



Joint Departmental / ISMB Retreat
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Oral presentations abstracts



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Flavin-containing monooxygenases: genetic variation and drug metabolism

Asvi A. Francois and Elizabeth A. Shephard

Flavin-containing monooxygenase (FMO) proteins play an important role in the metabolism of foreign chemicals including therapeutic drugs. Consequently polymorphic variation in *FMO* genes can lead to changes in drug metabolism, which in turn can cause adverse drug reactions or lack of drug efficacy. Five members of the FMO family of proteins, FMOs 1, 2, 3, 4 and 5 are known to metabolise drugs. Nearly one-third of the world's population is infected with *Mycobacterium tuberculosis* with pulmonary tuberculosis being the most common form of the disease. Tuberculosis is especially common in the world's poorer areas including Africa. This talk will concentrate on the FMO2 protein and how genetic variation in the *FMO2* gene influences the metabolism of an important class of drugs used to treat tuberculosis. The FMO2 protein is of particular interest because in the *FMO2* gene of Asians and Causcasians there is a nonsense mutation that causes premature truncation of translation. Thus these individuals do not make FMO2 protein. In contrast, 25% of the population of sub-Saharan Africa have a wild type *FMO2* gene and thus produce a catalytically active protein. Results will be presented to show that thiacetazone, a cheap and commonly prescribed anti-tuberculosis drug in Africa, is a substrate for FMO2. The consequences of this finding for drug therapy and/or potential adverse reactions will be discussed.

ImmunoGrid: Towards a Clinically Relevant Systems Biology Model for the Human Immune System

Dr Mark Halling-Brown

Abstract:

The aim of the ImmunoGrid (EU Project) is to establish an infrastructure for the simulation of the immune system at the natural scale that integrates processes at molecular, cellular and organ levels.

At the current stage of development the simulator is able to mimick the dynamics of the most important immune cells reacting against a generic pathogen (virus and bacteria among these). Moreover it also handle specific processes for HIV-1 infection and for mammary carcinoma of HER-2/neu transgenic mouse. The use of key grid technologies provides the opportunity to perform simulations which are orders of magnitude more complex than the current models. In my presentation I will provide a general overview of the ImmunoGrid project together with some result of previous simulations with a similar version of the code in the area of cancer immunoprevention.



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Analyzing the oral metagenome

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The microflora of the oral cavity is one of the most diverse in the human body with greater than 800 bacterial species estimated to exist there. The oral cavity consists of distinct ecological niches (such as the surface of the tongue or the dental plaque) that harbour different bacterial species. Approximately half of oral species can not be cultured and, therefore, molecular approaches will be used to investigate this community.

Bacteria adhere to both oral surfaces and to each other to form biofilms. Bacteria will generally exist in equilibrium with the human host; however, some organisms are opportunistic and may cause disease. An understanding of adhesion mechanisms will provide insight into how such bacteria attach to human tissues and cause oral disease. A comprehensive metagenomic study of the oral microflora is being used to investigate the diversity and adhesion mechanisms of this unique bacterial community.

Previous studies of the oral metagenome have reported that up to 60% of clones in a BAC library contain human DNA. Therefore preferential isolation of prokaryotic DNA was desirable for the construction of an oral metagenomic library. High quality DNA is required for the production of metagenomic libraries and, therefore, a range of DNA extraction methods have been investigated. Differential centrifugation and selective lysis of human cells have been investigated as methods of removing human DNA from samples prior to library production. Field inversion gel electrophoresis and real-time PCR have been used to assess the molecular weight of DNA and quantity of human DNA respectively. The final sampling method, a combination of the two methods, was chosen accordingly. Whole genome amplification will be used to produce enough DNA for the production of metagenomic libraries.

Novel adhesins of the oral microbial community will be identified using phage display libraries. Phage display libraries are constructed by inserting DNA fragments into a bacteriophage host, which allows the bacterial peptide to be expressed on the surface of the phage particle. The library can be screened for peptides and protein domains that adhere to known ligands, which can be directly linked to the DNA sequence. Optimisation of methods for the production of fully representative phage display libraries have been, and will be further, investigated. Fosmid libraries will be used to complement phage display to obtain complete gene sequences and determine the species responsible for adhesion mechanisms identified.



Design and Synthesis of a non-radioactive probe for mannosyltransferase activity

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Glycoproteins are highly relevant as drugs and drug targets in the biomedical field. On one hand, aberrant glycosylation is the hallmark of a number of disease states (e.g. cancer): screening tools are required to identify new drug targets. On the other, new glycosylation strategies are crucial for efficient therapeutic proteins manufacturing.¹

Analogues of monosaccharides can be incorporated into glycopeptides² and mimetics from activated donors (e.g. GDP-mannose), using glycosyltransferases which can be previously subjected to rounds of directed evolution to tolerate a variety of substrates.³ In particular, we have developed a chemoenzymatic synthesis of two unnatural GDP-mannose analogues by exploiting the ability of a promiscuous enzyme⁴ to process unnatural substrates. These compounds bear an azide group that can be selectively reacted with a probe (e.g. by means of the Staudinger ligation,² or “click” chemistry⁵). This protocol will allow for a non-radioactive high throughput screening for mannosyltransferase activity, and therefore will offer a useful tool to trace and monitor protein glycosylation and its alterations.

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TIP48 and TIP49: united in mitosis or poles apart?

Andrew Niewiarowski

Abstract:

TIP48 and TIP49 are two homologous AAA+ ATPases and are individually essential in all eukaryotes. They have been implicated in a multitude of broad cellular processes including transcriptional regulation, oncogenesis and DNA repair. It is established that TIP48 and TIP49 are found together as components of multi-protein nuclear complexes, including several chromatin remodelling complexes; their chromatin remodelling activities may explain the variety of nuclear functions. It is still unclear whether a simple underlying mechanism of TIP48 and TIP49 links these diverse processes and how these different functions are coordinated. Moreover, it is essential to establish if TIP48 and TIP49 have independent roles and whether these roles are regulated by oligomerisation. We are currently investigating the biochemical properties and sub-cellular localisation of TIP48 and TIP49, with focus on the assembly of TIP48 and TIP49 into their active forms. Several recent reports have suggested that TIP48 and TIP49 are important factors in mitosis and cytokinesis, further increasing their broad repertoire of functions. In contrast with their nuclear activities, current data suggest TIP48 and TIP49 operate independently during mitosis. Investigating the various mitotic roles of TIP48 and TIP49 and how these are regulated is of central importance, and finding an underlying mechanism that connects the multitude of processes in which TIP48 and TIP49 are essential, remains an interesting conundrum.



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Structure, Free Energy, Enthalpy and Entropy in Protein-Ligand Interactions: A Database Approach

Tjelvar Olsson

Abstract:

Interactions of proteins with small molecules are central to many biological processes. Developing a detailed understanding of protein-ligand interactions is therefore fundamental to the molecular life sciences. An understanding of how structural perturbations correlate with the thermodynamics of binding forms a cornerstone of biophysical investigation.

To investigate how structure relates to the thermodynamics of binding, we have created a database of experimental data on protein-ligand interactions that have been characterized by both structural and calorimetric methods (<http://www.biochem.ucl.ac.uk/scorpio/scorpio.html>). The structures were analyzed in terms of buried apolar- and polar- surface area. These structural parameters were then correlated to the Gibbs free energy-, the enthalpy-, and entropy- of binding.

Contrary to common belief we find that there is no general relationship between the apolar surface area buried in forming a protein-ligand complex and the entropy. However, there is a significant correlation between the buried apolar surface area and the Gibbs free energy ($r^2=0.64$). Furthermore, we find that the buried polar surface area remains relatively constant for ligands of a wide range of sizes. The findings are discussed in terms of the evolution of selectivity and affinity in binding sites.

A comparative analysis of the methods and databases used in the study of protein-protein interactions

Eleni Rapsomaniki

Abstract:

High-throughput data (e.g. from yeast-two-hybrid and co-immunoprecipitation experiments) are believed to be of particularly low quality, with a high false-positive rate. Bioinformatics evidence (such as the measurement of co-expression and annotation similarity) is widely used to identify potential false-positive interactions in low-quality data sets.

We have extended existing approaches to the evaluation of protein interaction data by covering more species, by using a wider range of bioinformatics evidence, and by using sophisticated statistical techniques to handle missing data. One interesting result of our analysis is that, in contrast to popular belief, interactions stored in many databases are often more likely to be false-positives than interactions found uniquely in a single database.



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The Semi-Synthetic Production of Human Erythropoietin

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Erythropoietin (EPO) is a glycoprotein of 166 amino acids containing 3 *N*-linked carbohydrate groups (at Asparagines 24, 38 and 83) and a single *O*-linked group at Serine 126. The attached carbohydrate moieties serve to prolong circulating half-life and subsequent activity *in vivo*. EPO is produced in the body in response to hypoxia and stimulates the maturation of erythrocytes (red blood cells) from erythroid progenitor cells. Consequently, purified formulations of human EPO are routinely administered in the clinical setting to treat anaemia resulting from HIV infection, cancer and renal failure. Presently, commercial methods of EPO production rely upon large-scale eukaryotic tissue culture systems that are both inefficient and costly. Critically, the EPO produced using this approach exists as a heterogeneous mixture of glycosylated forms, much of which exhibits limited biological activity *in vivo*.

Here we present a novel semi-synthetic strategy for the assembly of human EPO that relies upon the chemical synthesis of short (28 and 32 amino acid) N-terminal regions that contain unique acetylenated cysteine derivatives. Protein ligation between these chemically derived fragments and larger bacterially expressed C-terminal fragments result in a semi-synthetic full length EPO protein amenable to site-specific *in vitro* glycosylation with chemically defined sugars and oligosaccharide mimics, thereby circumventing the molecular heterogeneity associated with natural glycosylation processes.

Structural Analysis of the Saf Pilus by Electron Microscopy and Image Processing

Osman Salih

Abstract:

Bacterial pili are virulence factors important in pathogenesis. Pili are involved in initiating attachment to host cells, which is a key step required to establish successful infections. Here we studied *Salmonella* atypical fimbriae (or Saf pili). The organisation of the subunits in this atypical fibrillum is unknown. In our study, we have used negative stain electron microscopy (EM) and single-particle image analysis to determine the three-dimensional (3D) structure of the *Salmonella typhimurium* Saf pilus formed by SafA, the major pilus subunit. We have found that structures of the Saf atypical fibrillum present globular subunits (~ 49 Å long, ~ 25 Å deep and ~ 22 Å wide) arranged in a row. The subunits are linked to each other through thin, short stretches of density giving a 'beads on a string'-like appearance. Quantitative and objective fitting of the atomic resolution structure of SafA into the electron density maps, linker modelling and energy minimisation have enabled analysis of subunit arrangement and interactions in the Saf pilus. The combined results demonstrate the inherent flexibility of the Saf pilus about the linker regions, which act as molecular hinges allowing a large range of movement between consecutive subunits in the fibre. These results explain why atypical fibrillae are flexible and appear unstructured on bacterial surfaces.

Validating gene expression clusters using the Gene Ontology and other external evidence

Stathis Sideris

Abstract:

A method was developed to allow the use of knowledge on protein functions and protein-protein interactions to identify coherent sets of co-regulated genes suggested by the clustering of gene expression profiles. This was achieved through the development of a gene expression clustering quality metric, which judges the tightness and separation of gene expression clusters, thus providing a quality measure on a clustering or a per-cluster basis. Cluster tightness and separation are assessed by harnessing the manual annotations provided by the Gene Ontology, enriched using integrated biological information available through an in-house data warehouse (BioMap). The metric was tested on a human B-cell gene expression dataset and refined on the basis of the results produced.



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Biophysical Characterisation of SH2 Domains: a semisynthetic strategy

Satpal Virdee

Abstract:

Src Homology 2 (SH2) domains are protein modules of ~100 aa and play an integral role in eukaryotic cell signaling. Cell receptors can respond to extracellular messages by the autophosphorylation of intracellular Tyr residues. These phosphorylated Tyr (pTyr) residues are then recognised by SH2 domain-containing signaling proteins. The propagation of extracellular messages by SH2 domains leads to the regulation of essential cellular processes such as cell growth, cell differentiation, cytoskeletal rearrangement and gene activation. Extensive structural and biophysical analysis of the SH2 domain of the Src kinase has been achieved by crystallography/NMR and titration calorimetry respectively revealing the mechanism of the sequence specific binding of pTyr motifs. By adopting a semisynthetic approach we have used expressed protein ligation (EPL) and native chemical ligation (NCL) to construct an SH2 domain from three of its constituent fragments. This will allow the insertion of small synthetic cassettes containing non-standard amino acids into the SH2 domain. Initially we aim to probe the significance of the sidechain length of a Lys residue by inserting Lys derivatives with progressively shorter aliphatic side chains. However, any non-standard groups can be inserted allowing the study of SH2 domains to be conducted with unprecedented flexibility.