4-year PhD Interdisciplinary Programme in Structural, Computational and Chemical Biology at UCL/Birkbeck/NIMR

First year rotations projects

2010-2011

Contents

Introductory notes 2

Structural and Molecular Biology 3

Chemical Biology 14

Computational Biology.. 19

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 11 October 2010 - Friday 14 Jan 2011	Top three preferences for the first rotation should be submitted to Jamie Baker, the programme coordinator, by 5pm on Thursday 7 October (j.r.baker@ucl.ac.uk)
Rotation 2	Monday 24 January 2011 - Friday 15 April 2011	Wednesday 05 January 2011 at 5pm
Rotation 3	Monday 16 May 2011 – Friday 05 Aug 2011	Wednesday 23rd March 2011 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: 10-ST035, 10-CP010 and 10-CH002 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.

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

Structural and Molecular Biology

EPR analysis of the key NF-κB modulator IKKγ .
Dr Tracey Barrett, Department of Biological Sciences, Birkbeck (t.barrett@mail.cryst.bbk.ac.uk)
Ref: 10-ST001

Project description not available.

The intracellular trafficking of NHE6
Dr Kate Bowers, Research Department of Structural & Molecular Biology, UCL
(katherine.bowers@ucl.ac.uk)
Ref: 10-ST002

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 mammalian NHE6, in particular its intracellular trafficking, using a combination of molecular and cell biological techniques.

http://www.smb.ucl.ac.uk/department-staff/index.php

Mutagenesis of Na+/H+ Exchanger Proteins (NHEs) based on molecular modelling
Joint project: Dr Kate Bowers, Research Department of Structural & Molecular Biology, UCL
(katherine.bowers@ucl.ac.uk)
Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
(martin@biochem.ucl.ac.uk)

Ref: 10-ST003, Linked with 10-CP009

Sodium/ proton exchangers of the NHE family are large, multi-spanning membrane proteins that transport one sodium ion for one proton across cellular membranes. We are interested in the intracellular subfamily of NHE proteins, particularly human NHE6, human NHE8 and yeast Nhx1p, which are all found on endosomal compartments. This project will use the molecular models/ structural predicitons created in the linked project "Modelling of Na+/H+ Exchanger Proteins (NHEs)" with Andrew Martin to determine key residues to mutate. Site-directed mutagenesis, in combination with functional assays, will allow us to test the structural models and gain insight into NHE function. Functional assays will be performed in mammalian tissue culture cells or yeast, as necessary.

The direction of this rotation will depend on the outcome of the linked rotation project, and thus the linked project should be completed (by the same student or another student) first.

http://www.smb.ucl.ac.uk/department-staff/index.php

Crystallisation trials with recombinant yeast mitochondrial cytochrome c oxidase
Dr Snezana Djordjevic, Research Department of Structural & Molecular Biology, UCL
(snezana@biochem.ucl.ac.uk)

Ref: 10-ST004, Linked with 10-ST0017

Cytochrome c oxidise is a large transmembrane protein complex found in mitochondria. It is the last enzyme in the electron transport chain and it contains a multitude of redox-active metal sites that are engaged in electron transport. This project follows on from the rotation project of Peter Rich on purification of a histidine-tagged form of mitochondrial cytochrome c oxidase from baker's yeast by nickel affinity chromatography. The aim of this rotation is to crystallise recombinant cytochrome c oxidase in order to produce crystals of sufficient quality for subsequent 3D structure determination. The longer terms aims will be to solve the 3D structure by X-ray crystallography and to explore enzyme mechanism with biochemical/biophysical studies of wild-type and mutant forms. Hence, these two linked rotation projects can form the basis for a subsequent Ph.D. programme.

http://www.smb.ucl.ac.uk/molecular-microbiology/dr-snezana-djordjevic.html

Exploration of death receptor death domain folding stability through chimaera design and cyclisation
Prof Paul Driscoll, Division of Molecular Structure, MRC NIMR (pdrisco@nimr.mrc.ac.uk)
Ref: 10-ST005

A subset of the TNF receptor superfamily is implicated in the extrinsic pathway of programmed cell death. These receptors (TNFR1, CD95/Fas, TRAIL-R1/R2) include a cytoplasmic domain comprising a homologous ~100 residue 'death' domain (DD) that makes critical homotypic interactions with the downstream adaptor proteins, e.g. FADD. Whilst the CD95-FADD DD-DD interaction is tractable, to date the DD's of TRAIL-R1 and -R2 do not yield stable proteins. The TRAIL receptors represent potential therapeutic targets for monoclonal antibody treatments in cancer. On the basis of a detailed sequence analysis we will explore the potential for obtaining stable folded TRAIL-R DD's using the principles of knowledge-based protein design and a high throughput protein preparation/NMR folding screen recently established in our lab. In addition we will test the potential for intein-based protein cyclisation to enhance the folding stability of both already tractable and more difficult DD targets.

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

Towards development of a protein-based small molecule biosensor based upon a rare folding topology
Prof Paul Driscoll, Division of Molecular Structure, MRC NIMR (pdrisco@nimr.mrc.ac.uk)
Ref: 10-ST006

The enzyme dimethylaminoarginine dimethylaminohydrolase (DDAH) derives from a wider family of proteins that are generally implicated in the metabolism of biological molecules related to the amino acid arginine. These enzymes have a relatively 3D molecular architecture rare involving a five-(beta-beta-alpha-beta)-bladed propeller topology. The active site is situated in the core of the protein, along what would be axis of the propeller shaft. Serendipitously we have found evidence from NMR dynamics investigation that the DDAH active site is enclosed upon substrate binding by a peptide 'flap'. The DDAH structure therefore appears to lend itself to the development of a general framework as a potential fluorescence-based biosensor of small molecule binding. This project will explore to this potential by Cys-coupling of fluorogenic agents to the 'flap' region and assessing the effect of ligand binding by NMR, fluorescence and potentially FRET-based measurements.

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

Structure and dynamics of the active site of histone deacetylase 8 by paramagnetic NMR spectroscopy
Dr Flemming Hansen, Institute of Structural and Molecular Biology (ISMB), UCL/Birkbeck
(d.hansen@ucl.ac.uk)
Ref: 10-ST007

Histone deacetylases (HDACs) modify the structure and function of chromatin and are crucial for transcriptional repression and epigenetic landscaping. The active site of HDACs consists of a metal ion coordinated to a histidine and two aspartic acids. Interestingly, the HDAC enzyme is active with a paramagnetic metal ion in the active site, which opens up for an exciting insight into the structure and dynamics of the catalytic site by paramagnetic NMR spectroscopy.

The project involves expression of isotope labelled HDAC8 enzyme and NMR studies of resonances in the immediate vicinity of the active site. Thus, we will use paramagnetic NMR to begin to characterise the active catalytic site, which may also involve an introduction to enzyme kinetics and theoretical calculations.

Structure-function analysis of a Chlamydia inclusion protein

Dr Richard Hayward, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (r.hayward@mail.cryst.bbk.ac.uk)

Ref: 10-ST008

Chlamydia trachomatis is a major human pathogen responsible for sexually transmitted diseases in the Western world and blindness (trachoma) in Developing nations. It is an obligate intracellular bacterium that survives by building a specialised replicative compartment within infected host cells, termed an 'inclusion'. Inclusion biogenesis requires the action of a family of >40 bacterial 'inclusion proteins' (Incs), likely to subvert host intracellular trafficking, apoptosis and cytokinesis. Incs share no obvious homology to either prokaryotic or eukaryotic proteins and surprisingly little is known about their structure and function.

The project will involve cloning a previously uncharacterised inclusion protein from C.trachomatis into plasmids to allow expression of the protein in laboratory E.coli and mammalian cells. Using microbiological techniques, the student will investigate conditions to express and purify the recombinant hydrophobic protein in E.coli. In parallel, cultured cells will be transfected and protein localisation examined by fluorescence microscopy. The project will provide training in recombinant DNA, aseptic microbiology, and mammalian cell culture techniques, and allow the student to gain experience of fluorescence and confocal microscopy.

Growth and characterisation of Pseudomonas Aeruginosa: Electron Paramagnatic Resonance studies of the whole cells

Joint project: Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL
(c.kay@ucl.ac.uk)
Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL
(j.santini@ucl.ac.uk)

Ref: 10-ST009

Bacteria use many different and unusual molecules as energy and carbon sources. An example is the pathogenic bacterium Pseudomonas Aeruginosa that is able to use ethanol as its sole source of carbon via an enzyme – Quinoprotein Ethanol Dehydrogenase (QEDH) which uses an unusual redox coenzyme - Pyrroloquinoline quinone. Whether this compound is also a vitamin for mammals has been hotly debated recently. Typically, enzymes are expressed in E. coli and isolated before characterisation. In this project we will perform the characterisation of the QEDH in the living cells – literally in vivo – using EPR spectroscopy. Growth of Pseudomonas Aeruginosa cell cultures using ethanol as sole carbon source will be carried out in the Santini Lab. Subsequent, quantification and characteristion of ethanol dehydroganse in the whole cells by EPR & ENDOR spectroscopy will be performed in the EPR Facility under the guidance of Dr Kay.

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

Elucidating RNA - RNAP/Ribosome interaction by magnetic resonance and site-directed spin labelling

Joint project: Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL
(c.kay@ucl.ac.uk)
Dr John Christodoulou, Research Department of Structural & Molecular Biology, UCL
(j.christodoulou@ucl.ac.uk)

Ref: 10-ST010, Linked with 10-CP003

RNA behaviour is not understood in detail due to its mobility and flexibility. To observe and understand RNA behaviour we will apply Nuclear Magnetic Resonance (NMR) and Electron Paramagnetic Resonance (EPR) in combination with site-directed spin labelling.

This enables us to detect relaxation changes via NMR experiments yielding information about the larger enviroment, whereas EPR elucidates the vicinity of the spin label or the distance between two labels. This project also includes the application of novel spin labels, which will enhance orientation selective EPR measurements. In a linked project the obtained data will be used to model the measured complexes.

A good understanding of physical principles is recommended as we will perform many different complex experiments with NMR and EPR.

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

Structural and Biophysical Analysis of Rpf Inhibitors

Prof Nick Keep, Department of Biological Sciences, Birkbeck (n.keep@mail.cryst.bbk.ac.uk)
Ref: 10-ST011

Resucitation promoting factors have been shown to control exit from dormancy - the state in which M.Tuberculosis resides in the lung in many fit individuals until they become weakened and TB develops. Chemical inhibition may act as a novel therapy for TB particularly in vunerable patient groups. (Keep et al., (2006). Wake up! Peptidoglycan lysis and bacterial non-growth states. Trends Microbiol. 14: 271-276)
Compounds generated by a virtual screening rotation last year will be investigated for experimental binding to Rpfs by co-crystallisation in our recently solved crystals of M.tuberculosis RpfC and by thermal denaturaion assay and in HSQC shifts of 15N labelled RpfB. This will give experience of both X-ray crystallography and NMR techniques. The compounds will also be assayed in a resuscitation assay using the non-pathogenic model organism Micrococcus luteus.

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

Structural analysis of Insect Odorant Binding Proteins

Prof Nick Keep, Department of Biological Sciences, Birkbeck (n.keep@mail.cryst.bbk.ac.uk)
Ref: 10-ST012

Insects have a very acute and specific sense of smell and can detect a partner or food plant over large distances. Odorant detection is a two stage process in insects. Firstly the odorant binds to an odorant binding protein (OBP) a diverse range of small proteins found in the antenna. These then deliver the protein to the odorant receptors in the nerve cells. There is evidence that the complex of the OBP and odorant is the stimulant for the receptor rather than the OBP just transporting the odorant to the receptor. Genome studies of aphids indicate that they have a reduced number of OBPs compared to flies or moths while still having great specificity for certain plant species. In collaboration with Rothamsted this project will purify and crystallise and possibly solve the structure of aphid OBPs and attempt to complex them with the known stimulating chemicals. Expression clones and some proteins are available.

This project is in collaboration with Dr Jing-Jiang Zhou and Prof Lin Field from Rothamsted Research

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

Probing structure and function in kinesin motor proteins in malaria (2)

Dr Carolyn Moores, Department of Biological Sciences, Birkbeck (c.moores@mail.cryst.bbk.ac.uk)
Ref: 10-ST013, Linked with 10-CP023

Malaria infects over 300 million people a year, killing more than 1 million. The complex life cycle of the malaria parasite, along with emerging drug resistance, means that novel drug targets are desperately needed. Kinesins are ATP-dependent microtubule-based molecular motors and are potential targets for anti-malarial drugs. The objective of the rotation is to characterise malaria kinesin motors using a combination of biochemistry and structural biology. The motor domains of the putative kinesin motors will be cloned and expressed in E. coli, and their biochemical activity will be characterised. In particular, kinesin ATPase activity and the way the motors interact with microtubules in vitro can provide considerable insight into their functions in vivo. Future work will involve cryo-electron microscopy of kinesin-microtubule complexes, screening for small molecule inhibitors and in vivo studies to understand the precise roles of these motors in the parasite.

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

Structural study of the capsid from the mutant SPP1 bacteriophage

Prof Elena Orlova, Department of Biological Sciences, Birkbeck (e.orlova@mail.cryst.bbk.ac.uk)
Ref: 10-ST014

Bacterial viruses (bacteriophages or phages) are the most populated biological entity in the Biosphere. The SPP1 bacteriophage is a phage that infects Bacillus subtilis which has an icosahedral capsid and a tail. The capsids protect the viral genome from harsh environment when viral particles outside a host cell. The capsid shell has strong mechanical properties that is able to tolerate the internal pressure of 6 atmospheres created by packed inside DNA. That requires a strong interaction between protein subunits that form the capsid. We have a mutant of the SPP1 bacteriophage that has less DNA inside of the capsid and therefore reduced pressure within the capsid.
We have obtained the structure of the wild type capsid and now are intersted to see the changes within the capsid at the reduced internal pressure. Structural analysis will be performed using cryo electron and image processing.

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

Structural study of murine p53 tumor suppressor protein under ADP
Joint project: Prof Elena Orlova, Department of Biological Sciences, Birkbeck
(e.orlova@mail.cryst.bbk.ac.uk)
Dr A. Okorokov, Wolfson Institute for Biomedical Research, UCL (a.okorokov@ucl.ac.uk)
Ref: 10-ST015

The p53 tumour suppressor protein is a classic gatekeeper of cellular fate. p53 initiates cell cycle arrest, senescence or apoptosis via pathways involving transactivation of p53 target genes. However, the exact molecular mechanism(s) of p53 activation as a transcription factor and/or participant in DNA repair pathways remains unknown. The activity of p53 depends of the presence of nucleotides. ADP allows to stabilize p53/DNA complexes and without DNA ADP apparently transfer p53 into the conformation when it is ready to interact with DNA. We would like to study this conformation of p53 using methods of cryo-electron microscopy and computational methods.
We have got the first structure of the murine p53 stabilised by ATP, and now our aim is to reveal a level of conformational changes in this important molecule. This study involves electron microscopy and image processing.

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

Structure-function studies of heparin/CRP binding to complement factor H
Prof Steve Perkins, Research Department of Structural & Molecular Biology, UCL
(s.perkins@medsch.ucl.ac.uk)
Ref: 10-ST016, Linked with 10-CH011

Complement factor H (CFH) of innate immunity is a key and very topical regulator of complement activity. CFH binds to heparan sulphate-like polyanionic oligosaccharides on host cell surfaces in order to protect these from complement attack. Following host protection, complement attacks pathogenic bacteria. Defects of heparin/CRP binding to CFH are associated with aHUS, a common cause of renal failure, and with age-related macular degeneration (AMD), a common cause of blindness in the elderly. The project will study heparin/CRP binding to CFH and its recombinant fragments in order to locate its binding sites and their affinities in the native full-length protein. CFH and its fragments will be purified, monitored by SDS-PAGE, then mixed with highly purified heparin/CRP and studied by sedimentation velocity analytical ultracentrifugation (AUC) and surface plasmon resonance (SPR) experiments. The results will be compared with predictions of heparin/CRP sites and heparin-CFH stoichiometry (see projects 10-CP012 & 10-CH011).

http://www.smb.ucl.ac.uk/department-staff/index.php

Large scale purification of histidine-tagged, yeast mitochondrial cytochrome c oxidase for crystallisation trials
Prof Peter Rich, Research Department of Structural & Molecular Biology, UCL (prr@ucl.ac.uk)
Ref: 10-ST017, Linked with 10-ST004

Cytochrome c oxidase is a major enzyme of the mitochondrial respiratory electron transfer chain. It converts electrochemical energy of oxygen reduction into a gradient of protons for subsequent ATP synthesis (Rich, P.R. and Maréchal. A. (2010) The mitochondrial respiratory chain. Essays in Biochemistry, 27, 1-23). A histidine-tagged form of mitochondrial cytochrome c oxidase from baker's yeast has been produced by transformation of the gene for one of the nuclear-encoded subunits. This allows facile purification by nickel column chromatography. The aim of this rotation is large-scale preparation of purified enzyme that is suitable for subsequent crystallisation trials in the linked rotation with Dr. Snezana Djordjevic. The longer term aim is to solve the 3D structure and to explore mechanism with biochemical/biophysical studies of wildtype and mutant forms. Hence, these projects can form the basis for a subsequent Ph.D. programme.

http://www.smb.ucl.ac.uk/department-staff/index.php

Functional studies of point mutations in yeast mitochondrial cytochrome c oxidase
Prof Peter Rich, Research Department of Structural & Molecular Biology, UCL (prr@ucl.ac.uk)
Ref: 10-ST018

Cytochrome c oxidase is a major enzyme complex of the mitochondrial respiratory electron transfer chain. It converts electrochemical energy of oxygen reduction into a gradient of protons for subsequent ATP synthesis (Rich, P.R. and Maréchal. A. (2010) The mitochondrial respiratory chain. Essays in Biochemistry, 27, 1-23). The yeast mitochondrial enzyme is highly homologous to its mammalian counterpart. We have constructed a histidine-tagged version of the yeast mitochondrial enzyme in order to allow facile purification of specific point mutant forms that are designed to explore aspects of the poorly understood mechanism by which the electron transfer/oxygen chemistry is coupled to proton transport. The project will involve purification of one or more of these mutant enzyme forms, followed by functional analyses using primarily vibrational mid-infrared spectroscopy, UV/visible spectroscopy and electrochemistry to assess effects on individual electron and proton transfer steps.

Parasite-host interactions in malaria-infected red blood cells

Joint project: Prof Helen Saibil, Department of Biological Sciences, Birkbeck
(h.saibil@mail.cryst.bbk.ac.uk)
Mike Blackman, Parasitology, MRC-NIMR

Ref: 10-ST019

Following invasion of an erythrocyte, the malaria parasite replicates within a membrane-bound parasitophorous vacuole (PV). During a process called egress, the enclosing PV and residual host erythrocyte membranes are sequentially ruptured, releasing new parasites which invade fresh erythrocytes. Egress involves a protease cascade, the target of which is thought to be complexes of the major surface protein that forms a layer of bristle-like fibres on the parasite surface.
The aim of the project is to understand the cellular and molecular changes in the parasite surface and host cell that lead to rupture of the PV and red cell membranes, and to infection of new red cells. The work will involve 3-dimensional analysis of the parasite-infected cells by electron tomography at different stages of egress, and analysis of the results in relation to the cell biology and biochemistry of parasite egress.

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

3D structure of the extracellular, infectious form of Chlamydia

Joint project: Prof Helen Saibil, Department of Biological Sciences, Birkbeck
(h.saibil@mail.cryst.bbk.ac.uk)
Dr Richard Hayward, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (r.hayward@mail.cryst.bbk.ac.uk)

Ref: 10-ST020

Chlamydia trachomatis is an obligate intracellular bacterial pathogen responsible for acute and chronic diseases, the principal cause of sexually transmitted disease and non-congenital infertility in the Western world, and of preventable blindness (trachoma) in developing nations. Chlamydia species share a unique biphasic developmental cycle. The extracellular form, termed elementary bodies (EBs), are spore-like and metabolically inert yet efficiently induce their own uptake into host cells. Once internalised into a specialised membrane-bound compartment, EBs differentiate into larger reticulate bodies (RBs) that replicate by binary fission. RBs subsequently re-differentiate into EBs and are released from the cell.
We wish to determine the 3-dimensional structure of the extracellular EB form of Chlamydia. EB surface structures are of particular interest because of their involvement in antigenicity, host cell adhesion, and protein secretion. The project will involve the isolation and purification of EBs from cultured mammalian cells and their analysis by cryo-electron tomography.

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

Transcriptional regulation of the arsenite oxidase genes

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

Ref: 10-ST021

The arsenite oxidase (Aro) in the model autotrophic arsenite-oxidising bacterium NT-26 is a heterotetrameric enzyme encoded by the genes aroB (encodes a Rieske-like 2Fe-2S cluster) and aroA (encodes the Mo-containing catalytic site and a 3Fe-4S cluster) (Santini & vanden Hoven, 2004; J. Bacteriol. 186:1614-1619). Both genes are essential for aerobic respiration with arsenite as electron donor. Arsenite oxidase gene expression is regulated by a two-component signal transduction system involving a sensor histidine kinase (AroS) and a response regulator (AroR). Expression of the aroB and aroA genes are induced by arsenite and downregulated by organic matter. A putative sigma54 promoter has been detected upstream of aroBA. The aims of this project are to 1) determine the aroBA transcriptional start site by primer extension and 2) purify AroR (and the sigma 54 factor) and demonstrate that it binds upstream of aroBA and if possible map the binding site by DNAse footprinting.

http://www.smb.ucl.ac.uk/department-staff/index.php

Studies of the respiratory arsenate reductase of Chrysiogenes arsenatis

Joint project: Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL
(j.santini@ucl.ac.uk)
Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL
(c.kay@ucl.ac.uk)

Ref: 10-ST022

Chrysiogenes arsenatis is an obligate anaerobic bacterium that couples the oxidation of acetate to the reduction of arsenate (AsV) to arsenite (AsIII) (Macy et al. 1996; Int. J. Syst. Bacteriol. 46:1153-1157). The enzyme responsible for the reduction of arsenate to arsenite, the arsenate reductase, is a periplasmic heterodimer consisting of two subunits, ArrA (contains molybdenum and a putative 4Fe-4S cluster) and ArrB (contains four putative iron-sulphur clusters) (Krafft & Macy, 1998; Eur. J. Biochem. 255:647-653). The objective of this study is to characterise the arsenate reductase. To do this the enzyme will be heterologously expressed in Escherichia coli and Electron Paramagnetic Resonance will be used to study the redox cofactors. This will be combined with site-directed mutagenesis to implicate amino acids involved in ligand binding and binding of redox cofactors. The overall aim of this project is to determine whether the Arr can be used as a biosensor for arsenate.

http://www.smb.ucl.ac.uk/department-staff/index.php

Functional and biophysical characterization of novel intrinsically unfolded proteins in the human genome

Joint project: Dr Renos Savva, Departement of Biological Sciences, Birkbeck
(r.savva@mail.cryst.bbk.ac.uk)
Dr John Christodoulou, Research Department of Structural and Molecular Biology, UCL
(j.christodoulou@ucl.ac.uk)

Ref: 10-ST023, Linked with CP006

This project is part of a coordinated approach to characterising some of the novel highly disordered proteins encoded in the human genome. Their functions include some of the most important biological processes but there is a critical lack of experimental information on these proteins. This project will involve a collaborative component wherein integrative computational analysis and protein simulations will be used to direct the expression and characterisation of novel human intrinsically disordered proteins. We will apply NMR and other techniques to collect as much functional and biophysical data as possible for a novel set of disordered proteins and disordered domains, obtained using parallel cloning and characterisation methods, feeding this data back to the modelling studies. In so doing, we will i) greatly enhance the current scant knowledge of this increasingly important class of protein, ii) contribute to new predictive modelling methodology informed by the results of the experimental studies.

This project has been piloted between two of the PIs, David Jones (linked) and Renos Savva with a limited set of computationally identified proteins. We aim to expand the number of targets using parallel cloning and expression approaches, and to focus on two specific areas: DNA-binding and metalloproteins, with a biophysics/NMR emphasis with John Christodoulou (joint PI), who also has expertise with the handling and study of intrinsically disordered proteins.

http://www.cryst.bbk.ac.uk/research/vpnai/vpnai_grp.html

Towards a structural understanding of the inhibition of uracil-DNA repair in HIV-1 infection and viability

Dr Renos Savva, Departement of Biological Sciences, Birkbeck (r.savva@mail.cryst.bbk.ac.uk)

Ref: 10-ST024

HIV-1 replication includes numerous complex virus-host interactions. The virally encoded protein Vpr, is involved in a variety of interactions with cellular proteins that enhance viral replication and fidelity while inducing G2 arrest and damage phenotypes on the cell. One cellular target is the human uracil-DNA glycosylase (UDG) isoform UNG2, which is recruited by Vpr and targeted for degradation. The WXXF motif of UDGs is the site of direct interaction with Vpr. Structures for both Vpr and the C-terminus of UNG2, where the binding site is located, are available from the PDB. Stable UDG-Vpr complexes have been expressed in the Savva lab, requiring purification and detailed structural and biophysical characterisation. Uracil-DNA repair degradation of HIV genomes is also blocked via the virally encoded protein vif, which complexes with cellular APOBEC-3G. Clones expressing this complex are also available. Structural data for either complex would lead to exploration of novel inhibitors of HIV-1.

http://www.cryst.bbk.ac.uk/research/vpnai/vpnai_grp.html

Mutating an interface of a dynamic assembly
Dr Christine Slingsby, Department of Biological Sciences, Birkbeck (c.slingsby@mail.cryst.bbk.ac.uk)
Ref: 10-ST025

The small heat shock proteins are large dynamic assemblies formed from ~20 KDa sequences containing one "alpha crystallin domain" (ACD). They are part of the stress response binding non-native proteins, and possibly disordered regions of network components. The 3D structure of alphaB-crystallin ACD shows that pockets within a single domain and a shared groove between a pair of ACDs are filled by sequence motifs from partner chains. Point mutations at these interface sites cause a range of neuromuscular diseases. It is hypothesized that these interface sites are protein substrate binding sites. This project will probe a key assembly contact by expressing a full-length alphaB-crystallin containing a point mutation at this interface. The impact of the mutation on the size and dynamics of the assembly will be tested using biophysical techniques. The idea is to see if assembly dynamics regulate access to functional binding sites.

http://www.cryst.bbk.ac.uk/research/crystallin/index.html

Probing the aggregation pathway of alpha-1 antitrypsin variants by means of ion mobility mass spectrometry
Joint project: Dr Konstantinos Thalassinos, Department of Biological Sciences/Structural and Molecular Biology, Birkbeck/UCL (k.thalassinos@ucl.ac.uk, K.Thalassinos@warwick.ac.uk)
Dr Bibek Gooptu, Department of Biological Sciences, Birkbeck
(b.gooptu@mail.cryst.bbk.ac.uk)

Ref: 10-ST026

Misfolding and aggregation of proteins can result in both loss of physiological function and also a gain of toxic function. One of the best characterised examples is polymerisation of α1-antitrypsin (A1AT) that causes early-onset emphysema and liver cirrhosis. The structures of the pathological conformers of A1AT are still not fully known and resolving these will aid in the development of new treatments.
The conformation and aggregation pathways of wild type and variant A1ATs will be analysed by means of ion mobility mass spectrometry (IMMS). IMMS is a leading-edge technique that separates ions based on their mass, shape and charge. It can provide valuable information regarding the conformation and oligomeric state of proteins. Combined with tandem mass spectrometry, the structural stability of the variants will also be examined.

http://www.smb.ucl.ac.uk/department-staff/index.php

Development of anti-obesity drugs
Dr Andrea Townsend-Nicholson, Research Department of Structural & Molecular Biology, UCL
(a.townsend-nicholson@ucl.ac.uk)
Ref: 10-ST027

Chemical Gastric Banding: The P2Y11 receptor is responsible for the slow relaxations needed for the stomach to accommodate a meal, making this receptor a therapeutic target for the development of anti-obesity drugs (1). The research project will investigate the functional properties of the P2Y11 receptor signalling pathways, using cells in culture and basic molecular and cellular biology techniques. A combination of techniques, including biochemical assays, fluorimetric (FLIPR) imaging assays and 35S-GTPγS assays to evaluate G protein-coupling, will be used to characterise the activity of different receptor antagonists at P2Y11 and to establish whether their activity differentially affects the cyclic AMP and calcium release signalling pathways.
(1) http://www.telegraph.co.uk/earth/earthnews/3334774/New-thin-pill-could-replace-surgery.html

Techniques: mammalian cell culture, calcium imaging, determination of de novo cAMP production, radioligand binding.

http://www.smb.ucl.ac.uk/department-staff/index.php

Bio-engineeering blood vessels
Dr Andrea Townsend-Nicholson, Research Department of Structural & Molecular Biology, UCL
(a.townsend-nicholson@ucl.ac.uk)
Ref: 10-ST028

Tissue Engineering of Vascular Structures: The use of electrohydrodynamic jetting (EHDJ), or bioelectrosprays, to create three-dimensional biological architectures such as blood vessels, using a patient's own cells, is an area of great research interest and therapeutic benefit (2). In collaboration with Dr Suwan Jayasinghe (UCL, Mechanical Engineering), mammalian endothelial and smooth muscle cells will be dissociated, cultured and biolelectrosprayed.

Initial experiments will use a number of techniques, including flow cytometry, proteomics, Q-PCR and assays of receptor function, to determine appropriate EHDJ conditions for the precision placement of these two constituent cell types, without affecting their biological properties. Further experiments will explore different strategies for electrospraying and electrospinning, to create vascular structures.
(2) http://www.scientificamerican.com/article.cfm?id=tissue-regeneration-matri

Techniques: primary cell culture, RNA and protein purification and characterisation, calcium imaging, polymerase chain reaction.

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Role of Hsp90 in yeast kinetochore assembly: structure determination of an Hsp90 complex by single particle electron microscopy

Dr Cara Vaughan, Department of Biological Sciences, Birkbeck (c.vaughan@mail.cryst.bbk.ac.uk)

Ref: 10-ST029, Linked with 10-CP025

The molecular chaperone Hsp90 plays an essential role in the assembly of multiprotein complexes in the cell. One such complex is CBF3 in the yeast kinetochore, a complex that binds the centromere DNA, and in doing so, acts as the foundation for most other kinetochore proteins to subsequently bind. We are interested in understanding how Hsp90 interacts with the proteins of CBF3 and what function it performs at a molecular level, in order to allow CBF3 to assemble into an active, centromere DNA-binding complex. To answer this question we will determine the structure of Hsp90 associated with proteins of CBF3 using single particle electron microscopy. The aim of this rotation project is to assemble the Hsp90-Sgt1-Skp1 complex (the first complex in the CBF3 assembly process) and, using negative stain EM, obtain the first images of this complex and classify these into class sums that will be suitable for subsequent structure determination.

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

Structural Studies of the Type IV Secretion Systems

Joint project: Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@mail.cryst.bbk.ac.uk, g.waksman@ucl.ac.uk)

Prof Elena Orlova, Department of Biological Sciences, Birkbeck
(e.orlova@mail.cryst.bbk.ac.uk)

Ref: 10-ST030

Type IV secretion systems (T4SSs) are nano-machines used for the transport of macromolecules (mostly virulence factors) 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 (published in Science). We have solved the crystallographic structure of its outer membrane part (published in Nature). 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 biochemical studies of the Usher outer-membrane assembly platform involved in pilus biogenesis

Joint project: Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@mail.cryst.bbk.ac.uk, g.waksman@ucl.ac.uk)

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

Ref: 10-ST031

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 orchestrates assembly and polymerization of subunits. For this rotation, we propose to use electron paramagentic resonance spectroscopy (EPR) to further our structural understanding of the usher. More specifically, we will focus the rotation work on the positioning of the usher plug domain relative to the chaperone-subunit complexes in the full-length usher-chaperone-subunit assembly complex.

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

Transforming a Sodium Channel into a Calcium Channel

Prof Bonnie Wallace, Department of Biological Sciences, Birkbeck (b.wallace@mail.cryst.bbk.ac.uk)
Ref: 10-ST032

Voltage-gated sodium channels are the causal agents in a wide range of neurological and cardiovascular diseases, and associated with chronic pain; as a result they are the target of a number of pharmaceutical drugs. NaChBac is a bacterial voltage-gated sodium channel which we can express in E Coli and purify in large quantities. This project will provide the student a wide exposure to structural molecular biology, computational biology and biophysics 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.

This is an interdisciplinary project crossing the boundaries of structural and computational biology.

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@biochem.ucl.ac.uk)
Ref: 10-ST033, Linked with 10-CH005

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 these 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/department-staff/index.php

Construction of a mutant firefly luciferase to create a luminescent GTP level sensor for living cells.

Prof John Ward, Research Department of Structural & Molecular Biology, UCL (ward@biochem.ucl.ac.uk)
Ref: 10-ST034, Linked with 10-CH012 and 10-CP011

Constructing a novel version of flirefly luciferase that will respond to (and hence measure) GTP instead of ATP would enable the new enzyme to be used as an intracellular reporter of GTP levels. GTP levels may change with various stimuli and be permanently altered in some disease states. However science does not yet have a general method of measuring GTP in vivo.
Firefly luciferase with modeled amino acid changes that would allow the potential for GTP acceptance will be made. A GST-Luciferase will be constructed then mutations made using oligonucleotide directed mutagenesis E. coli followed by expression in mammalian cells. The ratio of GTP/ATP specificity will be measured for the mutants contsructed
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The order of the linked projects is compulsory: 1) 10-CP011 2) 10-ST034 3) 10-CH012

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Mapping the interaction network of the RNAP-Spt4/5 elongation complex

Joint project: Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL
(werner@biochem.ucl.ac.uk)
Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
(martin@biochem.ucl.ac.uk)

Ref: 10-ST035, Linked with 10-CP010 and 10-CH002

Spt4/5 is a universally conserved transription factor that associates with its cognate RNAP and modulates its elongation properties. We have recently characterised the structure of Spt4/5 and carried out a preliminary functional analysis. One surprising result was that the Spt5 NGN domain alone was sufficient to facilitate recruitment to RNAP and subsequent activation. Now we need to map the interactions between Spt4/5 and the DNA/RNA nucleic acid scaffold of the RNAP ternary elongation complex (TEC) in a comprenhensive fashion by using sophisticated biophysical tools including FRET proximity mapping, and photo-cross linking approaches. X-ray structural information is often hampered by its static nature, whereas establishing proximity maps using a combination of X-ray structures, homology models and proximity maps obtained in solution are superior to investigate the dynamic nature and molecular mechanisms of large and complex targets such as the TEC.

http://www.smb.ucl.ac.uk/department-staff/index.php

Do Archaea employ two distinct classes of RNA polymerase? Structure and function of C34

Joint project: Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL
(werner@biochem.ucl.ac.uk)
Dr John Christodoulou, Research Department of Structural and Molecular Biology, UCL
(j.christodoulou@ucl.ac.uk)

Ref: 10-ST036

All eukaryotes utilise at least three distinct classes of RNA polymerases (I, II and III), whereas prokaryotes employ only one type of RNAP. Recently a novel RNAPIII-specific subunit (C34) has been identified in the archaea, which challenges this view by implying that there are two distinct subpopulations of these enzymes with the potential of transcribing specific subsets of genes reminiscent of the eukaryotic systems. This projects aims at a functional and structural characterisation of C34 in a range of molecular interaction and transcription assays adressing (i) RNAP binding of C34, (ii) incorporation of C34 into the DNA-TBP-TFB-TFE-RNAP preinitiation complex and (iii) effect of C34 on transcription. Our laboratory is at the forefront of researching RNAPs and we have extensive experience in a wide range of biochemical and biophysical approaches. This project is linked to the NMR solution structure determination of C34 with the Christodoulou laboratory at SMB.

http://www.smb.ucl.ac.uk/department-staff/index.php

Interactions and inhibition of an essential bacterial cell division protein

Dr Mark Williams, Department of Biological Sciences, Birkbeck (m.williams@mail.cryst.bbk.ac.uk)

Ref: 10-ST037

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. An N-acetylmuramyl-L-alanine amidase enzyme that is conserved in all Gram- (and possibly all free-living) bacteria, becomes localized to the site of division and cleaves the peptidoglycan component of the cell wall to enable daughter cells to separate. The aim of this rotation is to identify small-molecule binding partners of these enzymes by NMR and other biophysical methods. Specifically we hope identify their specific peptidoglycan substrate, the molecular mechanism of localization and to perhaps discover inhibitors which stop cell division.

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

Chemical Biology

New Chemical Reagents for the Modification of Protein Therapeutics
Dr James Baker, Chemistry, UCL (j.r.baker@ucl.ac.uk)
Ref: 10-CH001

Selective protein modification by inserting chemical reagents into disulfide bonds is a hugely appealing prospect. Of particular interest are protein therapeutics, the majority of which contain accessible disulfides, and the possibility of inserting PEG (polyethylene glycol) to provide increased in vivo stability.
We have recently discovered a series of reagents that are ideal for this purpose. This rotation project will involve: 1) The synthesis of new reagents to insert into disulfides. 2) The application of these reagents to therapeutic proteins of interest, such as somatostatin and insulin. The weighting of the research on these two areas is flexible and can be decided jointly by the student and supervisor. This research has the potential to make a major impact on our ability to modify, and thus improve, protein therapeutics.

Ideal for students interested in doing some synthetic chemistry with application to protein therapeutics. The amount of synthetic chemistry vs protein manipulation can be decided on discussion between the student and PI.

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

Design, synthesis and functional testing of novel cleavable immobilisation tags for recombinant proteins
Joint project: Dr James Baker, Chemistry, UCL (j.r.baker@ucl.ac.uk)
Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL (werner@biochem.ucl.ac.uk)

Ref: 10-CH002, Linked with 10-CP001 and 10-ST035

The analysis and nanomanipulation of higher order complexes consisting of recombinant proteins and nucleic acids is often aided by the engineering of derivatisation sites such as cysteine-SH and p-azido phenylalanine residues and subsequent coupling to e.g. fluorescent and nitroxide spin probes or affinity tags. Conventionally these derivatisations are covalent, and irreversible. However, in many experimental setups it is highly desirable to remove these tags during the later stages of the experimental strategy. In order to achieve this goal we are currently evaluating novel reagents, developed in the laboratory of Dr Jamie Baker, which are cleavable using high concentrations of reducing agents. This collaboration aims at the design, synthesis and testing of these novel compounds using a wholly recombinant multisubunit RNA polymerase from the hyperthermophilic archaeon M. jannaschii. In one example, it would be beneficial to develop an affinity tag that allows purification of the assembled RNA complex but which can subsequently be chemically cleaved. In another example, a reagent is envisaged which allows simultaneous fluorescent labelling and affininity tag modification. Subsequent to protein purification, the affinity tag could be removed chemically yielding a selectively, fluorescently labelled protein.

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

Structural and functional characterisation of hydrolases involved in cell wall peptidoglycan recycling of Mycobacterium tuberculosis.
Joint project: Dr Sanjib Bhakta, Department of Biological Sciences, Birkbeck (s.bhakta@bbk.ac.uk)
Prof Nick Keep, Department of Biological Sciences, Birkbeck (n.keep@mail.cryst.bbk.ac.uk)

Ref: 10-CH003

Design of new pilicides inhibiting virulence factors in bacterial pathogens

Joint project: Dr Helen Hailes, Chemistry, UCL (h.c.hailes@ucl.ac.uk)
Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@ucl.ac.uk)

Ref: 10-CH004

This project aims to design inhibitors of pilus biogenesis. 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 orchestrates assembly and polymerization of subunits. In this rotation, we would like to design compounds that will target the polymerizaton mechanism of subunits with a view to inhibiting it. Subunit polymerization is known to occur via a zip-in-zip-out mechanism starting at a well-defined site on the chaperone. By designing compounds that will target this site, we hope to inhibit the entire process of pilus biogenesis. We have already obtained compounds that target that site, from small molecule compound libraries and modelling studies. With the synthesis of modified compounds and conjugates we will probes this further to identify small molecule selective inhibitors.

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

Novel Alkaloid Synthesis and Biosynthesis

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

Ref: 10-CH005, Linked with 10-ST033

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. In this rotation project we will use NCS enzymes from John Ward's laboratory to investigate enzyme substrate specificity using dopamine, 4-hydroxyphenylacetaldehyde and analogues. Chemical syntheses of the isoquinolines will be carried out using a novel reaction we have recently discovered, and screening for the formation of the isoquinolines by NCS will be achieved using the synthetic samples prepared as controls. Using synthetic routes and the NCS as a biocatalyst a range of new benzylisoquinolines will be prepared.

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

Biosynthetic Studies of DNA-Binding Natural Products

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

Ref: 10-CH006

My group is studying the biosynthetic pathways towards the DNA binding anticancer natural products azinomycins A and B, using a combination of organic chemistry and molecular biology techniques. The aims are to understand the pathways and enzyme mechanisms involved in making these metabolites, and to develop methodology to produce modified metabolites that may have improved biological activity.
This project may involve one of the following experiments: synthesis of an isotopically labelled intermediate in the biosynthesis of the unusual aziridine-containing fragment followed by spectroscopic analysis of its incorporation into the end product; or synthesis and feeding of unnatural naphthoic acid intermediates to the producing bacteria and analysis of fermentations for novel metabolites.

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

Synthesis of Disruptors of PapG Function

Joint project: Prof Charles Marson, Chemistry, UCL (c.m.marson@ucl.ac.uk)
Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@ucl.ac.uk)

Ref: 10-CH007

The purpose is to synthesise small molecules that bind to PapG protein. Skills in basic organic chemistry must be in hand, or capable of being acquired rapidly. The Waksman group (Cell 2001, 105, 733) has obtained crystal structures of the natural tetrasaccharide Gb04 bound to PapG. It is hoped to disrupt binding of Gb04, a requirement for the attachment of bacteria to human kidney cells which leads to kidney infections. Molecular modeling has identified various quinazolines (Fig.) as suitable drug-like molecules with satisfactory affinity for PapG.

A general synthetic strategy to such compounds has already been worked out in the Marson group, and the student will apply this in order to prepare suitably substituted quinazolines. Possibly during this project, PapG disruptors will be evaluated in Prof. Waksman's laboratory where a PapG binding assay has been set up based on surface plasmon resonance (SPR) as implemented by Biacore.

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

A Systematic Approach to the Discovery of New Methods for Protein Modification and Novel Bio-Orthogonal Reactions
Dr Tom Sheppard, Chemistry, UCL (tom.sheppard@ucl.ac.uk)
Ref: 10-CH008

In this project we will develop a systematic method for identifying new chemical methods for protein modification. Such reactions must be compatible with both mild aqueous conditions and with the other functional groups present on protein surfaces. The reactivity of a chosen set of suitable chemical reagents and catalysts will be studied in combination with small sets of N/C protected amino acids (in deuterated water as solvent). The reaction outcomes will be determined via NMR spectroscopy and LCMS. Novel reactive combinations of reagents/amino acids will be identified, and then applied to the selective modification of small peptides to demonstrate their viability. The disocovery of new methods to modifiy rarer residues (e.g. pSer) will be of particular interest. Similarly, bio-orthogonal combinations of reagents/catalysts will also be identified with a view to developing new alternatives to existing bio-orthogonal reactions (e.g. Staudinger ligation or 'Click' cycloadditions).

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

Protein-small molecule bioconjugates as inhibitors of VirB11 self-assembly
Joint project: Dr Alethea Tabor, Chemistry, UCL (a.b.tabor@ucl.ac.uk)
Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@ucl.ac.uk)

Ref: 10-CH009

The VirB11 ATPase is a key component of the Type IV secretion systems (T4SSs) used by Gram- bacterial pathogens to export virulence factors. The Waksman group has studied the structure of this enzyme and demonstrated that it forms a hexameric pore structure (EMBO J., 22, 1969 (2003)). We have designed and synthesised small molecule inhibitors of the VirB11 ATPase which occupy the ATP binding site. In this project we will combine these small molecule inhibitors with a subunit of the VirB11 ATPase to give bifunctional bioconjugates which will simultaneously block the ATP binding site and prevent the self-assembly of the VirB11 complex. The project will involve over-expression of subunits of the VirB11 ATPase, followed by chemical modification of key residues in this subunit with the small molecule inhibitors, using modern techniques for bioconjugation such as click chemistry. Solid-phase synthesis of smaller peptide subunits of the VirB11 ATPase, followed by bioconjugation of the small molecule inhibitors, binding studies of the bioconjugates to the wild-type protein, and molecular modelling of the bioconjugates, may also be possible during this rotation.

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

How does nisin recognise lipid II?
Joint project: Dr Alethea Tabor, Chemistry, UCL (a.b.tabor@ucl.ac.uk)
Prof Paul Driscoll, Division of Molecular Structure, MRC NIMR (pdrisco@nimr.mrc.ac.uk)

Ref: 10-CH010

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. As a result of this selective binding, nisin may be a promising lead for the next generation of antibiotics. Nisin has a complex structure featuring multiple thioether bridges (from the residues lanthionine and methyl lanthionine), and it is believed that the N-terminus (rings A and B) is responsible for the selectivity of lipid binding. We have have recently developed powerful solid-phase methodology for synthesising lantibiotics. We have used this to synthesise analogues of ring B and of the entire N-terminus, and have shown that ring B on its own adopts two conformational states. In this project we will synthesise variants of ring B of nisin containing unnatural amino acid bridges and variant amino acids, analyse the conformations of these cyclic peptides and compare these with the wild-type peptide. The student will learn a number of techniques including synthetic organic chemistry, peptide synthesis and purification, and NMR analysis.

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

Stoichiometry of heparin/CRP binding to complement factor H

Dr Konstantinos Thalassinos, Department of Biological Sciences/Structural and Molecular Biology, Birkbeck/UCL (k.thalassinos@ucl.ac.uk, K.Thalassinos@warwick.ac.uk)

Ref: 10-CH011, Linked with 10-ST016 or 10-CP012

Complement factor H (CFH) of the innate immune system is genetically linked with age-related macular degeneration (AMD). The way in which CFH binds to heparan-sulphate/C-reactive-protein (CRP) on host cell surfaces is a very exciting topical topic relating to the protection of host cells. Two heparin/CRP binding sites are well known on SCR7 and SCR20, but others have been proposed at SCR9 and SCR13. The stoichiometry of heparin/CRP binding to CFH is important for undertanding its molecular mechanism. Our ultracentrifugation (AUC) work shows that multiple heparin/CRP molecules may bind to CFH. The aim of this project is to perform mass spectrometry of complexes of highly purified heparin/CRP with CFH. The change in molecular weight will provide important information on the maximum number of heparin/CRP molecules that can bind to CFH. The results will be compared with AUC and SPR data on heparin/CRP-CFH complexes and predictions of heparin/CRP sites in CFH (see projects 10-CP012 & 10-ST016).

http://www.smb.ucl.ac.uk/department-staff/index.php

Expression and testing of a mutant firefly luciferase to create a luminescent GTP level sensor for living cells.

Dr Geraint Thomas, Cell & Developmental Biology / Structural & Molecular Biology, UCL (G.Thomas@ucl.ac.uk)

Ref: 10-CH012, Linked with 10-ST034 and 10-CP011

Potential GTP sensors (G-luciferases) created by mutating firefly luciferase will be screened for suitability as GTP level sensors using wild-type luciferase as a reference. GST fusion proteins will be over-expressed in Cos cells, purified from lysates and assayed against nucleotides (ATP, GTP, TTP, CTP, UTP, XTP and corresponding diphosphates). The most selective fusion proteins will then be re-screened, un-purified, in the original lysates. Endogenous nucleotides will be removed by gel-filtration on standard "desalting" columns and then added back in combinations. Measurements will also be made in the crude lysate using the endogenous nucleotides. Lastly luminescence assays with candidate G-luciferases will be conducted in real-time in individual living cells using a luminescence Imaging Photon Detector and the IMP dehydrogenase inhibitor mycophenolic acid to specifically deplete GTP but not ATP. The effects will be reversed via the "salvage pathway" using guanosine. Native, ATP selective, luciferase will be serve as a control.

For rapid throughput I have a thermostated luminescence plate reader with automated sample injection. The Imaging Photon Detector is available through my affiliation to the Research Department of Cell & Developmental Biology. Results in live cells will be compared to predictions made with my new dynamic model of cellular purine metabolism.

The order of the linked projects is compulsory: 1) 10-CP011 2) 10-ST034 3) 10-CH012

http://www.cdb.ucl.ac.uk/research/thomas/index.shtml

Probing lateral molecular clustering in lipid membranes

Dr Salvador Tomas, Department of Biological Sciences, Birkbeck (s.tomas@sbc.bbk.ac.uk)

Ref: 10-CH013, Linked with 10-CP020

The aim of this project is to test the utility of lipid-porphyrin conjugates towards the measure of lateral interaction of membrane components, starting with the measure of cholesterol-cholesterol lateral clustering. To do so, we will measure the clustering of porphyrin-cholesterol conjugates using optical spectroscopy methods. The tendency of the cholesterol component towards clustering will be isolated by comparison against the clustering of porphyrin conjugates with phospholipids (see fig). The project will be developed in two stages:

- Synthesis and purification of porphyrin conjugates with di-glicerides 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

Chemical synthesis of deuterated unsaturated and oxidised phospholipids
Dr Salvador Tomas, Department of Biological Sciences, Birkbeck (s.tomas@sbc.bbk.ac.uk)
Ref: 10-CH014, Linked with 10-CP021

Exposure to the ubiquitous gaseous air pollutant ozone, O3, leads to decreased lung function and an increased risk of death. Inhaled ozone reacts with the unsaturated lipids and proteins found in pulmonary surfactant, the material that lowers the surface tension of the air-water interface of the lung. The aim of this project will be to synthesise and fully characterise some selectively deuterated phospholipids present in lung surfactant either initially or after exposure to ozone, that are not commercially available. The deuterated compounds synthesised will be used in neutron reflection experiments that, although not part of this rotation project, could be incorporated into a full PhD project.

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

Correlating structural changes with function of a DNA helicase
Dr Martin Webb, Physical Biochemistry, NIMR (mwebb@nimr.mrc.ac.uk)
Ref: 10-CH015

PcrA is a monomeric DNA helicasae with one role in asymmetric plasmid replication. We have previously investigated the translocation of this PcrA in association with an initiator protein, RepD, responsible for directing PcrA to the origin of replication (Slatter et al, Biochemistry, 2009). Several crystal structures of PcrA have been obtained by Wigley (Subramanya et al, Nature 1996, Soultanas et al, 2000) and one area of our research is to relate such structural changes to the biochemistry. This rotation project will use protein fluorophore labeling, following introduction of cysteines by mutation at specific locations on the protein surface. This labelled adduct will be used to examine the large conformation change produced by interaction with DNA and how this change may be modulated by interaction with RepD.The project will include protein chemistry as well as stopped-flow rapid reaction techniques, fluorescence spectroscopy.

Computational Biology

Molecular simulation of polyglutamine expansion proteins using PROFASI
Dr Kevin Bryson, Computer Science, UCL (K.Bryson@cs.ucl.ac.uk)
Ref: 10-CP001

There are currently nine human diseases, including Huntington's, that are caused by the unstable expansion of polyglutamine repeats. Beyond a certain critical length, stretches of polyglutamine can cause proteins to form amyloid inclusions, with the early oligomers implicated as toxic. The aim of this project is to examine the formation of those early oligomers using an established simulation tool called PROFASI. This tool has already been used to analyze the early oligomerization of amyloid tau protein in Alzheimer's disease, but currently nobody has applied it to simulate the structural effects of polyglutamine expansion. Such simulation is computational expensive and so the UCL Legion supercomputer will be employed.

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

Systems biology modelling of APP processing in Alzheimer's Disease
Dr Kevin Bryson, Computer Science, UCL (K.Bryson@cs.ucl.ac.uk)
Ref: 10-CP002

Proteolytic cleavage of the beta-amyloid precursor protein (APP) results in a number of cleavage products including the beta-peptide that fibrillizes into amyloid plaques implicated in Alzheimer's disease. Recent intense research in this area has revealed a complex processing pathway for APP involving different secretases. The aim of this project is to take information from recent reviews (e.g. Neurol. Med., 2010, 12:1-12) and create a systems biology model using either JDesigner/MATLAB. In silico experiments would include considering the relative changes in beta-amyloid production given brain region and also different mutations in APP and its processing enzymes. The study will be in collaboration with members of John Hardy's group at the Institute of Neurology, including model validation employing a unique data set from one of the largest and most comprehensive studies on exon-specific gene expression within the human brain (10 brain regions in over 90 individuals).

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

Experimental constraints in steered molecular dynamic simulations
Joint project: Prof Peter Coveney, Chemistry, UCL (p.v.coveney@ucl.ac.uk)
Dr Chris Kay, Research Department of Structural & Molecular Biology, UCL (c.kay@ucl.ac.uk)

Ref: 10-CP003, Linked with 10-ST0010

Biological magnetic resonance experiments yield many different clues concerning the structure of a macromolecule. However, the experimental data often is insufficient for gaining an understanding of the overall structure/function of biological macromolecule. Using NMR and EPR data we will model RNA - Ribosome/RNAP interactions. Especially for large systems we will use coarse-grained methods to simulate the overall behaviour of the RNA and the binding partner. This combination of experimental and theoretical techniques gives us a fast in-detail understanding of very large complexes.

Students should have experience with the methods proposed. A student with a good grounding in physics or biophysics from their first degree would be at an advantage. It is advisable to choose this project after having done the linked project as this one will use the data produced by this project.

http://www.chem.ac.uk/people/coveney/

Modelling the evolution of viruses
Dr Richard Goldstein, Division of Mathematical biology, MRC NIMR (rgoldst@nimr.mrc.ac.uk)
Ref: 10-CP004

Despite Sir McFarland Burnett's 1962 claim that 'By the end of the Second World War it was possible to say that almost all of the major practical problems of dealing with infectious disease had been solved.', infectious diseases remain a continuing, and in many cases, increasing risk to human health. We wish to understand the history of these pathogens, how they have emerged and re-emerged, what has been the process behind past and present pandemics, how pathogens evolve to avoid the immune system and drug treatments, and how they transfer from one host species to another. These particular events have left their mark on the sequences of these pathogens; we need to construct mathematical and computational models to decipher this evolutionary record.

http://www.nimr.mrc.ac.uk/research/richard-goldstein/

Modelling protein evolution
Dr Richard Goldstein, Division of Mathematical biology, MRC NIMR (rgoldst@nimr.mrc.ac.uk)
Ref: 10-CP005

Most models of protein evolution consider proteins as a series of letters, and mathematically model the rate at which one letter changes to another. In reality, proteins have structures and functional and thermodynamic attributes, while amino acids have physical and chemical properties. How can we develop evolutionary models that are informed by the properties of proteins and the amino acids that they contain? How can we interpret our evolutionary behaviour in terms of protein properties? How can we develop models that allow us to use evolutionary information - such as the available databases of related sequences - to tell us something about the evolving macromolecules? How do the answers to these questions depend on the type of protein?

http://www.nimr.mrc.ac.uk/research/richard-goldstein/

Computational, functional prediction of novel intrinsically unfolded proteins in the human genome
Prof David Jones, Computer Science / Research Department of Structural & Molecular Biology, UCL (dtj@cs.ucl.ac.uk)
Ref: 10-CP006, Linked with ST023

Intrinsically unfolded proteins are now believed to be an important aspect of higher organism protein function. Rather than having a single well defined "fold", these proteins are completely or partially unfolded for much of their lifetime, but fold upon recognizing their cognate ligand (e.g. ds-DNA, metals or other proteins). See figure for an example of a DNA binding example. How the controlled transition from the unfolded to the folded state is regulated, and how this regulation relates to the cellular function of the proteins is one of the current hot areas of research in modern biology. In this linked rotation project, the overall aim will be to try to improve the prediction of gene function by a detailed analysis of disordered regions in human sequences. As a linked project, in this "part" the specific aim will be to make use of computational techniques to identify a small number of examples of functionally distinct proteins which are predicted to make use of controlled order-disorder transition on binding to carry out their function. In the linked project, some of these selected targets will be evaluated in the lab. NOTE: this rotation would obviously need to be done before the experimental rotation.

This project has been piloted between two of the PIs, David Jones (linked) and Renos Savva with a limited set of computationally identified proteins. We aim to expand the number of targets using parallel cloning and expression approaches, and to focus on two specific areas: DNA-binding and metalloproteins, with a biophysics/NMR emphasis with John Christodoulou (joint PI), who also has expertise with the handling and study of intrinsically disordered proteins.

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

Inferring Novel Human Disease-Gene Associations
Prof David Jones, Computer Science/Research Department of Structural & Molecular Biology, UCL (dtj@cs.ucl.ac.uk)
Ref: 10-CP007

For around half of human diseases with a known genetic component, it is not known which genes are associated with the disease. In this rotation project, the overall aim will be to try out some ideas for linking diseases to human genes. We have collected a very large amount of data for every human gene e.g. sequence similarity, gene co-expression, predicted gene fusions and so on. So far we have used this data to predict the biological functions of functionally uncharacterised genes with a lot of success. The idea here would therefore be to try to use this large data set to link genes not just to biological functions (i.e. their role in the healthy organism) but to potentially related diseases (i.e. effects on the organism of perturbing the genes in some way). A very interesting approach we are keen on trying is to model relationships between disease symptomology and gene function and in this way back calculate which genes might be linked to specific symptom combinations.

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

Computational Neuroscience
Prof Zhaoping Li, Computer Science, UCL (z.li@ucl.ac.uk)
Ref: 10-CP008

Student may choose from two choices, one is a modeling project, another is a psychophysics experimental project. The PI will choose the project with the student depending on the skills of the student. Interested student should make an appointment via email (z.li@ucl.ac.uk) to discuss interests and feasibilities.

www.cs.ucl.ac.uk/staff/Zhaoping.Li/

Modelling of Na+/H+ Exchanger Proteins (NHEs)

Joint project: Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
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Dr Kate Bowers, Research Department of Structural & Molecular Biology, UCL
(katherine.bowers@ucl.ac.uk)

Ref: 10-CP009, Linked with ST-003

Eukaryotic NHE proteins are 12-helix transmembrane proteins vital for cellular homeostasis and of critical importance in disease. There are 9 human NHE isoforms, one of which has been modelled based on the known structure of the bacterial homologue, NhaA. Modelling is difficult as the sequence identity is very low. This project will use a combination of trans-membrane prediction, sequence alignment and comparative modelling methods to model the TM region of other members of the NHE family as well as investigating the use of fold recognition and threading to examine the large non-membrane C-terminal domain, for which no homologous structures are known.

This project is suitable for non-specialists.

The NHE proteins are the subject of research in the Bowers group. This project will model the proteins and the linked project in the Bowers group will perform mutagenesis experiments on the same protein potentially guided by the outcome of the modelling.

http://www.smb.ucl.ac.uk/department-staff/index.php

Structural Modelling of the complete M. jannaschii 12-subunit RNA polymerase

Joint project: Dr Andrew Martin, Research Department of Structural & Molecular Biology, UCL
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Dr Finn Werner, Research Department of Structural & Molecular Biology, UCL
(werner@biochem.ucl.ac.uk)

Ref: 10-CP010, Linked with 10-ST035 and 10-CH002

RNA polymerases are molecular machines that are responsible for DNA-dependent transcription in all life forms. They consist of 12 subunits and have a highly complex architecture, which combines a rigid core with flexible domains that are necessary for catalysis and the dynamic interactions with DNA template, RNA transcript and transcription factors. The understanding of RNAP function requires good structural models of RNAP. This project aims at building a 12-subunit model of the complete RNAP from the hyperthermophilic archaeon M. jannaschii. It will involve the use of sequence alignments and MODELLER together with the available medium to high-resolution structural data from archaeal and eukaryotic RNAPs to assemble a complete model. We have recently successfully applied these methods to model the complete structure of the Spt4/5 transcription elongation factor (NAR 38[12]:4040-51). The model will then be tested in the linked project with Dr Werner.

http://www.smb.ucl.ac.uk/department-staff/index.php

Analysis of firefly luciferase to create a luminescent GTP level sensor for living cells

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

Ref: 10-CP011, Linked with 10-ST034 and 10-CH012

Changes in guanine nucleotide levels accompany differentiation of some cell types especially those characteristic of some leukaemias; differentiation can be induced by compromising GTP synthesis. Our understanding is restricted by the absence of a real-time reporter of cellular GTP levels preventing screening of other known differentiating agents for changes in GTP. Also we do not know if changes in GTP levels occur naturally in the life cycles of cells, during differentiation or in the course of disease. Thus we aim to modify firefly luciferase such that it becomes GTP (rather than ATP) specific creating a GTP sensor which can be used in living cells. In this rotation, the student will analyze available structures for firefly luciferase in order to understand the ATP binding pocket and residues responsible for ATP specificity. Mutations will then be suggested that could change the specificity to GTP; these mutations will be modelled and GTP will be docked into the mutant binding sites. Comparisons will then be made with docking of ATP to propose the most selective mutants.

No programming is required for this project. The student will first work in Andrew Martin's lab to determine likely sites for mutation. He or she will then move to John Ward's lab to generate mutants and finally to Geraint Thomas's lab to perform expression and testing.
The order of the linked projects is compulsory: 1) 10-CP011 2) 10-ST034 3) 10-CH012

http://www.smb.ucl.ac.uk/department-staff/index.php

Prediction of heparin/CRP binding sites in complement factor H

Prof Steve Perkins, Research Department of Structural & Molecular Biology, UCL
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Ref: 10-CP012, Linked with 10-CH011

Complement factor H (CFH with 20 SCR domains) is a major protein genetically linked with age-related macular degeneration (AMD) which causes loss of central vision in the elderly (0.5 million in the UK). A common AMD-risk polymorphism in SCR7 at Tyr402His is located in the same SCR domain as heparin/CRP binding sites. Another heparin/CRP binding site is near SCR20 and may relate with renal disease. An understanding of these sites in CFH is crucial to understand function. This project will predict the heparin/CRP binding sites in SCR7 and SCR20 and search for more candidate sites in CFH. Bioinformatics analyses will be performed to compare crystal, NMR and homology structures for all 20 SCR domains in CFH with known structures for heparin/CRP, and rank these in order of probability. The results will be compared with AUC, SPR and mass spectrometry data of the heparin/CRP-CFH stoichiometry (see projects 10-CH011 and 10-ST016).

http://www.smb.ucl.ac.uk/department-staff/index.php

Does the bacterial content of the gut flora change in Fmo knockout mice as they age?

Joint project: Prof Elizabeth Shephard, Research Department of Structural & Molecular Biology, UCL
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Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL
(j.santini@ucl.ac.uk)

Ref: 10-CP013

A Fmo knockout mouse line has an unusual phenotype. The mice stop putting on weight as they age. In contrast, wild-type mice continue to increase in weight. The knockout mice eat the same amount of food as wild-type animals, but their fat deposits diminish substantially. The reasons for this are not known. The knockout mice have altered energy balance, which is important in metabolic disorders such as type 2 diabetes and hypermetabolism. Recently, altered gut flora has been linked to metabolic syndrome (Vijay-Kumar et al 2010, Science 328:228-231). The project will examine whether the gut flora is altered as a consequence of the inactivation of an Fmo gene, if this proves to be the case this will provide evidence for a link between gut flora and energy balance. DNA will be isolated from the faeces of knockout and wild-type mice. The 16S rDNA genes will be amplified by PCR, cloned and sequenced. Bioinformatic analyses will compare the sequences obtained with those of all known bacterial 16S rDNA sequences.

http://www.smb.ucl.ac.uk/department-staff/index.php

Trimethylaminuria: gut flora and severity of disorder

Joint project: Prof Elizabeth Shephard, Research Department of Structural & Molecular Biology, UCL
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Dr Joanne Santini, Research Department of Structural & Molecular Biology, UCL
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Ref: 10-CP014

Trimethylaminuria (TMAuria) is a disorder of body odour caused by excretion of dietary-derived trimethylamine (TMA) in the urine, sweat and breath. Gut bacteria cleave the TMA group from choline (in e.g. meat, soya). Affected individuals have a mutated FMO3 gene. FMO3 converts TMA to TMA N-oxide, which does not smell. Manipulation of gut flora to eliminate the choline cleavage enzyme is an attractive therapeutic possibility. First we must establish if the gut flora is different in TMAuria. The mouse offers an animal model. At 6 weeks of age the FMO3 gene is switched off in the livers of male (but not female) mice, causing TMAuria. The project will examine the gut flora of young and mature male and female mice and indicate whether this is altered as a consequence of the inactivation of the FMO3 gene. The 16S rDNA genes will be amplified by PCR, cloned and sequenced. Bioinformatic analyses will compare the sequences obtained with those of all known bacterial 16S rDNA sequences.

http://www.smb.ucl.ac.uk/department-staff/index.php

Prediction of the location of discontinuous B-cell epitopes for influenza A

Joint project: Dr Adrian Shepherd, Department of Biological Sciences, Birkbeck (a.shepherd@mail.cryst.bbk.ac.uk)
Prof David Moss, Department of Biological Sciences, Birkbeck (d.moss@mail.cryst.bbk.ac.uk)

Ref: 10-CP015

Effective epitope prediction would greatly reduce the number of experiments required for vaccine design. However, generic (multi-pathogen) prediction methods have proved largely ineffective, with the notable exception of T-cell epitope prediction for common MHC Class I alleles.
In this project we will develop machine learning tools (using artificial neural networks and/or SVMs) dedicated to the prediction of discontinuous B-cell epitopes in influenza A. Training and evaluation
data will be acquired by combining experimental data from the Immune Epitope Database with our own analyses of the epitope-shaped clusters of mutations that develop on the surface of influenza A haemagglutinin molecules (as the virus evolves to counteract the effect of existing antibodies within the human population). Performance of the pathogen-specific tools will be compared to that of generic tools (e.g. DiscoTope and BEpro).

http://people.cryst.bbk.ac.uk/~ubcg60a/

Towards the gene regulatory network that specify otic progenitors.

Dr Alona Sosinsky, Department of Biological Sciences, Birkbeck (a.sosinsky@mail.cryst.bbk.ac.uk)

Ref: 10-CP016

The sense of hearing is crucial for communication with our environment. Given its complexity, it has been a major challenge to unravel the signalling mechanisms as well as the intracellular factors controlling these processes in a temporal and spatial manner. Our own efforts have concentrated on the early events that gradually commit multipotent progenitor cells to otic fate. To identify novel candidates for congenital deafness and factors that specify otic progenitors, we compared the expression profiles of four cell populations representing different states of otic specification using microarray analysis. This project aims to use bioinformatics approaches to predict regulatory motifs common to the newly identified otic genes as well as the transcription factors that bind these motifs. This will be the first step towards the establishing gene regulatory network for otic specification.
Project will use our in-house method for analysis of cis-regulatory regions in eukaryotic genes and does not require advanced programming skills.

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

Understanding expression mechanisms of odorant receptor genes.

Dr Alona Sosinsky, Department of Biological Sciences, Birkbeck (a.sosinsky@mail.cryst.bbk.ac.uk)

Ref: 10-CP017

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 the sub-groups of olfactory receptor genes can be regulated by the same sets of transcription factors and therefore their non-coding regions can share similar binding sites for transcription factors. The project will attempt computational discovery and analysis of these shared regulatory inputs with the help of our in-house computational tool EDGI. EDGI allows discovery of short conserved motifs (putative binding sites) and their clusters in the sets of related sequences. Project involves programming on Perl and mining genomic data bases.

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

Structural constraints in the architecture of transcription regulatory modules.

Dr Alona Sosinsky, Department of Biological Sciences, Birkbeck (a.sosinsky@mail.cryst.bbk.ac.uk)

Ref: 10-CP018

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 for certain TFs. 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. In agreement with this observation our recent computational analysis of eukaryotic genomes has revealed that there are strong preferences for the length of DNA sequence that separate putative binding sites for certain pairs of TFs in genomic sequences. Our working hypothesis is that these factors have rigid structure and therefore strong space constraints for interaction with their partners. The project will involve sequence and structure analysis of theses TFs with existing bioinformatics software.

Design of novel protein folds (computational and structural)
Prof Willie Taylor, Mathematical Biology, MRC NIMR (wtaylor@nimr.mrc.ac.uk)
Ref: 10-CP019

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/research/willie-taylor/

Computational study of receptor clustering in lipid membranes
Dr Katherine Thompson, Department of Biological Sciences, Birkbeck (k.thompson@bbk.ac.uk)
Ref: 10-CP020, Linked with 10-CH013

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 (molecular mechanics and quantum mechanics) to characterise model systems in terms of binding energy, entropic changes (changes in cavity solvation and ligand solvation), structural changes and receptor clustering tendencies. The model systems will be a porphyrin–cholesterol and porphyrin-phospholipid conjugates in a phospholipid membrane for which experimental data should be available from a linked project.

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

Molecular dynamics simulations of oxidised monolayers of lung surfactant
Dr Katherine Thompson, Department of Biological Sciences, Birkbeck (k.thompson@bbk.ac.uk)
Ref: 10-CP021, Linked with 10-CH014

Exposure to the gaseous air pollutant ozone is associated with increased risk of death. Ozone is known to react with pulmonary surfactant, a mixture of lipids and proteins present at the air:water interface of the lung, but the changes that occur to the composition and structure of the surfactant following exposure to ozone is not well understood. In this project molecular dynamics will be used to study lung surfactant monolayers composed initially of phospholipids and the lung protein SP-B that have undergone varying levels of oxidative damage to determine how the surface tension changes with oxidation and whether the oxidation products remain at the air-water interface or dissolve into the bulk aqueous or gas phases. The results will complement on-going experimental work in the group.

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

The architecture of the Type IV secretion system
Joint project: Dr Maya Topf, Department of Biological Sciences, Birkbeck (m.topf@mail.cryst.bbk.ac.uk)
Prof Gabriel Waksman, Department of Biological Sciences/Research Department of Structural & Molecular Biology, Birkbeck/UCL (g.waksman@ucl.ac.uk)

Ref: 10-CP022

Project description not available.

Probing structure and function in kinesin motor proteins in malaria (1)

Dr Maya Topf, Department of Biological Sciences, Birkbeck (m.topf@mail.cryst.bbk.ac.uk)
Ref: 10-CP023, Linked with 10-ST013

Malaria infects over 300 million people a year, killing more than 1 million. The complex life cycle of the malaria parasite, along with emerging drug resistance, mean that novel drug targets are desperately needed. Kinesins, which are ATP-dependent microtubule-based molecular motors, are potential targets for anti-malarial drugs. The objective of the rotation is to undertake a bioinformatic approach to investigate the structure and function of kinesin motor proteins in malaria. Sequence analysis will enable identification of malaria-specific modifications that could modulate kinesin function, potentially rendering the motors susceptible to inhibition by anti-malarial treatments. Homology models of the malaria kinesins will be generated based on the currently known atomic-resolution structures of kinesin motors from other species. High-resolution kinesin-microtubule cryo-EM reconstructions from the Moores group will also be used to refine and interpret these homology models in the context of their assembly with microtubules. Small-molecule docking would potentially be used to propose functional modifications.

http://people.cryst.bbk.ac.uk/~ubcg67a/personal-info.html

The structure of protein complexes - solving a jigsaw puzzle

Dr Maya Topf, Department of Biological Sciences, Birkbeck (m.topf@mail.cryst.bbk.ac.uk)
Ref: 10-CP024

Project description not available.

Assigning function to TPR domains through evolutionary structural and sequence relationships

Dr Mark Williams, Department of Biological Sciences, Birkbeck (m.williams@mail.cryst.bbk.ac.uk)
Ref: 10-CP025, Linked with 10-ST029

TPR (tetratricopeptide repeat) domains are one of the most common protein-protein interaction modules found in eukaryotic proteins. More than 80 such domains have been identified in the human genome. They are often found in scaffolding molecules which help assemble specific proteins into large functional complexes. Consequently, it is important to understand which proteins bind to which TPR domain. The specific target proteins for a number of TPR domains are now known and several structures (including one from my laboratory) have been solved. The aim of this project is to utilise the existing structural data, together with comparative information from a variety of eukaryotic genomes to identify evolutionary relationships between TPR domains and thus identify those having related functions. Of particular interest is analysis of those TPR domains that may help us understand the assembly of the protein complexes involved in chromosomal separation.

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