



***BBSRC 4-year PhD Interdisciplinary Programme
in Structural, Computational and Chemical Biology***

At UCL and Birkbeck

PhD projects 2010



The Development of a New Pro-Drug Strategy

Ref: BBSRC33

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This PhD will involve the synthesis and application of functionalized maleimides designed to cleave on entry into cells. Drugs that are inactive on entry into the body but become activated upon reaching their destination are known as pro-drugs. Pro-drugs are extremely important in cutting-edge drug-development (such as magic-bullet therapy) as they prevent many undesired side effects and maximise efficacy. Currently only a few chemical motifs have been developed to cleave on entry into cells and this limits the options available when designing drug-conjugates. This project will aim to develop a series of new pro-drugs and to prove that they cleave in cells. The research has the potential to make a significant contribution to the design of future therapeutic agents.

Rotation project 1: Design and synthesis of maleimide conjugates that will fluoresce upon cleavage in cells. (J.Baker's and S.Caddick's lab)

Rotation project 2: Treatment of various cells with the maleimide-conjugates synthesised in rotation 1. Following cleavage of the conjugates the cells will light up and this fluorescence will be measured, proving the potential for this new pro-drug strategy. (A.Tinker's lab)

Activation of the IKK kinase complex

Ref: BBSRC34

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The multi-subunit IKK kinase complex is a key component of the canonical NF- κ B transcriptional pathway whose persistent activation has been linked to the majority of human cancers and inflammatory diseases. Although the mechanism by which the IKK kinase complex becomes activated is unknown, it is thought to pivot on a conformational change in its regulatory subunit IKK γ . Having recently obtained the crystal structure of IKK γ in its activated form when bound to a viral oncoprotein vFLIP, this project will focus on IKK γ in its inactive state (using protein crystallography together with site directed mutagenesis) and the important transition between the two using electron spin resonance (ESR). The way in which naturally occurring IKK γ mutations implicated in cancer and certain immune disorders influence the conformational transition will also be investigated.

Rotation project 1: Optimisation of crystallisation conditions that have already been established for one IKK γ construct and the spin labelling of single cysteine containing constructs that are already available for ESR studies. (T. Barrett's lab)

Rotation project 2: Preliminary ESR analysis of the labelled samples will be performed. (C.Kay's lab)

Characterisation of NHE proteins and their role in protein trafficking

Ref: BBSRC35

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The eukaryotic NHE sodium/ proton exchangers are a large family of trans-membrane proteins involved in the electroneutral exchange of sodium (or potassium) ions for protons across membranes. These proteins exchange ions along concentration gradients in the cell and are involved in many diverse cellular processes including the regulation of intracellular pH and cell volume, absorption of sodium into epithelia, salt tolerance, cell adhesion, cell proliferation, organelle biogenesis and protein trafficking. Mutations in NHE6 can cause X-linked mental retardation, while NHE9 has recently been linked with autism. The project will involve further characterisation of the intracellular NHEs (mammalian NHE6, NHE7, NHE8, NHE9 and yeast Nhx1p) and a study of their role in protein trafficking in mammalian cells and yeast.

Rotation project 1: To build models of the structures of the intracellular NHEs using the known structure of bacterial NhaA, and to identify potential key residues.* (A.Martin's lab)

*Please note: a background in computational biology is not necessarily required for this project.

Rotation project 2: To use the models generated in project 1 to mutate residues important for intracellular NHE function and to test these using site-directed mutagenesis followed by functional assays. (K.Bowers' lab)

NMR Studies of the Co-translational Folding: Ribosome Nascent Chain complex of an ankyrin repeat

Ref: BBSRC36

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The fundamental process of protein folding is intricately linked to protein synthesis on the ribosome. *In vivo*, the newly synthesized 'nascent chain' emerges from the ribosome one amino acid at a time and explores conformations that eventually lead to its folded and functional structure. Biological NMR spectroscopy and mass spectrometry will be used to obtain snapshots of the process of formation of a simple alpha-helical motif that occurs in clinically important ankyrin repeats as it emerges from its parent ribosome. This will be compared to the available data of folding of the isolated domain which show a C-terminal nucleation folding mechanism to provide fundamental insights into how co-translational folding differs from *in vitro* refolding.

Rotation project 1: NMR spectra of ribosome nascent chain complexes (RNCs) of several lengths of the emergence of an ankyrin repeat will structure and dynamics of the folding of the nascent polypeptide during its synthesis. (J. Christodoulou's lab)

Rotation project 2: Hydrogen-deuterium exchange methods using mass spectrometry will be used on the RNCs of ankyrin to provide conformational information on the folding process. The conformations of the emergent protein will be probed by exposure of the RNC to deuterium and then 'trapped' by subsequent quenching and then released via antibiotics. (A. McKay's lab)

Synthesis of Perfluorinated lipids and characterisation by EM for MR imaging applications

Ref: BBSRC37

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Novel lipids and targeting peptides have been prepared for use in a highly efficient ternary synthetic vector. Structural features such as PEG shielding groups and cleavable linkers have been incorporated to enhance *in vivo* delivery properties. In current collaborations our aim is to prepare novel liposomes containing ^{19}F for MR imaging applications that have enhanced MR contrast properties but are suitable for use *in vivo*. To date, few syntheses of perfluorinated lipids have been described and in this work versatile, stereoselective synthetic routes will be developed. Importantly, vesicles, lipoplexes and lipopolyplexes generated using the fluorinated compounds will be studied using electron microscopy (both negative stain and cryo-EM) to directly visualise the physical effects of the introduced modifications. These studies will establish a relationship between delivery and imaging properties, macromolecular organisation and molecular structure.

Rotation project 1: Development of new methods for the synthesis of and characterisation of novel perfluorinated lipids (H.Hailes' lab)

Rotation project 2: Visualise novel liposomes by negative stain electron microscopy to characterise consequences of chemical modifications on liposome structure (C.Moores' lab)

Structure-function of Chlamydia inclusion proteins

Ref: BBSRC38

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes sexually transmitted disease and blindness (trachoma). It replicates within a specialized membrane-bound compartment ('inclusion') inside host cells that selectively intercepts secretory traffic but remains distinct from the endocytic pathway. *Chlamydia* delivers multiple hydrophobic virulence effectors into the inclusion membrane, which share no sequence homology with prokaryotic or eukaryotic proteins, likely to subvert host signalling, cytoskeletal dynamics and cytokinesis. Despite their critical role little is known about their structure or function. The project will involve characterizing the structure and function of selected inclusion proteins and their host targets using a combination of biochemical, cell biology and imaging techniques together with infection models.

Rotation project 1: To express candidate inclusion proteins in yeast and mammalian cell systems and characterize the arising phenotypes. (K.Bowers' lab)

Rotation project 2: To express corresponding inclusion proteins studied in project 1 in *E.coli* and develop purification protocols to allow biochemical and biophysical analysis. (R.Hayward's lab)

[A hybrid approach towards improving the prediction of protein structures](#)

Ref: BBSRC39

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Structural genomics initiatives aim at solving structures for representative members of all protein families, so that most of the remaining proteins can be modeled based on their similarity to the known structure. However, current protein structure modelling methods suffer from errors, especially when the homologs are only remotely related or if the protein has more than one domain. In this project, we propose to develop a new hybrid experimental/computational method to improve the prediction of the structures of proteins. A distance map of surface-accessible amino acid residues will be generated by extensive chemical cross-linking, followed by identifying cross-linked peptide sequences using high-resolution mass spectrometry. We will then model the structure of the protein by combining existing structural prediction methods with the generated experimental restraints. The method will be applied to a variety of proteins, in particular doublecortin which is involved in neurodegenerative diseases and for which the complete structure is not available.

Rotation project 1: The student will be trained in generating distance-map data for a model protein of biomedical relevance using cross-linking, proteolysis, isolation, and mass spectrometry (S.Howorka's lab).

Rotation project 2: The student will be trained in computational biology with focus on protein structure prediction methods (M.Topf's lab).

[Development of Novel Computational Approaches for Protein Engineering and Design](#)

Ref: BBSRC40

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The main aim of this project will be to develop novel tools for computational protein engineering and design. In particular we are interested in exploring the possibility of altering the thermostability and efficiency of enzymes, with the exemplar case here being the bacterial arsenite oxidases. In this project we will investigate a knowledge-based approach to the problem of computational protein design – using statistical techniques along with machine learning to quickly identify likely good designs which can then be quickly evaluated using more detailed atomic simulations. One particular area of interest is to use machine learning to analyse patterns of mutation in naturally occurring proteins within a family where a physical or chemical property varies across the family. A key aspect of the methodology we will develop will allow particular sequence changes to be correlated to changes in stability (e.g. thermostability). Other aspects of the methodology will extend recent work we have done to characterise “fold determinant residues” in protein families i.e. automatically identifying residues which are crucial for stabilising the overall fold of a protein.

Rotation project 1: Initial investigation of methods likely to be utilised in the final tool e.g. benchmarking of automatic identification of fold determining residues and residues correlated with natural adaptation across protein families (D.Jones' group)

Rotation project 2: Student will assist in the ongoing studies of the exemplar enzyme family (arsenite oxidase) e.g. purification and analysis of thermostability of naturally occurring enzymes (J.Santini's lab)

New Chemistry to Explore Selectivity in Chemically Induced Protein Thioesterification Ref: BBSRC41

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Native chemical ligation (NCL) is a powerful chemical reaction for the production of modified proteins. Recently we observed a novel reaction whereby peptide and protein thioesters (key components in the reaction) can be formed through selective chemical protein cleavage. The aim of this project is to combine organic synthesis and molecular biology to determine the parameters which make this an efficient process. Having optimized the reaction we will prepare a panel of novel peptides which will be refolded and tested for antimicrobial and chemotactic properties against pathogenic bacteria. A further aspect of this project is to use bioinformatics to analyse protein sequences for favourable "motifs" for this reaction and evaluate the incidence of such motifs in various organisms.

Rotation project 1: Synthesis of antimicrobial peptides by native chemical ligation (D. Macmillan's lab)

Rotation project 2: Basic programming in perl within ISMB as preparation for high-throughput protein sequence analysis. (K.Bryson's lab)

Synthesis and Biology of Disruptors of the E. Coli PapG Adhesin with the Human Kidney Receptor Ref: BBSRC42

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E. coli attach to human kidney cells, leading to kidney infections. The adhesin PapG at the tip of *E. coli* mediates attachment of the bacteria to the tetrasaccharide GbO4 (Figure, left) on the human kidney (Waksman *et al.*, *Cell* 2001, 105, 733). The aim is to synthesise and evaluate the first synthetic disruptor (Figure, right) that will bind in the cleft of PapG, thereby preventing bacterial recognition. The Marson group has identified a potent novel ligand (Figure, right) that should displace GbO4. The three segments of the ligand will be synthesised and evaluated independently, before combination to give the complete ligand. Iterative optimisation of the segments and ligand will furnish a drug-like disruptor of PapG function supported by crystallography.

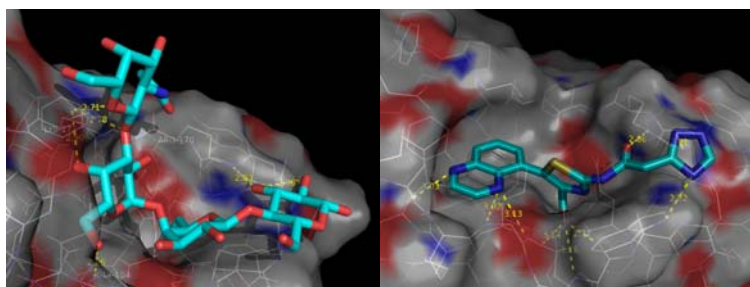


Figure. Docked and minimised binding to PapG of (a) GbO4 (b) a lead inhibitor.

Rotation project 1: The three major segments ('needles') of the novel ligand (Figure, right) will be synthesised (C. Marson's lab).

Rotation project 2: The 'needle' fragments will be evaluated in a PapG-binding assay based on surface plasmon resonance (SPR) as implemented by Biacore (G. Waksman's lab).

Analyzing and predicting metal binding sites in proteins

Ref: BBSRC43

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Metal binding is critical to the structural integrity and/or function of many proteins including key enzymes. Understanding metal binding will allow engineering of metal binding sites and prediction is key for low resolution structures (such as from structural genomics) where binding sites may not be resolved. This project will build on our previous work on predicting metal binding sites. We will create an automatically maintained database of metal binding sites in the PDB and, through analysis and machine learning, will implement novel prediction methods. In collaboration with Steve Perkins, who has identified a zinc binding site at an interface in Factor H, a particular focus will be on binding sites at chain interfaces, especially important where the complex is unsolved.

Rotation project 1: The student will become familiar with the existing tools and analyses and extend that work to predict metal binding sites in Factor H. (Martin group)

Rotation project 2: The student will test the results of the prediction work using site-directed mutagenesis and analytical ultracentrifugation on Factor H (Perkins lab)

Kinesin motor proteins in human parasites and their role in malaria

Ref: BBSRC44

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Kinesins are a superfamily of ATP-dependent, microtubule-based nano-motors that have essential, diverse roles within cells and are potential targets for anti-parasite drugs. The aim of this project is to investigate the function of kinesin motors in *Plasmodium*, the parasite that causes malaria and kills more than 1 million people a year. The recently sequenced genomes of several malaria species have enabled identification and preliminary characterisation of 10 putative kinesins. The project will involve computational, biochemical and structural characterisation - including cryo-electron microscopy - of *Plasmodium* kinesins; additional collaborations will enable dissection of kinesin functions in parasites. The project will provide insight into the roles of kinesins in *Plasmodium*; further development of the work may lead to potential drug targets for malaria treatment.

Rotation project 1: Malaria kinesins will be characterised biochemically and prepared for cryo-electron microscopy studies of kinesin-microtubule complexes. (C.Moores' lab)

Rotation project 2: Bioinformatic methods will be used to investigate malaria kinesins, identifying malaria-specific modifications that could render the motors susceptible to inhibition by anti-malarial treatments. (M.Topf's lab)

Predicting ligand-binding promiscuity from protein structure

Ref: BBSRC45

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The ability of a protein molecule to bind small ligands is a critical feature in biology, being involved in cellular processes essential for life and in the design of drugs. Although ligand binding sites are traditionally thought of as being highly specific, there is no “one protein – one ligand” mapping, and proteins exhibit a wide range of specificities in ligand binding^{1,2}. This binding “promiscuity” has important ramifications for evolution, drug design, biotechnology and the prediction of protein function. We propose here the development of a computational method that will predict the degree of ligand-binding promiscuity of a single binding site from characteristics derived from the structure of the site. Whilst the method will be developed based on existing biochemical and structural data, we will also verify its ability to predict binding events using crystallographic and biophysical methods.

1. Hult K, Berglund P (2007) Enzyme promiscuity: mechanism and applications. Trends Biotechnol.

2. Nobeli I, Favia AD, Thornton JM (2009) Protein promiscuity and its implications for biotechnology. Nat Biotechnol 27(2): 157-167.

Rotation project 1: This first rotation project will examine the extent to which simple physicochemical characteristics of a binding site correlate with ligand binding promiscuity using linear and non-linear statistical models. (I.Nobeli's lab)

Rotation project 2: This project will involve training the student in biophysical and crystallographic methods for looking at protein-ligand interactions. We have recently determined the structure of a yeast (*Rhodotorula graminis*) D(+)-mandalate dehydrogenase (MANDH), both in the presence and absence of its coenzyme, NAD. We have a number of possible ligands for this enzyme and the student would both determine the binding affinity of these compounds for MANDH using isothermal calorimetry and solve the structures of the ligand-bound enzyme by X-ray crystallography. (D.Moss' lab)

Bridging Computational and Experimental Biology to Reveal Functional Networks in B-Cell Signalling

Ref: BBSRC46

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Whilst the genome initiatives have given us the sequences of millions of proteins and the experimental technologies of functional genomics have provided insights into their functions, understanding how these proteins assemble and function together in a complex or pathway is still a major challenge. This project will develop improved algorithms for predicting protein associations and new approaches for integrating the predictions to generate functional networks. It will be applied to the study of B-cell signalling and differentiation being studied in the Kellam Group. Recently, it has been shown that specific B-cell pathways are subject to mutations in their participating genes causing different cancers. Functional networks which have components which can be targeted with known drugs will be of particular interest.

Rotation project 1: Apply existing in-house protein association prediction pipeline (FuncNet) to identify novel genes associated with B-Cell signalling pathways. (C.Orengo's lab)

Rotation project 2: Identify putative B-cell genes which have homologues known to bind one or more of the drugs characterised in the DrugBank resource and experimentally test their effect. (P.Kellam's lab)

Predicting protein function with hierarchical phylogenetic profiles: the Gene3D Phylo-Tuner method applied to eukaryotic genomes. Ranea JA, Yeats C, Grant A, Orengo CA. (2007) PLoS Comput Biol. Vol 3:e237.

Predicting protein function from sequence and structure. Lee D, Redfern O, Orengo C. (2007) Nat Rev Mol Cell Biol. Vol 8:995-1005.

Gene3D: comprehensive structural and functional annotation of genomes. Yeats C, Lees J, Reid A, Kellam P, Martin N, Liu X, Orengo C. (2008) Nucleic Acids Res. 36:D414-418.

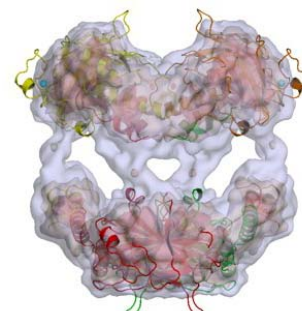
Structural-functional relationship of the human p53 tumour suppressor

Ref: BBSRC47

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Environmental pressures and exposure to various toxic agents keeps our cells under the constant threat of mutations that may induce cancer. Eukaryotic cells have developed defence mechanisms to eliminate mutated cells exploiting a number of proteins called tumour suppressors. p53 transcription factor is one such protein and has been termed the "guardian of genome". 50% of human cancers are associated with mutations in the p53 gene. While biomedical and biochemical studies of p53's function equipped us with a wealth of knowledge, the underlying mechanism of its response to DNA damage remains obscure. Revealing the mechanism(s) of p53 function(s) is essential for the development of novel therapeutic approaches. We have recently solved the first 3D structure of the full-length murine p53 tetramer (*EMBO J*, 2007). The structure has revolutionised existing concepts of p53's molecular organisation.



The new project will be focused on structural analysis of the human p53 (hp53) and in a complex interaction with its specific DNA target. It will involve biochemical experiments with recombinant proteins and analysis of the complexes by electron microscopy and structural analysis.

Rotation project 1: Learn principles of structural analysis by electron microscopy (E.Orlova's lab).

Rotation project 2: Expression/purification systems and protein biochemical analysis (Okorokov lab).

Structure-function studies of heparin binding to complement factor H

Ref: BBSRC48

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Complement factor H (CFH) is currently a very topical crucial regulator of innate immunity. CFH binds to heparan sulphate-like polyanionic oligosaccharides on host cell surfaces in order to protect these from complement, while it attacks pathogenic bacteria. Defective host cell protection is associated with a common cause of renal failure, and with age-related macular degeneration, a common cause of blindness in the elderly. Using state-of-the-art instrumentation, the project will study heparin interactions with CFH and its recombinant fragments in order to identify its binding sites and their affinities in the native full-length protein, crystallising these if possible. The results will be compared with docking predictions of heparin sites and bioinformatics analyses of heparin-protein interactions using the Protein Data Bank.

Rotation project 1: CFH will be purified, mixed with heparin fragments and studied by analytical ultracentrifugation and surface plasmon resonance to determine affinities. (S.Perkins' lab)

Rotation project 2: Mass spectrometry of complexes of highly purified heparin fragments with CFH will provide important information on the number of heparin sites on CFH. (A.McKay's lab)

[The roles of radical amino acids in enzymatic catalysis studied by vibrational infrared spectroscopy and electrochemistry](#)

Ref: BBSRC49

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Radical forms of amino acids are thought to play key roles in the catalytic mechanisms of many types of proteins. Mid-infrared vibrational (IR) spectroscopy offers a means to detect them and to characterise their properties and functions. However, to date there is very little information on the infrared spectra of such radical amino acids. In this project we will generate radical forms of amino acids by electrochemical methods and measure their IR characteristics. This information will be used to detect and characterise radical forms of amino acids in selected proteins whose mechanisms may involve such species.

Rotation project 1: Training in mid-IR protein spectroscopy and collection of mid-infrared data on proteins thought to utilise radical states of amino acids. (P.Rich's lab)

Rotation project 2: Training in electrochemical methods and application to generation of radical forms of specific pure amino acids.

[Chaperone-substrate interactions](#)

Ref: BBSRC50

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The AAA+ protein superfamily consists of ATPases that exert force to produce conformational changes in their substrate proteins or nucleic acids. They typically unfold or unwind their targets as part of regulated protein degradation, disassembly of complexes or gene expression. The Hsp100 chaperone members of this superfamily dissolve aggregated proteins by threading them through hexameric rings, providing tolerance to heat stress in bacteria and yeast, and also support transmission of yeast prions. A major question is the mechanism of substrate interaction – are substrates captured by the hexameric rings, or do the Hsp100 subunits assemble around the substrate? The project will involve native mass spectrometry and cryo EM to study the structure and assembly states of Hsp100 complexes with model substrates.

Rotation project 1: The student will gain some familiarity with EM and image processing, and with handling the Hsp100 chaperones. They will be trained in the principles and practice of single particle analysis. (H.Saibil's lab)

Rotation project 2: Students will be trained in the principles and application of mass spectrometry, in particular under conditions in which large complexes are analysed in their fully assembled states. (A.McKay's lab)

Wendler, P., Shorter, J., Plisson, C., Cashikar, A.G., Lindquist, S.L. & Saibil, H.R. (2007) Atypical AAA+ subunit packing creates an expanded cavity for disaggregation by the protein remodelling factor Hsp104. *Cell* 131, 1366-1377.
Hernandez, H. and Robinson C.V. (2007) Determining the stoichiometry and interactions of macromolecular assemblies from mass spectrometry. *Nature Protocols* 2, 715-726.

[Arsenite Oxidase as a Novel Biosensor](#)

Ref: BBSRC51

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Arsenic is a notorious poison to most organisms yet prokaryotes have evolved mechanisms to metabolise it by either using arsenate as an electron acceptor or arsenite as an electron donor. The arsenite oxidase consists of two heterologous subunits and contains molybdenum, iron and sulphur as redox cofactors. This project involves studying the arsenite oxidase from the model arsenite-oxidising bacterium NT-26 with a view to engineering a new and better biosensor for arsenite. The project will use a variety of molecular (e.g. site directed mutagenesis) and biophysical (e.g. electron spin resonance) techniques to understand the mechanism of arsenite oxidation. Questions to be addressed are: 1) what amino acids are involved in binding the arsenite, 2) how is the molybdenum bound to the protein, 3) can the substrate binding be changed so that the enzyme binds other metals/metalloids?

Rotation project 1: Optimising expression of the heterologously expressed arsenite oxidase and enzyme purification (J.Santini's lab)

Rotation project 2: To develop an understanding of ESR using the heterologously expressed arsenite oxidase (C.Kay's lab)

[Investigating the links between B-cell differentiation and disease using text and data mining techniques](#)

Ref: BBSRC52

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B-cells are the antibody-producing cells of the body and have the potential to form B-cell lymphomas when errors occur in their differentiation pathway. Herpes viruses can also manipulate these pathways, again leading to lymphomas. Vast amounts of literature, gene expression and protein interaction datasets exist for normal and malignant B cells. However the ability to mine such data from the literature to generate new conceptual links, especially to clinical data, is not well advanced.

This project will involve: identifying text- and data-mining techniques that are effective in our chosen domain (including the development of novel information retrieval strategies where necessary); and the evaluation of knowledge discovery techniques for making conceptual links that are both new and meaningful.

Rotation project 1: Development of a pipeline to facilitate the systematic application and evaluation of text- and data-mining techniques to a subset of the data (A.Shepherd's lab)

Rotation project 2: Over-expression of virus interferon regulatory factors and ChIP to identify human genes that are able to bind the viral transcription factors (P.Kellam's lab)

Development of Selective Phosphate-Binding Small Molecules and their Application to the Study of Hsp90/Cdc37 Interactions

Ref: BBSRC53

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This project will involve the design and synthesis of small molecules to selectively bind phosphorylated residues. These small molecules will then be further functionalised to incorporate fluorescent tags to provide a new method for accurate detection/quantification of phosphorylated sites, which will then be applied to the study of the Hsp/Cdc37 system. The cochaperone Cdc37 is phosphorylated on Ser13 and Tyr4 *in vivo*, and these modifications are thought to be essential for the activation of Hsp90-dependent kinases. The structural consequences and mechanistic role of pSer13 is not understood, however preliminary *in-vitro* experiments suggest it may strengthen the interaction between Hsp90, Cdc37 and kinase during the Hsp90-dependent kinase activation process. Similarly, the kinase responsible for the phosphorylation of Tyr4 is unknown.

Rotation project 1: Synthesise a variety of potential phosphate-binding agents and examine their interaction with serine phosphates via simple NMR studies. (T.Sheppard's lab)

Rotation project 2: Prepare a phospho-mimetic version of pTyr4-Cdc37 in which Tyr4 is mutated to aspartate or glutamate (i.e. Y4D- or Y4E-Cdc37). (C.Vaughan's lab)

Partners in cancer: the interplay of small shock heat proteins and kinases

Ref: BBSRC54

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The small heat proteins (shsps) are new drug targets for a wide range of cancers where they are upregulated and confer cytoprotection. They likely block apoptosis in a way that involves their own phosphorylation, which in turn has an effect on signaling kinases upstream of the mitochondrion. In this project a phosphorylated low assembly form of an shsp will be subject to 3D structure determination by crystallography. The phosphorylated shsp will be used to pull down potential partners from extracts of various cancer cell lines. Proteomics will be used to analyze the range of potential partners from pulled down complexes. Candidate components for binary interactions will be taken through biophysical characterization and 3D structure determination. Interface features will be used to guide drug design.

Rotation project 1: The student will be trained in techniques of bacterial overexpression of suitable constructs of shsps and their crystallization. (Slingsby lab)

Rotation project 2: Proteomics (PI to be confirmed). The student will be trained in how to determine protein components, and their modifications, from complex mixtures.

Institute of Cancer Research: The student will have access to cancer cell lines and will gain expertise in the preparation of phosphorylated shsps, the binding of designed small molecules to shsps and fragment based screening for drug discovery, molecular pharmacology and assays of chaperones and the stress pathway, as well as gaining familiarity with the drug discovery process.

Elucidating mechanisms of neural induction: systems biology approach

Ref: BBSRC55

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This project aims to elucidate the mechanisms responsible for inducing the vertebrate nervous system. During neural induction, the embryonic neural plate is specified and set aside from other parts of the ectoderm by signals emitted from a structure called the organizer. We attempt to reveal inducing signals emitted by organizer as well as a set of induced genes. The ultimate goal is to draw a network of events that induce neural fates in embryonic ectoderm. Proposed project includes computational sequence analysis of regulatory regions for a set of organizer-induced genes by in-house developed methods, prediction and validation of regulators (transcription factors) and analysis of their expression patterns, as well as inferring regulatory network that control neural development. The understanding of conditions/factors that regulate normal neural induction would allow us to make a real progress towards producing of certain neuronal subtypes from cultured stem cells. If successful, this approach can be used to treat neurodegenerative diseases.

Rotation project 1: Computational analysis of regulatory regions for a set of known organizer-induced genes. (A. Sosinsky's lab).

Rotation project 2: Experimental validation of predicted regulatory regions (C.Stern's lab).

Chemistry and biology of Hepcidin: Master regulator of iron metabolism

Ref: BBSRC56

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There is a strong connection between iron metabolism and healthy living. Iron levels are controlled by hepcidin- a small regulatory peptide. Hepcidin binds to and induces the degradation of Ferroportin (an iron efflux protein) involved in dietary intestinal iron absorption, maternal fetal iron transport across the placenta, efflux of iron from liver, and iron recycling. Dysfunction of the hepcidin pathway and the resulting iron imbalance might play an important role in anaemia. Understanding the interactions between ferroportin and hepcidin is important to develop drugs that can maintain healthy levels of iron. In this project we will synthesize hepcidin and determine which aspects of its structure are important in regulation the levels of iron and whether these structural features can be developed into drugs for the treatment of iron loss.

Rotation project 1: Total synthesis, folding and characterisation of hepcidin (D.Macmillan's lab)

Rotation project 2: Optimisation of intracellular iron retention and efflux assay for hepcidin activity and introduction to the use of surface plasma resonance to determine binding kinetics (K.Srail's lab)

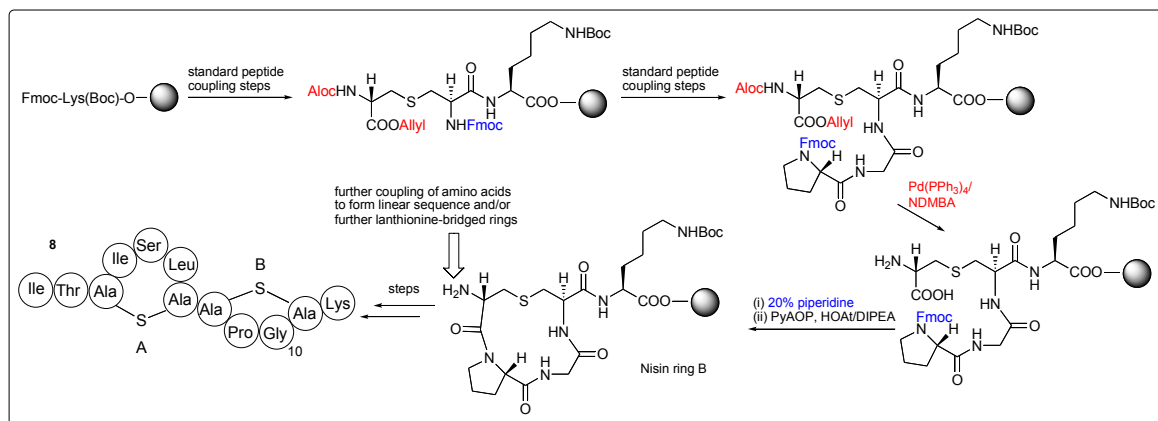
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Additional collaborator: **Paul Driscoll (NIMR/ISMB)**

The aim of this project is to investigate why the lantibiotic peptide, nisin, has a high affinity and unprecedented selectivity for binding lipid II in bacterial membranes.¹ Nisin has a complex structure featuring multiple thioether bridges (from the residues lanthionine and methyl lanthionine), which has posed a major challenge for synthetic chemists. The Tabor laboratory has recently developed powerful methodology for synthesising lantibiotics such as nisin.² In this project we will synthesise variants of the N-terminus of nisin containing unnatural amino acid bridges. We will prepare lipid II variants using a combination of synthetic and biosynthetic techniques.³ We will then carry out structural studies by NMR to characterise the binding of the nisin analogues to the lipid II analogues within biologically relevant membrane models, and we will also assess their bioactivity. We aim to understand what factors govern this unprecedented recognition between a peptide and a glycolipid, and also aim to prepare nisin analogues with even higher affinity and selectivity, with potential as lead structures for a new generation of antibiotics.

Rotation project 1: We have recently prepared an analogue of the N-terminus of nisin (residues 1-12) (D. C. Nicolau, unpublished). Using the same approach, we will prepare further analogues, keeping the sequence of ring B intact and varying the sequence and bridge structure of ring A. (Tabor lab)



Rotation project 2: Nisin and available analogues will be used to set up an assay for the bacteriocidal activity of nisin and derivatives. Both Gram positive and Gram negative species will be screened to develop a sensitive and wide ranging assay. The biosynthesis of some lipid II analogues with variant prenyl chain lengths and intermediates will be carried out using bacterial vesicles and cloned MurG.³

1. E. Breukink, B. de Kruijff. Lipid II as target for antibiotics. *Nature Rev. Drug Disc.* 5, 321 (2006).
2. S. Bregant, A. B. Tabor Orthogonally Protected Lanthionines: Synthesis and Use for the Solid-Phase Synthesis of an Analogue of Nisin Ring C. *J. Org. Chem.* 70, 2430-2438 (2005).
3. E. Breukink et al. Lipid II is an Intrinsic Component of the Pore Induced by Nisin in Bacterial Membranes. *J. Biol. Chem.* 278, 19898 (2003).

Subunit ordering in pilus assembly

Ref: BBSRC58

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P pili are surface fibers of uropathogenic *E. coli* that are involved in bacterial infection by mediating recognition to the host kidney epithelium. These fibers are formed by the noncovalent assembly of six different homologous subunit types in an array that is strictly defined in terms of both the number and order of each subunit type. Despite the importance of controlled subunit ordering for the production of functional pili, the mechanism determining specificity of subunit ordering remains unclear. The aim of this project is to simulate the mechanism leading to the specific subunit ordering in pilus biogenesis.

Rotation project 1: The student will develop a mathematical model to simulate subunit ordering and will incorporate experimental data into this model (M.Topf's lab)

Rotation project 2: Different hypotheses about the factors controlling pilus assembly from the computational work will be tested functionally (G.Waksman's lab)

The Structural Consequences of Phosphorylation of the Hsp90 cochaperone Cdc37

Ref: BBSRC59

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Cdc37 is a cochaperone of the molecular chaperone Hsp90. It is essential in the activation of a wide range of kinases that are central components in cellular signalling pathways. As such it can contribute to the kinases disregulation when these pathways go awry, for example in cancer. Phosphorylation of its N-terminal domain regulates Cdc37 function however the molecular details of the consequences of these modifications, both in terms of structure and interaction with partner proteins, are unknown.

The aim of this project is to investigate the effects of phosphorylation of Cdc37 using biophysical and structural (both crystallography and NMR) methods. This will involve generation of a semi-synthetic Cdc37 in order to incorporate phosphorylation in the N-terminal domain of the protein.

Rotation project 1: Cloning and expression of the constructs required for native chemical ligation reactions. (C.Vaughan's lab)

Rotation project 2: Peptide synthesis of phosphorylated peptides of the N-terminal region of Cdc37 and native chemical ligation of these peptides with the remainder of the protein prepared in rotation 1 (D.Macmillan's lab).

Engineering Novel Alkaloid Biosynthesis by Synthetic Biology

Ref: BBSRC60

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Alkaloids are a large and diverse family of nitrogen-containing compounds and many are used as pharmaceuticals. Recently a key enzyme in the biosynthesis of benzyloisoquinoline alkaloids has been characterised. This is (*S*)-norcoclaurine synthase (NCS) which carries out the coupling step of an arylethylamine and an aldehyde in pathways that leads to over 2,500 alkaloids. There are two different NCS enzymes and synthetic genes that have been codon optimised for *E. coli* will be used to explore the range of products able to be synthesised. Mutagenesis of each NCS, together with modelling of active mutants based on the NMR structure of the smaller NCS will attempt to correlate the activity with alterations to the side chains.

Rotation project 1: The student will receive training in the growth and expression of different NCS synthetic genes, and test biotransformations with purification/characterisation (J.Ward's lab)

Rotation project 2: The student will carry out chemical syntheses of the isoquinolines and structure determination of biotransformation products together with purification/characterisation (H.Hailes' lab)

Structure and function of the transcription elongation factor Spt4/5

Ref: BBSRC61

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Multisubunit RNAPs carry out the transcription of all cellular genomes. This projects aims at characterising the molecular mechanisms of Spt4/5, a universally conserved transcription elongation factor. We have established assays that measure the nucleic acid binding and elongation activities of Spt4/5, and obtained the x-ray structure of the core factor. Now we need to (i) determine the complete structure of Spt4/5 by solution mapping (using ESR and NMR) (ii) investigate the interactions between RNAP subunits and Spt4/5 (using fluorescently labelled factors) and (iii) carry out an extensive functional analysis (using mutagenesis and transcription activity assays) to determine the molecular mechanisms underlying the modulation of RNAP activity by Spt4/5 .

'Molecular Mechanisms of RNA polymerase - the F/E complex is required for high processivity in vitro'; Hirtreiter, Grohmann and Werner, NAR 2009 Nov 11 (in press)

'Using Fluorescence Anisotropy to monitor RNAP assembly in vitro', Grohmann, Hirtreiter and Werner Biochem J. 2009 Jul 15;421(3):339-43.

Rotation project 1: Cloning, expression, purification of recombinant Spt4/5 and RNAP variants; functional analysis of mutants

Rotation project 2: Derivatisation of Spt4/5 variants with nitroxide spin labels and EPR spectroscopy

Mechanism and Inhibition of Bacterial Cell Division

Ref: BBSRC62

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The cell wall that encases all bacteria is essential to their survival and the enzymes which create it are the target of many current antibiotics. Resistance is developing to many of these drugs. However, the cell wall is rigid, and so has also to be continually broken down (and remade) in order to allow growth and division of bacterial cells. Inhibition of the enzymes involved in this break down may provide a route to new antibacterial therapies. The N-acetylmuramyl-L-alanine amidase family of enzymes is among the most important of these peptidoglycan remodeling enzymes and are essential to division in many bacteria. The aim of this project is to determine the structures of these proteins, to identify precisely their natural substrates and to discover inhibitors which stop cell division.

Rotation project 1: We are already able to express one member of the family in quantity. Initial work would focus on structural study and screening of potential substrates and inhibitors.

Rotation project 2: To carry out computational screening for potential small-molecule binders based on knowledge of homologous enzymes and the chemistry of the bacterial cell wall.

Understanding the gas-phase topology of DNA

Ref: BBSRC63

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The linear double helical structure of DNA is an iconic image from the modern age, even appearing on a commemorative £2 coin. However, variations on this basic structure have important biological consequences. We will use ion mobility-mass spectrometry (IM-MS)(2) to probe the gas-phase structure of large DNA molecules. IM-MS is a technique related to mass spectrometry, which allows the separation of charged molecules or 'ions' according to their shape, or "collision cross section". Novel AFM techniques will be developed in collaboration with Bart Hoogenboom (UCL, LCN) to determine the structure of the DNA molecules and the location of charge. This will be performed before and after electrospray to determine the localisation of charge introduced into these molecules during ionization.

The aim of this project is to develop novel IM-MS based methods to probe the gas phase structure and dynamics of DNA molecules.

Aims of Rotation projects

1. Determine the mobility of ions, created by nanoelectrospray ionisation of large DNA molecules.
2. Carry out AFM measurements to determine structure and location of charge on DNA before and after the electrospray process.

Computational study of receptor clustering in lipid membranes as a function of ligand valancy

Ref: BBSRC64

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Many biomolecular processes involve the binding of a ligand to a receptor (protein) located in a lipid membrane. This project involves elucidating the underlying energetics of this deceptively simple act using computational methods (quantum mechanics) combined with biophysical measurements to fully characterise a model system in terms of binding energy, entropic changes (changes in cavity solvation and ligand solvation), structural changes and receptor clustering tendencies. The binding modes of a range of multivalent nitrogen ligands (di-valent, trivalent etc) with simple artificial models of membrane bound receptors, metalloporphyrins with cholesterol “anchors” to hold them in lipid micelles will be studied as a function of receptor clustering.

Rotation project 1: Use combined quantum mechanics and molecular dynamics (Oniom) calculations to elucidate the structure of micelles formed from the Zn-metalloporphyrin receptors. Characterisation of the Zn-binding site in terms of geometry, degree of solvation (compared to free receptor in water), and spacing between receptor sites.

Rotation project 2: Measurement of the binding constants for a range of divalent ligands with free and micelle bound Zn-metalloporphyrins, and thus determine the extent of receptor cluster formation following ligand binding.