition to the recognition of their importance through the PRSA, we should explore what more we can do for them.

To address this, we recently undertook a consultative exercise to map all the support, opportunities and events that we provide to our postdocs and research staff. Our main goals were to create both a useful guide for what is available, but also identify any obvious gaps for improvement. This led us to realise that we do already provide excellent support but we are not very good at articulating what we do, and it could of course be improved.

To fix this, we have developed a new structured postdoc programme, outlined in Box 1, overleaf. Many of its elements have been available for some time. For example, we run a successful and much appreciated mentoring scheme, through which postdocs are paired with both a senior academic and peers at similar career stages. Teaching opportunities, both in practicals, lecturing and tutorial settings, have also always been available, albeit not always well advertised.

But we are also introducing new initiatives. One area where we realised we could do better is supporting the transition to independence. We are establishing two new initiatives to support our postdocs and research staff keen to develop their career in the direction of applying for independent PI positions. New 'Pathway to Independence' grants aim to provide modest but valuable research funding. These pump-priming grants (up to £10k) provide resources to acquire preliminary data and develop independent ideas, as well as help build a grant portfolio, all of which are essential when applying for future funding and faculty positions. Established on a temporary basis as part of our post-COVID career support, after two very successful rounds, we are pleased to announce that this is now a permanent annual scheme.

Leadership skills are also important for those transitioning to more senior roles of any kind. We are therefore developing a leadership skills programme, which will be available to all in the Dunn School, but primarily focused on postdocs and research staff. We are keen to take suggestions on the types of sessions we should include, and would welcome ideas from both present and past members of the department.

Our overall message is that the Dunn School takes very seriously its responsibilities to our postdoctoral and research staff. We also recognise that warm words about how much we value them, and wanting to be leaders in this regard, are not sufficient: we need to think hard about how we can do better. Ultimately, we want to provide an exceptional programme of support and opportunities that will allow all our research staff to launch the next step in their career, whether that is in academia or elsewhere. The new Dunn School postdoc programme is a significant step towards that goal.

BOX 1: The Dunn School postdoc programme

The Dunn School postdoc programme highlights our commitment to providing a launching pad for the careers of our postdocs and research staff. Of course, the epicentre of a postdoc's research life is the group that they join and their supervisor. But the Department can add great value, reflecting our vision that everyone who works or studies here feels as much a member of the Dunn School, as of their individual group. We encourage all our postdocs and research staff to consider taking advantage of these opportunities, in discussion with their supervisor and mentors.

Transition to independence

Funding success is particularly important at the transition to independence stage. To help our postdocs build their funding portfolio, we run regular workshops on how to apply for funding and explain the various routes to independence; provide comprehensive financial and administrative support for applications, as well as proposal feedback and mock interviews provided by more senior members of the department; and provide a regular email roundup to summarise upcoming internal and external funding opportunities.We also offer our own internal pump-priming grants ('Dunn School Pathway to Independence grants') of up to £10k, allowing postdocs the funds to develop independent ideas and acquire preliminary data.

Leadership development

We are developing a leadership skills training programme, open to all, to provide training in a range of skills required for those moving to more senior positions. We also provide a variety of opportunities to gain experience of positions of influence and to develop citizenship and leadership skills, particularly through membership of the PRSA committee, and the opportunity to organise our annual postdoc symposium. Postdocs are also encouraged to chair departmental symposia sessions, our internal progress seminars, and to host external speakers.

Teaching Experience

Teaching is not required or expected from our postdocs, but it can be useful to gain experience and enhance CVs. There are many opportunities as part of departmental and university teaching:

- Mentoring and co-supervising of DPhil, masters and undergraduate students
- Contributing to practical classes and lectures
- Access to tutorial teaching experience

Mentoring

Primary mentorship is the role of a researcher's group leader. In addition, all postdocs and research staff are encouraged to join our departmental-wide mentoring scheme, which includes small group peer- and PI-led mentoring. Each mentoring cycle runs for 12 months, allowing for several mentoring experiences over a Dunn School career.

Training

All postdocs and research staff have the right to 10 days of leave a year for training and career development. As employees of the University of Oxford, they have access to a very wide range of courses and training sessions, some organised at departmental level, others in the central University. As a department, the Dunn School enhances these opportunities by making available grants to support attendance of external courses not available locally (Guy Newton Training grants).

Writing, Presenting and Communicating

These are core skills that underpin success in any professional career track and we offer many ways of gaining experience. Presentation skills, both posters and talks, are developed as part of progress seminars and the annual postdoc symposium. Joining our news team is a great opportunity to improve both scientific and public-facing writing, and we occasionally host writing workshops with author and science journalist Roger Highfield (a Dunn School visiting professor). Public engagement is also encouraged, and there are opportunities for projects in the local community (e.g. through local schools) as well as internal grants to support new initiatives.

Dunn School community

We foster an open door, consultative and collegiate culture. Social events help foster this environment, from regular coffee breaks organised by the PRSA to the departmental monthly Dunn Drinks, Christmas and summer parties, as well as opportunity for refreshments and informal networking after our seminars. The 'Get to know you' discussion series provides and informal and candid environment to hear from more senior colleagues on their career trajectory and experience. We also place real emphasis on providing a family-friendly environment. Grants are available for caring support, for example to help attend scientific conferences or other career development opportunities. In addition, the Dunn School provides all staff with a range of other measures (work flexibility, core hours for meetings, access to university nurseries, etc) aimed at helping the complex balance between professional and home life.

Careers, inside and outside academia

The Dunn School aims to provide a launch pad for the careers of all our research staff, whether or not they want to pursue an academic route. Beyond the wide-ranging opportunities for academic mentorship, and the University's Careers Service, we host seminars with external speakers including, very often, former Dunn School members, who have pursued different career paths. Our departmental mentoring scheme also includes mentoring opportunities for those considering careers outside academia. In addition, and extending the theme that we are willing to provide targeted financial support to support career development, Guy Newton Research Fund personal grants are available for career development opportunities.

Beyond the Brick Walls: An Encounter with Oxford's Medical History through Portraits

Ashley Younger

In the previous issue of Fusion we introduced our ongoing project to digitize and catalogue our growing collection of portraits. In this issue, Dr Ashley Younger (currently a second year MSc student in Women's and Reproductive Health) shares her experience of working on this project over the last year.

Taking a sharp left onto Sherrington Road, I navigate the labyrinth of alleys threading through the science buildings. Passing the imposing liquid nitrogen canisters, which create small mountains of ice, I turn right onto Sherard Road and find myself in front of the Sir William Dunn School of Pathology. It's astonishing to think that I can simply stroll up the left side of the external staircase to the main door (in the 1920s, a privilege only granted to the University's first professor of Pathology, Professor Dreyer), where my Bod card magically grants me access to this hall of medical history. The walls across all three floors of the main building, as well as in the EPA building, are lined with photographic portraits, all framed in similar rectangular black frames. These portraits date back to the 1930s and continue to include incoming DPhil students.

I first learned about the Dunn School portraits from Catarina Vicente, the Head of Scientific Strategy and Projects, during a Reuben College dinner. She mentioned that this treasure trove of undigitized history needed to be cataloged before the centenary celebration of the Dunn School. When she asked for help to build the digital archive, I jumped at the chance to be involved. Arriving in Oxford as a postgraduate can often make you feel like an outsider, confined to your department or lab, with the mysteries of other buildings hidden behind their facades. Opportunity to not only enter into such an important place in Oxford's scientific community, but to contribute, was a real gift in feeling included in preserving history.

Over the past year, while working on the portraits project, my daughter contracted scarlet fever due to a Group A streptococcus infection. She was treated with penicillin and improved drastically within hours of the first dose. In the early twentieth century, before antibiotics, scarlet fever was a leading cause of death in children, and those who survived often faced long-term complications such as rheumatic fever and arthritis. When I walked into the Dunn School that week to work on another set of portraits, I was overwhelmed with gratitude for the discoveries made in this building, discoveries that still directly impact my family today. I imagined Florey and his colleagues rubbing mould spores into their clothes in case they had to abandon their work during a World War II invasion. I pictured researchers biking through University Parks with penicillin for the Radcliffe Infirmary, then riding back to the Dunn School with collected urine to extract any unused medicine. I expressed my gratitude to the "Penicillin Girls," who, despite not being featured on the wall of portraits, played a crucial role in advancing the research.

Carefully, I removed the portraits from the wall one by one, cleaned their dusty frames, logged information into a database, escorted them to the Bodleian for digitizing, and finally returned them to their rightful place of honor. This work granted me access to a space I would not normally have entered and instilled in me a profound sense of reverence for those who came before. Balancing my own research, I often worked on the portrait project late into the evening. As I closed up the small office, which had once been part of the men's cloak rooms, I would walk through the halls as daylight faded, sensing the presence of those who had worked there in the past.

After a year of archiving, I am passing the baton to Tadhg Goodison, who will continue the preservation work and further illuminate the portraits with his expertise as a historian. I would like to thank Sarah Barefield, Nick Cistone, Philip Cobden, Martin Smith, Wayne Swan, the café staff and the Raff lab. A special thank you to Cat, an incredible mentor who enabled me to create my own history in the Dunn School. If you have the opportunity, I recommend reading "The Mould in Dr. Florey's Coat" by Eric Lax. And next time you pass the portraits, take a moment to remember who walked these very same hallways.

Philanthropy supporting the Dunn School community

Catarina Vicente

The Dunn School is very fortunate to benefit from the generosity of two research funds established in 1967: The Guy Newton Research Fund and the Edward Penley Abraham Research Fund. The history of these two funds is described in some detail in Fusion 17 (2018). Briefly, while penicillin was never patented by the Dunn School, by the time Newton and Abraham had developed cephalosporins (to this day, the most widely used antibiotics world-wide) lessons had been learnt and royalty payments (and the extraordinary generosity of Edward Abraham and Guy Newton) led to the establishment of these funds.

While modest Guy Newton grants have been available to Dunn School PIs for many years, in 2022 we formalised a series of internal schemes that are open for applications from anyone in the department. The impetus for this initiative came from our experience supporting the department during the pandemic. At the time, we established a fund to help mitigate the impact of COVID on career development and work productivity. 51 personal grants were awarded, supporting members of the department in a variety of ways. Following the success of this fund, and consultation in the department, we decide to formalise the various funds described below. Each fund was set up to address either an area of strategic importance, or a gap in available funding.

We always welcome feedback on how we can maximise the direct impact of philanthropic giving. You can share your thoughts with Cat Vicente, Head of Scientific Strategy and Projects: catarina.vicente@path.ox.ac.uk

Training Grants
Supporting the attendance of training costs, workshops and other career development opportunities.

Carers Career Grants
Covering extraordinary caring costs which, if not covered, would prevent someone with caring responsibilities to take advantage of opportunities for career development or improve work efficacy.

Public Engagement Grants
Encouraging small public engagement with research projects.

Academic Visitors Grant
Supporting short sabbatical visits by leading academics.

Student Enhancement Scholarship
Enhancing the funding of visiting external masters or PhD students.

Speaker Grants
Fostering research exchange and collaborations by encouraging external speakers in addition to those invited for departmental seminars.

Pathway to Independence Grants
Providing postdocs and research staff with seed funding to support their career development, e.g. to acquire preliminary data that will support a future grant application.

The lasting legacy of Norman Heatley, the unassuming penicillin pioneer who changed the course of medicine

'…without Heatley, no penicillin' – Sir Henry Harris

This article was originally published on the Oxford Giving Medium channel, which focuses on fundraising at the University: https://medium.com/@oxfordgiving

Dr Norman Heatley

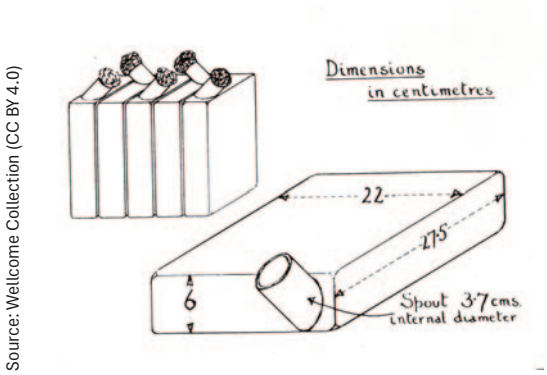
Norman Heatley is often described as the unsung hero of the penicillin story. A recent PhD graduate with a genius for invention, he became a pivotal member of the Oxford team that developed the miracle drug in the early 1940s.

But while his colleagues at the Sir William Dunn School of Pathology – Professor Howard Florey and Ernst Chain — were awarded the Nobel Prize for their efforts, for decades Norman's contributions went unrecognised. His daughter Rose reflects on his incredible legacy and explains how she plans to make her own mark on the future of human health by leaving a gift to the Dunn School in her will.

The great survivor

'Penicillin was the height of my father's career. He was very young – 30 years old,' says Rose Heatley, Norman's eldest daughter. She was born after the drug's development and doesn't recall him talking about it when she was growing up. 'My awareness of the penicillin story and his work didn't come until there were the anniversaries of the discovery. Daddy was always the one interviewed because all the other people involved had died, so he became the great survivor.'

Even then, she says, 'he was so modest. He used to say: I'm not that special.' Many would strongly disagree with that assessment, of course. In an interview with the BBC in 2010, medical historian and author Dr Eric Sidebottom described him as 'the key technical man' in the Oxford team. He devised a clever assay for measuring the activity of penicillin, established appropriate conditions under which it was stable, and pioneered a multi-stage technique to isolate and concentrate it.



Source: Wellcome Collection (CC BY 4.0)

The initial sketch for the pottery culture flask designed by Norman Heatley

Norman also designed the ceramic vessel used to grow the *Penicillium notatum* mould from which penicillin was extracted in the necessary quantities for clinical trials. 'I've got one here,' says Rose, taking it down from a shelf in her living room. Perched on top are two little vials, which read: 100,000 Oxford units, penicillin, sodium salt, store below 10 degrees centigrade. 'I think maybe he kept his paperclips in them,' she laughs.

Norman commissioned a company to make 1,000 of these vessels and by early 1941 the team felt as though they had enough penicillin to start treating humans. On 12 February that year, 43-year-old policeman Albert Alexander became the first patient to receive the drug. He was being cared for at the Radcliffe Infirmary in Oxford and although his condition initially improved, supplies of the penicillin ran out before the cure could be completed. He succumbed to his infection shortly after.

Albert Alexander in uniform
Courtesy of Linda Willason
(CC BY-ND 4.0)

The team then changed their approach to administering the drug and, following a series of treatment successes and publication of their findings, were able to secure its mass production in the US (Norman, who had travelled there with Howard Florey in 1941, stayed on for some time as an adviser in the research laboratories). Within just a few years penicillin was being used to treat Allied soldiers fighting in the Second World War, significantly reducing the number of deaths and amputations caused by infected wounds. It's estimated that over 500 million lives have now been saved by the drug.

A quintessential family man

Norman may have played a pivotal role in one of the greatest medical advances of the 20th century, but it was only in his

Two of the six women recruited to 'farm' penicillin at the Dunn School. They were nicknamed the 'penicillin girls'

later years that he began to receive wider recognition for the contribution he made. In 1990 he was awarded an extremely rare Honorary Doctorate of Medicine from Oxford University, and two decades later, a Blue Plaque was unveiled at his home of more than 60 years.

Photo by Bill Nicholls (CC BY-SA 2.0)

It was here, and in his role as a family man, that Rose remembers her father most fondly. He was exceptionally practical, she says, making over 20 bookcases for the house as well as a slide that fastened to the stairs, allowing her and her siblings to whizz down them onto a pile of cushions below. He was also 'far from a gender stereotype', doing all of the family's washing, making curtains and lampshades, altering clothes and growing vegetables in the garden. And he was very kind, too, regularly offering lifts to people waiting at the bus stop as he and his family drove past in their car.

Before her father's death in 2004, Rose helped to set up the Norman Heatley Lecture at the Dunn School in honour of his work there. The annual event gives members of the University the chance to hear from and meet some of the world's top scientists. After Rose inherited some money from her godmother, she made a further gift towards their maintenance. 'It just felt like a very worthy cause, bringing people together of international renown in the field of medical research, some of them Nobel Prize winners,' she says.

Source: US National Archives and Records Administration, ID: 515170

WWII advertising poster for penicillin

Rose makes a point to attend every year, returning to the place she remembers visiting as a child: she and her father would have a sandwich lunch together every Friday in his lab, before he took her to ballet class in the afternoon. It was this personal connection that initially sparked an interest in leaving the Dunn School a gift in her will – a decision that was cemented by the incredible impact of the work taking place there now.

Despite enormous past research successes, the department has never rested on its laurels. Today, close to 300 scientists from more than 30 countries work collaboratively to uncover the molecular and cellular mechanisms that underpin human health and disease. Areas of focus include the identification of novel therapies and diagnostic strategies for cancer, and the development of vaccines for bacterial and viral pathogens.

Many challenges still to be solved

Rose didn't follow in her father's scientific footsteps. Instead she studied Chinese at SOAS in London before embarking on a career at the BBC, starting with the Chinese section of the World Service. Her interest in the country's language and culture remains today, and she points to the Chinese Taoist emphasis on the importance of knowing what is 'enough' as a guide for her giving. 'It's over 2,000 years old,' she says, 'and a real prompt to donate.'

As the Dunn School approaches its centenary in 2027, donations of any size, including those left as gifts in wills, have a critical role to play. 'Penicillin has saved millions of lives,' says Rose, 'but there are still many challenges to be solved. It's money that could lead to new drugs and very necessary new drugs for all kinds of illnesses, which will benefit potentially millions of people — how can that not be good thing?'

The Sir William Dunn School of Pathology

Rose Heatley

Further reading: Take a closer look at the international team of scientists and medics that came together in Oxford to develop penicillin, including the 'penicillin girls', pictured above: www.mhs.ox.ac.uk/backfromthedead/exhibition/team-penicillin

Molecular Organization of Cell Junctions in epithelial tissues

Karina Pombo-García

Dr Karina Pombo-García, a new group leader at the Rosalind Franklin Institute, is the Dunn School's inaugural Visiting Group Leader. This new scheme allows academics in other institutions to associate with the Dunn School for a period of time to explore and develop scientific synergies, sponsored by an existing research group. In this issue, Karina discusses her vision for her new group, as well as the benefits of her association with the Dunn School.

Understanding how functional order emerges from individual components is an unsolved major question. How does life emerge and self-organize from a pool of biomolecules? An essential part of this process must have been the role of epithelial cells. It is fascinating to understand how epithelia cells organized their components in space and time to shape a robust tissue allowing life.

Epithelial cells have developed cell-cell adhesion complexes that keep them together, forming barriers that protect us from the external world. During the cell adhesion process, cells also acquire a characteristic apical-basal polarity that drives the membrane compartmentalization and the order of biomolecules. The loss of either cell polarity or cell adhesion leads to cell and tissue disfunction. Several diseases have been associated with loss of tissue barrier function provided via the cell adhesion complexes or the dysregulation of cell polarity, including processes such as pathogen entry, cancer progression and inflammation However, it is still very difficult to pinpoint the direct molecular mechanism between these diseases and cell adhesion. Interestingly, despite being an important therapeutic target that affect many diseases, there are still very few small molecule drugs targeting cell junctions.

I am interested in the clinical applications and links to disease in this area, but I am also fascinated by the fundamental cell biology and biophysics of cell-cell junctions.

During my postdoc I asked the following question: how do tight junctions form a close belt that seals our cavities? We discovered that protein drops spread on the membrane to form the tight junction (Pombo-Garcia *et al*., *Nature* 2024). We initially observed that cells form membrane condensates via liquid-liquid phase separation, nucleating the tight junction when two cells touch (Beutel *et al*, *Cell* 2019). Later, we showed that condensates elongate when in contact with the membrane, forming a layer of protein similar to a water droplet spread over glass. In physics this is known as a wetting transition. We concluded that membrane prewetting by condensates promotes tight-junction belt formation allowing the tissue to seal (Pombo-Garcia *et al*., *Nature* 2024) (Figure 1). We used theoretical modeling based on thermodynamics of surface growth to recapitulate our experimental dynamic data.

Interestingly, using STED super-resolution microscopy, we were able to map at the nanoscale scale an interface between the cell apical top and the condensates on the lateral membrane, suggesting cells use the polarized organization of the epithelia cell membrane to spatio-temporally position and shape the condensates. This is an elegant example of how cells use condensates to form functional mesoscale assemblies.

What next?

My newly established lab at the Rosalind Franklin Institute (Harwell) seeks to combine quantitative cell biology, chemical biology and biophysics to understand the spatio-temporal organization of cell junctions in the gut and its dysregulation during inflammation and infection. Our primary technique is super-resolution STED microscopy which allows us to visualize the dynamics of nanoscale adhesion complexes inside cells. Our current set-up using adaptive optics also allows us to visualize organoids and human tissues in 3D, which has been a significant challenge due optical aberrations in thick samples. We hope the visualization of these processes in space and time at high resolution in 3D tissue samples will bring us a step closer to understanding the biology and link to the pathology.

In the lab, we are interested on approaching biological questions as multiscale processes from molecules to tissues. At times, the underlying mechanisms of self-organization of molecules and tissues might follow the same rules. For example, are tissues following wetting processes in the body to shape their structures? We also want to push the understanding of condensate formation in tissues developing different cutting-edge techniques that will allow us to follow the spatio-temporal dynamics of these processes in the human gut. We want to move beyond proteins and understand how other biomolecules such as lipids and sugars play a role on the organization of the cell membrane during adhesion and polarity. As we face a new era on the physics of life, it is fascinating to keep exploring how physical rules, geometrical constraints and mechanics play a role on organizing life beyond genes.

It is an exciting time to ask questions, (it has always been). However, it still requires asking the right question and the team to make it happen.

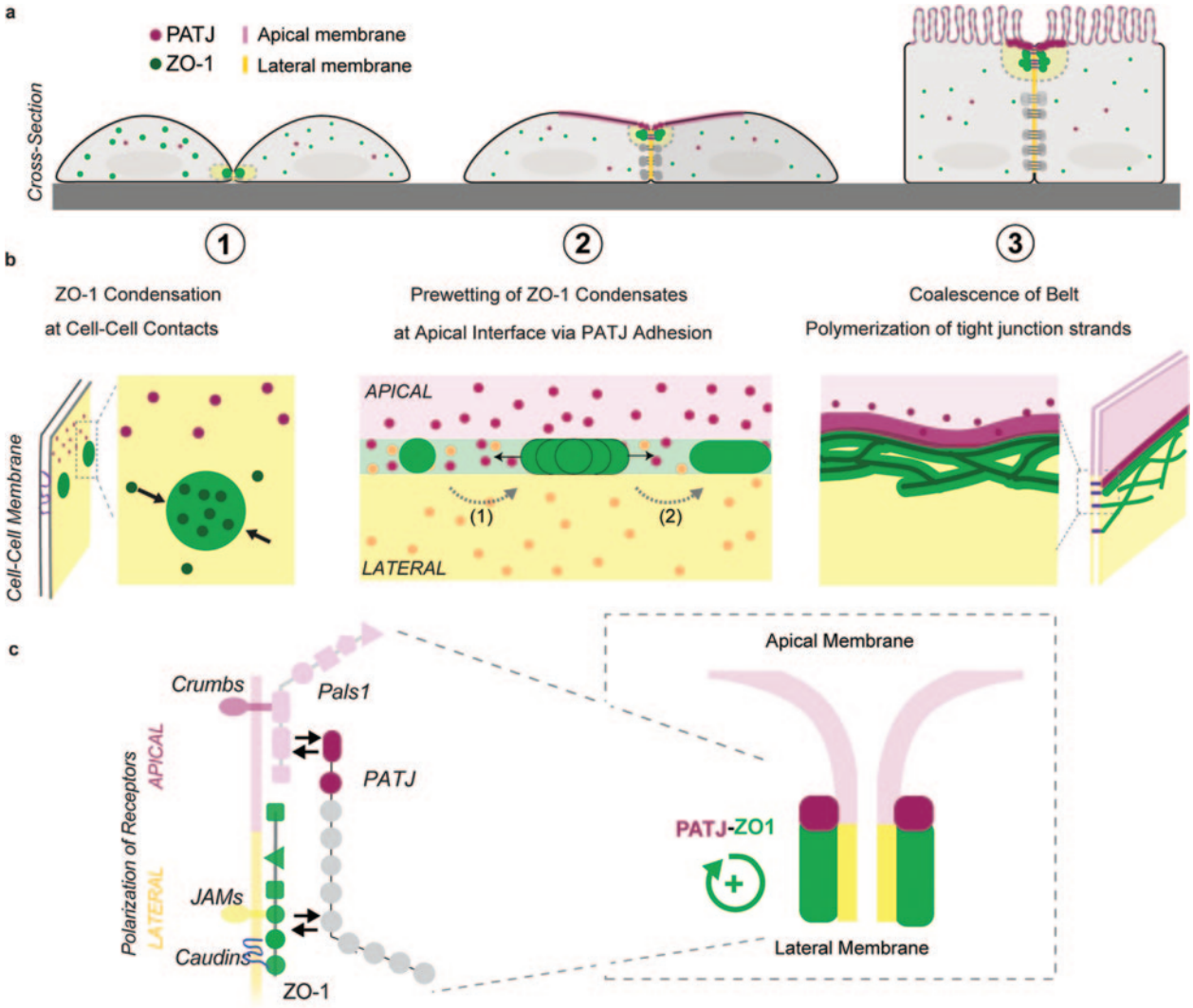


Figure 1. Model of tight junction belt formation by prewetting of junctional condensates along the apical membrane interface. (a) tight junction formation phases on the cellular level. (1) condensates nucleate at cell-cell contact sites. (2) Cells polarize and nucleated surface condensates grow around the apical interface (3) Cell-cell contacts elongate, and the tight junction belt closes and seals the tissue. (b) Mesoscale events during tight junction belt formation. (1) Nucleation of condensates leads to partitioning of junction proteins. (2) Interaction of nucleated condensates with the apical interface induces condensate growth along the interface via a prewetting transition. (3) Polymerization of tight junction strands establishes the tight transepithelial barrier. (c) Molecular interactions underlying prewetting of condensates along the apical interface. Figure taken from Pombo-Garcia *et al.*, *Nature* 2024

Being a collaboration partner with the Dunn School allows my team to ask risky questions in systems out of our comfort zone like pathogen entry or viral infection. For example, we are currently discussing with Jordan Raff the function of condensates and how their physical properties affect function. Meanwhile we are exploring with Chris Tang how different bacterial glycans interact with epithelial cells to disrupt the barriers.

It is very exciting to be part of the Dunn School! It's a very welcoming atmosphere that really promotes scientific discussion and we are looking forward to collaborating with other groups..

Brief biography

Karina completed her PhD in Chemistry between Helmholtz (HZDR), Technische Universität Dresden (TUD), Germany and Monash University, Australia on the development of radiopharmaceuticals and nanomaterials for molecular imaging for early cancer diagnosis. Later, she shifted to do a postdoc in cell biology and biophysics at the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden with Prof. A. Honigmann & Prof. T. Hyman. During her time there, she made a key discovery understanding the process by which epithelial cells connect via wetting of liquid-condensates of tight junctions (Beutel *et al.*, *Cell* 2019, Pombo-Garcia *et al.*, *Nature* 2024). Exciting collaborations also allowed her to work on lipid imaging (Iglesias *et al*, *Bioarxiv* 2024), the neuropathology of stress granules (Yan *et al.*, *Bioarxiv* 2023) and small drugs on transcriptional condensates, all using super-resolution microscopy (Basu *et al.*, *Nat. Cell Chem* 2023).

References

[1] Pombo-Garcia *et al.*, *Nature* 2024, https://doi.org/10.1038/s41586-024-07726-0.
[2] Beutel *et al.*, *Cell* 2019, https://doi.org/10.1016/j.cell.2019.10.011.
[3] Yen *et al.*, *Bioarxiv* 2023 10.1101/2024.01.23.576837.
[4] Basu *et al.*, 2023 *Nat Str and Mol Bio* https://doi.org/10.1038/s41594-023-01159-5.
[5] 5.Iglesias *et al.*, 2024 https://doi.org/10.1101/2024.05.14.594078.

UNIVERSITY OF OXFORD

THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
We are a department of the University of Oxford within its Medical Sciences Division

HEAD OF DEPARTMENT
Prof Matthew Freeman FRS,
Sir William Dunn School of Pathology,
South Parks Road
Oxford OX1 3RE, U.K.
matthew.freeman@path.ox.ac.uk

EDITOR
Catarina Vicente
Head of Scientific Strategy and Projects
Tel: +44 (0)1865 275597
catarina.vicente@path.ox.ac.uk

From the *Fusion* Archives...

The following is a selection of excerpts, chosen by the Editor from past editions of *Fusion*, that give a flavour of events and topical issues within the Dunn School during years gone by...

Fifteen years ago...

In the 2009 issue of Fusion, Dr Alvin Volkman writes about his time in the Dunn School in the 1960s, working with Jim Gowans. He particularly recollects meeting Nat Smith, a Dunner with very direct connections to Broadway and Hollywood...

"*Smith paused before pouring a second cup of tea; spout poised a few inches above the rim of my cup and asked, "Is my daughter, Margaret...Maggie, well regarded in America?" I was lost for a moment. Why on earth should I know anything about his daughter? I asked myself. Maggie Smith? Then the penny dropped: I remembered from conversations with theatre-going friends that they had been impressed with an English actress named Maggie Smith and that she had received excellent reviews in her first appearance on Broadway in 1956. [...] In 1961 most of the future Dame Maggie's great triumphs of stage and screen were yet to be. My skimpy knowledge about Maggie's standing in America seemed to disappoint Nat but not too many years would pass before he would no longer need to ask anyone about Maggie's accomplishments.*"

*****

Twenty years ago...

The third issue of Fusion (2004) mentions the first of what is now an annual Dunn School tradition, as well as an important part of our students' training: the Graduate Student Symposium. Impressively, this has been uninterrupted since 2003 (including during the COVID years, when it took place online).

"*The Department held its inaugural Graduate Students' Symposium on the 30th June, 2003. Modelled on a scientific conference and attended by the entire Department, this annual symposium provides training in vital communication skills as well as critical scientific feedback. A panel of judges evaluated all contributions and awarded a prize for the best poster to Laura Briggs ('Insight to flagellar protein function from studies with trypanosomes') and for the best oral presentation to Namir Hassan ('Regulation of the immune response through interactions of CD6')*."

*****

Curious? See page 24

Fusion is produced by the Medical Informatics Unit, NDCLS, University of Oxford. Telephone +44 (0)1865 222746.
Ref: Fusion2024/500