Poster 1

Experimental Data Tracking at the PSI Structural Biology Knowledgebase

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Experimental Data Tracking is one of the features available through the PSI Structural Biology Knowledgebase (SBKB: http://sbkb.org) that outlines the current status of projects among the various structural genomics efforts. The resource consists of two databases: a target registration database (TargetDB: http://targetdb.sbkb.org), and an expanded target tracking database that includes experimental protein production trial histories and protocols (PepcDB: Protein expression, purification, and crystallization DataBase http://pepcdb.sbkb.org). To date, the two databases hold over 270,000 structure targets and 750 experimental protocols. The main purpose of TargetDB is to provide information about the experimental status of protein targets from structural biology centers worldwide, including the four high-throughput centers, nine membrane protein centers, and ten biology partnership groups comprising the current PSI:Biology network of investigators. The database can be searched by target identifier, contributing center name, experimental status, protein sequence, and accession numbers of popular databases including GenBank, PDB, PFAM, and UniProt. PepcDB expands the information provided by TargetDB for each target with detailed descriptions of protein production experiments and the protocols used, such as for cloning, expression, and purification. PepcDB query options include searching by protocol keywords, experimental status (successful or failed), experiment type, or special target categories such as biomedical, membrane protein, and community nominated. The database query and report options make the TargetDB and PepcDB useful tools for experimental design and data mining, and are two of the data elements returned when performing a protein sequence or PDB ID search on the SBKB. Both searches will return any related target and protocol information related to the sequence or PDB ID used in the query. Going forward in PSI:Biology, these two distinct target tracking databases and web portals will be merged to provide both services in one location. Included in the merger will be an update to the data collected, such as better support for complex structures and including cryoEM related experimental status values. Also planned for the new service is a web interface to allow for small scale target registration. Funding is provided by the NIGMS

Poster 2

X8 PROSPECTOR: A Maintenance-Free X-ray System for Macromolecular Crystallography

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The number of life science investigators utilizing crystallography increases every year. Synchrotrons are an invaluable resource for structural work but not everyone has unlimited access to a beamline. Having a highly reliable in-house diffraction system to characterize samples and collect data can increase the productivity of any lab. Until recently, the standard choice for an in-house X-ray source was a rotating anode generator which provides high performance but requires a significant amount of maintenance resulting in higher costs and down-time. The BRUKER X8 PROSPECTOR utilizes an Incoatec Microfocus Source (IìSTM) and a high sensitivity APEX II CCD to provide a very low maintenance, high performance system that can be used to collect data on a wide range of protein crystals. A description of the X8 PROSPECTOR and experimental results will be discussed.

Poster 3

xtalPiMS: a tool for managing your crystallization experiments

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CrystalPiMS (xtalPiMS) is a tool for managing crystallization experiments, and in particular the large numbers of images that can be produced by automated imaging systems. xtalPiMS is built on top of the Protein Information Management System (PiMS), a laboratory information management system for protein production. xtalPiMS groups images taken from particular plates at particular times into "inspections". It adds specialized and intuitive interfaces to PiMS for finding plates and inspections, and a viewer that allows scientists to rapidly scan through the set of images in an inspection. Each image can be scored from a drop-down menu or using keyboard shortcuts. xtalPiMS is optimized for 96-well plates but is capable of handling plates of different sizes and also multiple crystallization trials per well. xtalPiMS is installed in the Oxford Protein Production Facility (OPPF) and York Structural Biology Laboratory, where it has been integrated with automated imaging systems from Formulatrix, Veeco and BioTom. xtalPiMS manages >72,000,000 crystal trial images from > 33,000 trial plates in the OPPF. xtalPiMS underpins the OPPF's new facilities in the Research Complex at Harwell, adjacent to Diamond. Like PiMS, xtalPiMS is a web-based application that runs on widely-available open source software. xtalPiMS is accessed using a web browser, typically requiring no additional software to be installed on the client. xtalPiMS is available under the same terms as PiMS, including to licensed users of the CCP4 suite. To try xtalPiMS, visit http://www.pims-lims.org:8080/xtalpims/ (user name "demo", password "demo").

Poster 4

Comprehensive annotation of protein domain boundaries

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The choice of protein domain boundaries for expression constructs can strongly impact the ability of proteins to be soluble and crystallize. Computational approaches to infer optimal domain boundaries for expression can be unreliable and frought with uncertainty. On the other hand, existing experimental approaches are difficult to apply with sufficient resolution on a genome-wide scale. We show that split GFP measurement of soluble protein at the level of single cells, coupled with flow sorting and sequencing, promises to provide the needed quantitation, depth, and coverage to rapidly identify protein domain boundaries. Throughput is high enough and cost low enough to make the process economical for large ORF collections, cDNA libraries, and whole genomes.

Poster 5

Using the LabDB crystallographic laboratory information management system with laboratory hardware and biochemical experiments

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The LabDB LIMS (laboratory information management system) is a component-based, database-driven tool for tracking crystallographic experiments from cloning to structure solution. LabDB is widely used by several structural genomics projects to track tens of thousands of proteins, including the Center for Structural Genomics of Infectious Diseases, the New York Structural Genomics Research Consortium, the Midwest Center for Structural Genomics, and the Enzyme Function Initiative. LabDB now interacts with the Minstrel HT crystallization observation robot (Rigaku Americas) to automatically extract crystallization data (images and annotations). LabDB also integrates with the AKTA family of liquid chromatography systems (GE Healthcare) to record chromatograms and samples collected. We are expanding the interface to work with mobile touchscreen devices such as Android and iPad tablets. Most significantly, the newest modules of LabDB now track functional experiments on proteins, such as kinetic assays. These tools make it possible to associate functional experiments with crystallographic work, which is critical in a high-throughput pipeline for selecting ligands for co-crystallization and soaking experiments.

Poster 6

Crystal freezing quenches protein motions and biases hydrophobic packing

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Over the last 30 years, data collection for macromolecular X-ray crystallography has almost completely transitioned from at room temperature (273-298K) at low-power sources to cryogenic temperatures (90-100K) at brilliant synchrotron beamlines. Although cryopreservation is an invaluable tool for limiting radiation damage when determining structures, few studies have examined how the freezing process might bias the functional interpretation of structural results. Previously, we found that Ringer sampling of room temperature, but not cryogenic, electron density revealed a network of alternative side chain conformations essential for catalysis the proline isomerase CypA. Motivated by this temperature-dependent difference, we compared 30 proteins with both high-resolution room and cryogenic temperature datasets using newly developed tools to evaluate protein core packing, refine multiple conformations, and sample electron density in real space. Our results confirm that freezing remodels the crystal lattice and reduces protein volume. Surprisingly, freezing also artificially drives improved packing and changes the distribution of side chain conformations in both exposed and buried regions. As with CypA, we find that temperature-sensitive networks of alternative side chain conformations are correlated with chemical exchange detected by NMR experiments. For example, in H-Ras electron density sampling discovers an intrinsic allosteric side chain network, supported by NMR data, in the room temperature, but not cryogenic, electron density maps. Accounting for how cryogenic X-ray data collection compresses proteins and quenches internal fluctuations may lead to improvements in protein structure prediction, functional assignment, and design.

Poster 7

Understanding Signaling Networks through the Covariance Analysis of NMR Chemical Shifts

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Allostery is a fundamental mechanism of regulation in biology and it is critical for the control of signaling networks. The residues at the end points of long-range allosteric perturbations are commonly identified by the comparative analyses of structures and dynamics in apo and effector-bound states. However, the networks of interactions mediating the propagation of allosteric signals between the end points often remain elusive. We will show that the covariance analysis of NMR chemical shift changes caused by a set of covalently modified analogs of the allosteric effector (i.e. agonists and antagonists) reveals extended networks of coupled residues. Unexpectedly, such networks reach not only sites subject to effector-dependent structural variations, but also regions that are controlled by dynamically driven allostery. In these regions the allosteric signal is propagated mainly by dynamic rather than structural modulations, which result in subtle but highly correlated chemical shift variations. The proposed chemical shift covariance analysis (CHESCA) [1] identifies inter-residue correlations based on the combination of agglomerative clustering (AC) and singular value decomposition (SVD) [2]. AC results in dendrograms that define functional clusters of coupled residues, while SVD generates score plots that provide a residue-specific dissection of the contributions to binding and allostery. The CHESCA approach was validated by applying it to the cAMP-binding domain of the exchange protein activated by cAMP (EPAC) and the CHESCA results are in full agreement with independent mutational data on EPAC activation. Overall, CHESCA is a generally applicable method that utilizes a selected chemical library of effector analogs to quantitatively decode the binding and allosteric information content embedded in chemical shift changes.

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Poster 8

SAXS - A tool for gleaning structural information when conventional methods aren't enough

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X-ray crystallography is a fundamental technique for obtaining atomic resolution structural information for macromolecules. However the requirement for diffraction quality crystals often limits its efficacy for studying protein complexes or systems with inherent structural disorder. In such cases where crystallographic methods have failed, small angle X-ray scattering (SAXS) provides a complementary tool for extracting structural information from biological systems. In particular, SAXS proves ideal for studying partially disordered macromolecules, for monitoring structural changes in response to environmental perturbations and for monitoring conformational changes due to ligand binding. It also provides a useful tool for probing for the "crystallization slot". Here we introduce the first commercial system specifically designed for high-throughput solution scattering experiments with macromolecules, the BioSAXS-10001. Comprised of specially designed focusing optics and a Kratky block, the BioSAXS-1000 system eliminates smearing issues common to traditional Kratky cameras. The system includes a high sensitivity pixel array detector and intuitive data collection software that includes full automation of the Kratky alignment hardware. Together, the many features of the BioSAXS-1000 system allow for synchrotron-quality SAXS data from a home laboratory source.

Poster 9

A New Method for Epitaxial Growth of Protein Crystals

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A device was invented for protein crystallization by sandwiching the liquid droplet between two surfaces, in which the hydrophilic surfaces can be used as crystallization substrates. It was applied to the epitaxial growth of protein crystals on mica surface. The epitaxial growth of trichosanthin (TCS) crystals on mica surface was observed and was identified by X-ray crystallography. We demonstrated for the first time the epitaxial growth of the TCS crystal on a hydrophilic mica surface with a precise match of the lattices and molecular structures. It was found that the metal ions K+ played a critical role in inducing the epitaxial crystal growth of TCS on mica surface, by binding both the TCS and the mica substrate. The experimental results provide new insights into the molecular mechanism of epitaxial growth and the epitaxial growth induced by metal ions may become a new method for the epitaxial crystal growth of proteins and other organic molecules.

Poster 10

Parallel approaches towards structural characterisation of lysosomal degradation network

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Lysosomes are organelles central to degradation and recycling processes in human cells. They contain over 40 acid hydrolases (proteases, lipases, glycosidases) that degrade endocytosed macromolecular substrates in a highly regulated manner. Inside the lysosomes hydrolases does not function as an individual enzyme but are usually organized in a complicated networks and/or cascades. Different enzymes may compete for the same substrate. A combination of different enzymes may exhibit synergistic or antagonistic affect on the degradation pattern of a single substrate or a mixture of different substrates. Recent progress in development of high-throughput (HTP) techniques for following multi-step kinetic events using mass spectrometry has enabled us to monitor relationships between enzymes and substrates in a multi-component systems usually found inside the lysosomes. However a major bottleneck still remains the availability of sufficient amounts of proteins needed for such studies. We have decided to develop a platform dedicated to the expression and characterization of lysosomal hydrolytic enzymes and Staphylococcal antigens as model substrates. Standardized cloning and expression protocols enable parallel cloning and small-scale expression analysis of several tens of protein targets using E.coli, insect cell and mammalian expression systems. In the first round approximately 50 human targets, mostly lysosomal hydrolases and 30 microbial antigens were selected, cloned and expressed at different scale. Highly automated two step purification scheme allows rapid purification of sufficient amounts of crystallization-quality proteins like cathepsin C, legumain, lysosomal chitobiase, alpha fucosidase 1, lysosomal neuroaminidase, acid ceramidase and different Staphylococcal antigens. The platform provides a solid foundation for further structural and biochemical characterization of molecular pathways inside the lysosome.

Poster 11

PSPF - A protein sample production facility for structural biologists

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The Protein Sample Production Facility (PSPF) was established in 2007 as the result of an international review of the major bottlenecks and needs for the structural biologists within the Helmholtz Association. The platform is dedicated to develop and provide infrastructure for the production of biomacromolecules for structural biology research. The decentralized facility is shared between the Helmholtz Centre for Infection Research (HZI) in Braunschweig and the Max-Delbrück-Center (MDC) in Berlin. Since 2010, the PSPF is additionally integrated within the ESFRI project INSTRUCT, providing infrastructure and expertise in the field of sample production for the whole European structural biology community. The PSPF dedicates fifty percent of its resources to cooperation and offers services for researchers at German and European academic institutions to produce proteins for subsequent structural analysis by X-ray crystallography, NMR spectroscopy and electron microscopy. The PSPF also trains external researches in its laboratories. Core activity of the PSPF at the MDC is high-throughput protein production in E. coli including construct design, cloning, expression and solubility testing, and protein purification. Additionally, crystallization and structure determination is offered. At the HZI, large scale protein sample production in eukaryotic expression systems such as yeast, baculovirus/insect cells and mammalian cells is performed, including labeled proteins. Furthermore, a junior research group is focussing on the development of new and fast strategies for creating stable mammalian expression cell lines. The PSPF currently accepts the submission of research proposals (http://www.pspf.de). All projects will be reviewed by a scientific committee prior acceptance.

Poster 12

Production of recombinant human multi-protein transcription factor complexes

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The production of sufficient quantities of homogenous protein is an essential prelude not only for structural investigations but represents a rate limiting step for many human functional studies. We present here our strategies for the production of multi-subunit human transcription factors such as nuclear hormone receptor complexes or the basal transcription/DNA repair factor TFIIH using the baculovirus expression system. Selected examples illustrate recent developments and their impact on the structural biology of human protein: (i) HTP mini expression screening, (ii) use of fluorescent proteins as makers and for quality control (iii) vector development for parallel cloning and (co-)expression of multiple constructs for a single target, (iv) single virus co-expression of multi-subunit complexes. Aricescu, R. et al (2006) Eukaryotic expression: developments for structural proteomics.Acta Crystallogr.D.Biol.Crystallogr. 1114-1124. Abdulrahman W et al. A set of baculovirus transfer vectors for screening of affinity tags and parallel expression strategies.Anal Biochem. Anal Biochem. 2009 Feb 15;385(2):383-5. Fouillen L et al. Analysis of recombinant phosphoprotein complexes with complementary mass spectrometry approaches. 2010 Anal Biochem. 2010 Dec 1;407(1):34-43. Abdulrahman W et al. Pipeline approaches for multiprotein complex expression using the baculovirus/insect cell expression system In prep.

Poster 13

A high-throughput immobilized bead screen for stable proteins and multi-protein complexes

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We describe an in vitro, colony screen for E. coli expressing soluble proteins and stable, assembled multiprotein complexes. Proteins with an N-terminal 6-His tag and C-terminal GFP S11 tag are fluorescently labeled in situ by complementation with a GFP 1-10 detector fragment. After partial colony lysis, soluble proteins diffuse through a supporting filtration membrane and are captured on affinity beads immobilized in agarose. Images of the fluorescent colonies and beads are used to guide the selection of optima. After the assay, colonies retain sufficient viability for picking and propagation, eliminating the need to make replica plates. We show that the assay can be used to identify full-length domains in a gene with no a priori knowledge of the domain's boundaries. To demonstrate this, we screened a randomly fragmented library of the p85 gene for the full length BCR domain. The assay also clearly distinguished clones expressing soluble, stable multi-protein complexes from those that are soluble, but unstable due to missing subunits. A library of fully assembled E. coli YheNML and non assembled YheML was used to demonstrate this application.

Poster 14

Protein production as an entry point for human biology: the future of structural genomics?

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Genomic sequencing has delivered a view of the entire complement of human genes and non-coding sequences, and enabled genome-wide studies of germ-line and somatic variation associated with disease, as well as mapping expression patterns and regulatory networks. Yet, this avalanche of data has not driven more detailed research into new proteins; in fact, recent scientific literature is disproportionately focussed on targets identified before the genome era (e.g. 65% of papers from 2009 refer to 50 of the 530 protein kinases in the genome that were already heavily studied in the 1990s). This is at least partly the result of a lack of powerful reagents to study the function of most newly prioritized genes. The primary goal of the Structural Genomics Consortium (SGC) was to produce high-quality structural data of >1000 human disease-related proteins for immediate deposition in the public domain. An important additional outcome has been the production of an even larger number of recombinant human proteins whose structure has not yet been solved. This compendium of proteins provides a key resource for initiating research into new targets of interest. Utilization of this resource by the SGC has involved establishing a wide network of collaborations involving both individual proteins and strategic initiatives. The latter include a pilot study for systematic generation of affinity reagents (monoclonal and recombinant antibodies) and the development of small-molecule chemical probes of chromatin-interacting proteins. Here we present a pilot study aiming to effectively generate purified protein domains of new targets selected based on disease relevance. We apply the package of informatic analysis, expression systems and vectors to quickly identify the best route to produce each protein with yield and purity suitable for antibody generation and further research. The results exemplify a possible mode of research whereby proteins are generated to promote research into new targets by a variety of routes, not limited to structure determination.

Poster 15

Nanobodies with G protein like properties stabilize a G Protein-Coupled Receptor Active State

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The complex behavior of GPCRs in respons to natural or synthetic ligands and proteins can be attributed to the receptor's structural plasticity manifested in multiple functionally distinct conformational states. Obtaining crystals of GPCRs in an agonist bound, active-state has proven to be challenging due to the instability of this state in the absence of G protein. Here we describe the generation of camelid single-domain antibody fragments (nanobodies) that have G protein like properties towards the human β2 adrenergic receptor (β2AR). To generate receptor specific nanobodies, a llama was immunized with purified agonist bound β2AR reconstituted at high density into phospholipid vesicles. A library of nanobody clones was generated and screened against agonist bound β2AR. Seven clones were identified that recognize the agonist bound but not the inverse agonist bound receptor. One of these nanobodies was found to increase the affinity of β2AR for agonists 30-fold and to induce conformational changes at TM6, two effects that are indistinguishable from those observed upon adding G protein to the receptor. This nanobody that faithfully mimics the effects of Gs binding was used to obtain diffraction quality crystals and to solve the first structure of an active agonist-bound state of the human β2 adrenergic receptor.

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Poster 16

A Versatile and Efficient High-Throughput Cloning Tool for Structural Biology

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Methods for the cloning of large numbers of open reading frames into expression vectors are of critical importance for challenging structural biology projects. Here we describe a system termed fragment exchange (FX) cloning that facilitates the high-throughput generation of expression constructs. The method is based on a class IIS restriction enzyme and negative selection markers. FX cloning combines attractive features of established recombination- and ligation-independent cloning methods: It allows the straightforward transfer of an open reading frame into a variety of expression vectors and is highly efficient and very economic in its use. In addition, FX cloning avoids the common but undesirable feature of significantly extending target open reading frames with cloning related sequences, as it leaves a minimal seam of only a single extra amino acid to either side of the protein. The method has proven to be very robust and suitable for all common pro- and eukaryotic expression systems. It considerably speeds up the generation of expression constructs compared to traditional methods and thus facilitates a broader expression screening.

Poster 17

Approaches to promote crystallization of challenging antigen/antibody complexes: Crystallization of the quaternary complex of TLR3 ECD with three non-competing Fabs

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The mechanism of action of therapeutic antibodies can be elucidated from the three-dimensional crystal structures of their complexes with antigens, but crystallization remains the primary bottleneck to structure determination. We present strategies for the successful crystallization of TLR3 ECD in complex with three non-competing anti-TLR3 antibody Fab fragments. We found that fine purification of the quaternary complex of TLR3 with three Fab fragments combined with microseed-matrix screening and additive screening promoted crystal formation and allowed the structure determination of the 250 kDa complex and thus define the epitopes and paratopes of the respective Fabs. Fine purification entailed the application of a very shallow gradient in anion exchange chromatography, resulting in the resolution of two separate complex peaks, which had different crystallizability. We found that both seeding and use of additives was essential to generating diffraction-quality crystals. We also propose that co-crystallization with multiple non-competing Fab fragments, when available, may be a viable path when an antigen complex with a single Fab proves to be recalcitrant to crystallization.

Poster 18

Predicting success of protein solubility in heterologous expression systems using PROSO II web server

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Many fields of science (structural biology, protein biochemistry and biophysics etc.) and industry (enzymes and antibodies production etc.) depend on efficient production of active protein using heterologous expression in Escherichia coli. Protein solubility upon expression is an individual trait of proteins which, under a given set of experimental conditions, is determined by their amino acid sequence. Prediction of solubility from sequence is instrumental for many application including setting priorities on targets in large-scale proteomics projects, optimizing efficiency of protein production etc. Our aim was to build a server predicting which proteins have the best/worst chances to be soluble once heterologously expressed in Escherichia coli. We present a machine-learning based model called PROSO II to assess the chance of a protein to be soluble upon heterologous expression in Escherichia coli. The classification algorithm is based on protein a k-mer frequencies and sequence similarity. It was organized as a two-layered structure in which the output of a primary logistic classifier for composition and a Parzen window model for sequence similarity serves as input for a second level logistic classifier. Experimental progress information from the pepcDB database and the PDB were used as the source of data for training and evaluation. In comparison with previously published methods we built our model based on currently the biggest dataset consisting of 82000 proteins. As estimated by 10-fold cross-validation our method achieved an accuracy =71%, area under Receiver Operating Characteristic curve =0.785 , MCC (Matthew's correlation coefficient) =0.421, sensitivity =0.754, specificity =0.666, precision (soluble)=0.693, gain (soluble)=1.383, precision (insoluble)=0.73 and gain (insoluble)=1.46. When tested on the separate holdout set not used at any point of method development our server attained the following performance values: accuracy =75.4%, MCC =0.39, sensitivity =0.731, specificity =0.759, precision (soluble)=0.377, gain (soluble)=2.263, precision (insoluble)=0.934 and gain (insoluble)=1.121. We believe that our server is unique in providing reliable solubility predictions based on the largest currently available experimental dataset and cutting edge machine learning technologies. PROSO II web-site is provided at: http://mips.helmholtz-muenchen.de/prosoII/prosoII.seam

Poster 19

An old topic: the mechanism of protein folding and unfolding

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We have determined a series of structures of a protein at present of varieties of urea concentrations: 2.4M, 4.0M, 5.6M, 7.2M, and 8.3M. We found 105 individual urea molecule binds to the protein through one mode of interaction: Hydrogen bond. Further urea competing experiments also showed hydrogen bonds play critical roles in the stabilization of tertiary structure of proteins. We conclude that the disruption of hydrogen bonds is the main reason that urea unfolds protein. The importance of hydrogen bonds in the protein structure leads us to a series of protein folding experiments starting from native form samples and inclusion bodies. Our data showed that proteins could be refolded in unique condition. We are trying to derive a general protocol to refold all proteins. I hope that our results may solve one of the bottlenecks in the field: protein production. From critical factors that lead to the successful protein folding, we are trying to derive the main driving force in protein folding so as to the general protein folding mechanism. We found that protein folding seems follow a very simple pathway.

Poster 20

Salvage and Storage of Previously Uncrystallized Infectious Disease Protein Targets in the SSGCID High Throughput Crystallization Pathway Using Microfluidics

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Emerald BioSystems, Emerald BioStructures

The MPCS Plug Maker is a microcapillary-based protein crystallization system for generating diffraction-ready crystals from nanovolumes of protein. Crystallization screening using the Plug Maker was used as a salvage pathway for retired proteins from the Seattle Structural Genomics Center for Infectious Disease (SSGCID) in an attempt to salvage high quality diffraction and crystallization leads. These screens generated successful results in spite of previously unsuccessful standard screening and a “hybrid” screening with a screen of only 16 crystallants Further, the CrystalCards used to store the crystallization experiments set up by the Plug Maker are shown be a viable container for long-term storage of protein crystals without discernable loss of diffraction quality with time. Use of the Plug Maker with SSGCID proteins is demonstrated to be an effective crystal salvage and storage method.

Poster 21

Expression strategies for protein complex production

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It is well known that many vital biological functions carried out by the cell are more often performed by multiprotein complexes rather than by single proteins. A major challenge in the field of structural biology is to obtain atomic resolution of proteins in the context of the multiprotein assemblies whereby they carry out their task. Protein complexes, especially those found in eukaryotic hosts, tend to be present only in very low quantities in the cell. Since relatively large quantities of homogeneous material are required for analysis by biophysical methods such as X-ray crystallography, heterologous overproduction is often necessary. In our group, we have developed the ACEMBL and MultiBac baculovirus expression systems to recombinantly produce multiprotein complexes in either E. coli or insect cells. We also addressed parallelization and automation of gene assembly for multiprotein complex expression by developing robotic routines for multigene vector generation. We introduced several improvements of baculovirus expression system performance including modifications of transfer plasmids, methods for generating composite multigene baculoviral DNA and simplified and standardized expression procedures. In order to address the problem of stoichiometrically imbalanced subunits, we developed a polyprotein expression strategy for producing multiprotein complexes. Together, these developments provide a useful toolbox for tackling challenging protein complexes.

Poster 22

Large-scale Production of Soluble Proteins in Wheat Germ Cell Free (WGCF) Systems as Rescue Paths for Structural Genomics Projects for Infectious Disease Drug Targets

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The goal for the Seattle Structural Genomics Center for Infectious Diseases (SSGCID) is to determine protein structures from NIAID Category A, B, and C agents, as well as the emerging and re-emerging infectious disease organisms. The SSGCID pipeline follows a multi-pronged serial escalation approach that supports the application of alternative strategies for rescuing targets that has failed the initial structure determination efforts. Cell free expression has been widely used in structural genomic studies in recent years. The wheat germ cell-free system has advantages in large scale protein productions for many applications including structural genomics projects. Two types of systems from Cell Free Sciences were employed for protein production, the Protemist DT II and Protemist XE. However, high expression yield often caused proteins to form aggregates during the production stage. NV10, a linear carbohydrate polymer with a molecular weight of 5 kDa, was used to in the translational mixture. It had enhanced the solubility of various protein targets in the soluble form. Efforts in combination of using the Protemist XE protein synthesis robot with polymer reagent to achieve soluble proteins in a rescue path for bacterial expression system will be discussed. (This research was funded under Contract HHSN272200700057C from the National Institute of Allergy and Infectious Diseases, National Institutes of Health - Department of Health and Human Services.)

Poster 23

Designing and creating custom optimization screens using the E-Screen Builder and the Opti Matrix Maker desktop instrument

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Emerald BioSystems, Emerald BioStructures

Designing crystallization screens is a task all crystallographers must perform, but generating the conditions and pipetting is labor-intensive and time consuming. A free online software module has been created that does the work of designing optimization screens for commercial screens from Emerald BioSystems and other vendors. Select the conditon to optimize and the software automatically generates the formulations for a 96-well optimization screen for protein crystallization. You can change the components and choose variations with a few simple clicks. The resulting formulations can be used in several ways: a) download the condition information in spreadsheet form at no cost, b) order a 96-well screen online from Emerald BioSystems, c) transfer the information to the new Opti Matrix Maker instrument to create any screen containing up to 10 stock solutions right on your lab bench. This tool is called the E-Screen Builder and is located at http://www.emeraldbiosystems.com/escreentoolkit/escreen.html.

Poster 24

Molecular tools for facilitating multi-gene manipulations and protein expression

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Molecular manipulations, including DNA cloning and mutagenesis are basic tools used on a routine basis in many individual laboratories as well as in structural genomics centers. Over the last decade new methodologies have emerged that facilitated DNA manipulations. Efficient and new developments are required for maintaining robust pipelines, and are a prerequisite to any heterologous expression system. Ligation Independent Cloning (LIC) techniques were developed and replaced the classical Ligation Dependent Cloning (LDC) platform. In recent years, we have adapted, at the Israel Structural Proteomics Center (ISPC), the Restriction Free (RF) cloning platform, an LIC strategy based on previously described principles of foreign DNA integration into an expression vector by whole-plasmid amplification of the insert and the plasmid. We developed new applications for DNA cloning and mutagenesis based on the RF cloning platform. The new applications include simultaneous cloning of multi-DNA fragments into distinct positions within an expression vector, simultaneous multi-component assembly, and parallel cloning of the same PCR product into series of expression vectors. In addition, we have expanded the applications for multiple alterations of the target DNA, including simultaneous multiple-site mutagenesis and simultaneous introduction of deletions and insertions at different positions. We demonstrate the usefulness and robustness of the new applications for facilitating protein engineering and expression of recombinant proteins including for therapeutic applications.

Poster 25

Random microseeding: a theoretical and practical exploration of the Microseed Matrix Screening (MMS) method, with new recommendations for achieving crystallization success.

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Douglas Instruments has completed theoretical and practical studies of Microseed Matrix Screening (MMS), a powerful approach to protein crystallization, introduced by Ireton and Stoddard in 2004 [1]. The method was automated by D'Arcy et al. [2], who first used seeding with random screening kits. Experience shows that MMS with random screens generates more hits or better-diffracting crystals in about 75% of cases where at least one crystal can be obtained. Our studies included analysis of the stability of seed crystals suspended in unconventional stock solutions and the effectiveness of seed crystals from novel sources. This has given rise to new recommendations for maximizing the number of hits, reducing the prevalence of salt crystals, increasing the diversity of crystal forms, and harvesting seed crystals from microfluidic devices. We also investigated nucleation with microporous glass, zeolites, precipitates and mixtures of crushed crystals of proteins that were unrelated to the target protein. These were less effective than conventional MMS, but may still be useful - they can of course be used before the first hits have been found. Throughout the project, the statistical significance of experiments was increased by focusing on "pregnant" conditions - defined as conditions that reliably gave crystals when seeds were present, but which otherwise gave no crystallization.

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Poster 26

Automated Ligand Identification in PHENIX

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Automated interpretation of unknown electron densities of small-molecule ligands is a crucial step towards high-throughput protein crystallography. We have developed a computational program to help achieve this task under the PHENIX[1] framework. This program first attempts to fit a library of ligands to the unknown density of interest using the Resolve ligand fitting algorithm [2]. The fitting results are scored, ranked, and presented to the user. The scoring algorithm takes into consideration of density correlations as well as non-bonded interactions between fitted ligand and the input protein model. A default ligand library of the 180 most commonly observed ligands from the Protein Data Bank is automatically used when no information of the potential ligands is provided. When users input biochemical information related to the ligands or the protein molecules, custom ligand libraries are generated on the fly for the subsequent fitting and ranking processes. This program also takes advantage of multi-thread computational environment. Examples and results will be presented.

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Poster 27

ACMI: Towards Automatic Crystallographic Map Interpretation at Low Resolution

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One bottleneck in high-throughput protein crystallography is interpreting an initial error-prone or low-resolution electron-density map. This activity is particularly time-consuming at resolutions worse than about 3 Å. We have developed ACMI (Automatic Crystallographic Map Interpreter), with a new algorithm that uses a probabilistic model to infer an accurate protein backbone layout and uses particle filtering, a sampling technique, to produce a set of all-atom protein models. We have recently extended the method to utilize supplemental information, including bioinformatics predictions of disordered regions and use of direct searches for secondary structural elements in the electron density map. We have tested our algorithm on a number of poor-quality experimental density maps. We show that our approach generally produces a more accurate model than three leading methods—Textal, Resolve and ARP/WARP—in terms of main chain completeness, sidechain identification and crystallographic R factor on initial stages of refinement. Source code and experimental density maps available at www.cs.wisc.edu/acmi This work supported by NIH PSI GM074901, NLM LM-008796 and NLM training grant T15 LM007359.

Poster 28

The practical aspects of sulfur phasing using a chromium anode X-ray source

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The number of structures solved by a single-wavelength anomalous scattering (SAD) has increased about 100-fold during the past 10 years. Many advancements in detector technology, experimental protocols, data reduction and structure determination software have been critical for this growth. Despite that progress, structure determination using single-wavelength anomalous scattering of sulfur atoms (S-SAD) is still a considerable challenge, as the total number of novel structures solved by S-SAD is only a small fraction of all SAD structures. The use of longer X-ray wavelengths enhances the sulfur anomalous signal but at the same time increases the degree of absorption effects. Thus the use of 2.29 Å wavelength radiation generated by a Cr rotating anode, combined with newly developed absorption correction algorithms, has led to the determination of a significant number of novel structures. To broadly explore the feasibility of this approach, we analyzed diffraction data of 22 different proteins and attempted to solve each by S-SAD technique. The selected data represent a broad range of crystals; they varied by space group, diffraction limit, diffraction quality and solvent content. Of the 22 data sets collected, 15 structures were solved. We will present and discuss some unique strategies that were used to determine these structures, as well as the possibilities for general guidelines for sulfur SAD phasing experiments.

Poster 29

Iodide Phasing in a Structural Genomics Setting

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SSGCID; Emerald BioStructures and Seattle BioMed

The Seattle Structural Genomics Center for Infectious Disease (SSGCID) focuses on the structure elucidation of potential drug targets from infectious disease organisms. Most SSGCID targets have homologs in other organisms that are validated drug targets, and for which structures are known. Thus, the majority of SSGCID targets may be solved by molecular replacement (MR). Reflective of this, all proteins are expressed in native form. However, targets that have a conformational flexibility, e.g. kinases, or targets without a homologous structure, e.g. targets requested by the community, require experimental determination of phases. Anomalous dispersion experiments using selenomethionine labeled protein currently is the most common route for de novo structure determination. However, this method requires labor-intensive expression and purification of labeled protein for which crystallization conditions frequently have to be re-optimized. For SSGCID, the first approach for de novo phasing of protein structures has become the iodide soak. For this technique, existing native crystals or at least available native protein can be used. At CuKα wavelength, the anomalous signal of iodide is stronger than the anomalous signal of Se at its peak wavelength. Despite its simplicity the method works convincingly well: since the implementation of the technique in November 2009, we could determine amost 20 structures this way. In our data analysis pipeline, we frequently can generate an initial model within hours after data collection. Here we present our practical and computational methodology for in-house iodide phasing and highlight in a few case studies the use of this straightforward and powerful method in a high-throughput structural genomics environment.

Poster 30

Combining 1H–1H distance constraints derived in supercooled water with residual dipolar couplings for protein structure refinement

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Highest resolution protein structures are of importance to (i) assess conformational preferences in proteins, (ii) calibrate molecular dynamics force fields and (iii) validate structure refinement procedures. Nowadays, cryo X-ray crystallography performed at temperatures from -150 to -170 °C routinely delivers protein structures of very high resolution (< 1.5 Å). However, flash-cooling of the crystals may affect the protein conformation, in particular the protein surface. Hence, methodology yielding highest resolution NMR structures is also required in order to identify subtle changes arising from crystal formation and possibly from flash-cooling. NMR-based structural biology of proteins can be pursued in supercooled water well below the freezing point of water [1]. Since flipping rates of aromatic rings are greatly reduced at such low temperatures [2], additional 1H-1H distance constraints can be detected for structure refinement [3]. For the 6kDa protein Bovine Pancreatic Trypsin Inhibitor (BPTI), those were combined with constraints previously reported for 36°C [4], and comparison with a 1.0Å crystal structure [5] shows that the thus refined NMR structure exhibits both significantly increased precision and accuracy. We measured polypeptide backbone 1H–15N and 1H–13Cα residual dipolar couplings (RDCs) at ambient temperature (~20/25°C) for BPTI aligned in C8E5 and glucopone[6]. Orientational constraints derived from the RDCs enabled us to further increase precision and accuracy of the BPTI NMR solution structure. This finding exemplifies the impact of combining 1H–1H distance constraints derived in supercooled water with residual dipolar couplings for obtaining highest resolved NMR solution structures.

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Poster 31

Canadian Macromolecular Crystallography Facility – a Suite of Fully Automated Beamlines

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The Canadian Light Source (CLS) is a 2.9 GeV national synchrotron radiation facility located on the University of Saskatchewan campus in Saskatoon. The small-gap in-vacuum undulator illuminated beamline 08ID-1 together with a second bending magnet beamline, 08B1-1, constitute the Canadian Macromolecular Crystallography Facility (CMCF). The CMCF provides service to more than 60 Principal Investigators in Canada and the United States. Up to 25% of the beam time is devoted to commercial users and the general user program is guaranteed up to 55% of the useful beam time and is run under a peer-review proposal system. The CMCF staff provides “Mail-In” crystallography service to the users with the highest scored proposals. Beamlines are equipped with very robust end-stations including on-axis visualization systems and Rayonix 300 CCD series detectors. They are each complemented with a Stanford automounter (SAM). An average exposure at the ID beamline is 1s and on the BM beamline is five time longer. MxDC, an in-house developed beamline control system, is integrated with a data processing module, AutoProcess, allowing full automation of data collection and data processing with minimal human intervention. It also allows remote control of experiments through interaction with a Laboratory Information Management System (LIMS) that was developed at the facility.

Poster 32

I03: automation and pathogenic sample handling at Diamond Light Source

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Beamline I03 is a macromolecular crystallography beamline at the UK synchrotron, Diamond Light Source. The beamline is tunable and is equipped with a Rigaku ACTOR sample changer and a recently installed Pilatus 6M detector, allowing extremely rapid sample screening and data collections. The beamline and software have been developed to streamline the data collection process for the user and automated procedures are in place to determine the best data collection strategy; provide image analysis during the data collection; process the data quickly at the end of the experiment; and also process the data using a more thorough approach ready for further downstream processing. All the experiment details and subsequent processing results are stored in the experimental database, ISpyB, and a constantly updated web-page gives a clear overview of all the experiments performed during the visit. In addition to the standard MX experiments, I03 has been designed to provide a safe-working environment for pathogenic samples up to ACDP Hazard Group 3. The containment measures include a negative-pressure air-handling system with HEPA filtered extract air and a permanently installed decontamination system based on hydrogen peroxide. The pathogenic samples will be contained within crystallisation plates and experiments will be done in-situ at room temperature. The current status of the automation systems and pathogenic sample handling at Beamline I03 will be presented.

Poster 33

Advanced and automated methods in Structural Biology and Structural Genomics: Better, faster, simpler: data collection with PILATUS pixel detectors

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The PILATUS pixel detector has revolutionized protein crystallography and biological small- and wide-angle scattering by combining noise-free counter properties with highest data acquisition rates. These features enable optimized data acquisition modes and new experimental techniques. The PILATUS detector is a modular two-dimensional hybrid pixel array detector with a pixel size of 0.172 mm, which operates in single-photon counting mode (Brönnimann et al., 2006). Different detector dimensions and read-out speeds allow for a perfect adaptation to the specific experimental requirements. PILATUS detectors offer a number of outstanding features: frame rates of 12 to 300 Hz at a full frame readout time of 2.3 ms, no readout noise and dark current in combination with a counter depth of 20 bit, a detector point-spread-function corresponding to one pixel, where the overexposure of a pixel does not influence the neighboring ones as well as the possibility to suppress fluorescence by means of an energy threshold (Kraft et al., 2009). The PILATUS 6M detector was developed at the Paul Scherrer Institut specifically for protein crystallography. Following its introduction at the SLS beamline X06SA in summer 2007, currently 6 systems are in operation at high performance beamlines. More than 10 PILATUS 1M and 2M detectors are implemented at synchrotron SAXS facilities. Besides static and time-resolved biological SAXS, these detectors enable previously impossible data acquisition protocols such as scanning-SAXS and X-ray ptychography. The properties of the PILATUS pixel detectors allow for a significant increase of the beamline throughput and enable novel automation features. The short readout and fast framing rate enable collection of diffraction data in fine-phi-slicing mode with continuous sample rotation (Hülsen et al., 2006), resulting in shortest possible data collection times and providing improved data quality. Similarly, diffraction based alignment and grid scanning is ideally supported by the high framing rate (Aishima et al., 2010). In SAXS experiments, the noise-free read-out and the high frame rate allow radiation damage to be assessed easily. Time resolved SAXS/WAXS experiments are enabled by the gating possibility of the detectors. Results from various protein crystallography and SAXS/WAXS experiments will be presented. Optimal data collection strategies for protein crystallography will be discussed in terms of oscillation angle, data redundancy and beam focusing properties as well as dose rate effects in room temperature data collection. The possibility to automate the whole data acquisition sequence from sample alignment to data reduction will be outlined.

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Poster 34

Heavy Metal Soaking Revived: a Fast, Cheap, Efficient Route to Routine High-Throughput Phasing

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The power of experimental phasing by soaking heavy atom compounds is evident in that it was yielding phases for macromolecular crystals long before cryocooling, synchrotrons and automatic detectors. Nevertheless, over the last two decades, selenomethionine phasing has established itself as the method of choice for experimental phasing, in both high-throughput and other labs: though neither cheap nor robust - new protein must be grown, purified and crystallized, and the anomalous signal is weak and sensitive to radiation decay - it has two dominating strengths: the existence of selenium is very likely can be robustly characterized; and it provides a route to the phase problem by harnessing molecular biology and wet lab protocols, which, following long battles to obtain crystallizing protein, experimenters typically are typically far more familiar with than crystal handling. We have therefore revisited heavy atom soaking in order to establish it as a routine phasing approach, specifically by considering the strengths of selenomethionine, making use of recently reported developments in soaking (Joyce et al. 2010, Acta D66), while also exploiting the great sensitivity of instrumentation and methodology developed to exploit the weak anomalous dispersion of selenium. Our protocol comprises five elements: 1. one-time preparation of frozen aliquots of aqueous heavy atom stocks, to reduce the energy barrier to handling; 2. confirmation of covalent binding to free protein by mass-spectrometry; 3. standardized quick-soak treatment of isolated crystals with heavy atom solution (5-60min, 5mM or higher); 4. (optional) in-house crystal testing (automated) for diffraction and intensity changes; 5. standardized data collection of one or more 360° low-dose datasets, generally requiring no wavelength tuning and little optimization. The procedure has yielded a 70% success rate, and revealed that from the bewildering range of heavy atom compounds, two usually suffice, both highly soluble: Thiomersal and K2PtCl4. We present a catalogue of situations and metrics by which not only success but also failure can be rapidly identified: the latter is an essential feature of a high-throughput procedure, so that heavy atom soaking now serves as a first pass at phasing, which can be attempted as soon as acceptable native diffraction has been established and if any unharvested crystals remain. Indeed, thanks to its simplicity and power, it is now our method of choice even for targets when molecular replacement is likely to work only with complications, i.e. with remote homologs or at low resolution: the quality of the experimental phases means that model building can be completed in a fraction of the time.

Poster 35

NMR-related resources at PDBe

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The Protein Data Bank in Europe (PDBe, pdbe.org), located at the European Bioinformatics Institute (EMBL-EBI), is the European partner in the wwPDB organisation, which manages the PDB archive. We collaborate with the other wwPDB partners (RCSB, BMRB and PDBj) on deposition, annotation, remediation, validation and dissemination of macromolecular structures and the associated experimental data (mainly X-ray, NMR, EM). To simplify deposition of NMR data to the PDB and BMRB, PDBe has developed an Entry Completion Interface (ECI) for CCPN projects. For individual NMR entries, PDBe provides several kinds of value-added information, including validation of archived chemical shifts through coordinates (VASCO), analysis of clustering and domain organisation and identification of the most representative conformer (OLDERADO) as well as links to other NMR resources such as BMRB, NMR Restraints Grid (NRG; a database of remediated restraints) and NRG-CING validation reports. PDBe is currently developing an interactive graphical tool for analysis of NMR entries, which allows visualisation of the information produced by VASCO, CING and OLDERADO on an ensemble or individual models. Finally, PDBe hosts several databases of recalculated NMR structures: RECOORD, which compares ensembles calculated by CYANA and CNS with and without water refinement, and LOGRECOORD, which uses log-normal potential to interpret the NOE data. These and other resources can be accessed from the URL pdbe.org/nmr.

Poster 36

PSI SBKB Technology Portal: A Structural Biology Web Resource

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The Technology Portal of the Protein Structure Initiative Structural Biology Knowledgebase (PSI SBKB; http://technology.lbl.gov/portal/) is a web resource providing methods and tools that can be used to relieve bottlenecks in many areas of structural genomics research. Several useful features are available on the web site, including multiple ways to search the database of over 225 technological advances, a link to videos of methods on YouTube, and access to a technology forum where scientists can connect, ask questions, get news, and develop collaborations. The Technology Portal is a component of the PSI SBKB (http://sbkb.org), which presents integrated genomic, structural, and functional information for all protein sequence targets selected by the Protein Structure Initiative. Created in collaboration with the Nature Publishing Group, the SBKB offers an array of resources for structural biologists, such as a research library, editorials about new research advances, a featured molecule each month, and a Functional Sleuth for searching protein structures of unknown function. An overview of the various features and examples of user searches will highlight the information, tools, and avenues for scientific interaction available through the Technology Portal. The PSI SBKB Technology Portal is funded by the NIGMS.

Poster 37

The Structural Biology Knowledgebase: a Portal to Protein Sequences, Structures, Functions, Methods, and More

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The Protein Structure Initiative's Structural Biology Knowledgebase (SBKB, URL: sbkb.org) is an open web resource designed to turn the products of the structural genomics and structural biology efforts into knowledge that can be used by the biological community to understand living systems and disease. Here we will present examples on how to use the SBKB to enable biological research. For example, a protein sequence or Protein Data Bank (PDB) structure ID search will provide a list of related protein structures in the PDB, associated biological descriptions (annotations), homology models, structural genomics protein target status, experimental protocols, and the ability to order available DNA clones from the PSI:Biology-Materials Repository. A text search will find publication and technology reports resulting from the PSI's high-throughput research efforts. Web tools that aid in research, including a system that accepts protein structure requests from the community, will also be described. Created in collaboration with the Nature Publishing Group, the Structural Biology Knowledgebase monthly update also provides a research library, editorials about new research advances, news, and an events calendar to present a broader view of structural genomics and structural biology.

Poster 38

Structural genomics of histone tail recognition

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The structural genomics of histone tail recognition web server is an open access resource that presents within mini articles all publicly available experimental structures of histone tails in complex with human proteins. Each article is composed of interactive 3D slides that dissect the structural mechanism underlying the recognition of specific sequences and histone marks. A concise text html-linked to interactive graphics guides the reader through the main features of the interaction. This resource can be used to analyze and compare binding modes across multiple histone recognition modules, to evaluate the chemical tractability of binding sites involved in epigenetic signaling and design small molecule inhibitors.
http://www.thesgc.org/resources/histone_tails/

Poster 39

Structure shortcuts to function prediction

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Knowledge of protein structures is essential to our understanding of known functions and can assist prediction of unknown activities. Like sequence-structure relationships, structure-function relationships are also very complex, hindering the direct predictions of protein function from structure. This is arguably the main reason of why functional annotation of new proteins is trailing several years behind their structural characterisation. The progress in function discovery for genomic proteins of unknown function so far has been achieved mainly through bioinformatics inference enhanced by protein structure data. Protein structure is a hub for the integration of all kinds of data on the protein superfamily. It facilitates multiple structural and functional predictions for its known and probable members, including specified activities of uncharacterised protein families. One of the most successful integrated approaches to function prediction is the matching of structurally characterised proteins of unknown function and "hypothetical functions of unknown protein". There is circumstantial biochemical evidence for the existence of many yet unexplored metabolic pathways. Some of these pathways are deduced from known chemical structures of the end products, others provide probable routes between identified intermediates or suggest new variations of established pathways. There are associated enzymatic activities, many of which were partly purified in the past but not sequenced at the time. These "hypothetical" enzymes probably account for a substantial fraction of genomic proteins of unknown function. Many of them may have already been characterised by Structural Genomics, although their identities remained unknown. Selected examples of functional annotations of various Structural Genomics targets by this and similar integrated approaches, including our confirmed and new functional predictions, will be presented.

Poster 40

A Structure Prediction Method for Remote Homologs in the Absense of Sequence Signal, Using Supersecondary-Structure Building Blocks and Basic NMR Data

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In recent years, structural modeling approaches have seen dramatic successes in predicting protein structures based on sequence data alone. However, these methods have limited success rates when the sequence signal of the protein to be characterized is low. As a result, predicting the structures of such proteins requires additional information besides the sequence. Here, we present a method that takes chemical shift and residual dipolar coupling data (both of which are easily obtainable from NMR experiments) to generate backbone structures without relying on the sequence signal. Ultimately, this method shows promise in modeling query proteins with little sequential resemblance to existing solved structures. The basis for this method is the library of supersecondary structure building blocks (smotifs), each of which consists of two successive secondary structures connected by a loop. It has been shown that the current smotif library contains all possible paired secondary structure orientations, which leads to the conclusion that the structure of any given protein can be assembled from this finite set of building blocks [1]. Thus, this method selects smotifs from this library and assembles them into candidate backbone structures for the query protein, similar to the Molecular Fragment Replacement approach [2], but with several important modifications. Before running the algorithm on a given structure, artificial chemical shift fingerprints are generated for each smotif in the library, using the program SPARTA [3]. In addition, the angles determining the relative orientation of the two secondary structures in each smotif are calculated and stored in the library. This pre-processing, although computationally expensive, is a one-time calculation. For a given query protein, the sequence is parsed to identify regions of secondary structure, which is readily done through PSIPRED [4]. After identifying the number and location of putative secondary structures, a two-stage process selects suitable smotif building blocks from the library. First, residual dipolar coupling (RDC) data from multiple alignment media can provide an estimate of the angle between successive secondary structures. For alpha-helices, data from N-H bonds can be used to approximate vectors running parallel to the helix, whereas for beta-strands, averaged data from backbone C-N bonds is necessary to approximate the principal strand axis vector. Based on this initial RDC-based analysis, the entire library of smotifs is filtered by the relative orientation of their secondary structures (which has been pre-calculated). After this filtering step, the experimental chemical shift data are compared to the artificially-generated chemical shift fingerprint for each smotif to identify the closest matches. This yields a set of potential building blocks to build the protein structure. After suitable smotif candidates have been selected, they are joined to generate a full backbone model of the query protein. This is easily done by superimposing the overlapping secondary structures of successive smotifs. Once a set of candidate backbone models has been assembled, they can be scored using a customized neural network-based scoring function that takes as inputs the radius of gyration, a statistical contact potential [5], hydrogen bond potential and an implicit solvation potential [6], and returns a measure of how ‘native-like’ or ‘feasible’ the given structures are. This method was validated on calmodulin, thioredoxin and sensory rhodopsin as test cases. Teh resulting models are within 2-3A RMSD from the solution structure (considering the entire structure for RMSD calculation). These preliminary results show that, given sufficient chemical shift and RDC data, this method is able to generate structures for unsolved proteins without the need for sequence signals.

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Poster 41

Using superimposition of protein-chemical complex structures to reveal new targets for established drugs

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In the presented study, we explore the network of 3 dimensional structures of proteins-chemical complexes to predict undocumented relationships between molecules. The underlying principle is that existing interactions between molecules can be used to predict new interactions. For pairs of proteins sharing a common ligand, we use protein and chemical superimpositions combined with fast structural compatibility screens to predict whether additional compounds bound by one protein would bind the other. The method reproduces 84% of complexes in a benchmark, and we make many predictions that would not be possible using conventional modeling techniques. Within 19,578 novel predicted interactions are 7,793 involving 718 drugs, including filaminast, coumarin, alitretonin and erlotinib. The growth rate of confident predictions is twice that of experimental complexes meaning that a complete structural drug-protein repertoire will be available at least ten years earlier than by X-ray and NMR techniques alone.

Poster 42

SCOP revisited

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The Structural Classification of Proteins (SCOP) project was established in 1994 to annotate all known structural and probable evolutionary relationships amongst proteins of known structure. This was implemented by creating the SCOP database, in which protein domains are organised on several hierarchical levels in a tree-like fashion. This domain-centric hierarchical scheme was thought to reflect the paradigm of protein structure and function evolution. It was successfully applied to the classification of new structures since. As the number of known protein structures has grown with the advent of Structural Genomics, their classification analysis revealed many new, more subtle pathways of protein evolution, as well as new types of structural relationships, not envisaged by the original scheme. We have tried to accommodate these new findings in the existing scheme, but these attempts resulted in some inconsistencies in the classification. The time has come for fundamental changes to the classification principles. We shall present a prototype of the SCOP2.0 database that we have developed recently. SCOP2.0 is designed to provide a more advanced framework for protein structure annotation and classification. The new classification scheme retains some basic features of the original SCOP hierarchy, but it differs in several key aspects. It is protein-centric and is based on representative sequences and structures. The relationships between proteins are represented by a network of nodes. Each node represents relationship of particular type (family, superfamily etc) and is exemplified by a region of protein structure. There is no underlying hierarchical tree, that is, each node can have many parental nodes. Different nodes are also connected by non-hierarchical interrelationships that describe either a given evolutionary event or a common structural feature such as motif, repeat etc.

Poster 43

Database integration for expanded search options for protein structure identification

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The users of the Structural Biology Knowledgebase (SBKB) constitute a diverse group who require searches for protein structures based on a variety of information. To enable advanced text queries by the varied user base, we have initiated a project to map and assemble the annotations that are expected to facilitate the searches. As an initial step, annotations were assembled that included the descriptions of the small molecule interactions of the protein structures, the cellular and biochemical pathways in which the structures participate, and the structural classes of the protein structures. An online tool was created to separate the structures retrieved by text query according to the resource in which they are annotated and according the different annotation categories within each of those resources. The tool can be used in conjunction with the SBKB after text search is initiated there and as a separate application. The structures retrieved by a text query are ranked according to their relative content of both the text and non-text attributes that are associated with the text query. Similarly, categories of annotations, such as cellular pathway participation, are ranked according to their relevance to the text query. As a guide, the values of the ranks are depicted as colors within the visual spectrum where structures ranked highest are colored red and those lowest are violet. The aim of the described tool is provide a means for users to quickly identify structures of interest and the annotations most relevant to their queries.

Poster 44

Detecting Helitrons using the Local Combinational Variable Approach

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Helitrons represent a new class of transposable elements recently uncovered in animals and plants. One remarkable feature of Helitrons is their ability to capture gene sequences, which makes them of considerable potential evolutionary importance. Unlike other DNA transposable elements, Helitrons possess low conservation in terms of sequence or structure features. Conventional sequence alignment method proves effective on those conservative motifs. However, Helitrons are only conservative at two terminals and consensus model from one species are not general enough to be used as searching criteria for Helitrons in other species, which results in the inability to detect Helitrons in other species. Thus identifying Helitrons remains a challenge. We employed a Local Combinational Variable (LCV) approach based on its successful applications in protein structure research. Over four thousand high quality Helitron sequences from maize genome were taken as source of LCVs. We scooped out all potential Helitron segments with relatively high scores of LCVs. The scoring scheme of LCVs can make full use of information from every significant fraction of known Helitron sequences. Unknown nucleotide segments are measured by how many LCVs they cover. Those with higher scores than a preset expected threshold are putative Helitrons. We have been successfully identified thousands of Helitrons from other species, such as sorghum, soybean, and poplar using LCVs derived from maize Helitrons.

Poster 45

The Protein Model Portal – towards Continuous Automated Model EvaluatiOn (CAMEO)

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The Protein Model Portal (PMP) has been developed to foster effective use of molecular models in biomedical research by providing convenient and comprehensive access to structural information for a specific protein. For the first time both experimental structures and theoretical models for a given protein can not only be searched simultaneously, but also analyzed for structural variation. The current release allows searching 15.5 millions of model structures for 3.8 million distinct UniProt (UP) entries (UP release 2011_04).

Ultimately, the accuracy of a structural model determines its usefulness for specific applications. Model quality estimation tools allow evaluating the accuracy of generated models to indicate their usefulness for specific applications in biomedical research. Here, we present new developments in Protein Model Portal supporting model validation and quality estimation, which consist of (1) service interfaces to several established modeling and model quality estimation tools and (2) CAMEO (Continuous Automated Model EvaluatiOn) designed for the continuous evaluation of the modeling and model quality estimation tools included in PMP. The comprehensive view on structural information offered by the Protein Model Portal will allow a unique opportunity to apply consistent assessment and validation criteria to the complete set of structural models available for a specific protein. Further, the continuous assessment of the modeling and quality estimation services registered with CAMEO will provide an objective view on method developments within the modeling community.

Feel free to visit us at the PSI SBKB Booth!

Poster 46

Binding Ligand Prediction by Comparing Local Surface Patches of Potential Pocket Regions

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Function of a protein, specifically, the type of ligand that bind to a protein, can be predicted by finding similar local surface regions of known proteins. We developed an alignment free local surface comparison method for predicting the type of ligand that is likely to bind to a query protein. The algorithm, named Patch-Surfer, represents a binding pocket as a combination of segmented surface patches, each of which is characterized by its shape, the electrostatic potential, the hydrophobicity, and concaveness. Representing a pocket by a set of patches is effective to absorb difference of global pocket shape while capturing local similarity of pockets. The shape and the physicochemical properties of surface patches are represented using the 3D Zernike descriptor (3DZD), which is a series expansion of mathematical 3D function. The 3DZD allows a compact and rotation invariant representation of 3D objects. Two pockets are compared using a modified weighted bipartite matching algorithm, which matches similar patches from the two pockets. The benchmark studies showed that Patch-Surfer's prediction performance is superior to existing methods, including the Pocket-Surfer method developed previously in our group (Chikhi, Sael, Kihara, Proteins 2010), which represents a pocket as a whole. On a dataset of 100 non-homologous proteins that bind one of nine different ligands. 84.0% of the binding ligands were predicted correctly within the top three scores using the shape and pocket size information. The performance was further improved to 87.0% when surface properties, i.e. electrostatic potential and hydrophobicity, were added. Overall, we show that proposed method is powerful in predicting the type of ligand a protein binds even in the absence of homologous proteins in the database. (Sael & Kihara Int J Mol Sci, 2010). This work is supported by NIH (R01 GM075004).

Poster 47

An automatically updated set of predictions of functional associations across all known protein structures

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The assembly and integration of the characteristics of protein structures provides a means to predict novel functional associations. Patterns within the measurements and annotations of the protein structures can be associated with specific biological functions. Given a protein that has incomplete set of measurements and annotations, data mining techniques can accurately predict the corresponding functional association. We have recently reported the development of a data mining tool that generates predictions of protein function. The tool addresses the requisite technical challenges to generate accurate predictions with probability estimates across all known protein structures. Here we describe the automation of that process so as to update the set of predictions in coordination with availability of new protein structures along with their measurements and annotations. To show applications of the tool, novel predictions of protein function are highlighted for structures of unknown function that were solved through the Protein Structure Initiative. These predictions provide leads for further experimental characterization of the proteins.

Poster 48

Could we eliminate experiments from structural genomics? A computational experiment using structure prediction metaservers.

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Structural genomics(SG) and computational structure prediction are two alternative approaches to the structural characterization of the protein universe. In this work, we investigate whether computational modeling methods could be used in place of SG, focusing on a set of about 200 proteins that have been targets of the PSI-2 programs. We found that automated blind structure prediction using publicly available metaservers would have accurately predicted the structures of about one fourth of the targets (\"accurately\" defined as placing more than 70% of the atoms in the model within 2A of the positions determined experimentally). Almost all of the targets that could be modeled with this accuracy had a template in the Protein Data Bank (PDB) with more than 25% sequence identity. However, the vast majority of the SG targets that had no match to PDB templates (above 25% sequence identity) would not have been predicted by the metaservers with this accuracy. As expected, models created by participants in the CASP competition were significantly better than those produced by the metaservers. Our results show that at the present stage of the development of modeling tools available for biomedical researchers, they cannot replace the experimental SG pipeline.

Poster 49

iSee: Disseminating Structural Biology Data To The Masses

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Making structural biology data accessible to non-structural biologists is key to ensure that structural information is considered by biologists as well as during a chemical probe development or drug discovery project. In collaboration with MolSoft LLC, the SGC has developed an intuitive and interactive platform called iSee that enables the integration of annotated structural biology data and visualisations into a 3D document. These documents can be shared and viewed via web pages, an offline browser, or within Microsoft Office documents. The platform and our data in the iSee format is freely available online and is now used for publishing structural biology data in a number of journals such as NSMB and PLoS ONE/Biology.

Poster 50

Computational analysis of protein kinases over-expressed in triple negative breast cancer

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Despite the development of novel therapeutic agents, breast cancer represents a major cause of death among women [Jamal et al., 2010]. Among breast cancer patients, triple negative (TN) breast cancer (TNBC) represents approximately 15% of cases [Cleator et al., 2007]. TNBC is clinically the most aggressive form of breast cancer and the less responsive to standard treatment leading to poor prognosis [Speers et al. 2010]. TNBC is characterized by the absence of the estrogen receptor, the progesterone receptor as well as the HER2 protein kinase. Recently, it has been shown that a subset of 26 protein kinases (TNVT set) are over-expressed in TNBC. Furthermore, their inhibition in siRNA knockdown experiments leads to varying levels of growth inhibition in TN and sometimes non-TN cancer cell lines [Speers et al., 2010; Bianchini et al., 2010]. These studies validate TNVT set kinases as potential therapeutic targets. The aim of this project is to characterize the binding site of TNVT set kinases using different computational methods developed in our research group and to determine which protein kinases of this subset could be more likely to bind similar ligands as part of a poly-pharmacological approach. We generated homology models for the 15 kinases in the TNVT set with unknown structure using Modeller [Eswar et al., 2008] followed by molecular dynamics relaxation using Gromacs [van de Spoel, et al., 2005]. We calculated global sequence similarities and binding-site sequence similarities using the MSAID program [Chenard et al., in preparation] and 3D atomic binding-site similarities using the IsoCleft program [Najmanovich et al., 2008] for the TNVT set of kinases. In order to visualize the relationship between the different similarity matrices, we used a visualization tool developed in our group called MC-heatmaps [Chenard et al., in preparation]. This analysis shows that binding-site sequence similarities somehow reflect global sequence similarities. Binding-site 3D atomic similarities reflect binding-site sequence similarities but are more widespread. This may have potential functional consequences in terms of small-molecule molecular recognition. Such similarities can potentially lead to cross-reactivity effects but they can also be exploited in the development of multi-functional poly-pharmacological drugs. Recently, the dissociation constants (Kd) of 38 small-molecule inhibitors for 290 protein kinases (including 17 kinases in the TNVT set) were calculated [Karaman et al., 2008]. These experimental binding-profiles were used to define a measure of functional profile similarity using Kendall rank correlations (τ) [Kendall, 1938; Adler, 1957]. We will present results using our docking program FlexAID [Najmanovich, 2004] for the 38 small-molecules tested by Karaman et al. against the 26 kinases in the TNVT set. Similar to experimental binding-profiles, the docking scores can be used to define docking binding-profiles similarities using τ rank correlations. For the 17 kinases in the TNVT set with experimental binding-profiles, we compare experimental and docking τ correlations. Docking binding-profile similarities are then used to cluster the 26 kinases in the TNVT set. Clusters represent subsets of kinases within the TNVT set with functionally similar binding-sites. Finally, we compare functional docking profile similarities to the sequence and 3D atomic similarities discussed above. This analysis will allow us to detect subsets of kinases in the TNVT set for which it may be possible to develop multi-functional inhibitors. Additionally, the methods presented here can be readily applied to study family-wide structure-function relationships in other protein families.

Poster 51

From PyMOL session files to Jmol state scripts: The Proteopedia PyMOL2Jmol Translator

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Jmol is an open-source Java viewer for chemical structures in 3D. It is used in Proteopedia, the online collaborative 3D encyclopaedia of biomolecules (http://www.proteopedia.org), to permit 3D visualization of molecular structures on virtually all pages of Proteopedia via the WWW. Complementing Proteopedia's user-friendly scene authoring tools, which make it easy to create Jmol scenes, we now introduce Proteopedia's "PyMOL2Jmol Translator", which converts scenes from the very widely used 3D structure visualization program PyMOL into its equivalent representation in Jmol. This tool allows PyMOL scenes to be shared with Jmol users and to be published on the web through the Jmol web applet. Seamless integration of the PyMOL2Jmol Translator with Proteopedia enables rapid web-publication of PyMOL-created content (Fig 1).

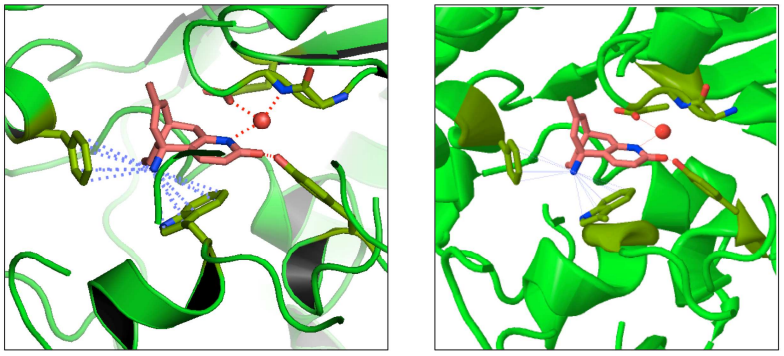

Fig. 1 Example of the conversion of a PyMOL session file ("pse") to Jmol via PyMOL2Jmol Translator.

Poster 52

Solution NMR Structures of De Novo Designed “Ideal Structure” Proteins

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Structural characterization of designed proteins is a critical step in validating computational design methodology. Many of the groups involved in computational protein design have limited resources for 3D structure determination, and structural genomics platforms are ideally suited for collaborative projects aimed at accelerating the field. Four ideal folds have been targeted for de novo design: Ferredoxin-like, Rossmann 2x2, Flavodoxin-like (Rossmann 2x3), and Rossmann 3x3 folds. Protein candidates with different primary sequences were computational designed and pre-selected based on computational energy at University of Washington. Unlabeled or 15N labeled protein samples were prepared for selected protein candidates and were further screened by NESG at Rutgers using 1D NMR or 2D [15N-1H] - HSQC. Suitable protein candidates were then selected for structure determination by NMR or/and X-ray. To date, solution NMR structures have been determined for five targets. The Ferredoxin-like protein (NESG ID OR15); Rossmann 2x2 fold protein OR16; Flavodoxin-like proteins OR28 and OR36 Rossmann 3x3 fold protein OR32. One target (OR16) has also provided crystallization hits. The experimental NMR structures of OR15 and OR16 are in excellent agreement with their designed models. However, structures of the three proteins OR28, OR36 and OR32 turn out to be P-loop NTPase fold structures that have two □-strands swapped compared to designed models. These NMR experimental structures provide unique valuable information on how to improve the protein designed strategies.

Poster 53

Crystal Structure of the first domain of the human PolyC binding protein: Insight into the specificity of nucleotide recognition by the KH domains

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Poly-C binding proteins are ubiquitous oligonucleotide-binding proteins in eukaryotic cells that play fundamental role in regulation of gene expression via interaction with C-rich oligonucleotides. PCBP-1 (αCP-1 or hnRNP E1) is involved in the post- transcriptional regulation of mRNA by binding to the 3'-UTR and has been found to interact with a variety of mRNA such as Androgen Receptor, αGlobin, Tyrosine Hydroxylase and Lipoxygenase. PCBP binding to RNA can result in both silencing and enhancement of translation through a diverse set of mechanisms. PCBPs have also been found to be important for the replication of viral RNA which utilise them in both the translation and replication of the viral genome of picornaviruses. In addition to its RNA binding properties, PCBP-1 has been shown to bind ssDNA. Such interactions play a role in transcriptional regulation with PCBP identified as the ssDNA binding protein underlying proximal promoter activity of mouse μ-opioid receptor. The aim of this work understands the molecular basis of DNA/RNA recognition by PCBP-1 using biophysical methods including Surface Plasmon Resonance and X-ray Crystallography. Here, we present the crystal structure of the first domain of the human PCBP1 in complex with a 6-mer nucleotide. The structure reveals the key contacts between the protein and the nucleotide accommodated in the binding cleft. We used SPR to compare the binding affinities of different DNA sequence. Both data suggest a clear preference for cytosine bases in the binding cleft.

Poster 54

SeSEAM: a Systematic Mutagenesis-Driven Strategy for Site-Resolved NMR Studies of Supramolecular Assemblies

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It is becoming increasingly feasible to use solution NMR spectroscopy to analyse the function, local structure, intermolecular interactions and dynamics of large protein assemblies of up to 1 MDa. However, due to the size-limitations of standard NMR assignment strategies, the process of obtaining sequence-specific assignments of such systems remains a considerable challenge. We have fully assigned isoleucine (δ1,γ2), alanine, and leucine and valine (proR and proS) methyl groups of a 468 kD dodecmeric aminopeptidase complex using an approach based on site-directed mutagenesis. We produced a library of single-site mutants each of which is designed to "turn off" a resonance in 2D methyl correlation spectra. The library was expressed using appropriate methyl-labelling protocols and each mutant was analysed using 2D methyl TROSY experiments. The missing peak in each spectrum can accordingly be assigned to a specific methyl group in the mutated residue. A key attraction of this approach is the simplicity of the assignment process; complicated NMR experiments and time-consuming specialized data analysis is not necessary. The concept of resonance assignment by mutagenesis is not new. However, modern automated laboratory techniques (mutagenesis, cloning, expression, purification, etc) and advances in NMR spectrometer technology (increased sensitivity, sample changers, small volume probes, etc) make it possible to implement this simple approach in a substantially more comprehensive, systematic and straightforward way. Each step in the protocol (library construction, protein expression and purification, data acquisition and analysis) can be conveniently automated which makes this methodology ideally suited to laboratories familiar with higher-throughput techniques. The SeSAM approach, therefore, offers an alternative, general and convenient method for overcoming the hurdle of NMR assignment in larger protein systems.

Poster 55

Identification and structural - functional characterization of protein-protein complexes of the E. coli phenylacetate utilization pathway

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The phenylacetate catabolic pathway is present in one sixth of sequenced genomes where it forms a catalytic core for degradation of the wide variety of aromatic compounds (Luengo et al., 2001). Eleven enzymes participate in this pathway and they are all encoded within the single paa operon. Biochemically, opening of the aromatic ring can occur either under anaerobic or aerobic conditions by two different mechanisms. The paa pathway contains features of both these pathways and employs an unusual ring-opening chemistry. Phenylacetate is activated by binding to the coenzyme A (CoA), its aromatic ring becomes oxygenated to yield 1,2-epoxyphenylacetyl-CoA and rearranged to the seven member ring O-heterocyclic enol ether, an oxepin-CoA. This isomerization is followed by a hydrolytic ring cleavage and â-oxidation. We have performed a comprehensive search for protein-protein interactions among the enzymes of the paa pathway by coexpression of various combinations of paa enzymes in which only one subunit was tagged. Purification of these mixtures by IMAC and analysis of the elution fraction allowed us to identify the PaaABCE and PaaFG complexes. The PaaABCE belongs to a new family of bacterial multicomponent monooxygenases, the enzyme group which contains methane monooxygenase and toluene monooxygenase. Reconstitution of the reaction in vitro allowed us to show that the absence of any of one of PaaA,C,B or E subunits prevents catalysis. The PaaE component is a reductase which shuttles electrons from NADPH through FAD and a Fe-S cluster to the terminal oxygenase subunit (Grishin et al., 2011). The structure of the PaaAC subcomplex bound to the natural substrate phenylacetyl-coenzyme A proved that PaaAC represents the oxygenase component with several unique structural features. The low resolution structure of PaaABC combined with SAXS analysis showed that PaaB subunit serves as a bridge connecting two PaaAC heterodimers forming the binding site for the PaaE reductase. Determination of the whole PaaABCE complex structure is in progress. The structure of the PaaFG complex showed that both subunits belong to the crotonase fold. In complex the homohexamer of PaaF is sandwiched between two trimeric discs of PaaG. The low solubility of PaaF, when expressed alone, allowed us to suggest that the interaction with PaaG is beneficial for the stabilization of PaaF. The structures of PaaF and PaaG provide clues on the catalytic mechanisms of these enzymes, in particular the rearrangement of the 1,2-epoxyphenylacetyl-CoA to the oxepin-CoA.

Poster 56

New strategy for the use of NMR spectroscopy in the crystallography-centric Joint Center for Structural Genomics

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The Joint Center for Structural Genomics (JCSG) has implemented an approach to use solution NMR spectroscopy for expanding structure coverage of the protein universe by solving first structure representatives of large protein families that could not yet be successfully tackled by X-ray crystallography. This new strategy is based on previously established orthogonality between protein sequence and structure determination success rates either by NMR spectroscopy or by X-ray crystallography, and uses the following four-step protocol: (i) Sequence-based bioinformatics to prioritize protein orthologues for high NMR structure determination success rate. (ii) Preparation of structure-quality protein solutions guided by 1D 1H-NMR and 2D [15N,1H]-correlation NMR-profiling. (iii) Extensively automated NMR structure determination. (iv) Interactive structure refinement and validation. So far, this approach has provided structural representatives for more than 3000 protein sequences, which are members of 10 distinct pfam families. The resulting repertoire of three-dimensional architectures shares low sequence identity with proteins deposited in the PDB, and includes both new folds and well-characterized structure families.

Poster 57

Rapid comparison and multimeric protein complex fitting for low-resolution electron microscopy data

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The electron microscopy (EM) is an emerging technique in structural biology, which can often obtain protein structure data for cases that are difficult by X-ray crystallography, e.g. structure of protein complexes. However, structure data by EM is often at a resolution as low as 10-20 Angstroms or even lower. Thus, the computational challenges include how to efficiently and accurately compare low-resolution EM density map to known structures and how to fit known atomic-detailed structures to EM map. Here, we present novel methods for addressing the two challenges. First, we developed a novel method for rapid comparison of a query EM density map to proteins in PDB. The method uses the 3D Zernike Descriptor (3DZD), which is a mathematical series expansion of 3D function (i.e. the EM density map). The 3DZD enables fast structure database searches, taking advantage of its rotation invariance and compact representation. We examined EM maps of various resolutions and found that the method has good performance in identifying the structures of the same fold even for EM maps at 15-20 Angstrom resolution (Sael, Kihara, BMC Bioinformatics, 2010). Next, we applied the 3DZD for fitting multiple component proteins into an EM map. The method starts with generating a large number of docking conformations of the multiple component proteins. The multiple docking is performed by the Multi-LZerD algorithm, which combines the pairwise docking results of each pair of proteins. Then the fitness of the multiple docking decoys and the EM map is quantified by using the 3DZD. The method obtained docking results with a root mean square deviation (RMSD) of 1.1 to 3.3 Angstrom to the native structures in all the cases we tested. Overall, we show that the 3DZD is powerful in comparing EM data for comparison and multiple-docking guided by the low-resolution data. This work is supported by NIH (R01 GM075004).

Poster 58

αB-crystallin: How solid-state NMR can help to study difficult biological systems

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Small heat shock proteins (sHSP) help to maintain protein homeostasis by interacting with partly folded substrates to prevent cell damage (1-3). The ATP-independent chaperone αB-crystallin (αB, 20 kDa, 175 residues) is an archetypal example (4). Discovered as a highly abundant protein in the eye lens that plays a critical role in maintenance of lens transparency, the known biological roles of αB continue to expand. The protein is expressed in many tissue types, notably muscle and brain, in a stress-inducible manner, where it presumably serves as a chaperone for misfolded cellular proteins. Consistent with such a role, αB is implicated in a growing number of diseases that includes cardiac myopathies and neurodegenerative diseases such as Alexander's and Alzheimer's disease (5-7). Furthermore, αB has been shown to play a protective role and can reverse symptoms of multiple sclerosis (8). Thus, a full structural description of αB is an important step towards understanding its mode(s) of action. As all sHSPs, αB is organized in three domains: 1) an N-terminal domain of approximately 60 residues, 2) a central α-crystallin domain (ACD) of about 90 residues involved in dimerization , a C-terminal domain of 25 residues containing the IXI-motif, a motif, usually comprised of two Ile residues separated by an intervening residue, that is highly conserved in sHSPs. Recent studies including our work have yielded the first atomic-level structures of α-crystallin domains (ACDs) from polydisperse mammalian sHSPs determined by using solid-state NMR and X-ray crystallography (9-11). Furthermore we observe a pH-dependent modulation of the interaction of the IXI motif with β4/β8 consistent with a pH dependent regulation of the chaperone function. We present a model for full-length αB including the N-terminal domain as a tetrahedrally symmetrical 24mer based on the published EM map of αB (12) and structural restraints from solid-state NMR. Next we use small-angle X-ray scattering (SAXS) to measure αB's heterogeneity in solution and together with new EM analysis we propose a model in which additional dimeric units can fill existing openings in a 24mer to create higher-order multimers that look alike in negatively-stained preparations in electron microscopy (13). 1. Bukau B, Weissman J, & Horwich A (2006) Molecular chaperones and protein quality control. Cell 125(3):443-451. 2. Haslbeck M, Franzmann T, Weinfurtner D, & Buchner J (2005) Some like it hot: the structure and function of small heat-shock proteins. Nat Struct Mol Biol 12(10):842-846. 3. Ecroyd H & Carver JA (2009) Crystallin proteins and amyloid fibrils. Cell Mol Life Sci 66(1):62-81. 4. Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci U S A 89(21):10449-10453. 5. Vicart P, et al. (1998) A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. Nat Genet 20(1):92-95. 6. Goldstein LE, et al. (2003) Cytosolic beta-amyloid deposition and supranuclear cataracts in lenses from people with Alzheimer's disease. Lancet 361(9365):1258-1265. 7. Kato K, et al. (2001) Ser-59 is the major phosphorylation site in alphaB-crystallin accumulated in the brains of patients with Alexander's disease. J Neurochem 76(3):730-736. 8. Ousman SS, et al. (2007) Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination. Nature 448(7152):474-479. 9. Jehle S, et al. (2010) Solid-state NMR and SAXS studies provide a structural basis for the activation of alphaB-crystallin oligomers. Nat Struct Mol Biol 17(9):1037-1042. 10. Bagneris C, et al. (2009) Crystal structures of alpha-crystallin domain dimers of alphaBcrystallin and Hsp20. J Mol Biol 392(5):1242-1252. 11. Laganowsky A, et al. (2010) Crystal structures of truncated alphaA and alphaB crystallins reveal structural mechanisms of polydispersity important for eye lens function. Protein Sci 19(5):1031-1043. 12. Peschek J, et al. (2009) The eye lens chaperone alpha-crystallin forms defined globular assemblies. Proc Natl Acad Sci U S A 106(32):13272-13277. 13. Jehle S, et al. (2011) The N-terminal domain of aB-crystallin provides a conformational switch for multimerization and structural heterogeneity. Proc Natl Acad Sci U S A (accepted for publication)

Poster 59

The GD1A Glycan is a Cellular Receptor for Adenoviruses Causing Epidemic Keratoconjunctivitis

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Adenovirus type 37 (Ad37) is a leading cause of epidemic keratoconjunctivitis (EKC), a severe and highly contagious ocular disease. Whereas most other adenoviruses infect cells by engaging CD46 or the coxsackie and adenovirus receptor (CAR), Ad37 binds to sialic acid-containing cell surface molecules. However, the identity of the Ad37 receptor has remained unknown. Using glycan array screening, we found that the receptor-recognizing knob domain of the Ad37 fiber protein specifically binds to a branched hexa-saccharide (the carbohydrate portion of the GD1a ganglioside) that features two terminal sialic acids. Soluble GD1a glycans and GD1a-binding antibodies efficiently prevented Ad37 virions from binding to and infecting human corneal cells. X-ray crystallographic studies reveal that the two terminal sialic acids dock into two of three previously established sialic acid-binding sites in the trimeric Ad37 knob. Based on the fact that the fiber knob protein contains three sialic acid binding sites a trivalent inhibitor was designed. This glycoconjugate efficiently inhibited binding of Ad37 to human corneal cells as well as Ad37 infection. The co-crystal structure of the Ad37-inhibitor complex demonstrates that the inhibitor engages now all three binding sites of the Ad37 fiber protein. Thus, biological competition experiments and structure-function analysis of the virus-inhibitor interaction show that such molecules may be suitable for topical treatment of EKC.

Poster 60

Crystal Structure of the Dengue Virus Methyltransferase Bound to a 5'-capped Octameric RNA

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The N-terminal domain of the flavivirus NS5 protein functions as a methyltransferase (MTase). It sequentially methylates the N7 and 2'-O positions of the viral RNA cap structure (GpppA-7meGpppA-7meGpppA2'-O-me). The same NS5 domain could also have a guanylyltransferase activity (GTP+ppA-RNA-GpppA). The mechanism by which this protein domain catalyzes these three distinct functions is currently unknown. Here we report the crystallographic structure of DENV-3 MTase in complex with a 5'-capped RNA octamer (GpppAGAACCUG) at a resolution of 2.9 A. Two RNA octamers arranged as kissing loops are encircled by four MTase monomers around a 2-fold non-crystallography symmetry axis. Only two of the four monomers make direct contact with the 5- end of RNA. The RNA structure is stabilised by the formation of several intra and intermolecular base stacking and non-canonical base pairs. The structure may represent the product of guanylylation of the viral genome prior to the subsequent methylation events that require repositioning of the RNA substrate to reach to the methyl-donor sites. The crystal structure provides a structural explanation for the observed trans-complementation of MTases with different methylation defects.

Poster 61

Crystal structure of Toxoplasma gondii porphobilinogen synthase: Insights on octameric structure and porphobilinogen formation

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Porphobilinogen synthase (PBGS) is essential for heme biosynthesis in the apicomplexan parasite Toxoplasma gondii, an opportunistic human pathogen. As a result of its unique evolutionary origin T. gondii porphobilinogen synthase (TgPBGS) is located in the apicoplast and is distinct from its human counterpart with respect to metal ion dependence and quaternary structure dynamics. We present the crystal structure of TgPBGS which contains an octamer in the crystallographic asymmetric unit; this structure was determined as a part of the Seattle Structural Genomics Center for Infectious Disease's (SSGCID's) community request program. TgPBGS contains structures for its N-terminal and C-terminal regions that have not been seen in ~40 previously solved PBGS crystal structures. A TgPBGS-specific C-terminal extension forms an intersubunit β-sheet that secures a pro-octamer dimer and prevents hexamer formation, which can form in human PBGS. Crystallized in the presence of substrate, each TgPBGS active site contains one molecule of the product porphobilinogen. Unlike prior structures from human and yeast PBGS, which contain a substrate-derived heterocycle directly bound to an active site zinc ion, the product-bound TgPBGS active sites do not contain a metal ion, although putative ligands for a postulated active site magnesium ion are present and ordered. This puts to question the long-hypothesized notion that some PBGS use an active site zinc while others use an active site magnesium. Unlike human PBGS, the TgPBGS octamer contains eight allosteric magnesium ions that lie at the eight intersections between pro-octamer dimers. Insights obtained from TgPBGS structure inform us on possible structure function relationship in Plasmodium and other apicomplexan PBGS, especially with respect to the role of the C-terminal extension seen throughout this phyla. The unique aspects of TgPBGS quaternary structure have promise for development of parasite selective PBGS inhibitors.

Poster 62

Structure of hemagglutinin from a pandemic 2009 H1N1 influenza virus and implication for viral infection

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Influenza virus infects host cells through membrane fusion, a process directed by the viral surface glycoprotein hemagglutinin (HA). Cleavage of the HA precursor protein HA0 into the subunits HA1 and HA2 by cellular proteases is essential for virus entry into host cells. We have characterized recombinant HA proteins from 2009 pandemic (KOR01) and seasonal influenza viruses isolated from patient nasopharyngeal swabs. The pandemic HA is identified as a monomer in solution, and exhibits increased susceptibility to proteolytic cleavage by cellular proteases than the seasonal HAs. We have also determined the crystal structure of the pandemic H1N1 HA protein at 2.7 Å resolution. The pandemic HA surprisingly reveals a monomeric conformation in the crystal as well, and unique features that involve head-to-head and tail-to-tail arrangements of monomeric HA molecules, creating a star-shaped hexagram of a tubular assembly with an empty channel of 60-70 Å in diameter. Electrostatic interactions are important in the head-to-head interfaces, and the stem regions adopted two parallel coiled-coil helices resembling the leucine zipper structure. The head region of KOR01 HA was found to be rotated clockwise by ~20° relative to the stem region, and a constructed trimeric model of KOR01 HA based on superposition of stem regions adopts a relatively open and blown-out conformation, compared to a typical trimer. This novel structural arrangement may suggest an additional critical role of HA1 which is known to separate from HA2 during membrane fusion.

Poster 63

Solution NMR structure of MED25(391-543) - the viral transcription activator-interacting domain of human mediator subunit 25

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The Mediator complex is an essential part of the eukaryotic RNA polymerase type II (RNAP II) machinery for gene transcription [1]. 747-residue subunit 25 of the human mediator (MED25) was identified as the target of the acidic transactivation domains of the Herpes simplex type 1 protein VP16 [2], and the Varicella-zoster virus major transactivator protein IE62 [3]. Both transactivation domains bind to the polypeptide segment of MED25 comprising approximately residues 390 to 540, called activator-interacting domain (ACID). We present the solution NMR structure of MED25(391-543), comprising the ACID domain of subunit 25 of the human mediator. The structure is similar to the beta-barrel domains of the human protein Ku and the SPOC domain of human protein SHARP, and provides a starting point to understand the structural biology of initiation of HSV-1 and VZV gene activation.

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Poster 64

Crystal structures of murine norovirus RNA-dependent RNA polymerase complexes

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Norovirus, a member of Caliciviridae family is the leading cause of viral gastroenteritis worldwide. Bovine and murine norovirus-1 (MNV-1) are the common infective forms found in animals. MNV-1 is used as the surrogate for HNV research since it is the only from that can be grown in cellular culture. Crystal structures of recombinant MNV-1 RNA dependent RNA polymerase (RdRp) has separately been determined in complex with its protein primer Vpg and known inhibitors ribavirin and 5-fluorouracil (5FU). The overall structure has typically similar right hand fold as that of RNA polymerases. Crystals with C2 symmetry revealed a dimer with half a dimer in the asymmetric unit and the protein exists predominantly as a monomer in equilibrium with a smaller population of dimers, trimers and hexamers in solution. The binding mode of ribavirin and 5Fu in the active site confirms the conservation of enzyme catalysis in viral RdRp. The extensive network of interactions mediated by water and metal ions stabilizes the inhibitor in the active site. The protein primer Vpg which is predicted to be an intrinsically disordered protein has been modeled near the base of thumb domain. These complex structures will lead us a step forward in understanding the replication of this virus.

Poster 65

Combining in vitro and in silico studies: structural genomics of Schistosoma mansoni redox proteins

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Parasitic diseases are a major threat to human health, especially in tropical and sub-tropical countries, where access to treatment is extremely limited. In particular, helminthic infections are devastating in terms of morbidity and mortality. Moreover the number of current available drugs is very poor, thereby the identification of new macromolecular targets is of utmost importance. The advances in genomics and proteomics have prompted this task to more rational and interdisciplinary strategies. The research carried out at the Dept. of Biochemical Sciences is a structural genomics project focussed on the thiol-mediated detoxification pathway of Schistosoma mansoni, the major causative agent of human schistosomiasis. This metabolic pathway shuttles electrons from NADPH to reactive oxygen species (ROS) through several interacting proteins, all of whom are expressed throughout the complex parasitic life cycle. Among these antioxidant enzymes, some are highly conserved between the parasite and the host, while a couple are unique to the parasite (at least in their active site), hence good candidates as new targets. We use molecular, biochemical and bioinformatics tools to functionally and structurally characterise these redox proteins, in order to obtain as much information as possible, later to be used in a challenging structure-based drug design, starting from known lead compounds [1,2]. Here I shall present what we are learning about Glutathione Peroxidase (Gpx) and Thioredoxin Glutathione Reductase (TGR). Gpx is at the bottom of the enzymatic cascade reducing phospholipid peroxides [3], while TGR is the acceptor of NADPH electrons [4].

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Poster 66

Exploring the druggability of Hsp90 from protozoan parasites

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Heat shock protein 90 (Hsp90), named after its apparent molecular weight, is a molecular chaperone whose functions include assisting protein folding and maturation. It is composed of three distinct domains, an N-terminal ATP binding domain, a middle domain and a C-terminal domain involved in substrate binding and dimerization. Its chaperone activity is coupled with ATP hydrolysis that triggers conformational changes, including a transient association of the N-terminal domains. The relationship between the ATPase and chaperone activities is quite complex in part due to the fact that several accessory proteins could modify the Hsp90 activity. Nevertheless, it has been shown that interfering with the ATPase activity, either by mutation or pharmacological means, severely compromises Hsp90 functions. Therefore the majority of Hsp90 inhibitors targets the ATP binding site in the N-terminal domain. Several of these inhibitors have reached clinical stages as anticancer therapeutics, showing high selectivity and low toxicity profiles. In the case of infectious diseases, some of these inhibitors have shown anti-parasitic activities, validating Hsp90 as an attractive drug target. In parasites, the chaperons has an additional role contributing to the survival under drug exposure which is directly linked to Hsp90's involvement in stress responses and represents an important source of phenotypic resistance. Therefore, hampering the chaperone activity will reduce the pathogen's ability to combat the drug effects and may limit the occurrence of resistant strains. To fully explore the 'druggability' potential of parasitic chaperones at the SGC we are characterizing Hsp90 from seven protozoan parasites, which include the causative agents of malaria, sleeping sickness and leishmaniasis. Our approach comprises the chemical profiling of each parasitic chaperone against a panel of known Hsp90 inhibitors, through a variety of biophysical and enzymatic techniques, such as DSF, inhibition assays and ITC. Despite the limited size of our library, clear substrate preferences have emerged for each parasitic Hsp90 tested. Furthermore, a compound has been identified with a marked selectivity for several parasitic chaperones with respect to their human counterparts. Crystal structures of the N-terminal domain of these parasitic Hsp90 in complex with several inhibitory compounds, including the one showing the most promising selectivity, have revealed the structural plasticity present at the ATP-binding site. The structural results together with our chemical profiling provide a strong foundation for the implementation of a medicinal chemistry program to optimize this initial compound as an inhibitor of parasitic Hsp90s. Besides the potential of compounds like this as scaffolds to explore in a drug discovery program, they can also serve as starting point for the development of chemical probes, specific reagents to explore the biological roles of parasitic Hsp90s.

Poster 67

Characterization of essential kinases from protozoan parasites

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Calcium-dependent protein kinases (CDPKs) play pivotal roles in the calcium-signaling pathway in plants, ciliates and apicomplexan parasites, but are absent in animals or fungi. Several CDPKs were found to be essential at key life cycle phases in malaria parasite belonging to the species Plasmodium. In the N-terminus, CDPKs contain a CaMK-like kinase domain, which is regulated by a calcium-binding domain in the C-terminus. We recently determined the first structures of inactivated and calcium-activated CDPKs from Apicomplexa. Calcium binding triggered a large conformational change, representing a new mechanism in calcium signaling and a novel EF-hand fold, thus we set out to determine if this mechanism was universal to all CDPKs. We solved additional CDPK structures, including several from the species Plasmodium. We highlight the similarities in sequence and structure across apicomplexan CDPKs, and strengthen our observations that this novel mechanism could be universal to most canonical CDPKs. Our new structures demonstrate more detailed steps in the mechanism of calcium activation and possible key players in regulation. Because of their essentiality in key infectivity and proliferation stages, we are also working on characterizing the structure and activity of several Plasmodium CDPKs with the aim to identify potential inhibitors.

Poster 68

Surprises from Structural Genomics: Structural differences between two proteins from the DUF3349 superfamily, Mycobacterium tuberculosis (Rv0543c) and Mycobacterium smegmatis (MSMEG_1066), identify a potential biological and structural diversity within th

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A superfamily of proteins with a "Domain of Unknown Function", DUF3349, is present predominately in Mycobacterium and Rhodococcus bacterial species suggesting that these proteins may have a biological function unique to these bacteria, and consequently, may prove to be an attractive drug target to combat tuberculosis. Recently, the first structure for a member of this superfamily, Rv0543c, was determined as a community request by the Seattle Structural Genomics Center for Infectious Diseases (SSGCID) using NMR-based methods (Arch. Biochem. Biophys., 506:150-156). The structure of the protein (2KVC) consists of a bundle of five α-helices α1 (M1 - Y16), α2 (P21 - C33), α3 (S37 - G52), α4 (G58 - H65) and α5 (S72 - G87) held together by a largely conserved group of hydrophobic amino acid side chains. Both size exclusion chromatography and NMR spectroscopy show that Rv0543c is a monomer in solution while circular dichroism spectroscopy indicates that Rv0543c irreversibly unfolds upon heating with an estimated melting temperature of 62.5ºC. More recently, a crystal structure was determined for another protein in the DUF3349 superfamily, Mycobacterium smegmatis, a protein with 38% identity and 61% similarity with Rv0543c. Like Rv0543c, the structure of MSMEG_1066 (3OL4) also contains a bundle of five α-helices. However, except for the first N-terminal two helices, the orientation of the helices is drastically different in Rv0543c and MSMEG_1066. Furthermore, size exclusion chromatography and NMR spectroscopy suggest the protein is a dimer in solution, as observed in the crystal structure, while circular dichroism spectroscopy indicates the protein reversible refolds after heating to 80ºC. Binding assays show that MSMEG_1066 will not bind the same ligands that Rv0543c will bind. Taken together these results suggest that the amino acid sequences of the DUF3349 superfamily of proteins must be more closely examined as initial biophysical studies suggest this family of proteins perhaps should be subdivided into two subfamilies with distinct structural and physical properties. Understanding these differences are essential if proteins in the DUF3349 superfamily are to be used for rational structure-based drug design. This research was funded by the National Institute of Allergy and Infectious Diseases, National Institute of Health, Department of Health and Human Services, under Federal Contract number HHSN272200700057C.

Poster 69

Structural insight into the function of effector proteins – bacterial pathogenic factors orchestrating eukaryotic cell biology

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Many Gram-negative bacteria interact with human, animal, or plant hosts and inject a specific set of proteins called effectors into the cytosol of host cells through various (type III, IV and VI) secretion systems. While the specific function of the majority of effector proteins remains unknown collectively they share the ability to alter host cellular pathways/systems to promote the bacterial life-style and contribute to virulence. Distantly related bacterial pathogens may harbour closely related effectors, demonstrating that one pathogenicity mechanism can give rise to a multitude of diseases that range from bubonic plague in humans to fire blight in fruit trees. Thus, characterization of the specific functions of effectors represents a major task for research into bacterial pathogenesis. We have structurally and functionally characterised representatives of several common families of effectors, including IpaH, NleG and HopA1. Our results demonstrate that these bacterial proteins are able to mimic cellular functions otherwise specific to eukaryotic proteins and interfere with critical cell processes such as the ubiquitination signalling system.

Poster 70

Crystal Structure of Aspartate Racemase from Salmonella typhimurium in Complex with Substrate, Product and their Analogs.

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D-stereoisomers of amino acids, although much less common than L, are shown to play important roles both in Prokaryotes and Eukaryotes. D-stereoisomers are synthesized from L by amino acid racemases which catalyze a proton-transfer reaction to invert a chiral center Cα of an amino acid. There are two kinds of racemases, the pyridoxal 5'-phosphate (PLP)-dependent ones and PLP-independent ones. Aspartate racemase is a representative of (PLP)-independent amino acid racemase. In eubacteria, it is important potential drug target since its product D-aspartic acid is essential component of peptidoglycan layer in bacterial cell wall protecting the organism from osmotic lysis. Although there are a few homologous structures, they have relatively low sequence identities (36% and lower). At the Center for Structural Genomic of Infectious Diseases (CSGID), using the high throughput tools developed in the center, we determined the crystal structure of initially putative aspartate racemase from Salmonella typhimurium. We confirmed the enzyme activity by finding both L- and D-aspartic acids in the active site after co-crystallization of the enzyme with L-aspartic acid. Aspartate racemase structures with several different ligands have also been determined. The crystal structure, the mode of ligand binding as well as the mechanism by which the enzyme converts various ligands from L to D-stereoisomer will be discussed based on comparison of enzyme apo-form and complexes of this enzyme with L-aspartic, D-asparctic, L-glutamic, succinic and malic acids. This work was supported by National Institutes of Health, the Contract No. HHSN272200700058C and by the U.S. Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

Poster 71

Structure of Neisseria meningitidis outer membrane protein PorB and a method in membrane protein structure determination

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PorB is the second most prevalent outer membrane protein in Neisseria meningitidis. PorB is required for neisserial pathogenesis and bacterial survival, but also has been attributed to play a role in elicitation of a Toll-like receptor mediated host immune response. During infection PorB likely binds host mitochondrial ATP, which is thought to be important for mitochondrial cellular apoptosis. We have determined the x-ray crystal structure of PorB at 2.3 Å resolution. Structural analysis and co-crystallization studies suggest three distinct putative solute translocation pathways through the channel pore. Co-crystallization with the ATP analog AMP-PNP suggests that binding of nucleotides regulates these translocation pathways both by partial occlusion of the pore and by restricting the motion of a putative voltage gating loop. PorB, located on the surface of N. meningitidis, can be recognized by receptors of the host innate immune system. We propose that features of PorB suggest Toll-like receptor mediated recognition of outer membrane proteins may be initiated by a non-specific electrostatic attraction. Here we also present a strategy for both α-helical and β-barrel type of membrane protein expression, purification and crystallization. Reference 1. M. Tanabe, CM. Nimigean, TM. Iverson, Proc Natl Acad Sci U S A. 107, (2010), 6811-

Poster 72

Antibody Constant Region Effects on Fine Specificity

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A new therapeutic approach against many inflammatory diseases as well as cancers involves the passive administration of antibodies (Ab). One such disease for which experimental passive Ab therapy has shown promise is cryptococcosis. The efficacy of passive Ab therapy against C. neoformans is dependent on the Ab constant region. Antibodies, or immunoglobulin molecules are products of the adaptive immune response that function as antimicrobial proteins with two domains, a constant region (C) shown to confer effector properties and a variable region (V) shown to be responsible for antigen (Ag) binding. Historically, the C and V regions were considered to be functionally independent, with the structure of the V region thought to confer Ag specificity directly without interference from the CH domains since they are separated by a flexible hinge. Here, we used four murine monoclonal antibody isotypes, IgG1, IgG2a, IgG2b and IgG3 of the 3E5 family that share identical variable region sequences against the C. neoformans capsular polysaccharide, glucuronoxylomannan (GXM). Previous studies from our group indicate that within the 3E5 mAb set, differences in isotype translate into differences in fine specificity. Our objective is to identify specific residues and structures of an Ab C region that influence the conformation and specificity of their V regions using biophysical and structural tools. For example, tryptophan fluorescence spectroscopy studies revealed that the emission wavelength maxima are blue-shifted for each of the four isotypes upon binding GXM. Isotype-related differences in the magnitude of this shift, suggest different electronic states that are also consistent with and support differences in paratope. Furthermore, Circular Dichroism spectroscopy showed that the secondary structure composition was different for the native structures of the isotypes, though when bound to native Ag, the changes in secondary structure revealed a pattern that did not correlate with primary sequence homology. Small and Wide Angle X-Ray Scattering has been completed on the isotypes both with and without a peptide mimetic of GXM; P1, that should provide information such as the size, envelope shape, flexibility, hinge angle, oligomerization and surface-to-volume ratio. Lastly, we have been able to obtain the X-ray structure of the 3E5 IgG3 Fab. These techniques are expected to provide structural information to inform the observation that constant region type can affect Ab fine specificity for Ag, an effect that presumably results from constraints imposed on V region structure by adjacent C region sequences.

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Structural characterization of microcin immunity protein MccF from Bacillus anthracis

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Microcins are small ribosomally synthesized antibiotic peptides produced by Enterobacteria. Microcins are secreted into the media to control the level of closely related bacterial species. Microcin biosynthesis in bacteria requires several enzymes and proteins involved in toxin maturation, export and self-immunity. In E. coli, the translation inhibitor microcin C7 (MccC7) is a linear heptapeptide, whose N-terminus has been replaced by an N-formyl group and C-terminus has been replaced by a phosphodiester of 5'-adenylic acid and N-aminopropanol. Five genes, mccABCDE, are required to produce mature extracellular microcin. The sixth gene, mccF, encodes a specific self-immunity protein. In the Bacillus species, microcins are poorly characterized and their role is not fully understood. In an effort to better understand microcins from Gram positive bacteria, we have determined a 2.10 Å crystal structure of the MccF microcin immunity protein from Bacillus anthracis str. Ames. The asymmetric unit contains two molecules, which are associated into a homodimer. Fold analysis and sequence alignment with close structural homologs (serine peptidase, LD-carboxypeptidase) of the MccF protein shows conservation of the hydrolytic triad Ser-His-Glu, suggesting that the MccF protein may display the same activity. Structural details and analysis will be presented. This work was supported by National Institutes of Health, the Contract No. HHSN272200700058C and by the U.S. Department of Energy, Office of Biological and Environmental Research, under contract DE-AC02-06CH11357.

Poster 74

Plasticity of Vibrio and A. baumanii genomes: structural genomics for mobile gene functions

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The wide extent of lateral gene transfer (LGT) presents challenges to genomics of micro-organisms: no one cell line is representative of the gene content of a species as a whole. Instead, prokaryotic genomes harbour features such as pathogenicity islands, integrons or transposons in which are arrayed highly novel genes for niche adaptation and host defence. In many cases, specific function cannot be readily assigned due to lack of sequence homologues within annotated genomes. These bacterial gene targets have proved to be highly amenable to the cloning and screening procedures of structural genomics. I present our output of protein crystal structures from genes derived from Vibrio integron arrays and genomic islands of new Acinetobacter baumanii strains. Our structures reveal new enzyme variants (glyoxalase, acetyltransferase), drug transport/binding proteins and antibiotic resistance factors which all clearly confer fitness advantage. A significant component of our structural output also contains entirely novel protein folds.

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Towards the structure of the Pseudomonas aeruginosa alginate epimerase AlgG

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Pseudomonas aeruginosa is an opportunistic pathogen that forms chronic biofilm infections in the lungs of cystic fibrosis patients. These infections are the leading cause of morbidity and mortality in this patient population. The main component of the bacterial biofilm in the CF lung is the exopolysaccharide alginate, which is synthesized in the cytoplasm as a homopolymer of poly-mannuronate and exported across the inner membrane, periplasmic space and outer membrane by a putative biosynthetic complex consisting of ten proteins. Whilst in the periplasm the poly-mannuronate polymer can be either selectively acetylated at the O2' and/or O3' positions, or modified by the C5 epimerase, AlgG, to produce a random polymer of α1-4 linked βD-mannuronate and αL-guluronate. In addition to its role in epimerization, AlgG has also been suggested to be part of the biosynthetic complex, as mutant strains lacking algG, only produce shorter uronic acids that have been shown to be the by-products of degradation by the alginate lyase, AlgL. To gain insight into the role of AlgG in alginate biosynthesis at the molecular level we have cloned and heterologously expressed in Escherichia coli both Pseudomonas aeruginosa AlgG and its P. syringae sp homologue. These proteins share 61% sequence identity. A four-step purification protocol involving metal ion affinity chromatography, removal of the histidine-tag with thrombin (an optional step), followed by size exclusion and then ion exchange chromatography has been developed. This protocol produces ~9 mg protein/L culture of each protein that is greater than 95 % pure as judged by SDS-PAGE. Correct folding of AlgG was confirmed using circular dichroism spectroscopy. A site-directed mutant of P. aeruginosa AlgG that abrogates its activity has also been cloned, expressed and purified. Crystallization trails of the P. aeruginosa and P. syringae apo-proteins, and the D324A mutant in complex with a poly-mannuronate trimer are in progress. As limited proteolysis experiments suggest the presence of a smaller stable core, in situ proteolysis crystallization trails have also been setup. Our progress toward the structure determination of AlgG will be presented. This research is funded by grant from the Canadian Institutes of Health Research to P.L.H. & the National Institutes of Health to P.A.T.

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Structural Genomics Studies of the Proteins Involved in the Synthesis, Degradation, and Reception of c-di-GMP in Xanthomonas campestris

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Cyclic-di-GMP is a universal secondary messenger molecule that was first described as an allosteric activator of cellulose synthase in Gluconacetobcter xylinus, but is now known to be a crucial molecule that regulates a diverse range of important cellular functions, including virulence, biofilm formation, cellular morphology, and motility in most eubacteria. Its local concentration in the cell thus needs to be tightly regulated. To date, the synthesis, degradation, and functions of cyclic-di-GMP are issues that are being actively investigated. It is now well recognized that cyclic-di-GMP is synthesized from two molecules of GTP by diguanylate cyclase (DGC) containing the GGDEF domain and degraded by phosphodiesterase (PDE) containing the EAL-domain or HD-GYP-domain. Cyclic-di-GMP is also able to bind with a number of effector proteins such as Clp from X. campestris, PelD regulator from P. aeruginosa, FleQ regulator from P. aeruginosa and various proteins with a PilZ domain to regulate a wide variety of downstream gene expression. Furthermore, cyclic-di-GMP is also found to be regulated by the quorum sensing mechanism, which leads to expression of virulence genes. Since anti-virulence has been proposed as a superior strategy for combating the drug resistance problem of pathogenic bacteria, understanding the structures and functions of c-di-GMP related proteins will help develop next generation antibiotics toward this goal. Xanthomonas campestris is a pathogen that contains more than 30 proteins that are related to the metabolism of c-di-GMP, hence it is an ideal organism for structural genomics studies. In recent years, we have solved a number of proteins in this respect; 1) The RpfF structure that is responsible for synthesizing signals for quorum sensing; 2) One monomeric PilZ and one tetrameric PilZ noncanonical proteins; 3) The first transcription factor CLP that binds c-di-GMP; 4) A HAMP-GGDEF domain protein that exhibit a novel competitive inhibition mode for c-di-GMP synthesis; 5) An EAL domain protein that contain a novel c-di-GMP binding motif. The structures and functions of these proteins will be discussed in this meeting.

Reference: Chin K-H, et al (2010) The c-AMP receptor-like protein Clp is a novel c-di-GMP receptor linking cell-cell signaling to virulence gene expression in Xanthomonas campestris. J Mol Biol 396, 646-662.

Poster 77

Early structural studies of potential Burkholderia pseudomallei antigens

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Burkholderia pseudomallei, a Gram-negative soil bacterium, causes Melioidosis an infection affecting both humans and animals. This illness was first described in 1912, but since 2000, Melioidosis has become endemic in tropical areas of Southeast Asia and Northern Australia. Its diagnosis is difficult since the symptoms mimic different infections, such as Tuberculosis. This polymorphism and the high resistance of B. pseudomallei (listed as a biological threat agent of class 3) are responsible for the high rate of mortality. Since antibiotic therapies are scarcely effective against Melioidosis and does not lend itself to administration in developing countries, vaccination is an attractive approach against this threat. It is known that the classical approach to vaccine development is time-consuming, and only allows the identification of few antigens candidates. More recently, a new strategy called the ‘Reverse Vaccinology approach’ (RV) has been introduced. Antigen candidates are thus selected using complementary and synergistic methods, such as functional genomics, protein microarrays, bioinformatics/computational biology. In the search for potential vaccine candidates, RV cannot stand alone and must be supported by further studies, including expression, purification of selected antigens for immunization trials. It is well established that not all included antibodies are protective. In this context, we aim to study several B. pseudomallei potential antigens by X-ray crystallography, with the aim of identifying structural features of protective antigens that discriminate them from non-protective antigens. To date, 21 constructs have been designed for heterologous expression in E. coli as GST or His-tagged fusions proteins. The progress made to date is here-reported, together with the methodology of our approach.

Poster 78

Mycobacteria tuberculosis: from a structural genomics to systems biology

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Tuberculosis is a re-emerging major threat of global health because of the increasing occurrence of hyper multiple drug resistance and a major problem of lethality in HIV positive patients. The knowledge of the complete genome of Mycobacterium tuberculosis provides an outstanding opportunity to systematically image the protein components associated and ligand complexes at near atomic resolution. At the European Molecular Biology Laboratory, we have set up a pipeline for rapid and reliable determination of more than 60 structures of M. tuberculosis targets, primarily using X-ray crystallography. A number of specific developments in the area of sample preparation have aided to accelerate this process [1,2]. The targets we are investigating can be basically divided in three categories: function known, function can be predicted, function unknown. Therefore, there is a major challenge to complement structural data with functional data, based on purified proteins, in mycobacterial cultures, cell cultures and animal models. Some of our concepts, to tackle this challenge, have been illustrated in high light examples [3,4]. In a new project to to establish an integrated system biology framework on Mycobacteria tuberculosis [http://www.systemtb.org/], structural biology is now used as a high-end tool for quantitative interpretation of various "omics" data. We report about recent mostly unpublished advances to combine high resolution structural biology with proteomics [5], metabolomics, lipidomics and transcriptomics. Our ultimate aim is to demonstrate structural biology as a tool to facilitate the development of new drugs, vaccines and markers against tuberculosis. [1] Poulsen et al. (2010) Stoichiometric protein complex formation and over-expression using the prokaryotic native operon structure. FEBS Lett. 2010 Feb 19;584(4):669-74. [2] Noens et al. (2011) Improved mycobacterial protein production using a Mycobacterium smegmatis groEL1DeltaC expression strain, submitted. [3] Ma et al. (2006) The Mycobacterium tuberculosis LipB enzyme functions as a cysteine/lysine dyad acyltransferase. Proc Natl Acad Sci U S A. 103:8662-7. [4] Due et al. (2011) Bisubstrate specificity in histidine/tryptophan biosynthesis isomerase from Mycobacterium tuberculosis by active site metamorphosis. Proc Natl Acad Sci U S A. 2011 Feb 14. [5] Poulsen et al. (2010) Proteome-wide identification of mycobacterial pupylation targets. Mol Syst Biol. 6:386.

Poster 79

Structural characterization of the receptor-binding domain of the D and C/D mosaic neurotoxins produced by Clostrodium botulinum

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The most lethal natural protein toxins for humans are the botulinum neurotoxins (BoNTs) produced by different strains of the bacterium Clostridium botulinum, the etiological agent responsible for the deadly neuroparalytic disease botulism. A group of four immunologically distinct serotypes (A, B, E, and F) are associated with the human disease while two serotypes (C and D) are responsible for deadly botulism outbreaks afflicting animals. The mechanism of neurotoxin entry into neuronal cells, using both protein and ganglioside receptors on the neuronal cell surface, is well understood for serotypes A, B, E, F and G. However, the entry mechanism for serotypes C and D remains unclear. To obtain structural insights into the mechanism of neuronal cell entry for BoNT-C and BoNT-D, the crystal structures for the heavy chain receptor-binding domain (HCR) of BoNT-D (S863-E1276) and the BoNT-C/D mosaic (S867-E1280) were determined, with assistance of the Seattle Structural Genomics Center for Infectious Disease (SSGCID), to 1.65 and 1.56 Å resolution, respectively. In general, the crystal structures of BoNT-D (3OGG, Biochem. Biophys. Res. Commun., 401:498-503) and BoNT-C/D (3PME, Biochem Biophys. Res. Commun. 404:407-412) are similar to the two sub-domain organization observed for other BoNT HCRs: an N-terminal jellyroll barrel motif and a C-terminal β-trefoil fold. However, some subtle structural differences are observed that may be related to biological differences. These structural differences will be presented in detail and discussed in conjunction with liposome-binding experiments demonstrating that BoNT-C/D-HCR binds phosphatidylethanolamine liposomes much stronger than BoNT-D-HCR. This research was funded by the National Institute of Allergy and Infectious Diseases, National Institute of Health, Department of Health and Human Services, under Federal Contract numbers HHSN272200700057C and award number U01AI081895.

Poster 80

Crystal structures of penicillin-binding protein 3 from Pseudomonas aeruginosa in both apo- and acyl-enzyme forms reveal the molecular basis of therapy.

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Pseudomonas aeruginosa is an opportunistic pathogen responsible for severe life-threatening infections especially in patients suffering from chronic respiratory diseases such as cystic fibrosis. Compared to many other Gram-negative bacteria, P. aeruginosa is highly resistant to antibiotics and, as with many bacterial pathogens, resistance increases with repeated use of antibiotics. Penicillin binding protein 3 (PBP3) is a therapeutic target in P. aeruginosa and we have determined structures of this PBP in its native form and in complex with several beta-lactams, including ceftazidime, a frontline antibiotic for treating pseudomonas infections. We present the similarities and differences between the structures and discuss structural features that might be exploited to derive novel inhibitors of therapeutic value.

Poster 81

A quest for novel antibiotics against MRSA

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Antibiotics can be considered as one of the most important discoveries of the 20th century and are saving millions of lives worldwide every year. Most of the present antibiotics in the market are simple modifications of older products which allow pathogens to gain resistance quickly and evolve as a superbug. MRSA (methicillin-resistant Staphylococcus aureus) caused 18,650 deaths in the United States alone in 2005. In the UK, MRSA cost 1593 lives from 100,000 infections in 2007. There is therefore an urgent need to develop new antibiotics to fight against this deadly pathogen. The modes of action of present antibiotics target protein synthesis, nucleic acid synthesis, cell-wall synthesis or folate synthesis in bacteria. None of the clinically approved antibiotics targets bacterial cell division, an essential process in the bacterial life cycle. Inhibition of cell division has proven to have an immediate bactericidal effect in cocci shaped Staphylococcus aureus, whereas in rod shaped bacteria Bacillus subtilis cells are less viable. The aim of the project is to undertake a structural investigation of FtsZ protein from a cocci-shaped bacteria MRSA and rod-shaped bacteria Vibrio cholerae, followed by fragment based inhibitor design to inhibit ftsZ. FtsZ is an essential cell division component which is localized specifically to the midcell to form a Z-ring structure during cell division and is also well conserved among bacterial species with very few exception such as Chlamydia and some archeal species. In 2008, it was discovered that the synthesized compound PC190723 shows inhibition against MRSA_ftsZ and prevents cell growth. Unfortunately, there is no structure of MRSA_ftsZ or complex with PC190723 till to date. Both genes from MRSA and Vibrio cholerae (VC) have already been cloned, expressed, and the proteins have been purified. An initial crystal hit of MRSA-ftsZ diffracted at low resolution and is currently under optimization. Initial crystals of VC-ftsZ are also under optimization. Co-crystallization of MRSA_ftsZ and PC190723 produced some micro-crystals and optimizations are ongoing. A high resolution complex structure of MRSA_ftsZ and PC190723 will give us an exclusive prospect to make further modifications of PC190723 to make a valid drug against MRSA in the very near future.

Poster 82

Bacterial Surface Lipoproteins Provide Novel Antimicrobial Targets

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Host-restricted bacterial pathogens share the ability to display proteins anchored to the extracellular surface, surface lipoproteins (SLPs), that function in essential processes including, immune evasion and nutrient uptake. These SLPs elicit bacteriocidal antibodies and have been targeted for vaccine development to provide coverage against pathogens such as Neisseria meningitis a causative agent of meningitis. A good example of an SLP is the Transferrin (Tf) binding protein, TbpB. Not only does TbpB possess a lipidated amino terminus that anchors it to the cell surface of Neisseria, it also binds the host glycoprotein Tf. TbpB functions to recruit iron-loaded Tf to the surface of the bacterial outer membrane, providing the essential nutrient iron to the bacterial cell during host colonization. Herein we describe structures of TbpB and define its binding interface with Tf. Surprizingly, the Tf binding surface on TbpB is not conserved in structure or sequence, rather it is in a hyper-variable region of the protein that is undoubtedly necessary to prevent immune detection. Despite this hyper-variability of TbpB, the overall interaction interface between TbpB and Tf is maintained. Our structures explain the mechanism of Tf:TbpB binding and provide insight into the development of SLP based vaccines.

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Substrate capture mechanism provides a mode for inhibition

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Mycobacteria tuberculosis (Mtb), the causative agent of tuberculosis, is responsible for more death in the world today than any other bacteria. As part of the Tuberculosis Structural Genomics Consortium (TBSGC), our research group previously determined the structure of anthranilate phosphoribosyl transferase (AnPRT) from Mtb. AnPRT is the second enzyme in the tryptophan biosynthetic pathway and was identified as a potential drug target through gene knockout experiments, which resulted in a strain of Mtb that was essentially avirulent even in immunodeficient mice. AnPRT catalyses a reaction between anthranilate and phosphoribosylpyrophosphate (PRPP), and the crystal structure of Mtb-AnPRT was originally determined with and without PRPP (PDB ID: 1ZVW and 2BPQ, respectively). In silico docking was used to predict the binding motif of anthranilate, the second substrate, surprisingly predicted two sites despite a 1:1 reaction ratio with PRPP. Previously, 165 compounds were screened for inhibitory action against Mtb-AnPRT. The most potent of these compounds was co-crystallized with Mtb-AnPRT and PRPP. One compound had a bianthranilate character and the 2.0 Å resolution structure of this inhibitor bound to Mtb-AnPRT (PDB ID: 3QQS) was determined by molecular replacement using the Mtb-AnPRT structure without PRPP bound (PDB ID: 1ZVW) as a search model. Interestingly, the structure revealed multiple binding motifs for the inhibitor, two of which were consistent with the previously predicted binding motifs for anthranilate. Forty analogues of this potent Mtb-AnPRT inhibitor were subsequently assayed for activity against the enzyme, several of which showed were found to be more potent inhibitors. This new series of inhibitors were docked into the 3QQS structure, providing insights for the development of more potent inhibitors. Such techniques will continue to drive design of increasingly potent inhibitors against Mtb-AnPRT for future development of a new anti-tuberculosis agent.

Poster 84

Structural Genomics and Homology Analysis of Proteins on the Salmonella Pathogenic Island 6: an Emerging Picture of Early Host-Pathogenic Signaling

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A surprisingly large percentage of bacterial proteins still remain uncharacterized, even decades after the complete sequencing of their genomes. Many of these proteins have been shown by genome analysis or high throughput experimental approaches to be integral in important processes, such as virulence in pathogenic bacteria. Using Salmonella typhi as a model pathogen, we focus on a subset of uncharacterized proteins in pathogenic island 6 (SPI-6) that are predicted to be secreted to the extracellular space or surface of the outer membrane. We have assigned functions to these proteins through distant homology and analysis of both modeled and experimental 3D protein structures. Additionally, we combine existing information from high-throughput proteomic, co-expression, and protein-protein interaction data to contextualize the role of these proteins in the presence of a host. The emerging picture suggests that SPI-6 proteins are involved a complex interaction network with the host, similar to the host-bacteria interactions between symbiotic, mutualistic or commensal organisms. These insights provide a better background to study specific pathogenic adaptations of S. typhi and understand the very early stages of infection, before activation of the late-stage virulence mechanisms.

Poster 85

Structure-based drug development for the inhibition of antibiotic resistance

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Antibiotic resistance is a serious and growing public health concern. Resistance is conferred by multiple mechanisms, including reduced drug accumulation through efflux out of the bacterial cell, alteration of the target site, or chemical modification/destruction of the antibiotic. The antibiotic kinases aminoglycoside phosphotransferases (APHs) deactivate aminoglycoside drugs by phosphorylation using ATP in a substrate- and site-specific manner, such that they are less effective at inhibiting bacterial protein translation. We are interested in developing inhibitors of APHs as possible therapeutic interventions for antibiotic resistance. To that end, we are carrying out a structure genomics program to crystallize APH enzymes and in the presence of their substrates and potential inhibitors. This work is in collaboration with Dr. Gerry Wright's laboratory at McMaster University, Hamilton, Ontario. In this poster, we present one structural basis of the inhibition of APH enzymes. We present the structure of APH(2'')-IVa with quercetin, a flavanoid compound that is a general eukaryotic protein kinase inhibitor. We also present structure-activity-relationship data that explores flavanoid inhibition of APH(2'') enzymes. This class of inhibitor forms interactions mostly with the backbone of the APH(2'')-IVa enzyme. It is possible that the flavanoid backbone could be modified to increase specificity for APH enzymes. We also present the structure of APH(3')-Ia in complex with ATP; this structure will be important for that rational design and understanding of the mechanism of ATP-competitive inhibition. It is our belief that structures will be pivotal in the rational development of therapeutic interventions for the alleviation of aminoglycoside drug resistance.

Poster 86

Fine-tuning of the Reverse Vaccinology Approach by X-ray Crystallography: From Genome to Antigen

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One of the main obstacles for vaccine development is the identification of antigens that stimulate an immune response. Over the last decade, a genome-based in silico method called Reverse Vaccinology (RV) has facilitated the selection of secreted or surface-exposed protein antigens from many pathogens, including bacteria. As the immunogenic capacity of an antigen is determined by epitope formation, which, in turn, depends on its three-dimensional structure, stability and related dynamics, RV ideally should be reinforced by structural biology-related studies, that will help drive antigen construct selection and the rational design of epitope-based vaccines. Identification of the precise structural properties that render an antigen protective i.e. capable of eliciting the production of bactericidal antibodies, is the biggest challenge challenge. In this context, we carry out the crystallisation and X-ray diffraction analysis of protective and non-protective antigens from Group A and Group B Streptococci and, more recently, Burkholderia pseudomallei. The aim is to determine the 3D structures of these antigens in order to develop criteria that can distinguish protective from non-protective antigens, prior to immunization tests, with the ultimate aim to accelerate the antigen selection process and hence, vaccine development. The multidisciplinary approach used in our medium-throughput, structural genome to antigen project is here-described, together with the crystal structures of some of our antigen targets, one of which is an actual vaccine candidate.

Poster 87

A structural profiling of the exctracellular ligand binding domains of Human Metabotropic Glutamate Receptors (groups I, II and III)

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L-Glutamate is a major excitatory neurotransmitter in the mammalian nervous system. It acts via gating in two classes of receptors - ion channels (ionotropic) and G-protein coupled (metabotropic) receptors. The metabotorpic Glutamate receptors (mGluR's) function in variety of neurological processes and are validated targets for drug design. According to sequence homology, associated second messenger systems, and pharmacological properties the eight human mGluR's are divided into three subgroups (Groups I, II, and III) We report a comparative structural study of the mGluR extracellular ligand binding domains that covers all three groups. Both members of Group I are presented by structures of mGluR1 complexed with LY341495 antagonist (PDB 3KS9) and mGluR5 with glutamate (PDB 3LMK). Group II is represented by a low resolution structure of mGluR3, and that of group III by mGluR7 with LY341495 antagonist (PDB 3MQ4). The proteins form homodimers with either "active" (A) or "resting" (R) conformation at the dimeric interface. Binding of an agonist mediates a "closed" conformation of the protomer while an antagonist stabilizes an "open" conformation. The dimer can therefore adopt a spectrum of conformations: open-open/A, closed-closed/A, open-open/R, closed-closed/R. According to the currently adopted equilibrium model, the open-open/A conformation is energetically disallowed. Surprisingly, mGluR1 with bound antagonist adopts this "disallowed" conformation. This indicates that the equilibrium model needs critical re-evaluation.

Poster 88

Crystal structures of human tankyrase in complex with novel inhibitors

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Tankyrases (TNKS1/PARP5a/ARTD5 and TNKS2/PARP5b/ARTD6) belong to a family of ADP-ribosyltransferases (PARPs), enzymes that catalyze ADP-ribosylation of a number of nuclear and cytosolic proteins. ADP-ribosylation is a reversible post-translational modification that affects a variety of fundamental cellular processes, such as transcriptional regulation and maintenance of genome stability. Tankyrases are better known for their role as positive regulators of telomere elongation. Tankyrases also act as positive regulators of Wnt signaling through regulation of axin levels. Inhibitors of tankyrase catalytic activity have a potential for use as therapeutic agents in cancers and also as research tools in the study of Wnt-signaling and DNA repair pathways. We assembled a ~200 compound collection of previously known PARP inhibitors and chemically related molecules. We used surface plasmon resonance and thermal shift assays to profile these compounds against human PARP catalytic domains in general and the tankyrases in particular. A number of verified hit compounds were assayed for their effect on autocatalytic activity of tankyrases. The result is a selectivity profile of some widely used research tools. In addition, we solved crystal structures of TNKS2 in complex with six compounds from this collection. These structures point to important structural features of tankyrase-ligand interactions, and they can serve as a basis for rational design of more potent and selective inhibitors.

Poster 89

Inhibition of PDZ domain mediated protein-protein interactions

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PDZ domains are among the most common protein interaction domains. More than 450 PDZ domains in close to 200 human proteins are known and play a key role in cellular signaling e.g. Dishevelled-PDZ (Dvl-PDZ) domain and in organization of multi-protein complexes e.g. Shank-PDZ domain. PSD-95, DlgA and ZO-1 are the three founding members of the family of PDZ domain containing proteins. Dvl proteins are intracellular scaffolds, and there is mounting evidence that the Dvl-PDZ domain is critical for Wnt signal propagation. Dvl transduces Wnt signals from the receptor Frizzled (FZ) to downstream components in canonical and noncanonical Wnt signaling pathways. Up-regulation and overexpression of Dvl proteins have been reported in many cancers, including those of breast, colon, prostate, mesothelium and lung. An example of a protein network mainly organized by PDZ-domain interactions, is the post synaptic density (PSD) in the synapse. The central scaffolding protein of the PSD is Shank which interlinks clusters of glutamate receptors. Mutations in the Shank gene have been ascertained in neuronal disorders e.g. mental retardation, typical autism and Asperger syndrome. Therefore we selected the Dvl3-PDZ and Shank3 PDZ domain as biologically attractive targets for the development of small molecule PDZ ligands.

Poster 90

Application of a protein characterization platform in achieving higher structural genomics success

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The 3D structures of human therapeutic targets are enabling for drug discovery. However, their purification and crystallization remain rate determining. In many cases, ligands have been used to increase the success rate of protein purification and crystallization. In the Structural Genomics Consortium (SGC), we have established a platform to characterize large numbers of purified proteins. This includes screening for ligands by differential scanning fluorimetry and differential static light scattering, enzyme assays, peptide arrays and peptide displacement in a 384-well format. We will report application of this platform in identifying high affinity ligands to generate high resolution x-ray structures of proteins. These structures are essential in developing chemical probes for targeted proteins. The chemical probes are expected to inhibit a protein selectively in a cell and can be a powerful tool to probe the protein target, investigate its biological role and assess its suitability as a pharmaceutical target.

Poster 91

Structural biology on Legionella

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Legionnaires' disease is a severe type of pneumonia with a high fatality rate caused by Gram-negative bacteria of the genus Legionella. With over 600 outbreaks and more than 32.000 cases of Legionnaires' disease between 1995 and 2005 worldwide, the pathogen has become a significant public health risk. Although a limited number of virulence proteins have been identified and partially characterized, structural information on proteins from Legionella is still scarce. Especially the lack of structures of macromolecular assemblies limits our understanding of the intracellular life cycle of Legionella, which is regulated through a complex network of protein-protein interactions of pathogen and host-cell components. At the Institute of Biochemistry in Lübeck we are using X-ray crystallography to determine structures of virulence proteins of Legionella. Additional unknown proteins important for pathogenicity are identified by employing a differential proteomics approach. Resulting 3D-protein structures are used for rational design of mutant proteins to correlate structure and function and rational structure-guided development of new antibiotics. We are especially interested in large protein assemblies and complexes with host-cell proteins. In an effort to identify new factors contributing to virulence in a proteomics approach the protein expression level of L. pneumophila wildtype and a FlaR-deficient strain was analysed. Five proteins with significantly changed expression profiles could be identified: HtpB, PilN, trigger factor and two additional proteins of unknown function. As a first target for structural characterization the type-IV pilus biogenesis protein PilN essential for pilus assembly was chosen, purified and crystallized. The full structure determination of PilN will help clarify the role of the pilus during infection of host cells. Furthermore, we have determined the 3D-structures of three important virulence proteins from Legionella: Mip (macrophage infectivity potentiator protein), a dimeric protein expressed on the cell surface, exhibits a peptidyl prolyl cis-trans isomerase activity and is essential for the initiation of the infection. Based on the structure using in-silico screening we recently identified promising small molecule inhibitor hits. HtrA (heat shock requirement A) is a periplasmatic protein with protease and chaperone function, its activity is regulated by oligomerization. We have determined the structure of the HrtA homolog in Legionella, DegQ, which forms a 580 kDa dodecameric complex that encapsulates a large internal cavity. The structure led us to propose a model for regulation of the catalytic activity of DegQ. FeoB, a polytopic transmembrane GTPase involved in bacterial ferrous iron acquisition, contributes to virulence in a number of intracellular pathogens. We determined the structure of the cytosolic domain of FeoB in free and substrate-bound form.

Poster 92

Insights into CARM1 methylation: design of selective inhibitors and peptide mimics. A structure based approach.

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Post-translational methylation of arginine is a widespread epigenetic modification found in eukaryotes that is catalyzed by the protein arginine methyltransferases (PRMTs). PRMTs have been implicated in a variety of biological processes, such as regulation of transcription, translation and DNA repair. At least nine members of PRMTs (PRMT1 to PRMT9) have been identified and classified into two main classes. Coactivator-associated arginine methyltransferase 1 (CARM1, also known as PRMT4) was identified as an enhancer of the transcriptional activation by several nuclear hormone receptors. CARM1 is a crucial protein involved in many biological processes including the regulation of chromatin structure and transcription via methylation of histones and many transcriptional cofactors. As such, understanding the detailed mechanism of action of this protein at the structural level is important and has implications ranging from pure structural information to potential way of regulating gene expression via inhibitor design. The work presented here combine chemistry, molecular modeling and X-ray crystallography with the aim to address two challenges in the field. The first one is to understand at the atomic level the mode of binding of substrate/product arginine-containing peptides, reflecting states prior and subsequent to methylation. The second one is to design, synthesize and improve by structure based-drug design compounds that can inhibit CARM1 methylation activity. We have previously solved several crystal structures corresponding to three isolated modules of CARM1. Crystal structures of the CARM1 catalytic module revealed large structural modifications and have shown that the NH2-terminal and the COOH-terminal end of CARM1 catalytic module contain molecular switches that may inspire how CARM1 regulates its biological activities by protein-protein interactions. Moreover, our recent structural and functional studies have shown that peptides outside the catalytic core of CARM1 are essential for substrate binding and recognition. Full detailed analysis of new structures of CARM1 in the presence of substrates mimics will be presented. We will also present our last results concerning the development of news selective inhibitors of PRMTs.

Poster 93

Structural basis for compound C inhibition of the human AMP-activated protein kinase α2 subunit kinase domain

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AMP-activated protein kinase (AMPK) functions as a sensor to maintain energy balance, and is therefore a potential target for drug design against metabolic syndrome. We have determined the crystal structure of the complex between the phosphorylated-state mimic T172D mutant AMPK α2 kinase domain and a selective inhibitor, compound C, and revealed the unique inhibitor-binding mode of this protein kinase.

Poster 94

Small Molecules Targeting Bromodomains

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Bromodomains recognize acetylated lysines and play important roles as readers for epigenetic modifications. There is emerging evidence that the acetyl lysine binding pocket in bromodomains is druggable. The SGC Epigenetics chemical probe project aims to develop tool compounds targeting bromodomains, which will not only give tools to dissect the biological function of acetyl lysine signaling, but also provide new areas for drug discovery. Here we present the small molecule hits that we have discovered for several diverse sub-families of bromodomains by biochemical screening of compounds selected from two different approaches, virtual ligand and fragment screening. These hits have been validated by biophysical methods and X-ray co-crystal structures, and represent excellent starting points for developing selective chemical probes for bromodomains.

Poster 95

Aminocoumarin Biosynthesis: One Pathway, Many Paths to Structure Determination

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The aminocoumarin antibiotics are natural products of soil-dwelling bacteria called Streptomycetes. They are potent inhibitors of DNA gyrase, an essential bacterial enzyme and validated drug target (1), and thus have attracted considerable interest as potential templates for drug development. To date, aminocoumarins have not seen widespread clinical application on account of their poor pharmacological properties. Through studying the structures and mechanisms of individual biosynthetic enzymes we will be better informed to redesign of these compounds through rational pathway engineering. Novobiocin, the simplest compound, requires at least 17 gene products to convert primary metabolites into the mature antibiotic (2). We have solved the crystal structures of five of these biosynthetic enzymes (3-7) The structure determinations have ranged from the trivial to the challenging and have necessitated a variety of different approaches. The path from crystal to structure will be described for each enzyme. Rerefences 1. Maxwell, A. & Lawson, D. M. (2003). The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. Curr. Top. Med. Chem. 3, 283-303. 2. Steffensky, M., Mühlenweg, A., Wang, Z. X., Li, S. M. & Heide, L. (2000). Identification of the novobiocin biosynthetic gene cluster of Streptomyces spheroides NCIB 11891. Antimicrob. Agents Chemother. 44, 1214-1222. 3. Jakimowicz, P., Tello, M., Meyers, C. L., Walsh, C. T., Buttner, M. J., Field, R. A. & Lawson, D. M. (2006). The 1.6-A resolution crystal structure of NovW: a 4-keto-6-deoxy sugar epimerase from the novobiocin biosynthetic gene cluster of Streptomyces spheroides. Proteins 63, 261-265. 4. Keller, S., Pojer, F., Heide, L. & Lawson, D. M. (2006). Molecular replacement in the "twilight zone": structure determination of the non-haem iron oxygenase NovR from Streptomyces spheroides through repeated density modification of a poor molecular-replacement solution. Acta Cryst. D62, 1564-1570. 5. Gómez García, I., Freel Meyers, C. L., Walsh, C. T. & Lawson, D. M. (2008). Crystallization and preliminary X-ray analysis of the O-carbamoyltransferase NovN from the novobiocin-biosynthetic cluster of Streptomyces spheroides. Acta Cryst. F64, 1000-1002. 6. Gómez García, I., Stevenson, C. E., Uson, I., Freel Meyers, C. L., Walsh, C. T. & Lawson, D. M. (2010). The crystal structure of the novobiocin biosynthetic enzyme NovP: the first representative structure for the TylF O-methyltransferase superfamily. J. Mol. Biol. 395, 390-407. 7. Metzger, U., Keller, S., Stevenson, C. E., Heide, L. & Lawson, D. M. (2010). Structure and mechanism of the magnesium-independent aromatic prenyltransferase CloQ from the clorobiocin biosynthetic pathway. J. Mol. Biol. 404, 611-626.

Poster 96

Structure-based drug design of novel Aurora kinase A inhibitors: Structural basis for potency and specificity

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Aurora kinases have emerged as attractive targets for the design of anti-cancer drugs. Through structure-based virtual screening, novel pyrazole hit 8a was identified as Aurora kinase A inhibitor (IC50 15.1 μM). X-ray co-crystal structure of 8a in complex with Aurora A protein revealed the C-4 position ethyl carboxylate side chain as a possible modification site for improving the potency. Based on this insight, bioisosteric replacement of the ester with amide linkage and changing the ethyl substituent to hydrophobic 3-acetamido-phenyl ring, led to the identification of 12w with a ~450-fold improved Aurora kinase A inhibition potency (IC50 33 nM), compared to 8a. Compound 12w showed selective inhibition of Aurora A kinase over Aurora B/C, which might be due to the presence of a unique H-bond interaction between the 3-acetamido group and the Aurora A non-conserved Thr217 residue, which in Aurora B/C is Glu and found to sterically clash with the 3-acetamido group in modeling studies.

Poster 97

Role of Solubilization in Soaking Low-Affinity Compounds for Generating Co-Crystals

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The fragment-based approach to compound development has gained popularity in recent years due to its generic nature, which makes it directly relevant to genomics-based initiatives such as development of chemical probes against families of proteins. A crucial ingredient is obtaining structural information on binding, and of the various ways to generate co-crystal structures, soaking crystals with compounds is by far the most convenient and cheapest, provided a soakable crystal form can be identified. However, even if feasible, soaking is not guaranteed to be effective, a problem exacerbated by the low binding affinity of fragments, raising the need for generic ways to improve success rates. The BAZ2B bromodomain is a readily soakable crystal system yet in a first pass yielded successful binding for only 3% of soaked fragment molecules, leading us to investigate compound solubilization as factor of success, based on a systematic screen of solubility parameters such as temperature, solvent miscibility, the sequence of adding solvent or mother liquor, compound concentration, and required protein-ligand ratio. A model is presented to rationalise the results in terms of local concentration of active sites.

Poster 98

A two-pronged approach to finding novel calpain inhibitors

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Calpains are a family of Ca2+-dependent cysteine proteases that help mediate Ca2+ signaling in the cell. They catalyze the limited proteolysis of many proteins to modulate the activity of their targets. Elevated calpain activity is a complication of various human diseases, such as ischemic injury, neurodegenerative disorders (Alzheimer, Huntington, and Parkinson diseases) and muscular dystrophies. In addition, type 2 diabetes has been linked to a specific calpain isoform. Inhibition of calpain activity has emerged as a potential therapeutic target for the treatment of these and other pathophysiological conditions. Although significant efforts have been made in the past and recent years to develop calpain inhibitors that could be used as therapeutics, the currently available inhibitors of calpain have either low affinity or poor specificity. The specificity issue is a consequence of calpain being structurally similar in the core of the active site cleft to other cellular cysteine proteases (like the cathepsins). However, more distal sub-sites in the cleft are formed by calpain-specific features where there are opportunities for calpain selectivity. Unlike the cathepsins, calpain is a complex multidomain protein (110 kDa) with a large and small subunit that is activated by calcium. Up to ten calcium ions bind cooperatively to the enzyme to activate it with significant movement of the domains relative to each other. Thus it is likely that there are binding sites unique to calpain outside the catalytic cleft that provide an opportunity for specific inhibitor design. We have taken a two-pronged approach to screen for novel calpain inhibitors. In one, we use high throughput screening (HTS) of compound libraries to detect inhibitors of calpain in a highly sensitive FRET-based peptide cleavage assay. The other detects ligand binding using diferential static light scattering or DSLS (StarGazer) to measure the aggregation temperature (Tagg) of a catalytically inactive mutant calpain. Tagg values were determined with and without ligands to calculate the stabilizing effect of the ligand (ƒ´Tagg). When 11,000 non-peptide small molecules were screened in the HTS enzymatic assay, nine structures significantly inhibited calpain 2-induced degradation of the FRET substrate when compared to the vehicle control. Three of these structures also substantially inhibited (>40% at 10 ƒÝM) the autolysis of calpain 2. In the ligand-based approach, we screened 2040 fragments (Structural Genomics Consortium, Toronto) against inactive recombinant calpain 2 and identified promising fragment hits that showed significant ƒ´Tagg values of up to 10 oC. Leads from the two assays are being cross-checked. These compounds would serve as platforms for developing potent and specific calpain inhibitors. Funded by CIHR

Poster 99

Crystal structure of metarhodopsin II

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Rhodopsin is a G-protein-coupled receptor (GPCR), in which the inactivating ligand 11-cis-retinal is covalently bound in the seven-transmembrane helix bundle of the apoprotein opsin. After light-induced cis/trans-isomerization of the ligand, the Schiff base linking retinal and Lys296 deprotonates to form the active Meta II state. To overcome the problem of subsequent rapid Schiff base hydrolysis and all-trans-retinal dissociation, we formed Meta II for structural analysis by soaking opsin crystals with all-trans-retinal. Here we present the 3.0 Å and 2.85 Å crystal structures, respectively, of Meta II alone or in complex with a C-terminal fragment derived from the α-subunit of the heterotrimeric G protein. The Gα fragment binds in a large crevice at the cytoplasmic side and identifies the reversibly formed Meta II as the active, G-protein-interacting state. By comparison with rhodopsin and early photoproducts we propose how retinal translocation and rotation induces the gross-conformational changes characteristic for Meta II. The structures can now serve as models for the large GPCR family.

Poster 100

Structural membrane proteomics using the hyperthermophilic eubacterium Aquifex aeolicus

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Abstract Proteins from hyperthermophilic organisms are considered to be more stable and more rigid than their mesophilic counterparts. Therefore, the chance to obtain stable, homogeneous and crystallizable membrane protein complexes should be better with complexes from (hyper)thermophilic organisms than with complexes from mesophilic ones. Aquifex aeolicus is such a hyperthermophilic eubacterium with a known genome sequence (Deckert, et al., 1998). It grows at 95°C, was first isolated and described in 1992 (Huber, R. et al.). It has a rather small genome of 1,551,335 bps (1/3 that of Escherichia coli), which makes the task of purifying the membrane protein and protein complexes relatively easy. We purify and characterize as many proteins and protein complexes from membranes of Aquifex aeolicus as possible using conventional biochemical techniques. The proteins are identified by MALDI TOF mass spectrometry, and subjected to crystallization trials. So far we have identified 84 protein chains which belong to 50 membrane proteins and protein complexes. These are primarily involved in metabolism and in energy transduction. Very few transporters of the inner membrane have been found, only one secondary active transporter has been identified. We have purified and characterized the NADH:quinone oxidoreductase (complex I), a supercomplex consisting of the cytochrome bc1 complex and of the cytochrome c oxidase, the cytochrome c oxidase with an alternative function, the F1Fo-ATP synthase, sulfide-quinone oxidoreductase, DMSO reductase, flavocytochrome c:sulfide dehydrogenase and several complexes of “hypothetical proteins”, partly functioning as porins. We have started crystallization attempts with 12 of them. All of them could be crystallized, and five of them diffract X-rays to 3 Á or better. The structures of the sulfide:quinone oxidoreductase (Marcia, et al. 2009) and the hydrophilic domain of complex I have been solved. Despite the limited amounts of the individual proteins available this structural proteomic project has been highly successful. References Deckert, G., Warren, P.V., Gaasterland, T., Young, W. G., Lenox, A. L., Graham, D. E., Overbeek, R., Snead, M. A., Keller, M., Aujay,M., Huber, R., Feldman, R. A., S Short, J. M., Olsen, G. Short, J. M., Olsen, G. J., and Swanson, R. V. (1998) Nature 392, 353-358 Huber, R; Wilharm, T; Huber, D.; Trincone, A.; Burggraf, S.; Koenig H.; Rachel, R.; Rockinger, I.; Fricke, H.; and Stetter, K. O. (1992) System. Appl. Microbiol. 15, 340-351 Marcia, M., Ermler, U., Peng, G., and Michel, H. (2009) PNAS 106, 9625-9630

Poster 101

Human integral membrane protein structural biology at the SGC

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Integral membrane proteins represent approximately 25% of the genes in the human genome and are the targets for more than 50% of all therapeutic drugs. The number of structures available for this important class of proteins is however still very limited due to the difficulty of expressing, purifying and crystallising these hydrophobic proteins. Human membrane proteins are particularly under-represented with only 21 structures solved. At the SGC we are tackling this challenging area by putting together a pipeline for production of human membrane proteins using the baculovirus expression system. To date we have screened over 200 human integral membrane proteins for expression and purification in the Baculovirus system. For each protein we start with a series of constructs, screen for proteins that can be purified on a small scale, test a variety of detergents to see which gives stable, monodisperse protein and then we purify the protein on a 3L scale. To date we have obtained over 25 proteins that can be purified on a scale of at least 50 ug per litre and we have crystals of three targets. We have recently obtained diffraction to beyond 3.4A from crystals of a human ABC transporter and the structure has been phased using a mercury derivative, giving interpretable maps. The initial structure and interpretation will be presented and discussed.

Poster 102

Crystallizing Membrane Proteins Utilizing High-Throughput Methods

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Structural characterization of membrane proteins is a vital component in efforts to understand the processes behind many disease states. The crystallization of a membrane protein is hampered by the requirement of detergent, which is used to extract the protein from the lipid bilayer to form a protein-detergent complex (PDC). We recently implemented a 1536-cocktail crystallization screen for membrane proteins based on detergent phase partitioning studies, utilizing different combinations of polyethylene glycol, salts, and detergents (1). As membrane proteins have been observed to form crystals near the phase boundary of the detergent used to form the PDC, the empirically derived phase partitioning data serve as the foundation of the screen. The high-throughput crystallization screening and optimization pipeline at the Hauptman-Woodward Medical Research Institute has been developed to serve both the structural biology and structural genomics communities. Since its deployment in August 2009, the phase boundary-based crystallization cocktails have been utilized to screen over 170 unique membrane proteins (and >300 membrane protein samples total). An overview of the crystallization screening services available to investigators, as well as recent developments focusing on optimization, visualization, and the suitability of the cocktails for cryopreservation will be presented. Further information on the facility is available at http://www.hwi.buffalo.edu. (1) Koszelak-Rosenblum et al., Protein Science, 18:1828-1839 (2009). We gratefully acknowledge the financial support of NIH U54 GM074899, NIH U54 GM094611, NIH U54 094597, the Cummings Foundation, the John R. Oishei Foundation, and the Margaret L. Wendt Foundation.

Poster 103

The Overexpression of Human Integral Membrane Proteins in HEK-293S Cells, and its Successful Application in the Structure Determination of Human RHCG

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One, of the numerous, challenges associated with the structure determination of integral membrane proteins is in obtaining the milligram quantities of protein necessary for crystallization screening. The recombinant expression of human integral membrane proteins, in particular, remains as a significant bottleneck to structure determination given that these proteins often require eukaryotic cellular machineries for their proper post-translational processing. Herein, we describe the methods that we have developed to overexpress human integral membrane proteins in modified human embryonic kidney-293S (HEK-293S) cells. This expression system was recently used in our 2.1 Å… x-ray crystal structure determination of human rhesus C glycoprotein (RhCG), a trimeric integral membrane protein essential for ammonia excretion and renal pH regulation.

Poster 104

Automated high-throughput crystallization suited for membrane proteins at the ESFRI Instruct Core Centre Frankfurt

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Within ESFRI (European Strategy Forum on Research Infrastructures) and INSTRUCT (Integrated Structural Biology Infrastructure for Europe) we operate the Core Centre Frankfurt, which is devoted to membrane proteins. Here we try to understand membrane proteins based on an accurately known structure. Our work emphasizes on the structure determination of proteins and protein complexes integrated into the cellular membranes of living organisms. The importance for life of membrane proteins can be deduced from a huge variety of duties, for the communication of the cell with its environment, like signal exchange, as well as energy transduction and specific uptake and/or release of substrates of all kind. Within the Core Centre four areas of protein and crystal handling are installed: Verification of the protein by mass spectrometry, thermal stability tests with a calorimetric approach or a Thermofluorassay, fully automated crystallization and analysis of the crystals by X-ray diffraction. Mass spectrometry has been especially successful because we were able to determine twice database errors in the amino acid sequence of endogenous proteins so far. A Thermofluorassay with small sample quantities has been improved for membrane proteins using the dyes Sypro Orange and CPM. This method allows to quickly enhance the starting conditions for crystallization. Membrane protein crystallization still remains a challenging task. The Rigaku CrystalMation™ system was setup to fully automate the crystallization process while dealing with sample volumes of 100 nl per experiment. Three different crystallization strategies are applied to offer a broad range of crystallization conditions for membrane proteins: First commercially available membrane protein crystallization screens and second a series of in-house produced screens are provided. These are one pH screen and screens depending on tolerated pH and used detergent, covering a set of PEGs and salts which generate conditions close to phase separation. The third method applied is the fully automated setup of cubic phase crystallization with a Zinsser ProCrysMeso robot connected to the CrystalMation system. Additionally we are using various temperatures to suit the need of the individual membrane protein and this enables us to provide a quite diverse range of crystallization conditions. Identification of crystallization hits is simplified by UV detection combined with conventional imaging.

Poster 105

Challenges and Opportunities in Structure Determination of Membrane Proteins

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Membrane proteins are the supreme example where more effort is needed in structural biology. In spite of their abundance and importance, of the more than 60,000 protein structures in the Protein Data Bank, only around 200 of these structures represent unique membrane proteins. To facilitate structural studies on membrane proteins, The Membrane Protein Laboratory at Diamond Light Source Ltd (Diamond-MPL) was created in a collaboration between Imperial College London and Diamond, funded by the Wellcome Trust. The MPL is a research and training state-of-the-art user facility open to scientists from laboratories anywhere in the world interested in solving the 3-dimensional structures of membrane proteins by X-ray crystallography. Because membrane proteins are unstable, hard to crystallise and crystals difficult to handle. More systematic approaches and technical developments are needed to improve the success rate of the structure determination of membrane proteins. The MPL has a formal collaboration with I24 microfocus beamline at Diamond to develop new techniques for crystallisation and structural determination of membrane proteins, including prototype systems for high throughput methods, improving handling of small and delicate crystals, and methods for collecting and merging data from a large number of small crystals. The beamline is unique in its ability to deliver a tunable X-ray beam (6 – 25keV) of variable size between 5 μm and 50 μm on to crystal samples. This versatility is coupled with a state-of-the-art pixel array detector.

Poster 106

Recent Advances in Cell-free and Cell-based Protein Production at the Transmembrane Protein Center

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The Transmembrane Protein Center (TMPC) is a part of the NIGMS-funded Protein Structure Initiative, PSI:Biology. Our operations philosophy is to merge the automated cell-free production of membrane proteins for structure determination with the power of functional characterizations. Center expertise includes advanced LIMS, bioinformatics, cell-free and cell-based expression, scale-up production, and biophysical characterization of membrane proteins; expert collaborators carry out functional studies with all membrane proteins studied and structure determination. The TMPC has a primary goal of determining founding structures for several classes of multi-pass integral membrane proteins and enzymes. Our emphasis on small-scale purification screening and automation offers unique opportunities for rapidly testing combinations of lipids, detergents, and membrane scaffolding proteins during membrane protein translation. When appropriate, cell-based expression methods are incorporated into the project workflow. Process design also facilitates simple and efficient approaches to membrane protein purification. Results from the operation of our membrane protein production and structure determination pipeline will be presented. Protocols, plasmids and other information produced from this project can be obtained from the PSI:Biology-Materials Repository (http://psimr.asu.edu/) and the StructuralBiology KnowledgeBase (http://kb.psi-structuralgenomics.org/). Researchers interested in testing our cell-free translation and other approaches with their membrane proteins should use the KnowledgeBase Community-nominated Targets Proposal system (http://cnt.sbkb.org/CNT/targetlogin.jsp) or project email (cesginfo@biochem.wisc.edu). To learn more about these techniques researchers may also participate in a hands-on cell-free workshop in fall of 2011 (http://www.uwmembraneproteins.org). This work is funded by the NIGMS Protein Structure Initiative, PSI:Biology Network grant 1 U54 GM094584 (BG Fox, Project Director).

Poster 107

Progress in High-Throughput Production and Crystallisation of Integral Membrane Proteins at the SGC

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Production of mammalian membrane proteins for structural determination remains highly challenging due to their instability and hydrophobic nature. However, as more than 50% of all drugs target integral membrane proteins (IMPs), it is imperative that we develop technologies for producing and solving the structures of these proteins. In June 2009, the IMP group at the Structural Genomics Consortium began to apply our a high-throughput approach of expressing and purifying multiple constructs using standard parallel methods to membrane proteins, with the aim of generating a pipeline of proteins for crystallisation and ultimately structural determination. Initially, using the baculovirus/insect cell expression system, we tested on a 3 ml scale, a series of constructs for each target from the G-protein coupled receptor (GPCR), channel, solute carrier, ABC transporter and enzyme families for expression and purification with n-dodecyl-beta-D-maltoside (DDM). Out of 212 targets tested, 170 were confirmed as soluble on detergent extraction by Coomassie SDS-PAGE or Western blotting. A second screen against a panel of detergents and cholesterol hemisuccinate identified alternative detergents for extracting 127 of these targets. So far, 56 targets have progressed to scale up expression and 29 have been successfully purified. Using an analytical gel filtration system, these proteins were screened for conditions which give monodisperse protein, resulting in 24 progressing to crystallisation trials. So far, 3 targets from different families are consistently producing crystals and 2 give diffraction beyond 4Å. This pipeline has therefore delivered the throughput and quality control needed for production of protein for structural studies.

Poster 108

Structural and Functional Insights into Membrane Transporters and Sensors by Solid-State NMR

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Membrane proteins are among the most attractive goals for structural studies, due to their biochemical and medical importance. However, they are often not amenable to studies by the traditional structural methods, such as crystallography and solution NMR, because of the difficulties with functional expression, solubilization, and crystallization. Solid-state NMR is an attractive emerging alternative, which can be applied to these important proteins after reconstitution into native-like membrane-mimicking environment. Recently, we have demonstrated that solid-state NMR can yield valuable information on the secondary structure, dynamics, and environment of functionally important sidechains for a 7TM helical membrane protein, proton-pumping proteorhodopsin (1,2), which resists crystallization. Next, we could refine the X-ray model for another 7TM protein, Anabaena sensory rhodopsin (3), in the lipid environment, using torsional and distance constraints obtained by solid-state NMR. We revealed the structure of fragments disordered or distorted in crystals, and, using site-specific monitoring of H/D exchange, obtained unique information on the protein dynamics and its location in the membrane. Finally, we recently showed that such methodology can be extended to eukaryotic membrane proteins of similar size and architecture, using expression of Leptosphaeria rhodopsin in methylotrophic yeast Pichia pastoris as an example (4).

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Poster 109

The structures of p53, p63 and p73 reveal significant changes in the DNA-binding L2 loop

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The p53 family of transcription factors comprises p53, p63 and p73. At times of stress, p53 functions as a tumour suppressor inducing cell cycle arrest and apoptosis, whereas p63 and p73 are important for development. In most human cancers p53 activity is lost. Mechanisms are under investigation to re-activate p73 to induce apoptosis in p53-/- cell lines. Paralogs p63 and p73 share 60% sequence identity with p53 and bind many p53 response elements and interaction partners. To better understand their different activities we determined the structure of the DNA-binding domain of p73 refined at 1.8Å resolution. While the p53 fold and DNA-contact residues were highly conserved, the conformations of the L2 and L3 loops were diverged. The packing of the L2 loop was significantly altered by a 2-residue insertion leading to a small displacement of the H1 helix. Interactions of the p53 cancer hotspot residue R175 were also changed with the loss of the R175-D184 salt bridge. These effects may contribute to the different properties reported for p73, including an increased thermal stability and reduced binding to ASPP2.

Poster 110

Targeting the human cancer pathway protein interaction network by structural genomics

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Structural genomics provides an important approach for characterizing and understanding systems biology. As a step towards better integrating protein three-dimensional (3D) structural information in cancer systems biology, Northeast Structural Genomics Consortium (NESG) (www.nesg.org) have constructed a Human Cancer Pathway Protein Interaction Network (HCPIN) by analysis of several classical cancer-associated signaling pathways and their physical protein-protein interactions. Many well-known cancer-associated proteins play central roles as “hubs” or “bottlenecks” in the HCPIN. The HCPIN website (http://nmr.cabm.rutgers.edu/hcpin) provides a comprehensive description of this biomedical important multi-pathway network, together with experimental and homology models of HCPIN proteins useful for cancer biology research. NESG is targeting > 1,000 human proteins from the HCPIN for sample production and 3D structure determination. The long-range goal of this effort is to provide a comprehensive 3D structure-function database for human cancer-associated proteins and protein complexes, in the context of their interaction networks. Through Feb 2010, NESG has cloned >1,000 HCPIN protein and protein domain targets. Some 60 3D structures of these human proteins have been determined and deposited into the PDB. Many intermediate results, such as expression constructs, biochemical reagents, and biophysical characterization data generated in these ongoing structural genomics efforts are freely available to the cancer biology community. The some 60 structures of human proteins determined by NMR or X-ray crystallography include TLR2/MYD88 TIR domains, Retinoblastoma Binding Protein 9 (RBBP9), the ARID domain of ARID3A, Deleted-In-Oral-Cancer (DOC-1), UNC119, Epidermal Growth Factor (EGF)-like domains, and other biologically important proteins and protein-protein complexes. New functional insights have been identified, and will be discussed in this presentation.

Poster 111

Structural Changes in Soluble Sensory Rhodopsin Transducer upon the Interaction with DNA

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Anabaena sensory rhodopsin (ASR) is a recently discovered membrane photosensor with a unique signal transduction cascade. It interacts with a soluble tetrameric transducer (ASRT), regulating expression of several genes related to the utilization of light energy. Even though the X-ray crystal structure of ASRT is available, the mechanism of its interaction with DNA is still unknown. We used solution NMR to characterize structure of ASRT tetramer in solution and to understand the mechanism of the DNA-binding. Both X-ray crystal structures of ASRT and solution NMR data reveal seven beta-strands forming a rigid scaffold (beta-face) and a flexible, partially disordered alpha-face, comprised by the C-terminal parts and loops. We found that conformation of the alpha-face in solution is very different from that in the crystals. While C-termini of crystalline ASRT are either alpha-helical or disordered, half of ASRT monomers in solution feature additional C-terminal beta-strand, whereas another half have their C-tails as random coils. Titration of ASRT solution with a 20 bp fragment of the pec operon promoter showed that this structural heterogeneity may be functionally meaningful, as only monomers with beta-structured C-tails bind the DNA. The DNA binding occurs with submicromolar affinity and 1:1 stoichiometry (DNA:ASRT tetramer), and results in a significant ordering of the alpha-face of the interacting monomers, which involves the extension of the C-terminal alpha-strand and reorganization of the first loop. Changes in NMR signals of the ASRT sidechains suggest that specific Arg and Asn/Gln residues are involved in the interaction with DNA. Acknowledgements S.W. is supported by the Canadian Institutes of Health Research (CIHR) fellowship.

Poster 112

A dual binding mode for RhoGTPases in plexin signalling

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Plexins are cell surface receptors for the semaphorin family of cell guidance cues. The cytoplasmic region comprises a Ras GTPase-activating protein (GAP) domain and a RhoGTPase binding domain. Concomitant binding of extracellular semaphorin and intracellular RhoGTPase triggers GAP activity and signal transduction. The mechanism of this intricate regulation remains elusive. We present two crystal structures of the human Plexin-B1 cytoplasmic region in complex with a constitutively active RhoGTPase, Rac1. The structure of truncated Plexin-B1-Rac1 complex provides no mechanism for coupling RhoGTPase and Ras binding sites. On inclusion of the juxtamembrane helix, a trimeric structure of Plexin-B1-Rac1 complexes is stabilised by a second, novel, RhoGTPase binding site adjacent to the Ras site. Site-directed mutagenesis combined with cellular and biophysical assays demonstrate that this new binding site is essential for signalling. Our findings are consistent with a model in which extracellular and intracellular plexin clustering events combine into a single signalling output.

Poster 113

Structural and Functional Characterization of Pathogen Effector—Host Protein Interactions

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The interface between pathogenic bacteria and the host cells they infect is defined by numerous secreted effector proteins that interact with host-cell biomolecules to modulate cell function along a trajectory that varies in time and space. Understanding the molecular details of these interactions will enhance understanding of infectious bacterial diseases. Previous research in our laboratory has identified many novel putative secreted effector proteins in Salmonella Typhimurium, and we are now identifying their host cell protein targets and characterizing these interactions and their functional consequences in the PSI:Biology project Program for the Characterization of Secreted Effector Proteins. Our approach combines high resolution mass spectrometry with chemical cross-linking and affinity pulldown experiments to discover and validate host target proteins of effectors in Salmonella as well as other bacterial pathogens. We are also conducting in vivo transfections and transductions to express effector proteins in macrophage and epithelial cell lines, together with proteomic analysis to identify host phenotypes elicited by the pathogen. These experiments will allow identification of potential interacting host proteins for uncharacterized effectors, and will enable development and testing of functional hypotheses for these effectors. Complementing these studies are high resolution structures of pathogen effectors, host targets, and in ideal cases, the resulting protein complexes determined by our PSI:Biology partners, the Midwest Center for Structural Genomics. In this presentation we will describe our approaches to target identification and selection, identification of interacting proteins, and functional characterization of these interactions, and we will present results from the initial set of targets from Salmonella Typhimurium to be tested with our protocols.

Poster 114

Structural Biology of Selective Autophagy

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Autophagy is an intracellular bulk degradation system in which isolation membranes enclose a portion of cytoplasm to form a double membraned vesicle called an autophagosome and deliver its inner constituents to the lytic compartments1. Atg8 and its mammalian homolog LC3 are ubiquitin-like modifiers that are localized on isolation membranes as a conjugate with phosphatidylethanolamine and play crucial roles in the formation of autophagosomes. Although autophagy is in principle a non-selective, starvation-induced response, recent studies have also shed light on another mode of autophagy that selectively and constitutively degrades aggregated proteins, surplus or damaged organelles, and even invasive bacterial cells. Defects in autophagy cause the accumulation of ubiquitin-positive protein inclusions, leading to severe liver injury and neurodegeneration. p62 functions as a receptor to link such protein inclusions to LC3, thus playing a pivotal role in their efficient engulfment into autophagosomes. This process is similar to the cytoplasm-to-vacuole targeting (Cvt) pathway in yeast; a model of selective autophagy in which Atg19 functions as a receptor to link a huge assembly of vacuolar enzymes, aminopeptidase I and α-mannosidase to Atg8, thus playing a pivotal role in their efficient engulfment into Cvt vesicles. Using NMR and X-ray crystallography, we report here the structural basis for LC3-p62 and Atg8-Atg19 interactions. Remarkably, LC3 and Atg8 were shown to interact with p62 and Atg19, respectively, in a quite similar manner; they recognized the side-chains of Trp and Leu in a four-amino acid motif, WXXL, in p62 and Atg19 using hydrophobic pockets conserved among Atg8 homologs. Together with mutational analyses, our results show the fundamental mechanism that allows Atg8 homologs, in association with WXXL-containing proteins, to capture specific cargo molecules, which endows isolation membranes and/or their forming machineries with target selectivity. We also showed that WXXL motif is used to recruit Atg8/LC3 to facilitate efficient conjugation reaction with phosphatidylethanolamine.

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Poster 115

Induced-fit upon Ligand Binding Revealed by Crystal Structures of the Hot-dog Fold Thioesterase in Dynemicin Biosynthesis

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Dynemicins are structurally related 10-membered enediyne natural products isolated from Micromonospora chernisa with potent antitumor and antibiotic activity. The early biosynthetic steps of the enediyne moiety of dynemicins are catalyzed by an iterative polyketide synthase (DynE8) and a thioesterase (DynE7). Recent studies indicate that the function of DynE7 is to off-load the linear biosynthetic intermediate assembled on DynE8. Here, we report crystal structures of DynE7 in its free form at 2.7 Å resolution and of DynE7 in complex with the DynE8-produced all-trans pentadecen-2-one at 2.1 Å resolution. These crystal structures reveal that upon ligand binding, significant conformational changes throughout the substrate-binding tunnel result in an expanded tunnel that traverses an entire monomer of the tetrameric DynE7 protein. The enlarged inner segment of the channel binds the carbonyl-conjugated polyene mainly through hydrophobic interactions, whereas the putative catalytic residues are located in the outer segment of the channel. The crystallographic information reinforces an unusual catalytic mechanism that involves a strictly conserved arginine residue for this subfamily of hot-dog fold thioesterases, distinct from the typical mechanism for hot-dog fold thioesterases that utilizes an acidic residue for catalysis.

Poster 116

A Structural Genomics Approach to the Structure Determination of Macrophage Proteins

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Macrophages are cells differentiated from circulating blood monocytes that represent the first line of defense against pathogen invasion. Macrophages are widely distributed throughout the body and are particularly abundant at the route of pathogen entry. They play a critical role in immune defense by initiating, promoting, preventing, suppressing or terminating immune responses. We established a high-throughput pipeline at the University of Queensland to investigate the structures and functions of novel macrophage proteins [1]. My project began with the selection of 12 novel, biologically interesting and crystallization-feasible targets that were then designed into 96 different constructs. Processing of the 96 constructs was performed in parallel using simple automated applications of ligation-independent cloning, small-scale bacterial expression and purification, and solubility assessment. After processing these 12 targets, I found that 16 constructs of 3 targets (25%) yielded soluble protein. From the three soluble targets, I have spent most time on two proteins BinCARD and Fam96a. Bcl10 interacting CARD protein (BinCARD) is a CARD-domain containing protein that interacts with Bcl10 to downregulate NF-κB transcription factor activation [2]. Bcl10 is an intracellular signalling protein that also contains a CARD domain. The primary function of Bcl10 is to interact with CARD-domain containing proteins through CARD-CARD interactions to regulate its activity in the NF-κB signalling pathway [3]. The crystal structure of BinCARD solved at 1.5 Å resolution revealed six anti-parallel α-helices, suggesting that this protein is similar to other CARDs of known structures. Family with sequence similarity 96, member A (Fam96a) is a novel DUF59 domain containing protein that belongs to a group of diverse proteins with no function characterised yet. Evidence has shown that exploration of these proteins is an important area for further studies [4]. The crystal structure of Fam96a at 1.8 Å resolution revealed two different types of domain swapped-dimer conformation. Interestingly, Fam96a and its homologue Fam96b are the only two DUF59 domain containing proteins to be expressed in mammalian cells. It has been reported that Fam96b and cytosolic iron-sulfur protein assembly 1 (Ciao1) are part of the MMXD protein complex involved in chromosome segregation [5]. We have confirmed by chemical crosslinking that Fam96a also formed a complex with Ciao1. Crystals of the Fam96a and Ciao1 complex were obtained and diffracted to 7.0 Å resolution. Approaches to improve the crystal diffraction are currently being applied.

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Poster 117

The structural basis for selective binding of non-methylated CpG islands by the CFP1 CXXC domain

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CFP1 is a CXXC domain-containing protein and an essential component of the SETD1 histone H3K4 methyltransferase complex. CXXC domain proteins direct different chromatin-modifying activities to various chromatin regions. Here, we report crystal structures of the CFP1 CXXC domain in complex with six different CpG DNA sequences. The crescent-shaped CFP1 CXXC domain is wedged into the major groove of the CpG DNA, distorting the B-form DNA, and interacts extensively with the major groove of the DNA. The structures elucidate the molecular mechanism of the non-methylated CpG-binding specificity of the CFP1 CXXC domain. The CpG motif is confined by a tripeptide located in a rigid loop, which only allows the accommodation of the non-methylated CpG dinucleotide. Furthermore, we demonstrate that CFP1 has a preference for a guanosine nucleotide following the CpG motif.

Poster 118

The role of calmodulin in the regulation of TRPV5/6 channels

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The epithelial Ca2+ channels TRPV5/6 (transient receptor potential vanilloid 5/6) constitute the apical entry gate for Ca2+ reabsorption in the kidney and intestine, respectively. TRPV5/6 channels activity is thoroughly regulated in order to fine-tune the amount of Ca2+ reabsorption. Besides the regulation by various hormonal stimuli, the channels harbor a negative feedback mechanism preventing excessive Ca2+ influx. The Ca2+ influx through TRPV5/6 induces rapid channel inactivation, which is controlled by the C-terminus of the channel. Calmodulin negatively modulates TRPV5 activity by binding to the C-terminal fragment. This effect can be reversed by PTH-mediated channel phosphorylation, which disturbs the calmodulin binding. We used high-resolution NMR spectroscopy and isothermal titration calorimetry to explore the details of calmodulin binding to the TRPV5/6 C-terminal fragments. Interestingly, calmodulin binds to the C-terminal TRPV5/6 peptides in a mode distinct from the usual 1:1 mode. Here, we present the detailed information about this binding and develop a model of possible physiological role it has in the regulation of the channels.

Poster 119

Structure determination and comparative analysis of novel human ubiquitin-like domains.

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The ubiquitin fold acts as a signaling modulator associated with regulating, trafficking, and degrading proteins. The Human genome contains ~250 ubiquitin-like domains, of which a couple dozen may act as covalent modifiers. Ubiquitin and ubiquitin-like domains have been implicated in a number of malignancies, neuromuscular disorders, neurodegenerative disorders and other human illnesses. Identifying the structural effects of sequence variations between different ubiquitin-like homologues will provide insight into their varied functional pathways, since the role of ubiquitin-like modifiers is typically mediated by protein-protein interactions. Structure determination and analyses of ubiquitin-like homologues facilitates residue mapping and comparative analysis of protein-protein interaction sites, which provide insight into the many roles that ubiquitin-like homologues play in cellular processes. Collaborative efforts involving the NorthEast Structural Genomics consortium (NESG) and the Structural Genomics Consortium (SGC) have generated the structures of 15 ubiquitin-like domains using nuclear magnetic resonance (NMR) experiments and X-ray crystallography. We aim to get complete structural coverage of all human ubiquitin-like domains. Comparative analysis of structurally characterized ubiquitin-like folds has revealed potential interaction partners with regions similar to known ubiquitin and SUMO interacting domains. Comparative analysis of structural features of all ubiquitin-like homologues will facilitate further studies into the mechanisms of the ubiquitylation system, in addition to identifying pathways associated with uncharacterized ubiquitin-like domains.

Poster 120

The structure of the Klf4 DNA-binding domain links to self-renewal and macrophage differentiation

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Krueppel-like factor 4 (Klf4) is a C2H2-type zinc-finger transcription factor that is indispensable for terminal maturation of epithelial tissues. Furthermore, it is part of a small set of proteins that are used to generate pluripotent embryonic stem cells from differentiated tissues. We describe that the expression of a Klf4 zinc-finger domain mutant induces self-renewal and block of maturation in primary hematopoietic cells, while wild-type Klf4 induces terminal macrophage differentiation. Moreover, we solved the crystal structure of the zinc-finger domain of Klf4 bound to its target DNA, revealing that primarily the two C-terminal zinc-finger motifs are required for site specificity. Lack of those two zinc fingers leads to deficiency of Klf4 to induce terminal macrophage differentiation. The N-terminal zinc finger, on the other hand, inhibits the otherwise cryptic self-renewal and block of differentiation activity of Klf4.

Poster 121

The mechanism of the formation of hairpin telomeres

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Crystal structures of the TelAg from Agrobacterium tumefaciens, a plant pathogen which costs mulitmillion dollars lose each year in US, in complex with different substrate DNA molecule analogues. These structures reveal the process of the TelAg dimer cleaving substrate DNA duplexes into overhang intermediate and subsequently facilitate the formation of two hairpin products from the overhang intermediates. The structures also show that the hairpinning reaction is irreversible by a unique-shape pocket formed by the TelAg dimer that prevents re-ligation of the cleaved DNA substrate.

Poster 122

Biophysical Studies of Type II Diabetes-Causing Mutations in the SUR1 L0 Linker

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ATP-sensitive potassium (KATP) channels couple glucose metabolism to membrane potential and insulin release in pancreatic beta-cells. KATP channels contain four copies of the pore-forming Kir6.2 protein and four copies of the regulatory sulfonylurea receptor 1 (SUR1). SUR1 is an ATP binding cassette (ABC) protein and, as such, contains two transmembrane-spanning domains (MSDs) fused to two cytosolic nucleotide-binding domains (NBDs). Transmembrane helices in the MSDs extend into the cytoplasm and are connected by short coupling helices that contact the NBDs. SUR1 contains an additional MSD (MSD0) that is connected to MSD1 by the cytoplasmic L0 linker. Unlike most ABC proteins, SUR1 does not function as a transporter, but strictly as a regulator of KATP channel gating. ATP binding and hydrolysis at the SUR1 NBDs results in channel opening from a closed state. Further, the L0 linker controls the open probability of the channel, likely through interactions with the cytoplamsic domains of Kir6.2 and the SUR1 NBDs. Proper regulation of KATP channel activity by SUR1 is critical. Normally, under high glucose conditions, KATP channels are closed, which ultimately leads to insulin release. Gain-of-function mutations in the SUR1 NBDs and L0 linker result in failure of KATP channel closure even when glucose levels are high. Insulin secretion is prevented, leading to type II diabetes. Thus, studies of the SUR1 NBDs and L0 linker are critical to understanding the molecular basis underlying KATP channel gating under normal conditions and of altered KATP channel function in type II diabetes. The present work is focused on studies of the L0 linker of SUR1 in wild type and mutant states. Preliminary data on changes in the structure and stability of the L0 linker will be presented and discussed.

Poster 123

Structural insight into transcriptional coactivation of LRH-1 by beta-catenin

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The crystal structure of a complex of the armadillo repeat region of beta-catenin and the nuclear receptor liver receptor homolog 1 (LRH-1 or NR5A2) is solved at 2.8 Å resolution. The surface of beta-catenin for interaction of LRH-1 partly overlaps defined contact sites for peptide segments of beta-catenin partners, including T-cell specific transcription factor 4 (TCF-4). The surface of LRH-1 that engages beta-catenin is comprised of helices 1, 9 and 10 and is distinct from known interaction surfaces of LRH-1 and proteins, including the corepressor and coactivator binding site associated with activation function 2 (AF-2). Targeted mutagenesis of amino acid residues forming both sides of the LRH-1/beta-catenin interface reveals that it is essential for forming stable interactions between these proteins in mammalian cells and GST-pull down assay in vitro. Mutagenesis of the analogous binding site on the androgen receptor (AR) affects its association with beta-catenin, providing evidence that the observed LRH-1/beta-catenin interaction may be prototypic. The structure of LRH-1/beta-catenin opened new insight into transcriptional regulation of nuclear receptor by beta-catenin.

Poster 124

Recognition and specificity determinants of the human Cbx chromodomains

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The eight mammalian Cbx proteins are chromodomain containing proteins involved in regulation of heterochromatin, gene expression and developmental programs. They are evolutionarily related to the Drosophila HP1 (dHP1) and Pc (dPc) proteins that are key components of chromatin associated complexes capable of recognizing repressive marks such as trimethylated Lys9 and Lys27, respectively on histone H3. However, the binding specificity and function of the human homologs, Cbx1-8, remain unclear. To this end, we employed structural, biophysical and mutagenic approaches to characterize the molecular determinants of sequence contextual methyllysine binding to human Cbx1-8 proteins. While all three human HP1 homologs (Cbx1,3,5) replicate the structural and binding features of their dHP counterparts, the five Pc homologs (Cbx2,4,6,7,8) bind with lower affinity to H3K9me3 or H3K27me3 peptides and are unable to distinguish between these two marks. Additionally, peptide permutation arrays revealed a greater sequence tolerance within the Pc family and suggest alternative nonhistone sequences as potential binding targets for this class of chromodomains. Our structures explain the divergence of peptide binding selectivity in the Pc subfamily and highlight previously unrecognized features of the chromodomain that influence binding and specificity.

Poster 125

Insights into the modular organization of plakin domains in solution

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The spectrin family of proteins are critical for correct functioning in a wide variety of cells. They are flexible rod-like proteins which play important roles in conferring shape and stability of metazoan cells. Mutations within spectrin members cause a variety of muscular, blood, heart and skin diseases. The spectrin repeat is the main structural platform for the spectrin family which consists of a triple-helical coiled coil, with a repetitive heptad pattern. The structural organization of large spectrin proteins (25kDa+) in solution is currently unknown. The desmoplakin plakin domain which is a member of the spectrin family was used to investigate the modular organization of spectrin repeats in solution. A variety of full length plakin domain and subdomain proteins from desmoplakin were expressed. Mutations which cause Arrhythmyogenic Right Ventricular Cardiomyopathy (ARVC) in desmoplakin were also investigated. Finally, models of spectrin repeats from plakin proteins were created based on known structures. Small-Angle-X-ray-Scattering (SAXS) analysis (a solution based low resolution biophysical technique) of the desmoplakin plakin domain revealed extended conformations with apparent flexibility. The subdomains were also analysed by SAXS which gave similar envelopes to their crystallized homologues confirming physiological relevance of both the crystal and solution forms. Using N15 labelled samples we showed by Nuclear Magnetic Resonance (NMR) that missense mutations cause alterations to their local structural environments, significant enough to cause ARVC. Using a variety of techniques this study provides insight into the collective behaviour of spectrin repeats in a physiological context and provides greater understanding into biophysical implications of ARVC mutations.

Poster 126

Protein Structure Initiative:Biology-Materials Repository: A Biologist's Resource for Protein Expression Plasmids

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The PSI:Biology-Materials Repository (MR; http://psimr.asu.edu) provides centralized archival and distribution of the annotation and samples for plasmids created by the Protein Structure Initiative (PSI). The MR has developed an informatics and sample processing pipeline that manages this process for thousands of samples per month from nearly a dozen PSI centers. Plasmid annotations, which include the full length sequence, vector information, and associated publications, are stored in the freely available, searchable database DNASU (http://dnasu.asu.edu). Plasmids link to external resources, including the PSI Structural Biology Knowledgebase (http://sbkb.org), which facilitates cross-referencing plasmids to additional protein annotations and experimental data. To simplify plasmid distribution, the MR invented an Expedited MTA network, where researchers from network institutions can order and receive PSI plasmids without any institutional delays. As of February 2011, nearly 40,000 PSI plasmids and 78 empty vectors are available upon request from DNASU icsg(http://dnasu.asu.edu). Overall, the MR's repository of expression-ready plasmids, its automated pipeline, and the rapid process for receiving and distributing these plasmids more effectively allows the research community to dissect the biological function of proteins whose structures have been studied by the PSI.

Poster 127

Biophysical studies of the first nucleotide binding domain of SUR2A

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ATP-sensitive potassium (KATP) channels are found in various cell types, including vascular smooth muscle, heart, and pancreas. By sensing the cellular concentrations of ADP and ATP, KATP channels link cellular metabolism to membrane potential and excitability, leading to crucial roles in several biological processes. KATP channels are comprised of four pore-forming potassium channel subunits (Kir6.1 or Kir6.2) and four sulfonylurea receptors (SUR1 or isoforms of SUR2). The SUR proteins are members of the ubiquitous ATP-binding cassette (ABC) superfamily. However, unlike most ABC proteins, SURs do not transport substrates but function strictly as regulators of KATP channel activity. ATP binding at the Kir6 subunits results in closed KATP channels, whereas ATP binding and hydrolysis at the SUR nucleotide binding domains (NBDs) opens the pore. Mutations in the nucleotide binding domains (NBDs) of SUR2A that disrupt regulation of KATP channel gating are associated with several cardiovascular disorders, making studies of the NBDs essential in understanding regulation of KATP function in normal states and pathophysiology of disease. Currently, studies into the molecular basis by which various mutations in SUR2A cause disease are limited. This is primarily a consequence of poor solubility of the isolated SUR2A NBDs, as is typical for many eukaryotic NBDs. By employing structure-based sequence alignments, predictions of disordered regions, and biophysical studies, we determined domain boundaries for SUR2A NBD1 that enabled, for the first time, NMR studies of NBD1. Our biophysical studies demonstrate that the isolated SUR2A NBD1 is folded and exhibits ATP binding activity. Additional studies are now possible to examine the effects of disease-causing mutations on structure, dynamics, and interactions of NBD1 and some of these data will also be presented.

Poster 128

Cas1, Cas2, Cas3…: structure and activity of the core CRISPR nucleases

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and their associated proteins (Cas) comprise a novel microbial defence system, a potential functional analog to the eukaryotic RNA-silencing machinery. Six Cas protein families (Cas1-6) represent the core group of CRISPR-associated proteins, which are predicted to function as nucleases. However, very little is known about their biochemical activities and structures. Here we show that Cas1, Cas2, and Cas3 proteins from various organisms represent three novel families of nucleases with different substrate specificities. Cas1 can cleave single stranded DNAs and RNAs, as well as branched DNA substrates; Cas2 is an endoribonuclease with a preference for the U-rich sequences, whereas Cas3 is an exonuclease active against single stranded DNAs. The crystal structures of these proteins were solved revealing the different protein folds and potential active sites, which were further characterized using site-directed mutagenesis. Our structural and biochemical data provide insight into the molecular mechanisms of activity of the core Cas proteins, which play a critical role in the CRISPR mechanism.

Poster 129

Structural basis of IscS-mediated protein-protein interactions for iron-sulfur cluster assembly and tRNA thiolation

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The cysteine desulfurase IscS is a highly conserved master enzyme initiating sulfur transfer via persulfide to a range of acceptor proteins involved in Fe-S cluster assembly, tRNA modifications, and sulfur-containing cofactor biosynthesis. IscS-interacting partners including IscU, TusA, ThiI, and rhodanese RhdA are sulfur acceptors. Other proteins, such as CyaY/frataxin and IscX, also bind to IscS, but their functional roles are not clear. We have solved the crystal structures of the IscS-IscU and IscS-TusA complexes providing the first insight into their different modes of binding and the mechanism of sulfur transfer. Exhaustive mutational analysis of the IscS surface allowed us to map the binding sites of various partner proteins and to determine the functional and biochemical role of selected IscS and TusA residues. IscS interacts with its partners through an extensive surface area centered on the active site Cys328. The structures indicate that the acceptor proteins approach Cys328 from different directions and suggest that the conformational plasticity of a long loop containing Cys328 is critical for the ability of IscS to transfer sulfur to multiple acceptor proteins.

Poster 130

4E-BP1 pre-structures eIF4E into a 5'-cap receptive conformation and controls eIF4E mRNA export activity

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The eukaryotic initiation factor 4E (eIF4E) is a key translation and mRNA export factor that controls the expression of genes involved in cell growth, proliferation, and apoptosis. Binding of eIF4E to the 5'-cap structure on mRNA is required to initiate protein synthesis and promote the export of specific mRNAs. Multiple regulators, including PML, eIF4G and 4E binding proteins (4E-BPs), alter eIF4E's affinity for the 5'cap and this serves as a mechanism to control eIF4E's overall activity in the cell. Regulating cap affinity is an important aspect of understanding the priming step in translation and mRNA export. Here, we present the crystal structures of cap free human eIF4E and eIF4E-BP1 peptide complex. Additional NMR data and the crystal binary complex suggest that the 4E-BP1 peptide pre-structures eIF4E into a cap recognition conformation. Our cell based studies also demonstrate a role for 4E-BP1 in the nucleus, showing that it modulates the nuclear mRNA export activity of eIF4E. Overall, we discuss 4E-BP1 as having multiple modes of controlling gene expression, via eIF4E, at the nuclear and cytoplasmic levels.

Poster 131

Structural Proteomics of Bacterial Protein Complexes at the BSGI

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Bacterial proteins participate in numerous transient and more stable associations (complexes) in order to perform a variety of biochemical processes. Both high-throughput interactome and individual investigator-initiated studies have provided a wealth of data on protein complexes in bacteria, particularly for E. coli. For the past few years the BSGI has focused efforts on the preparation, crystallization, structural analysis and functional characterization of a number of protein complexes, mainly from E. coli. Various approaches for protein expression, complex formation and stabilization were optimized for each of the systems under study. The structures determined include those involved in Fe-S cluster assembly, dihydroxyacetone metabolism and type II cohesin-scaffoldin interactions. In some instances, NMR titration experiments and small angle X-ray scattering have also been utilized to gain further structural insights. Using the structures as a starting point, mutagenesis combined with in vitro and in vivo functional assays have provided new biological insights into these processes. Research supported by CIHR.

Poster 132

The Joint Center for Structural Genomics

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The Joint Center for Structural Genomics (JCSG; www.jcsg.org) high-throughput (HTP) gene-to-structure pipeline has delivered over 1100 novel structures to the community by both x-ray crystallography and NMR, on a wide range of targets from bacteria to human, with a sustained output rate of >200 structures/year in PSI-2. Our approach involves processing large numbers of targets through an extensive combination of bioinformatics and biophysical analyses to efficiently characterize each target in order to optimize its path through our pipeline. As a PSI:Biology Center for High-Throughput Structure Determination, our primary mission is to adapt and extend this robust and flexible HTP platform to meet the challenges and embrace the new opportunities that arise from Biology Partnership projects, future PARS, and the community. On the one hand, in close collaboration with our PSI:Biology Partnerships and a more focused approach, the JCSG is leveraging its HTP platform to address challenging targets and capitalizing on our extensive experience to develop the best strategies to enhance the chances of success. On the other hand, we continue to process other categories of targets in a HTP mode. These target sets are initially focused on structural coverage of the protein universe and on our Biological Theme project in metagenomics, more specifically, on the microbial communities that inhabit specific niches and environments of the human body. The human microbiota is dominated by poorly characterized bacterial phyla, which contain an unusually high number of uncharacterized proteins and remain largely unstudied. Their influence upon human development, physiology, immunity, and nutrition are only starting to surface, and, thus, represents an exciting new frontier for structural genomics and high-throughput structural biology where we can investigate the contributions of these microorganisms to human health, as well as to disease. Supported by NIGMS: U54-GM094586.

Poster 133

NIGMS PSI:Biology Initiative – Enabling High-Throughput Structural Biology and Structural Genomics

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The National Institute of General Medical Sciences has announced PSI:Biology to continue the development of high-throughput structural biology methods and apply them to important biological problems. This will be accomplished by establishing a network of collaborations between centers for structure determination and biologists with interests in problems involving particular proteins or collections of proteins that would benefit from structural information. These awards are named Consortia for High-Throughput-Enabled Structural Biology Partnerships and successful applicants will help to define targets for structure determination by the centers and will receive funds for functional studies in the applicants' laboratories. This mechanism provides an on-going opportunity for the wider biomedical research community to obtain funding to participate in the PSI through collaboration with the high-throughput structure determination centers and with the centers for membrane structure determination.

Poster 134

The Mitochondrial Protein Partnership

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The Mitochondrial Protein Partnership (MPP), located in the Center for Eukaryotic Structural Genomics (CESG) at the University of Wisconsin-Madison, has the goal of applying technologies developed by the Protein Structure Initiative (PSI) to problems of interest to the community of biologists and biochemists who investigate the role of mitochondria in human health and disease. The MPP is directed by Drs. John Markley and Dave Pagliarini, and is supported by an NIH grant entitled, "Partnership for High-Throughput Enabled Biology of the Mitochondrial Proteome" (U01GM094622). A major goal of the MPP will be to screen all human and mouse mitochondrial open reading frames to determine whether they can be used to produce protein samples by an automated in vitro protein production system that utilizes a cell-free wheat germ extract. All proteins produced by this system will be analyzed for solubility, molecular mass, aggregation/dispersity, and thermal stability. Protein samples made in this way will be subject to functional investigations, including protein-protein and protein-small molecule interactions, enzymatic analyses, and mitochondrial bioenergetic measurements. The MPP solicits nominations from the scientific community for mitochondrial protein targets of biological/biomedical interest that would be suitable for collaborative study. Once nominations have been approved, collaborative arrangements have been set up, these targets are given high priority, and collaborators are informed of progress made in producing and characterizing the protein in determining structures. To become a collaborator with the MPP, an investigator must first nominate a mitochondrial protein or proteins in an email addressed to mpp_collaborations@biochem.wisc.edu, with "MPP Nomination" in the subject line. To read more about the goals and strategies of the MPP, please visit our website at: http://www.mitoproteins.org

Poster 135

Structural and functional genomics of a model organism Thermus thermophilus HB8: toward functional discovery of functionally unknown proteins

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The ultimate goal of our research is to understand all of the biological phenomena in the cell at an atomic-resolution, on the basis of the structures and the functions of all of the molecules, and to predict all of the biological phenomena in the cell. To achieve this goal, we chose the extremely thermophilic organism, Thermus thermophilus HB8, as a model organism, because the essential 2,200 genes (proteins) encoded in its genome have been selected during evolution and are common to many organisms, including human. Furthermore, as this strain is thermostable, this model organism is a good candidate for X-ray structural analyses of its macromolecules. The total number of protein structures from this organism is now more than 490, including 100 or so solved by other groups. This number is about 22% of the total number of proteins of this organism, which is the highest among all organisms. Surprisingly, our genome analysis revealed that as many as 1/3 of the 2,200 total proteins (genes) are functionally uncharacterized, although many of them are essential and common to many organisms, including human. In order to interpret the whole-cell phenomena, the identification of the functionally-uncharacterized (especially hypothetical) proteins is essential. Therefore, we identified their functions (1) by using the structural data obtained from structural genomics, and (2) by genome-wide functomics data obtained from mRNA analysis (transcriptomics), from protein analysis (proteomics), and from metabolite analysis (metabolomics), and then (3) we confirmed the function of each isolated protein in vitro. The plasmids for protein expression and for gene disruption are available to the public through the RIKEN BioResource Center (see http://www.thermus.org). Some of the information about protein production (protein expression and purification), crystallization, structure, and functomics are also available from our database (http://www.thermus.org) and public databases. In this presentation, we will show examples of classification of several functionally uncharacterized proteins focusing on their characteristics of transcriptional regulation. For instance, cyclic AMP receptor protein (CRP), which is one of the global transcription factors that regulate multiple genes, positively regulates 22 genes, including three hypothetical genes. These genes may be involved in DNA/RNA metabolism including clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) proteins that are components of a novel host defense system against invading foreign replicons, such as phages. In total, we have elucidated the functions of seven transcriptional regulators out of about 70 regulators most of which are functionally unknown, and found that 84 genes, including 28 functionally unknown [clusters of orthologous groups of proteins (COG) code S or non-categorized in COG] genes could be categorized based on their transcriptional regulatory mechanism.

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