DPhil/MSc Projects available for 2022 entry

List of projects/project areas:

1. **Evolutionary cell biology of chromosome segregation** (supervisor: Dr Bungo Akiyoshi) (DPhil/MSc)
2. **Dissecting the pathways of chromosome mis-segregation and micronucleation in cancer** (supervisor: Prof Francis Barr) (DPhil only)
3. **Nanomachines and the Bacterial Cell Envelope** (supervisor: Prof Ben Berks) (DPhil only)
4. **Maintenance of genomic stability and DNA repair in humans** (supervisor: Associate Professor Martin Cohn) (DPhil/MSc)
5. **Ageing and cell senescence** (supervisor: Associate Professor Lynne Cox) (DPhil only)
6. **Analysis of the human antibody repertoire induced by novel vaccines against the leading PfRH5 blood-stage malaria antigen** (supervisor: Prof Simon Draper) (DPhil only)
7. **Regulation of the regulators** (supervisor: Dr Paul Elliott) (DPhil/MSc)
8. **Mechanisms and molecular structure of the splicesome during pre-mRNA splicing** (supervisor: Dr Sebastian Fica) (DPhil only)
9. **Social interactions in microbes and other group living species** (supervisor: Prof Kevin Foster) (DPhil/MSc)
10. **Molecular Characterisation of the Human Cold Shock Response: "Cooling the Cellular Clock"** (supervisor: Associate Professor Andre Furger) (DPhil/MSc)
11. **Post-translational regulation of centrosome function** (supervisor: Dr Fanni Gergely) (DPhil/MSc)
12. **Genome stability and its regulatory signalling mechanisms** (supervisor: Dr Ian Gibbs-Seymour) (DPhil only)
13. **cbEGF containing proteins in health and disease** (supervisor: Prof Penny Handford) (DPhil/MSc)
14. **Structural studies of host-parasite interactions** (supervisor: Prof Matt Higgins) (DPhil only)
15. **Innovating protein nanotechnologies for cancer analysis and immune activation** (supervisor: Prof Mark Howarth) (DPhil only)
16. **Epigenetic control of centromere assembly and inheritance** (supervisor: Associate Professor Lars Jansen) (DPhil only)
17. **Deciphering the molecular choreography within bacterial cell envelopes** (supervisor: Prof Syma Khalid) (DPhil only)
18. **Discovering how epigenetics regulate gene expression in stem cells** (supervisor: Prof Rob Klose) (DPhil only)
19. **Detection, Signalling and Repair of DNA Damage** (supervisor: Prof Nick Lakin) (DPhil/MSc)
20. ***Drosophila* as a model to study neurodegeneration and investigating the interaction between *Leishmania* and its insect vector** (supervisor: Prof Petros Ligoxygakis) (DPhil/MSc)
21. **SMC complex function in chromosome condensation, segregation and regulation of gene expression** (supervisors: Prof Kim Nasmyth and Dr Madhusudhan Srinivasan) (DPhil/MSc)
22. i. **Structural basis for receptor mediated trafficking in the early secretory pathway**

ii. **Structural basis for lysosomal protein function in Parkinson's disease**
iii. **Molecular mechanisms controlling glycosylation and protein acetylation in the ER and Golgi** (supervisor: Prof Simon Newstead) (DPhil only)

23. **Evolution of epigenetic mechanisms: implications for cancer** (supervisor: Associate Professor Peter Sarkies) (DPhil/MSc)
24. **Advanced cellular imaging to study functional nuclear organization** (supervisor: Dr Lothar Schermelleh) (DPhil only)
25. **Imaging how bacteria adapt to stress: From DNA damage to mutations** (supervisor: Associate Professor Stephan Uphoff) (DPhil only)
26. **Transcriptional and post-transcriptional regulation of gene expression in eukaryotes** (supervisor: Dr Lidia Vasilieva) (DPhil/MSc)
27. **Replication blockade and genome stability** (supervisor: Prof Matthew Whitby) (DPhil/MSc)
28. **Computational approaches to receptor dynamics and ligand binding** (supervisor: Prof Phil Biggin) (DPhil only)
29. **Functional ramifications of outer membrane organisation in Gram-negative bacteria** (supervisor: Prof Colin Kleanthous) (DPhil only)
30. **From structure to function: characterizing three novel putative lipid transport proteins and their role in intracellular lipid flux** (supervisor: Prof Benoit Kornmann) (DPhil/MSc)
31. **Compartmentalisation via liquid-liquid phase separation in cells** (supervisor: Dr Tim Nott) (DPhil only)
32. **Proteins at the centre of human health and disease** (supervisor: Associate Professor Jason Schnell) (DPhil/MSc)

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1. Evolutionary cell biology of chromosome segregation

Supervisor: Dr Bungo Akiyoshi

We are interested in revealing the mechanism of chromosome segregation in eukaryotes. Our focus is on the kinetochore, a highly complicated macromolecular protein complex that assembles onto centromeric DNA and interacts with spindle microtubules. Even a simple yeast kinetochore contains more than 40 different proteins. Although it was previously assumed that all eukaryotes possess yeast-like kinetochores, we discovered an exception to this rule in *Trypanosoma brucei*.

Trypanosoma brucei is an experimentally-tractable eukaryotic parasite that branched early in eukaryotic history and is well known as the causative agent of African sleeping sickness. We recently identified 25 kinetochore proteins and found that these proteins are well conserved among kinetoplastid species (e.g. *T. cruzi* and *Leishmania*) (1–4). However, they do not have significant homology to conventional kinetochore proteins present in other eukaryotes, suggesting that kinetoplastids build up kinetochores using a unique set of proteins. We are currently trying to reveal their function using a variety of techniques ranging from biochemistry to biophysics (NMR spectroscopy, X-ray crystallography, and cryoEM). By understanding how trypanosomes segregate their chromosomes, we aim to understand fundamental principles of eukaryotic segregation machinery. We also aim to target the unique kinetochore for novel therapeutics against sleeping sickness and leishmaniasis.

The aim of this project is to characterize the interaction between two unconventional kinetochore proteins called KKT7 and KKT10. KKT10 is a protein kinase that has been shown to be an attractive drug target (5). Understanding the regulation of KKT10 is therefore of significant interest. Although we previously showed that KKT10 directly interacts with the N-terminal fragment of KKT7 (6), it remains unknown how they form the binding interface and how the interaction may be regulated.

To better understand the function and dynamics of the KKT7-KKT10 interaction, at the level of individual residues, you will express and purify an isotopically-labelled ($^{13}C/^{15}N$) recombinant KKT7 protein fragment as well as unlabelled KKT10 protein from *E. coli* (in Akiyoshi lab). The sample will be analyzed using NMR spectroscopy and sequence-specific backbone assignments will be performed and backbone dynamics probed in the absence and presence of KKT10 (in Redfield lab); comparison of NMR parameters in these two states will provide information about residues in KKT7 involved in the binding site for KKT10. You will also aim to determine the structure of the complex using X-ray crystallography.

Reference

1. Akiyoshi B and Gull K. (2014) ***Cell*** 156 (6): 1247-58
2. Nerusheva OO and Akiyoshi B. (2016) ***Open Biology*** 6: 150206
3. Nerusheva OO, Ludzia P, and Akiyoshi B. (2019) ***Open Biology*** 9: 190236
4. Ludzia P, Lowe ED, Marcianò G, Mohammed S, Redfield C and Akiyoshi B (2021) ***Structure***
5. Saldivia et al. (2020) ***Nature Microbiology*** 5(10):1207-1216
6. Ishii and Akiyoshi (2020) ***Journal of Cell Science*** 133, jcs240978

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2. Dissecting the pathways of chromosome mis-segregation and micronucleation in cancer

Supervisor: Prof Francis Barr

Mounting evidence shows that in many cancers, chromosome segregation errors linked to micronucleation underpin solid tumour evolution by promoting cellular heterogeneity within tumours, ultimately leading to metastasis. Micronuclei arise when a small number of chromosomes are not incorporated into the main nucleus of the progeny cell, which is different to classical chromosome instability where all chromosomes are captured in one intact nucleus. Due to their size and mechanism of formation, micronuclei are structurally and functionally defective and do not efficiently compartmentalise DNA away from the cytoplasm or ensure its error-free replication. These are crucial differences explaining why micronuclei are the major site of chromothripsis, a chromosome shattering process that acts as a catalyst for cancer evolution. To defend against this danger, cells possess an innate immune surveillance mechanism for cytosolic DNA which initiates an interferon and NF-kB signalling response upon detection of micronuclei. This process involves the cyclic GMP-AMP synthase (cGAS) and the interferon response adaptor stimulator of interferon genes (STING).

Despite this knowledge, there are few cases where the mechanism of cancer-associated micronucleation is understood, thus hampering progress in the area of cancer biology. One tractable example is the Aurora A- PP6 oncogene-tumour suppressor axis, discovered by our labs, which is crucial for efficient bipolar spindle formation and chromosome segregation. Aurora A is a mitotic kinase and classical oncogene frequently amplified in cancers whose activity is regulated through auto-phosphorylation counteracted by protein phosphatase 6 (PP6). Cancer-associated loss of function "*driver*" mutations in PPP6C, the catalytic subunit of PP6, trigger chromosome mis-segregation with micronucleation. This is most prominent in melanoma, where PPP6C loss-of-function

"driver" mutations are found in >12% of cases. However, whether the cGAS/STING response is triggered by these micronuclei or other changes to genome integrity is currently not known. In this project, we will investigate how the different classes of chromosome segregation errors and micronucleation events seen following dysregulation of the Aurora A-PP6 axis link to cGAS/STING signalling. Additionally, we will explore the potential for evasion of the cGAS/STING response in PP6-mutant and Aurora A amplified tumour cells. Our immediate research aims are to follow the formation of individual micronuclei in PP6-deficient cells, and determine whether they are immediately recognised by the cGAS/STING pathway, or whether this occurs only after multiple aberrant cell divisions.

The project combines molecular biology, cell cycle analysis, genomics, classical biochemistry and advanced microscopy approaches with the option to perform immunological assays to measure innate immune signalling. There will therefore be extensive training opportunities in all these areas by experts in the field.

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3. Nanomachines and the Bacterial Cell Envelope

Supervisor: Prof Ben Berks

The cell envelope of bacteria comprises the cell wall and either one or two membranes, and provides a formidable barrier to the movement of macromolecules between the bacterial cytoplasm and the external environment. Our group aims to understand the molecular mechanisms by which proteins, nucleic acids, and mechanical force are transferred across and along these barriers. As part of this work we characterise the dedicated nanomachines that carry out these processes.

We use a wide range of methodologies in our work, in some cases via collaboration. These approaches include protein purification and characterisation, bacterial cell biology, bacterial genetics, live cell single molecule fluorescence imaging, bioinformatics, and structural biology.

Specific research areas in which projects could be offered include:

- **Protein transport.** We study the transport of folded proteins across the bacterial inner membrane by the **Tat transport system** and protein export across the outer membrane by the recently discovered **Type 9 Secretion System**. Both systems are important for bacterial pathogenesis.
- **DNA transport.** We study the mechanisms by which genes move between bacteria, thereby contributing to antibiotic resistance and other adaptive traits. We are interested in the processes of **Transformation** (natural competence), in which the bacterium takes up naked DNA molecules directly from their environment and **Conjugation** in which DNA is transferred between bacteria either by direct contact or via a retractile pilus.
- **Gliding motility** in which bacteria move rapidly across solid surfaces using surface adhesins running on mobile tracks located in the cell envelope.
- **Physical properties of the cell envelope.** In particular, we are interested in the functional properties of the periplasm, which is the compartment lying between the inner and outer membrane of Gram-negative bacteria and which contains the cell wall.

Projects in new cell envelope topics are also likely to be available. Please contact me!

Example references showcasing some of our technical approaches:

Hennell James *et al.* (2021) Structure and mechanism of the proton-driven motor that powers type 9 secretion and gliding motility. *Nat Microbiol* **6:** 221.
Lauber *et al.* (2018) Type 9 secretion system structures reveal a new protein transport mechanism. *Nature* **564:** 77.
Alcock *et al.* (2016) Assembling the Tat protein translocase. *Elife* **5:** e20718.
Alcock *et al.* (2013) Live cell imaging shows reversible assembly of the TatA component of the twin-arginine protein transport system. *PNAS* **110:** E3650.
Silale *et al.* (2021) The DNA transporter ComEC has metal-dependent nuclease activity that is important for natural transformation. *Mol Microbiol* doi: 10.1111/mmi.14720.

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4. Maintenance of genomic stability and DNA repair in humans

Supervisor: Associate Professor Martin Cohn

Integrity of the genome is critical to both the development and health of humans. Our chromosomes are constantly exposed to various types of DNA damage, which if unrepaired can cause diseases such as cancer. To meet these challenges, several DNA repair pathways have evolved. Our laboratory is focused on understanding how these pathways work in human cells. We have discovered several new proteins playing important roles in these pathways. By applying state-of-the-art techniques in biochemistry, molecular biology and cell biology, combined with world-class mass spectrometry and high-resolution live-cell imaging and structural biology, we are continuously elucidating the role of new components of the DNA repair pathways. Examples of methods used are recombinant protein purification, in vitro assays, CRISPR/Cas9 genome engineering, various cell-based assays including sophisticated live-cell imaging, and cryo-EM. Please contact Martin Cohn (martin.cohn@bioch.ox.ac.uk) for details on the newest and most interesting ongoing projects.

Recent papers published by DPhil students from our group:

Liang. C-C. and Cohn, M.A. (2021). Purification of DNA repair protein complexes from mammalian cells. **STAR Protoc.** 2(1):100348.

Socha, A., Yang, D., Bulsiewicz, A., Yaprianto, K., Kupculak, M., Liang. C-C., Hadjicharalambous, A., Wu, R., Gygi, S.P. and Cohn, M.A. (2020). WRNIP1 is recruited to DNA interstand crosslinks and promotes repair. **Cell Rep.** 32(1):107850.

Lopez-Martinez, D., Kupculak, M., Yang, D., Yoshikawa, Y., Liang, C-C., Wu, R., Gygi, S.P. and Cohn, M.A. (2019). Phosphorylation of FANCD2 inhibits the FANCD2/FANCI complex and suppresses the Fanconi anemia pathway in the absence of DNA damage. **Cell Rep.** 27(10):2990-3005.

Liang, C-C., Li, Z., Lopez-Martinez, D., Nicholson, W., Venien-Bryan, C. and Cohn, M.A. (2016). The FANCD2-FANCI complex is recruited to DNA interstrand crosslinks prior to monoubiquitination of FANCD2. **Nature Commun.** 7:12124.

Schwab, R., Nieminuszczy, J., Shah, F., Langton, J., Lopez Martinez, D., Liang, C-C., Cohn, M.A., Gibbons, R., Deans, A. and Niedzwiedz, W. (2015). The Fanconi anaemia pathway maintains genome stability by coordinating replication and transcription. **Mol. Cell.** 60(3):351-61.

Liang, C-C., Zhan, B., Yoshikawa, Y., Haas, W., Gygi, S.P. and Cohn, M.A. (2015). UHRF is a sensor for DNA interstrand crosslinks and recruits FANCD2 in the Fanconi Anemia pathway. **Cell Rep.** 10(12):1947-56.

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5. Ageing and cell senescence

Supervisor: Associate Professor Lynne Cox

Background:
Human ageing is accompanied by progressive loss of tissue homeostasis and diminished health, and accumulation of senescent cells. Cellular senescence is induced by cell stress, and results in essentially irreversible proliferation arrest accompanied by characteristic morphological and metabolic changes, together with secretion of a pro-inflammatory and pro-tumorigenic secretome. Recent data suggest senescence is causative in age-related diseases, since senescent immune cells drive ageing of other tissues [1], local injection of senescent cells leads to development of premature age-related pathologies across the body [2], and removal of senescent cells from ageing animals significantly improves health [3], even increasing survival following infection with coronavirus [4, 5]. In addition to contributing to age-related diseases (including bone fragility, GI-tract dysfunction, Alzheimer's and Parkinson's disease), senescence is also thought to promote premature ageing following treatment with genotoxic cancer therapies (chemo- and radio-therapy).

We have recently shown that it is possible to reverse (and even potentially to prevent) some detrimental senescent cell phenotypes by modulation of mTOR and Rho signalling pathways [6, 7], and our preliminary data suggest that such reversal is accompanied by global changes that may restore cells to a 'youthful' molecular state. It is therefore important to explore these changes and investigate which changes are actually driving the apparent rejuvenation.

Aims of DPhil project:

- To conduct extensive -omics analysis of primary human cells (eg RNAseq, proteomics, phosphoproteomics) in order to identify consistent patterns of molecular changes that occur with senescence, and those that 'rejuvenate' on drug treatment.
- To conduct detailed phenotypic studies including high resolution imaging to investigate the factors found to change on senescence and senomodifying treatment.
- To determine which factors are drivers of such changes, through genetic manipulation (eg CRISPR knock-out/knock-in).

Potential outcomes: This project should lead to identification of factors contributing to cell senescence, as well as biochemical pathways critical for cell rejuvenation.

Training: The project will involve training in a range of cell and molecular biology techniques including primary cell culture, fluorescence microscopy, genome editing and -omics, as well as experimental design, scientific writing and presentation, and outreach.

References
[1] Yousefzadeh *et al.* (2021) An aged immune system drives senescence and ageing of solid organs. *Nature* 594, 100–105 (2021). https://doi.org/10.1038/s41586-021-03547-7
[2] Xu *et al.* (2018) Senolytics improve physical function and increase lifespan in old age. Nat Med. 2018 Aug;24(8):1246-1256. doi: 10.1038/s41591-018-0092-9.

[3] Baker *et al.* (2016) Naturally occurring $p16^{Ink4a}$-positive cells shorten healthy lifespan. *Nature* 530, 184–189 (2016). https://doi.org/10.1038/nature16932
[4] Camell et al (2021) Senolytics reduce coronavirus-related mortality in old mice. *Science* 16 Jul 2021: 373(6552), eabe4832 DOI: 10.1126/science.abe4832
[5] **Cox** and Lord (2021) Coronavirus: Targeting aging cells improves survival. *Science* 16 Jul 2021: 373(6552), 281-282 DOI: 10.1126/science.abi4474
[6] Walters, Deneka-Hannemann, **Cox** (2016) Reversal of phenotypes of cellular senescence by pan-mTOR inhibition. Aging (Albany NY). 2016 Feb;8(2):231-44
[7] Walters and **Cox** (2021) Intercellular transfer of mitochondria between senescent cells through cytoskeleton-supported intercellular bridges requires mTOR and Cdc42 signalling. *Oxid Med and Cellular Longevity* Vol 2021 Article ID 6697861, https://doi.org/10.1155/2021/6697861

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6. Analysis of the human antibody repertoire induced by novel vaccines against the leading PfRH5 blood-stage malaria antigen

Supervisor: Prof Simon Draper

Traditionally the analysis of human antibody responses following vaccination has involved global approaches, with assays measuring the total polyclonal serum antibody response in terms of titre, concentration, subtype response or avidity. Although such measures remain useful readouts, the development of highly effective vaccines against difficult and complex pathogens, such as the *Plasmodium* parasite that causes human malaria, requires a much greater understanding of the fine specificity of the vaccine-induced antibody response. In recent years, significant advances have been made in terms of ability to analyse the human antibody repertoire generated in response to vaccination, and to assess the identification of key neutralising epitopes via structural biology.

The aim of this project will be **to analyse the human antibody response induced by novel candidate vaccines against the leading PfRH5 blood-stage malaria antigen in Phase I/IIa clinical trials undertaken in Oxford.** This will build upon on-going research in the Draper Group to develop vaccines against this critical target within the parasite and to better understand its biology. A variety of techniques will be used to isolate antigen-specific B cell subsets from volunteers immunised with the PfRH5 antigen using different vaccine delivery platforms in humans. The isolated B cells will be used to analyse the human antibody response via sequencing and cloning of the B cell receptor gene repertoire. Key analyses will include variable region gene usage, mutation, and clonality of the response over time post-immunisation. Identified sequences will be used to generate human monoclonal antibodies (mAbs) which will be assessed for functional anti-parasitic activity, affinity and epitopes mapped on the antigen using structural biology and other immunological approaches. The outputs of this work should identify key epitopes and determinants within PfRH5 that are recognised following human vaccination with novel vaccine delivery platforms, leading to the structure-guided design of improved next-generation vaccine immunogens for onward clinical development. The project will be benefit from the group's extensive experience of clinical immunology, B cell immuno-monitoring and parasitology, as well as from strong collaborations with leading structural biologists.

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7. Regulation of the regulators

Supervisor: Dr Paul Elliott

Post-translational modifications, such as ubiquitination, offer a rapid and versatile mechanism to control complex signalling pathways and cellular processes through the generation of defined codes. The Elliott lab utilises a host of biochemical, biophysical and structural techniques to investigate how the complex ubiquitin code is regulated. The ubiquitin code is produced, interpreted and destroyed through respective activity of ligases (writers), ubiquitin-binding domains (readers), and deubiquitinating enzymes (erasers). Importantly, the ubiquitin code needs to be tightly regulated and assembled at the right location for a defined length of time to generate the appropriate response. To achieve such a tightly regulated response, the readers, writers and erasers are often modified themselves through additional post-translational modifications.

This project investigates how post-translational modifications of deubiquitinating enzymes (DUBs) control the activity, and in some cases, specificity of the DUB. The specific project will be derived from discussion with the student and will be tailored to their interests and build upon their research expertise.

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8. Mechanisms and molecular structure of the splicesome during pre-mRNA splicing

Supervisor: Dr Sebastian Fica

Mammalian genes are transcribed into precursor messenger RNAs (pre-mRNAs), from which non-coding introns are spliced out in the nucleus before the mRNA is exported to the cytoplasm and translated into proteins. Introns are excised by the spliceosome – a dynamic assembly of RNA and proteins and splicing errors are implicated in up to 30% of human diseases.

The spliceosome assembles *de novo* on each pre-mRNA and catalyses two sequential transesterifications at a single RNA-based active site to excise a specific intron and ligate the flanking exons into mRNA[1]. During catalysis, several trans-acting ATPases modulate the transitions between different conformations spliceosome. These ATPases promote the exchange of reactants at the active site, allow exchange of protein factors that stabilize each conformation, and proofread fidelity of splice site choice[2]. Following mRNA synthesis, the mRNA is released by the action of the ATPase Prp22, while the ATPase Prp43 disassembles the resulting intron-lariat spliceosome (ILS) to release the excised intron for degradation.

In the last six years, structures of different conformation of the yeast spliceosome have provided a molecular view of the basic mechanism of splicing in yeast, showing how the splice sites are recognised and how specific factors stabilize each catalytic conformation[2]. The structure of the yeast post-catalytic spliceosome (P complex) suggested a mechanism by which Prp22 releases the mRNA, while the structure of the yeast ILS provided some structural insights into disassembly of the spliceosome by Prp43 and its associated co-factors. Importantly, in yeast, proofreading of correct splice site usage by Prp22 is coupled to a discard pathway in which Prp43 disassembles spliceosomes that utilise incorrect splice sites and are rejected by Prp22. The location of Prp43 in the human ILS remains unclear, as does the composition of the associated complex responsible for discard and disassembly in mammals.

Although the active site and basic splice site recognition is conserved from yeast to humans, additional ATPases associate with the human spliceosome and have been implicated in splicing fidelity[3]. Indeed, in mammals fidelity of splice site choice often relies on recognition of only 1-2 nucleotides around highly variable splice sites, which must be balanced with alternative splicing. Thus, how Prp22, Prp43, and other mammalian ATPases act to safeguard splicing fidelity remains poorly understood.

We aim to establish a biochemical system in vitro, and potentially in vivo, to study proofreading of splice site use and spliceosome disassembly in humans. We wish to identify the specific complexes involved in these processes and to use this system to trap intermediates during proofreading, discard, and disassembly. Electron cryomicroscopy will then be employed to obtain molecular insights into the mechanism of action of Prp22, Prp43, and associated co-factors and elucidate the mechanisms underlying proofreading of correct splice site choice.

References

1. Wilkinson, M. E., Charenton, C. & Nagai, K. RNA Splicing by the Spliceosome. *Annual Review of Biochemistry* **89**, 1–30 (2019).

2. Fica, S. M. & Nagai, K. Cryo-electron microscopy snapshots of the spliceosome: structural insights into a dynamic ribonucleoprotein machine. *Nature structural & molecular biology* **24**, 791–799 (2017).

3. Fica, S. M. Cryo-EM snapshots of the human spliceosome reveal structural adaptions for splicing regulation. *Current Opinion in Structural Biology* **65**, 139–148 (2020).

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9. Social interactions in microbes and other group living species

Supervisor: Prof Kevin Foster

Microbial communities contain many evolving and interacting species, which makes them difficult to understand and predict. The Foster lab combines molecular and cellular biology with ecological and evolutionary approaches to break down this complexity. Using both theory and experiment, we study how bacteria cooperate and compete in order to succeed in their communities. The lab also studies the ecological networks formed by interacting bacteria, with the goal of predicting and manipulating gut communities for better health. We have a range of projects on offer within the remit of the lab, which can be tailored to the student's interests. See our website for more details of our research: https://zoo-kfoster.zoo.ox.ac.uk/

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10. Molecular Characterisation of the Human Cold Shock Response: "Cooling the Cellular Clock"

Supervisor: Associate Professor Andre Furger

Background

Despite the widespread use of controlled cooling in clinical and emergency settings and for organ preservation, we know surprisingly little about the impact that cold temperatures have on human cells at the molecular level. Our research aims to understand how cells respond to biotic and environmental

stressors and adapt to the challenges they cause. As part of this research effort, we recently began to address the deficit in our understanding of the cellular cold shock response and we characterised the gene expression programs and the structural changes triggered by exposing human cells to a range of sub-physiological temperatures.

The Project
We discovered that cold exposure prompts temperature specific responses in human cardiomyocytes that include changes to the chromosomal architecture and the resetting and synchronisation of the circadian clock. The circadian clock is an intrinsic time keeping mechanism present in almost all cells in all organisms and enables the alignment of cell physiology and organism behaviour with the earths day and night cycle. At the molecular heart of the circadian clock are the core clock genes and their products that create transcription-translation feedback loops and so regulate rhythmic expression of their own and thousands of other genes. These links regulate the rhythm of many cellular processes and contribute to the healthy function of tissues and organs. Identifying the cues and understanding the underlying molecular process that can influence the cellular circadian clock, is thus of great importance.

Aim of the DPhil project:
The DPhil project aims to understand how structural changes to the chromosome architecture and changes to the expression of the core clock genes in response to cooling in cardio myocytes are linked, and how they coordinate and force the resetting of the circadian clock.

Aim of MSc project:
In a separate avenue of research in our lab, we use our expertise in RNA metabolism and RNA structure and function to design RNA therapeutics with an opportunity for a MSc by research.

What do the projects offer?
The projects offer training in a wide range of state of the art methodologies including, super resolution microscopy, high through-put sequencing technologies, bioinformatics, *in vitro* mRNA production, tissue culture, cell biology and classic biochemistry and molecular biology techniques.

A fully funded Oxford Percival Stanion Studentship linked to Pembroke College Oxford is also available in the Furger lab. More details can be found here.

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11. Post-translational regulation of centrosome function

Supervisor: Dr Fanni Gergely

Background:
Centrosomes are small cytoplasmic organelles that act as mitotic spindle poles, template cilia formation, and contribute to signalling, trafficking and organelle positioning. They are essential for proliferation of normal cells; indeed, congenital mutations in centrosomal genes cause growth failure syndromes.
Centrosomes are complex multiprotein assemblies that undergo dynamic changes during the cell cycle. These include a once-per-cell cycle assembly of their core structure followed by a massive recruitment drive of components involved in microtubule production during mitosis and subsequent removal of these proteins. Our central hypothesis is that the microtubule-nucleating capacity of centrosomes is controlled via phosphorylation of centrosome components. Although several kinases

including Aurora-A, Nek2, Cdk1 and Polo-like kinases 1, 2 and 4, have been all implicated in regulating centrosome functions, a comprehensive list of their targets in the organelle is still lacking. We are currently undertaking an unbiased proteomic survey to elucidate centrosomal targets for these kinases.

Aims:
The ultimate goal of this DPhil project is to improve our understanding of how centrosomal microtubule production is controlled during the cell cycle.
First, we will functionally characterise newly identified phosphorylation sites in centrosomal proteins with an established role in microtubule nucleation. By developing tools such as specific antibodies against phosphorylation sites along with cell lines where the sites are genetically edited, we will obtain mechanistic insight into how these post-translational modifications contribute to centrosome function. Second, we will ask if these phosphorylation sites are present across multiple cell types, and if so, whether their impact on microtubule nucleation is conserved. Unpublished results from our laboratory suggest that certain cell types of the hematopoietic lineage do not expand their centrosome during mitosis. In these cell types, post-translational modifications of centrosome components could play an even more important part in controlling microtubule production. This hypothesis will be tested with the tools generated in the first aim, and proteomic survey of centrosomes from these unusual cell lineages will provide further insight. Finally, armed with knowledge from the first two aims, we will ask whether and how centrosomal microtubule nucleation capacity impacts on cellular processes such as trafficking, signalling and spindle formation.

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12. Genome stability and its regulatory signalling mechanisms

Supervisor: Dr Ian Gibbs-Seymour

Background:

Research in the lab is focussed on understanding the mechanisms of DNA repair, which is a cellular process that functions to maintain the integrity of DNA and, in doing so, prevents the transformation of a normal cell into a cancer cell. Importantly, we know that failure to properly regulate DNA repair leads to various cancers, so understanding this regulation is essential. One class of enzymes that plays a major role in DNA repair regulation is deubiquitinating enzymes or DUBs. The lab has recently identified a novel DUB class that functions in DNA repair. However, there are still key gaps in our mechanistic understanding of this DUB that we aim to address using an integrated approach of biochemistry and cell biology. Promisingly, targeting DUBs with small molecule inhibitors is an emerging and attractive therapeutic avenue in cancer research.

There are currently opportunities for students to join our cell biology efforts to either work on the novel DUB that we have recently discovered or explore new cancer-relevant avenues in ubiquitin-dependent signalling.

A project may involve one of three areas of on-going research in the lab.

(1) Use advanced single-cell high-content microscopy approaches in combination with engineered cell lines to understand the steps in a cellular ubiquitin-dependent signalling process regulated by the recently discovered DUB, as well as use a range of cellular assays available in the lab.

(2) Follow-up on hits from genome-wide CRISPR-Cas9 screen that we have performed to explore the mechanistic basis for synthetic lethal interactions using advanced cell biology, genetics and proteomics tools available in the lab.

(3) Confirm a predicted paralog synthetic lethality in the ubiquitin system using tools and cell lines generated in the lab and then explore this further using genome-wide CRISPR-Cas9 screens and/or base editing approaches to understand which cellular pathways are particularly susceptible to this synthetic lethal interaction, followed by mechanistic dissection of these observations.

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13. cbEGF containing proteins in health and disease

Supervisor: Prof Penny Handford

We have made substantial progress in developing structural and functional methods to study proteins rich in EGF-like domains, such as Notch and its ligands. Combined use of these techniques has resulted in i) new insight into the specificity of the Notch-ligand interaction and its regulation by O-glycosylation ii) in the identification of both flexible and rigid EGF-like domain interfaces in the N-terminal EGF4-13 extracellular region which binds ligand iii) identification of a membrane component contributing to a ternary receptor/ligand complex which has implications for tuning of the Notch complex in different physiological contexts. Projects for 2022 will include structure-informed functional studies of the C2 lipid binding domain of Jagged 2 in health and disease. This 3 part project will involve i) establishing a eukaryotic expression system for the extracellular portion of Jagged 2 ii) introducing disease-causing/protein engineered mutations to probe structure/function relationships using established biochemical/cell biology and biophysical methods iii) creating CRISPR -variants based on extracellular domain studies to probe biological function.

References

Coppens et al. (2021) Am J of Hum Genet A form of muscular dystrophy associated with pathogenic variants in JAG2,108, 840-856,ISSN 0002-9297, https://doi.org/10.1016/j.ajhg.2021.03.020.

Martins T, Meng Y, Korona B, Suckling R, Johnson S, Handford PA*, Lea SM*, Bray SJ*. The conserved C2 phospholipid-binding domain in Delta contributes to robust Notch signalling. EMBO Rep. 2021 Aug 4:e52729. doi:10.15252/embr.202152729. Epub ahead of print. PMID: 34347930.

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14. Structural studies of host-parasite interactions

Supervisor: Prof Matt Higgins

The Higgins lab study host-parasite interactions of human infective parasites that cause diseases such as malaria, sleeping sickness and amoebiasis. We use structural biology techniques, including crystallography and cryo-electron microscopy and protein design. Project can be designed with prospective students and may address aspects of the fundamental biology of the host-parasite interface or may be more translational, involving vaccine immunogen design using structure-guided approaches. For more information about the group, see higginslab.web.ox.ac.uk

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15. Innovating protein nanotechnologies for cancer analysis and immune activation

Supervisor: Prof Mark Howarth

The general theme of our research is innovating protein technologies for immune activation. We have a number of potential projects for DPhil students, adapted according to their interests and experience. These projects generally involve engineering of novel protein platforms and then development in cell systems to establish therapeutic potential. We commonly employ rational protein engineering and protein evolution and work with a range of collaborators with expertise in different cellular and disease models. Particular projects include multi-functional proteins for control of T cell behaviour, to enhance the success of cancer vaccines and CAR-T cells. For cell therapy we are advancing our NeissLock technology for irreversible cell surface modification. We have established proteins that are resistant to degradation by the proteases of the stomach and small intestine, towards protein therapeutics that can be eaten and then inhibit toxins or inflammatory damage in the GI tract. We have done extensive work on vaccine development, leading to a candidate in clinical trials, based on modular assembly of virus-like particle vaccines via spontaneous isopeptide bond formation. We have a project on protein and nanoparticle modification, to bias the induction of neutralising antibodies and overcome limitations of vaccines in the most challenging targets like influenza, HIV and malaria.

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16. Epigenetic control of centromere assembly and inheritance

Supervisor: Associate Professor Lars Jansen

The centromere is a unique chromosomal locus that forms the anchorage point for mitotic spindle microtubules and is essential from chromosome segregation during cell division. Centromeres are defined by a highly unusual chromatin domain in which canonical nucleosomes are replaced by variants bearing the H3 like protein CENP-A. This centromere-specific chromatin forms the foundation for the assembly of the centromere complex.

We have discovered that CENP-A nucleosomes are stably inherited across cell division and assembly of new CENP-A chromatin is dependent on pre-existing CENP-A (Bodor et al MBoC 2013 and Falk et al Science 2015). This CENP-A cycle forms an epigenetic feedback mechanism where centromere inheritance is largely driven by the centromeric chromatin proteins rather than DNA sequence elements. Intriguingly, we previously discovered that assembly of new CENP-A occurs only once per cell cycle upon mitotic exit in early G1 phase. Central to this cell cycle control mechanism are the major cell cycle kinases, Cdk 1 and 2 that we found to inhibit CENP-A assembly in S, G2 and M phases of the cell cycle (Silva et al., Developmental Cell 2012). Loss of Cdk1 and 2 activity in early G1 triggers the assembly process. Further, we found that the targets of these kinases are two key CENP-A assembly factor complexes (Stankovic et al., Molecular Cell 2012).

Key open questions remain. First, once targeted to centromeres how does new CENP-A incorporate into chromatin and, second, how is this process turned off to ensure the centromere does not expand too far into chromatin and third, how is CENP-A marked for stable transmission into subsequent cell division cycles? The DPhil project will revolve at answering these key questions. Key methodologies include mammalian cell culture, microscopy-based fluorescent pulse labeling technologies to visualize both ancestral as well as nascent pools of CENP-A histones, chromatin immunoprecipitation (ChIP),

NGS experiments, mass spectrometry as well as CRISPR-based genome editing to establish cell biological tools.

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17. Deciphering the molecular choreography within bacterial cell envelopes

Supervisor: Prof Syma Khalid

The cell envelope that surround Gram-negative bacteria is a complex tripartite architecture composed of two membranes and the periplasm. The cell envelope provides protection in various forms, against dangers from the external environment. In order to achieve these protective functions, molecular organisation and communication within each of the compartments as well as across all three compartments must be coordinated and regulated. While we know many of the structures of the proteins, lipids and glycans that are involved in these processes, less is known about how they work together.

The Khalid group use computational methods (mostly molecular dynamics simulations) in collaboration with experimental groups (e.g. working in structural biology and biophysics) both within Oxford and outside to study the molecular interactions within the cell envelope of Gram-negative bacteria. We typically construct computational models of the cell envelope by taking structures/homology models of proteins, embedding them in a model of their native environment (membrane or aqueous) and adding key molecules such as the cell wall, osmolytes, water and ions. We then use molecular dynamics to animate the model. The underlying biochemistry is combined with classical physics by the computer program to predict how the molecules should move. The resulting movie is then analysed to explore key molecular interactions. Molecular graphics and images play a big role in our work.

A typical DPhil project would involve working on one or more proteins (often in collaboration with the team that determined the structure of the protein(s)) to understand how the conformational dynamics of the individual proteins and also how they interact with other proteins. The ability to write code or previous experience of molecular dynamics simulations is not a requirement; it is however important that you are enthusiastic about the structures and dynamics of proteins and happy to work with computers.

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18. Discovering how epigenetics regulate gene expression in stem cells

Supervisor: Prof Rob Klose

Controlling how our genes are expressed is fundamental to cell function and development. While the molecular biology revolution of the mid-twentieth century defined the central dogma which states that DNA instructs the production of RNA and then protein, the mechanisms that unpin how the earliest steps of this cascade are controlled, namely how DNA is read and translated into the RNA remains very poorly understood. In eukaryotes, the ability to read DNA sequence is profoundly influenced by histones that package DNA into chromatin, and chromatin constitutes a central epigenetic regulator of RNA production and gene expression. However, our understanding of the mechanisms that enable epigenetic systems to control gene expression remain rudimentary at best.

To address this fundamental problem, we use embryonic stems cells as a model to study how chromatin and epigenetic systems regulate gene expression to ensure stem cells remain pluripotent and can also support cellular differentiation and organismal development.

In the context of this overarching focus of the laboratory, a project is available to examine how proteins that function at CpG island elements (epigenetically defined elements associated with gene promoters in mammals) contribute to regulation of gene expression. The student will use and develop (via CRISPR-based genome engineering) embryonic stem cell lines containing degrons to rapidly deplete epigenetic regulators. They will then use genomic approaches coupled with next generation sequencing (ChIP-seq, RNA-seq, etc.) and/or live-cell imaging to determine how these systems affect chromatin modifications and gene expression. In particular, this will be applied to components of either the Polycomb repressive or Trithorax activator systems, which are paradigms of epigenetic gene regulation in mammals. As the project progresses there will also be an opportunity to dissect how these systems are used to regulate gene expression during cellular differentiation. The student will benefit from extensive support and training in the approaches necessary to tackle these fascinating problems, and will be at the forefront of uncovering how the epigenome shapes gene regulation and genome function.

For more details, see Kloselab.co.uk or our recently published papers:

(1) BAP1 constrains pervasive H2AK119ub1 to control the transcriptional potential of the genome. Fursova NA, Turberfield AH, Blackledge NP, Findlater EL, Lastuvkova A, Huseyin MK, Dobrinić P, Klose RJ. Genes and Development, 2021.

(2) Live-cell single particle tracking of PRC1 reveals a highly dynamic system with low target site occupancy. Huseyin MK and Klose RJ. Nature Communications, 2021.

(3) Cohesin Disrupts Polycomb-Dependent Chromosome Interactions in Embryonic Stem Cells. Rhodes JDP, Feldmann A, Hernández-Rodríguez B, Díaz N, Brown JM, Fursova NA, Blackledge NP, Prathapan P, Dobrinic P, Huseyin MK, Szczurek A, Kruse K, Nasmyth KA, Buckle VJ, Vaquerizas JM, Klose RJ. Cell Reports, 2020.

(4) PRC1 Catalytic Activity Is Central to Polycomb System Function. Blackledge NP, Fursova NA, Kelley JR, Huseyin MK, Feldmann A, Klose RJ. Molecular Cell, 2020.

(5) Synergy between Variant PRC1 Complexes Defines Polycomb-Mediated Gene Repression. Fursova NA, Blackledge NP, Nakayama M, Ito S, Koseki Y, Farcas AM, King HW, Koseki H, Klose RJ. Molecular Cell, 2019.

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19. Detection, Signalling and Repair of DNA Damage

Supervisor: Prof Nick Lakin

Background

Genomes are under continual assault from a variety of agents that cause DNA damage. Preserving genome integrity through repair of this damage is critical for human health and defects in these pathways leads to a variety of pathologies including neurodegeneration and cancer. Therefore,

understanding the mechanistic basis of DNA repair will provide insights into the causes of these conditions and, importantly, strategies for their treatment. Our research aims to understand the mechanistic basis of DNA repair, with specific reference to how a family of proteins called Poly(ADP-ribose) polymerases (PARPs) regulate these processes. Our long-term vision is to exploit this knowledge to treat a variety of diseases including neurodegeneration and cancer.

Research Projects
PARPs are a cornerstone of the DNA damage response that promote repair of breaks in the DNA helix by modifying proteins at the damage site with ADP-ribose - a process known as ADP-ribosylation. These pathways are critical to maintain genome integrity and are attractive clinical targets, with PARP inhibitors being used to treat breast and ovarian tumours. However, despite the success of PARP inhibitors in the clinic, the mechanistic basis of how PARPs regulate DNA repair is unclear, representing a fundamental barrier to refine and broaden the application of these agents in the clinic. The overall goal of our research is to address these fundamentally important questions by defining the mechanistic basis of how PARPs regulate a variety of DNA repair mechanisms. DPhil projects are available that integrate cutting edge genome engineering, proteomics and cell biology to address the following:

a) How do PARPs become activated in response to DNA damage?
b) What proteins do they modify at sites of DNA damage?
c) How do these modifications regulate DNA repair?

These multidisciplinary hypothesis-driven research projects will increase our understanding of how PARPs regulate DNA repair and provide critical information to develop novel strategies that target PARPs to treat a variety of pathologies.

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20. *Drosophila* as a model to study neurodegeneration and investigating the interaction between *Leishmania* and its insect vector

Supervisor: Prof Petros Ligoxygakis

A. Sandflies transmit *Leishmania* parasites which cause leishmaniasis to humans: The second deadliest parasite, behind only *Plasmodium* (malaria). Expanding our understanding of how *Leishmania* avoid the insect vector's immune response, how they progress through their life cycle in the vector and how they pre-adapt for transmission to a human are key to determining how best to block transmission. This is particularly pertinent, as the areas affected by leishmaniasis are defined by the vector range – a range that is expanding in Europe, the Americas, and Southern Asia due to climate change. The project will generate *Leishmania* mutants with a molecular "barcode" and then we will test them for their ability to establish infection in sandflies.

B. Identifying DNA sequence variation in immune genes has allowed us to better predict the chances of an individual developing neurodegenerative disease such as the Late Onset Alzheimer's Disease (LOAD). These risk genes are connected to inflammation in the immune cells of the brain, called microglia. Nevertheless, separating cause and consequence is still an intractable problem since neurodegeneration itself increases inflammation while how the immune system responds to such changes may determine whether neurons live or die. For rapid progress in assessing genetic risk, we will use the fruit fly *Drosophila*, which has a 2-month lifespan. We will test loss or alteration of function in fly homologues of human LOAD risk genes in glia, which has the same immune and

neurological properties as mammalian microglia. We will do this in 1) healthy-aged flies and 2) flies genetically predisposed to higher levels of inflammation. We will ask how risk genes influence increasing levels of age-dependent neurodegeneration going from condition 1 to 2 by looking at lifespan, age-dependent brain damage and neurological phenotypes in locomotion, sleep patterns, and circadian rhythm. Given the conservation in immunity and basic brain development between flies and mammals, we are hoping to uncover mechanisms underscoring LOAD and provide space for testing drugs at the whole animal level.

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21. SMC complex function in chromosome condensation, segregation and regulation of gene expression

Supervisors: Prof Kim Nasmyth and Dr Madhusudhan Srinivasan

To ensure accurate chromosome segregation, monumental topological problems posed by the sheer size and physical properties of DNA are overcome by a set of highly conserved ring-shaped DNA motors, namely condensin and cohesin. Chromosomal DNA is weaved into discrete chromatids during mitosis by condensin, this keeps the DNA free from entanglements. To ensure their equal segregation, replicated sister chromatids are held together by cohesin from S phase until their disjunction in anaphase. In addition to its role in sister chromatid cohesion, cohesin also organizes the interphase chromatin into discreet domains, a process that is important for transcriptional regulation, DNA repair and generation of immunoglobulin diversity. Our lab focuses on understanding the molecular processes that result in chromosome organization and sister chromatid cohesion.

To weave DNA into chromosomes as well as to organize interphase chromatin, condensin and cohesin are thought to trap small DNA loops and progressively enlarge them in an ATP hydrolysis dependent manner. This activity, called Loop Extrusion (LE) has been recently reconstituted in vitro using both cohesin and condensin, this opens avenues for in depth understanding of the mechanism of LE. One of the most popular models for LE, the so called “scrunching model” supposes that the Smc complex undergoes dramatic conformational changes involving cycles of folding and unfolding of the cohesin/condensin molecules to inchworm along the chromosome. Our current research is focused on understanding the molecular mechanism of DNA loop extrusion by cohesin and condensin. To do so, we use biochemical reconstitution, Cryo-EM, Single molecule biophysical and microscopy assays, and in vivo validation in living yeast cells using protein crosslinking and DNA topology analysis.

Sister chromatid cohesion is established and maintained by co-entrapment of sister DNAs within cohesin rings. We have recently shown that cohesion is established during DNA replication by two different pathways, each of which depend on a different set of proteins associated with the replication fork. We have recently demonstrated that one of the two pathways for cohesion establishment works by converting chromosome associated cohesin molecules into cohesive structures during DNA replication. To understand the molecular mechanism of this ‘cohesin conversion pathway’, we are developing an in vitro single molecule assay to study cohesion establishment during replication using the Xenopus cell free system. We have purified and fluorescently labelled the Xenopus cohesin complex and are planning to analyse the fate of the DNA associated cohesin molecules upon encounter with the replication fork containing fluorescently labelled MCM helicase. In parallel, by combining DNA replication and cohesin loading with purified components, we intend to reconstitute cohesion establishment in vitro. Our objective is also to visualize the intermediate steps of cohesion establishment by cryo-EM.

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22. i. Structural basis for receptor mediated trafficking in the early secretory pathway
ii. Structural basis for lysosomal protein function in Parkinson's disease
iii. Molecular mechanisms controlling glycosylation and protein acetylation in the ER and Golgi

Supervisor: Prof Simon Newstead

Project 1 – Structural basis for receptor mediated trafficking in the early secretory pathway. This project will seek to understand the molecular basis for receptor mediated trafficking, including cargo recognition, signal discrimination and receptor regulation. We currently have a mature project focused on the KDEL receptor, with both a PDRA and DPhil student working on this protein. We wish to recruit a DPhil student to study either the Erv41/46 receptor, responsible for non-KDEL mediated protein retrieval from the Golgi or SURF4, responsible for trafficking newly synthesised proteins from the ER to the Golgi. The student will be trained in state-of-the-art structural biology, cellular imaging, and membrane protein biochemistry techniques. A key goal of the project will be the selection of synthetic nanobodies for structural and cellular imaging. We are developing novel ways to utilise nanobodies to track receptor movement in live cells and correlate this with structural and biophysical information.

Relevant papers:
Brauer P. et al. (2019) Structural basis for pH-dependent retrieval of ER proteins from the Golgi by the KDEL receptor. Science 363, 1103-07.
Gerondopoulos A. et al. A signal capture and proofreading mechanism for the KDEL receptor explains selectivity and dynamic range in ER retrieval. eLife 2021;10:e68380.

Project 2 – Structural basis for lysosomal protein function in Parkinson's disease. The aim of this project is to address the molecular mechanisms underpinning the role of channels and transporters in Parkinson's disease (PD). PD and frontotemporal dementia/amyotrophic lateral sclerosis (FTD/ALS) are insidious and incurable neurodegenerative diseases that represent a significant burden to affected individuals. Both PD and FTD/ALS are defined at postmortem by the presence of protein aggregates and the loss of specific subsets of neurons. Lysosome dysfunction has been identified as playing an important role in the aetiology of PD, particularly with respect to dysregulation of calcium, potassium and amino acid homeostasis. However, the molecular mechanisms linking lysosomal dysregulation, protein aggregation and neuronal cell death have not been firmly established. Using a combination of structural biology (cryo-EM) and targeted nanobody binders, we will use in cell biochemical techniques to dissect out the roles of lysosomal channels and transporters. The student will join a current DPhil and PDRA studying these systems.
Relevant papers:
Wallings RL. Et al. Lysosomal Dysfunction at the Centre of Parkinson's Disease and Frontotemporal Dementia/Amyotrophic Lateral Sclerosis. Trends Neurosci.42. 899-912 (2019).

Project 3 – Molecular mechanisms controlling glycosylation and protein acetylation in the ER and Golgi. This project seeks to understand how sugar molecules are transported within human, fungal and parasite cells. Several pathogenic organisms use sugary coats to evade our immune system causing widespread diseases. Several fungal species, in particular Candida albicans and Aspergillus fumigatus can establish infections in patients undergoing organ transplant or chemotherapy. Yeast infections cause several million deaths each year worldwide and can establish chronic yeast infections

in healthy patients, known as thrush. In the developing world, several species of parasites, called trypanosomes, use similar sugar coats to hide from immune cells in humans and cattle, causing devastating diseases in both. Fortunately, the type of sugar that these organisms need to create these sugary defence systems are not present in human cells, making them attractive targets for drug development. This project builds on previous research in the group reporting the first structure for a fungal nucleotide sugar transporter, Vrg4. Using state of the art structural and biochemical methods we are developing small molecule inhibitors and antibodies targeted at fungal NSTs. Our aim is to establish a novel antifungal agent to fight the growing threat of fungal disease.

Relevant papers:

*Parker JL, **Newstead S**. (2017) Structural basis of nucleotide sugar transport across the Golgi membrane. Nature 551, 521-524*

*Parker JL, Corey RA, Stansfeld PJ, **Newstead S.** (2019) Structural basis for substrate selectivity and regulation of nucleotide sugar transporters in the lipid bilayer. Nat Commun. 10, 4657.*

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23. Evolution of epigenetic mechanisms: implications for cancer

Supervisor: Associate Professor Peter Sarkies

DNA methylation is a highly conserved epigenetic modification which, in mammals, is fundamental to establishing and maintaining cell identity during development. However, DNA methylation evolves very rapidly across different species- most strikingly DNA methylation has been lost altogether is several independent lineages including model organisms such as the nematode worm C. elegans, the fruit fly Drosophila melanogaster and yeast (Saccharomyces cerevisiae). Intriguingly, rapid evolution of DNA methylation levels and genome-wide distribution is also seen in human cancer, with many cancers displaying markedly reduced DNA methylation (hypomethylation). Recently we discovered that a potential reason why DNA methylation is so frequently lost across evolution is that DNA methyltransferase enzymes carry a "cost". In addition to their usual activity introducing 5-methyl-cytosine, DNA methyltransferases have the propensity to damage DNA, introducing alkylation damage in the form of the highly toxic DNA lesion 3-methyl-cytosine. DNA methyltransferases co-evolve with the DNA repair enzyme ALKB2 across species, because ALKB2 repairs 3-methyl-cytosine in DNA (Rosic et al., Nature Genetics, 2018; Lewis et al., PLoS Genetics 2020). In this project the student will explore the implications of this finding for human cancer, using Acute Myeloid Leukemia (AML) as a model. We will test the hypothesis that DNA hypomethylation arises due to the danger posed by DNA methyltransferase activity, by following the evolution of DNA methylation levels in human cancer cells in the lab following deletion of ALKB2 using CRISPR technology. We will also use CRISPR technology to induce varying levels of ALKB2 reduction, enabling us to quantitatively compare the effect on DNA methyltransferase activity over time. Additionally we will explore the potential relevance of this to cancer treatment by testing the sensitivity of cancer cells with varying levels of DNA methyltransferase activity to alkylating agents. The student will learn cell culture, CRISPR-Cas9 mediated genome editing, high-throughput sequencing and mathematical modelling, using all these techniques to gain potential insight into the causes and consequences of epigenetic dysfunction in cancer.

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24. Advanced cellular imaging to study functional nuclear organization

Supervisor: Dr Lothar Schermelleh

Three-dimensional (3D) chromatin organisation plays a crucial role in regulating mammalian genome functions such as RNA transcription, replication and DNA repair. Population-based sequencing approaches (e.g. Hi-C) have highlighted the compartmentalisation of chromatin into 0.5-1 MB sized topologically associating domains (TADs). However, many of the physical features at the single-cell level are still underexplored. Our primary research objective is to identify principles and underlying mechanisms of functional chromatin organisation in mammalian cells. Specifically, we aim to understand the interplay between biophysical forces, epigenetic memory, and cohesin complex activity to modulate cell-type-specific transcriptional programmes by directly visualising dynamic nuclear organisation and gene activity in living or 3D-preserved cells. To this end, we employ a combination of genetic editing with innovative *in vivo*/*in situ* fluorescence labelling and super-resolution imaging approaches. Our activities are closely linked to the Micron Oxford Advanced Bioimaging Unit and supported by our well-established ties to leading chromatin and epigenetic research groups within the Department and across Oxford.

For a PhD project, we seek (an) enthusiastic, proactive and adventurous student(s) eager to immerse in the latest imaging technologies to study topographical and biophysical aspects of gene regulation in an interdisciplinary environment. The topic of the project can be along the lines of either (1) studying the effect of directed phase separation on mesoscale domain organisation and transcriptional modulation, (2) examining mechanisms of gene reactivation during pathological de-differentiation processes (e.g. liver fibrosis, EMT), or (3) examining enhancer-promoter interactions e.g. in the alpha-globin locus, using multiplexed RNA-DNA-Immuno-FISH and correlative 3D super-resolution light end electron microscopy. The details of any project will be subject to personal preferences and be worked out closer to start date.

Main techniques: Mammalian tissue culture, molecular cloning, transfection, immunofluorescence labelling, fluorescence in situ hybridisation (DNA/RNA FISH), super-resolution structured illumination microscopy, single-molecule imaging, focussed ion beam scanning electron microscopy (FIB-SEM), computational image analysis.

Relevant papers:
Rodermund L,…, Schermelleh L#, Brockdorff N#. 2021. Time-resolved structured illumination microscopy reveals key principles of Xist RNA spreading. *Science*. 372: eabe7500
Miron E, …, Schermelleh L. 2020. Chromatin arranges in chains of mesoscale domains with nanoscale functional topography independent of cohesin. *Science Advances* 6, eaba8811.
Ochs F, …, Schermelleh L#, Lukas J#, Lukas C. 2019. Stabilization of chromatin topology safeguards genome integrity. *Nature*, 594: 571-574.
Schermelleh L et al. 2019. Super-resolution microscopy demystified. *Nat Cell Biol* 21: 72-84.
Schermelleh L et al. 2008. Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. *Science*. 320: 1332-6.

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25. Imaging how bacteria adapt to stress: From DNA damage to mutations

Supervisor: Associate Professor Stephan Uphoff

Fast and accurate repair of DNA damage is essential for the survival and genome stability of all organisms. Indeed, DNA damaging agents are highly effective as antibiotic treatments against

bacterial pathogens and as cancer therapies. However, a major side effect of the DNA lesions caused by these treatments is the formation of mutations that promote disease progression and eventually lead to drug resistance. Research in the Uphoff lab aims at understanding this process from the molecular scale to the cellular level, ultimately describing and dissecting the mechanisms that drive evolution of cell populations.

A key aspect of our work is the development of advanced fluorescence microscopy techniques to visualise molecular processes inside living cells. For example, we use single-molecule imaging to track the movement of DNA repair enzymes as they search for damage sites, and we use fluorescent markers to "see" the creation of mutations in living cells. We also apply microfluidic technologies to trap and manipulate individual cells.

The adaptation of cells to stress conditions is a multifaceted process, involving many aspects of cell biology. Rather than studying particular mechanisms in isolation, our projects aim at understanding how DNA repair and mutagenesis function in the context of the cell, and in relation to gene regulatory responses, DNA replication, metabolism, and the cell cycle. Our research focuses on bacteria. Beyond their usefulness as tractable model organisms, bacteria play crucial roles in human health and the environment. Bacterial infections and rising antibiotic resistance are major burdens to society, impacting millions of lives and causing significant economic loss worldwide.

The Uphoff group is a multi-disciplinary team with diverse expertise in biochemistry, molecular biology, genetics, and physics. DPhil projects typically involve the development of new research methods to address fundamental biological questions. DPhil students in the group gain experience in a range of skills beyond classical biochemistry, such as super-resolution microscopy, microfluidics, and programming for quantitative data analysis. The exact project focus will be discussed and matched to the interests and experience of the applicant.

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26. Transcriptional and post-transcriptional regulation of gene expression in eukaryotes

Supervisor: Dr Lidia Vasilieva

Recent technological advances have revealed a plethora of diverse long non-coding (nc) RNA molecules produced from eukaryotic genomes. Mutations in non-coding regions of the genome and altered expression of ncRNAs underpins a number of pathologies including cancer. Yet, very little is known about mechanisms involved in production of ncRNAs preventing us from understanding their role in health and disease. Our previous work lead to discovery that in contrast to mRNAs, nc transcripts rely on distinct and poorly understood mechanisms that control their RNA polymerase II (Pol II) transcription. As a result, ncRNAs are non-polyadenylated and targeted by the cellular RNA degradation machinery, RNA exosome.
The PhD project aims to fill the key gaps in our understanding of the transcriptional mechanisms involved in regulation of ncRNA. This will be achieved through the Aims 1-3. A PhD student will identify and characterise transcription complexes linked to production of ncRNA biochemically (Aim 1) and investigate how these complexes are recruited to Pol II during transcription and how they control biogenesis of ncRNA in human cells using state-of-the-art genomic approaches (Aim 2 and 3).

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27. Replication blockade and genome stability

Supervisor: Prof Matthew Whitby

A hallmark of ageing is the accumulation of genomic mutations and rearrangements through mistakes made during the normal processes of DNA replication, repair and chromosome segregation. It is thought that this gradual corruption of the genome results in gene regulatory changes, which cause cellular degeneration and functional decline that ultimately drives ageing and its associated diseases. Accordingly, the pace of genomic deterioration is likely to be a key determinant of healthy lifespan, which is strongly influenced by both environmental and genetic factors. Through a complete understanding of how mutations and genome rearrangements arise, as well as the factors that mitigate their occurrence, we will be better placed to develop new approaches to improve the healthy ageing of humankind.

Conflicts between replication forks and single-strand DNA breaks (SSBs) and protein-DNA complexes (PDCs) are a major threat to genome stability through their potential to cause fork collapse and failure of complete genome duplication. By exploiting state-of-the-art fission yeast genetics, advanced microscopy, protein biochemistry, advanced proteomics and genomic approaches, we aim to elucidate the different pathways that limit genome instability arising from replication fork-SSB/PDC conflicts, and how pathway choice is influenced by the nature and context of the SSB/PDC. This work will make a seminal contribution to our understanding of how genome deterioration, and consequent ageing and age related disorders, is driven by problems that arise during S phase.

For more information about the Whitby lab see: https://whitbylab.com

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28. Computational approaches to receptor dynamics and ligand binding

Supervisor: Prof Phil Biggin

Projects are likely to be formulated around transporter proteins and or ion channels. Interested students should contact Prof Biggin directly for advice that depends on their specific backgrounds.

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29. Functional ramifications of outer membrane organisation in Gram-negative bacteria

Supervisor: Prof Colin Klanthous

The outer membrane (OM) of Gram-negative bacteria adapts to a changing environment, supports cellular integrity, adheres to host cells during pathogenesis and is a major factor in antibiotic resistance. The textbook description of the OM is as a well-mixed asymmetric bilayer in which lipids and proteins are freely interspersed. Recent work from our laboratory on the model organism *Escherichia coli* has shown that this is far from reality. Using bacteriocins as specific labels, we have shown that outer membrane proteins (OMPs) are instead organised both temporally and spatially into supramolecular islands (Rassam et al (2015) *Nature*; Rassam et al (2018) *Nat Commun*). OMP islands move as tectonic plates towards the old poles of growing cells where OMP biogenesis is absent. As a result, the bacterium replenishes the protein composition of its OM simply by division. What is less well

understood however is how such organisation impacts other aspects of the organism's biology.

The aims of this project are to use experimental and computational methods to probe the functional consequences of OMP organisation in bacteria. The Kleanthous & Khalid labs have collaborated extensively using similar integrated approaches (e.g. Szczepaniak et al (2020) *Nat Commun*). Specifically, the project will investigate how OMP island formation impacts antibiotic expulsion through TolC-efflux channels and cell envelope stabilisation via the peptidoglycan-binding protein, OmpA. Experimental approaches will use a combination of photoactivated crosslinking coupled with LC/MS-MS, and super-resolution fluorescence microscopy in live bacteria (e.g. White et al (2017) *PNAS*). Computational approaches will exploit recently developed coarse grain and atomistic molecular dynamics simulations of the bacterial cell envelope (e.g. Khalid et al (2019) *Acc Chem Res*).

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30. From structure to function: characterizing three novel putative lipid transport proteins and their role in intracellular lipid flux

Supervisor: Prof Benoit Kornmann

Csf1, Fmp27 and Ymr117w are three proteins for which very little is known. They are conserved in animals and fungi; in humans they are involved in diseases; yet, their molecular function remains elusive. Homology searches and AI-based protein structure modelling suggests these are lipid-transport proteins, that is, proteins that exchange lipid molecules between two membranes, using a large hydrophobic tunnel at their core. The project will consist in studying them in yeast. Where are they localized, and between which membranes? What lipid do they transport? What happens to the cell when these proteins are missing? The goal will be to glean molecular understanding of the function of these factors to understand the pathophysiology of their associated diseases. This project will require learning state-of-the-art technologies ranging from microscopy, lipid mass spectrometry and functional genomics using next-generation sequencing.

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31. Compartmentalisation via liquid-liquid phase separation in cells

Supervisor: Dr Tim Nott

A central organising principle of eukaryotic cells is the compartmentalisation of biochemical reactions. The most widely known method for generating cellular compartments is to use membranes as boundaries. However, not all processes are organised in this fashion. Cells also contain a variety of compartments such as nucleoli, Cajal bodies, P-granules and nuage that lack a membrane boundary. Often spherical in appearance and predominantly associated with processing nucleic acids, these membraneless organelles are highly dynamic, and can rapidly assemble and dissolve with changes to the cellular environment.

It has recently been discovered that certain membraneless organelles behave as liquid-like droplets of concentrated protein and RNA, and form through the process of liquid-liquid phase separation. Importantly, the interiors of membraneless organelles are unique solvent environments, and display surprising, emergent biochemical properties. For example, synthetic membraneless organelles

engineered from intrinsically disordered proteins can selectively absorb and melt nucleic acids without the input of ATP or conventional enzymatic protein domains.

Overall, the Nott Lab aims to study this little explored but incredibly widespread form of compartmentalisation in cells. This work will begin to describe how organisms make use of solvent phases present in the interior of membraneless organelles that are fundamentally distinct from bulk-water. Drawing on aspects from cell biology, structural biology, polymer theory and advanced fluorescence microscopy we will gain fundamental insight into how cells spatially regulate and process genetic and epigenetic information. More info at: www.nottlab.com. Project areas include:

1. Protein and nucleic acid structure inside membraneless organelles
Structural biology-oriented projects will focus on understanding how protein structure and interactions are modulated inside phase-separated membraneless organelles. Protein structure will be probed using intramolecular FRET between fluorophores at specific amino acid positions or by using differentially stable GFP derivatives that fluoresce only when folded. Protein-protein or protein-nucleic acid interactions will be measured using intermolecular FRET and compared to measurements made outside the organelle environment. Structural biology techniques such as NMR, ESI-mass spectrometry, ITC and SEC-MALLS will complement microscopy experiments.

2. Partitioning and biochemical properties of membraneless organelles
The biochemical consequences of performing reactions inside proteinaceous solvent environments are only just beginning to be explored. Projects in this area will involve characterising the liquid-liquid phase transitions that underpin membraneless organelle formation and measuring how fluorescently labelled biomolecules are partitioned and trafficked into/out of model membraneless organelles. The work will be interdisciplinary and involve characterisation of membraneless organelle proteins using biophysical techniques, fluorescence microscopy and developing novel enzyme assays.

3. Tuning the properties of membraneless organelles
Just as the primary amino acid sequence of globular proteins encode the necessary information for the polypeptide to fold into a specific three-dimensional structure, the primary sequence of some intrinsically disordered proteins encode functional liquid-liquid phase separation behaviour. Through mutagenesis and varying environmental conditions, we will investigate how the biochemical activity of model membraneless organelles can be tuned and modified.

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32. Proteins at the centre of human health and disease

Supervisor: Associate Professor Jason Schnell

Our laboratory seeks to understand the molecular structure and function of proteins, including those involved in human health and disease. Projects in two main areas are possible. (1) We have a long-standing in interest in determining the molecular mechanisms by which Influenza virus proteins function. We are studying the protein-protein and lipid interactions of ‘flu’ proteins to better understand their role in the virus life cycle and to identify potential therapeutic targets. (2) More recently, we have begun to study the structure and function of chaperone proteins, especially J-domain proteins, to understand how they interact with misfolded/unfolded clients and with the HSP70 machinery.

A central technique of our laboratory is solution nuclear magnetic resonance (NMR) spectroscopy, which allows atomic-level studies of protein structures and their interactions. NMR can be uniquely

informative in situations where the molecular conformations or interactions are dynamic or heterogeneous. However, we also use a wide variety of other biochemical and biophysical tools to support our investigations. We also have collaborations with various research groups around the world including virologists, cell biologists, and computational biologists.

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