Coffs Harbour 12-14 July 2007

A joint initiative by the Queensland Protein Group and the Sydney Protein Group

Programme

Thursday July 12th

3pm – 7pm	Registration and *Welcome drinks*
7-8pm	*Plenary: Speaker Nick Dixon*
8pm - later	Buffet style dinner

Friday July 13th

8:30 – 10:30am *Symposium 2*

M. Bathi, Exploring the LIM code for motor neuron development - The interaction between Isl-1 and Lhx-3 (20+5 minutes)

Ranajeet Ghose, A Molecular Anchor for Intramembrane Proteolysis? (20+5 minutes)

Andrea Bugarcic Secretion of rotavirus glycoprotein NSP4 from polarised epithelial cells (20+5 minutes)

Brett Hambly, Phosphorylation of myosin binding protein-C altered in hypertrophic cardiomyopathy (15+5 minutes)

Geoff Birrell, Analysis and identification of Australian elapid venom proteins (15+5 minutes)

10:30- 11am Morning Tea – coffee, tea, snack.

11am – 1pm *Symposium 3*

Dmitri Mouradov, Chemical cross-linking, mass spectrometry and molecular modelling techniques to determine structures (20+5 minutes)

Charikleia Ioannou, The loading of the helicase in *Bacillus subtilis* (20+5 minutes)

C.K. Liew, Investigating the DNA-binding properties of THAP domains (20+5 minutes)

Janelle Hancock, Aprataxin has a unique role in DNA damage repair (15+5 minutes)

R. E. Mansfield, Investigating the RNA-binding specificity of splicing factor ZNF265 (15+5 minutes)

1pm Lunch – sandwiches, fruit, tea, coffee

4pm – 5pm *Plenary: Speaker Ashley Buckle*

5pm – 6pm *Symposium 4*

J.S. Mylne, Towards a solution structure for Arabidopsis DNA binding protein VERNALIZATION1 (15+5 minutes)

Gregor Guncar, Crystal structures of flax rust avirulence proteins AvrL567-A and AvrL567-D (15+5 minutes)

Itamar Kass, The structure and dynamic of class I Hydrophobin EAS at an air water interface (15+5 minutes)

6:30 – 8pm *Poster & Trade Display*

Beer, wine, juice, soft drink.

8pm - later *Conference Dinner* – buffet style BBQ

Saturday July 14th

8:30 – 10:30am *Symposium 5*

Jonathan M. Harris, The perils of positional scanning: Putting protease peptide library screening in perspective (20+5 minutes)

Christophe Schmitz, Automatic NMR resonance assignment of methyl groups using paramagnetic lanthanides (20+5 minutes)

Elizabeth Skippington, An automatic and scalable approach to model whole genome evolution (20+5 minutes)

Gordon King, Towards High Throughput Protein Crystallisation: Condition Screening (15+5 minutes)

Tim Grune, Protein Crystallography at the Australian Synchrotron (15+5 minutes)

10:30 – 11am Morning Tea – coffee, tea, snack

11am – 1pm *Symposium 6*

Stacy Scott, Investigation into Human Galectin-1 Function and Structure (20+5 minutes)

Jack Flanagan, Interactions between the sigma class glutathione transferase and non-glutathione ligands (20+5 minutes)

Mary Marfori, Structural basis of recruitment of tandem hot dog domains in acyl-coA thioesterase 7 and its role in inflammation (20+5 minutes)

Norelle Daly, No structural requirement for the P3' proline in the minimized SFTI-1 scaffold (15+5 minutes)

Joakim Swedberg, Substrate-Guided Design of a Potent and Specific KLK4 Protease Inhibitor (15+5 minutes)

1pm Lunch – sandwiches, fruit, tea, coffee

2pm End of meeting

Sponsorship Acknowledgements

The organising Committee gratefully acknowledges the support of all of the following companies and organisations:

Australian Society for Biochemistry and Molecular Biology

The Queensland and Sydney Protein Groups

Queensland Cyber Infrastructure Foundation

Australian Synchrotron

GE Healthcare

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Plenary lecture:

A Molecular Mousetrap Determines Polarity in Termination of DNA Replication

Nicholas E. Dixon
Department of Chemistry, University of Wollongong, NSW 2522

In the final stage of synthesis of the circular Escherichia coli chromosome, DNA replication forks are blocked in the terminus region by complexes of the Tus terminator protein with Ter DNA sites when they approach from one direction, but not the other. The first arriving fork is therefore trapped between a pair of oppositely-oriented Ter sites, where it stalls, awaiting the arrival of the second fork. The underlying basis for the polarity of the Tus-Ter replication fork block has now been shown to depend on DNA strand separation by the replicative DnaB helicase at the fork. Approach of the replication machinery from one (the permissive) direction leads to rapid dissociation of Tus from Ter, while strand separation on approach from the other (non-permissive) direction results in formation of a remarkably stable “locked” complex. “Lock” formation specifically requires a particular cytosine residue in Ter, which moves 14 Å from its normal position to bind in a cytosine-specific pocket on the surface of Tus; see Mulcair et al., *Cell*, **125**, 1309-1319 (2006). The "Tus-Ter Lock" is sufficiently stable to be exploited for practical applications in biotechnology.

Plenary lecture:

Making GABA with two GADs

Ashley Buckle
The Department of Biochemistry and Molecular Biology
School of Biomedical Sciences, Faculty of Medicine &
Victorian Bioinformatics Consortium (VBC)
Monash University, Clayton, Vic 3800
Australia

In mammals, the two isoforms of Glutamic Acid Decarboxylase (termed GAD65 and GAD67) function to produce GABA from glutamate, and control fundamental processes such as neurogenesis, movement and tissue development. Despite high sequence identity, GAD65 and GAD67 display strikingly different enzymatic and antigenic properties. Whereas GAD67 is responsible for production of a basal pool of GABA for neurotransmission, GAD65 is a much poorer enzyme but is activated to produce extra GABA when required. Further, GAD65, but rarely GAD67, is auto-antigenic, with autoantibodies characteristically detectable in type I diabetes as well as neurological disorders such as stiff person syndrome. In order to understand the structural basis for their contrasting properties, we have determined the crystal structures of N-terminal truncations of both GAD65 and GAD67. I will discuss three major outcomes of this work: (1) a mechanism for GABA/glutamate homeostasis; (2) an explanation for the requirement of two GAD isoforms in mammals; (3) the structural basis for the contrasting antigenicities of GAD65 and GAD67.

Symposium 1-1:

Exploring the LIM code for motor neuron development - The interaction between Isl-1 and Lhx-3

M. Bhati, A. Nancarrow, V. Craig, M. Lee, J. M. Guss, J. M. Matthews
School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW, Australia

LIM-HD (LIM homeodomain) proteins are essential for defining cell fate, especially in the developing central nervous system. Isl-1 and Lhx-3 are two LIM-HD proteins implicated in neuronal development that form the basis of regulatory complexes in two adjacent cell types in the ventral spinal chord, V2 interneurons and motor neurons. Both cell types express Lhx-3 and the nuclear adaptor protein Ldb1, however, Isl-1 is only expressed in postmitotic motor neurons. In the two complexes, the two LIM domains of Lhx-3 mediate different protein:protein interactions that appear to be critical for the regulation of the two different cell types. In V2 interneurons, this involves a direct interaction with the LIM interaction domain (LID) of Ldb1, whereas in motorneurons Isl-1 interacts directly with Ldb1-LID and Lhx-3 binds instead to Isl-1. We are interested in characterising these interactions with the overall goal of understanding their role in neuronal development.

Using yeast two hybrid analyses, we have identified a 30-residue region of Isl-1 used for interaction with the Lhx-3 LIM domains (LIMs). We have also identified residues in the Isl-1 minimum binding domain critical for interaction with Lhx-3 through alanine scanning mutagenesis. Complexes of Ldb1:Lhx-3LIMs and Isl-1:Lhx-3LIMs were subjected to chemical denaturation by guanidine hydrochloride monitored by tryptophan fluorescence for comparison of complex stability. We have used protein crystallography to determine the mode of interaction between Lhx-3 and Isl-1. This data, together with structural information from previously studied LIM complexes, suggests a general mode of binding with LIM domains.

Symposium 1-2:

A Molecular Anchor for Intramembrane Proteolysis ?

Armando Del Rio, Kaushik Dutta, Jose Chavez, Iban Ubarretxena-Belandia & Ranajeet Ghose*
The City University of New York, New York, USA New York Structural Biology Center, New York, USA

Rhomboids are ubiquitous integral membrane proteases that release cellular signals from membrane-bound substrates through a general signal transduction mechanism known as regulated intramembrane proteolysis (RIP). We present the NMR structure of the cytosolic N-terminal domain (NRho) of P. aeruginosa Rhomboid. NRho consists of a novel α/β fold and represents the first detailed structural insight into this class of intramembrane proteases. We find evidence that NRho is capable of strong and specific association with detergent micelles that mimic the membrane/water interface. Relaxation measurements on NRho reveal structural fluctuations on the μs-ms timescale in regions including and contiguous to those implicated in membrane interaction. This structural plasticity may facilitate the ability of NRho to recognize and associate with membranes. We suggest that NRho plays a role in scissile peptide bond selectivity by optimally positioning the Rhomboid active site relative to the membrane plane.

Symposium 1-3:

Secretion of rotavirus glycoprotein NSP4 from polarised epithelial cells

Andrea Bugarcic and John Taylor
School of Biological Sciences, University of Auckland, NZ
Institute of Glycomics, Griffith University, Gold Coast

Rotavirus is a non-enveloped dsRNA virus and is associated with severe dehydrating diarrhea in infants and young children throughout the world. Rotavirus targets the fully differentiated epithelial cells lining the tips of the small intestinal villi. Although different aspects of the rotavirus replication cycle have been researched extensively the precise method by which rotavirus is able to induce diarrheal disease have not been fully elucidated.

Recent studies suggest involvement of viral non-structural protein NSP4 in the pathophysiology of rotavirus-induced diarrhea by acting as an enterotoxin. To mediate enterotoxic effects in vivo, NSP4 needs to be secreted or released from rotavirus-infected cells in a soluble form, but studies indicate that this is a transmembrane glycoprotein located in the ER membrane.

This study reveals that NSP4 is actively secreted into the culture medium of polarised Caco-2 preferentially from the apical surface. Also, secretion was found to be inhibited by addition of BFA and monensin, suggesting a Golgi-dependent pathway is involved in release of this protein. In accordance with this finding, the secreted NSP4 isoform was found to undergo additional post-translational modification and is partially resistant to deglycosylation by EndoH and PNGaseF. Investigation into a carrier vehicle for NSP4 release from infected cells produced no evidence that this protein was packaged in exosomes but further examination of the secreted NSP4 isoform by negative staining and transmission electron microscopy revealed compact circular structures of ~10nm in diameter.

Symposium 1-4:

Phosphorylation of myosin binding protein-C altered in hypertrophic cardiomyopathy

Cecily Oakley#, Joo-Mee Hwang, Louise Brown§, Murat Kekic, Piotr Fajer# & Brett Hambly
Pathology and Bosch Institute, University of Sydney; #Biological Sciences, Florida State University; §Chemistry, Macquarie University

Cardiac myosin binding protein-C (cMyBPC) is a large regulatory protein within the sarcomere. cMyBPC phosphorylation increases systolic tension, and dissociates the N-terminal region of cMyBPC from the S2 neck region of myosin. cMyBPC mutations are associated with familial hypertrophic cardiomyopathy (FHC). We have cloned, expressed and purified the N-terminal region (immunoglobulin motifs C1 to C2) that encompasses the tri- phosphorylation sites (defined as sites A to C). Using in vitro mutagenesis we have also generated four FHC mutant forms of C1-C2. Four FHC-causing mutations are located in the phosphorylatable linker between Ig motifs C1 and C2; G278E, G279A, R326Q and L352P. The effect of these mutations on phosphorylation with PKA was investigated. The G279A mutation yielded the same rate of phosphorylation as the WT C1-C2, while the G278E mutant was phosphorylated more slowly. Surprisingly, the R326Q and L352P constructs were phosphorylated more rapidly than WT. In all cases the phosphorylation order was the same as WT. Structure prediction provides insights into the mechanisms underlying the changes in phosphorylation rate. Together these data suggest that an alteration in cMyBPC phosphorylation rate may underlie the pathogenesis of FHC caused by these mutations, although paradoxically the rate can be increased or decreased.

Symposium 1-5:

Analysis and identification of Australian elapid venom proteins

Geoff W. Birrell, Stephen T. H. Earl, Tristan P. Wallis, Paul P. Masci, John de Jersey, Jeffrey J. Gorman, and Martin F. Lavin
Queensland Institute of Medical Research and Universtity of Queensland

Australian snake venoms are lethal cocktails affecting several mammalian physiological processes. We hypothesise that Australian elapid venoms are a potential source of new human therapeutics. The aim of this study was to systematically examine the venoms of 20 Australian elapid snakes using proteomic methods such as 2-dimensional gel electrophoresis (2D PAGE) with tandem mass spectrometry (MS) and de novo peptide sequencing to identify all venom components and select novel venom proteins with potential for development as new human therapeutics. When separated by 2D PAGE, each venom showed approximately 100-200 protein spots, varying in molecular weight from 7 to over 100 kDa with pIs from 3-10. Using MS, previously characterised venom proteins such as PLA2 enzymes, neurotoxins and prothrombin-activating proteins have been identified, along with proteins not previously reported in Australian elapid venoms. A number of novel proteins have been identified and selected as potential therapeutic candidates. In addition, we have used several enzymes, protein stains and specific antisera to examine different post-translational modifications amongst Australian elapid venom proteins. This work represents the most comprehensive analysis and identification of Australian elapid venom proteins yet undertaken. Birrell et al, Mol Cell Proteomics 2007 (6): 973-986.
Supported by QRxPharma Pty/Ltd and the Australian Research Council.

Symposium 2-1:

The loading of the helicase in Bacillus subtilis

Charikleia Ioannou,[1, 2] Patrick M. Schaeffer,[3, 4] Nicholas E. Dixon[2, 3] and Panos Soultanas[1]

[1]Centre for Biomolecular Sciences, School of Chemistry, University of Nottingham, University Park, Nottingham, NG7 2RD, UK
[2]Department of Chemistry, University of Wollongong, NSW 2522
[3]Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia [4]Department of Biochemistry and Molecular Biology, James Cook University, Australia

During DNA replication, the DNA double helix needs to be unwound if the genetic information is to be accessible to the replication machinery. The task of separating the two strands is performed by the DNA helicases, which encircle DNA and drive the replication fork. Although certain helicases are capable of self-loading onto ssDNA, efficient loading requires the helicase loader.

In *Bacillus subtilis* the helicase loader is DnaI, a primosomal protein that delivers the replicative ring helicase DnaC onto DNA during initiation of DNA replication. Here, we reveal that DnaI consists of two domains: a smaller N-terminal domain (Nd) that interacts with the helicase and a C-terminal domain (Cd) with ATPase and DNA-binding activities. Although DnaI is unable to bind ssDNA, a cryptic DNA-binding site on the Cd is exposed upon removal of the Nd. DnaI Cd also binds ATP and exhibits low ATPase activity. DnaI is sufficient to load the replicative helicase from a complex with six DnaI molecules without the requirement for a dual helicase loader system. Upon delivery of the helicase on the DNA substrate, ATP hydrolysis triggers conformational changes that allow dissociation of DnaI.

Based on the above findings a speculative model has been devised to accommodate and translate these data into biologically significant events during the process of helicase loading. This theoretical model describes DnaI as a ‘molecular matchmaker’ that brings the helicase in contact with DNA. DnaI realises a cycle of actions that leave the helicase delivered on the replication bubble.

Symposium 2-2:

Chemical cross-linking, mass spectrometry and molecular modelling techniques to determine structures

Dmitri Mouradov[1], Jade K. Forwood[1], Gordon King2, Ian Ross[3], David Hume[3], Jennifer L. Martin[2,1], Bostjan Kobe[1,2] and Thomas Huber[1]
[1] School of Molecular and Microbial Sciences, The University of Queensland. [2] Institute for Molecular Bioscience, The University of Queensland. [3] CRC for Chronic Inflammatory Diseases, Institute for Molecular Biosciences, The University of Queensland

Solving the structures of protein complexes by x-ray crystallography is a difficult and challenging process, which in a large number of cases fails to produce results. New approaches are being explored to identify interaction interfaces of proteins. One such approach aims to derive a set of sparse distance constraints using chemical cross-linkers to map out residues in the protein interaction interface, and then determine the orientation of the proteins in the complex structure by computational means. This approach is particularly powerful when partial structural information is available, for example, the structures of individual proteins in a complex or individual domains in a multi-domain protein. In such cases, this technique allows low-resolution structure determination even with a limited number of approximate distance constraints. Here we present a new technique that we have developed to greatly improve identification of cross-linked peptides and demonstrate how we applied it to produce models of a protein complex and the domain organization of a multi-domain protein.

Symposium 2-3:

Investigating the DNA-binding properties of THAP domains

C.K. Liew, M. Tan, H.R. Nicholas, M. Crossley, J.P. Mackay
School of Molecular and Microbial Biosciences, University of Sydney

C-terminal binding proteins (CtBPs) are a family of transcriptional co-repressors that are highly conserved amongst vertebrates and invertebrates. CtBPs play critical roles in differentiation, apoptosis, development and oncogenesis. Gene repression mediated by CtBP occurs through different mechanisms, one of which involves recruitment of histone deacetylase enzymes (HDACs). CtBPs appear to lack any intrinsic DNA-binding ability and are recruited to the DNA by promoter bound transcription factors. Interestingly, sequence analysis of CtBP from C. elegans reveals an N-terminal domain not found in CtBPs from other organisms. This domain was identified as a THAP domain and members of this domain family have been shown to bind DNA. THAP domains are a recently discovered class of zinc-binding domains, originally identified in the protein THAP1 (Thanatos-associated protein-1, where Thanatos is death, after the Greek God of death). These domains (~90 residues in size) contain the signature (Cys-X2-4-Cys-X35-53-Cys-X2-His). Our aim is to understand the function the THAP domain in C. elegans CtBP and the role that it plays in the context of the whole protein. To this end, we determined the solution structure of the C. elegans CtBP THAP domain and showed that it is very similar to a human THAP domain but with some distinct features. We also showed that this domain is capable of binding DNA containing a core AGTG motif and NMR spectroscopy was used to localise its DNA-binding surface. Current efforts are focused on determining the affinity and the specificity of this interaction with DNA

Symposium 2-4:

Aprataxin has a unique role in DNA damage repair

Janelle Hancock1,2, Olivier Becherel2, Martin Lavin2,3
1- Queensland Institute of Medical Research
2- Queensland University of Technology
3- University of Queensland

The protein Aprataxin is mutated in the neurological disease Ataxia with Oculomotor Apraxia Type 1 (AOA1). AOA1 patient cells are hypersensitive to DNA damaging agents and patient brain sections show elevated levels of oxidative DNA damage, suggesting a role for Aprataxin in DNA repair. Aprataxin contains three functional domains: a Forkhead Associated (FHA) phosphoprotein interaction motif, a C2H2 zinc finger and a Histidine Triad (HIT) nucleotide hydrolase domain. We show that Aprataxin's zinc finger binds double stranded DNA, and that its HIT domain hydrolyses adenosine derivatives. We also show that DNA competitively inhibits HIT domain activity, demonstrating crosstalk between the HIT and zinc finger domains. Furthermore we show that Aprataxin binds and hydrolyses 5' adenylated DNA, a DNA repair intermediate resulting from abortive ligations. Given the oxidative DNA damage present in AOA1 brain sections, we examined wether the presence of oxidative damage near a single strand break could trigger accumulation of 5' adenylated DNA as a consequence of abortive ligations. We found that oxidative damage to the 3' terminus of a DNA nick causes inhibition of ligation and accumulation of 5' adenylated DNA. As DNA ligases have an absolute requirement for a 5' phosphate terminus, this 5' adenylate must be hydrolysed to generate a 5' phosphate moiety if repair is to occur. We show that Aprataxin is the only protein in mammalian cells which can repair 5' adenylated DNA, suggesting that Aprataxin has a critical role in the cell, to prevent accumulation of unligatable DNA ends.

Symposium 2-5:

Investigating the RNA-binding specificity of splicing factor ZNF265

R. E. Mansfield, F. E. Loughlin, P. Setiyaputra, J. P. Mackay
School of Molecular and Microbial Biosciences, University of Sydney

The human protein ZNF265 is a novel splicing factor that has been shown to interact with other known splicing components and direct alternative splicing in vitro (1). The domain structure of ZNF265 resembles SR proteins, with a C-terminal RS (Arg-Ser-rich) domain and two RNA-binding zinc finger motifs in the N-terminal region. These zinc finger domains have been shown to specifically bind to a single-stranded RNA motif that is essentially identical to the mammalian 5' splice site consensus sequence (AGGUAA). We have used a combination of fluorescence anisotropy binding assays and ^{15}N-HSQC titrations to investigate the binding affinity and sequence preferences for each of the two zinc fingers in ZNF265. The results of this study should prove useful for the manipulation of specific 5' splice site sequences to further assess the molecular mechanism of action of ZNF265. (1) Adams D. J., Van der Weyden L., Mayeda A., Stamm S., Morris B. J. and Rasko J. E. (2001) J. Cell. Biol. 154, 25-32

Symposium 3-1:

Towards a solution structure for Arabidopsis DNA binding protein VERNALIZATION1

J.S. Mylne [1], D.J. Craik [1], J.M. Hill [2]
[1] Division of Chemical and Structural Biology, Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD, 4072, AUSTRALIA
[2] School of Molecular and Microbial Sciences, The University of Queensland, St. Lucia, QLD, 4072, AUSTRALIA

Vernalization is a physiological process whereby prolonged cold (namely winter) accelerates flowering. In model plant Arabidopsis, this is predominantly achieved by repressing MADS box floral repressor FLOWERING LOCUS C (FLC). Five proteins have been identified to date as required to repress and maintain repression of FLC in response to cold treatment. Of these five proteins, VERNALIZATION1 (VRN1) is the only one that is plant-specific and appears only to have dicotyledonous homologs. VRN1 possesses two B3 DNA binding domains, PEST and nuclear localization sequences [refs 1, 2]. We are using NMR spectroscopy to study the 3D solution structure of VRN1 protein domains. To begin we have focused on a 17 kDa region that includes the second B3 domain and a region upstream of this which is highly conserved among VRN1 homologs and predicted to be structured. Through these structural studies we hope to gain a greater understanding of how VRN1 functions in DNA binding and whether regions of VRN1 are likely to interact with other proteins involved in vernalization. Hypotheses arising from the structural work will be tested by functional studies such as mutagenesis, DNA binding and rescue of vrn1 mutants.

1. Mylne, Barrett, Tessadori, Mesnage, Johnson, Bernatavichute, Jacobsen, Fransz, Dean. (2006) LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC. Proc. Natl Acad. Sci. U.S.A. 103:5012-5017.

2. Levy, Mesnage, Mylne, Gendall, Dean. (2002) Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. Science. 297:243-246.

Symposium 3-2:

Crystal structures of flax rust avirulence proteins AvrL567-A and AvrL567-D

Gregor Guncar[a,b], Ching-I A. Wang[a], Jade K. Forwood[a,b], Trazel Teh[a], Ann-Maree Catanzariti[d], Gregory J. Lawrence[c], Horst Joachim Schirra[b], Peter A. Anderson[e], Jeffrey G. Ellis[c], Peter N. Dodds[c] and Bostjan Kobe[a,b,f]

[a]SMMS, University of Queensland, Brisbane [b]IMB, UQ, Brisbane [c]CSIRO, Canberra [d]University of California, Berkeley, USA [e]SBS, Flinders University of South Australia, Adelaide [f]SRC for Functional and Applied Genomics, UQ, Brisbane

Gene-for-gene model of plant disease resistance involves recognition of pathogen avirulence proteins by plant resistance proteins. Flax rust AvrL567 avirulence proteins and the corresponding flax L5, L6 and L7 resistance proteins interact directly. We determined the three-dimensional structures of two members of the AvrL567 family, AvrL567-A and AvrL567-D. The structure of AvrL567-A was solved by SAD technique, using the in-house Cu X-ray source, taking advantage of a bound Co2+ ion and allowed us to solve the structure of AvrL567-D by molecular replacement. The structures of both proteins are very similar and reveal β-sandwich fold with no close known structural homologues. The polymorphic residues in the AvrL567 family map to the surface of the protein and polymorphisms in residues associated with recognition differences lead to significant changes in surface chemical properties. Analysis of single amino acid substitutions in AvrL567 proteins confirm the role of individual residues in conferring differences in recognition and suggest that recognition specificity of the L proteins for the AvrL567 family members results from the cumulative effects of multiple amino acid contacts. The structure also provides insights into possible pathogen-associated functions of AvrL567 proteins, with nucleic acid binding activity demonstrated in vitro, suggesting a possible role in influencing host gene expression during infection. Our studies provide some of the first structural information on avirulence proteins that bind directly to the corresponding resistance proteins, allowing an examination of the molecular basis of the interaction with the resistance proteins as a step towards designing new resistance specificities.

Symposium 3-3:

The structure and dynamic of class I Hydrophobin EAS at an air water interface

Itamar Kass* Rima Gupte* Alan E. Mark*^
***School of Molecular and Microbial Sciences, The University of Queensland ^Institute for Molecular Bioscience, The University of Queensland**

Hydrophobins are small proteins secreted by fungi that coat the aerial structures of these microorganisms with a hydrophobic water-repellent layer. More generally, hydrophobins self-assemble on a variety of surfaces including at an air-water interface. At surfaces hydrophobins form an amphiphilic film that acts to invert the hydropathy of a surface. Such films can reverse the wettability of a surface such Teflon and are considered to have great potential as biomaterials. However, despite considerable efforts, our knowledge of the structure of hydrophobins and the mechanism through which they form rodlets is far from complete. In this research, we apply MD simulation techniques in the study of the structure and dynamics of hydrophobin EAS and truncated EAS from Neurospora crassa at an air-water interface. In the simulations the peptides are highly flexible in solution and readily attach to an air-water interface. The simulations have been used to investigate whether the protein binds using a unique subset of residues and to how they may further assemble into a robust film.

Symposium 4-1:

The perils of positional scanning: Putting protease peptide library screening in perspective

Jonathan M. Harris, Thomas Takayama, Joakim Swedberg
Institute for Health and Biomedical Innovation Queensland University of Technology Kelvin Grove Queensland

Positional scanning of Synthetic Combinatorial Libraries (PS-SCL) dominates the field of protease inhibitor and substrate design. This technique uses combinatorial chemistry to create pools of related peptides that are then screened for proteolytic activity with recombinant proteases. Typically pools of hundreds to thousands of fluorogenic peptides are present in each pool and libraries can encompass up to a million individual species. This diversity coupled with the technique's comparative ease of use, high throughput format and history of apparent successes have ensured that it is the method of choice for the protease enthusiast. However, the recent failure of a series of Matrix Metallo-Protese (MMP) inhibitors in phase III clinical trials have lead to a reassessment of the technique's strengthens and weaknesses. Here we present a comparison of PS-SCL with substrate guided inhibitor design and serial peptide synthesis which dramatically highlights the strengths and flaws of both approaches.

Symposium 4-2:

Automatic NMR resonance assignment of methyl groups using paramagnetic lanthanides

Christophe Schmitz[1], Michael John[2], Ah Young Park[2], Nicholas E. Dixon[3], Gottfried Otting[2], Thomas Huber[1]
[1]University of Queensland, School of Molecular and Microbial Sciences;
[2]Australian National University, Research School of Chemistry;
[3]University of Wollongong, Department of Chemistry

Methyl groups are excellent probes for the study of proteins by NMR spectroscopy due to their favourable relaxation properties and intense ^{1}H NMR signals. Buried methyl groups report on the packing of side chains in the protein core and therefore provide important restraints for protein-fold determination. On the protein surface, methyl groups can serve as hydrophobic probes of protein-protein and protein-ligand interactions. The resonance assignment of methyl groups in ^{13}C labeled proteins is a difficult and expensive task, especially for large sized proteins. Here we show that pseudocontact shifts induced by site-specifically bound paramagnetic lanthanide ions provide fast access to sequence-specific resonance assignments of methyl groups in proteins of known three dimensional structure. The algorithm POSSUM (*p*aramagnetically *o*rchestrated *s*pectral *s*olver of *u*nassigned *m*ethyls) was developed to automatically obtain sequence-specific assignments of the cross-peaks both in the paramagnetic and diamagnetic spectra of a structurally determined protein. POSSUM was first tested on perfect synthetic data generated for the N-terminal exonuclease domain ε 186 of *E. coli* DNA polymerase III. Random noise was then added to test the robustness of the approach. Finally, POSSUM was used with experimental NMR data, and the result compared with the reference assignment obtained by established NMR techniques.

Symposium 4-3:

An automatic and scalable approach to model whole genome evolution

Elizabeth Skippington and Thomas Huber
School of Molecular and Microbial Sciences, The University of Queensland St Lucia, Brisbane Qld 4072 Australia

The extent to which lateral genetic transfer has shaped organismal evolution is a contentious issue which has been stimulated by the recent increase in the number of complete sequenced genomes that have allowed previously unsuspected candidates for lateral gene transfer to be determined. The purpose of my research is the the pursuit of incongruent phylogenetic distributions with the view to identifying phylogenetic anomalies including LGT. Whole-genome BLASTP (protein vs protein) comparisons of all proteins for every possible pairing of organisms that make up a sample of 640 complete genome sequences available from the Joint Genome Institute's Integrate Microbial Genome system will be used to craft distributions representing the amino acid identity of genes between two genomes. The assumption is that via statistical analysis of these distributions insight can be gained into the evolutionary mechanisms which have been employed. Here, I will present the implementation of a tool to compare whole genomes and its application to delineate lateral from horizontal gene transfer in microbial evolution.

Symposium 4-4:

Towards High Throughput Protein Crystallisation: Condition Screening

Gordon King and Jenny Martin
Institute for Molecular Bioscience, University of Qld

The production of protein crystals that are capable of providing high resolution structural models is a major challenge in structural biology. The availability of commercial instruments capable of automating the setup of crystallisation conditions using small quantities of protein has made high throughput screening of a large number of crystallisation conditions a practical possibility for university-based laboratories. This talk will discuss the approaches used in high throughput crystallisation condition screening and introduce the crystal screening service available through the ARC Special Research Centre for Functional and Applied Genomics at the Institute for Molecular BioScience, University of Queensland.

Symposium 4-5:

Protein Crystallography at the Australian Synchrotron

Tim Grune Julian Adams Ruth Plathe Andy Broadbent
Protein Crystallography Australian Synchrotron

The Australian Synchrotron is the new 3rd generation synchrotron source currently being built in Victoria, Australia. This 3GeV machine will start to come on-line for users in 2007. Initial funding covers the construction of the machine and nine beamlines of which two are dedicated to Protein Crystallography. Details of the construction and commissioning progress for the two protein crystallography beamlines will be presented.

Symposium 5-1:

Investigation into Human Galectin-1 Function and Structure

Stacy Scott, Helen Blanchard
Griffith University, Gold Coast Campus

Galectins are a type of carbohydrate-binding protein (lectin) that are characterized by a conserved carbohydrate recognition domain (CRD) and specific affinity for glycoconjugates that contain β-galactosides. Human galectin-1, a non-covalently bound homodimer approximately 32 kDa in size, is expressed ubiquitously within the adult body and is involved in a wide range of regulatory processes. The over-expression of galectin-1 by many human cancers also suggests that galectin-1 plays a role(s) in tumourigenesis, which is reason for the interest in the development of potent galectin-1-specific therapeutics. Here we present two unreported crystallographic structures of human galectin-1 complexed with lactose, and one crystallographic structure of apo human galectin-1. Structural differences are evident in the novel crystallographic structures when compared to the lactose bound human galectin-1 crystallographic structure published by Lopez-Lucendo et al (2004). Additionally, novel dynamic light scattering analysis of human galectin-1, lactose bound and apo, is reported. Together with solution studies, the novel crystallographic structures provide greater insight into the function of human galectin-1, so that more potent galectin-1 inhibitors may be designed.

Symposium 5-2:

Interactions between the sigma class glutathione transferase and non-glutathione ligands.

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The glutathione transferases are phase II detoxication enzymes that catalyse conjugation of the tripeptide glutathione (GSH) to many non-polar compounds. This facilitates the metabolism and clearance of many xenobiotics. The biological roles of this superfamily were recently expanded with some isoforms modulating inflammatory and oxidative stress responses. They are functional as dimers, with one active site per monomer that is divided into a GSH binding site, formed from the N-terminal thioredoxin-like domain, and a second ligand binding site defined by the all helical C-terminal domain. Characterisation of ligand binding in these enzymes is primarily through biochemical and crystallographic analysis of non-specific ligands. Although isoform specific inhibitors using GSH analogues targeting the well defined GSH binding site exist, there are few, if any, targeting the second ligand binding sites. As little attention has been given to the application of docking calculations in defining binding modes for these enzymes, we have identified a series of non-GSH sigma class GST inhibitors and characterized possible active site interactions using docking calculations and crystallography. GOLD best reproduced the binding mode of a high affinity inhibitor using Chemscore a no-hydrogen model of the protein and GSH as the negatively charged thiolate species formed in many GST active sites. This system could also rank order known actives, but could not separate closely related non-actives. An all-hydrogen model gave more “unlikely poses” indicating that rescoring using a different method should be applied to the results.

Symposium 5-3:

Structural basis of recruitment of tandem hot dog domains in acyl-coA thioesterase 7 and its role in inflammation

Mary Marfori[1], Jade Forwood[1,2], Anil Thakur[1], Gregor Guncar[1,2], Dmitri Mouradov[1], Weining Meng[1], Jodie Robinson[2], Thomas Huber[1], Stuart Kellie[1,2], Jennifer Martin[1,2], David Hume[1,2], Bostjan Kobe[1,2]

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Acyl-CoA thioesterases (Acots) are a group of ubiquitously expressed proteins that catalyse the hydrolysis of fatty acyl-CoAs to their corresponding free fatty acids and coenzyme A, thereby regulating lipid metabolism and cellular signalling. These enzymes are present in all three branches of life, with bacterial proteins possessing one acyl-CoA hydrolase domain (BFIT_BACH), and mammalian enzymes boasting two copies of the BFIT_BACH motif. Each domain exhibits the "hot-dog fold" comprising of an antiparallel β-sheet "bun", enveloping an α-helical "sausage", arranging in a hexameric/ trimeric quaternary structure in bacterial and mammalian proteins respectively. The structures of the separate N- and C-terminal domains of the mouse Acot7 enzyme were recently determined, and the structure of the full length protein was consequently inferred using a combination of chemical cross-linking and mass spectrometry techniques. Site directed mutagenesis studies successfully identified the presence of two potential active sites at the domain interface of the mouse protein. The results highlight the necessity of domain cooperativity to produce a functional enzyme, with catalytic residues being contributed from both N- and C-terminal hot-dog domains of the protein. Significantly, it was revealed that half of the active sites in the eukaryotic macromolecular assembly are not catalytically active. Additionally, the enzyme displayed greatest thioesterase activity towards arachidonoyl-CoA, and was shown to be over-expressed in LPS treated macrophage cell lines. Together, these results suggest roles of Acot7 in eicosanoid metabolism and identify the enzyme as a candidate drug target in inflammatory disease.

Symposium 5-4:

No structural requirement for the P3' proline in the minimized SFTI-1 scaffold

Norelle L. Daly, Yi-Kuang Chen, Fiona M. Foley, Paramjit S. Bansal, Rekha Bharathi, Richard J. Clark, Christian P. Sommerhoff[¶] and David J. Craik.
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SFTI-1 is a small cyclic peptide from sunflower seeds that is one of the most potent trypsin inhibitors and is related to the Bowman-Birk family of inhibitors (BBIs). BBIs have potential as cancer chemopreventive agents, and at only 14-amino acids in size SFTI-1 is thought to be a highly optimised scaffold of the BBI active site region. In the current study a suite of 12 alanine mutants of SFTI-1 has been synthesized and their structures and activities determined. SFTI-1 incorporates a binding loop that is clasped together with a disulfide bond and a secondary loop making up the circular backbone. We show here that the secondary loop stabilizes the binding loop to the consequences of sequence variations. In particular, full length BBIs have a conserved cis proline that has previously been shown to be required for well-defined structure and potent activity but we show here that the SFTI-1 scaffold can accommodate mutation of this residue and still have a well-defined native-like conformation and nanomolar activity in inhibiting trypsin. Amongst the Ala mutants the most significant structural perturbation occurred when Asp14 was mutated and is thus a key residue in maintaining the highly constrained structure of SFTI-1. This aspartic acid residue is thought to be involved in the cyclization mechanism of SFTI-1. Overall, this mutational analysis of SFTI-1 clearly defines the optimised nature of the SFTI-1 scaffold and demonstrates the importance of the secondary loop in maintaining the active conformation of the binding loop.

Symposium 5-5:

Substrate-Guided Design of a Potent and Specific KLK4 Protease Inhibitor

Joakim E. Swedberg1, Thomas takayama2, Sarah Kruger3, Jonathan Harris1

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Abstract Human Kallikrein 4 (hKLK4) is a trypsin-like serine protease. Recent studies showing that it is over-expressed in prostate cancer and has a potential role in Epithelial to Mesenchymal Transition suggest it is a target for prostate cancer therapy. Here we demonstrate that KLK4 is sensitive to inhibition by Sunflower Trypsin Inhibitor (SFTI), and use this cyclic peptide as the basis for the design of KLK4 inhibitors with enhanced specificity and potency. SFTI comprises 14 amino acids with a cyclized backbone bisected by a single disulphide bond producing a bicyclic system containing a conserved β-hairpin found in all Bowman-Birk type inhibitiors, with residues Arg2, Thr4 and Lys5 inserting into the trypsin P1-P4 sub site pockets. This feature is responsible for enzyme-inhibitor contacts whilst the second loop is responsible for conformational stability. We reasoned that short peptides complementary to the P1-P4 sub sites of KLK4 (as opposed to trypsin) could be selected on the basis that they would also be good proteolytic substrates for the enzyme. Accordingly, we synthesised a panel of 125 para-nitroanilide (pNA) peptide substrates and assessed the proteases' catalytic activity with these compounds. We found KLK4 expressed highest amidolytic activity with the sequence FVQR-pNA and an SFTI variant was constructed with the substitutions Arg2→Phe2, Thr4→Gln 4 and Lys5→Arg5 based on this substrate. This variant was found to potently inhibit KLK4 (IC50 135 nM) whilst lacking the ability to inhibit the proteases thrombin or trypsin.

poster presentation:

Computational studies of the vibrational spectrum of an alpha helical protein in terahertz range

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Center for Biomolecular Engineering, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland

Terahertz (THz) spectroscopy is an emerging technology that will become a useful tool for numerous aspects of physical chemistry. In terms of frequency, THz spectroscopy covers the region from ~0.1 THz to 10 THz, which lies in the border between far infrared and microwave. Due to the nature of THz radiation, the spectra represent information on intermolecular vibration as well as the translations and liberation of the molecule. Driven by many foreseeable applications, the knowledge of THz spectroscopy had been greatly expanded over the last few years. Supported by the Australian Research Council, a national THz spectroscopy facility had been established by our collaborators in the University of Adelaide. The interest of our group is to investigate the effects that structural and environmental changes imposed on the THz spectrum, and for biomolecular systems computationally predict the frequency spectrum in the THz range. Computationally, we used two approaches in order to extract the spectrum information. One is molecular dynamics simulation followed by principle component analysis to calculate the vibration spectrum, and alternatively, we performed a normal mode analysis on the modelled structure to approximate the spectrum. Here, we will present preliminary results obtained on a small peptidic test system; a surfactant that is composed of 21 amino acids. This peptide can be switched between an alpha-helical structure and random coil structure by varying environmental conditions, such as pH, salt concentration and temperature, and thus allows comparative analysis of THz spectra in different preferred structural states.

poster presentation:

Molecular Dynamics on a Grand Scale: Towards large scale atomistic simulations of self-assembling biomolecular systems

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The challenge in modelling structure formation using molecular dynamics (MD) simulation techniques is to accurately describe the properties and behaviour of the system in terms of the interactions between explicit atoms. The size and complexity of biomolecular systems, such as proteins and nucleic acids, together with the time scales that must be reached necessitates the use of extensive computing resources. The demand for realism in computer games has lead to modern Graphics Processing Units (GPUs), the central microchips on video cards in personal computers, comprising hundreds of processing engines. These may be programmed to perform complex, general-purpose operations on large data sets, providing an effective computational output an order of magnitude greater than the latest, similarly priced Central Processing Units (CPUs). This project used the GPU to accelerate the calculation of the solvent-solvent interactions, which account for most of the computation in MD simulations. We present the implementation on the GPU and the differences in the performance of the GPU and CPU running these calculations. Issues such as accuracy and data transfer to and from the video card are also discussed.

poster presentation:

Differential Oligomerisation of LIM Domain-Binding Proteins 1 and 2.

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LIM domain-binding protein one (Ldb1) is a nuclear adaptor protein that is able to bind to LIM-homeodomain and LIM-only proteins to form larger transcriptionally active complexes. Several domains are conserved between Ldb proteins from different species: a nuclear localisation sequence; a LIM interaction domain (LID); and, a so-called homodimerisation domain that mediates self-association. The dimerisation domain was previously defined, by yeast two hybrid[1] and co-immunoprecipitation[2] analysis, as the 200 amino-terminal residues. We want to further characterise this domain in Ldb1 and its homologue Ldb2. Despite many similarities Ldb2 is not sufficient to compensate for Ldb1 in *Ldb1*- knockout mice.

We have designed slightly shorter constructs for the dimerisation domains. These designs were selected based on both the structured region of the domains (determined by limited proteolysis in combination with mass spectrometry), and the minimum region capable of self-association (determined by yeast two-hybrid analysis). The domains have now been expressed as fusion proteins with several different protein tags. Preliminary data based on size exclusion chromatography combined with multi-angle laser light scattering indicates that Ldb1 is a dimer. However, similar experiments on Ldb2 indicate that this protein is not dimeric, but instead forms a stable tetramer. These different oligomeric states imply that the two proteins can form different transcription complexes to achieve different biological functions.

(1) Breen J. J., Agulnick A. D., Westphal H., and Dawid I. B. (1998). J. Biol. Chem. 273(8): 4712-4717.
(2) Jurata L. W. and Gill G.N. (1997). Mol. Cell. Biol. 17(10):5688-5698.

poster presentation:

An Analysis of RNA binding RanBP Zinc Finger Domains

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Many structural classes of zinc fingers exist and these domains can have different functions in various contexts. Different members of one class of zinc ribbon type zinc fingers called RanBP zinc fingers, are able to bind proteins like ubiquitin, RanGTP/RanGDP and also RNA and ssDNA.
Currently only the RanBP zinc finger domains from TLS and ZNF265 are known to bind RNA. The residues of the second finger in ZNF265 that are important in interacting with RNA, have been mapped out using NMR methods (Fionna Loughlin, unpublished data). With this knowledge in hand, a sequence alignment of all RanBP zinc fingers in the human genome reveals a conserved set of residues, that are present in what appears to be a subclass of RanBP zinc fingers, including those found in RBM5, RBP56, RBM10, TEX13a and EWS.
Using fluorescence anisotropy and NMR methods I hope to reveal whether these proteins do in fact have RNA-binding activity and to determine whether the mechanisms of RNA binding are conserved. These data will allow us to discover whether there really is a subclass of RNA binding RanBP zinc fingers and will provide useful functional information for several of these poorly characterised proteins.

poster presentation:

Does FOG-1 recruit Sin3b to regulate gene expression?

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FOG-1, through its interaction with GATA-1, is required for the normal differentiation of platelets and erythrocytes. The essential nature of FOG-1 is illustrated by FOG-1 knockout mice, which die at embryonic day 11.5 due to severe anaemia. FOG-1 also has a GATA-1 independent role during blood cell development and is likely to have a range of protein binding partners. Despite all the evidence implicating FOG-1 as being essential during hematopoiesis, the mechanisms through which FOG-1 acts as a transcriptional regulator are not well understood. Using a combination of pull-down experiments and peptide mass fingerprinting we have discovered that a particular domain of FOG-1 (residues 100-254) interacts with Sin3b, a global regulator of transcription and genomic stability. We have solved the structure of this domain by NMR and found it resembles a SET domain, a motif found in proteins associated with the regulation of gene expression. We have shown the colocalisation of FOG-1 and Sin3b by confocal microscopy and investigated the effects of the interaction on transcriptional regulation. The recruitment of the co-repressor Sin3b via the SET domain of FOG-1 may explain the regulatory role FOG-1 plays during blood cell development.

poster presentation:

Detection of human CBG phosphorylation through the use of a phosphoprotein detection dye

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Protein phosphorylation is a common post-translational modification (PTM) event, and the identification of this modification on the polypeptide chain of human corticosteroid-binding globulin (CBG) has provided additional evidence that further supports the active roles of this protein in steroid hormone action. CBG is a serum transport glycoprotein which binds and regulates the bioavailability as well as the bioactivity of the steroid hormone cortisol. Till now only glycosylation of N-linked oligosaccharide chains were identified to impart physiochemical properties in the synthesis and activity of human CBG. PNGase-F treatment resulted in complete deglycosylation of human CBG as evident on a 1D gel and subsequent isoform profiling on a 2D polyacrylamide gel identified three distinct isoforms. These three isoforms of human CBG of similar molecular mass were found to have isoelectric point charges of 5.3, 5.4, and 5.5, and from this 2D gel profile evidence was obtained to suggest that an additional form of PTM existed on the polypeptide chain of human CBG. Phosphoprotein detection dye, Phos-tag 540 was used to determine the presence of phosphorylation. PNGase-F treated human CBG separated using 2D-PAGE revealed for the first time, phosphorylation of the human CBG polypeptide chain.

poster presentation:

Investigating the Folding Dynamics of Cyclotides: A Simulation Approach

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Cyclotides are cyclic polypeptides that contain an embedded cyclic cystine knot and are found in at least two plant families (Rubiaceae and Violacaea). In addition to their unusual structural topology, cyclotides have attracted much attention due to their anti-HIV and anti-microbial activity. A major question in regard to cyclotides is how these structures fold and whether any of the disulfide-bonds are required for the peptide to adopt the structure as determined by NMR[1]. For example, non-native disulfide bonding patterns emerge during the process of folding to the native state[2], it is not known if these represent intermediates on the folding pathway. A 2-disulfide intermediate of MCoTI-II was isolated and recently the structure was solved using NMR. Cemazar *et al*[2] established that the intermediate could fold into the native without the need for any chaperone. In order to better understand the mechanism of folding of cyclotides, atomistic Molecular Dynamic (MD) simulations involving MCoTi-II have been performed.

1. Saether, O. *et al*. Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1. *Biochemistry* **34**, 4147-58, 1995
2. Cemazar, M. *et al*. Knots in rings. The circular knotted protein Momordica cochinchinensis trypsin inhibitor-II folds via a stable two-disulfide intermediate. *J. Biol. Chem.* **281**, 8224-32, 2006

poster presentation:

Standing out of the multitude: MED-1 zinc fingers recognize an atypical DNA sequence

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Zinc fingers are a common super class of transcription factor domain that can bind to DNA, RNA and proteins. The C. elegans protein MED-1 and its homologue MED-2 are zinc finger containing proteins that are required for activating the expression of mesoderm-specific target genes in the early stages of development. Unlike most DNA binding GATA-type zinc fingers which bind to the consensus sequence (A/G/T)GATA(A/G), the zinc finger of MED-1 binds to a unique and more defined sequence $(A/G)_3$GTATAC. On the basis of multidimensional NMR data, a model for the interaction between the MED-1 zinc finger and its target DNA sequence has been proposed. The model provided shows that the domain unexpectedly forms an extended helix in its basic tail upon binding to DNA. In order to corroborate this model, we have made mutations in a number of surface residues in the protein and measured the effects of the mutations on DNA-binding ability.

poster presentation:

Location and orientation of ligand molecules by paramagnetic NMR techniques

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The presence of paramagnetic metal ions has been exploited in nuclear magnetic resonance experiments to yield valuable structural information on metalloproteins. Labeling techniques, where lanthanide binding peptides (LBP) are attached to a target protein can be used to introduce paramagnetic effects into proteins that do not bind metal ions. We present a computational approach to determine the optimal LBP attachment sites on a target protein in order to determine the precise location and orientation of ligand molecules. This approach considers the effects of paramagnetic line broadening and the elimination of degenerate ligand solutions due to the symmetric X-tensor. Molecular dynamics (MD) simulations were performed to characterise dynamical behaviour of the LBP and to analyse LBP induced structure alteration in the protein to which it is attached. From a total of 162 residues in the test protein T4 lysozyme, the labelling protocol determines 8 residues suitable for the attachment of a LBP. For two of these sites the LBP can be attached in only one of the two preferred disulphide dihedral angles. It is shown that the combination of two paramagnetic labels in a docking protocol is sufficient to determine the unique location and orientation of a ligand molecule in agreement with a reference position. We also show that both the flexibility and the induced structure perturbation of the LBP can be partly attributed to the intrinsic domain motions of T4 lysozyme. We provide a tool to solve for the location and orientation of a ligand molecules using LBP labelled proteins.

poster presentation:

EXPLORING THE MOLECULAR BASIS OF THE LIM CODE

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The LIM-HD (LIM Homeodomain) protein family is responsible for cell type specification in a wide variety of tissues. Unique combinations of LIM-HD proteins expressed in developing tissue are believed to form “LIM-codes” that direct cell fate. The homologous pairs of Isl1/Isl2 and Lhx3/Lhx4 represent a fundamental example of developmental regulation by the LIM code in the ventral neural cord. With the essential cofactor, Ldb1, combinations of these proteins can regulate interneuron or motorneuron development. In the absence of the Isl proteins, the tetrameric complex of 2Ldb1:2Lhx3 or 2Ldb1:2Lhx4 dictates V2 interneuron fate. In the presence of Isl1 or Isl2, a hexameric complex directs motor neuron differentiation[1]. Thus, in the alternate complexes, the Lhx3/Lhx4 LIM domains mediate different protein-protein interactions that may be critical for the regulation of the two distinct developmental pathways.

Yeast two-hybrid analyses have defined a 30-residue segment in both Isl1 and Isl2 that acts as a LIM interaction domain (LID) for association with both Lhx3 and Lhx4. An alanine mutagenic screen of the Isl-LIDs is currently being employed to identify residues responsible for mediating each of the interactions between these proteins. Further work by Y2H has allowed us to discover novel interaction partners among the 12 LIM-HD proteins.

1. Thaler, J. P., S. K. Lee, *et al.* (2002). "LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions." *Cell* **110**(2): 237-49.

poster presentation:

Interactions of Tal-1 and E2A in T-cell acute lymphoblastic leukaemia

Sandra Wissmueller and Jacqueline M. Matthews
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Acute lymphoblastic leukaemia (ALL) is the most common form of childhood leukaemia, and also affects adolescents and adults. T-cell acute lymphoblastic leukaemia (T-ALL) accounts for 10-15 % of ALL and is characterized by the accumulation of immature T-lymphocytes. It has a worse prognosis than the common B-cell ALL. The transcription factor Tal-1 is overexpressed in more than 50 % of cases of T-ALL and is believed to trigger the onset of leukaemia by sequestering the E2A gene products, E12 and E47. These two proteins bind to DNA as homodimers, but can also form heterodimers with Tal-1 that preferentially target different DNA sequences to up- and down-regulate the expression of different genes. The inhibition of an interaction between Tal-1 and E2A would form a good starting point in the development of potential treatments of T-ALL. This type of inhibitor development requires a detailed characterization of the interaction, which forms the subject of this work. The specific aims of this project are 1. To characterize the interaction between Tal-1 and E2A proteins. 2. To investigate the molecular interaction of Tal-1/E2A heterodimers with DNA. 3. To assess the roles of Tal-1/E2A complexes in cells. 4. To determine the structure of a Tal-1/E2A/DNA complex. These experiments will provide new insights into the transcriptional regulatory network of Tal-1 and will be the first step in developing new treatments for T-ALL leukaemia.

poster presentation:

Tension caused by changes to the EAS line up

Paul Vukasin[1], Ingrid Macindoe[1], Matt Templeton[2], Joel Mackay[1], Ann Kwan[1] and Margie Sunde[1]

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Hydrophobins are a family of small hydrophobic proteins that play a vital role in growth and reproduction of filamentous fungi. These proteins are secreted as monomers and self-assemble into an amphipathic monolayer at hydrophilic-hydrophobic interfaces. This monolayer is composed of substructures known as rodlets, which reduces surface tension at air-water interfaces. In fungi, the rodlets coat structures such as spores and reverse the wettability of fungal surface structures. EAS is a hydrophobin from the fungus Neurospora Crassa and its structure has been solved by NMR. The monomer structure displays a distinct segregation of hydrophobic and charged residues onto opposing faces of the protein.

We have used the structure of EAS to identify residues on the charged and hydrophobic faces for mutagenesis studies, as these residues may be responsible for the proteins high surface activity. We will characterise the folding and structure of the EAS mutants by NMR and the polymerized mutant rodlets by electron microscopy and thioflavin T fluorescence binding assay. Surface activity will then be assessed using contact angle measurements. These studies will provide insights into the structural determinants of hydrophobin functions and allow us to engineer novel hydrophobin-based molecules with enhanced surface activity.

poster presentation:

Effect of heterogeneous nucleating agents in protein crystallization experiments

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In the field of protein structure determination by X-ray crystallography, the crystallization step is considered to be a major bottleneck. Successful crystallization requires both the formation of nuclei that are capable of supporting crystal growth, and a subsequent crystal growth thereon. In a typical protein crystallization experiment that uses a sparse matrix screening approach, crystal growth occurs spontaneously in a supersaturated solution due to the phenomenon of homogenous nucleation. However the sparse matrix crystal screens commonly used in these typical crystallization experiments are not sampled extensively for protein and precipitant concentrations, and as a result the suitable supersaturation conditions for nucleation are often missed. In this study, we have tested and employed the approach of heterogeneous nucleation, to try and increase the chances of protein crystallization by screening various heterogeneous nucleants and have seen that the heterogeneous nucleating agents have a positive effect in protein crystallization experiments.

poster presentation:

Molecular Analysis of the THAP Domain from C. elegans C-terminal binding protein (CtBP)

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Many eukaryotic proteins are composed of multiple domains, and one way to understand the functions of these proteins is to define the functions of their constituent domains. One protein domain for which we currently have only a limited understanding is the THAP (Thanatos-associated protein) domain. THAP domain containing proteins from Caenorhabditis elegans have been implicated in various biological processes such as vulval development, meiotic chromosome segregation and cell-cycle transitions. One such protein is the C-terminal binding protein (CtBP), a transcriptional co-repressor that is involved in development and oncogenesis. Work in our lab has demonstrated that the C. elegans CtBP THAP domain (CeCtBP-TD) binds to DNA containing a core AGTG motif. Furthermore, NMR spectroscopy has been used to determine the structure of CeCtBP-TD (1) and to localise its DNA-binding surface. Using this data, we mutated key surface residues that are likely to be involved in THAP-DNA interactions. The binding affinities of these mutants for DNA were then measured using fluorescence anisotropy. This enabled us to identify the specific residues on CeCtBP-TD that mediate its interaction with DNA. (1) Liew, C. K., Crossley, M., Mackay, J. P. and Nicholas, H. R. (2007). Solution Structure of the THAP Domain from Caenorhabditis elegans C-terminal Binding Protein (CtBP). Journal of Molecular Biology, 366: 382-390.

poster presentation:

Structure and stability of Pyrin PYD and mutations associated with autoinflammatory disease

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Pyrin domain (PYD)-containing proteins are key components of pathways that regulate inflammation, apoptosis and cytokine processing. Their importance is further evidenced by the consequences of mutations in these proteins that give rise to autoimmune and hyperinflammatory syndromes. Being the latest addition to the death domain superfamily, the PYD is the least well-characterised of all four subfamilies, which also includes the death domain, death effector domain and the caspase-recruitment domain. The death domain fold classically boasts six antiparallel helices in a Greek key architecture. These domains communicate via homotypic interactions and facilitate the assembly of multi-protein complexes to serve as molecular switches of signalling cascades. The PYD was first discovered in the MEFV gene encoding Pyrin, a protein implicated in the inflammatory process probably as a natural inhibitor. Amino acid substitutions R42W and A89T in the N-terminal PYD of Pyrin and several mutations identified in the C-terminal B30.2 domain have been associated with the onset of Familial Mediterranean Fever (FMF), an autoinflammatory disease characterised by recurrent bouts of fever and serositis. Interestingly, the R42W mutation occurs in the α2-α4 region where high sequence and structural variability has been observed for PYDs. Here, we present NMR and circular dichroism (CD) studies of the wild-type and FMF-associated forms of Pyrin PYD. A comparison of the structure and stability of these proteins using NMR and CD spectroscopy has revealed some new and interesting insights into PYD structure and the molecular basis of FMF.

poster presentation:

Structural studies of the second PHD finger from Mi2beta and its binding to histone tail

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Mi2beta is a protein found to be integral to the nucleosome remodeling and deacetylatase (NuRD) complex and in particular, the two PHD fingers (plant homeodomain) have been shown to be essential for the interaction between Mi2beta and the deacetylase component HDAC1. PHD fingers are small protein domain of ~100 residues characterised by a Cys_4-His-Cys_3 zinc-binding motif. These domains are commonly found in proteins involved in transcription and have an array of functions.
Recently, several studies of PHD fingers from a number of transcription factors have revealed that these domains can recognize histone tails with specific modifications. We therefore wondered if the PHD fingers of Mi2beta can also bind histones. Using NMR titration studies, we are able to show that the second PHD domain from Mi2beta (Mi2beta-P2) specifically recognizes a peptide corresponding to the histone H3 dimethylated at lysine 9 (H3K9me2). Here, the structure of Mi2beta-P2 alone and in complex with H3K9me2 will be presented and the similarities and differences in its mode of binding to other known PHD:histone peptide complexes will be discussed.

poster presentation:

The RNA-binding properties of RanBP2-type zinc fingers

Paula M. Vaz, Robyn E. Mansfield, Fionna E. Loughlin, Joel P. Mackay
School of Molecular and Microbial Biosciences, University of Sydney, NSW, 2006

In alternative splicing, the different exons that make up a pre mRNA are included in the final transcript as a result of the recognition of different sets of splice sites by the splicing machinery. Many proteins are involved in the regulation of 5' and 3' splice site selection, including the SR (Ser-Arg rich) family of proteins. ZNF265 is an SR-like protein comprising a C-terminal Ser-Arg rich domain and two RanBP2-type zinc finger domains at the N-terminus. Previous work suggests that this protein has a role in the regulation of splicing. This type of zinc finger is found in a variety of proteins that have differing functions and the fingers themselves are able to bind both proteins and nucleic acids. Recent work in our lab has shown that ZNF265 can bind single-stranded RNA containing an AGGUAA motif, and that the binding specificity is mediated by specific residues in the two RanBP2-type zinc fingers. Many of the residues identified by our lab to be essential in the RNA-binding interaction are conserved in the RanBP2 zinc finger of TLS, a protein that was originally identified as a component of a fusion protein present in a number of cancers. Like ZNF265, it has been implicated in splicing, as it both associates with splicing factors and regulates splicing in in vitro splicing assays. We have implemented SELEX and fluorescence anisotropy binding assays to analyse the interaction of the zinc fingers in these two proteins with RNA.

poster presentation:

The interaction of Myelin Transcription Factor-1 with DNA

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Myelin transcription factor-1 (Myt1) is a transcription factor expressed in the developing central nervous system (CNS); it activates the proteolipid protein (PLP) gene in Schwann cells and their precursors, leading to the production of the myelin sheath around axons. Myt1 contains 6-7 zinc fingers, depending on splicing, and these zinc fingers are known to recognize double stranded DNA. The target sequence (AAGTT) of Myt1 in the PLP promoter is known and we have shown that the fifth zinc finger (F5) of Myt1 can interact with this sequence. The structure of a F5:DNA complex has subsequently been calculated on the basis of NMR titration data, and in order to test the veracity of the structure, we constructed a series of point mutants in the F5 protein and tested their ability to bind DNA using surface plasmon resonance. Our data will contribute to a general understanding of how this unusual class of ZnF proteins recognizes DNA.

poster presentation:

Proteomic and structural characterization large cytoplasmic protein complexes from macrophages

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Until recently, tools for structural analysis (NMR, X-ray crystallography) have been limited to study of small proteins or the component parts of larger complexes. Recently the use of electron microscopy (EM) in combination with single particle analysis has opened the possibility of analyzing large cellular complexes. It is anticipated that such complexes will mediate essential cellular functions.
To determine the ultrastructure of such protein assemblies isolated from macrophage cytoplasm we have employed multiple strategies, particularly the combination of a rapid and as simple as possible biochemical purification of large complexes together with identification of their individual subunits by mass spectrometry, and finally, ultrastructural determination by EM and single particle analysis.
A cytoplasmic fraction was purified from the macrophage cell line RAW264 by nitrogen cavitation lysis followed by analytical sucrose gradient separation. Proteomic screening by peptide finger-print mass spectrometry (MALDI/MS) identified a list of novel candidate proteins involved in large protein assemblies, including CAD (trifunctional protein with the enzymatic activities during pyrimidine biosynthesis), CML66 (tumor-associated antigen), and p600 (retinoblastoma-binding protein). EM of negatively stained samples from selected fractions in combination with image analysis and 2D averaging allowed the identification of distinguishable classes of different “particles” (protein complexes).
The combination of different approaches for biochemical purification of large cytoplasmic protein complexes, for proteomic identification of individual proteins within the complex and the ultrastructure determination by electron microscopy and single particle analysis (negatively stained and frozen-hydrated samples) will provide insights into the structural organization of those complexes and their biological roles and functions.

poster presentation:

Dissecting the copper dependent redox behaviour of the Prion protein

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The prion protein (PrP) is an extracellular glycoprotein normally associated with the pre-synaptic membrane of neuronal cells. Misfolding of this protein has been shown to cause diseases such Creutzfeldt-Jacob disease in humans, bovine spongiform encephalitis in cows and scrapie in sheep. Accumulation of misfolded PrP to form amyloid plaques leads to significant neurodegeneration. Oxidative stress is a hallmark of prion diseases, and cells lacking PrP appear to be highly susceptible to reactive oxygen species. Oxidative damage is considered a major contributor to neuronal damage observed in PrP diseases. PrP is thought to act as a synaptic antioxidant and the fact that PrP is a cuproprotein is a major facet of any antioxidant ability. PrP can bind up to six Cu2+ ions in the unstructured N-terminal region between residues 51 and 111. Copper can redox cycle between Cu2+ and Cu+ at a biologically relevant potential and this cycling is important for removing reactive oxygen species. In this work we investigate the redox properties of peptides corresponding to the major copper binding regions. Using a range of electrochemical techniques such as redox potentiometry and voltammetry, along with circular dichroism and NMR spectroscopy we show that significant redox diversity exists within the PrP copper binding sites. Whereas some sites show pseudo-reversible Cu2+/+ redox activity, suggesting binding of Cu2+ and Cu+, other sites show only Cu2+ binding. Additionally, we have evidence that some sites can also bind Cu3+, and the alkene decomposition products formed may have significant implications for PrP folding and misfolding.

poster presentation:

Structural basis for the interplay between Vps26A and Vps26B incorporation into the retromer protein complex

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Retromer is a heteropentameric complex with important roles in endosomal membrane trafficking, most notably in the retrograde transport of lysosomal hydrolase receptors from endosomes to the Golgi. The core of retromer is comprised of three subunits Vps35, Vps29 and Vps26. In mammals there are two paralogues of the medium subunit Vps26, Vps26A and Vps26B, both of which have unknown functions. We find that Vps26A and Vps26B are incorporated into distinct retromer complexes in vitro and in vivo, and each binds to Vps35 with similar nanomolar affinity. We have determined the crystal structure of mouse Vps26B and show it to be composed of two β-sandwich sub-domains. Intriguingly Vps26 proteins share a striking and unexpected similarity to the arrestin family of proteins that regulate the signalling and endocytosis of G-protein coupled receptors. Using structure- based mutagenesis we show that both Vps26A and Vps26B are incorporated into retromer complexes via binding of Vps35 to a highly conserved region within the C-terminal sub-domain and that this interaction is required for endosomal recruitment of the proteins.

poster presentation:

Congenital heart disease caused by mutations in the transcription factor Tbx20

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Formation of the mammalian heart involves a precisely orchestrated series of molecular and morphogenic events and is regulated by cardiac transcription factors. Perturbations in this process can have a huge impact in the form of congenital heart disease. The recently discovered T-box transcription factor Tbx20 is one of these key cardiac control proteins. It is believed to function through multiple interactions with other regulatory proteins and target genes. Tbx20 has been shown to be essential for normal cardiovascular development. The developing heart is a primary site of Tbx20 expression but Tbx20 mRNA is also observed in the adult mouse heart, where it has a key role in adult cardiac function. Richard Harvey and colleagues have recently found mutations in Tbx20 that are associated with atrial or ventricular septal defects and other cardiac anomalies. We have characterised the effect of mutations within the T-box DNA binding domain of human TBX20 that are associated with a family history of congenital heart disease. We have analysed the structure and stability of the mutant domains and measured the affinity of the mutant T-box domains for the T-box DNA binding site. The I152M mutation results in reduced affinity for the DNA binding site, while the Q195X and T209I mutations appear to destabilise the domain. Our findings are the first to link TBX20 mutations to human disease.

poster presentation:

Structure and function of the death effector domain is dependent on a conserved “charge triad”

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Death effector domains (DEDs) and other members of the death domain superfamily are characterised by a highly conserved six helix bundle fold, and typically form homotypic interactions with structurally similar binding partners. The importance of DED-containing proteins is highlighted by their involvement in key cellular processes including apoptosis and cell proliferation. As a result, intense efforts have been directed at understanding their structure and molecular mechanisms of interaction. Despite a similar three-dimensional structure, the amino acid sequence composition of DEDs is highly variable. A notable exception is the presence of an RxDL motif in helix 6 and a conserved acidic residue in helix 2. Structural studies of the DEDs of PEA-15 and the viral caspase inhibitor MC159 have revealed that these three conserved charged residues form a salt bridge network. This “charge triad” is likely to play a vital role in both function and structural stability as it links two functionally important regions of the DED. To further investigate the role of the charge triad, we introduced several point mutations into the PEA-15 DED to disrupt sections of the salt bridge. The structural integrity, stability and activity of the mutants were compared with the wild-type protein using CD and NMR spectroscopy, guanidinium chloride- and urea-induced denaturation and functional assays. Mutation of any part of the charge triad dramatically reduced the stability of PEA-15 and consequently impaired its function. In contrast to suggestions of a non-structural role [J. Biol. Chem. (2006) 281: 2960], our results indicate that the charge triad found in most DEDs makes an important contribution to protein stability.