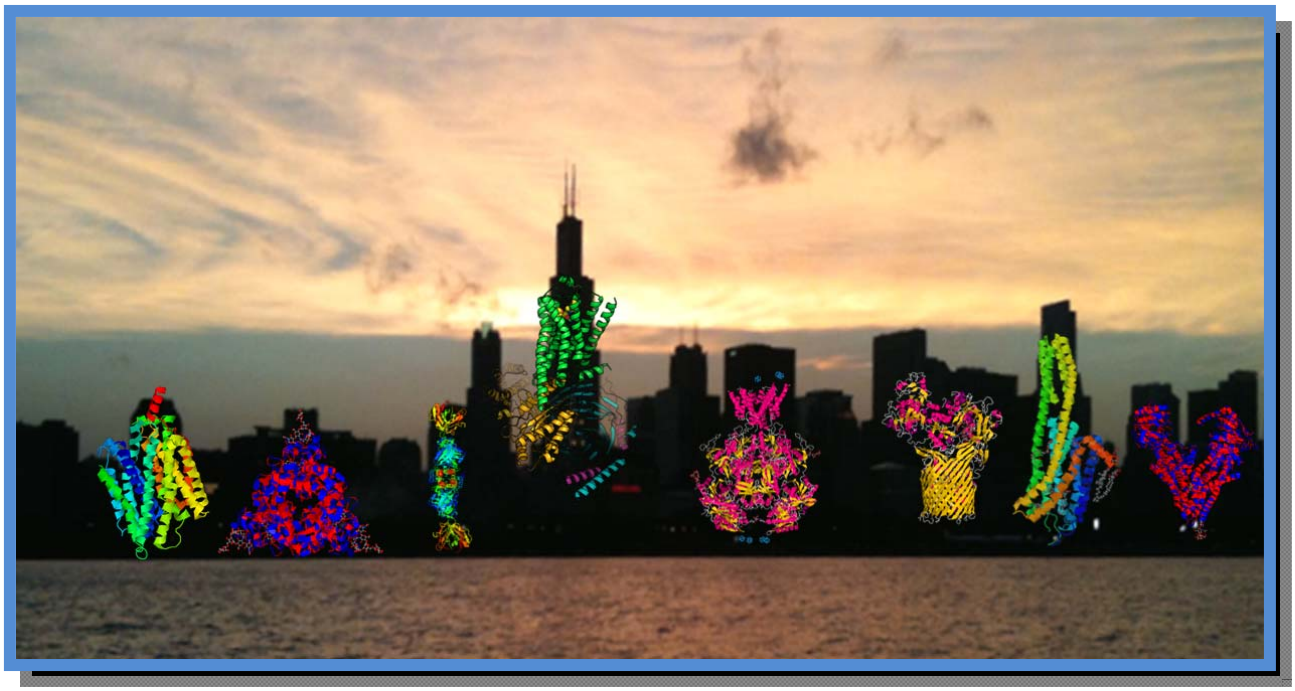
Membrane Protein Structures
2015 Meeting



April 9 – 12, 2015

Advance Photon Source
Argonne National Laboratory
Lemont, IL 60439

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Introduction

The Membrane Protein Structures (MPS) 2015 symposium is the 5th in a series of very successful meetings that have focused on the technologies, reagents and materials that are critical for membrane protein production and structure determination. The program will focus on a variety of recent transformative technologies, and on the illuminating membrane protein structures they have enabled.

This two and a half day symposium will be held at the Advanced Photon Source Conference Center at Argonne National Laboratory from Friday morning, April 10, through Sunday noon, April 12, 2015. Special features of the MPS 2015 meeting include the following activities:

- Five workshops will precede the meeting on Thursday, April 9, and cover a wide range of topics relevant to membrane protein structural biology.
- A Welcome Reception is scheduled for the evening of April 9.
- Poster sessions and receptions will be held in the vendor area on Friday and Saturday before dinner, April 10 and 11, to provide additional time to interact.
- Two series of after-dinner "Lightning Talks" on Friday & Saturday, April 10 & 11, should stimulate questions and discussion.

Meetings in this series have been supported by the NIH Common Fund/Roadmap Initiative in Structural Biology (http://commonfund.nih.gov/structuralbiology/index#) for 10 years, from 2004-2014. Subsequently, investigators funded by the NIH-NIGMS Protein Structure Initiative in membrane protein structure determination have participated. The dates, locations and chairs of these meetings are listed on the next page

On behalf of the Scientific and Local Organizing Committees, we welcome you and hope you have an enjoyable and rewarding meeting.

Local Planning Committee
Bob Fischetti, Argonne National Laboratory, Chicago
Michael Becker, Argonne National Laboratory, Chicago
Surajit Banerjee, Northeastern Collaborative Access Team, Chicago
Steve Corcoran, Argonne National Laboratory, Chicago
Sheila Trznadel, Argonne National Laboratory, Chicago
Cyndi Salbego, Northeastern Collaborative Access Team, Chicago

Scientific Organizing Committee
Bob Stroud, University of California, San Francisco
Ray Stevens, University of Southern California, Los Angeles
James Love, Albert Einstein College of Medicine, New York
Bob Fischetti, Argonne National Laboratory, Chicago
Jean Chin, NIGMS, NIH, Bethesda

The five meetings in this series are listed below:

- November, 2007 at The Scripps Research Institute, La Jolla, CA: Ray Stevens
- March, 2009 at Mission Bay, University of California San Francisco: Bob Stroud
- November, 2010 at a La Jolla hotel, La Jolla, CA: Ray Stevens
- November, 2012 at a San Francisco hotel, San Francisco, CA: Bob Stroud
- April, 2015 at APS-ANL in Chicago, IL: Stroud, Stevens, Love, Fischetti and Chin

Logistics and Argonne

APS WEB Site: http://www.aps.anl.gov/

MPS 2015 WEB Site: http://aps.anl.gov/mps2015

Visitor Information: http://www.aps.anl.gov/About/Visiting/

Transportation (see also the Visitor Information link above):

- United Limousine 800-331-9037
- A-1 Limousine 630-833-3788

Currency:

- The hotels and banks near Argonne do not exchange currency. Plan on using major credit cards or exchanging currency at the airport.

Accommodations:

- On-site Argonne Guest House www.anlgh.org
- Off-site Aloft www.aloftbolingbrook.com

Food:

- Conference lunches and dinners will be served at the Argonne Guest House restaurant for those who pre-paid during registration
- The Argonne Guest Restaurant servers
  - Breakfast
    - Monday – Friday: 6:45 AM – 8:30 AM
    - Saturday – Sunday: 7:00 AM – 11:00 AM
  - Lunch
    - Monday-Friday: 11:00 AM – 1:30 PM
  - Dinner
    - Monday-Friday: 5:00 PM – 8:30 PM
- The Argonne cafeteria is open Monday through Friday from 6:30 a.m. to 1:30 p.m., (closed weekends)
- The 401 Grill in the APS Conference Center is open
  - Monday-Friday: 11:00 AM – 2:00 PM and 4:30 PM – 9:00
  - Saturday and Sunday: 11:30 PM – 7:00 PM
- There are many nice restaurants a short drive from Argonne that are listed at http://www.aps.anl.gov/About/Visiting/Restaurants/

<u>Workshops Schedule</u>
April 9, 2015

Please note each workshop will run in the morning and be repeated in the afternoon so attendees can participate in two workshops.

7:30 AM	Registration opens at 7:30 AM in the APS Atrium
8:30 AM	Morning Workshop Sessions
10:00 AM	Coffee
10:30 AM	Morning Workshop Sessions - continued
12:00 PM	Lunch at Guest House
1:30 PM	Afternoon Workshop Sessions
3:00 PM	Coffee
3:30 PM	Afternoon Workshop Sessions - continued
5:00 PM	Workshops end
6:00 PM	Dinner at Guest House
7:30 PM	Welcome Reception and Registration at Guest House
9:00 PM	Registration Closes

Workshop Abstracts

Beamline Tours and Innovative Micro-crystallography Tools

Hosts: **Michael Becker** and **Surajit Banerjee**
Location: Bldg. 436, Sector 23 (GM/CA@APS) and Sector 24 (NE-CAT)

Description: Staff at APS Sector 23 (GM/CA@APS) and Sector 24 (NE-CAT) will host beamline tours, with particular emphasis on demonstrating tools that enable micro-crystallography on challenging macromolecular crystals, such as with membrane-protein crystals grown in mesophase. These will include hardware features, such as mini-beam collimators and a laser system for Second Order Nonlinear Imaging of Chiral Crystals (SONICC), and software features for different scanning techniques – diffraction based centering, raster and vector data collection along with the automated data processing pipeline, RAPD (Rapid Automated Processing of Data).

Lipidic Cubic Phase and Serial Crystallography

Host: **Vadim Cherezov** and **Wei Liu**
Location: Bldg 401 A1100

Description: Serial crystallography, in which data are collected from many small crystals intersecting with X-ray beam at random orientations, has recently demonstrated a great success at X-ray free-electron lasers as well as at third generation synchrotron sources. Lipidic Cubic Phase (LCP) is a membrane-mimetic material that supports membrane protein crystallization and makes an ideal matrix for delivery of membrane and soluble protein crystals for data collection by serial crystallography. The workshop will focus on sample preparation in LCP and will address other questions related to serial crystallography.

Round table on expression/purification

Host: **James Love**
Location: Bldg 401 Auditorium

Description: The major bottleneck in working with membrane proteins remains expression and purification of 'good quality', functionally active material for structural and other studies. Technologies have advanced to make more and more targets tractable and this round table will focus, in a very informal manner, on what methods are working in different labs together with useful troubleshooting and money saving tips.

(Workshops continued)

Data analysis for membrane proteins

Host: **James Holton**
Location: Bldg. 401 E1100

The central problem of membrane protein crystallography is that you generally can't get good enough data from just one crystal. This is not a new problem in crystallography (in fact, it is the oldest), but it has definitely been recalcitrant to automation. The large combinatorial explosion of possible ways to merge data from more than a dozen or so crystals is mostly to blame, but the phenomenon of non-isomorphism is particularly exacerbating. Fortunately, in recent years, several critical algorithmic advances have been made, mostly in the arena of XFEL data processing, but the gap between 3rd and 4th generation sources is now being bridged. Automated procedures are on the horizon, but in the meantime a simple re-visiting of the fundamental principles laid down by the pioneers of crystallography can go a long way to getting your particular project organized and streamlined to make the best use of the crystals that you've got. We will review these principles, and showcase the latest software for addressing the problems of multi-crystal crystallography.

Protein Science at the Advanced Protein Characterization Facility

Host: **Andrzej Joachimiak**
Location: Bldg. 446

The APCF is a new facility recently added to the structural biology portfolio at the Argonne National Laboratory. The physical co-location of APCF with the Advanced Photon Source allows for rapid structural characterization of proteins, and is a unique feature at US light sources.
The APCF utilizes high-throughput methods and has expanded capacity to clone genes, express, produce and crystallize proteins and other macromolecules. Crystals are grown at nanoliter scale from hundreds of conditions and are delivered to APS beamlines where data are collected and three-dimensional structures are often determined in near real time. The biochemical functions of proteins can be deciphered through a combination of structural and bioinformatics data and can be experimentally validated by functional assays.
The workshop will include an introduction to the facility and a tour of the APCF laboratories. Presentations will include several examples of important drug targets and their complexes with ligands, and protein-protein and protein-DNA complexes. Strategies for engineering and optimization of proteins used in our structural and biochemical studies will be discussed.

Meeting Schedule

Friday April 10, 2015

7:30 AM Registration, Coffee and Breakfast Rolls in the APS Atrium and Gallery

8:00 AM Opening – Bob Fischetti, GM/CA Manager & XSD Assoc. Division Director
8:05 AM Welcome – Stephen Streiffer, ALD for Photon Sciences & APS Director

Transporters and Channels – Bob Fischetti and Bob Stroud, session chairs

8:10 AM Bob Stroud, UCSF, *Wiggle wiggle not a trickle: transporters in action*
8:40 AM Liang Feng, Stanford, *Structures of bacterial homologues of SWEET transporters in two distinct conformations*
9:10 AM TBA
9:40 AM Ron Kaback, UCLA, *It Takes Two to Tango--Structure/Function Studies Yield a Mechanism for Symport by LacY*

10:10 AM Coffee Break in the Atrium and Gallery

Lipid Protein Interactions – Jean Chin, session chair

10:30 AM Steve Sligar, UIUC, *Nanodisc Platform for Determining the Structure and Function of Membrane Proteins*
11:00 AM Ann Kenworthy, Vanderbilt, *Targeting proteins to lipid rafts: mechanisms and consequences*
11:30 AM Lukas Tamm, UVA, *Structure and Function of Prokaryotic Outer Membrane Proteins: From Nanopores to Antibiotic Resistance*
12:00 PM Daniel Rosenbaum, UTHSC Dallas, *Structural biology of disease-related membrane signaling proteins*

12:30 PM Lunch at the Guest House

Expression/Purification – Ian Hunt, session chair

2:00 PM Liz Carpenter, SGC, *The SGC pipeline for production of human membrane proteins for structural biology*
2:30 PM James Love, AECOM, *Production of human membrane proteins in mammalian cells*
3:00 PM John Burg, Garcia Lab, Stanford School of Medicine, *Structural basis for chemokine recognition and activation of a viral G protein-coupled receptor*

3:30 PM **Conference Photo** & Coffee Break in the Atrium and Gallery

(Friday April 10, 2015, Continued)

NMR – Ann McDermott, session chair

4:00 PM	Chad Rienstra, UIUC, *Phospholipid and sterol interactions by solid-state NMR: Roles in blood clotting and antifungal drug mechanisms*
4:30 PM	Francesca Marassi, Sanford Burnham MRI, *Influence of the Membrane Environment on Protein Structure and Function*
5:00 PM	Mei Hong, Massachusetts Institute of Technology, *Solid-State NMR Investigations of Membrane-Curvature Induction by Viral Proteins*
5:30 PM	Gerhard Wagner, Harvard, *Design and application of new nanodiscs for membrane protein structure and function*
6:00 PM	Poster session, reception in the Gallery
7:00 PM	Dinner in the Guest House Restaurant
8:30 PM	"Lightning Talks" and open discussion in the Guest House Restaurant (Cash bar)

Saturday April 11, 2015

7:30 AM Registration, Coffee and Breakfast Rolls in the APS Atrium and Gallery

Receptors – Ray Stevens, session chair

8:00 AM Tracy Handel, UCSD, *Structure and implications of the metastasis promoting/HIV entry receptor, CXCR4, in complex with a chemokine*

8:30 AM Beili Wu, SIMM, *Structural studies of purinergic receptors P2Y12R and P2Y1R*

9:00 AM Gyorgy Snell, Takeda Pharmaceutical, *High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875*

9:30 AM Krys Palczewski, Case Western Reserve U. *Interfacial catalysis: chemistry of vision*

10:00 AM Coffee Break in the APS Atrium and Gallery

Complexes, Enzymes, Pumps – Bob Stroud, session chair

10:30 AM Poul Nissen, DANDRITE, *Structure and mechanism of sodium dependent transporters*

11:00 AM Jue Chen, Rockefeller Univ., *Crystal structure of a polypeptide processing and secretion transporter*

11:30 AM Thomas Tomasiak, UCSF, *Cryo microscopy and structures of ABC transporters*

12:00 PM Eduardo Perozo, UoC, *Mechanisms and regulation of channels*

12:30 PM Lunch at the Guest House

Crystallization – James Love, session chair

2:00 PM Nick Noinaj, Purdue Univ., *The role of BamA in the biogenesis of β-barrel outer membrane proteins*

2:30 PM Samuel Gellman, Univ. Wisconsin, *Development of new detergents for membrane protein manipulation and crystallization*

3:00 PM Vadim Cherezov, USC, *GPCR crystallography at XFELs*

3:30 PM Coffee Break in the APS Atrium and Gallery

EM/Hybrid Methods – Jean Chin and Bob Fischetti, session chairs

4:00 PM Bridget Carragher, NYSBC, *EM as a Tool for Understanding the Structure of Membrane Proteins*

4:30 PM Yifan Cheng, UCSF, *Structures of TRP Channel by Single Particle CryoEM*

5:00 PM Adam Frost, UCSF, *Structure of the Mitochondrial Outer Membrane Fission Machinery*

5:30 PM Ann McDermott, Columbia, *Functional Studies of an Ion channel by NMR in native membranes: Transmembrane Allostery as a Basis for Inactivation*

6:00 PM Poster session, reception in the APS Gallery

7:00 PM Dinner in the Guest House Restaurant

8:30 PM "Lightning Talks" and open discussion in the Guest House Restaurant (Cash bar)

Sunday April 12, 2015

7:30 AM Registration, Coffee and Breakfast Rolls in the APS Atrium and Gallery

Innovative Methods – Ward Smith and Jean Chin, session chairs

8:00 AM Wayne Hendrickson, Columbia U., *SAD Phasing Analysis of Native Membrane Protein Structures*

8:30 AM Tamir Gonen, HHMI, *MicroED – Three Dimensional Electron Crystallography*

9:00 AM Stefan Vogt, APS, Argonne, *Opportunities with Diffraction Limited Storage Rings*

9:30 AM Petra Fromme, ASU, *Serial Femtosecond Crystallography: Dawn of a new Era in Structural Biology of Membrane Proteins*

10:00 AM Coffee Break in the APS Atrium and Gallery

10:30 AM Bob Fischetti, GM/CA and APS, *Innovative approaches to x-ray microcrystallography (DLSR)*

11:00 AM Senyon Choe, SALK, *High-speed NMR structure determination of human membrane proteins: In silico drug search*

11:30 AM Ray Stevens USC, *Integrative Methods to Understanding Human GPCR Signaling*

12:00 PM CLOSING

Speaker Abstracts

Wiggle wiggle – not a trickle: Transporters in action

Robert M. Stroud

UCSF, USA

Transmembrane channels and facilitators can be highly selective in mediating the transport of nutrients and ions across membranes 'downhill', down their concentration gradient. Transporters can drive and concentrate nutrients or ions 'uphill' (energetically) across membranes achieving gradients of >1000:1 driven by 'downhill' movement of other coupled ions, protons, metabolites or electrochemical gradients[1,2,3,4]. How do such 'secondary transporters' accomplish this? Using three examples[1,2,4] we seek to understand the mechanisms by which this coupling or two equilibria achieves the necessary structural transitions that drive uphill transport. In each case we derive mechanistic insight into the basis for how this is accomplished. What emerges is already surprising in many ways. For example it might seem that to concentrate ions or nutrients against a 1000:1 gradient, the affinity for the nutrient should change from high-affinity on one side to low on the other. But this is not so; the affinity is the same from either side. How do they achieve concentration without either getting blocked by a bound nutrient 'stuck' in the sequestered state? – and how do they not leak substrates, driving ions, or even protons across the membrane?

1. Pedersen BP, Kumar H, Waight AB, Risenmay AJ, Roe-Zurz Z, Chau BH, Schlessinger A, Bonomi M, Harries W, Sali A, Johri AK, Stroud RM (2013) Crystal structure of a eukaryotic phosphate transporter. *Nature.* **496:** 533-6.
2. Waight AB, Pedersen BP, Schlessinger A, Bonomi M, Chau BH, Roe-Zurz Z, Risenmay AJ, Sali A, Stroud RM (2013) Structural basis for alternating access of a eukaryotic calcium/proton exchanger. *Nature.* **499:** 107-10.
3. Pak JE, Ekendé EN, Kifle EG, O'Connell JD 3rd, De Angelis F, Tessema MB, Derfoufi KM, Robles-Colmenares Y, Robbins RA, Goormaghtigh E, Vandenbussche G, Stroud RM. Structures of intermediate transport states of ZneA, a Zn(II)/proton antiporter. Proc Natl Acad Sci U S A. (2013) **110:**18484-9.
4. Kumar H, Kasho V, Smirnova I, Finer-Moore J, Kaback RH, Stroud RM. (2014) Structure of Sugar-Bound LacY. *Proc Natl Acad Sci U S A*. **111:** 1784-1788.

Structure and mechanism of SemiSWEET sugar transporter

Liang Feng

Molecular and Cellular Physiology, Standford School of Medicine, USA

Sugars serve as the principal energy source, basic building material and important signal molecules in both animals and plants. Their distribution throughout the body is controlled by sugar transport across the membrane, which is mediated by sugar transporters. The recently discovered SWEET family proteins are monosaccharide and disaccharide transporters and play crucial roles in many physiological and developmental processes in plants and animals. SWEET homologs in prokaryotes, called SemiSWEET, are among the smallest natural transporters. We have solved high-resolution structures of SemiSWEETs in different conformational states, which reveal the architecture of this transporter family and provide structural basis to understand their transport mechanism.

Mechanisms of substrate translocation and phosphorylation in an EIIC saccharide transporter

Jason McCoy, Zhenning Ren, Matthias Quick, and **Ming Zhou**

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX

The bacterial phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS) is a unique sugar uptake pathway. Sugar transport is accomplished by three components of the system: Enzyme I (EI), the histidine-containing phosphocarrier protein (HPr) and Enzyme II Complex (EII), which is composed of protein subunits EIIA, B and C. An extracellular sugar molecule is taken up by EIIC, translocated across the cell membrane so that it is accessible from the intracellular side, and phosphorylated by EIIB before it is released into the cytosol. The phosphate group originates from PEP, and is transferred sequentially through EI, HPr, EIIA and EIIB. Covalent modification of the sugar during transport, known as group translocation, makes the mechanism of EIIC distinct from that of other families of sugar transporters. Full understanding of this mechanism has been hampered by the lack of atomic resolution crystal structures of EIIC. We have solved crystal structures of two EIIC homologs from *Bacillus cereus*, and by comparing the two structures we were able to deduce conformational changes required for substrate translocation and phosphorylation.

It takes two to tango--Structure/function studies yield a mechanism for symport

H. Ronald Kaback

Departments of Physiology and Microbiology, Immunology & Molecular Genetics, Molecular Biology Institute, University of California Los Angeles, Los Angeles, California 90095.

Lactose permease (LacY), a paradigm for the largest family of membrane transport proteins, catalyzes the coupled translocation of a galactoside and an H^+ across the *Escherichia coli* membrane (galactoside/H^+ symport). Initial x-ray structures reveal N- and C-terminal domains, each with six largely irregular transmembrane helices surrounding an aqueous cavity open to the cytoplasm. Recently, a structure with a narrow periplasmic opening and an occluded galactoside was obtained, confirming many observations and indicating that sugar binding involves induced-fit. LacY catalyzes symport by an alternating access mechanism. Experimental findings garnered over 45 years indicate that: (i) The limiting step for lactose/H^+ symport in the absence of the H^+ electrochemical gradient ($\Delta\tilde{\mu}_H$+) is deprotonation, whereas in the presence of $\Delta\tilde{\mu}_H$+, the limiting step is opening of apo LacY on the other side of the membrane. (ii) LacY must be protonated to bind galactoside (the pK for binding is ~10.5). (iii) Galactoside binding and dissociation--not $\Delta\tilde{\mu}_H$+--are the driving force for alternating access. (iv) Galactoside binding involves induced fit causing transition to an occluded intermediate that undergoes alternating access. (v) Galactoside dissociates, releasing the energy of binding. (vi) Arg302 comes into proximity with protonated Glu325 causing deprotonation. Accumulation of galactoside against a concentration gradient does not involve a change in K_D for sugar on either side of the membrane, but the pK_a (the affinity for H^+) decreases markedly. Thus, transport is driven chemiosmotically, but contrary to expectation, $\Delta\tilde{\mu}_H$+ acts kinetically to control the rate of the process.

The Nanodisc Platform for Determining the Structure and Function of Membrane Proteins

Stephen G. Sligar

Swanlund Endowed Chair, University of Illinois Urbana-Champaign, USA

Membrane proteins are involved in numerous vital biological processes, including transport, signal transduction and the enzymes in a variety of metabolic pathways. Integral membrane proteins account for up to 30% of the human proteome and make up more than half of all currently marketed therapeutic targets. Unfortunately, membrane proteins are inherently recalcitrant to study using the normal toolkit available to scientists, and one is most often left with the challenge of finding inhibitors, activators and specific antibodies using a denatured or detergent solubilized aggregate. Often, since membrane proteins are inherently insoluble and prone to aggregation and oligomerization in solution, the active state of interest is obscured. The Nanodisc platform circumvents these challenges by providing a self-assembled system that renders typically insoluble, yet biologically and pharmacologically significant, targets such as receptors, transporters, enzymes, and viral antigens soluble in aqueous media. Because Nanodisc constructs provide a native-like bilayer environment that maintain a target's functional activity, they are a versatile tool in the study of ion channels, biased signaling by GPCRs, integrins, cytochrome P450s, various toxins and viral entities as well as a plethora of pharmaceutical targets. Importantly, the Nanodisc also provides a controlled surface for protein – membrane recognition as is involved in RAS and integrin signaling and blood coagulation. In addition to these opportunities, Nanodiscs provide a nanometer scale vehicle for the *in vivo* delivery of amphipathic drugs, therapeutic lipids, tethered nucleic acids, imaging agents and active protein complexes. In my presentation I will summarize the numerous recent successes using the Nanodisc platform.

Targeting proteins to lipid rafts: mechanisms and consequences

Anne Kenworthy

Vanderbilt School of Medicine, Nashville, TN

How proteins and lipids cooperate to form functional domains remains a major question in biology. Here, I will discuss two examples of how proteins interact with raft-associated lipids and the functional consequences of these interactions. First, I will discuss studies of the 99 residue transmembrane C-terminal domain (C99) of the amyloid precursor protein. Association of C99 with cholesterol-rich membrane raft domains is thought to promote cleavage of C99 to release the Alzheimer's disease-promoting amyloid-β (Aβ) polypeptide. By reconstituting C99 into GUVs composed of coexisting raft-like liquid ordered (Lo) domains and liquid disordered (Ld) domains, we are currently testing the hypothesis that C99 has an intrinsic affinity for raft-like liquid ordered (Lo) domains imparted by its ability to specifically bind to cholesterol. Second, I will discuss how cholera toxin, the causative agent of cholera, targets raft lipids as a mechanism to enter cells via endocytosis. The membrane binding subunit of cholera toxin, CTxB, is a homo-pentamer that is known to bind to 5 copies of its glycolipid receptor, GM1. Using living cells as a model system, we are currently examining a potential role for toxin-induced raft crosslinking in induction of membrane curvature and toxin uptake.

Structure and Function of Prokaryotic Outer Membrane Proteins: From Nanopores to Antibiotic Resistance

Lukas K. Tamm

University of Virginia, Charlottesville, VA, USA

Solution NMR has contributed substantially to elucidating structures of membrane proteins over the past 14 years. In addition to solving structures, great efforts have been made to also characterize the dynamics of membrane proteins on multiple time scales by NMR and relate these findings to function. Moreover, NMR offers unique opportunities to study specific and general lipid interactions of membrane proteins and elucidate how these interactions contribute to the function of membrane proteins.
Our lab has focused on outer membrane proteins (OMPs) of Gram-negative bacteria. We have solved the structures of OmpA and OmpG of *Escherichia coli* and OprH and OprG of the highly pathogenic bacterium *Pseudomonas aeruginosa*. From the beginning OmpA has served as a fantastic system to study the folding and solve the structure and detailed dynamics of a model membrane protein (1-3). OmpG is of great interest to engineering nanopores for biosensing. The determination of concerted loop dynamics by detailed PRE measurements and ensemble calculations have contributed a great deal to understand its pH-dependent pore gating (4-6). OprH contributes to the antibiotic resistance of *P. aeruginosa* presumably by tightly binding to lipopolysaccharides in the outer membrane, i.e. an interaction that we have closely monitored by NMR (7). We found that OprG is responsible for the uptake of small amino acids and are undertaking efforts to understand this transport and its resistance to antibiotics by novel NMR approaches (8 and unpublished results).

1. Arora A, Abildgaard F, Bushweller JH, and Tamm, LK (2001) Structure of the outer membrane protein A transmembrane domain by NMR spectroscopy. *Nature Struct. Biol.* 8:334-338.
2. Hong H, Szabo G, and Tamm LK (2006) Electrostatic side-chain couplings in the gating of the OmpA ion channel suggest a mechanism for pore opening. *Nature Chem. Biol*. 11:627-635.
3. Liang, B, Arora A, and Tamm LK (2010) Fast-time scale dynamics of outer membrane protein A by extended model-free analysis of NMR relaxation data. (Special issue: dynamics of membrane proteins by NMR) *Biochim. Biophys. Acta* 1798:68-76.
4. Liang B and Tamm LK (2007) Structure of outer membrane protein G by solution NMR. *Proc. Natl. Acad. Sci. USA* 104:16140-16145.
5. Zhuang T, Chisholm C, Chen M, Tamm LK (2013) NMR-based conformational ensembles explain pH-dependent opening and closing of OmpG channel. *J.A.C.S.* 135:15101-15113. PMC3863726
6. Zhuang T and Tamm LK (2014) Control of engineered nanopore conductance by concerted loop motions. *Angewandte Chemie (Intl. Ed.)* 53:5897-5902. PMC4091679

Structural biology of disease-related membrane signaling proteins

Daniel Rosenbaum

UT Southwestern Medical Center, Dallas, TX, USA

We are studying how human integral membrane proteins undergo conformational changes that convert sensing of hormones, lipids, and other stimuli into activation of intracellular signaling cascades. We developed methods that enabled structure determination of G protein-coupled receptors (GPCRs), which comprise a majority of the hormone and neurotransmitter receptors in mammals. Using these methods, we solved the first high-resolution structures of the orexin receptors, which respond to neuropeptides in the central nervous system to regulate sleep and other behavioral functions. These structures reveal how clinically approved orexin receptor antagonists bind and inhibit the protein, and provide clues to the mechanism of hormone activation. We are also investigating the mechanism of cholesterol homeostasis mediated by integral membrane proteins of the SREBP signaling pathway. These molecules are similar to GPCRs in that they convert the binding energy of specific ligands into conformational changes across membranes that trigger downstream signaling. Our long-term goal is to use this structural and mechanistic knowledge to design therapeutic compounds for human diseases.

The SGC pipeline for production of human membrane proteins for structural biology

Andrew Quigley, Chitra Shintre, Yin Yao Dong, Mariana Grieben, Simon Bushell, Ashley C.W. Pike, Alexandra MacKenzie, Belinda Faust, Leela Shrestha, Solenne Goubin, Claire Strain-Damerell, Pravin Mahajan, Shubhashish Mukhopadhyay, Nicola A. Burgess-Brown, **Elisabeth P. Carpenter**

Structural Genomics Consortium, Nuffield Department of Medicine,
University of Oxford, Old Road Campus Research Building, Roosevelt Drive,
Headington, Oxford, OX3 7DQ, England.

Structural studies of human integral membrane proteins (IMPs) is a field that is advancing rapidly. New technologies for producing proteins and new methods for solving structures are beginning to have an impact and this has lead to the deposition of over 50 structures. At the Structural Genomics Consortium in Oxford we work with ion channels, solute carriers, ABC transporters, enzymes and other IMPs that are associated with genetic diseases. We use primarily use expression in insect cells although we are also testing the use of mammalian systems for protein production. We have screened over 300 proteins to determine which can be purified from insect cell cultures and we have succeeded in producing 10% of these proteins at a level where they are suitable for crystallisation. To produce stable proteins we use a range of constructs, purification methods, detergents and lipids, with size exclusion chromatography profiles and thermostability as the main readouts of protein quality. We also use a range of crystallisation methods and, for the proteins we have crystallised to date, vapour diffusion has been the most successful technique for crystallisation of our targets. From the 30 proteins we have purified, we have obtained lead crystals for twelve proteins and solved 5 structures, with examples from each of the protein families we are are working with.

We recently solved structures of two human ion channels from the K2P family, TREK-1 and TREK-2. These polymodal potassium channels are responsive to a series of activatory and inhibitory stimuli, including stretch, temperature, pH and a range of small molecules. We have solved TREK-2 in two different conformations and as a complex with an inhibitor. These structures, together with mutagenesis and electrophysiology data have given us an understanding of the regulation of these polymodal channels, which will help with future drug design programs for these potential pain targets.

Production of human membrane proteins in mammalian cells

James Love

Albert Einstein College of Medicine. NY.

Integral membrane proteins are key targets in the understanding of health and human disease, yet producing functional material in great enough quantities for structural studies remains a formidable task. This talk highlights the expression technologies and novel devices under development that will greatly aid high-throughput efforts to produce functional human membrane proteins, specifically GPCRs and transporters, for a plethora of downstream studies.

Structural basis for chemokine recognition and activation of a viral G protein-coupled receptor

John S. Burg

Molecular and Cellular Physiology, Standford School of Medicine, USA

G protein-coupled receptors (GPCRs) engage a diverse set of ligands ranging from small molecules to proteins. The structures of GPCRs bound to small molecule ligands have revealed much about receptor structure and activation. However, proteins comprise a significant fraction of GPCR ligands for which structural information is lacking. Chemokines are protein GPCR ligands that function as immune modulators. Several viruses also encode chemokines and chemokine receptors in order to hijack the host immune response. To address the question of how chemokine receptors engage their protein ligands, we purified and solved the X-ray crystal structure of a complex between the human cytomegalovirus GPCR US28 and the chemokine domain of human CX3CL1 (fractalkine). We expressed both US28 and CX3CL1 in HEK293 cells using the Bacmam system, in which baculovirus is used to transduce mammalian cells. US28 and CX3CL1 were first purified separately, and then immobilized CX3CL1 was used to purify US28 by ligand affinity. We used the purified US28/CX3CL1 complex to produce an immunized alpaca nanobody cDNA library, allowing yeast display selection of nanobodies directed against the US28/CX3CL1 complex to use as crystallization aids. We solved two crystal structures of US28 in complex with CX3CL1 using lipidic cubic phase crystallization. The nanobody-bound structure was solved at a resolution of 2.9 Å and the other without a nanobody at 3.8 Å. These structures reveal a paradigm for chemokine binding applicable to chemokine-GPCR interactions more generally. Furthermore, the structure of US28 in both crystal forms suggests that this viral GPCR has evolved a highly stable active state in order to achieve efficient agonist-independent constitutive signaling.

Phospholipid and sterol interactions by solid-state NMR: Roles in blood clotting and antifungal drug mechanisms

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Atomistic information about lipids is challenging to obtain by most experimental methods. Magic-angle spinning solid-state NMR enables new insights into the structure, dynamics and functional molecular interactions of phospholipids and sterols. In this talk, first I will present approaches to examine the assembly of phosphatidylserine in the presence of calcium into complexes that accelerate blood coagulation. We have developed semi-synthetic isotopic labeling strategies, combined with assembly in Nanodiscs and NMR measurements to report detailed structural information in this context. Second, I will describe our studies to elucidate the mechanism of action of the antifungal drug amphotericin, which is the gold standard small molecule drug for treatment of life-threatening fungal infections. For over half a century, this powerful but also highly toxic small molecule has evaded development of microbial resistance. Understanding how amphotericin kills yeast is key to guide the development of derivatives with an improved therapeutic index. Through a series of NMR and functional studies, we demonstrate that amphotericin exists primarily as large, extramembranous aggregates that kill yeast by extracting ergosterol from lipid bilayers. This new mechanistic understanding is guiding development of amphotericin B analogs with improved efficacy.

"Amphotericin forms an extramembranous and fungicidal sterol sponge", T.M. Anderson, M.C. Clay, A.G.Cioffi, K.A. Diaz, G.S. Hisao, M.D. Tuttle, A.J. Nieuwkoop, G. Comellas, N. Maryum, S. Wang, B.E. Uno, E.L. Wildeman, T. Gonen, C. M. Rienstra, M.D. Burke. *Nat. Chem. Bio.* 2014, *10*, 400-406.

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NMR-restrained structure determination of membrane proteins in lipid bilayer membranes.

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The biological functions and molecular structures of proteins are highly dependent on the physical and chemical properties of the surrounding environment. Just as water is essential for supporting the native states of soluble proteins, the lipid bilayer is critical for preserving the functional and structural integrity of membrane proteins.
The principal advantage of NMR spectroscopy as a method for structure determination is its ability to examine proteins in samples that are very close to their functional environments. NMR is well suited for studying soluble proteins in water and membrane proteins in lipid bilayer environments.
Here we describe recent progress on NMR structure determination of membrane proteins in lipid bilayers, with examples that include a bacterial virulence factor and a human cyto-protective oncoprotein. We show that samples can be optimized for parallel structural and activity studies and we present new computational methods designed specifically for proteins in membranes.

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Solid-State NMR Investigations of Membrane-Curvature Induction by Viral Proteins

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Many proteins generate membrane curvature for function. Examples include viral fusion proteins and proteins that mediate virus budding and release. How protein structure underlies membrane-curvature generation is so far poorly understood. We are investigating the conformation, dynamics, and lipid interactions of two curvature-inducing membrane proteins: the fusion protein of the paramyxovirus PIV5, which catalyzes virus-cell membrane fusion, and the influenza M2 protein, which mediates membrane scission during virus release. ^{13}C and ^{15}N chemical shifts indicate that the fusion peptide (FP) and transmembrane domain (TMD) of the PIV5 fusion protein adopt dramatically different conformations in different lipid membranes. ^{31}P NMR lineshapes and correlation spectra reveal that these conformations differ in their abilities to induce membrane curvature and dehydration, which are necessary for high-energy fusion intermediates, thus the multiple conformations have different functional relevance. We characterize the nature of the membrane curvature by solid-state NMR, supplemented by small-angle X-ray scattering, and propose possible FP and TM conformations involved in the hemifusion intermediate and post-fusion states.

To investigate how the influenza M2 protein generates membrane curvature for virus budding, we developed novel relaxation and correlation approaches involving oriented bicelles under static and off-magic-angle-spinning conditions. The data show that an amphipathic helix in M2 causes high membrane curvature and M2 is localized in this high-curvature domain. To determine the conformation of the cytoplasmic domain that encompasses the amphipathic helix, we have adopted a two-pronged approach, assigning and simulating the spectra of uniformly ^{13}C, ^{15}N-labeled full-length protein, as well as measuring site-specifically labeled proteins produced using chemical ligation. The results consistently indicate that the cytoplasmic domain is highly disordered, suggesting that binding to other virus proteins or membrane domains may play a significant role in M2 function.

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Design and use of new nanodiscs for studies of membrane protein structure and function

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Membrane protein studies depend crucially on finding sample conditions that are close to the native membrane environment and suitable for structural and functional studies. In the past we have used phospholipid nanodiscs for NMR studies of small integral membrane proteins. While we could record good NMR spectra of small membrane proteins in nanodiscs and also determine a structure there were obvious problems with this approach. These include nanodisc size and heterogeneity. In particular, the distribution of nanodisc diameters was rather wide. As a consequence, the number of membrane proteins incorporated couldn't be controlled precisely, and the quality of NMR spectra was suboptimal due to the heterogenety [1,2]. To address this problem we have developed protocols for covalently linking the termini of the membrane scaffolding protein (Msp). Indeed, the circularization creates an extremely narrow distribution of nanodisc diameter. Using this approach we have designed nanodiscs ranging from 8 to 80 nm in diameter. While the smaller nanodisc are ideal for NMR studies of integral membrane proteins, the larger particles allow incorporation of bigger membrane proteins or membrane protein complexes, which are suitable for structural studies with electron microscopy.

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Lessons from the Structure of the Chemokine Receptor, CXCR4

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Chemokine receptors are G Protein-Coupled Receptors (GPCRs) best known for their role in controlling cell migration in the context of immune system function. However, inappropriate regulation of chemokine-mediated processes contributes to the pathology of many diseases, the most validated of which is HIV where CCR5 and CXCR4, serve as co-receptors for viral entry into cells. Chemokines/receptors are also associated with a multitude of inflammatory diseases such as asthma, rheumatoid arthritis, and multiple sclerosis. For these reasons, they have become widespread targets in the pharmaceutical industry where high-resolution structures would be extremely valuable for drug design. In the last several years, numerous structures of G Protein-Coupled Receptors (GPCRs) have finally succumbed to crystallization including two chemokine receptors in complex with small molecule antagonists. However, the natural ligands of chemokine receptors are small proteins, and protein:protein complexes involving membrane proteins tend to be particularly challenging structural targets. In this presentation I will describe our strategy for determining the structure of the chemokine receptor, CXCR4, in complex with a chemokine ligand and what we have learned from the structure.

Structural studies of purinergic receptors $P2Y_1R$ and $P2Y_{12}R$

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In response to adenosine 5′-diphosphate, purinergic receptors P2Y1R and P2Y12R regulate platelet activation and thrombus formation, and thus serves as important antithrombotic drug targets. Here we report the crystal structures of human $P2Y_{12}R$ in complex with its non-nucleotide reversible antagonist AZD1283, with a full agonist 2-methylthio-adenosine-5'-diphosphate (2MeSADP), and with the corresponding ATP derivative 2-methylthio-adenosine-5'-triphosphate (2MeSATP). The $P2Y_{12}R$ structures reveal a distinct straight conformation of helix V, which sets $P2Y_{12}R$ apart from other known class A G protein-coupled receptor (GPCR) structures. Agonist and non-nucleotide antagonist adopt different orientations in the $P2Y_{12}R$, with only partially overlapped binding pockets. The agonist-bound $P2Y_{12}R$ structure answers long-standing ambiguities surrounding $P2Y_{12}R$-agonist recognition, and reveals interactions with several residues that had not been reported to be involved in agonist binding. As a first example of a GPCR where agonist access to the binding pocket requires large scale rearrangements in the highly malleable extracellular region, the structural studies therefore will provide invaluable insight into the pharmacology and mechanisms of action of agonists. We also report the crystal structures of human $P2Y_1R$ in complex with a nucleotide antagonist MRS2500 at 2.7Å resolution, and with a non-nucleotide antagonist BPTU at 2.2Å resolution. The $P2Y_1R$ structures reveal two distinct ligand binding sites, providing atomic details of $P2Y_1R$'s unique ligand binding modes. MRS2500 recognizes a binding site within the seven transmembrane bundle of $P2Y_1R$, which, however, is different in shape and location from the nucleotide binding site in the $P2Y_{12}R$ structure. BPTU binds to an allosteric pocket on the external receptor interface with the lipid bilayer, making it the first structurally characterized selective G protein-coupled receptor (GPCR) ligand located entirely outside of the helical bundle. These high-resolution insights into $P2Y_1R$ should enable discovery of new orthosteric and allosteric antithrombotic drugs with reduced adverse effects.

High-resolution structure of the human GPR40 receptor bound to allosteric agonist TAK-875

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Human GPR40 receptor (hGPR40), also known as free fatty-acid receptor 1 (FFAR1), is a G-protein-coupled receptor that binds long chain free fatty acids to enhance glucose-dependent insulin secretion. Novel treatments for type-2 diabetes mellitus are therefore possible by targeting GPR40 with partial or full agonists. TAK-875, or fasiglifam, is an orally available, potent and selective partial agonist of the hGPR40 receptor, which reached phase III clinical trials for the potential treatment of type-2 diabetes mellitus. Data from clinical studies indicate that TAK-875, which is an ago-allosteric modulator of hGPR40, demonstrates improved glycaemic control and low hypoglycaemic risk in diabetic patients. Here we report the crystal structure of hGPR40 receptor bound to TAK-875 at 2.3Å resolution. The co-complex structure reveals a unique binding mode of TAK-875 and suggests that entry to the non-canonical binding pocket most probably occurs via the lipid bilayer. The atomic details of the extensive charge network in the ligand binding pocket reveal additional interactions not identified in previous studies and contribute to a clear understanding of TAK-875 binding to the receptor. The hGPR40–TAK-875 structure also provides insights into the plausible binding of multiple ligands to the receptor, which has been observed in radioligand binding and Ca^{2+} influx assay studies. Comparison of the transmembrane helix architecture with other G-protein-coupled receptors suggests that the crystallized TAK-875-bound hGPR40 complex is in an inactive-like state.

Interfacial catalysis: Chemistry of Vision

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Retinal photoreceptor cells can respond to light throughout our lives because they continuously regenerate a light-sensitive chromophore and certain essential structures. Defects in many proteins involved in these processes cause photoreceptor degeneration. Each mammalian ROS consists of a pancake-like stack of 500–2,000 distinct disk membranes enclosed by the plasm membranes (PM). The major protein component (>90%, 5 mM) of ROS disc membranes is rhodopsin (Rho), a member of GPCR family A. Rho is also present at lower density in the PM. With its covalently bound ligand 11-*cis*-retinal, Rho undergoes changes upon photoactivation that ultimately trigger phototransduction, a biochemical cascade of reactions that amplifies the signal triggered by light and converts it to an electrophysiological response. Regeneration of rhodopsin requires a constant production of 11-*cis*-retinal through visual cycle with the key retinoid isomerase, RPE65. The absence of structures of RPE65 in complex with retinoids represented the main bottleneck to understanding its catalytic mechanism, with poor aqueous solubility of the natural substrates and products the major factor that prevented the formation of high-occupancy complexes for crystallographic studies. To overcome this inherent limitation, we identified retinoid-like molecules with improved solubility characteristics. We showed that emixustat, a modulator of visual cycle activity that has entered clinical trials for treatment of AMD, efficiently inhibits RPE65, and enhanced its structural similarity to the retinoid carbon backbone by substituting a β-ionone ring for the cyclohexyl moiety, creating a hybrid molecule referred to as MB-001. RPE65 was then successfully crystallized in complex with emixustat or MB-001, and the corresponding structures were determined to 1.8 and 2.3 Å resolution, respectively.The bound inhibitors were unambiguously identified in the electron density maps, and the additional residual electron density in an adjacent hydrophobic pocket within the active site cavity was assigned to a Fe-bound palmitate molecule. Based on these structures, we proposed a detailed catalytic mechanism, which involves polarization of the ester group of the natural substrate with Fe acting as a Lewis acid, breakage of the C15-O bond yielding a retinyl cation-palmitate ion pair, and nucleophilic attack of the solvent on C15.

Structure, function and mechanism of Na+ dependent transporters

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Na+,K+-ATPase is an active membrane transporter of the P-type ATPase family. It fulfills a key role in all animal cells by establishing and maintaining the electrochemical gradient for Na+ across the plasma membrane and it is also critically involved in K+ homeostasis and clearance. Na+,K+-ATPase is an electrogenic transporter that for each ATPase cycle extrudes three Na+ ions in return for two K+ ions. A unique site 3 for Na+ most likely undergoes protonation/deprotonation as part of the Na+/K+ release processes. Furthermore, the enzyme is heavily influenced by the lipid environment and is a key target for cardiotonic steroids, such as digoxin and ouabain. We have determined structures of Na+ and K+-bound states and also investigated inhibitory complexes with cardiotonic steroids. Combined with biochemical and electrophysiological studies we have identified important functional sites, in particular with attention to site 3 and a C-terminal ion pathway.
Secondary transporters of the neurotransmitter:sodium symporters (NSS) use the energy stored in the Na+ gradient for accummulative cotransport of solutes, such as the neurotransmitters glycine, GABA, dopamine, serotonin and norepinephrine in human. Recently we have obtained new insight of the functional cycle of NSS through new structures determined of the bacterial NSS proteins MhsT and LeuT in the inward-facing, Na+ and substrate-occluded state (Trp bound MhsT) and the outward-facing, occluded return state (Na+ and substrate-free LeuT). The new structures point to an important interplay between the substrate sites, an extracellular vestibule and the intracellular interface in control of inward-outward switching.

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Crystal structure of a polypeptide processing and secretion transporter

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Bacteria secrete peptides and proteins to communicate, to poison competitors, and to manipulate host cells. Among the various protein-translocation machineries, the peptidase-containing ATP-binding cassette transporters (PCATs) are appealingly simple. Each PCAT contains two peptidase domains that cleave the secretion signal from the substrate, two transmembrane domains that form a translocation pathway, and two nucleotide-binding domains that hydrolyze ATP. In gram-positive bacteria, PCATs function both as maturation proteases and exporters for quorum-sensing or antimicrobial polypeptides. In gram-negative bacteria, PCATs interact with two other membrane proteins to form the Type I secretion system. In this paper, we present crystal structures of PCAT1 from *C. thermocellum* in two different conformations. These structures, accompanied by biochemical data, show that the translocation pathway is a large α-helical barrel sufficient to accommodate small folded proteins. ATP binding alternates access to the transmembrane pathway and also regulates the protease activity, thereby coupling substrate processing to translocation.

Cryo-EM structure of an ABC exporter

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ABC transporters couple ATP hydrolysis to the transport of numerous biologically important substrates, including lipids, peptides, and xenobiotic molecules. Structural studies of ABC exporters have revealed several details of their architecture, catalytic mechanism, and clues to their transport process but connecting the diverse array of conformational states has proven to be a challenge. TmrAB is a likely lipid transporter with a heterodimer arrangement. We determined the structure of TmrAB to 8.2Å resolution with details <5Å in the transmembrane region using newly developed cryo-EM technologies including direct electron detectors, maximum likelihood based reconstruction, and drift correction coupled with an antibody fragment assisted approach. The structure and subsequent cysteine cross-linking reveal an inward open conformation with nucleotide binding domains (NBDs) in contact at their C-termini but otherwise in a conformation different from the inward states of many other ABC exporters. Density in the transmembrane domains (TMDs) may be substrate bound to a laterally open gate poised to accept hydrophobic substrates from the inner leaflet of the lipid bilayer. Taken together, these findings suggest a substrate translocation mechanism where substrate binding to a lateral gate coupled to conformational changes at the NBDs upon ATP binding initiates their rearrangement in a slide and twist mechanism to facilitate transport.

The Structural Basis of Charge Translocation in Voltage Sensing Domains

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The transduction of electric fields into protein motion plays an essential role in the generation and propagation of cellular signals. Voltage-sensing domains (VSD) in ion channels and enzymes carry out these functions through reorientations of discrete gating charges in the S4 helix. The voltage sensitive phosphatase from *C. intestinalis* (Ci-VSP) is controlled by a standard VSD with high sequence similarity to the S1-S4 segments found in Na^+ and K^+ channels. To address some of the fundamental questions regarding how membrane proteins sense transmembrane voltages, we have pursued structural and biophysical information on Ci-VSP's isolated VSD, under conditions that stabilize the Up and the Down conformations. An overview of the conformational transitions in VSDs from Ci-VSP and the potassium channel KvAP is provided on the basis of combined X-ray crystallography, spectroscopy and electrophysiology analyses. Additional spectroscopic analyses of the human proton channel hHV1 show that the secondary structure of transmembrane segments is generally consistent with the expected VSD topology. Solvent accessibility measurements revealed that the gating Arginines (205, 208 and 211) are located on the lower part of S4, the internal leaflet of the bilayer. The presence of a narrow solvent occluded region strongly suggests a short proton conduction pathway, and does not seem to be compatible with the idea of a long water wire across membrane. Furthermore, the Hv1 represents a resting state VSD at 0 mV according to its G-V curve. These results help define an explicit mechanism for voltage sensing and set the basis for electromechanical coupling in voltage-dependent enzymes and ion channels.

The role of BamA in the biogenesis of β-barrel outer membrane proteins

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β-barrel membrane proteins are essential for nutrient import, signaling, motility, and survival. In Gram-negative bacteria, the β-barrel assembly machinery (BAM) complex is responsible for the biogenesis of β-barrel membrane proteins, with homologous complexes found in mitochondria and chloroplasts. Here we describe the structure of BamA, the central and essential component of the BAM complex, from two species of bacteria: *Neisseria gonorrhoeae* and *Haemophilus ducreyi*. BamA consists of a large periplasmic domain attached to a 16-strand transmembrane β-barrel domain. Three structural features speak to the mechanism by which BamA catalyzes β-barrel assembly. First, the interior cavity is accessible in one BamA structure and conformationally closed in the other. Second, an exterior rim of the β-barrel has a distinctly narrowed hydrophobic surface, locally destabilizing the outer membrane. And third, the β-barrel can undergo lateral opening, evocatively suggesting a route from the interior cavity in BamA into the outer membrane.

Development of new Amphiphiles for Manipulation and Crystallization of Membrane Proteins

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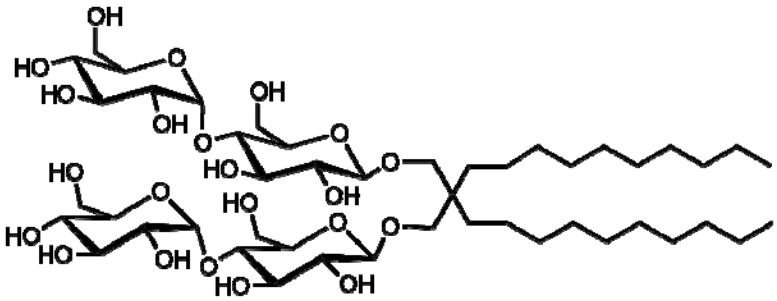
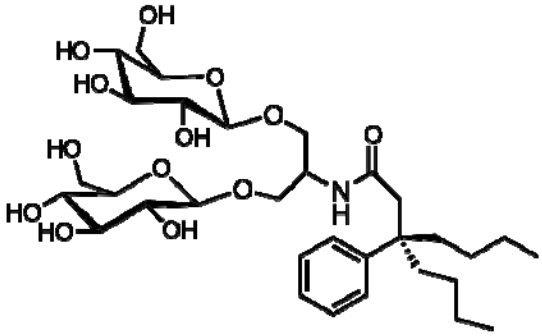
The detergents that are used to enable solubilization and crystallization of membrane proteins generally feature very simple structures, with a non-ionic polar headgroup and a linear alkyl tail. Some time ago my group became interested in exploring new amphiphilic molecules with somewhat more complex structures for this purpose. Our efforts led to the development of tripod amphiphiles and to the "neopentyl glycol" (NG) detergents. The former group has not been widely adopted by the membrane protein research community (at least so far), but NG detergents have contributed to some very significant crystallographic successes.



NG Amphiphile



Tripod Amphiphile

GPCR crystallography at XFELs

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Structural studies of G protein-coupled receptors (GPCRs), and other biomedically relevant membrane proteins and complexes, are hampered by challenges related to growing sufficiently large crystals capable of withstanding radiation damage and yielding high-resolution data. We have developed a new approach of using a membrane mimetic gel-like matrix known as lipidic cubic phase (LCP) for growth and delivery of membrane protein microcrystals for data collection by serial femtosecond crystallography (SFX) at X-ray free electron lasers (XFEL) (Liu at al, 2013, Science 342, 1521). Microcrystals are delivered to the intersection point with an XFEL beam using a specially designed LCP injector (Weierstall et al, 2014, Nat Commun, 5, 3309), allowing to adjust LCP flowrate and minimize crystal consumption. LCP-SFX uses highly intense 40-fs XFEL pulses to minimize radiation damage and collect room temperature high-resolution data from sub-10 μm crystals. Protein consumption is reduced by 2-3 orders of magnitude compared to previously used liquid injector, making the LCP-SFX method attractive for structural studies of challenging membrane and soluble proteins, and their complexes. Recent applications of this method lead to solving difficult structures of the human δ-opioid receptor in complex with a bifunctional peptide ligand (Fenalti et al, 2015, Nat Struct Mol Biol, doi: 10.1038/nsmb.2965), a novel GPCR (Zhang et al, 2015, Cell, accepted), and a major GPCR signaling complex (Kang et al, 2015, under review).

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Using EM to understand the dynamics of molecular machines.

Bridget Carragher

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The last few years have witnessed several dramatic improvements in the progress of Molecular Electron Microscopy (EM), a set of techniques and approaches used to analyze the structure of macromolecular machines using a transmission electron microscope (TEM). New detectors and image processing software have enabled reconstruction of atomic resolution maps, at least for large well ordered macromolecules. High levels of automation in image acquisition and processing have enabled the reconstruction of multiple different states of molecular machines from a single sample. This provides the opportunity to understand structures that may be highly heterogeneous and/or dynamic. We will present a brief overview of the new technology and illustrate its application to the understanding of dynamic molecular machines.

Structures of TRP ion channels by single particle cryo-EM

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The mammalian transient receptor potential (TRP) superfamily of cation channels is large and functionally diverse, second only to that of potassium channels. Structure determination of TRP channels, or more general of mammalian integral membrane proteins, has always been a very challenging and difficult task compared to that of soluble proteins. The vast majority of membrane protein structures determined to date have utilized X-ray or electron crystallography. With these crystallographic methods, obtaining well ordered three-dimensional (3D) or two-dimensional (2D) crystals is an absolute necessity and still remains a major bottleneck.

As a versatile tool in structural biology, single particle electron cryo-microscopy (cryo-EM) has achieved milestones of determining near atomic resolution three-dimensional (3D) reconstructions of a broad range of proteins complexes, including integral membrane proteins, without the need of crystals. Such achievements were enabled by recent technological breakthroughs in single particle cryo-EM, particularly the development of novel dose-fractionated image acquisition method based on CMOS direct electron detection camera, robust algorithms for correction of motion induced image blurring, and novel algorithms to classify homogenous subset of particle images from large datasets containing particles with heterogeneous conformations or compositions.

Our recent work of TRPV1 ion channel provided the first atomic structure of the entire TRP channel superfamily and provided a blueprint for understanding the unique structural and mechanistic aspects of TRP channel functions. It also demonstrated the potential of using single particle cryo-EM to determine atomic structures of integral membrane proteins.

Molecular Mechanism of ESCRT-III Assembly

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The ESCRT pathway mediates a series of important cellular membrane remodeling and fission events, including HIV-1 budding and cytokinetic abscission. While the components of the ESCRT pathway are known, the mechanisms underlying membrane remodeling and fission are not yet well understood. During these remodeling processes, ESCRT-III family proteins form filaments that appear to constrict membranes and facilitate fission. We have used electron cryomicroscopy to determine the near-atomic resolution structure of helical, double-stranded filaments formed by two homologous human ESCRT-III subunits that function in abscission, CHMP1B and IST1. The outer strand comprises closed, compact IST1 subunits, in a conformation that is very similar to the crystal structure of monomeric IST1. Surprisingly, the inner strand comprises interlocking CHMP1B subunits that adopt heretofore unseen extended, open conformations that we were able to model *de novo* based on the high quality charge density maps. In cells expressing exogenous CHMP1B and IST1, we observe analogous ESCRT-III filaments both in the cytoplasm and protruding from the plasma membrane. In vitro, the double-stranded CHMP1B-IST1 filaments can also form constricting conical spirals in which adjacent turns of the spiral interact through a continuum of related but inequivalent electrostatic interactions that allow the spiral to adjust its diameter continuously. We speculate that the ability to form conical spirals allows ESCRT-III filaments to draw membranes together to the point of fission, as occurs during the final stage of enveloped virus budding.

Functional Studies of an Ion channel by NMR in native membranes: Transmembrane Allostery as a Basis for Inactivation

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Ion binding in a potassium ion channel in membrane bilayers is studied by NMR, yielding insights into channel inactivation. At high potassium the selectivity filter is in a conductive form, and at low potassium it collapses into a long-lived nonconductive structure with reduced ion occupancy. New solid-state NMR methods are applied to full length wild type channel and to the inactivation-less mutants E71A and F103A, to test the hypothesis that an important channel-inactivation process has as its central step the evacuation of ions from the selectivity filter. To gain more insight into the role of the E71A mutation, we also characterize the protonation state of E71 and its hydrogen bond partner. We also show that ion affinity is strongly allosterically coupled to pH triggered opening of the channel.

SAD Phasing of Native Membrane Protein Structures

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Anomalous diffraction has proved to be highly effective for phase evaluation in macromolecular crystallography, and anomalous diffraction methods now predominate for *de novo* structure determination. Analyses from multi- and single-wavelength anomalous diffraction (MAD and SAD) experiments have produced more than 80% of all *de novo* macromolecular structures obtained so far in the 21st century, and SAD alone accounted for more than 70% of those reported in 2013 (Hendrickson, *Quart. Rev. Biophys.* **47**, 49-93, 2014). The New York Consortium on Membrane Protein Structure (NYCOMPS) has used a structural genomics approach to address the universe of membrane protein structures, and this focus on novelty has generated many crystals requiring *de novo* phase evaluations for structural analysis. These studies have presented several challenging problems based on selenomethionyl proteins or heavy-atom derivatizations. Such challenges have also motivated our development of methodology for phase evaluation directly from the light elements (Z = 15-20; P, S, Cl, K, Ca) that are intrinsic to and relatively prevalent in native membrane protein structures (Liu *et al*., *Science* **336**, 1033, 2012; Liu *et al*., *Acta Cryst. D* **69**, 1314, 2013; Liu *et al*., *Acta Cryst. D* **70**, 2544, 2014). We will present examples from the NYCOMPS experience.

MicroED - Three dimensional electron crystallography

Tamir Gonen

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My laboratory studies the structures of membrane proteins that are important in maintaining homeostasis in the brain. We focus mainly on membrane proteins that are found in the blood brain barrier and that are involved in ion, water, nutrient update, communication and waste removal. Understanding structure (and hence function) requires scientists to build an atomic map of every atom in the protein of interest, that is, an atomic structural model of the protein of interest captured in various functional states. We spearheaded a new method for structural biology in general and cryo EM in particular called MicroED. Here, a high powered cryo electron microscope is used in diffraction mode for structural analysis of proteins of interest from vanishingly small crystals. The samples we use are often a billion times smaller in volume than what is normally used for other structural biology methods like x-ray crystallography. As such samples that were previously unattainable can now be studied by MicroED. In this talk I will describe the MicroED method, and finish by using highlighting how this new method is helping us understand major brain diseases like parkinsons disease.

Unique opportunities with diffraction limited storage rings

Stefan Vogt

Advanced Photon Source, Argonne National Lab, and
Feinberg School of Medicine, Northwestern Univ., USA

The next generation of synchrotron light sources have the potential to revolutionize hard x-ray science. They provide (close to) diffraction-limited emittance and therefore can deliver several orders of magnitude increased brightness and coherent flux. This directly translates into vastly improved focusing capabilities, rapid data acquisition with micro- and nano-beams, and enables the use of lensless imaging approaches both in real as well as in reciprocal space. We will discuss some of the features of the proposed upgrade of the Advanced Photon Source in this context, and will speculate on their application.

Serial Femtosecond Crystallography: The Dawn of a New Era in Structural Biology

Petra Fromme

Department of Chemistry and Biochemistry and the Center for Applied Structural Discovery at the Biodesign Institute, Arizona State University, Tempe, Arizona, USA

Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction “snapshots” are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from the world's first high energy X-ray free-electron laser, the Linac Coherent Light Source. Photosystem I was used as the model system for the first serial femtosecond crystallography experiments that showed the proof of concept that diffraction of nanocrystals can be observed using femtosecond pulses that are 10^{12} stronger than 3rd generation synchrotron sources and destroy any material that is placed in its focus [1],[2]. By using femtosecond pulses briefer than the time-scale of most damage processes, femtosecond nanocrystallography overcomes the problem of X-ray damage in crystallography [3] and extends to atomic resolution [4],[5]. It is also applied to membrane proteins crystallized in lipidic environments [6],[7]. Femtosecond crystallography also opens a new avenue for determination of protein dynamics. First experiments on the proof of principle for time resolved serial femtosecond nanocrystallography have been performed on Photosystem I-ferredoxin [8] and Photosystem II nano-crystals [9] and conformational changes of the Mn_4Ca cluster and its protein environment were observed for the first time in the transition from the dark S_1 to the double excited S_3 state [10]. Very recently we were able to show that TR-SFX studies extend to atomic resolution using the photoactive yellow protein as a model system [11]. These pioneering work paves the way for the determination of molecular movies of the dynamics of membrane proteins "at work" in the future including the determination of a molecular movies of water splitting.

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Innovative approaches to X-ray microcrystallography

Robert F. Fischetti

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Recent advances in microcrystallography at beamlines on 3rd-generation storage-ring-sources (ie. synchrotron sources) have enabled atomic-resolution structure determination for many important biological problems. Some of the recent developments that have improved data quality, reduced primary radiation damage at liquid nitrogen temperatures, or improved user friendliness include: user-selectable micro-beams, raster mapping, vector data collection, shutter-less data collection, multiple crystal strategy and SONICC alignment of crystals. The Linac Coherent Light Source, an XFEL, has also driven new developments, such as “serial crystallography”, which are now being implemented on storage-ring-source beamlines. Another very exciting but controversial topic is the potential for outrunning secondary radiation damage at room temperature. In the near future, 4th generation storage-ring sources based on multibend achromatic (MBA) lattices will increase brightness by at least two orders of magnitude. In this talk, I will present the current state of microcrystallography and the potential game-changing future for structural biology enabled by the APS-MBA source.

High-speed structure determination of human membrane proteins by NMR and in silico drug discovery

Senyon Choe

Professor, Drug Discovery Collaboratory, Qualcomm Institute, UCSD, USA

Nearly half of today's major pharmaceutical drugs target human integral membrane proteins (hIMPs). A major hurdle to study hIMPs, however, lies in methods of protein production for biochemical and structural characterization. By a combination of Cell-Free (CF) expression and Combinatorial Dual isotope-Labeling (CDL) strategy to facilitate high-speed structure determination of hIMPs by solution NMR spectroscopy (Maslennikov et al., 2010; Klammt et al., 2012, Nature Methods), we have devised CNDY (Cell-free, NMR spectroscopy, Molecular Dynamics) strategy to search for small molecule leads (Maslennikov & Choe, 2013). The CNDY strategy can offer a significant discovery toolset specially when combined with computational methods (Lindert et al., 2014).

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Integrative Methods to Understand G Protein-Coupled Receptor Structure and Function

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G protein-coupled receptors (GPCRs) constitute one of the largest protein families in the human genome and play essential roles in normal cell processes, most notably in cell signaling. After 20 years of technology innovation, the benefits of the technology efforts are now paying off. Delivering GPCR structure-function data in close collaboration with the community experts on specific receptor systems is of immense value to the basic science community interested in cell signaling and molecular recognition, as well as the applied science community interested in drug discovery. To date, we have determined high resolution structures of 18 of the 26 – notably the adrenergic, adenosine, histamine, dopamine, chemokine, S1P, opioid, serotonin, AT1, LPA1, class B (glucagon), C (mGluR1) and F (smoothened) receptor systems with new structures continuing to be deposited at a strong rate.

This work is being followed up with additional integrative biophysical characterization methods including ^{19}F NMR spectroscopy/HDX/single molecule flourescence/EM studies to understand signaling pathways. We are now observing both common and different structural features in these receptors. The scope of structural diversity of GPCRs at different levels of homology provides insight into our growing understanding of the biology of GPCR action and their impact on drug discovery. The latest high resolution structures are providing new insight including allosteric control of the receptors by cholesterol and sodium, as well as novel hypotheses of the receptors having channel or transporter activity. The rapidly expanding repertoire of GPCR structures provide a solid framework for experimental and molecular modeling studies, and helps to chart a roadmap for comprehensive structural coverage of the whole superfamily and a more thorough understanding of GPCR chemistry and biology.

This work is supported by NIGMS PSI grant U54GM094618.

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Poster Abstracts

P-1

Crystallographic studies of the bacterial Membrane Associated O-Acyltransferase protein DltB

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Membrane Associated O-Acyltransferases **(**MBOATs) form a superfamily of proteins that are conserved from bacteria to human and add an acyl group to the oxygen atom of a metabolite or a serine and threonine side chain of a protein. These polytopic integral membrane proteins play important metabolic roles and several human members such as Diacylglycerol acyltransferase 1 (DGAT1), Ghrelin O-acyltransferase (GOAT) and Hedgehog acyltransferase (HHAT) have emerged as important drug targets in metabolic diseases and cancer. The bacterial MBOAT protein, DltB, participates in the biosynthesis of a major component of gram-positive bacterial cell wall and therefor represents a drug target for gram-positive bacterial pathogenesis. In contrast to histone acetyltransferases and protein N-acetyltransferases, MBOATs are poorly understood at both the biochemical and structural level. We screened more than a dozen recombinant MBOAT proteins from bacteria to human to identify an MBOAT protein that was amenable to purification and crystallization. This lead to the preparation of several different crystal forms of the bacterial DltB MBOAT protein, The best crystals grow to a typical size of 0.15 x 0.15 x 0.05 mm and diffract to a resolution of 7~8 Å. The crystals form in space group P2 or $P2_1$ with unit cell dimensions a=136.5 Å, b=162.8 Å, c=165.5 Å and β = 108°. A Matthews Coeffecient of 2.43 would predict 16 protomers and 49% solvent in the asymmetric unit cell, suggesting that the crystals potentially contain 4 DltB tetramers in the asymmetric unit cell. We are currently using several strategies to improve these crystals. We anticipate that a structure of the DltB MBOAT will provide novel molecular insights into the structure-function of the MBOAT family, will pave the way to address their substrate specificities and will provide important insights into MBOAT-specific probes and inhibitors for therapy.

P-2

Molecular basis of transition metal transport and regulation

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Abstract
Divalent metals are required for a wide range of cellular function, including electron transport, catalytic processes, and transporting oxygen in plasma. On the flip side, many transition metals are toxic at elevated levels, thus requiring stringent regulation for normal homeostasis.

Here we report the crystal structures of a bacterial transition metal transporter in both the outward- and inward-facing states. The metal transporter adopts the major facilitator superfamily fold, and comparisons of the two structures reveal that the protein undergoes an intra-domain conformational rearrangement during the transport cycle. We identified a substrate-metal binding site, based on structural and mutational analyses, and a putative regulatory mechanism, with detailed insights to be discussed.

P-3

ABCs in Adaptive Immunity and Viral Immune Evasion

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The recognition and elimination of virally or malignantly transformed cells is the pivotal task of the adaptive immune system. For immune surveillance, a snapshot of the cellular proteome is displayed on major histocompatibility class I molecules (MHC I) as immunodominant epitopes for recognition by cytotoxic T-lymphocytes. The knowledge about the track from the equivocal protein to the presentation of peptides has greatly expanded, leading to an astonishingly elaborated understanding of the MHC I peptide loading pathway. I will report on the structure and function of heterodimeric ABC transporters. The contribution of the individual proteins and subcomplexes as well as the architecture and dynamics of the peptide-loading complex will be discussed, including mechanisms of viral immune evasion.

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P-4

Anatrace: Advancing Technologies for Membrane Protein Research

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In the thirty years that have elapsed since the publication of the first membrane protein structure, we have witnessed many significant strides in this field; however, challenges in membrane protein production, purification, and crystallization still persist. Attracted by the challenge and the tremendous biological importance of membrane proteins, and encouraged by the rapid recent growth in membrane protein structures, more crystallography laboratories are venturing into the field, seeking know-how, tools, and reagent support. Throughout this time, Anatrace Products, LLC has been a trusted resource in membrane protein research, due to their large portfolio of detergents and lipids, and unsurpassable standards in quality and reproducibility. Extending their product portfolio, Anatrace acquired Microlytic North America, Inc., offering unique and productive solutions for protein crystallography.

We will review our customers exciting recent successes in membrane protein crystallization using the Microlytic Crystal Former, a capillary-based diffusive-mixing device. Additionally, we will also introduce the Analytic line of products, aimed at supporting membrane protein research in both established laboratories and new-comers to the space. Lastly, we are excited to introduce new detergent offerings with demonstrated success in membrane protein stabilization and crystallization.

P-5

Rapid screening of membrane protein expression in transiently transfected insect cells

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Abstract
Membrane proteins play critical roles in many biological processes and are the focus of intense biomedical research. One bottleneck for studying membrane proteins is the difficulty in expressing correctly folded and stable proteins, which often requires extensive protein engineering and multiple rounds of optimization, a time and resource intensive process. Here, we describe a method for rapidly screening membrane protein expression in insect cells. The method uses a green fluorescent protein (GFP) covalently fused to target membrane proteins and the resulting fusion proteins are then transiently expressed in insect cells. This approach enables us to dramatically reduce the time and resources required for expression screening by eliminating the need to create recombinant baculovirus. We show that transiently expressed membrane proteins can be directly monitored for their subcellular localizations by fluorescence microscopy. Moreover, their expression levels, approximate molecular mass, and stability can be evaluated with nanogram levels of unpurified proteins by ultrasensitive fluorescence-detection size exclusion chromatography (FSEC). We present our proof of principle studies using a homotrimeric ion channel (ASIC3) and a heterodimeric transporter (SLC7A5/SLC3A2) as examples, and demonstrate the utility of transient expression coupled with FSEC in optimizing membrane protein expression.

Protein Expression and Purification. 2013 Mar; 88(1):134-42.

P-6

Structural prerequisites for G protein activation by the neurotensin receptor

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Neurotensin (NTS) is a 13-amino acid peptide that functions as a neurotransmitter and a hormone in the nervous system and in peripheral tissues. NTS has a wide range of biological activities and has important roles in antinociception, cancer cell growth, and the pathogenesis of schizophrenia. Most of the known agonist effects of NTS are mediated through the G protein-coupled receptor (GPCR) neurotensin receptor 1 (NTSR1). Previously, we determined the structure of NTSR1 bound to $NTS_{8\text{-}13}$ in an active-like conformation with six thermostabilizing mutations providing insight into the binding mode of a peptide agonist. This receptor bound G protein but was unable to catalyze nucleotide exchange at the Gα subunit, indicating that some of the stabilizing mutations may have restricted the ability of NTSR1 to relate agonist binding to the activation of G protein. Here we analyzed the effect of three of those six mutations (E166A$^{3.49}$, L310A$^{6.37}$, F358A$^{7.42}$) on G protein activation and present structures of active-like NTSR1 that are able to activate G protein. Unique to these structures is the presence of a phenylalanine at position$^{7.42}$ causing the conserved W321$^{6.48}$ to adopt a side chain rotamer conformation parallel to the lipid bilayer, which has not been seen in any GPCR structures to date. The W321$^{6.48}$ residue also seals the top of the collapsed Na^+ ion binding pocket. Importantly, NTSR1 residues W321$^{6.48}$ and F358$^{7.42}$ are central to linking the agonist peptide, bound closer to the extracellular surface, with residues in the lower part of NTSR1 that are implicated in conformational changes for GPCR activation. In the intracellular receptor half, the bulkier L310$^{6.37}$ side chain dictates the position of R167$^{3.50}$ of the highly conserved D/ERY motif. This, together with the presence of the neighboring E166$^{3.49}$ provides the determinants for G protein activation.

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P-7

Crystal structures of translocator protein 18 kDa (TSPO) and identification of a cholesterol binding enhancement motif

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Transport of cholesterol into mitochondria is the first and rate limiting step of steroid hormone synthesis. Translocator protein 18 kDa (TSPO) is recognized as a key player in this process and is highly expressed in steroidogenic tissues, metastatic cancer, Alzheimer's and Parkinson's diseases, and inflammatory conditions. A cholesterol recognition amino acid consensus sequence (CRAC) was first identified in TSPO as a general cholesterol binding motif. Various other ligands that bind to TSPO, including benzodiazepine drugs, have been extensively used as diagnostic agents in PET imaging and treatment options. However, conflicting data and the lack of high resolution structure has led to much controversy concerning the precise role of this ancient conserved protein. We utilized the purified TSPO from *Rhodobacter sphaeroides* (*Rs*TSPO), a bacterial homolog that shares a conserved function with the mammalian protein, to investigate the properties of TSPO biochemically and crystallographically. We determined high resolution crystal structures of *Rs*TSPO wild-type and a mutant equivalent to the human single polymorphism (A147T) related to anxiety disorders and reduced pregnenolone production. The crystal structures provide new insight into ligand interaction sites and reveal significant structural changes in the cholesterol binding region caused by the mutation. A three amino acid sequence adjacent to the CRAC and the A147T sites was identified as a cholesterol binding enhancement motif, by mutation of the bacterial TSPO to the human sequence, resulting in an increase in binding affinity for cholesterol of >1000 fold. These results provide insights into the function of TSPO at a molecular level and strongly support a role for TSPO in cholesterol metabolism. (NIH R01GM26916, MSU Center for Mitochondrial Science and Medicine, SF-M)

P-8

Lipidic cubic phase serial millisecond crystallography using synchrotron radiation

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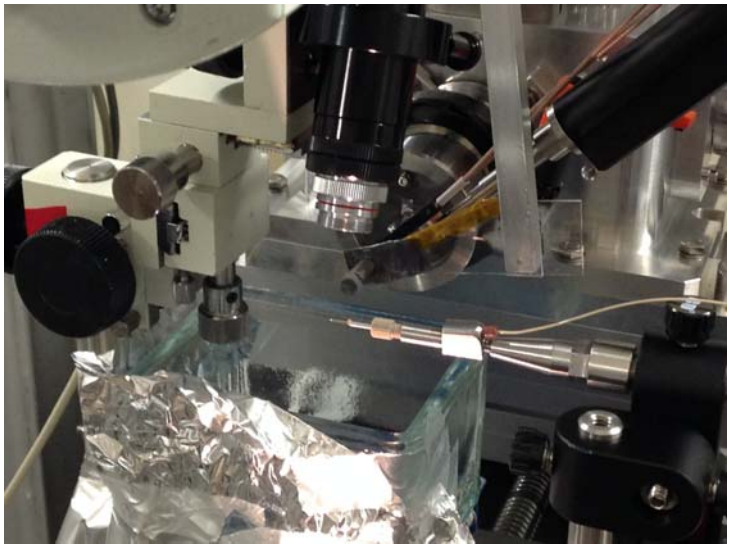
Lipidic cubic phases (LCPs) have emerged as successful matrixes for the crystallization of membrane proteins.Moreover, the viscous LCP also provides a highly effective delivery medium for serial femtosecond crystallography (SFX) at X-ray free-electron lasers (XFELs). Here, the adaptation of this technology to perform serial millisecond crystallography (SMX) at more widely available synchrotron microfocus beamlines is described. Compared with conventional microcrystallography, LCP-SMX eliminates the need for difficult handling of individual crystals and allows for data collection at room temperature. The technology is demonstrated by solving a structure of the light-driven protonpumpbacteriorhodopsin (bR) at a resolution of 2.4Å



P.Nogly, et al, *Lipidic cubic phase serial millisecond crystallography using synchrotron radiation.* **IUCrJ 2 (2015) (open access)**

P-9

A successful screening strategy for solving structures of human solute carriers, leading to the structure of the sugar transporter GLUT-3 (SLC2A3).

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The Integral Membrane Proteins Group at the SGC has developed a rigorous screening process to quickly identify the protein constructs and conditions most suitable for crystallization based around expression in *Spodoptera frugiperda* cells. We are currently using this process to screen a range of Solute Carriers (SLCs) with 64 proteins currently under review. Ten proteins have been produced in sufficient quantity for extensive crystallization studies and has resulted in crystals for two SLCs and a structure for SLC2A3 (GLUT-3). GLUT-3 is a member of the major facilitator superfamily (MFS) and was originally designated as the neuronal glucose transporter. GLUT transporters are responsible for facilitating glucose transport across the membrane. Substrates include glucose, manose, galactose, xylose and dehydro-ascorbic acid. Our Apo structure, solved to 2.75Å using x-ray crystallography is in an open outwards conformation. With no substrate bound to GLUT-3 there is a conformational change resulting in a more accessible open outward state than that seen in the partially occluded D-xylose bound structure of XylE (PDB: 4GBY).

P-10

Structure of the proton-pumping transhydrogenase from *Thermus thermophilus* reveals a highly asymmetric dimer

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The nicotinamide nucleotide transhydrogenase couples the proton motive force to hydride transfer between NADH and $NADP^+$,

$$H^+_{out} + NADH + NADP^+ \rightarrow H^+_{in} + NAD^+ + NADPH$$

where "in" and "out" refer to the electrically negative and positive sides, respectively, of the bacterial cytoplasmic membrane or mitochondrial inner membrane. In the mitochondrion and in most prokaryotes, the enzyme uses the proton motive force (positive outside) to drive the reaction to favor the reduction of NADPH which is required for the reduction of glutathione and consequent removal of reactive oxygen species. As part of our effort to use hyperthermophilic organisms as a source of membrane proteins for structural studies, we cloned, expressed and have determined the structure of the transhydrogenase from *Thermus thermophilus.*

The transhydrogenase is a homodimer, and each half of the homodimer consists of three domains. Domain I (dI) is hydrophilic and contains the binding site for $NADH/NAD^+$. Domain III also hydrophilic and contains the binding site for $NADPH/NADP^+$. Domain II is hydrophobic and contains the pathway for proton translocation across the membrane. There are no prosthetic groups or redox metals required for enzyme function, and direct hydride transfer is catalyzed from NADH to $NADP^+$ across the interface between dI and dIII. The structures of the hydrophilic domains $(dI)_2$ and dIII, as well as a heterotrimeric complex, $(dI)_2dIII$, have been previously determined. The current work has provided the 2.8 Å X-ray structure of the dimeric membrane domain $(dII)_2$, as well as the holo-enzyme to 6.9 Å resolution.

The membrane domain contains two copies of dII, each with 12 transmembrane helices and a putative proton channel. A highly conserved histidine resides midway within this putative channel. Each half of the holo-enzyme dimer consists of one copy of dIII sandwiched between domains dI and dII. Remarkably, in one half of the dimer dIII is oriented with the $NADP^+$ binding site facing the NADH site in dI, but in the other half of the dimer dIII is oriented in an inverted position with the $NADP^+$ binding site at the interface with domain dII. Both conformations have been confirmed by crosslinking.

The data suggest a dynamic functional model in which domain dIII flips between two orientations, one in which hydride transfer occurs (via dI) and the second where proton translocation occurs (via dII). Furthermore, the structure enforces an asymmetry such that the two halves of the dimer must operate out of phase with each other. More work is needed to test this model and to obtain higher resolution structural data.

P-11

Cell-free translation systems for biophysical and biochemical characterization of proteins and protein complexes

[1,2]**Coleman M. A.,** [1]Bourguet F. A., [1]Ly S. S., [2]He, W., [1]Blanchette C. D., [1]Fischer N. O. [1]Laurence T. A., and [1]Jackson P. J.

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Cell-free systems have been utilized to produce difficult to express proteins, such as toxic and membrane bound proteins. Here we discuss how our laboratory has incorporated cell-free technologies to produce labeled proteins, protein complexes and trans-membrane proteins in yields that are more than sufficient for biophysical and biochemical characterization. Because cell-free translation is an open process, we have adapted commercial systems for multiple high-throughput screening techniques dependent on the suspected proteins function. For example, labels and co-factors can easily be added to synthesis reactions, such as BODIPY-labeled $tRNA^{Lys}$, fluorescent protein tags, peptides, SNAP-tags, small molecule co-factors, nanolipoproteins, lipids and fluorescently-labeled lipids. We also demonstrate that co-translation of multiple proteins is achievable and this is one of the most effiecient processes for solubilizing membrane bound proteins. We have also shown that protein complexes of up to three polypeptides can be co-translated within a single reaction. A wide range of active enzymes and receptors such as rhodopisins, G-protein coupled receptors (GPCRs), kinases, cytokines, antibodies, proteases and cell wall hydrolases, secretion system complexes have been studied. After purifying, proteins and protein complexes were used for biophysical and biochemical characterization using fluorescence correlation spectroscopy (FCS), circular dichroism (CD), electron microscopy (EM) and kinetic analysis. Overall, cell-free represents a unique solution to address multiple bottlenecks in the production, purification and characterization of proteins that in the past have been difficult to previously obtain.

P-12

Internal Lipid Architecture of the Hetero-Oligomeric Cytochrome b_6f Complex

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The role of lipids in the assembly, structure, and function of hetero-oligomeric membrane protein complexes is poorly understood. The dimeric cytochrome b_6f complex, a 16-mer consisting of eight distinct subunits with 13 trans-membrane helices per monomer, catalyzes trans-membrane proton-coupled electron transfer for energy storage[1]. Based on a 2.5 Å crystal structure of the dimeric complex, 23 distinct lipid binding sites per monomer have been identified[2]. Annular lipids are proposed to provide a connection for super-complex formation with (i) the photosystem-I reaction center, and (ii) the LHCII kinase enzyme for trans-membrane signaling. Internal lipids mediate (iii) cross-linking to stabilize the domain-swapped iron-sulfur protein subunit, (iv) dielectric heterogeneity within inter-monomer and intra-monomer electron transfer pathways, (v) mediation between the conserved core of the complex and the four peripheral single membrane spanning subunits, and (vi) dimer stabilization through lipid-mediated inter-monomer interactions. This study provides the most complete structure-analysis thus far obtained of lipid-mediated functions in a multi-subunit membrane protein complex, and reveals lipid-sites at positions essential for assembly and function.

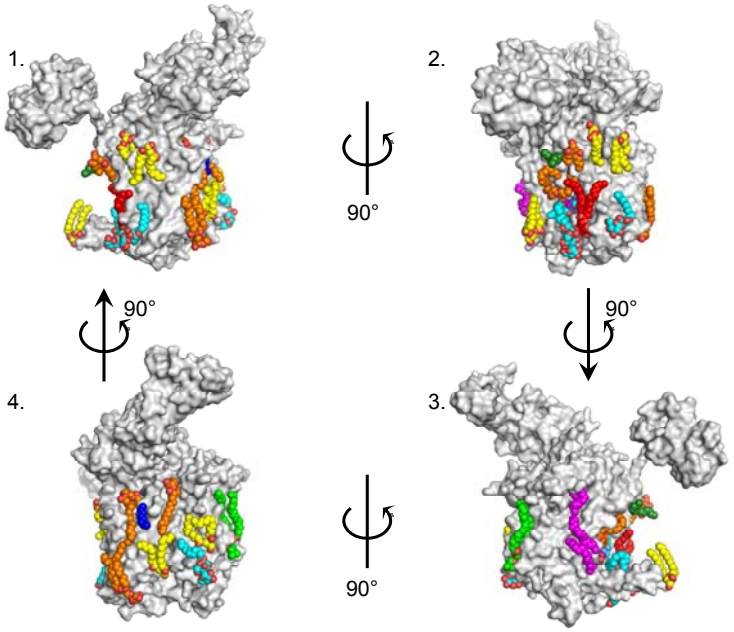

Figure. Lipid binding sites in the cytochrome b_6f complex (PDB ID 4OGQ). For simplicity, the cytochrome b_6f monomer is shown as a light-gray surface, and lipids are shown as colored space-filling spheres. Four views (1-4) are shown, each rotated by 90° from the previous view.

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P-13

Probing the TolC Trans-Periplasmic Pathway of the Cytotoxin, Colicin E1

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The pathway of cellular entry of colicins and viral DNA is a fundamental structure problem involving the structure and function of the inner and outer membranes of *E. coli* that is relevant to an understanding of the molecular basis of infectious diseases [1]. The cytotoxin colicin E1 uses the outer membrane/trans-periplasmic drug export protein, TolC, for its import. TolC consists of a 12 strand OM β-barrel connected to a 12 strand α-helical tunnel that defines a pathway through the peptidoglycan barrier in the periplasm to the cytoplasmic membrane in which the C-terminal domain of colicin E1 inserts and forms a depolarizing ion channel. Previous studies with planar bilayers showed that colicin E1 occludes TolC channels [2], as do the translocation [T]-domain peptides of certain colicins [3]. The nature of the interaction of the colicin with TolC the mechanism of its translocation through the TolC, across at least half of the periplasmic space [4], is not known.

In vivo protection of sensitive *E. coli* from colicin E1 by a series of N-terminal colicin peptides was used to probe the interaction of the colicin with TolC, with the goal of defining the sites of TolC-colicin interaction and the mechanism of colicin passage across at least half of the periplasm [4]. N-terminal segments '1-40','1-81', and '1-100' of the colicin did not provide cytotoxic protection, nor occlude TolC channels. Segments '1-120', '1-140', 1-190', as well as '41-190' and '57-190' protected efficiently *in vivo* and occluded TolC channels formed in planar bilayer membranes. Occlusion required a negative electrical potential relative to the trans-side of colicin addition, and was irreversible. Co-elution of the colicin peptides with TolC on a Superdex 200 column was shown for '41-190', but not for '1-81.' In addition to the correlation with protection *in vivo* from killing by colicin E1, occlusion efficiency also correlated with a basic nature of the colicin protein between residues 82 and 140.

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P-14

A Potential Problem with the Accuracy of Membrane Protein Structures

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Virtually all integral membrane proteins that span a biological membrane in a helical conformation are situated in the presence of trans-membrane electrical potentials of approximately 100-150 mV across a low dielectric (lipid) medium of approximately 30 Å, thus yielding an electric field intensity of approximately 3 x10^5 V/cm.

Atomic structures of > 500 independent integral membrane structures (1) have been obtained through crystallization and diffraction analysis in the absence of this field. The question arises as to whether the absence of this intra-membrane field and the resulting electrostatic interactions result in a significant change in the structures of membrane proteins, which are isolated in detergent, and crystallized in detergent, lipid cubic phase, or bicelles, environments in which the electric field is absent.

The possible effect of this E-field on membrane protein structure was tested with the large hetero-oligomeric active cytochrome b_6f complex, from which a high (2.5 Å) resolution crystal structure (2) has been determined. The protein complex was incorporated into liposomes across which a valinomycin-dependent Nernst diffusion potential = -118 mV (25°C), inside negative, was imposed. Characteristic times for formation and decay of the valinomycin-induced trans-membrane potential in liposomes were determined using a potential-sensitive fluorescence probe.

The dependence of the relative fluorescence yield and emission peak of tryptophan fluorescence emission from the liposome-embedded cytochrome complex was measured in the presence and absence of the – 118 mV trans-membrane Nernst potential. The emission peak of the fluorescence emission spectrum generated by the tryptophan ensemble of the cytochrome complex was found to undergo a blue shift and an increase in fluorescence yield upon addition of valinomycin in the presence of the K^+ gradient. These results suggest that the structure of the cytochrome complex is perturbed in the presence of the trans-membrane electrical field that results from physiologically relevant trans-membrane potentials. The details and magnitude of the potential-dependent structure changes of the cytochrome b_6f complex are presently not known.

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P-15

Construction of an ankyrin-based combinatorial library: a successful application

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A diverse DNA combinatorial library coding for ankyrin seven-repeat proteins (ANK-N5C) was designed and constructed by a PCR-based random-assembly strategy. We isolated a transcription blocker that prevents the activation of the *melAB* operon of *Escherichia coli*, so melibiose-dependent expression of α-galactosidase and melibiose permease is inhibited. High-resolution crystal structures reveal that the designed ANK-N5C protein has a typical ankyrin repeat fold, and the specific transcription blocker, ANK-N5C-281, forms a domain-swapped dimer. Functional tests suggest that the function of the *melAB* transcription activator MelR is inhibited by protein ANK-N5C-281. All ANK-N5C proteins are expected to have a concave binding area with negative surface potential, suggesting a new tool for discovery of binders recognizing structural motifs with positive surface potential.

P-16

Thermodynamic mechanisms of the phosphotransferase protein IIA^{Glc} binding to melibiose and lactose permeases

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Carbohydrate uptake in many bacteria is regulated by the phosphotransferase protein IIA^{Glc}, enabling *cells* to utilize glucose preferentially over other sugars. The Na^+-coupled melibiose permease (MelB) and the H^+-coupled lactose permease (LacY) are two of many sugar permeases regulated by IIA^{Glc}. We characterized the thermodynamic features of IIA^{Glc} binding to both permeases by applying chemical cross-link and isothermal titration calorimetry. We show that IIA^{Glc} directly binds to both permeases in the absence or presence of melibiose, exhibiting dissociation constants between 3-10 μM at a stoichiometry of one. Strikingly, permeases differ in their thermodynamic response to binding IIA^{Glc}. With MelB, which was crystallized in an outside-open conformation, the binding is driven by enthalpy change with compensation of entropy change; with LacY, which was captured in an inside-open conformation, the binding is driven by entropy change with compensation of enthalpy change. In both cases, the binding restrains the conformational entropy. Consistently, IIA^{Glc} inhibits the affinity of MelB or LacY for sugar substrates; i.e., IIA^{Glc} inhibits an induced-fit process, and blocks inducer entry into the cells. The regulatory protein IIA^{Glc} can serve as a useful tool for the study of permeases under its regulation.

P-17

High Throughput Screening for Optimal Extraction and Purification of Membrane Proteins Toward Structural Research Determination

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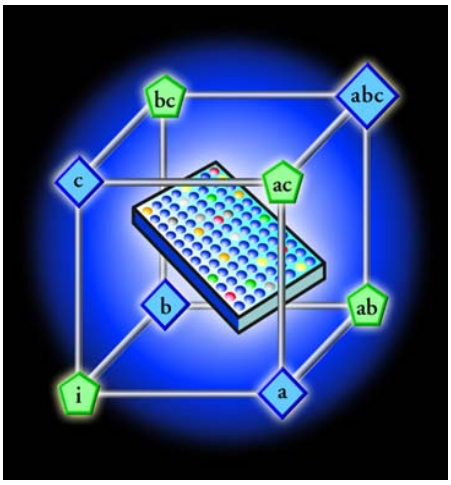
In spite of their importance as drug targets, to date less than 1% the solved protein structures in the Protein Data Bank (PDB) are membrane proteins. Because the hydrophobic transmembrane segments of membrane proteins are embedded in the lipid bilayer detergents are required to extract them. This creates technical challenges in producing large enough quantities for structural research. There isn't a one size fits all approach for membrane proteins and each protein needs to be individually optimized. The myriad of detergents and conditions available to be tested makes this a daunting endeavor. In an attempt to more rigorously explore parameters associated with membrane protein extraction we have incorporated high throughput (HTP) approaches using Design of Experiment (DOE) and robotics. These methods have allowed us to explore numerous detergents under various conditions collecting over 600 data points per protein in order to optimize our chances of success toward structural research determination. Robust data sets allow us to create surface response curves of protein desirability that we utilize to select the optimal conditions for extraction and purification. Details of the design and implementation of the approaches will be presented along with results from practical applications.

This work is done in collaboration with Structural Genomics Consortium Oxford (SGC).



P-18

Structure and Activity of Tryptophan-rich TSPO Proteins

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TSPO translocator proteins bind steroids and porphyrins, and they are implicated in many human diseases, for which they serve as biomarkers and therapeutic targets. They bind benzodiazepine compounds such as diazepam (Valium). TSPOs have tryptophan-rich sequences that are highly conserved from bacteria to mammals. We report crystal structures for *Bacillus cereus* TSPO (*Bc*TSPO) down to 1.7Å resolution, including a complex with the benzodiazepine-like inhibitor PK11195. We present a new and efficient iodine phasing method for crystals grown in lipidic cubic phase. We also describe BcTSPO-mediated protoporphyrin IX (PpIX) reactions, including catalytic degradation to a previously undescribed heme derivative. We used structure-inspired mutations to investigate reaction mechanisms, and we showed that TSPOs from Xenopus and man have similar PpIX-directed activities. Although TSPOs have been regarded as transporters, the catalytic activity in PpIX degradation suggests physiological importance for TSPOs in protection against oxidative stress. Thus, our biochemical findings provide new avenues for understanding the multiple physiological and pathological roles played by TSPO and our structures provide a solid basis for the design of precision medicines that can discriminate between $GABA_A$ and TSPO receptors.

P-19

Microfluidic Platform for Optimization of Protein Crystallization by Microseed Screening

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Membrane proteins are important targets for crystallization because they are transducers of material and signals across cell membranes and intracellular organelles, and their malfunction accounts for many diseases in humans. One of the bottlenecks to obtaining diffraction quality crystals of membrane proteins is the need to screen thousands of crystallization conditions while negotiating limited availability of protein, membrane protein instability in non-native lipids and detergents, and the long incubation times before the appearance of initial crystals. Upon successful crystallization, initial crystals are typically unsuitable for X-ray diffraction and require further optimization.

The practice of introducing protein microseeds to crystallization droplets enhances control over crystal quality by separating the events of nucleation and growth in supersaturated crystallization droplets. Ideally, this method is employed when setting up routine crystallization screens. Most laboratories do not use microseed matrix screening as it involves disturbing the crystallization drop to introduce microseeds by either a skill intensive and manually laborious procedure, or by microseeding robots.

Here, we present high-throughput X-ray compatible microfluidic chips to facilitate microseed matrix screening. This approach incorporates microseeds into microbatch crystallization experiments to enhance likelihood of crystallization success. The chips automate the mixing of variable nanoliter volumes of protein solution, precipitants, and microseeds in an array of 96 wells for thorough crystallization screening and optimization. X-ray analysis of crystals is accomplished via *in situ* room temperature serial crystallography of randomly oriented crystals throughout crystallization chips, where small wedges of data from dozens of crystals are merged to form a complete dataset. As proof-of-concept, large, single crystals of photoactive yellow protein were grown and analyzed on-chip. Further microfluidic microseeding efforts have resulted in optimized crystals of cytochrome bo_3 oxidase

P-20

Regulatory mechanisms of the sarcoplasmic reticulum calcium transport complex detected by FRET microscopy and microsecond simulation

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We have used live-cell fluorescence microscopy and all-atom molecular dynamics (MD) simulation to detect regulatory interactions of sarcolipin (SLN), phospholamban (PLB), and the Ca^{2+}-transport ATPase (SERCA) from sarcoplasmic reticulum. SERCA is a 110 kDa Ca^{2+} pump, whereas SLN and PLB are ~5 kDa inhibitory subunits that regulate SERCA activity and thus muscle and heart contractility. SLN and PLB are expressed differentially across human tissues and their inhibition is controlled by β-adrenergic-stimulated phosphorylation. When the three proteins (SERCA-SLN-PLB) are expressed in the same tissue, a "super-inhibited" complex is formed.

To determine the complex equilibria of oligomeric interactions between SERCA, SLN, and PLB, we used FRET microscopy. Proteins were tagged with genetically-encoded fluorescent probes (CFP, YFP) and expressed in baculovirus-infected insect cells. Protein-protein interactions (SLN-SLN, SLN-PLB, PLB-PLB, SERCA-SLN, SERCA-PLB) were assayed for binding affinity, oligomer number, and interprobe distance. Results indicate (a) SLN and PLB show high-affinity self-association into homo-oligomers and low-affinity cross-assembly as hetero-dimers, and (b) SERCA forms 1:1 binary complexes with SLN or PLB when co-expressed with either subunit individually, showing 3-fold higher affinity for SLN over PLB.

To determine the inhibitory mechanism of dephosphorylated PLB bound to SERCA, MD simulations and protein p*K*a calculations were performed in the absence of bound Ca^{2+} at physiological pH (PDB 4KYT). Microsecond-long MD simulations indicated that the transport sites of PLB-bound SERCA are completely exposed to the cytosol, and that 1-2 K^+ ion bind transiently (≤5 ns) and non-specifically (9 different positions) to the two transport sites, with occupancy time of 80%. p*K*a values of acidic residues in the transport sites indicated that PLB-bound SERCA populates a unique state protonated at residue E771 but deprotonated at E309 and D800. Principal component analysis of the inhibited SERCA-PLB dimer was compared to our published results for unregulated SERCA monomers (Ca^{2+}-bound, Ca^{2+}-free).

Our FRET and MD results identify structural mechanisms of SERCA regulation. We propose that SLN and PLB monomers bind independently to SERCA and that binary and ternary complexes of SERCA exist in dynamic equilibrium controlled by phosphorylation and expression levels specific to muscle type. We also propose that PLB binding to SERCA populates a novel protonated intermediate, $E1{\bullet}H^+_{771}$, which serves as a kinetic trap, inhibiting transmembrane structural transitions of Ca^{2+} binding while controlling ATPase headpiece dynamics.

Acknowledgments. This work was funded by grants to JMA (UMN College of Biological Sciences), LME-F (AHA-12SDG12060656), SLR (NIH R01-HL092321), and DDT (NIH R01-GM27906, P30-AR0507220, T32-AR007612).

P-21

Escherichia coli as host for membrane protein structure determination: a global analysis.

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Background. Over 450 unique membrane protein (MP) structures have been obtained so far, providing molecular details about how MP work in normal and pathological situations. Despite numerous breakthroughs in physical and biochemical methods, the structural biology of MP is hampered by their recombinant production in a functional state. Protein production is also a major issue in vaccine and drug development. Thus, there is a crucial need for rules to produce them at low cost.

Methodology and principal findings. A comprehensive analysis of protein databases and of protein production protocols in bacteria was performed. Articles citing Miroux and Walker for the use of the C41λ(DE3) and C43λ(DE3) bacterial hosts, Moffatt and Studier for the use BL21λ(DE3) host and Guzman *et al.* for the use of the arabinose expression system (2817 articles altogether) were parsed for keywords or a combination of regular expressions. We found that 213 unique MP structures have been obtained after production of the target protein in *E. coli*. The primary expression system used was the one based on the T7 RNA polymerase, followed by the arabinose and T5 promoter based expression systems. Importantly, two bacterial mutant hosts namely C41λ(DE3) and C43λ(DE3) have contributed to 28% of non *E. coli* MP structures. Analysis of expression protocols demonstrated a preference for a combination of bacterial host-vector together with a bimodal distribution of induction temperature and of inducer concentration.

Conclusions and perspectives. Our data show how to efficiently produce MP in E. coli and explain why strategies developed to improve expression systems have focused on limiting the toxicity for the bacterial host. Together with the practical rules revealed by this study, we propose a route to extend membrane protein expression in *E. coli* to eukaryotic sequences based on adapting codon optimality to the *E. coli* translation dynamic.

P-22

A Structural Mechanism for Calcium Transporter Headpiece Closure Studied by Molecular Dynamics Simulation.

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To characterize the conformational dynamics of sarcoplasmic reticulum (SR) calcium pump (SERCA) we performed molecular dynamics simulations beginning with several different high-resolution structures. We quantified differences in structural disorder and dynamics for an open conformation of SERCA versus closed structures, and observed that dynamic motions of SERCA cytoplasmic domains increased with domain-domain separation distance. The results are useful for interpretation of recent intramolecular Förster resonance energy transfer (FRET) distance constraints obtained for SERCA fused to fluorescent protein tags. Those previous physical measurements quantified the population of discrete structural substates and revealed that open conformations of SERCA are more dynamically disordered than compact conformations. The present simulations support this hypothesis and provide additional details of SERCA structural dynamics. Specifically, all-atoms simulations revealed large scale translational and rotational motions of the SERCA N-, A- and P-domains. We also observed a transition from an open to a closed headpiece conformation over the course of a 400 ns trajectory. Intermediate structures sampled during the open-to-closed transition were long lived, with >50 ns dwell times observed for each conformation. We observed well-resolved peaks in a histogram of calculated domain separation distances, consistent with saltatory transitions between discrete conformational substates. The open-to-closed conformational transition was mediated in part by an initial interaction of an N-domain loop (Nβ5-β6, residues 426-436) that formed dynamic H-bonds with residues 133-139 of the A-domain. The propensity for domain closure was increased by pre-alignment of H-bonding residues and decreased by mutation of these residues to Ala. Overall the data suggest that N-domain loop residues are particularly important for mediating the transition through several novel structural substates during the closure of the SERCA cytoplasmic headpiece.

P-23

Inside the 'primed' SecY translocon

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SecY constitutes the core of the highly conserved SecYEG translocon complex that governs the translocation or integration of proteins across or into cytoplasmic membranes. All available evidence suggests that nascent transmembrane (TM) helices simply partition between SecY and the surrounding membrane. The efficiency of TM helix insertion has been experimentally investigated and a "biological" hydrophobicity scale determined [1,2]. Although this scale correlates well with the Wimley-White octanol-based hydrophobicity scale [3], the hydrophobic solvation parameter measured for translocon-to-membrane partitioning has 1/3 the value 开 _ 开 _ between water and non-polar phases [4]. The hydrophobic effect arises from the energetics of the dehydration of non-polar surfaces, suggesting that the smaller solvation parameter observed for translocon/bilayer partitioning arises from an altered state of water in the translocon. To examine this question, we performed molecular dynamics simulations of SecYE from *Pyrococcus furiosus* [5] embedded in a POPC bilayer. SecYE in the *P. furiosus* structure is in a partially open 'primed' state. It conveniently remained open over the course of a 450 nsec simulation, perhaps due to the presence of lipids wedged between helices TM2B and TM7 (the lateral gate), thus allowing close examination of the water on an extended time scale throughout the length of hourglass-shaped channel, including the hydrophobic constriction ring. As the ring is approached, water molecules exhibited a behavior different from those in the bulk phase: rotational and translational water dynamics slowed, the water hydrogen-bond network was longer lived, and the interactions among water molecules strengthened. These features are characteristic of water molecules in close proximity to macromolecules. Within the channel, the water dipoles exhibit a preferred orientation, which affects the electrostatic (ES) potential. Average ES maps reveal a largely positive potential relative to the bulk aqueous phase within the channel except for a strong negative peak at the N-terminus of TM2b. This has implications for the entry of signal sequences into the translocon channel. Research supported by NIH grants RO1 GM74637 and PO1 GM86685.

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P-24

Structure of an H^+-coupled, substrate-bound MATE transporter yields mechanistic insights into multidrug extrusion

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Multidrug transporters pose preeminent clinical challenges by compromising the efficacy of existing and future antibiotics and therapeutic drugs. Multidrug and toxic compound extrusion (MATE) proteins constitute a ubiquitous family of multidrug transporters and couple the efflux of structurally dissimilar drugs to the influx of either Na^+ or H^+. The ~900 MATE transporters identified thus far can be classified into the NorM, DinF (DNA-damage-inducible protein F) and eukaryotic subfamilies based on amino-acid sequence similarity. Structures of Na^+-coupled, extracellular-facing NorM transporters had been determined, which revealed twelve membrane-spanning segments that are related by a quasi-twofold rotational symmetry as well as a multidrug-binding cavity situated near the membrane-periplasm interface. Here we report the crystal structures of an H^+-coupled MATE transporter from the DinF subfamily, with and without substrate, unveiling a surprisingly asymmetric arrangement of twelve transmembrane helices and a largely hydrophobic multidrug-binding chamber located in the middle of the lipid bilayer. Combining structural and biochemical analyses, we confirmed the biological relevance of the substrate-binding site and suggested a direct competition between H^+ and substrate during DinF-mediated drug transport. Furthermore, we modeled the intracellular-facing conformation and suggested a structure-based antiport mechanism to explain how a MATE transporter alternates between its extracellular- and intracellular-facing conformations to propel multidrug extrusion. Unexpectedly, we discovered that the arrangement of drug- and counterion-binding sites is different between the NorM and DinF transporters, unraveling the mechanistic diversity among MATE transporters that is not revealed by the sequence-based analysis alone. Collectively, our findings provided fundamental brushstrokes to the molecular picture depicting how a MATE transporter works and laid the groundwork for future experimental efforts aimed at overcoming multidrug resistance.

P-25

Expression, purification and reconstitution of active and thermally stable CFTR protein

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Knowledge of the mechanism of action of the unique ABC ion channel, CFTR, and the impact of cystic fibrosis causing mutations is severely limited by the lack of 3D structural information at high resolution. Efforts to generate purified CFTR protein of sufficient quality and quantity for crystallization have been limited by the lack of an adequate natural source and its behavior in heterologous expression systems. When expressed at relatively high levels in commonly used over-expression systems such as insect cells and yeast only a very small proportion of the molecules are active and most of the protein is highly aggregated and intractable. Small amounts of protein expressed in mammalian cells have been studied at low concentration by electron microscopy. Although the reasons that the protein self associates so strongly are not fully understood, its relatively low thermal stability seems likely to be one. Significantly, the major CF causing mutation, ΔF508, renders the protein very thermally unstable and therefore a great deal of attention has been paid to this property of CFTR. Multiple second site mutations of CFTR in NBD1 where F508 normally resides and small molecule binders of the domain increase the thermal stability of the mutant. These manipulations are not specific for ΔF508 CFTR but also stabilize other mutants and the wild-type protein. We now have applied ΔF508 “corrective” changes to generate wild-type constructs that express at much higher levels in scaled-up suspension cultures of mammalian cells. After purification to homogeneity and reconstitution into proteo-liposomes these proteins retain channel activity for hours above 37°C. Active non-aggregated protein can be maintained at 4°C in detergent or lipid for at least a week. Different stabilized constructs with primarily open, closed or actively gating states offer the possibility of eventually observing the structural differences between these states. (Supported by the NIH and CFF)

P-26

Exploring Membrane Protein Stability by nanoDSF

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nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins.

The fluorescence of tryptophans in a protein is strongly dependent on close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 150 mg/ml down to 5 µg/ml. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

Here, we present data of different projects to underline the performance and versatility of nanoDSF: Detergent screen for the membrane proteins and rapid and quantitative quality control and long-term stability tests for different protein classes.

P-27

Structure and Dynamics of an EIIC Sugar Uptake System

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The phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) is a sugar uptake system unique to bacteria. It is a multicomponent system consisting of several cytosolic proteins and a dimeric transmembrane protein (EIIC in most PTS systems), which transports extracellular sugar across the membrane. Although EIIC is a uniporter, it is able to drive concentrative transport of its ligand because the sugar is phosphorylated by a cytosolic protein, EIIB, while still bound to the transporter. Phosphorylation prevents the sugar from escaping the cell and primes it for consumption by the cell. Little is known regarding the mechanism of sugar translocation and phosphorylation. Currently, the only available crystal structure of an EIIC is that of the N,N'-diacetylchitobiose transporter, bcChbC (1). We have proposed a mechanism for sugar translocation and phosphorylation, and we will report our progress in characterizing the mechanism.

Reference

Cao Y, Jin X, Levin EJ, et al. Crystal structure of a phosphorylation-coupled saccharide transporter. Nature. 2011;473(7345):50-4.

P-28

X-ray structure of a membrane protein from the CDP-alcohol phosphatidyl-transferase family

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We present the 2.65 Å structure of the bifunctional enzyme CTP:inositol-1-phosphate cytidylyltransferase (IPCT) fused with CDP-inositol:inositolphosphotransferase (DIPPS) [1]. The cytoplasmic IPCT domain is homologous to nucleotidyltransferases and the membrane DIPPS domain belongs to the CDP-alcohol phosphatidyltransferase (CDP-AP) family. Most CDP-APs are involved in phospholipid biosynthesis across the three domains of life. They share a conserved sequence pattern and catalyze the displacement of CMP from a CDP-alcohol by a second alcohol. Phospholipids are not only major structural components of biological membranes but they also play key roles in cell physiology, regulation, and maturation of numerous cellular processes. Disruption of phospholipid homeostasis is associated with several human diseases and plays a crucial role in pathogen invasion, infectivity, and virulence. A better understanding of their metabolic pathways and regulation should help development of chemotherapeutic drugs against various infectious diseases.

The bifunctional protein IPCT/DIPPS dimerizes through the DIPPS domains, each comprising six transmembrane α-helices. The active site cavity is hydrophilic and widely open to the cytoplasm with a magnesium ion surrounded by four highly conserved aspartate residues from helices TM2 and TM3. We analyze the functional role of highly conserved amino acid residues by site-directed mutagenesis studies, dock substrates into identified pockets and propose a catalytic mechanism. This structure opens new avenues for homology modeling of other CDP-OH phosphotransferases, namely those with biomedical interest.

Reference

1- Nogly, P. et al. (2014) X-ray structure of a CDP-alcohol phosphatidyltransferase membrane enzyme and insights into catalytic mechanism. Nature Communications DOI: 10.1038/ncomms5169I: 10.1038/ncomms5169

P-29

NE-CAT Crystallography Beamlines for Challenging Structural Biology Research

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The NorthEastern Collaborative Access Team (NE-CAT) focuses on the design and operation of synchrotron X-ray beamlines for the solution of technically challenging structural biology problems and provides an important resource for the national and international research community. Currently NE-CAT operates two undulator beamlines: 24ID-C - tunable in the energy range from 6 to 22keV and 24ID-E - not-tunable, but optimized for Se SAD experiments. Both beamlines are equipped with state-of-the-art instrumentation. MD2 microdiffractometers installed at both beamlines provide very clean beams down to 5 microns in diameter and are capable of visualizing micron-sized crystals. Large area detectors (Pilatus-6MF), not only provide the best diffraction data, but also make possible to resolve large unit cells. Both beamlines are equipped with ALS style automatic sample mounters. Towards improving the diffraction from low resolution crystals, NE-CAT has installed humidity controlled device, HC1 in the 24ID-E hutch. Locally developed software suite RAPD provides data collection strategies, quasi-real time data integration and scaling and simple automated MR/SAD pipeline through 384 core computing cluster. Users of the beamlines are supported by experienced resident crystallographers.

To meet the needs technically challenging crystallographic projects, cutting-edge hardware and software ideas are implemented. A summary of beamline capabilities, technology, scientific highlights and details of availability will be presented. Funding for NE-CAT is provided through P41 grant from the NIGMS and from the NE-CAT member institutions.

P-30

Identification of a putative proton delivery network in a microsomal cytochrome P450 2B4 F429H mutant.

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The highly conserved Phe429, the first residue of the β-bulge on the proximal side of the heme of microsomal cytochrome P450 2B4, has been mutated to a histidine. The Phe429 His mutant of P450 2B4 has been crystallized with 4-chlorophenol imidazole bound to the iron (Yang et al, 2014). While the overall structure of the mutant is similar to that of the wild type protein in a closed conformation, a hydrogen bond has been noted between the δN of the histidine and the thiolate of the axial cysteine. Biochemical characterization of the mutant demonstrates a >90% loss of activity, an 87 mV increase in the redox potential, and an increase in the stability of the oxyferrous and hydro peroxo intermediates in the variant. The properties of the mutant are explained by the structure which demonstrates an electron-withdrawing hydrogen bond between the heme axial thiolate and a hydrogen on the δN of the histidine.

The structure was also determined of the mutant with the detergent, Cymal 5, bound in the substrate binding pocket ~ 7Å away from the heme iron. Surprisingly an unprotonated carbonyl of acetic acid was bound to the heme iron. The other acetate carboxyl oxygen is protonated and forms a hydrogen bond with the OH group of the conserved active site Thr302. Hydrogen bond formation with the axial acetate was a result of the conserved active site motif (AGXET) moving closer to the center of the porphyrin due to the absence of a bulky heme-bound inhibitor as in the 1-CPI structure discussed above. Analogous movements of the I helix likely occur in the course of a normal catalytic cycle when either water, oxygen, or OOH^- are the small sixth heme ligand. This structure reveals a putative proton delivery network on a microsomal P450 that is similar to that of P450 camphor.

Yang Y., Zhang H., Usharani D., Bu W., Im S., Tarasev M., Waskell L. (2014). Structural and functional characterization of a cytochrome P450 2B4 F429H mutant with an axial thiolate-histidine hydrogen bond. *Biochemistry,* 53(31), 5080-5091. Doi:10.1021/bi5003794.

P-31

Profiling GPCR-Ligand Complexes for Crystallization: Spotlight on the Nociceptin Receptor

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During the past decade there have been monumental advances in the field of G protein-coupled receptor (GPCR) crystallography. In order to accelerate the rate of GPCR structure determination, it is necessary to define the relationship between receptor-ligand complexes and their propensity for crystallogenesis. The nociceptin receptor (NOP) is a member of the opioid subfamily of GPCRs that possesses promising therapeutic potential and a distinct pharmacology from 'classical' opioid receptors. Intensifying drug discovery efforts targeting NOP aim to exploit its role in pain transmission, drug addiction, and anxiety through the development of NOP-selective analgesics, antidepressants, and other therapeutics. With a focus on NOP-antagonist complexes, we explore the relationship between successful GPCR-ligand co-crystallization and ligand properties such as size, potency, and lipophilicity.

Examining the properties of NOP-antagonist complexes and their propensity for crystallization, we observe that a ligand's ability to inhibit G protein mediated signaling is correlated with its ability to induce receptor stability and, consequently, crystallogenesis. Having identified the most promising candidate ligands for crystallization, we solved two new structures of the nociceptin receptor in complex with antagonists SB-612111 and Compound-35 (C-35), extending the findings of the previously reported antagonist-bound structure of NOP. The insights presented here not only contribute to our general understanding of the ligand-aided crystallogenesis of GPCRs, but also serve as a valuable resource in the structure-based design and development of antagonists for the important therapeutic target NOP.

Keywords
G protein-coupled receptor, membrane protein crystallography, nociceptin receptor, opioid receptor, LCP.

P-32

Catalysis and Inhibition of a Monotopic Membrane Enzyme in Lipid Bilayer Nanodiscs

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Cyclooxygenase-2 (COX-2) is the target of non-steroidal anti-inflammatory drugs (NSAIDs) such as Ibuprofen, and COX-2 specific inhibitors (coxibs) such as Celebrex. COX-2 is a monotopic membrane protein dimer that is anchored to the luminal leaflet of the ER membrane via four amphipathic alpha-helices in each monomer. While COX-2 is a sequence homodimer, various biochemical experiments have demonstrated that the enzyme functions with half-of-sites reactivity such that only one monomer is active at a given time. Furthermore, certain ligands can act allosterically by binding to one monomer to influence catalysis in the partner monomer. Chemical cross-linking experiments have delineated a region at the dimer interface that is proposed to undergo conformational change upon allosteric ligand binding. However, crystal structures with allosteric ligands have failed to reveal such structural rearrangements. In COX-2 crystal structures detergent molecules bind at the entrance of the active site and close to the region of proposed conformational change. Thus, it is likely that the restricted confines of the crystal lattice combined with detergent-protein interactions serve to mask the conformational motions underlying allosteric regulation between monomers of the COX-2 dimer. To investigate these potential issues we have incorporated COX-2 into lipid bilayer nanodiscs of controlled size and lipid composition. A dual affinity purification approach was developed to ensure isolation of COX-2:nanodisc complexes with high purity and monodispersity. Size exclusion chromatography and negative-stain EM confirmed that COX-2:nanodisc complexes had been properly formed and purified. Compared to detergent solubilized enzyme, nanodisc incorporated COX-2 had similar kinetic characteristics with various fatty acid substrates and COX inhibitors. The nanodisc incorporated COX-2 was also allosterically activated by palmitic acid in a similar fashion to detergent solubilized enzyme. We analyzed COX-2:nanodiscs formed with POPC, DOPC, POPS, or DOPS lipids and determined that lipid composition caused no significant alterations to COX catalysis or inhibition. Thus, detergent and nanodiscs maintain COX-2 in a similar catalytically active state. Functional reconstitution of COX-2 and other fatty acid oxygenase family members into lipid bilayer nanodiscs will serve as a future utility in investigating the structure, dynamics and function of these important enzymes in the lipid bilayer bound state.

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P-33

Exploring the Broad Utility of Fab Fragments as an Aid in Crystallization of Flexible Proteins

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Monoclonal antigen-binding fragments (Fabs) have been shown to facilitate the crystallization of membrane proteins in a number of ways:

Fabs promote the formation of rigid crystal contacts by binding soluble regions of the protein-detergent-complex (PDC) and thereby increasing its hydrophilic surface area. Fabs can raise PDC stability by binding flexible regions within it. The PDC may favour a particular conformation with the Fab bound. In this case, Fabs ensure conformational homogeneity of the protein solution.

Importantly, only point (1) above is relevant exclusively to membrane proteins. Since our lab is interested in bacterial effectors, we propose that Fabs may also facilitate their crystallization and structure determination. We have raised Fabs against a number of protein targets by the method of phage-display and analyzed their binding via ELISA, and pull-down experiments. Our integral membrane target is ExbB-ExbD, an inner membrane complex essential for TonB system functionality. Using the periplasmic domain of ExbD, we have been able to isolate a Fab which binds not only ExbD, but the whole ExbB-ExbD complex in the presence of detergent. We have also included several effector kinases in our biopanning experiments, yielding additional Fabs to study. Further efforts to characterize these Fabs are currently underway.

P-34

SONICC implementation at GM/CA@APS

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Second order nonlinear optical imaging of chiral crystals (SONICC), based on femtosecond laser scanning microscopy, has been implemented at GM/CA@APS undulator beamline 23ID-B for rapid protein crystal localization and centering. The technique is based on infrared laser light impinging on non-centrosymmetric crystals of proteins, which selectively may yield a frequency-doubled, visible signal generated by the anharmonic response of the electron cloud of the protein in response to the laser field. One aim of this method is to locate small crystals grown in opaque crystallization media for centering in X-ray beams of only a few microns or less in cross-section, such as for membrane-protein crystals grown in mesophase [1]. The optical system implemented at the beamline includes 'trans' and 'epi' detection of Second Harmonic Generation (SHG) signals [2]. In addition, scanning visible laser light across the sample and detecting two-photon excited UV fluorescence (TPE-UVF) provides complementary contrast based on the native fluorescence of proteins. Recent efforts towards providing safe, user-friendly capabilities will be described, including: increasing rates of data acquisition, providing bright-field laser imaging capabilities, and incorporation of laser-safety interlocks suitable for a general user program.

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Keywords: SONICC, synchrotron, microcrystals

P-35

Crystal structure of a polypeptide processing and secretion transporter

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Bacteria secrete peptides and proteins to communicate with each other or to poison competitors. In addition to the well-studied Sec translocation, prokaryotes use several other Sec-independent secretion systems, including the Type I Secretion System that employs an ABC transporter. In gram-positive bacteria, dedicated ABC transporters export quorum sensing or antimicrobial polypeptides. In gram-negative bacteria, the ABC transporter interacts with two other membrane proteins to assemble a continuous channel penetrating both the inner and outer membranes. Here we present the crystal structure of a Peptidase-Containing ABC transporter (PCAT), PCAT1, from Clostridium thermocellum that secretes a 66-residue peptide and concomitantly cleaves its N-terminal signal sequence. The transmembrane helices form a large helical barrel that traverses nearly the entire lipid bilayer and is large enough to accommodate compactly folded domains. The peptidase domains are located near the cytoplasmic surface of the membrane, with catalytic residues facing the opening of the helical barrel. As the first structure of an ABC transporter that both processes and transports its substrate, it offers a structural basis for understanding the Type I secretion system.

P-36

Methyl labeling and TROSY NMR spectroscopy of proteins expressed in the eukaryote *Pichia pastoris*

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^{13}C Methyl TROSY NMR spectroscopy has emerged as a powerful method for studying the dynamics of large systems such as macromolecular assemblies and membrane proteins in detergent micelles. Specific ^{13}C labeling of aliphatic methyl groups and perdeuteration are limited primarily to proteins expressed in *E. coli*, preventing studies of many eukaryotic proteins of physiological and biomedical significance. We demonstrate the feasibility of efficient ^{13}C Isoleucine δ1-methyl labeling in an established eukaryotic expression host, *Pichia pastoris*, and show that this method can be used to label a eukaryotic protein, actin, which cannot be expressed in bacteria. This approach will enable NMR dynamics studies of previously intractable targets.

P-37

Allosteric modulator as an additional tool for GPCR stabilization and crystallogenesis: case study of CC chemokine receptor 2

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Small molecule agonists and antagonists are crucial tools for successful crystallogenesis in GPCR structural biology by stabilizing the receptors into a homogeneous conformational state. Recent discovery of small molecule allosteric modulators for several chemokine receptors (CXCR1, CXCR2, CCR4, and CCR2) offers opportunities for developing new therapeutic treatments targeting diseases related to directed immune cell migration such as inflammation, neurodegeneration, diabetes, and cancer. Two of these allosteric modulators for CCR2, CCR2-RA-R and JNJ-27141491, were previously mapped into intracellular minor pocket of CCR2 formed by TM6, TM7, and helix 8. It behaves as a non-competitive inhibitor for CCR2 in terms of CCL2 induced [^{35}S]GTPγS binding. Interestingly, CCR2-RA-R can displace CCL2 completely whereas CCL2 can only displace CCR2-RA-R to 60% of its total binding. It is still unclear how CCR2-RA-R affects CCL2 binding to CCR2 at the molecular level. Here we show that significantly higher thermal stability for purified CCR2 with ICL3-T4L fusion is achieved in the presence of both orthosteric and allosteric antagonists than when either is present individually. This observation suggests a synergistic effect of the two types of antagonists and supports the concept that CCR2-RA-R is biasing the conformation equilibrium of CCR2 to an inactive state, unfavorable for agonist CCL2 binding. Binding of both types of antagonists likely locks the receptor in an inactive state, which holds promise for being a successful co-crystallization candidate. Effect of CCR2-RA-R on an antagonist viral chemokine vMIP-II binding kinetics is also interrogated with a cell based assay.

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P-38

Human membrane proteins overexpression, purification and thermostability assays with successful application in structure determination

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X-ray crystal structures of eukaryotic integral membrane proteins, while potentially being of extremely high impact, are highly underrepresented relative to prokaryotic membrane proteins. One key reason for this underrepresentation is that membrane proteins can be difficult to overexpress at a level, and at a quality, that is amenable for structural studies. Here we report a simplified, systematically developed robust strategy from small-scale screening to large-scale over-expression of human integral membrane proteins in mammalian expression system for structural studies. These expression method and assays have been utilized for determination of 2.1 Å X-ray crystal structure of human rhesus C glycoprotein (RhCG) (the first human membrane protein structure from human cells), another human rhesus glycoprotein structure determination, and to purify two Class B GPCRs to homogeneity for crystal trials. This methodology streamlines small scale screening of several different constructs utilizing Fluorescence Size Exclusion Chromatography (FSEC), generation of desired construct's stable clonal cell line, detergent screening using FSEC, implementing thermostability assays towards buffer and additives optimization for achieving stability and homogeneity and lastly large scale expression for crystallization. These techniques are designed to rapidly advance the structural studies of eukaryotic integral membrane proteins including human membrane proteins, in particular in those cases where heterologous expression in lower order expression systems is unsuccessful.

P-39

An integrated framework advancing membrane protein modeling and design

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Membrane proteins are critical functional molecules in the human body, constituting more than 30% of open reading frames. Unfortunately, a myriad of difficulties in overexpression, reconstitution into membrane mimetics, and structure determination severely limit our ability to determine their structures. Computational tools are therefore instrumental to bridging the gap between the large number of known sequences and the few known structures, consequently increasing our understanding of membrane protein function and their role in disease. Here, we describe a general framework facilitating membrane protein modeling and design that combines the scientific principles for membrane protein modeling with the flexible software architecture of Rosetta3. This new framework, called RosettaMP, provides a general membrane representation including scoring and conformational sampling routines that can be easily combined to create new protocols. To demonstrate the capabilities of this implementation, we developed four proof-of-concept applications for (1) prediction of free energy changes upon mutation, (2) high-resolution structural refinement, (3) protein-protein docking, and (4) assembly of symmetric protein complexes, all in the membrane environment. Preliminary data show that these algorithms can produce meaningful energies and structures. The data also suggest needed improvements to both sampling routines and score functions to elevate these tools to state-of-the-art software tools. Importantly, the applications collectively demonstrate the potential of combining the flexible nature of RosettaMP with the power of Rosetta algorithms to facilitate membrane protein modeling and design.

P-40

Membrane Protein Complex $ExbB_4$–$ExbD_1$–$TonB_1$ from *Escherichia coli* Demonstrates Conformational Plasticity

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Iron acquisition at the outer membrane (OM) of Gram-negative bacteria is powered by the proton motive force of the cytoplasmic membrane (CM), harnessed by the CM-embedded complex of ExbB, ExbD and TonB. Its stoichiometry, ensemble structural features and mechanism of action are unknown. By panning combinatorial phage libraries, periplasmic regions of dimerization between ExbD and TonB were predicted. Using overexpression of full-length *exbB*–$exbD_{His}$ and $tonB_{S\text{-}tag}$, we purified detergent-solubilized complexes of ExbB–ExbD–TonB from *Escherichia coli*. Protein-detergent complexes of ~230 kDa with a hydrodynamic radius of ~6.0 nm were similar to previously purified $ExbB_4$–$ExbD_2$ complexes. Significantly, they differed in electronegativity by native agarose gel electrophoresis. Stoichiometry was determined to be $ExbB_4$–$ExbD_1$–$TonB_1$. Single particle electron microscopy agrees with this stoichiometry. Two-dimensional averaging supported the phage display predictions, showing two forms of ExbD–TonB periplasmic heterodimerization: extensive and distal. Three-dimensional (3D) particle classification showed three representative conformations of $ExbB_4$–$ExbD_1$–$TonB_1$. Based on our structural data, we propose a model in which ExbD shuttles a proton across the CM *via* an ExbB inter-protein rearrangement. Proton translocation would be coupled to ExbD-mediated collapse of extended TonB in complex with ligand-loaded receptors in the OM, followed by repositioning of TonB through extensive dimerization with ExbD. Here we present the first report for purification of the ExbB–ExbD–TonB complex, molar ratios within the complex (4:1:1), and structural biology that provides insights into 3D organization.

P-41

Identifying conformationally selective, inhibitory Fabs for site-2 protease

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Regulated intramembrane proteolysis is a conserved signaling mechanism from bacteria to humans. An integral step of this mechanism is the site-specific cleavage of a peptide that spans the membrane. The cleavage, conducted by a membrane-embedded protease, occurs within the lipid bilayer. We are using site-2 protease (S2P), one of the first intramembrane proteases to be discovered, as a model protease for this protein class.

In order to achieve better knowledge of intramembrane protease structure, mechanism and function we are generating conformationally selective, inhibitory antibodies against S2P. Generally, intramembrane proteases are a very challenging class of proteins for antibody discovery due to their need to be incorporated in membranes to keep their native form. To overcome these difficulties, we embedded S2P in micelles of detergent solution and performed in-solution panning with indirect immobilization of the intramembrane protease. A human naïve Fab phage-display library was used to identify antibodies that specifically bind S2P (1).

To develop an activity assay for S2P that can be used to prioritize the Fab binders we are elucidating its substrate specificity with multiplex substrate profiling by mass spectrometry (MSP-MS) (2) using a chemically diverse, peptide library. Knowing the substrate specificity of S2P permits rational design of a sensitive and selective FRET substrate that can be used to identify inhibitory antibodies against S2P and determine their inhibitory potential. Generation of an inhibitory Fab against S2P, Fab(3F1), validates the approach.

We have developed a methodology to generate inhibitory antibodies against intramembrane proteases like S2P. These antibodies will accelerate the progress in both structural and functional studies of intramembrane proteases. The inhibitory antibody bound to S2P has the potential to reveal more details about the mechanism of this particular enzyme.

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P-42

Femto- and attosecond serial crystallography - time-resolved studies of dynamics in biological systems

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Serial Femtosecond X-ray nanocrystallography (SFX) is a recently developed technique for protein structure determination that is based on collection of X-ray diffraction from nano- and microcrystals using a free electron laser (FEL). SFX is especially valuable for challenging proteins as membrane proteins, because the time-consuming process of growing larger crystals can be avoided (1, 2). In general structural analysis with the use of free electron X-ray laser is a powerful method to overcome the radiation damage problem and to perform time-resolved structure analysis (1-4). Recent results of studies on the membrane protein complex Photosystem II using time resolved SFX (3) will form the basis for future time-resolved studies of biomolecules.

Furthermore an introduction of the concept of attosecond serial crystallography, which is currently under development, and its potential advantages over SFX will be given. Its capability for new insights in the water oxidation process in Photosystem II and its value for structural and functionals analysis of biological processes in general will be discussed.

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P-43

On the Lipid Dependence of the Stability of the Cytochrome b_6f Complex

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Structure-function studies of intra-membrane redox proteins have the particular advantage that function can be readily and quantitatively analyzed. Little is known, however, about the lipid dependence of structure parameters. Crystallization of the eight subunit hetero-oligomeric 270 kDa dimeric cytochrome b_6f complex is dependent on the presence of lipid (1, 2). At least 23 lipid binding sites per monomer are seen in the structure resolved to 2.5 A (3). The function of this lipid, which has been inferred to contribute to the heterogeneous dielectric constant of the complex (4), in the assembly and stabilization of the complex, is unknown.

A dependence of the thermal stability of the secondary structure of the cytochrome complex on state of lipidation is described. From thermal denaturation studies utilizing far-UV circular dichroism analysis, it was found that “melting” (i. e., loss of helical structure) of the b_6f complex starts from T= 45° F for the delipidated complex. The complex is more stable in the presence of anionic lipid, DOPG, for which a significant rate of melting starts at 49°C. The maximum rate of helix melting, determined from the first derivative of the amplitude at 222 nm of the specific molar ellipticityspectrum is 59° F for delipidated complex, while it is 62°F in the presence of DOPG. In the presence of the chemical oxidant, ferricyanide, the melting maximum at approximately 55° and 59°, respectively, for the delipidatedand DOPG-cytochrome b_6f complex.

The stability of the complex in the presence and absence of lipid was tested by clear native gel electrophoresis. Both delipidated and lipidated samples were heated, consecutively, to 30°, 40°, 50°, and 60°. In comparison to the lipidated b_6f complex, the rate of monomerization as a function of increasing temperature was enhanced in the delipidated complex.

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P-44

Mesophase-based Membrane Protein Crystallization Condition Screening on High-throughput Microfluidic Chips

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Membrane proteins serve key roles in cellular functions and their malfunction contributes to many diseases. Despite their physiological importance, the scientific community still lacks a thorough mechanistic understanding for most membrane proteins. A lack of high-quality 3-D structures of membrane proteins, which could reveal ligand binding sites and atomic resolution structural changes that contribute to disease states, hampers advances in structure-function research and rational drug discovery. Membrane proteins are generally intractable to conventional crystallization methods, leading to the rise of advanced techniques that account for low stability and amphiphilicity. Mesophase-based crystallization (generally called lipidic cubic phase [LCP] crystallization) has improved success rates of growing diffraction quality crystals, although has limited presence in structural biology laboratories due to the requirement of skilled labor and specialized equipment, or an expensive robot. Further, exhaustive screening is currently the only route to discover crystallization conditions for proteins of unknown structure, potentially requiring thousands of formulations for a trial-and-error screen. Manual harvesting of fragile crystals and cryo-cooling also negatively impact quality of diffraction data. An ideal crystallization platform would automate trial formulation, allow for growth of high-quality crystal, and enable X-ray analysis on undisturbed crystals.

To address these challenges we have developed high-throughput X-ray transparent microfluidic chips for *in meso* crystallization screening. The chips automate the formulation of up to 192 unique conditions in parallel, utilizing as little as 10 nL of protein solution per crystallization well. The mixing of protein and lipid is accomplished by *in situ* passive mixing, which enables the screening of multiple protein-lipid ratios. X-ray transparency, accomplished by assembling the chip with thin layers of high X-ray transmission materials, enables *in situ* diffraction to eliminate manual crystal harvesting. Additionally, the optical transparency of chips facilitates serial crystallography of dozens of randomly oriented, isomorphous crystals. Successful crystallization and structure determination of the wild type and a mutant of *R. sphaeroides* reaction center demonstrates the efficacy of the platform as a streamlined crystallography tool. Ongoing screening efforts focus on the crystallization of membrane proteins in the heme-copper oxidase superfamily.

P-45

A Membrane Protein Technologies Web Resource: The PSI SBKB Technology Portal

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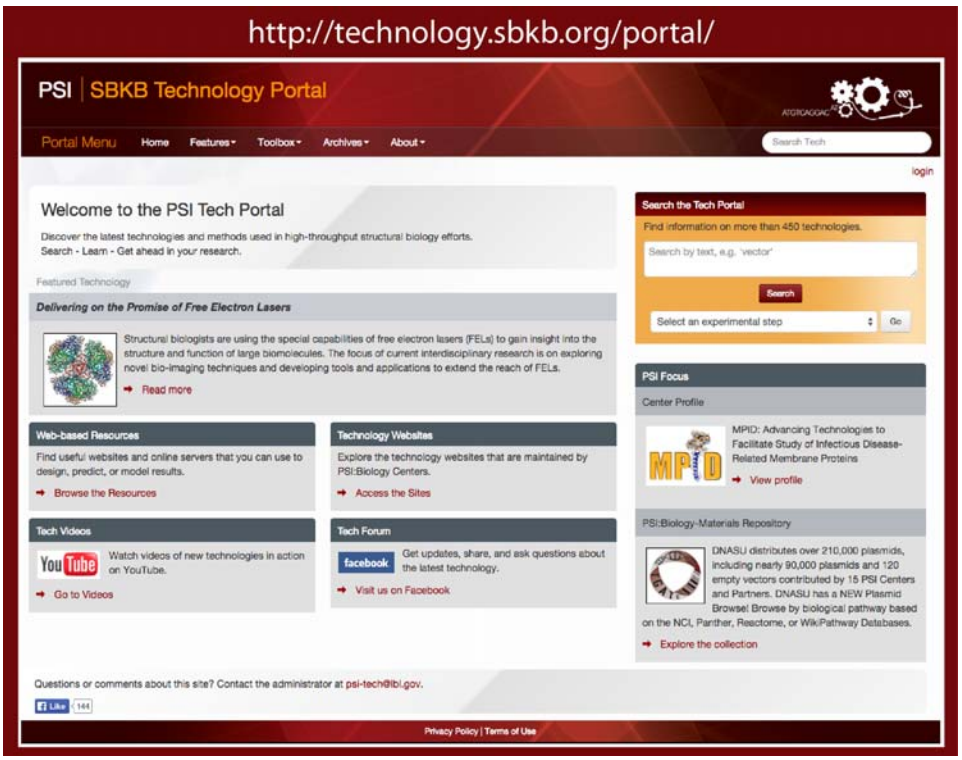
The Protein Structure Initiative (PSI) Technology Portal (http://technology.sbkb.org/portal/) provides access to information, methods, and tools that can be used to relieve bottlenecks in membrane protein structure determination. This web resource offers multiple ways to query the database of over 450 technological advances. Periodic articles offer an overview of cutting-edge methods in use by structural biologists (Featured Technology) and profile the research being performed at different PSI Centers. Other tools available are videos of new methods from the sbkbtech YouTube channel and the Facebook page, which serves as a forum for scientists to get news, connect, and ask questions. The PSI Tech Portal provides easy access to online web servers and technical websites that are maintained by the individual PSI Centers. Newly developed pages outline the structure determination platform of the Joint Center for Structural Genomics. The Technology Portal is connected to the Structural Biology Knowledgebase (http://sbkb.org), a portal of portals that houses integrated genomic, structural, and functional information for all protein sequence targets selected by the PSI. The information, tools, and opportunities for scientific interaction available through the PSI Tech Portal will be highlighted. For updates, "Like" the PSI Tech Portal on Facebook: https://www.facebook.com/techportal.



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P-46

Conformational Changes that Opens TrkH Ion Channel

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A high intracellular K^+ concentration is required for many physiological processes such as setting the membrane potential, enzymatic activity and osmoregulation. To carry K^+ through the lipid bilayer membrane, organisms must express K^+ transport proteins, such as proteins in the Superfamily of K^+ Transporters (SKT). TrkH, a member of SKT, is required for bacteria growth in low external K^+ environments. Previous studies showed that TrkH is an ion channel and ATP increases the channel activity through an associated cytosolic protein, TrkA. However, how TrkH is gated, and how ATP controls the gating of TrkH are still not clear. To understand this gating mechanism, we solved a structure of apo TrkH-TrkA complex from *Vibrio parahaemolyticus* by single particle cryo-electron microscopy.

P-47

Function of the Cytochrome b_6f Complex in Trans-Membrane Signaling

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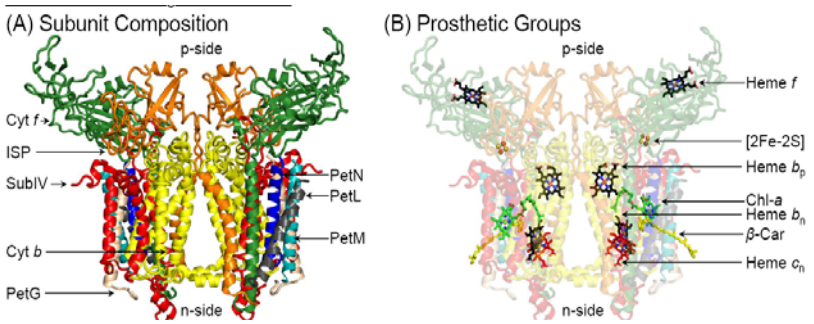
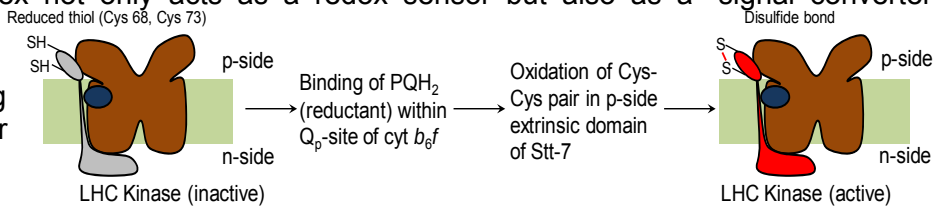
The hetero-oligomeric 8 subunit 270 kDa dimeric cytochrome b_6f complex (**Fig. 1**), currently seen at a resolution of 2.5 Å (1), catalyzes proton-coupled redox reactions of the lipid soluble substrate plastoquinone/plastoquinol (PQ/PQH_2), to generate a trans-membrane proton electrochemical proton gradient, utilized for ATP synthesis, through vectorial transfer of H^+ to the electrochemically positive (p-side) of the complex. The p-side PQH_2 deprotonation-oxidation (Qp) site is proposed to also function as a "redox-sensor" to control the distribution of the light-harvesting pigment proteins (2, 3). Binding of the quinol at the Qp site has been proposed to be the signal for the activation of a kinase ("Stt7") that phosphorylates the pigment-proteins, thus facilitating their light-dependent re-distribution (4- 6). The activation and catalytic sites of the kinase are located on the p and n-sides, respectively. This passage of trans-membrane information over a distance ≈ 30 Å from the cytochrome Qp -site to the Stt-7 kinase domain is caused by a presently unknown signalling mechanism and resultant conformational changes. A conserved p-side cysteine pair is involved in kinase activation/inactivation (5). Activation of the enzyme is proposed to be dependent on the bonded status of the conserved cysteine pair (5) and on mediation by thioredoxin (7). Here, it is proposed: (**a**) the relatively high level of p-side superoxide (O_2^-.) formation linked to quinol oxidation (8, 9) is relevant to the mechanism of kinase activation; (**b**) O_2^- undergoes dismutation to yield hydrogen peroxide in the presence of the organelle superoxide dismutase. (**c**) This peroxide reacts with the cellular thioredoxin system, leading to its oxidation, and consequently cysteine oxidation and disulfide bond formation in the kinase. Thus, the kinase would be activated to phosphorylate the light-harvesting protein, as summarized briefly in **Fig. 2**, The cytochrome b_6f complex not only acts as a redox sensor but also as a "signal converter", transforming the reducing signal of PQH_2 to an oxidizing signal required for kinase activation.





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P-48

A COMPARITIVE STUDY OF MACRO AND MICRO-CRYSTALLIZATION TECHNIQUES

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X-ray crystallography is the most effective and proliferate method for elucidating three-dimensional structure of macromolecules. It has played key role in our understanding of biological phenomena by providing accurate structural details to atomic resolution. Since the first protein structure was solved (J. C. Kendrew et.al, *Nature* **181**, 662-666, 1958), the field has made major advances, and yet, producing large, well-ordered crystals requires extensive experimentation, which can span years to decades for proteins that are difficult to crystallize (eg. membrane proteins). With the advent of free electron lasers, it is now possible to explore novel targets using femtosecond crystallography (H. N. Chapman et.al, *Nature* **470**, 73-77, 2011). But the concept is relatively new and needs considerable progress, including development of methods for crystal growth, before it can be widely applied.

Various methods exist for producing nano (0.1-1μm), micro (1-10μ) and macro (0.1-1mm) crystals. From dialysis to free interface diffusion, each approach has its merits and shortcomings. The knowledge of available techniques is essential while trying to make crystals of high quality and uniform size distribution and this poster attempts to summarize differences in techniques and conditions between macro, micro and nano-crystallography of Photosystem I and II, thylakoid membrane protein complexes isolated from *Thermosynechococcus elongatus*.

This work was supported by: The Center for Bio-Inspired Solar Fuel Production (an Energy Frontier Research Center funded by the DOE, Office of Basic Energy Sciences), The National Institute of Health and The US National Science Foundation.

P-49

Automated protein crystal optimisation with TTP Labtech's dragonfly

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The ability to crystallise proteins, nucleic acids or macromolecular complexes pose significant challenges to the protein crystallography community, from large scale screening assays for the determination of initial crystallization conditions, screen optimisation and final screen set-up.
Protein crystal optimisation is vital to ensure high quality X ray diffraction data for the solving of high resolution structure. This process involves the set-up of a series of complex screening combinations where the ratios of the individual components identified from primary crystallisation studies are varied.

In order to reduce the effort and tedium of this process, TTP Labtech have introduced dragonfly as an addition to their successful mosquito liquid handling portfolio for crystallisation screening.
This poster demonstrates that "dragonfly" is a valuable, compact, low cost addition to the crystallographer's bench. It eliminates lengthy and complicated plate set-up at the optimisation stage of crystallisation.

This poster demonstrates that TTP Labtech's dragonfly is a valuable compact low cost addition to the crystallographer's bench. It eliminates lengthy and complicated plate set-ups at the optimisation stage of crystallisation.

P-50

Structural Basis for Inhibitor Binding to the Yeast ATP Synthase

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The mitochondrial F_1F_o ATP synthase is a multimeric protein complex (>550,000 Da) that is responsible for synthesis of 90% of the cellular ATP under aerobic conditions. F_1 is a water-soluble complex, which contains the active site for ATP synthesis. F_o, partly comprised of a ring of 10 c-subunits, is embedded in the inner membrane of the mitochondrion and forms a proton turbine, which drives the synthesis of ATP. Oligomycin is a prototypical inhibitor of the mitochondrial ATP synthase having been first reported in 1958 by Henry Lardy. We have recently published the structure of the F_o c_{10}-ring with bound oligomycin. We report here the structures of oligomycin A, B, C, and 21-hydroxy bound to the c-ring of F_o from yeast *S. cerevisiae*. Using molecular dynamics simulations, we show that binding of the inhibitor to the c_{10}-ring is facilitated by a mixed hydrophilic/hydrophobic environment, indicating that oligomycin blocks the proton-loading sites in the c-ring that are exposed to the water-filled, proton channels across the F_o complex. We also report the IC_{50} for the oligomycin derivatives as well as those for miconazole, venturicidin, and TMC207. TMC207 is a recently approved drug used to treat multidrug resistant forms of *M. tuberculosis*. Analysis of the structural basis of oligomycin binding to the c-ring, the primary sequence comparison of c-rings from various species, and the sites identified that when mutated confer inhibitor resistance, we conclude that the oligomycin binding site frames the binding site for all of these inhibitors. Lastly, we suggest that V_o inhibitors bind to the vacuolar ATPase in the corresponding region.

P-51

Nonlinear optical correlation spectroscopy for characterizing translational diffusing motion of protein nanocrystals

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Serial femtosecond crystallography has been routinely employed in obtaining structural information of membrane proteins. During serial femtosecond crystallography, the diffraction pattern is pooled from thousands of individual protein nanocrystals. The increasing trend of using smaller and smaller protein crystals has introduced new challenges. One such challenge is the lack of tools to reliably detect and distinguish nanocrystals and small microcrystals from solution or amorphous protein material.

Second harmonic generation correlation spectroscopy (SHG-CS) has the potential to serve as a rapid, non-destructive and crystal specific method for protein nanocrystals characterization. Information like particle size and concentration can be retrieved from the intensity fluctuation of the signal by applying autocorrelation analysis on the SHG intensity data. Unlike dynamic light scattering, the intrinsic selectivity of the second order nonlinear optical process provides SHG-CS the ability to distinguish well-ordered structures like protein nanocrystals from conglomerate and amorphous substrate. SHG-CS also offers S/N advantages as random noise yields to a zero response in the correlogram. Custom built instruments and software with real time feedback have been developed to perform SHG-CS measurement. In the presented work, $BaTiO_3$ nanoparticles are selected for validation of the method and calibration of the instrument. SHG-CS has also been applied to photosystem I (PSI) nanocrystals.

P-52

Imaging local electric fields produced upon synchrotron X-ray exposure

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Second harmonic generation (SHG) microscopy implemented at GM/CA@APS beamline 23ID-B has enabled direct visualization of three dimensional electric field distributions produced within protein crystals and cryogenic glasses following X-ray exposure, which could play a contributing role in X-ray damage during diffraction data collection(1). Following X-ray exposure, the photoelectrons produced from X-ray absorption can travel several microns from the initial location of irradiation. This charge redistribution results in the growth of substantial local electric fields. Based on simulations of photoelectron trajectories, the electric field distributions were predicted to arise over locations within and significantly beyond the region exposed to X-rays during data collection. Because the large majority of protein crystals are symmetry-allowed for piezoelectricity, the fields could potentially produce local lattice distortions and negatively impact diffraction resolution. The simulations suggesting generation of large local electric fields were supported by electric-field induced second harmonic (EFISH) generation of cryogenic liquids, which yields a signal intensity proportional to the squared magnitude of the local static electric field. The polarization-dependent SHG intensity maps predicted based on the simulations agreed well with experimental measurements with no adjustable parameters, including the presence of nodes of zero-field at locations beyond the exposed regions. Changes in SHG could be easily observed even after low-dose exposures routinely used to center the crystals by X-ray diffraction raster scanning prior to data collection.

Keywords: X-ray damage, synchrotron, microcrystals

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P-53

Binding of Quaternary Ammonium Ions to a Potassium Channel

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We have used stopped flow spectrophotometry to measure the binding affinities of quaternary ammonium (QA) ions to the KcsA potassium channel. KcsA is a pH sensitive channel that activates at low pH and then inactivates rapidly. In membranes containing POPC:POPG at a 4:1 ratio, approximately half of the channels inactivate within one second. Stopped flow spectrophotometry allows rapid mixing of reagents and measurement of fluorescence on a millisecond time scale. Channel activity was measured by mixing a suspension of 100 nm proteoliposomes at neutral pH with a thallium containing buffer at a low pH, and monitoring the flux of thallium through open potassium channels via the quenching of a trapped fluorophore. Using this method, channel inhibition was measured with several species of QAs. Due to the hydrophobic nature of the drugs used, solubility of the long chain QAs became a limiting factor in our measurements. We found that binding affinity increased with alkyl-chain length from four to eight carbon atoms. Tetrabutylammonium (TBA) was measured to have an inhibition constant of ~120μM. The solubility limits of tetrahexylammonium (THA) and tetraoctylammonium (TOA) did not permit the measurement of a complete inhibition curve. At the solubility limits of THA (2μM) and TOA (100nM) the measured inhibition was ~70% and ~30%, respectively.

P-54

pJAZZ® Linear Cloning Platform for Multisubunit Protein Expression.

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We present a new platform for bacterial and eukaryotic cloning and expression of multigene systems, repeat units, and structurally unstable DNAs. pJAZZ is a unique linear cloning vector that tolerates nearly any DNA sequence, including AT-rich genes and highly repetitive sequences that are impossible to capture in typical circular vectors. pJAZZ has a cloning capacity of up to 40 kb, enabling stable assembly and expression of complex multigene systems such as tandem arrays, deleterious coding sequences, and other difficult targets. Mammalian versions of the vector have been used to express a 40-kb tetrameric IP_3-gated Ca^{++} channel and other diverse targets, such as highly repetitive silk genes and multi-protein complexes. We have adapted pJAZZ for light-inducible expression to enable precise spatiotemporal control over expression. The pJAZZ system is amenable to standard cloning and transfection techniques, and thus is an excellent platform for expression of multisubunit membrane protein complexes in eukaryotic cells.

P-55

X-ray structure of a mammalian stearoyl-CoA desaturase

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Stearoyl-CoA desaturase (SCD) is conserved in all eukaryotes and introduces the first double bond into saturated fatty acyl-CoAs. Since the monounsaturated products of SCD are key precursors of membrane phospholipids, cholesterol esters, and triglycerides, SCD is pivotal in fatty acid metabolism. Humans have two SCD homologs (SCD1 and SCD5), while mice have four (SCD1-SCD4). SCD1-deficient mice do not become obese or diabetic when fed a high-fat diet because of improved lipid metabolic profiles and insulin sensitivity. Thus, SCD1 is a pharmacological target in the treatment of obesity, diabetes, and other metabolic diseases. SCD1 is an integral membrane protein located in the endoplasmic reticulum, and catalyzes the formation of a *cis*-double bond between the 9th and 10th carbons of stearoyl- or palmitoyl-CoA. The reaction requires molecular oxygen, which is activated by a diiron center, and cytochrome b5, which reduces the diiron center. To better understand the structural basis of these characteristics of SCD function, we crystallized and solved the structure of mouse SCD1 bound to stearoyl-CoA at 2.6 Å resolution. The structure shows a novel fold comprising four transmembrane helices capped by a cytosolic domain, and a plausible pathway for lateral substrate access and product egress. The acyl chain of the bound stearoyl-CoA is enclosed in a tunnel buried in the cytosolic domain, and the geometry of the tunnel and configuration of the bound acyl chain provide a structural basis for the regioselectivity and stereospecificity of the desaturation reaction. The dimetal center is coordinated by a unique configuration of nine conserved histidine residues that implies a potentially novel metal center and mechanism for oxygen activation. The structure also illustrates a possible route for electron transfer from cytochrome b5 to the diiron center.

P-56

Rapid Characterization of Protein Quality Attributes on the LabChip® GXII Touch Micro-Capillary Electrophoresis Platform

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Perkin Elmer, Inc., Hopkinton, MA USA

To effectively develop biotherapeutic molecules and bring them to market, it is critical that researchers thoroughly understand the relationships between specific process parameters and product quality. *Design of Experiment* studies which can most effectively define these relationships through full examination of the range of potential variables affecting glycoprotein quality are dependent on the analysis of large numbers of samples. Traditionally, SDS-PAGE, HPLC, and CE based separation techniques have been used but with limited throughput. The LabChip GXII Touch system offers rapid analysis of seven glycoprotein quality attributes, thereby enabling significant expansion of process development studies and as products are transferred to production environments. Here we demonstrate the sensitivity, resolution, and reproducibility of the GXII Touch system using adalimumab as a reference protein.

P-57

De novo protein structure determination from near-atomic-resolution cryo-EM maps

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Single-particle cryo-electron microscopy (cryo-EM) has shown a great potential to be the method to determine structures from samples which are reluctant to crystalize, for example: membrane proteins. Recent advances even enable cryo-EM to determine membrane protein structures at near-atomic resolution (3–5 Å). However, determining protein structure from near-atomic resolution density data, when no homologous structures are known, is a difficult but important problem. At these resolutions, side-chain density is ambiguous, making correct assignment of sequence difficult. Here, we present an automated *de novo* model building approach that combines sequence-derived predicted backbone conformations (fragments) with side-chain density-fit in a Monte Carlo sampling framework to accurately assign sequence into near-atomic resolution density maps.

On nine experimental cryo-EM maps from 3.3 to 4.8 Å resolution, our method yields accurate full-length models in six cases with, on average, 1.6 Å Cα RMSD and 2.5 Å all-atom RMSD to the deposited structures. Two proteins from our set, TRPV1 and FrhB, are proteins with new folds solved recently by manually building models into cryo-EM density; our method automatically obtained completed models with sub-2 Å Cα RMSD for both. To test the resolution limit of our approach, a previously unpublished 4.8 Å resolution map of the 20S proteasome was used. Our method yields a model with 1.2 Å Cα RMSD (2.0 Å all-atom); native side-chain packing in the core is recovered.

Finally, we apply the approach to a previously unsolved cryo-EM map containing the 660-residue contractile sheath protein complex of type VI secretion system from *Vibrio cholera*, yielding a nearly complete model, resolving portions of the structure unsolved or in error in an independent manual tracing. These results suggest that it is sufficient to directly and automatically build atomic models from near-atomic resolution density maps. The method should enable rapid and reliable protein structure determination from near-atomic resolution cryo-EM maps.

Reference

Wang et al., 2015, *Nature Methods* (doi:10.1038/nmeth.3287)

P-58

EPR data support the existence of a symmetric BH3-in-groove homodimer in oligomeric BAK.

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The BAX or BAK oligomeric pore formation in the mitochondrial outer membrane is a critical regulatory step in apoptosis, which is not clearly understood. Czabotar *et al.* (Cell 2013, **152**, 519) reported a crystal structure of a water-soluble tetramer of the BAX fragment (helices α2-α5) fused to the green-fluorescent protein (GFP), in which the α2-α3 extended helices and the α5 helix, respectively, were juxtaposed to each other, in an anti-parallel orientation, forming a symmetric "BH3-in-groove homodimer (BGH)." We have constructed a GFP-BAK fusion protein using the α2-α5 helices of mouse BAK, designated as GFP-BAKα2-α5, which also forms a soluble tetramer. To determine whether the BGH exists in the BAK oligomers in the membrane or not, we spin labeled the C-terminal hexahistidine-tagged soluble form of mouse BAK (helices α1-α8) at residues 84, 122, 128 and 135 and the corresponding residues in GFP-BAKα2-α5. We then compared the continuous wave (CW) EPR spectra of the spin-labeled residues from the tetrameric GFP-BAKα2-α5 with those from the oligomeric BAK in membrane. Spin labeled residue 122R1, located in the loop interconnecting helices α4 and α5 in the homology model of the GFP-BAKα2-α5 tetramer, displayed a mobile lineshape. The corresponding residue in the oligomeric BAK also had a remarkably similar lineshape, indicating that the two residues are in similar structural environments. Residues 84R1, 128R1 and 135R1, located at the anti-parallel helical interfaces in the BGH also had remarkably similar immobile lineshapes both in the GFP-BAKα2-α5 tetramer and in the oligomeric BAK in membrane, further strengthening the above conclusion. The preliminary intra-dimer distances between 84R1 spin label pairs in the GFP-BAKα2-α5 and the oligomeric BAK, determined by the double electron electron resonance (DEER) method, also support this interpretation.

P-59

Recombinant Techniques for the Production of Stable Multiprotein Complexes

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Many cellular processes are carried out by molecular machines composed of multiprotein complexes. The MCSG pipeline has produced more than 1,800 structure deposits to date. However, these structures represent mostly homo-oligomeric (63%) and monomeric (37%) protein stoichiometries. The structural characterization of hetero-oligomers requires extensions to the existing pipeline. These include refined target selection and construct design strategies, cloning techniques with single or dual vector systems, identification stable complexes and the use of alternative purification and crystallization methods. Here we present computational methods for the selection of stable multiprotein complexes compatible with the HTP pipeline. We also discuss cloning strategies to precisely address stoichiometries using a single expression vector with a polycistronic message or co-transformation of multiple vectors with differing copy numbers. The purification of hetero-oligomeric complexes requires methods that depart from the well-established dual IMAC protocol used for the purification of tens of thousands of homo-oligomeric and monomeric proteins in the MCSG pipeline. Similarly to protein-ligand complexes, the purification and crystallization of multiprotein complexes is challenging. Often, only one subunit of the complex is present in the crystal. The robotic infrastructure is well suited to experiment with a variety of purification workflows. In summary, techniques to improve the production of soluble and stable protein complexes and their crystallization will be presented. Several purification strategies, their advantages and shortfalls will be discussed.

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P-60

Robust tools for successful membrane protein structure determination

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Advances crystallization methods (lipidic cubic phase and bicelle) have successfully yielded crystals of many membrane proteins, but most crystals grown by these methods are small, weakly diffracting, inhomogeneous, radiation sensitive, and optically invisible when mounted on a loop and cryo-cooled. These crystals require sophisticated hardware and software tools to facilitate structure determination.

At GM/CA we are world leaders in developing such sophisticated tools for micro-crystallography. Beamlines 23IDD and 23IDB, provide an intense, homogeneous beam down to 5μm, a goniometer with 1μm sphere-of-confusion, on-axis visualization and sample mounting robotics. A Pilatus3-6M detector with a 1.0 mm thick X-ray sensor allows shutterless data collection with high S/N. The detector specifications include 100 Hz frame rate, 10 MHz/pixel count rate, and high X-ray efficiency. Software capabilities include a “rastering” tool to search for invisible crystals, or assess quality and identify better ordered regions of a large crystal; a “vector” tool to collect data along a radiation sensitive rod-shaped crystal; automated scans of absorption edge for anomalous data collection; a variety of data acquisition modes; automated data collection strategy and automated data reduction capabilities. All of these tools are integrated into an intuitive, user-friendly, all-in-one interface, JBluice-EPICS. Remote connection through NOMACHINE NX or TeamViewer allows researchers to use all beamline capabilities remotely, thus effectively bringing the synchrotron beamline to the home lab. These tools have greatly contributed to the publication of many GPCR and other membrane protein structures.

Future developments: Beamline optics and endstation on 23IDD will be upgraded to provide a high intensity beam whose size can be varied rapidly in the range of 1 - 20 μm, a new air bearing goniometer with a sphere-of-confusion (SOC) of ~100 nm, a miniature sample XYZ stage that allows centering and scanning of a micron-sized crystal, and a new on-axis-visualization system that provides high resolution optical images of sample crystals. Plans are being developed to upgrade the Advance Photon Source storage ring with a Multi-Bend Achromat lattice. The source properties will be dramatically improved primarily by reducing the horizontal source size to be comparable to the vertical source size, resulting in a 2-3 orders of magnitude increase in source brightness. Both beamlines will be significantly improved by the source upgrade. Moreover the new microfocusing optics for 23ID-D will fully exploit the new source and could deliver a 500 nm (FWHM) beam with >2e13 photons/sec. This unprecedented flux density will provide new opportunities and challenges, and allow the study some of the most important problems in biology. Details of these developments will be presented.

Lightning Talk Speakers- Friday

April 10, 2015 – After Dinner

P-5 **Xin Huang, Amgen, Inc.**
Rapid Screening of Membrane Protein Expression in Transiently Transfected Insect Cells

P-8 **Isabel Moraes, Diamond Light Source & Imperial College London**
Lipidic Cubic Phase Serial Millisecond Crystallography Using Synchrotron Radiation

P-11 **Matthew Coleman, Lawrence Livermore National Lab**
Cell-Free Translation Systems for Biophysical & Biochemical Characterization of Proteins & Protein Complexes

P-9 **Andrew Quigley, University of Oxford**
A Successful Screening Strategy for Solving Structures of Human Solute Carriers, Leading to the Structure of the Sugar Transporter GLUT-3 (SLC2A3)

P-20 **Joseph Mike Autry, University of Minnesota**
Regulatory Mechanisms of the Sarcoplasmic Reticulum Calcium Transport Complex Detected by FRET Microscopy & Microsecond Simulation

P-7 **Fei Li, Michigan State University**
Crystal Structure of Translocator Protein 18 kDa (TSPO) and Identification of a Cholesterol Binding Enhancement Motif

P-2 **Mika Jormakka, University of Sydney**
Molecular Basis of Transition Metal Transport & Regulation

P-24 **Min Lu, Rosalind Franklin University of Medicine & Science**
Structure of an H+-Coupled, Substrate-bound MATE Transporter Yields Mechanistic insights into Multidrug Extrusion

P-25 **John R. Riordan, University of North Carolina, Cystic Fibrosis Center**
Expression, Purification & Reconstitution of Active & Thermally Stable CFTR Protein

P-12 **William A. Cramer, Purdue University**
Internal Lipid Architecture of the Hetero-Oligomeric Cytochrome b6f Complex

Lightning Talk Speakers- Saturday

April 11, 2015 – After Dinner

P-1 **Pingfeng Zhang, School of Medicine, University of Pennsylvania**
Crystallographic Studies of the Bacterial Membrane Associated O-Acyltransferase Protein DltB

P-22 **Nikolai Smolin, Loyola University**
A Structural Mechanism for Calcium Transporter Headpiece Closure Studied by Molecular Dynamics Simulation

P-18 **Youzhong Guo, Columbia University**
Structure & Activity of Tryptophan-Rich TSPO Proteins

P-19 **Ashtamurthy Pawate, University of Illinois at Urbana-Champaign**
Microfluidic Platform for Optimization of Protein Crystallization by Microseed Screening

P-10 **Robert Gennis, University of Illinois, Urbana**
Structure of the Proton-Pumping Transhydrogenase from Thermus thermophilus Reveals a Highly Asymmetric Dimer

P-15 **Lan Guan, Texas Tech University Health Sciences Center**
Construction of an Ankyrin-Based Combinatorial Library: A Successful Application

P-21 **Bruno Miroux, CNRS Paris-Diderot**
Escherichia coli as Host for Membrane Protein Structure Determination: A Global Analysis

P-17 **Debra Brennan, Boehringer-Ingelheim Pharmaceuticals**
High Throughput Screening for Optimal Extraction & Purification of Membrane Proteins toward Structural Research Determinination

P-23 **Stephen H. White, University of California at Irvine**
Inside the 'Primed' SecY Translocon

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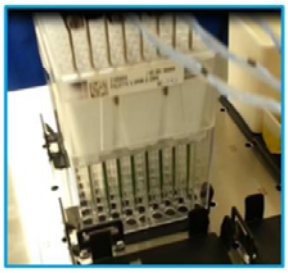

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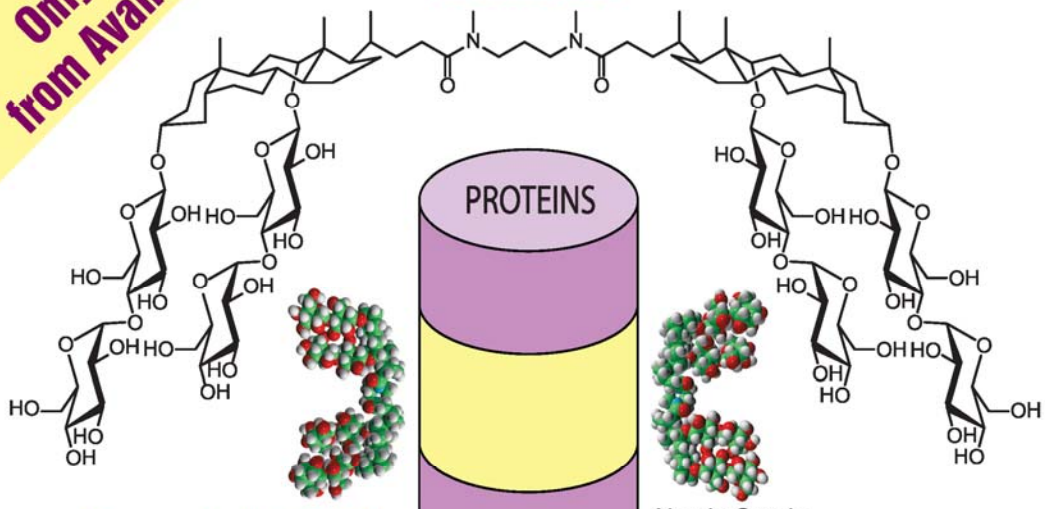

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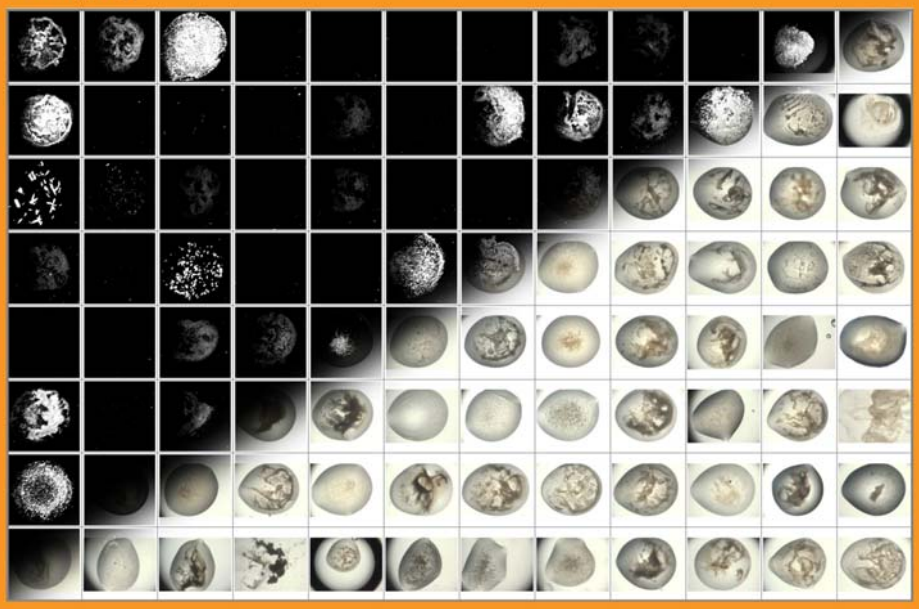

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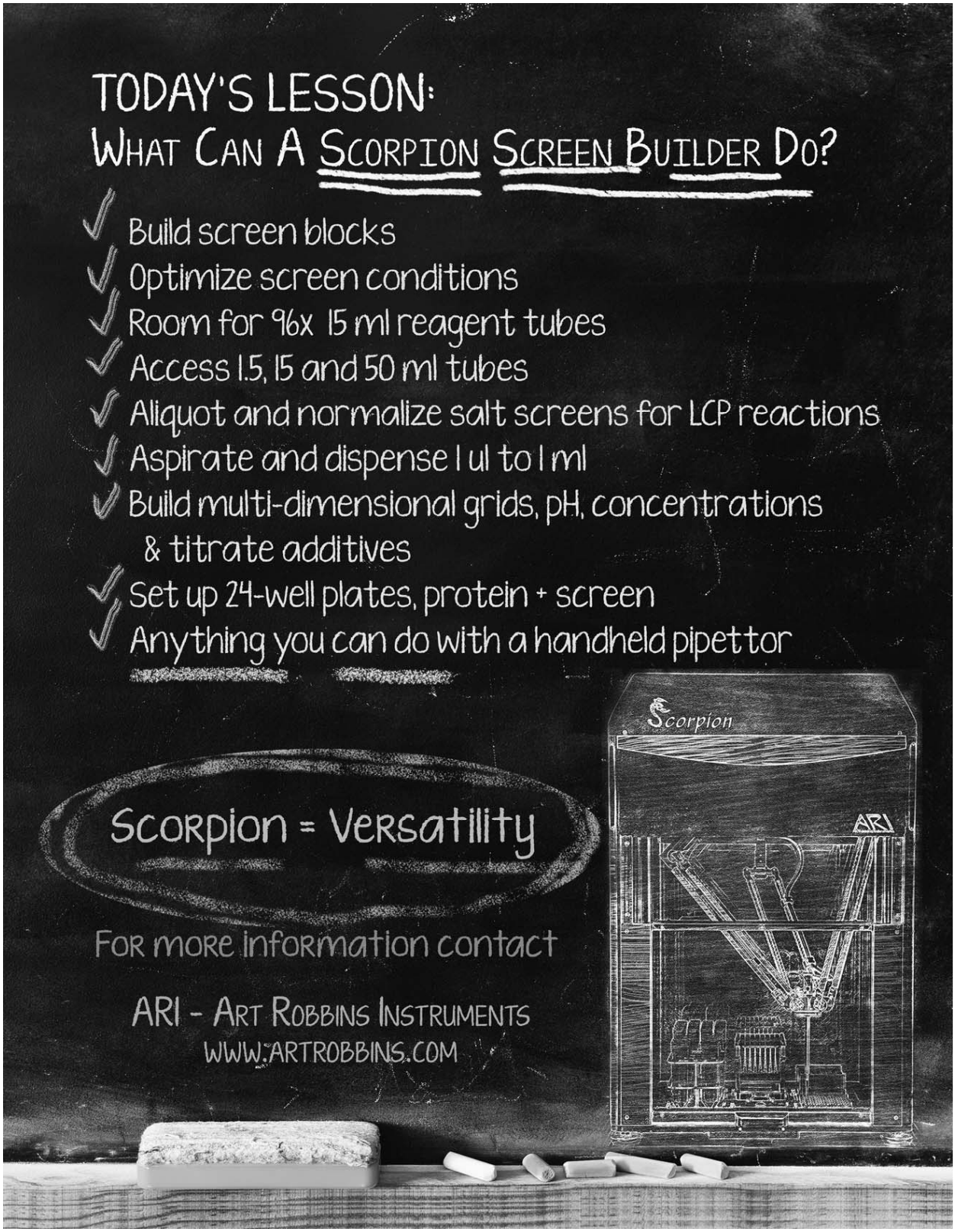
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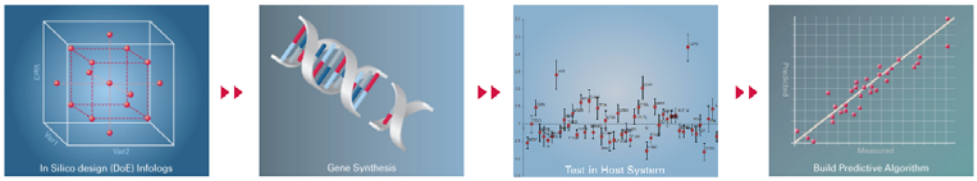

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Addendum

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Poster Abstracts

P-61

Surveying conformational landscapes at chemokine receptors using Nuclear Magnetic Resonance spectroscopy

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Far from acting as two-state systems, G protein-coupled receptors (GPCRs) adopt multiple inactive, intermediate, and active states as shown in a number of recent biophysical studies. The conformational heterogeneity demonstrated by GPCRs may underlie the ability of individual receptors to elicit diverse signaling outcomes following interactions with different types of ligands (i.e., inverse agonists, antagonists, partial and full agonists). The goal of this research is to identify conformational signatures associated with inactive, basal, intermediate-active, and fully active states at the chemokine receptors CXCR4 and CXCR7 using solution Nuclear Magnetic Resonance (NMR). Preliminary ^{13}C-Heteronuclear Single Quantum Coherence (^{13}C-HSQC) studies of selectively labeled receptors demonstrated multiple peaks, and individual cysteine mutagenesis allowed for the correspondence of two peaks with their native cysteine residues located at the N-terminus and second transmembrane domain (TM2) of CXCR7, respectively. We hypothesize that different classes of ligands achieve signaling diversity at chemokine receptors by differentially manipulating available *molecular switches* and that NMR analysis of selectively-labeled CXCR4 and CXCR7 complexes will reveal these distinct conformational profiles.

P-62

Structural Studies of the Transmembrane and Membrane Proximal Domains of HIV-1 gp41 by X-ray Crystallography

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The transmembrane subunit (gp41) of the envelope glycoprotein of HIV-1 associates noncovalently with the surface subunit (gp120) and together they play essential roles in viral mucosal transmission and infection of target cells. The membrane proximal region (MPR, residues 649-683) of gp41 is highly conserved and contains epitopes of broadly neutralizing antibodies. The transmembrane (TM) domain (residues 684-705) of gp41 not only anchors the envelope glycoprotein complex in the viral membrane but also dynamically affects the interactions of the MPR with the membrane. While high-resolution X-ray structures of some segments of the MPR were solved in the past, they represent the pre-fusion and post-fusion conformations, most of which could not react with the broadly neutralizing antibodies 2F5 and 4E10. Structural information on the TM domain of gp41 is scant and at low resolution.

Here we describe the expression, purification, biophysical characterization and structural studies of MPR-TM (residues 649-705) of HIV-1 gp41 by X-ray crystallography. MPR-TM was fused with maltose binding protein (MBP) to improve the overexpression. About 60 mg of MBP/MPR-TM recombinant protein was obtained from 1 liter of cell culture. Crystals of MBP/MPR-TM recombinant protein could not be obtained when MBP and MPR-TM were separated by a 42 amino acid (aa)-long linker but were obtained after changing the linker to three alanine residues. The crystals diffracted to 2.5 Å after crystallization optimization. The final structure demonstrated that MBP crystallized as a dimer of trimers, but the electron density did not extend beyond the linker region. SDS-PAGE and MALDI-TOF MS analysis of dissolved crystals confirmed that the crystal contained MBP only. As the MBP-AAA-MPR-TM protein is highly purified and is very stable, the cleavage may be induced by the process of crystallization. Based on comparison of the MBP trimer reported here with published trimeric MBP fusion structures and the fact that MBP on its own crystalizes as a monomer, it is indicated that MBP might form such a dimer of trimers under the effect of MPR-TM.

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