

First year rotations booklet

2008-2009



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Introductory notes

In Year 1 of the programme students will choose and undertake one lab Rotation Project in each of the three core areas of the programme:

- Structural biology
- Computational biology
- Chemical biology

During each lab rotation, which will last approximately 12 weeks, you will acquire the research skills and knowledge in which that lab is expert, contribute to journal clubs, decide whether you like the social environment of the lab, and assess whether you would like to study a PhD in that lab during years 2-4.

There are timetabled meetings with the Programme Coordinator during which you will discuss the choice of your Rotation Projects.

Rotations start and end dates as well as deadlines for submitting choices are gathered in the table below:

Rotation	Start and end dates	Deadline for submitting rotation choices
Rotation 1	Monday 6 October 2008 - Friday 16 Jan 2009	Top three preferences for the first rotation should be submitted to Alethea Tabor, the programme coordinator, by 5pm on Thursday 2nd October (a.b.tabor@ucl.ac.uk)
Rotation 2	Monday 26 January 2009 - Friday 01 May 2009	Wednesday 07 January 2009 at 5pm
Rotation 3	Tuesday 26 May 2009 - Friday 14 Aug 2009	Wednesday 22 Apr 2009 at 5pm

The projects presented in this booklet are listed by subject area. Please note the following points:

* Some projects are linked – which means that they are connected with each other. However ALL these projects may also be taken individually.

Example: ST011, CP007 and CH005 are linked projects. Students may choose to take the three linked rotations (for rotations 1, 2 and 3), or just one or two of them.

* Some projects refer to another project in the programme (Example: ST032 → See also CH012 and ST003). Those projects are NOT linked but will involve some collaborative work in this specific area.

* Most projects mentioned in the booklet could potentially be extended to PhD projects

Structural & Molecular Biology

Cloning and expression of human T-cell leukaemia virus Tax constructs

Dr Tracey Barrett, School of Crystallography, Birkbeck (t.barrett@mail.cryst.bbk.ac.uk)

Ref: ST001

The human T-cell leukaemia virus 1 (HTLV-1) is the main causal agent of adult T-cell leukaemia. In order to subvert host cells, HTLV-1 produces a number of proteins that deregulate the usually tightly controlled cellular mechanisms. One such oncoprotein is Tax that targets the NF- κ B transcriptional pathways. These have under their control genes that are essential for prolonging cellular survival/preventing apoptosis and thus offer a survival advantage to the virus when the pathways are constitutively activated. The project will involve the cloning and expression of various Tax constructs based on a domain shown to be essential for its interaction with IKK γ one of its host target proteins that is key to activation of the classical NF- κ B pathway. These constructs will subsequently be assessed for their capacity to form stable complexes with IKK γ using functional assays (pull-downs, gel filtration) and if time permits, crystallisation trials performed.

<http://www.cryst.bbk.ac.uk/research/tracey-home.htm>

ATP-dependant ligase in *Mycobacterium tuberculosis*

Dr Sanjib Bhakta, School of Biological and Chemical Sciences, Birkbeck (s.bhakta@bbk.ac.uk)

Ref: ST002

Mycobacterium tuberculosis is unique in its peptidoglycan architecture indicating the enzymes regulating the synthesis of the pivotal substrates as novel therapeutic targets. Mur enzymes (MurA – F) act during the initial cytoplasmic stages of peptidoglycan biosynthesis. Among them MurC, D, E & F are members of the ATP-dependant ligase family. These ligases contribute to the synthesis of UDP *N*-acetyl muramoyl pentapeptide through a sequential reaction and using a range of endogenous substrates. UDP-*N*-acetylmuramoyl-polypeptide ligase (MurF) acts at the final stage of UDP sugar linked polypeptide synthesis. The product of this reaction, UDP *N*-acetyl muramoyl pentapeptide, is the key substrate for the membrane bound transition stage of peptidoglycan biosynthesis.

The research aims to characterize ATP-dependant MurF ligase in *M. tuberculosis* H37Rv. Aim of the project will be met through following objectives:

1. Bio-informatics analysis of the *MraY* operon in *Mycobacterium tuberculosis*.
2. Expression, purification and determination of activity of the recombinant enzyme.

http://www.bbk.ac.uk/bcs/about_staff/bhakt

The folding of a nascent-chain during its synthesis on the ribosome – structural insights from NMR spectroscopy

Dr John Christodoulou, Research Department of Structural & Molecular Biology and School of Crystallography, UCL/Birkbeck (j.christodoulou@ucl.ac.uk)

(In collaboration with Dr Maya Topf, School of Crystallography, Birkbeck)

Ref: ST003

Protein synthesis occurs on ribosomes within all living organisms. The newly synthesized 'nascent chain' emerges from the ribosome one amino acid at a time and continuously explores conformations that eventually lead to its folded structure. This project aims to use structural methods, in particular NMR spectroscopy, to determine the structures of emerging nascent chains on their parent ribosomes (Structure and Dynamics of a ribosome-bound nascent chain using NMR spectroscopy Hsu, S-T. D., Fucini, P., Cabrita. L.D., Launay, H., Nierhaus, K., Dobson, C.M., Christodoulou, J., P.N.A.S. (2007), 104,16516-16521). In this way we will obtain snapshots of the process of protein folding during biosynthesis and enable an understanding of nascent chain misfolding, which can have devastating consequences.

<http://www.smb.ucl.ac.uk/structural-biology-molecular-biophysics/dr-john-christodoulou.html>

NMR studies of serpins: co-translational folding on ribosomes, misfolding and disease

Dr John Christodoulou, Research Department of Structural & Molecular Biology and School of Crystallography, UCL/Birkbeck (j.christodoulou@ucl.ac.uk)

(In collaboration with Dr Bibek Gooptu, School of Crystallography, Birkbeck)

Ref: ST004

Naturally-occurring mutations in the Serpin family of molecules can induce aberrant conformational change, misfolding and to the subsequent formation of aggregation-prone polymers, leading to devastating diseases including dementia, emphysema and thrombosis. This project will combine the use of biological NMR spectroscopy and biophysical methods to provide a structural description of conformational change in this group of proteins both on the ribosome as 'nascent chains' during protein synthesis and also as the mature proteins on their subsequent release. These data will be studied in the context of intervention strategies to prevent the misfolding of these proteins.

<http://www.smb.ucl.ac.uk/structural-biology-molecular-biophysics/dr-john-christodoulou.html>

Purification and characterisation of the oligomeric state of the full-length histidine kinase DosS – a signalling protein from *M. tuberculosis*

Dr Snezana Djordjevic, Research Department of Structural & Molecular Biology, UCL

(snezana@biochem.ucl.ac.uk)

Ref: ST005

Mycobacterium tuberculosis evades host immune response by entering a dormant state which in addition renders this bacterium insensitive to antibiotics. Proteins DosR, DosS and DosT form a signal transduction system that controls the major response of *M. tuberculosis* to hypoxia, which in turn is believed to be an important stimulus for entry into dormancy. We were the first to report that DosS is a haem-binding protein and following characterization of the domain involved in haem-binding we were able to postulate a model for DosS activation in hypoxic conditions or in a presence of nitric oxide. We now wish to determine molecular basis of the DosS-activated switch to dormancy of *M. tuberculosis* by the comprehensive characterization of the signalling mechanism of DosS. Within this research project a student will focus on characterisation of the oligomeric state of the multimeric full-length protein.

Aims:

- 1) To express in *E. coli* the full-length DosS protein.
- 2) To develop a reproducible protocol for purification of a homogenous homo-multimeric complex of DosS.
- 3) To characterise the oligomeric state of the protein by using analytical ultracentrifugation methods.

<http://www.smb.ucl.ac.uk/cell-signalling-metabolic-regulation/dr-snezana-djordjevic-2.html>

Characterisation of the redox properties of haem cofactor in a sensory domain of histidine kinase DosS

Joint project: Dr Snezana Djordjevic, Research Department of Structural & Molecular Biology, UCL (snezana@biochem.ucl.ac.uk)

Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL
(c.kay@ucl.ac.uk)

Ref: ST006

Mycobacterium tuberculosis evades host immune response by entering a dormant state which in addition renders this bacterium insensitive to antibiotics. Proteins DosR, DosS and DosT form a signal transduction system that controls the major response of *M. tuberculosis* to hypoxia, which in turn is believed to be an important stimulus for entry into dormancy. We were the first to report that DosS is a haem-binding protein and following characterization of the domain involved in haem-binding we were able to postulate a model for DosS activation in hypoxic conditions or in a presence of nitric oxide. We now wish to determine molecular basis of the DosS-activated switch to dormancy of *M. tuberculosis* by the comprehensive characterization of the signalling mechanism of DosS. This project will be carried out in collaboration with Dr Chris Kay and the student will focus on examining oxidation state of iron within the haem cofactor by using EPR spectroscopy.

Aims:

- 1) To express in *E. coli* the sensory domain of DosS as well as the full-length protein.

- 2) To measure EPR signal of the fully reduced and fully oxidised samples prepared under aerobic and under anaerobic conditions.
- 3) To determine the rate of iron oxidation and to examine buffer conditions that might affect this rate.

<http://www.smb.ucl.ac.uk/cell-signalling-metabolic-regulation/dr-snezana-djordjevic-2.html>

<http://www.london-nano.com/content/lcndirectory/christopherkay>

NMR studies of the death-inducing signalling complex core

Prof Paul Driscoll, Molecular Structure, MRC NIMR (pdrisco@nimr.mrc.ac.uk)

Ref: ST007

A major objective of our group is to describe, in three dimensional terms, the so-called death-inducing signalling complex (DISC) that forms at the cytoplasmic tails of the canonical CD95/Fas death receptor following ligation by apoptotic (programmed cell death) agonists. The rotation project will involve using novel strategies to ^{13}C , ^2H -isotope label a DISC core particle formed by homotypic association of the CD95 receptor and FADD adaptor protein death domains, followed by application of state-of-the-art heteronuclear NMR spectroscopy methods that can be applied to 'larger' proteins. Key project training outcomes: recombinant protein expression in *E. coli*; metal-affinity/size-exclusion chromatography; heteronuclear 2D NMR spectroscopy; NMR data processing/structural analysis.

<http://www.nimr.mrc.ac.uk/molstruct/driscoll/>

Molecular dynamics simulation of NMR parameters describing an enzyme active site 'lid'

Prof Paul Driscoll, Molecular Structure, MRC NIMR (pdrisco@nimr.mrc.ac.uk)

Ref: ST008

Over the past few years we have built up a portfolio of heteronuclear NMR-based observations of the segmental molecular dynamics (MD) of the bacterial enzyme dimethylarginine-dimethylamino-hydrolase (DDAH) in apo-, substrate, and product-bound states. The results can be parameterised in terms of local backbone motions that occur on either fast (pico-nanosecond) or intermediate (micro-millisecond) timescales. To obtain a picture of how such motions might appear in 3D, in particular to explore the amplitude of active site 'lid' motions, the rotation project will be to perform multi-processor computer-based simulations of the motion of DDAH with the GROMACS package. Key project training outcomes: Unix computing; MD simulation and analysis in terms of NMR observables.

<http://www.nimr.mrc.ac.uk/molstruct/driscoll/>

Structure/function analysis of mTOR beta: Molecular cloning mTORb into baculoviral expression vectors and generation of recombinant viruses

Prof Ivan Gout, Research Department of Structural & Molecular Biology, UCL

(i.gout@biochem.ucl.ac.uk)

Ref: ST009

The mTOR (mammalian target of rapamycin) is a central regulator of an evolutionary conserved signalling pathway which controls cellular metabolism, growth and proliferation. Deregulation of the mTOR-coordinated signalling has been associated with various human pathologies, including diabetes, inflammation and cancer. Rapamycin, a naturally occurring mTOR inhibitor, and its homologues have been currently examined as anti-cancer drugs in over 40 clinical trials. We have recently identified a novel mTOR splicing form, termed TOR β , which in contrast to the full length mTOR protein (mTOR α) has the potential to shorten significantly G1 phase of the cell cycle and to induce cell proliferation and survival. In agreement with these findings we also showed that mTOR β is an oncogene, capable to transform cells in cellular and animal models.

The structure of mTOR kinase has not been solved so far, despite major efforts in academic and industrial institutions. The task is complicated by the size of the protein (290kDa) and the inability to purify large quantities of endogenous protein or to obtain soluble protein (full length or kinase domain) in expression systems. The molecular weight of mTOR β is 80kDa, which makes it an attractive target for structural studies. Moreover, preliminary studies indicate that partially soluble mTOR β (fused to a solubility tag) could be expressed in bacteria.

The project offers the opportunity for gain a diverse range of experiences in molecular cloning, site-directed mutagenesis, tissue culturing, expression of recombinant proteins in bacterial and baculoviral systems, affinity purification and analysis of recombinant proteins, a range of techniques associated with synthesis and quality control of mTOR inhibitors.

Main stages:

1. Design of expression constructs;
2. PCR amplification and cloning of mTOR β full length sequence in baculoviral vectors as a non-tagged form or in frame with the His tag.
3. Restriction analysis and sequence verification of generated expression plasmids.
4. Generation of bacmids for produced constructs and production of recombinant baculoviruses in insect cells (Sf9 cells). Testing the expression and activity of recombinant mTOR β proteins under various experimental conditions (SDS-PAGE, Western blot analysis).

→ See also ST012 and CH004

<http://www.smb.ucl.ac.uk/cell-signalling-metabolic-regulation/professor-ivan-gout.html>

Mechanistic studies on the UvrABC pathway

Joint Project: **Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL**
(c.kay@ucl.ac.uk)
Dr Tracey Barrett, School of Crystallography, Birkbeck
(t.barrett@mail.cryst.bbk.ac.uk)

Ref: ST010

The UvrABC nucleotide excision repair pathway is the main mechanism for the correction of bulky DNA lesions in bacteria and involves three proteins UvrA, UvrB and UvrC. Despite extensive studies, several key aspects of the mechanism remain unresolved such as the oligomeric state of the major damage detection element UvrB and how this varies throughout the excision reaction. To probe these key issues, a combined structural and biophysical approach will be used. The project will first involve the mutagenesis and subsequent nitroxide spin labelling of key amino acids in UvrB that can be analysed using pulsed EPR to derive inter-label distances. The variation in these distances and changes in the label environments can then be analysed to determine how the oligomer is affected during the reaction which is pivotal to an understanding of the pathway.

<http://www.london-nano.com/content/lcndirectory/christopherkay>
<http://www.cryst.bbk.ac.uk/research/tracey-home.htm>

Structural Biology- Rpfs from *M. tuberculosis*

Dr Nick Keep, School of Crystallography, Birkbeck (n.keep@mail.cryst.bbk.ac.uk)
Ref: ST011 - Linked with CP007 and CH005

Tuberculosis (TB) results in approximately 3 million deaths each year, and the failure of the BCG vaccine to afford significant protection, the problems of multiple drug resistance, and the deadly combination of infection with *M. tuberculosis* and HIV have all served to stimulate a renewed drive to understand and tame this ancient disease. The WHO estimates that one third of the population harbours a latent tuberculosis infection and reactivation of the bacteria occurs in 10% of non-HIV patients and up to 30% of HIV patients, accounting for 13% of HIV-related deaths. Understanding and controlling dormancy is therefore a crucial development in anti-TB therapies.

Lead compounds from collaborators (including from linked rotations) will be assayed for biochemical activity using the HPLC assay for Rpf activity we are developing and the binding site delineated using HSQC shifts of an NMR sample. If a strong inhibitor is found then the compound will be tested in an *in vivo* growth assay for *Micrococcus luteus*. There will also be opportunities to work on structure determination of RpfC, for which we have preliminary crystals.

<http://people.cryst.bbk.ac.uk/~ubcg48a/>

Structure/function analysis of mTOR beta: The use of solubility tags for expressing soluble and active forms of mTORb kinase in insect cells

Prof Neil McDonald, School of Crystallography, Birkbeck (n.mcdonald@mail.cryst.bbk.ac.uk)

Ref: ST012

The mTOR (mammalian target of rapamycin) is a central regulator of an evolutionary conserved signalling pathway which controls cellular metabolism, growth and proliferation. Deregulation of the mTOR-coordinated signalling has been associated with various human pathologies, including diabetes, inflammation and cancer. Rapamycin, a naturally occurring mTOR inhibitor, and its homologues have been currently examined as anti-cancer drugs in over 40 clinical trials. We have recently identified a novel mTOR splicing form, termed TOR β , which in contrast to the full length mTOR protein (mTOR α) has the potential to shorten significantly G1 phase of the cell cycle and to induce cell proliferation and survival. In agreement with these findings we also showed that mTOR β is an oncogene, capable to transform cells in cellular and animal models.

The structure of mTOR kinase has not been solved so far, despite major efforts in academic and industrial institutions. The task is complicated by the size of the protein (290kDa) and the inability to purify large quantities of endogenous protein or to obtain soluble protein (full length or kinase domain) in expression systems. The molecular weight of mTOR β is 80kDa, which makes it an attractive target for structural studies. Moreover, preliminary studies indicate that partially soluble mTOR β (fused to a solubility tag) could be expressed in bacteria.

The project offers the opportunity for gain a diverse range of experiences in molecular cloning, site-directed mutagenesis, tissue culturing, expression of recombinant proteins in bacterial and baculoviral systems, affinity purification and analysis of recombinant proteins, a range of techniques associated with synthesis and quality control of mTOR inhibitors.

Main stages:

1. Designing of expression constructs, containing solubility tags at the N-terminus of mTORb (we will use two tags).
2. Generation of expression constructs by employing molecular cloning techniques. Production of recombinant viruses and expression studies.
3. Testing the expression and solubility of recombinant proteins with and without the solubility tags. Affinity purification of the fusion constructs. Cleavage of the solubility tag.
4. Examining the stability of affinity purified mTOR β constructs

At this stage, we should be able to select appropriate constructs for structural studies based on following criteria (expression level, solubility and stability, activity in *in vitro* kinase assay)

→ See also ST009 and CH004

<http://people.cryst.bbk.ac.uk/~ubcg30a/home.html>

Testing the "Fenn Effect" with a TIRFM sensor for inorganic phosphate

Prof Justin Molloy, Physical Biochemistry, MRC NIMR (jmolloy@nimr.mrc.ac.uk)

Ref: ST013

In the early 1920s, WO Fenn and AV Hill (working at UCL) made the important discovery that the rate at which muscle liberates heat (and consumes ATP) depends upon its work output. The way in which external load controls muscle biochemistry has been extensively studied in the intervening years – but we are now at the exciting point of being able to measure the phosphate produced by just a few myosin molecules. The aim of this research project is to measure the ATPase activity of acto-myosin under different mechanical loads in an *in vitro* motility assay. You will develop a sensitive, surface-based phosphate assay, using total internal reflection fluorescence (TIRF) microscopy that will give real-time readout of phosphate production as a single actin filament moves over a microscope coverslip. The assay will be of general applicability in biology and will give important information about how muscle generates force from ATP.

<http://www.nimr.mrc.ac.uk/physbiochem/molloy/>

Bacterial coat proteins: elucidating the structure of surface-layer lattices with electron microscopy

Dr Carolyn Moores, School of Crystallography, Birkbeck (c.moores@mail.cryst.bbk.ac.uk)

Ref: ST014 - Linked with CP016 and CH007

Surface layer (S-layer) proteins are a class of bacterial exoproteins which self-assemble into large two-dimensional crystalline lattices. The regularly shaped cell coats have important biological roles in archaea and many eubacteria including human pathogens. To address the shortage of high-resolution structural data, a combined approach will be pursued which integrates chemical cross-linking, electron-microscopy (EM), and computational modelling. The successful approach will expand our understanding of S-layer assembly and elucidate the structure at the atomic level.

Electron microscopy (EM) is an essential structural tool for examining large protein complexes. EM will be used to assess the impact of point mutations of SbsB on S-layer assembly and to analyse the structure of the S-layer lattice. Labels at specific sites on SbsB will also be located by analysis of EM images of the S-layer and correlated with the results from mass-spectrometry analysis.

<http://people.cryst.bbk.ac.uk/~ubcg62d/>

Kinesin-8 in action! Capturing kinesin-8 motors on microtubule end mimics

Dr Carolyn Moores, School of Crystallography, Birkbeck (c.moores@mail.cryst.bbk.ac.uk)

Ref: ST015

Kinesin-8 motors are involved in spindle positioning and chromosome segregation during mitosis. Kinesin-8s run along microtubules (MTs) and, upon reaching their ends, depolymerise them. The mechanism for their dual action is unknown. The aim of this project is to investigate the depolymerising activity of kinesin-8 using tubulin rings that mimic MT ends. The project will involve computational analysis of electron micrographs of kinesin-8-tubulin ring complexes in different nucleotide states. These results will be correlated with kinesin-8 ATPase assays so that, both biochemically and structurally, the basis for kinesin-8's unique function can be probed.

<http://people.cryst.bbk.ac.uk/~ubcg62d/>

Structural analysis of Mcm10 by cryo-electron microscopy

Dr Elena Orlova, School of Crystallography, Birkbeck (e.orlova@mail.cryst.bbk.ac.uk)

Ref: ST016

DNA replication is a central, fundamental biological process, essential for the cell's ability to pass genetic information to the next generation with its deregulation linked to chromosomal instability and a range of cancers.

Initiation of DNA replication starts with the assembly of the origin recognition complex, which with help of additional proteins is transformed into pre-replicative complexes. Loading of the Mcm10 protein triggers the next transition to the pre-initiation complex. Mcm10 serves as a structural connector between a helicase and DNA polymerase/primase complexes. Mcm10 is an essential part of the replication initiation machinery and important target for therapeutic intervention. The project is aimed to obtain the Mcm10 structure with a medium resolution (12-14Å) by image analysis of EM micrographs.

During this project a student will learn basics of electron microscopy, statistical image analysis, 3D reconstruction, and interpretation by fitting of atomic structures.

<http://people.cryst.bbk.ac.uk/~ubcg55a/>

Analysis of conformational changes within the tail of SPP1 bacteriophage induced by mutations in gp17.1 protein

Dr Elena Orlova, School of Crystallography, Birkbeck (e.orlova@mail.cryst.bbk.ac.uk)

Ref: ST017

Bacterial viruses (bacteriophages or phages) are the most populated biological entity in the Biosphere. The vast majority of known bacteriophages have an icosahedral capsid and a tail (order *Caudovirales*). Although much information has been obtained by biochemical methods, little is known about the structural basis of the infection process in which the phage tail is a key element. The release of DNA from the capsid is

induced by signal transmission through the bacteriophage tail due to sequential conformational changes of its subunits. Understanding the process of subunit interaction the detailed structures of two phage mutants are required: one where the tail is composed of only gp17.1 and another with the tail composed of 17.1* protein.

A student will learn during this project basics of electron microscopy, principles of single particle analysis and helical reconstruction.

<http://people.cryst.bbk.ac.uk/~ubcg55a/>

Localisation of the zinc binding SCR domains in complement factor H (CFH)

Prof Steve Perkins, Research Department of Structural & Molecular Biology, UCL

(s.perkins@medsch.ucl.ac.uk)

Ref: ST018

Complement factor H (CFH) is an important regulator of complement activity. This is inhibited by zinc and copper binding which causes CFH to oligomerise and leads to complement activation that will damage host cells. Large amounts of zinc are found in the eye, and may be implicated in the development of age-related macular degeneration (AMD). The aim of the project is to study several recombinant SCR domain fragments of CFH in the presence of zinc in order to determine which ones are responsible for the promotion of CFH oligomers. These fragments will be purified, monitored by SDS-PAGE and mass spectrometry, then zinc titrations will be performed by analytical ultracentrifugation sedimentation velocity experiments. The results will be compared with predictions of these metal sites.

→ See also CP011

<http://www.smb.ucl.ac.uk/bioinformatics/professor-steve-perkins.html>

Vibrational Infrared Spectroscopy of Electron Transfer Proteins

Prof Peter Rich, Research Department of Structural & Molecular Biology, UCL (pr@ucl.ac.uk)

Ref: ST019

Vibrational infrared spectroscopy, combined with visible spectroscopy and electrochemistry, will be applied to the cytochrome bc₁ complex, a central component of the mitochondrial electron transfer chain. The rotation project will be focused on resolution of the infrared properties of a substrate ubiquinone that remains bound to one of the catalytic sites in the purified protein complex. Electrochemistry will be used to convert the ubiquinone into its semiquinone form that is a natural catalytic intermediate. The infrared vibrational bands of bound ubiquinone and its semiquinone form will be compared to model compound spectra and interpreted in terms of structural factors that are important for binding.

<http://www.smb.ucl.ac.uk/structural-biology-molecular-biophysics/professor-peter-rich-5.html>

Histidine-Tagged Cytochrome c Oxidase from Yeast

Joint Project: Prof Peter Rich, Research Department of Structural & Molecular Biology, UCL (pr@ucl.ac.uk)

Dr Saul Purton, Research Department of Structural & Molecular Biology, UCL

(s.purton@ucl.ac.uk)

Ref: ST020

A histidine-tagged form of mitochondrial cytochrome c oxidase from baker's yeast, *Saccharomyces cerevisiae*, will be engineered by transformation of the gene for one of the nuclear-encoded subunits. The His-tagged enzyme will then be extracted and purified by nickel column chromatography. The aim of this project is to establish the groundwork for large-scale preparation of enzyme for crystallisation and spectroscopic (UV/visible and vibrational infrared) functional studies and for generation of site-directed mutant forms of mitochondrially-encoded subunits.

<http://www.smb.ucl.ac.uk/structural-biology-molecular-biophysics/professor-peter-rich-5.html>

<http://www.smb.ucl.ac.uk/molecular-microbiology/dr-saul-purton-5.html>

Allosteric mechanism and dynamics of ATP binding to GroEL

Prof Helen Saibil, School of Crystallography, Birkbeck (h.saibil@mail.cryst.bbk.ac.uk)

Ref: ST021 - Linked with CP017

Chaperonins are cylindrical-shaped macromolecular assemblies found in all three domains of life. They are essential for the folding of a significant number of proteins in the cell and therefore understanding how they work is an important problem in modern biology. GroEL is an *E. coli* chaperonin, containing two heptameric rings of identical subunits that enclose central cavities where non-native proteins are captured. Its allosteric cycle includes the formation of a complex with non-native substrate protein, ATP and a lid-like co-chaperonin GroES which facilitates protein folding, followed by ATP hydrolysis in the occupied ring which enables the release of GroES and folded substrate when new ATP binds to the opposite ring. Despite the extensive research by a variety of biochemical and structural techniques, the mechanism that advances the GroEL-GroES complex upon this ATP-driven folding cycle is still poorly understood. Recent work in the cryo electron microscopy (cryoEM) lab at the school of crystallography resulted, for the first time, in a number of distinct conformations of intermediate structures at different ATP states along the cycle. Unfortunately, the resolution of these structures, although at the sub-nanometer range, is still far from near atomic, which is required for a deeper analysis of the mechanism. We propose to extend the above work by adding a computational aspect. We will use image processing to improve the resolution of the current density maps, and our recently developed real-space refinement method to flexibly fit the known crystal structure of GroEL (in the apo state) into the lower-resolution maps of the different GroEL-ATP intermediates.

<http://people.cryst.bbk.ac.uk/~ubcg16z/index.html>

Electron microscopy studies of membrane pore-forming proteins

Prof Helen Saibil, School of Crystallography, Birkbeck (h.saibil@mail.cryst.bbk.ac.uk)

Ref: ST022

The cholesterol-dependent cytolysin family of bacterial toxins kill host cells by assembling large pores in their cell membranes. A related pore forming mechanism is used by the immune system protein perforin in cytotoxic lymphocytes for targeted killing of infected cells. These proteins are released as soluble monomers which assemble into large, membrane-inserted rings that puncture cellular membranes. We have defined the overall conformational changes involved in this conversion by electron microscopy and three-dimensional reconstruction. In this rotation project, the student will use negative stain EM to study pore assemblies of these proteins in lipid vesicles and monolayers. (Lukoyanova, N & Saibil, HR (2008) Trends Immunol. 29, 51-53)

<http://people.cryst.bbk.ac.uk/~ubcg16z/index.html>

Arsenite oxidation in a model thermophile

Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL

(j.santini@ucl.ac.uk)

Ref: ST023 - Linked with CP003

Arsenic is a notorious poison and is toxic to most cells. Prokaryotes however have evolved mechanisms to metabolise it by either using arsenate (As^{V}) as a terminal electron acceptor or arsenite (As^{III}) as an electron donor. The arsenite oxidase (Aro) catalyses the conversion of As^{III} to As^{V} coupled with the reduction of oxygen to water. The enzyme from three mesophiles has been purified and characterised and consists of two heterologous subunits that bind various cofactors (i.e. Mo, Fe, S). Homologues of the *aro* genes have been identified in various mesophiles (e.g. members of the *Proteobacteria*), thermophiles (e.g. *Thermus* spp.) and hyperthermophiles (i.e. three members of the Archaea). The mesophilic enzyme has been used as a biosensor for arsenite but improving its efficiency and thermal stability should lead to a better biosensor.

The overall objective of this project is to study the arsenite oxidase from the model thermophile, *Thermus thermophilus*. This will involve determining the optimum conditions for enzyme activity, cellular location and thermal stability. This should lead to the design of a suitable purification procedure.

<http://www.smb.ucl.ac.uk/molecular-microbiology/dr-joanne-santini-5.html>

The human WRNIP1 ATPase: binding and action on DNA

Dr Irina Tsaneva, Research Department of Structural & Molecular Biology and School of Crystallography, UCL/Birkbeck (tsaneva@biochemistry.ucl.ac.uk)

Ref: ST024

The Werner protein-interacting protein WRNIP1, a member of the AAA+ family of ring ATPases, plays a poorly understood role in DNA replication and rescue of stalled replication forks and has a suspected association with cancer development. WRNIP1 is highly conserved in eukaryotes and so is the bacterial orthologue RarA. Our preliminary data show that WRNIP1 binds specifically to branched and fork DNA substrates. In addition, it was shown recently that WRNIP1 has a ubiquitin-binding domain and undergoes ubiquitylation. The aim of this project is to compare the DNA-binding specificity of the human WRNIP1 to that of *E. coli* RarA and to characterise the enzymatic activities of the two proteins using synthetic DNA substrates.

<http://www.smb.ucl.ac.uk/molecular-cell-biology/dr-irina-tsaneva-2.html>

Structural Studies of the Bub1-Skp1 Complex: Controlling the Anaphase-Wait Signal

Dr Cara Vaughan, School of Crystallography, Birkbeck (c.vaughan@mail.cryst.bbk.ac.uk)

Ref: ST025

Bub1 is a mitotic checkpoint protein that is conserved from yeast to mammals. Its interaction with the kinetochore during mitosis is essential for an effective anaphase-wait signal. The molecular details of how Bub1 signals the checkpoint are not known, however its association with the kinetochore protein Skp1 is central to its function. Mutations in Bub1 have been linked to colorectal cancer and the chromosomal instability associated with this malignancy.

We are currently establishing conditions for the co-expression of the yeast homologues of Skp1 & Bub1 in *E. coli* in order carry out crystallographic studies of their complex. The rotation project will continue this work, combining many aspects of structural biology, from cloning constructs through to protein expression, purification and crystallisation trials.

<http://people.cryst.bbk.ac.uk/~ubcg72a/Home.html>

Myosin motors driving vesicle movement in vitro: Single molecule studies using fluorescence and mechanical techniques

Dr Claudia Veigel, Physical Biochemistry, MRC NIMR (cveigel@nimr.mrc.ac.uk)

Ref: ST026

Little is known about the mechanical properties of physiological ensembles of motor proteins in cells, such as ensembles of myosin and kinesin motors driving cytokinesis and intracellular traffic of membrane compartments, restructuring the cytoskeleton during cell movement or regulating the mechanics of signal transduction in hearing.

The aim of this project is to set up *in vitro* assays which will enable us to study the mechanical properties of tissue purified and reconstituted ensembles of myosin motors bound to their physiological cargo. As a model system we will study motor proteins on endocytic membrane vesicles. Using a variety of *in vitro* motility assays we will investigate purified clathrin coated native vesicles and reconstituted vesicles to which we will bind recombinant GFP-myosin VI and adaptor proteins recently identified to link myosin VI to membrane cargo. Vesicle movement along actin filaments will be studied using single molecule fluorescence and optical tweezers technology, established in our lab.

<http://www.nimr.mrc.ac.uk/physbiochem/veigel/>

Structural Studies of the Type IV Secretion Systems

Prof Gabriel Waksman, School of Crystallography/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@mail.cryst.bbk.ac.uk, g.waksman@ucl.ac.uk)

Ref: ST027

Type IV secretion systems (T4SSs) are machineries used for the transport of macromolecules across the bacterial cell envelopes of Gram-negative bacteria. T4SSs are highly versatile and have been found in many bacterial pathogens such as *Helicobacter pylori*, *Brucella suis*, and *Legionella pneumophila*. The secretion

system spans both bacterial membranes. The proposed rotation project is a collaborative project aiming at imaging a T4SS. We have already cloned, expressed, purified and imaged the T4SS core. We have crystallized the periplasmic part of this core. This rotation project aims at purifying a larger T4SS subassembly to gain a better understanding of protein secretion through T4SSs.

<http://people.cryst.bbk.ac.uk/~ubcg54a/>

Structural and computational studies of the Usher outer-membrane assembly platform involved in pilus biogenesis

Prof Gabriel Waksman, School of Crystallography/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@mail.cryst.bbk.ac.uk, g.waksman@ucl.ac.uk)

Ref: ST028

P and type 1 pili are responsible for the attachment of bacteria to the kidney and the bladder, respectively. P and type 1 pili are assembled by the highly conserved chaperone-usher (CU) pathway. Pilus subunits are produced in the cytoplasm, translocated to the periplasm by the Sec translocation machinery, and then taken up by a chaperone to cross the periplasmic space. At the outer-membrane, chaperone-subunit complexes are recruited to an outer-membrane assembly pore, termed "the usher" which orchestrate assembly and polymerization of subunits. For this rotation, we plan to use structural and computational tools to study the activation and gating of the usher pore and gain an understanding of usher pore function.

<http://people.cryst.bbk.ac.uk/~ubcg54a/>

Turning a Sodium Channel into a Calcium Channel

Prof Bonnie Wallace, School of Crystallography, Birkbeck (b.wallace@mail.cryst.bbk.ac.uk)

Ref: ST029

NaChBac is a bacterial voltage-gated sodium channel which we can express and purify in *E. Coli*. This project will provide a wide exposure to structural molecular biology methods: the student will use molecular biology techniques to make a mutant protein (changes to three residues in the pore region convert it from sodium-selective to calcium-selective), biochemical techniques to purify the protein, biophysical methods to characterise the protein (circular dichroism and fluorescence spectroscopy and drug binding, plus possibly crystallisation), and finally, bioinformatics and molecular modelling to examine the effects on the structure/function of the protein.

<http://people.cryst.bbk.ac.uk/~ubcg25a/>

Engineering novel Alkaloid Biosynthesis by Synthetic Biology

Prof John Ward, Research Department of Structural & Molecular Biology, UCL

(ward@biochemistry.ucl.ac.uk)

Ref: ST030 - Linked with CH006

Alkaloids are a large and diverse family of nitrogen-containing compounds largely synthesised by plants. Over 14,000 alkaloid structures are known. Many alkaloids and their derivatives are used as pharmaceuticals around the world and alkaloids represent a huge repository of functional chemical space. Recently some of the key enzymes in the biosynthesis of the major alkaloid classes have been cloned and characterised. One of the enzymes is (*S*)-norcoclaurine synthase (NCS) which carries out the key first coupling step in the pathway that leads to over 2,500 alkaloids including morphine, berberine and tubocurarine. There are two NCS enzymes: one is a small protein (210 amino acids) of the Bet v 1 pathogenesis related class and the other NCS type is a member of the 2-oxoglutarate family and is 352 amino acids. The project will take site directed and gene shuffled mutants of each NCS to determine their substrate specificity. Modelling of the active mutants based on the NMR structure of the smaller NCS will attempt to correlate the activity with alterations to the side chains.

<http://www.smb.ucl.ac.uk/molecular-microbiology/professor-john-ward-2.html>

Helicase translocation through DNA

Dr Martin Webb, Physical Biochemistry, MRC NIMR (mwebb@nimr.mrc.ac.uk)

Ref: ST031

Helicases are enzymes that drive the separation of double-stranded DNA into component single strands. This separation is a precursor of most DNA processing, including replication, recombination and repair. We are studying the mechanism and control of these proteins. This project will on one helicase involved in asymmetric plasmid replication, PcrA. This will involve cloning the appropriate parts of oriD sequences into plasmids, to provide termination signals for the helicase activity. Using methods developed in this laboratory for real time fluorescence measurements (for example, Dillingham et al (2008) Biophys. J. published on line, BioFast.), such plasmid constructs and oligonucleotide models will be used to measure translocation and examine how this process is terminated.

<http://www.nimr.mrc.ac.uk/physbiochem/webb/>

Molecular mechanisms of RNA polymerase: regulation of transcription elongation by Spt4/5

Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL

(werner@biochem.ucl.ac.uk)

Ref: ST032

The research in our laboratory focuses on the structure and function of multisubunit RNAPs and their regulation by transcription factors. This projects aims at unravelling the molecular mechanisms of Spt4/5, a universally conserved elongation factor. We have established assays that measure the nucleic acid binding and elongation activities of Spt4/5, and obtained crystals of the minimal active domain configuration. Now we need to investigate the interplay between RNAP, Spt4/5 and DNA/RNA to develop models of the modus operandi. Ref: Werner Mol Micro 65(5):1395-1404 (2007)

→ See also CH012 and ST003

<http://www.smb.ucl.ac.uk/molecular-microbiology/dr-finn-werner-2.html>

Molecular mechanisms of RNA polymerase: Analysing transcription with light

Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL

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Ref: ST033

Our laboratory investigates the structure and function of multisubunit RNAPs using a wholly recombinant enzyme that is reconstituted from its twelve subunits in vitro. This system allows us to incorporate pairs of fluorescent probes for Förster Resonance Energy Transfer (FRET) experiments at various strategic positions in the RNAP and subsequently analyse (i) complex formation of RNAP, DNA/RNA and transcription factors and (ii) conformational changes within transcription initiation and elongation complexes. We have hitherto labelled four RNAP subunits at various positions and already obtained intriguing insights into the transcription mechanism. Ref: Werner Trends in Microbiology 16(6):247-50 (2008)

→ See also CH012

<http://www.smb.ucl.ac.uk/molecular-microbiology/dr-finn-werner-2.html>

Toward a small molecule fragment library for protein function discovery

Dr Mark Williams, School of Crystallography, Birkbeck (m.williams@mail.cryst.bbk.ac.uk)

Ref: ST034 - Linked with CP008

One of the biggest challenges of the post-genomic era is the assignment of function(s) to the 60% of known protein sequences that have no known or only very distant homologues in the protein databases. In the case of proteins whose function depends on the binding of metabolites, a promising approach could be the use of small molecule fragments designed to probe protein function. Fragment-screening is playing an increasing role in drug discovery in industry and academia. This project aims to design and test a library of fragments from naturally occurring molecules for use in function discovery.

The fragment library will be tested on a diverse set of proteins for their ability to actually bind to and discriminate proteins of distinct function. Several biophysical techniques will be compared for detection and quantitation of binding of fragments – NMR, calorimetry and fluorescence-based methods. These experimental data will be used to further optimize the library design.

<http://people.cryst.bbk.ac.uk/~ubcg66a/>

Structure:function analysis of an essential bacterial cell division protein

Dr Mark Williams, School of Crystallography, Birkbeck (m.williams@mail.cryst.bbk.ac.uk)

Ref: ST035

The bacterial cell wall is single mega-macromolecule comprised of linear glycan chains interlinked by short peptides, which has to be modified to allow growth and division of bacteria. Among the most important enzymes in this process are the N-acetylmuramyl-L-alanine amidases which are responsible for cleaving the peptide crosslinks from the glycan chain. The overall aim of this project is to determine the structures of the enzymatic and peptidoglycan binding modules of these proteins, to identify precisely their natural substrates and to discover inhibitors of enzyme activity. Because the protein is highly dynamic, the principle methodology being employed is NMR. Some aspects of the project are at quite an advanced stage, and it may be possible to determine a structure during the rotation.

<http://people.cryst.bbk.ac.uk/~ubcg66a/>

Structural characterization of the Survivin-Aurora B interaction using NMR spectroscopy

Dr Xuemei Yuan, Research Department of Structural & Molecular Biology, UCL

(ucbcxuy@ucl.ac.uk)

Ref: ST036

In this 12-week project, we will produce suitable protein complex samples to facilitate NMR surface mapping studies of the interaction between two chromosomal passenger proteins, specifically, Survivin and Aurora B. Survivin is a homo-dimer with an elongated shape on its own thus precludes high-resolution structural studies using NMR spectroscopy. In the context of a 26 kDa ternary Survivin-Borealin-INCENP complex that we have produced, Survivin is a 16 kDa monomer therefore is readily suitable for NMR studies. The kinase domain of Aurora B is 28 kDa and is highly expressed in bacterial cells. The challenge in this project is to produce stable and soluble samples of the kinase domain of Aurora B, and our strategies include using N-terminal tags that have the potential to enhance the solubility of target proteins. Towards the end of the project, we aim to map the interaction surface of Survivin upon binding to Aurora B, using NMR chemical-shift perturbation studies.

<http://www.smb.ucl.ac.uk/structural-biology-molecular-biophysics/dr-xuemei-yuan.html>

Computational Biology

Employing systems biology to find commonality between polyglutamine repeat disease mechanisms

Dr Kevin Bryson, Computer Sciences, UCL (K.Bryson@cs.ucl.ac.uk)

Ref: CP001

There are currently 10 known polyglutamine repeat diseases and all share similar phenotypic features which are currently poorly understood at the molecular level. In this project, public microarray data will be integrated with biological pathway information, with the aim of detecting dysregulation shared between these different diseases at the network level. In doing so, the student will apply the latest statistical and machine learning techniques to -omics data within the context of human diseases.

<http://www.cs.ucl.ac.uk/staff/K.Bryson/>

Modelling Protein Evolution

Dr Richard Goldstein, Mathematical Biology, MRC NIMR (rgoldst@nimr.mrc.ac.uk)

Ref: CP002

We are interested in understanding how the evolutionary process yielded proteins with their observed structural, functional, and energetic properties characteristics, and how these characteristics can provide insight into the evolutionary process. Similarly, we would like to evaluate methods that have been developed to reconstructing phylogenetic relationships and evolutionary trajectories, but where the 'right' answer is generally unknown. By simulating protein evolution in a computer, we can explore the relationship between evolutionary process and resulting protein properties, while generating synthetic data for evaluating different methods where the evolutionary trajectory is known in complete detail.

<http://www.nimr.mrc.ac.uk/mathbio/goldstein/>

Computational redesign of arsenite oxidases

Prof David Jones, Computer Sciences / Research Department of Structural & Molecular Biology, UCL (dtj@cs.ucl.ac.uk)

Ref: CP003 - Linked with ST023

Arsenic is a notorious poison and is toxic to most cells. Prokaryotes however have evolved mechanisms to metabolise it by either using arsenate (As^{V}) as a terminal electron acceptor or arsenite (As^{III}) as an electron donor. The arsenite oxidase (Aro) catalyses the conversion of As^{III} to As^{V} coupled with the reduction of oxygen to water. The enzyme from three mesophiles has been purified and characterised and consists of two heterologous subunits that bind various cofactors (i.e. Mo, Fe, S). Homologues of the *aro* genes have been identified in various mesophiles (e.g. members of the *Proteobacteria*), thermophiles (e.g. *Thermus* spp.) and hyperthermophiles (i.e. three members of the Archaea). The mesophilic enzyme has been used as a biosensor for arsenite but improving its efficiency and thermal stability should lead to a better biosensor.

As a first step, 3-D models of representative arsenite oxidases will be built and examined to try to identify the sequence determinants which separate the enzymes from mesophiles from those of thermophiles. Based on this initial phylogenetic and modelling survey, an attempt will be made to computationally redesign the mesophilic enzyme by a making a minimum number of mutations to achieve high predicted thermostability and efficiency. This rotation project will involve protein sequence analysis, comparative modelling and novel computational design techniques using a method based on our *de novo* protein structure prediction algorithm.

<http://www.cs.ucl.ac.uk/staff/D.Jones/index.html>

Developing Improved Methods for Predicting Protein Interaction Sites

Prof David Jones, Computer Sciences / Research Department of Structural & Molecular Biology, UCL (dtj@cs.ucl.ac.uk)

Ref: CP004

In this rotation project, the overall aim will be to try to improve the prediction of functional binding sites in protein structures using new sequence and structure features. The initial part of the project will be to implement a basic Web server for predicting interaction sites. The initial model for this will be to read in a set of protein coordinates and analyse residue conservation and residue exposure using machine learning techniques. Once the baseline method has been implemented and tested, we will explore the addition of novel features such as predictions of protein disorder or novel measures of residue polarizability.

<http://www.cs.ucl.ac.uk/staff/D.Jones/index.html>

An automated LOVD parser for SAAPdb

Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
(martin@biochem.ucl.ac.uk)

Ref: CP005

SAAPdb is a database of protein mutations that we have developed over the last 5 years. It integrates mutation data from both SNPs and high penetrance disease-causing mutations with analysis of the likely local structural consequences of the mutations. This project will develop a parser for the new LOVD web-based standard for storing disease mutations; LOVD is very flexible and the parser needs to recognize and extract salient features automatically. This will allow many new sets of mutation data to be imported into SAAPdb. Programming skills will be required, but may be learned during the project.

<http://www.smb.ucl.ac.uk/bioinformatics/dr-andrew-c.r.-martin.html>

Modelling humanized anti-viral antibodies

Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
(martin@biochem.ucl.ac.uk)

Ref: CP006

This project will be performed in collaboration with the Defence Science and Technology Laboratory who are investigating the potential of developing humanized anti-viral antibodies as therapeutic agents. They have a series of antibodies with very similar sequence, some of which bind very well, while others have no activity at all. This project will build homology models of these antibodies using novel methods we are developing to see if we can get clues about key differences in structure. This information will later be compared to crystal structures. No programming skills are required.

<http://www.smb.ucl.ac.uk/bioinformatics/dr-andrew-c.r.-martin.html>

Virtual Screening against Rpf from *M. tuberculosis*

Dr Irilenia Nobeli, School of Crystallography, Birkbeck (i.nobeli@mail.cryst.bbk.ac.uk)

Ref: CP007 - Linked with ST011 and CH005

Tuberculosis (TB) results in approximately 3 million deaths each year, and the failure of the BCG vaccine to afford significant protection, the problems of multiple drug resistance, and the deadly combination of infection with *M. tuberculosis* and HIV have all served to stimulate a renewed drive to understand and tame this ancient disease. The WHO estimates that one third of the population harbours a latent tuberculosis infection and reactivation of the bacteria occurs in 10% of non-HIV patients and up to 30% of HIV patients, accounting for 13% of HIV-related deaths. Understanding and controlling dormancy is therefore a crucial development in anti-TB therapies.

Using the software GLIDE, a library of compounds will be virtually screened for binding to the NMR structure of RpfB for *M. tuberculosis* (Cohen-Gonsaud et al 2005; PDB1xsf). The GLIDE software has the ability to impose constraints on the binding position which makes it particularly suitable for the rather open pocket of the Rpf proteins. The use of alternative scoring functions and allowing some flexibility of the side chains in the binding site will also be explored in order to increase confidence in the *in silico* results.

<http://people.cryst.bbk.ac.uk/~ubcg71a/>

Toward a small molecule fragment library for protein function discovery

Dr Irilenia Nobeli, School of Crystallography, Birkbeck (i.nobeli@mail.cryst.bbk.ac.uk)

Ref: CP008 - Linked with ST034

One of the biggest challenges of the post-genomic era is the assignment of function(s) to the 60% of known protein sequences that have no known or only very distant homologues in the protein databases. In the case of proteins whose function depends on the binding of metabolites, a promising approach could be the use of small molecule fragments designed to probe protein function. Fragment-screening is playing an increasing role in drug discovery in industry and academia. This project aims to design and test a library of fragments from naturally occurring molecules for use in function discovery.

Cheminformatics methods will be employed to select a limited set of small molecules from commercially available datasets. The selection will be based on a number of physical criteria, quality of match to substructures occurring in natural metabolites, and inclusion of the reaction centre, followed by cluster analysis to reduce the number of fragments. The library will be optimized for function discrimination through *in silico* docking to representative protein structures.

<http://people.cryst.bbk.ac.uk/~ubcg71a/>

Computational protocols for analysing functional genomics data

Prof Christine Orengo, Research Department of Structural & Molecular Biology, UCL

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In collaboration with Dr Paul Kellam, Centre for Virology, UCL

Ref: CP009

Functional genomics experiments (e.g. microarray or proteomics analyses) typically produce long lists of hundreds of genes that could be associated with a particular biomedical system of interest. Since it is not possible to experimentally validate all the proteins, computational approaches are critical for filtering the data and selecting those proteins most likely to be truly associated with the biological process. The Orengo group have developed the BioMiner protocol which exploits evolutionary and functional information in the CATH-Gene3D database to predict functional networks of proteins involved in biological processes. This project will explore ways of improving this protocol and applying it to analyse microarray data being produced in Dr Paul Kellam's Group, on host-virus interactions.

<http://www.smb.ucl.ac.uk/bioinformatics/professor-christine-orengo.html>

Deciphering the metabolic diversity of a model arsenite-oxidising bacterium

Prof Christine Orengo, Research Department of Structural & Molecular Biology, UCL

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In collaboration with Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL

Ref: CP010

Arsenic is toxic yet organisms are isolated that thrive in arsenic-rich environments. Arsenite is the most mobile and toxic form of arsenic and can be used by members of the Bacteria as an energy source. The bacterium, NT-26 is a member of the *Proteobacteria* and gains energy from the oxidation of arsenite to arsenate which is linked to the reduction of oxygen to water. Recently, the NT-26 genome sequence has been determined. This project will involve searching the annotated genome sequence for putative metabolic proteins and their respective pathways. Once pathways are identified, the organism will be tested for its ability to use these specific compounds (e.g. elemental sulphur) for growth. Expression of the respective metabolic gene may also be determined under different growth conditions by isolating the RNA and performing RT-PCR.

<http://www.smb.ucl.ac.uk/bioinformatics/professor-christine-orengo.html>

Prediction of metal binding sites in complement factor H (CFH)

Prof Steve Perkins, Research Department of Structural & Molecular Biology, UCL

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Ref: CP011

CFH of the innate immune system is genetically linked with age-related macular degeneration (AMD) and causes loss of central vision in the elderly (0.5 million patients in the UK). High amounts of zinc are found in the drusen retinal deposits that accumulate in AMD patients. Under these conditions our ultracentrifugation work shows that CFH forms large aggregates, indicating at least two zinc-binding sites. Four types of zinc-binding sites are known in proteins. The aim of this project is to predict the zinc-binding sites in CFH. Bioinformatics analyses will be performed to compare crystal, NMR and homology structures for all 20 SCR domains in CFH with known experimental zinc-protein structures in the Protein Data Bank, and rank these in order of probability in order that these can be tested.

→ See also ST018

<http://www.smb.ucl.ac.uk/bioinformatics/professor-steve-perkins.html>

Investigating the links between B-cell differentiation and disease using text and data mining techniques

Dr Adrian Shepherd, School of Crystallography, Birkbeck (a.shepherd@mail.cryst.bbk.ac.uk) [PI]

Dr Paul Kellam, Centre for Virology, UCL (p.kellam@ucl.ac.uk) [Co-I]

Ref: CP012

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 differentiation 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 be a collaboration between Dr Adrian Shepherd and Dr Paul Kellam. It will involve developing new information retrieval strategies to address these challenges based on methods already being developed in the Shepherd group.

<http://www.cryst.bbk.ac.uk/~ubcg60a/>

Prediction and analysis of regulatory sequences for mammalian olfactory receptor genes

Dr Alona Sosinsky, School of Crystallography, Birkbeck (a.sosinsky@mail.cryst.bbk.ac.uk)

Ref: CP013

Hundreds of olfactory receptors, sensitive detectors of food, mates, and oviposition sites, are specifically expressed in the neurons of olfactory epithelium. Each of these neurons expresses only one or a few receptor genes. The mechanisms underlying this intriguing mode of gene expression are still poorly understood. Experimental data suggests that at least some regulatory sequences are shared by several olfactory receptor genes. The goal of this project is the computational discovery and analysis of these shared regulatory inputs with the help of our EDGI tool for discovery of short conserved motifs and their clusters in a set of related sequences.

<http://people.cryst.bbk.ac.uk/~ubcg70a/>

Distribution of binding sites for transcription factors in Drosophila genome

Dr Alona Sosinsky, School of Crystallography, Birkbeck (a.sosinsky@mail.cryst.bbk.ac.uk)

Ref: CP014

Transcription regulation of genes depends on sequence-specific binding of transcription factors (TFs) to their DNA binding sites within highly structured regulatory regions. Binding sites for cooperative TFs are often organized into functional groups called modules. The correct positioning of binding sites within regulatory module is shown to be functionally important. This is due to the requirement for TFs to maintain a particular position relative to each other on the DNA helix in order to juxtapose their respective protein-protein interaction domains and affect function. The project will involve prediction of specific arrangements

of cooperative binding sites within the regulatory modules from overrepresentation of pairs of corresponding candidate binding sites with particular spacing in a whole *Drosophila* genome.

<http://people.cryst.bbk.ac.uk/~ubcg70a/>

Design of novel protein folds (computational and structural)

Prof Willie Taylor, Mathematical Biology, MRC NIMR (wtaylor@nimr.mrc.ac.uk)

Ref: CP015

The prediction of protein structure reveals folds not found in Nature and for some of these, there is no apparent reason why they should not exist. This project will take novel predicted folds and design sequences for them. The best sequences will be synthesised and studied, either through external collaborators or, ideally, within the consortium. For sequences that fold, structural studies will be carried out, preferably by NMR (using the models to help solve the structure). The project will involve the development of software for protein design (building on current work in the group) along with molecular biology and structural studies with consortium partners.

<http://www.nimr.mrc.ac.uk/mathbio/taylor/>

Bacterial coat proteins: elucidating the structure of surface-layer lattices with computational modeling

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Ref: CP016 - Linked with ST014 and CH007

Surface layer (S-layer) proteins are a class of bacterial exoproteins which self-assemble into large two-dimensional crystalline lattices. The regularly shaped cell coats have important biological roles in archaea and many eubacteria including human pathogens. To address the shortage of high-resolution structural data, a combined approach will be pursued which integrates chemical cross-linking, electron-microscopy (EM), and computational modelling. The successful approach will expand our understanding of S-layer assembly and elucidate the structure at the atomic level.

The experimental data will be integrated into one computational framework. This approach will include the modeling of SbsB structure using bioinformatic methods, the fitting of the modeled structure into the electron-microscopy density maps, and the use of the cross-linking data as spatial restraints. In the absence of X-ray crystallography or NMR spectroscopy data, we hope that the incorporation of all the available low-resolution experimental data will improve the modeling process.

<http://people.cryst.bbk.ac.uk/~ubcg67a/>

Allosteric mechanism and dynamics of ATP binding to GroEL

Dr Maya Topf, School of Crystallography, Birkbeck (m.topf@mail.cryst.bbk.ac.uk)

Ref: CP017 - Linked with ST021

Chaperonins are cylindrical-shaped macromolecular assemblies found in all three domains of life. They are essential for the folding of a significant number of proteins in the cell and therefore understanding how they work is an important problem in modern biology. GroEL is an *E. coli* chaperonin, containing two heptameric rings of identical subunits that enclose central cavities where non-native proteins are captured. Its allosteric cycle includes the formation of a complex with non-native substrate protein, ATP and a lid-like co-chaperonin GroES which facilitates protein folding, followed by ATP hydrolysis in the occupied ring which enables the release of GroES and folded substrate when new ATP binds to the opposite ring. Despite the extensive research by a variety of biochemical and structural techniques, the mechanism that advances the GroEL-GroES complex upon this ATP-driven folding cycle is still poorly understood. Recent work in the cryo electron microscopy (cryoEM) lab at the school of crystallography resulted, for the first time, in a number of distinct conformations of intermediate structures at different ATP states along the cycle. Unfortunately, the resolution of these structures, although at the sub-nanometer range, is still far from near atomic, which is required for a deeper analysis of the mechanism.

We will use GroEL-GroES-ATP and ADP structures previously determined in the Saibil laboratories, which will be subjected to analysis by molecular dynamics and free-energy calculations, to unravel the mechanism of allostery in the GroEL-GroES ATP cycle. The proximity of the two labs will enable the student to work in

close contact with the people involved on the experimental side of this work, learning their methods and applying computational tools in order to improve their experiments.

<http://people.cryst.bbk.ac.uk/~ubcg67a/>

Computational haplotype reconstruction using indel mutations

Prof Ziheng Yang, Department of Biology, UCL (z.yang@ucl.ac.uk)

Ref: CP018

Determination of the phase or haplotype in DNA sequences from diploid individuals is important to many analyses in population genetics and genomics. When DNA is sequenced in both directions and when there is a heterozygous indel, haplotype can be inferred from a forward/reverse sequencing pair. The aim of this project is to develop a computer algorithm/programme for automatic haplotype reconstruction. The student should have good C/C++ programming skills and will develop skills of applying computational and mathematical techniques to solve important biological problems.

<http://www.ucl.ac.uk/biology/academic-staff/yang/yang.htm>

Chemical Biology

The synthesis of new beta-blockers

Dr James Baker, Department of Chemistry, UCL (james.baker@ucl.ac.uk)

Ref: CH001

In this project the student will carry out the synthesis of new beta-blockers. These compounds will be highly selective for the beta-2-adrenergic receptors. A fluorescent tag will then be attached to the beta-blocker that will allow us to visualize them when they bind to the receptors, providing us with important information on these medicinal targets.

<http://www.chem.ucl.ac.uk/people/Baker/index.html>

Investigating how the primary structure of PIKfyve affects its inhibition by YM201636

Dr Kate Bowers, Research Department of Structural & Molecular Biology, UCL

(katherine.bowers@ucl.ac.uk)

Ref: CH002

The type III phosphatidylinositol 3-phosphate 5-kinases (PIPkins) have a role in many cellular processes including: endomembrane homeostasis, glucose uptake, neural development and retroviral budding. The newly discovered drug YM201636 inhibits potently the murine but not the yeast type III PIPkin; however, the basis for this difference in potency is unknown. The aim of this project is to determine which structural features in the ATP-binding site of the type III PIPkins define sensitivity to YM201636. The project will require a comparison of PIPkins from yeast and mammals, and based on predictions from this, the expression of recombinant proteins with appropriate mutations. The sensitivity of these proteins to YM201636 will then be determined.

<http://www.smb.ucl.ac.uk/molecular-cell-biology/dr-kate-bowers.html>

Synthesis of Biologically Active Sulfonamide Libraries

Prof Steve Caddick, Department of Chemistry, UCL (s.caddick@ucl.ac.uk)

Ref: CH003

The synthesis of small molecules as pharmacological tools and as potential therapeutics is vitally important in fundamental biology and for the development of new treatments for disease. The SC group has been heavily involved in the development of new synthetic methods for the construction of diverse compound collections. The methods have been based upon novel approaches to sulfonamides and utilising all of the key aspects of modern synthetic science. In this rotation students will develop approaches to novel chemical entities which can then be screened against a wide range of biologically important targets. Recent examples of success using this approach include the development of novel inhibitors of HIV and of the methylarginine processing enzyme DDAH1.

Rotation students will learn the principles of organic synthesis, structural characterisation, library design and gain an appreciation of the scope and limitations of parallel synthesis, diversity oriented synthesis and combinatorial chemistry.

<http://www.chem.ucl.ac.uk/people/caddick/index.html>

Structure/function analysis of mTOR beta: Design of potential small molecule inhibitors towards mTOR kinase

Prof Steve Caddick, Department of Chemistry, UCL (s.caddick@ucl.ac.uk)

Ref: CH004

The mTOR (mammalian target of rapamycin) is a central regulator of an evolutionary conserved signalling pathway which controls cellular metabolism, growth and proliferation. Deregulation of the mTOR-coordinated signalling has been associated with various human pathologies, including diabetes, inflammation and cancer. Rapamycin, a naturally occurring mTOR inhibitor, and its homologues have been currently examined as anti-cancer drugs in over 40 clinical trials. We have recently identified a novel mTOR

splicing form, termed TOR β , which in contrast to the full length mTOR protein (mTOR α) has the potential to shorten significantly G1 phase of the cell cycle and to induce cell proliferation and survival. In agreement with these findings we also showed that mTOR β is an oncogene, capable to transform cells in cellular and animal models.

The structure of mTOR kinase has not been solved so far, despite major efforts in academic and industrial institutions. The task is complicated by the size of the protein (290kDa) and the inability to purify large quantities of endogenous protein or to obtain soluble protein (full length or kinase domain) in expression systems. The molecular weight of mTOR β is 80kDa, which makes it an attractive target for structural studies. Moreover, preliminary studies indicate that partially soluble mTOR β (fused to a solubility tag) could be expressed in bacteria.

The project offers the opportunity for gain a diverse range of experiences in molecular cloning, site-directed mutagenesis, tissue culturing, expression of recombinant proteins in bacterial and baculoviral systems, affinity purification and analysis of recombinant proteins, a range of techniques associated with synthesis and quality control of mTOR inhibitors.

In this rotation, in collaboration with Neil McDonald, we will design potential small molecule inhibitors using a combination of combinatorial and target oriented synthesis.

→ See also ST009 and ST012

<http://www.chem.ucl.ac.uk/people/caddick/index.html>

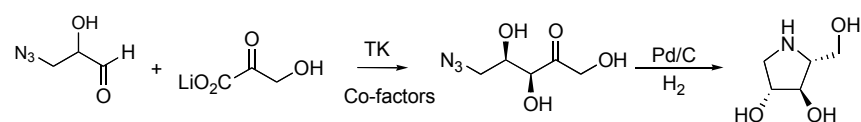
Synthesis of small molecule inhibitors against *M. tuberculosis*

Dr Helen Hailes, Department of Chemistry, UCL (h.c.hailes@ucl.ac.uk)

Ref: CH005 - Linked with ST011 and CP007

Tuberculosis (TB) results in approximately 3 million deaths each year, and the failure of the BCG vaccine to afford significant protection, the problems of multiple drug resistance, and the deadly combination of infection with *M. tuberculosis* and HIV have all served to stimulate a renewed drive to understand and tame this ancient disease. The WHO estimates that one third of the population harbours a latent tuberculosis infection and reactivation of the bacteria occurs in 10% of non-HIV patients and up to 30% of HIV patients, accounting for 13% of HIV-related deaths. Understanding and controlling dormancy is therefore a crucial development in anti-TB therapies.

The RpfB from *M. tuberculosis* shows homology to several glycoside hydrolases. Binding of RpfB to N-acetyl-glucopyranosyl amine (NAG, one of the two sugars that make up the polysaccharide of peptidoglycan) was not observed, but tri-NAG was found to influence chemical shifts of residues in the putative binding groove (S. Knap et al., J. Am Chem. Soc., 1996, 118, 6804). Therefore, one series of compounds to be used in screens that we propose synthesising are small molecule iminocyclitols that are potent glycosidase inhibitors: their efficacy is attributed to their mimicry of the transition state of enzymatic glycosidic cleavage. These can be synthesised using a reported biocatalytic route and the enzyme transketolase as outlined below.



Other compounds based on NAG will also be considered, such as azido-NAG (E. I. Petsalakis et al., Bioorg. Med Chem., 2006, 14, 5316) which could readily be modified using click chemistries.

<http://www.chem.ucl.ac.uk/people/hailes/index.html>

Use of Engineered Novel Alkaloid Biosynthetic Enzymes

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Ref: CH006 - Linked with ST030

Alkaloids are a large and diverse family of nitrogen-containing compounds, many are used as pharmaceuticals and they represent a huge repository of functional chemical space. Recently the enzyme

(S)-norcoclaurine synthase (NCS) has been cloned and characterised. NCS carries out the key first coupling step in the pathway that generates the tetrahydroisoquinolines via a Pictet-Spengler reaction between arylethylamines and aldehydes, generating a chiral centre. A codon optimised version for *E. coli* expression has been designed and obtained as a synthetic gene. In this rotation project we will use the recombinant NCS enzyme to investigate its substrate specificity using dopamine, 4-hydroxyphenylacetaldehyde and analogues. Chemical syntheses of the isoquinolines will be carried out using existing methods and screening for the formation of the isoquinolines by NCS will be achieved using the synthetic samples prepared as controls. The substrate specificity of mutant NCSs generated in John Wards group will also be explored (see linked rotation).

<http://www.chem.ucl.ac.uk/people/hailes/index.html>

Bacterial coat proteins: elucidating the structure of surface-layer lattices with chemical cross-linking

Dr Stefan Howorka, Department of Chemistry, UCL (s.howorka@ucl.ac.uk)

Ref: CH007 - Linked with ST014 and CP016

Surface layer (S-layer) proteins are a class of bacterial exoproteins which self-assemble into large two-dimensional crystalline lattices. The regularly shaped cell coats have important biological roles in archaea and many eubacteria including human pathogens. To address the shortage of high-resolution structural data, a combined approach will be pursued which integrates chemical cross-linking, electron-microscopy (EM), and computational modelling. The successful approach will expand our understanding of S-layer assembly and elucidate the structure at the atomic level.

The chemical analysis will focus on single-cysteine mutants of the S-layer protein SbsB from *Geobacillus stearothermophilus* PV72/p2. Surface-accessibility screens and cross-linking have identified cysteine residues at the outer surfaces of the lattice, and residues located at the contact sites between the subunits. Based on the existing single-cysteine mutants, the chemical rotation will exploit mass-spectrometric (MS) analysis of cross-linked peptides to map the subunit-subunit interface in the S-layer lattice.

<http://www.chem.ucl.ac.uk/people/howorka/index.html>

Biosynthetic Studies of DNA-Binding Natural Products

Dr Philip Lowden, School of Biological and Chemical Sciences, Birkbeck (p.lowden@bbk.ac.uk)

Ref: CH008

My group is studying the biosynthetic pathways towards the DNA binding anticancer natural products azinomycin B and anthramycin, using a combination of organic chemistry and molecular biology techniques. This project may involve one of the following experiments: synthesis of an isotopically labelled intermediate followed by spectroscopic analysis of incorporation into the end product; feeding of unnatural intermediates to the producing bacteria and analysis of fermentations for novel metabolites.

http://www.bbk.ac.uk/bcs/about_staff/lowden

Protein Mutagenesis to Explore Selectivity in Chemically Induced Thioesterification

Dr Derek Macmillan, Department of Chemistry, UCL (d.macmillan@ucl.ac.uk)

Ref: CH009

Native chemical ligation (NCL) is an extremely powerful chemical reaction which occurs in water, in the presence of all the functional groups present in naturally occurring proteinogenic amino acids, in high yield resulting in the formation of a native peptide (amide) bond at the site of ligation. Recently we observed that, under a specific set of reaction conditions, protein thioesters, which are key components in the reaction, can be formed through selective chemical protein cleavage. Our group is internationally known for its work in NCL chemical ligation. The aim of this project is to use a combination of organic synthesis and molecular biology to determine the parameters which make this an efficient process. Having developed optimal reaction conditions we will prepare a panel of mutant proteins using the polymerase chain reaction (PCR) and produce them in the bacterium *E. coli*, studying the generation of thioester intermediates from these protein precursors by LC-MS. 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.

<http://www.chem.ucl.ac.uk/people/macmillan/index.html>

Peptide Ligation to Explore Oligomerisation of the Regulatory Phosphoprotein Chk2

Dr Derek Macmillan, Department of Chemistry, UCL (d.macmillan@ucl.ac.uk)

Ref: CH010

Protein phosphorylation plays an important regulatory role in signaling pathways and transcription during cell-cycle progression. Interplay between many such phosphoproteins is required for execution of a successful and correctly timed program of responses to DNA damage. The aim of this project is to investigate the effect of specific phosphorylation events in the FHA domain on the structure and function of Chk2. The project is particularly suited to a chemical semi-synthetic approach since sufficient homogeneously phosphorylated material for analysis is not available from biological sources. In collaboration with the Smerdon lab at the National Institute for Medical Research (NIMR), who have over-expressed a fragment of the Chk2 FHA domain in a form such that the effects of phosphorylation can be investigated using expressed protein ligation we will complete the semi-synthesis of Chk2 for crystallisation trials and biological assay.

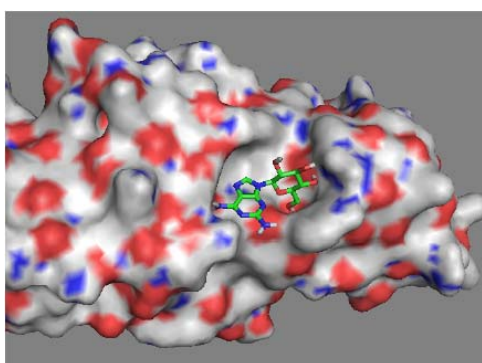
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Targeting the Action of E. Coli on the Human Kidney through the Synthesis and Biology of New Inhibitors of PapG

Prof Charles Marson, Department of Chemistry, UCL (c.m.marson@ucl.ac.uk)

Ref: CH011

The aim is to synthesise and evaluate of novel synthetic agents that act as competitive inhibitors for PapG-GbO4 (tetrasaccharide) receptor binding. Those inhibitors would minimise the attachment of *E. coli* bacteria to human kidney cells, thus being a potential treatment for kidney infections. The project will involve preparing the compounds in no more than two steps (and therefore suitable for a student who has done only limited compound preparation and purification). The student will combine the nitrogen ring segment (SW segment of ligand coloured green in the figure) with the sugar segment (NE segment). Under a collaboration with Prof G. Waksman, it is anticipated that the compound will be evaluated for affinity to the PapG protein using the Biacore technique. If the affinity is high it may be possible to attempt crystal growing of the ligand in the protein, for X-ray crystallography. (Ref: K. W. Dodson, J. S. Ponkner, T. Rose, G. Magnusson, S. J. Hultgren and G.Waksman, Cell 2001, 105, 733)



<http://www.chem.ucl.ac.uk/people/marson/index.html>

Oligomeric structure of the archaeal TIP complex

Dr Adam McKay, Department of Chemistry, UCL (adam.mckay@ucl.ac.uk)

Ref: CH012

The research in our laboratory focuses on analysing the structure and function of large multisubunit noncovalent complexes, primarily by 'native' mass spectrometry (MS) methods. Conventional MS denatures proteins using organic solvents and acids prior to analysis; here the proteins are ionized from volatile pH neutral buffers and transferred to the MS instrument using modified initial vacuum pressures all helping to

preserve tertiary and quaternary structure during transition from the solution to the gas-phase. Eukaryotic TBP-interacting proteins are involved in the regulation of gene expression and show interesting oligomerisation behaviour in in vitro studies. This projects aims to unravel the oligomeric populations of the TBP binding protein TIP from the archaeobacterium *Methanopyrus kandleri*. (Ref: Hernandez & Robinson, Nature Protocols, 2007, 2, 715-726)

→ See also ST032 and ST033

<http://www.chem.ucl.ac.uk/people/mckay/index.html>

Nano-scale Imaging of Supported Lipid Bilayers

Dr Susan Perkin, Department of Chemistry, UCL (susan.perkin@ucl.ac.uk)

Ref: CH013

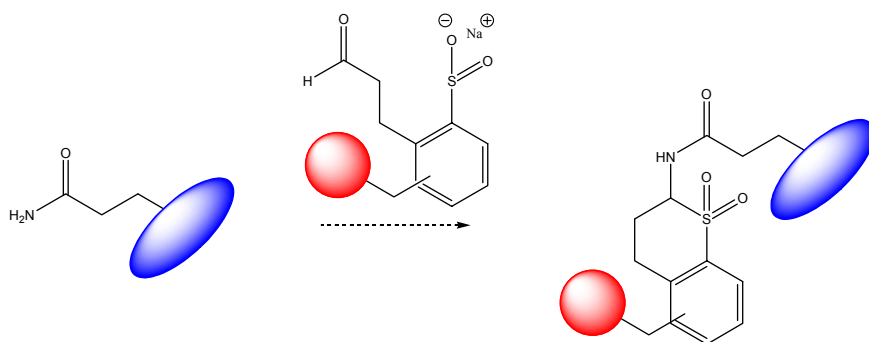
Lipids are a major class of biomolecules, performing the central task of encapsulating cell contents as well as creating compartments within cells. The different mixture of lipids occurring in different membranes results in variation in protein incorporation, flexibility, disorder, fusion, and many other properties. In order to study lipid membranes and their interactions with other molecules it is often useful to form a bilayer structure directly on a solid surface; the result is a so called 'supported lipid bilayer' or SLB. This project will aim to form SLBs using the method of vesicle deposition on a solid surface, and to study their properties using AFM (Atomic Force Microscopy) to create nano-scale images of the lipid layers.

<http://www.chem.ucl.ac.uk/people/perkin/index.html>

A Method for Selective Derivatisation of Glutamine and Asparagine Residues

Dr Tom Sheppard, Department of Chemistry, UCL (tom.sheppard@ucl.ac.uk)

Ref: CH014



There are many techniques for selectively modifying cysteine or lysine residues on protein surfaces with useful moieties such as fluorescent tags or binding groups. However, there are very few methods, for selectively targeting other residues via chemical methods. In this rotation project, we will investigate a new method to selectively functionalise the amide side-chains of glutamine and asparagine residues using a known chemical reaction of primary amides with aldehydes and sulfinates.

<http://www.chem.ucl.ac.uk/people/sheppard/index.html>

Structural studies of designed helical peptides arrayed on gold surfaces

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In collaboration with Dr Daren Caruana and Dr Abil Aliev, Department of Chemistry, UCL

Ref: CH015

There is currently major interest in being able to design protein motifs that can be arrayed on surfaces to produce designer materials with specific properties. We have been studying the electrochemical properties of a series of peptides, designed to be helical, and complexed to ruthenium chelates, that are arrayed on gold electrodes. CD studies of the peptide in solution have indicated that the peptides adopt either a PPII or an α -helical conformation, depending on the solvent. The aim of this project is to determine the conformation(s) that the peptide adopts on the gold support. The student will synthesise several alanine-

rich peptides and will study the conformation of the peptides by solid-state NMR, both uncomplexed and complexed to colloidal gold.

<http://www.chem.ucl.ac.uk/people/tabor/index.html>

Dendrimeric peptides binding siRNA

Dr Alethea Tabor, Department of Chemistry, UCL (a.b.tabor@ucl.ac.uk)

In collaboration with Dr Stephen Hart, ICH

Ref: CH016

Recently, small interfering RNA (siRNA) molecules have emerged as powerful tools for silencing genes, allowing the investigation and dissection of gene function. However, the major barrier to the use of siRNA as a tool is the difficulty of effectively delivering oligonucleotides in vivo. We have been investigating the use of bifunctional peptides comprising cationic dendrimeric sequences (to condense and package the siRNA) and a receptor-targeting sequence (allowing for cell-specific uptake of the resulting complex). In this project the student will synthesise a range of these bifunctional peptides and will investigate their binding to siRNA by gel-retardation assays in agarose gels. Techniques include: solid-phase peptide synthesis; peptide purification; analysis by mass spectrometry; gel-retardation assays.

<http://www.chem.ucl.ac.uk/people/tabor/index.html>

Probing lateral molecular clustering in lipid membranes

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Ref: CH017

Conjugates of water-soluble porphyrins and cholesterol embed efficiently into lipid membranes. Their tendency to form lateral clusters can be determined by monitoring changes in the porphyrin fluorescence and/or UV spectrum. The use of porphyrin conjugates with other lipids opens the possibility of determining the relative clustering tendencies of different membrane components. This project aims to prove this principle and will be developed in two stages:

- Synthesis and purification of porphyrin conjugates with di-glycerides and phosphate glycerides
- Determining the clustering tendency of the conjugates by optical spectroscopy measurements

The development of the project will require the use of traditional organic synthesis techniques and physico-chemical approaches to data treatment.

http://www.bbk.ac.uk/bcs/about_staff/tomas

Synthesis of β -Lactamase Antibiotics and their Analogues

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Ref: CH018

Our work has focused on the preparation of peptidomimetics, particularly aminosulfonic acid analogues of the peptide bond. During the course of this investigation, we have found that β -sultams (direct analogues of the β -lactams) can be prepared with relative ease. The synthesis involves 1,4-addition of an amine to an α -bromo-vinylsulfonate template followed by ring closure to give the α -bromo sultam. The proposed project will involve extension of our existing methodology to prepare more closely related analogues of the β -lactamase inhibitors. If time permits we will seek to add additional functionality at the α -position of the sulfonamide by manipulation of the alkyl bromide functionality. We envisage that this will be achieved by utilisation of an organometallic intermediate such as an organoindium, gallium, nickel, palladium or zinc species.

<http://www.chem.ucl.ac.uk/people/wilden/index.html>