MS85 BIOLOGICAL MOLECULES AS TARGETS FOR DRUG DESIGN
Chairpersons: Stefania Di Marco, William (Bill) Stallings

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AChBP Structures for Understanding Ligand Binding in Nicotinic Receptors
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Acetylcholine-binding protein (AChBP) from the mollusc Lymnaea stagnalis is at present the only high-resolution model for the ligand-binding domains of the ligand-gated ion channel family, which includes nicotinic acetylcholine, 5HT3, GABAA, GABAC and glycine receptors.

Here we present crystal structures from remote homologs from other molluscs that will define the variabilities in the binding sites. We will also explore a series of crystal structures of nicotinic receptor agonists and other ligands. These define how cation-pi interactions as well as remote electrostatic compensation contribute to ligand binding in the receptors. These structures also explain the many different data from ligand-binding studies on this pharmaceutically important class of neuronal receptors.

Comparison of these structures will be valuable for improving structure-function studies of ligand-gated ion channel receptors, including signal transduction, homology modeling and drug design.
Keywords: ligand-gated ion-channels, acetylcholine, toxin

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Classical and Non-classical Structure-based Drug Design
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Structure-based drug design is usually described as an iterative process in which knowledge of the three-dimensional structure of a receptor-ligand complex reveals details of the binding interface that can be improved by chemical modification of the ligand. These structure-based changes as evaluated by *in vitro* or *in vivo* assay, and improved ligands are subjected to additional cycles of structure determination, improvement and evaluation. Our studies on caspase-3, PTP-1B and other targets provide examples of such classical structure-based drug design. On occasion, however, structural studies lead to surprising results that produce unexpected effects on the inhibitor-development process. In both the caspase and PTP projects, early structures revealed that the apparent improvement in binding potency was inconsistent with program goals and this knowledge led to termination of the compound classes involved. In work on both nuclear receptors and kinases, knowledge of structure-based selectivity led to the design of novel assays that have effectively discriminated compounds on their biological properties. These studies demonstrate that structure-based drug design studies can not only lead to ligand optimization but to prioritization of compound classes and to the design of novel methods of discriminating among compounds based on their biological properties.
Keywords: structure-aided drug design, nuclear receptors, protein kinases

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Structure Based Drug Design of Novel Inhibitors of cGMP Phosphodiestearse, PDE5
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PDE5, a cGMP specific PDE, has been recognised in recent years as an important therapeutic target. It is composed of the conserved C-terminal, zinc containing, catalytic domain, which catalyses the cleavage of cGMP, and an N-terminal regulatory portion, which contains two GAF domain repeats [1]. Each GAF domain contains a cGMP-binding site, one of high affinity and the other of lower affinity [2]. PDE5 activity is regulated through binding of cGMP to the high and low affinity cGMP binding sites followed by phosphorylation, which occurs only when both sites are occupied [3]. PDE5 is found in varying concentrations in a number of tissues including platelets, vascular and visceral smooth muscle, and skeletal muscle. The protein is a key regulator of cGMP levels in the smooth muscle of the erectile corpus cavernosal tissue. Inhibition of PDE5 inhibits the breakdown of cGMP allowing the levels of cGMP, and hence smooth muscle relaxation, to be maintained [2]. Sildenafil, the active ingredient of Viagra® and a potent inhibitor of PDE5, has attracted widespread attention for the effective treatment of male erectile dysfunction.

We present here the application of the structures of PDE5 [4-9] to design novel inhibitors. The use of the complexes provides additional important structural information on the binding modes of multiple series of inhibitors. The structures also highlight the diverse chemical nature of inhibitors within this gene target and wider gene family, and the subtle structure activity relationships which assist the design of more potent and specific inhibitors to treat the many diseases where PDE's play a role.

[1] Beavo J. A., *Physiological Reviews*, 1995, **75**, 725-748. [2] Corbin J. D., Francis S. H., *Journal of Biological Chemistry*, 1999, **274**, 13729-32. [3] Thomas M. K., Francis S. H., Corbin J. D., *J. Biol. Chem.*, 1990, **265**, 14971-8. [4] Brown D.G. et al., *International Patent Application*, 2003, WO 2003/038080. [5] Sung B-J. et al., *Nature*, 2003, **425**, 98-105. [6] Huai Q. et al., *J. Biol. Chem*.,2004, **279**, 13095. [7] Zhang et al., *Mol. Cell*, 2004, **15**, 279. [8] Brown D.G. et al, *International Patent Application*, 2004, WO 2004/097010. [9] Card G.L. et al., *Structure*, 2004, **12**, 2233.
Keywords: structure based drug design, novel inhibitors, phosphodiesterase

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Structure-guided Drug Discovery for Protein Kinases Using Fragment-based Lead Identification/Lead Optimization
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Structural GenomiX, Inc. (SGX) has developed an integrated target-to-lead platform that combines high-throughput X-ray crystallography with a fragment-based approach to lead identification/optimization. The proprietary *FAST*™ (Fragments of Active Structures) process exploits crystallographic screening to detect, visualize, and identify small ligands (MW 150-200) that are bound to the target protein. Each member of the *FAST*™ fragment/scaffold library was designed to be amenable to rapid chemical elaboration at two or three points of chemical diversity using high-throughput organic synthesis. Initial lead optimization involves using our knowledge of the co-crystal structure of the target-fragment complex and advanced computational chemistry tools to guide synthesis of small focused linear (one-dimensional) libraries. These linearly elaborated fragments/scaffolds are then evaluated with *in vitro* biochemical and cellular assays and co-crystallography. Thereafter, optimal variations at each point of chemical diversity are combined to synthesize focused combinatorial (two- or three-dimensional) libraries that are again examined with assays and co-crystallography. (The potential chemical diversity of the fully elaborated *FAST*™ fragment/scaffold library far exceeds 160 million compounds.) These focused combinatorial libraries typically contain multiple novel compounds of low molecular weight (<350) that bind the target protein at low nM IC_{50} and already display considerable selectivity. Thereafter, compound series are prioritized for further medicinal chemistry and compound development efforts using the results of *in vitro* and *in vivo* ADME and *in vitro* toxicology studies in concert with structural information. Successful applications of the *FAST*™ fragment-based lead discovery/optimization process will be presented

for both protein kinases (Syk and Gleevec-resistant BCR-ABL) and proteases (Factor VIIa).

Keywords: fragment based drug discovery, structure guided drug discovery, protein kinase drug discovery

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Structural Basis of Multi-functional lipocalin-type Prostaglandin D_2 Synthase

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Prostaglandin (PG) D_2 is a natural somnogen inducing non-rapid eye moving (NREM) sleep and an immuno-modulator. PGD synthase (PGDS) is responsible for the production of PGD_2. We determined the crystal structures of lipocalin-type PGDS (L-PGDS) as the first enzymatic lipocalin by using SeMet-MAD phasing at 2.1 Å resolution [1]. L-PGDS has a catalytic architecture similar to the phylogenetically independent PGDS, hematopoietic PGDS, which belongs to a sigma class glutathione S-transferase [2]. L-PGDS is a multi-functional protein which also acts as a hydrophobic ligand-binding protein. The structures with different conformations in two crystal forms suggest the structural basis of the multi-functionalities as well as the mode of the catalytic action [3]. These proposed mechanisms were consistent with the extended site-directed mutagenesis. We present the structural and functional basis of L-PGDS as a multi-functional protein relevant to the biological actions including NREM sleep promotion in the prostanoid cascade [4].

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Keywords: prostaglandin D synthase, lipocalin, protein crystallography

MS86 PROGRAMMING ROBUST CIF AND XML INTO CRYSTALLOGRAPHIC SOFTWARE

Chairpersons: Herbert J. Bernstein, Brian McMahon

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CIF2CML - Automatic Processing of Chemical Crystallography in XML/CML

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The CIF data structure and hierarchy (CIF, dictionaries, DDL) is largely isomorphic with XML (document/DOM, schema, XMLSchema) and XML tools can therefore be configured to process data from CIFs. First our CIF2CML toolkits convert CIF documents to XML. The data are then validated structurally and semantically (against the dictionaries) and further converted to Chemical Markup Language (CML) (http://www.xml-cml.org).

The crystal structures in CML can then be stored, chemically validated and transformed using the JUMBO CML library and other CML-aware tools. Among the steps are (a) checks on chemical composition (b) treatment of disorder (c) application of symmetry (d) assignment of bonds and (e) molecules (f) unique chemical identification (IUPAC InChI) (g) calculation of 2D coordinates (h) storage in XML repository to create a structural knowledge base which can be searched for chemical and geometrical concepts.

The approach is highly modular with many hundred interoperable components, designed for use with WebServices (http://wwmm.ch.cam.ac.uk/gridsphere/gridpshere) and workflows such as Taverna (http://taverna.sf.net) and institutional repositories (http://eprints.soton.ac.uk/1633/) with Open data. We argue that Open source and Open data provide a robust high-throughput crystallographic semantic web whose prototype will be demonstrated.

Keywords: CIF, XML, CML

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The Role of Data Ontologies in CIF Deposition and Access

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For well over a decade crystallographic data have been routinely submitted to journals and databases as CIFs. During the deposition process, data contained within a CIF can be automatically checked and validated using electronic CIF dictionaries that contain the precise definitions of individual data items. When employed, these ontologies also serve an important role when archiving or accessing deposition data, and within or between crystallographic software applications.

This talk will describe how existing ontologies are employed in CIF deposition and access processes, and what software is currently available to utilize the DDL1 and DDL2 dictionaries during the reading and writing of CIFs. We will describe how the concept of ontological definitions can be extended to automatically provide executable functionality to validation and evaluation processes.

Many aspects of this talk are covered in detail in *International Tables for Crystallography Volume G*[1], which is being launched at this congress.

[1] International Tables for Crystallography, Volume G, *Definition and exchange of crystallographic data*, edited by S.R. Hall & B. McMahon. Heidelberg: Springer, 2005.

Keywords: CIF processing, ontologies, software design

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CIFFOLD: Managing Long Lines in CIF

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Until recently, information in Crystallographic Information File (CIF) format [1] was limited to 80 characters per line and there was no way to represent longer data items and comments faithfully. With the release of CIF version 1.1 [2], the maximum line size has been increased to 2048 characters and a protocol has been specified for folding and unfolding text fields and comments that exceed any given maximum line size. The C/C++ program CIFFOLD implements this line folding/unfolding protocol without loss of the semantic information in the files. This allows new, long-line CIF 1.1 files to be converted to a form suitable for processing by existing software for 80-character line CIF 1.0 files and to recover long-line CIF 1.1 files from CIFs produced by CIF 1.0 software. In addition to folding and unfolding, the software performs logical integrity checks and allows the user to set a variety of options providing control over the tradeoff between faithful versus compact representations. CIFFOLD is part of a package of CIF software for managing IUCr publications that is being upgraded from CIF 1.0 to CIF 1.1 specifications. All the new software in this package will be released under open-source software licenses. Parsers for CIF 1.1 written in C and in Fortran are included in this package.

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[1] Hall S.R., Allen F.H., Brown I.D., *Acta Cryst.*,1991, A**47**, 655-685. [2] http://www.iucr.org/iucr-top/cif/spec/version1.1

Keywords: CIF, mmCIF, software