ORAL PRESENTATION ABSTRACTS

Thursday March 10, 2016

Session I. Structure and Function of Cellular Receptors

Structure and function of Human GPCRs

Zhi-Jie Liu

iHuman Institute, ShanghaiTech University

Zhangjiang High Tech Park, Pudong New District, Shanghai 201210, China

Cell surface receptors and their related intracellular proteins are responsible for human cellular communications with each other and their environment, and are involved in a wide range of physiological activities. Such a central role in human biology makes cell signaling the target for intervention for tuning physiological responses and fighting numerous conditions and diseases. G protein coupled receptors (GPCRs) are involved in a wide range of physiological systems where they are responsible for around 80% of transmitting extracellular signals into cells.

In humans, GPCRs signal in response to a diverse array of stimuli including light molecules, hormones, and lipids, where these signals affect downstream cascades to impact both health and disease states. Yet, despite their importance as therapeutic targets, detailed molecular structures of only 30 unique GPCRs have been determined to date. A key challenge to their structure determination is adequate protein expression and crystallization. Here we report the quantification of protein expression in an insect cell expression system for all 826 human GPCRs using two different fusion constructs. Expression characteristics are analyzed in aggregate and among each of the five distinct subfamilies. These data can be used to identify trends related to GPCR expression between different fusion constructs and between different GPCR families, and to identify and prioritize lead candidates for future structure determination efforts.

Conformational Changes in Rhodopsin Photo-activation Revealed by Small-angle Neutron Scattering

Xiang-Qiang Chu, Utsab R. Shrestha, Debsindhu Bhowmik, Suchithranga M. D. C. Perera, Udeep Chawla, Andrey V. Struts, Vito Graziono, Sai Venkatesh Pingali, Shuo Qian, William T. Heller, Michael F. Brown

[1]Department of Physics and Astronomy, Wayne State University, USA; [2]Department of Chemistry and Biochemistry, University of Arizona, USA; National Synchrotron Light Source, Brookhaven National Laboratory, USA; [4]Biology and Soft Matter Division, Oak Ridge National Laboratory, USA.

Understanding G-protein-coupled receptor (GPCR) activation plays a crucial role in the development of novel improved molecular drugs. During photo-activation, the retinal chromophore of the visual GPCR rhodopsin isomerizes from 11-cis to all-trans conformation, yielding an equilibrium between inactive Meta-I and active Meta-II states. The principal goal of this work is to address whether the activation of rhodopsin leads to a single state or a conformational ensemble with detergent environment in solution. We use small-angle neutron scattering (SANS) technique to answer the above question. For the first time we observe the change in protein conformational ensemble upon photo-activation by SANS with contrast variation, which enables the separate study of the protein structure within the detergent assembly. Our study reveals the protein structural changes associated with GPCR activation in the case of visual rhodopsin.

Fast, accurate and reliable automation for protein crystallization and optimization

Soheila Vaezeslami, Paul Thaw, Joby Jenkins
TTP Labtech Ltd, Melbourn Science Park, Melbourn, Royston, Hertfordshire, SG8 6EE, UK

Automation of both soluble and membrane protein crystallization has significantly contributed to the rapid progress of crystallography-based drug design and structure-function studies. Modern crystallization screening methods require high throughput, accurate and reproducible dispensing of protein and screen drops of varying viscosities to generate initial crystal hits. Optimization of these initial hits is then vital to ensure high resolution of X-ray diffraction data and crystal structures. This time consuming process involves the set-up of many complex screening combinations, where the ratios of the individual components, identified from primary crystallization studies, are varied.

This presentation will describe the benefits of TTP Labtech's mosquito® LCP and dragonfly® positive displacement liquid handlers in accurately and reliably automating the protein crystallization workflows. We will see why the robust and versatile nature of the mosquito family of instruments has made them the most trusted instrument in use for protein crystallization, spanning academic, contract research and major pharmaceutical labs.

mosquito LCP true-positive displacement technology (8 channel) enables automating all crystallization methods, including but not limited to: vapor diffusion (sitting or hanging drop), microbatch, microseeding, additive screening, bicelle or LCP methods, without any changes to the instrument configuration.

dragonfly (5 or 10 channel) offers fast, automated and accurate crystal screen optimization down to 0.5 µL dispense volume, across a wide range of liquid viscosities. High dispense resolution (0.1 µL) and true-positive displacement technology of each independent channel ensure fine and reproducible pH and concentration gradient slicing, while providing a fast and cross-contamination free solution.

Both mosquito LCP and dragonfly offer "drag and design" easy-to-use software in order to make setting up low to high throughput experiments user friendly.

Working with CASIS to Utilize the International Space Station – National Laboratory

Marc Giulianotti,
Center for the Advancement of Science in Space (CASIS), Space Life Sciences Laboratory 505 Odyssey Way Exploration Park, FL

The mission of the Center for the Advancement of Science in Space (CASIS) is to maximize the utilization of the International Space Station National Laboratory for research and technology development aimed at benefitting humankind. CASIS supports collaboration with NASA, other government agencies, not for profit institutions, industry partners, and commercial entities committed to exploring the intellectual, technological and economic opportunities offered by space.

CASIS is actively engaged in supporting projects within the field of protein crystallization. In addition to supporting current flight projects CASIS is working with the user community to outline the basic science requirements for a long-term protein crystallization program aboard the International Space Station National Lab. The collaborative initiative is currently outlining the accessibility/timing, resources (flight/ground), education and funding needs required to create a sustainable program. Ultimately the work will lead to an ISS National Laboratory PCG initiative for repetitive, low-cost crystallization in microgravity providing a platform for human healthcare discovery to users across the discipline including commercial, other government agencies, academia and private research. In addition to these goals, CASIS is committed to inspiring the next generation through experiential learning opportunities.

Session II. Bioinformatics and computational modeling

Details matter! Building the outer membranes of Gram-negative bacteria

James C. Gumbart
Georgia Institute of Tech, North Ave NW, Atlanta, GA 30332

The ability of atomistic molecular dynamics simulations to model biological systems has increased dramatically in the past few years. This ability has come about through advances in hardware, software, and methodology. Recent examples include millisecond simulations of individual proteins, representations of membranes with intricate compositions, and even modeling of entire viruses at atomistic resolution. In this talk, I will describe the application of MD simulations to a specialized sub-cellular region, the second, outer membrane of Gram-negative bacteria. A relatively accessible region compared to the shielded inner contents of the cell, the outer membrane presents a number of attractive targets for antibiotics. With this in mind, I will relate our efforts to model the membranes of three highly pathogenic bacteria, Escherichia coli, Nesseria gonorrhoeae, and two Salmonella serotypes, as well as the outer-membrane proteins contained within.

In silico tools for understanding membrane protein genetic variants

Jeremy Prokop
HudsonAlpha Instutute for Biotechnology, 601 Genome Way, Huntsville AL 35806

Membrane proteins have long been understudied due to the complexities in bench top experiments. With the advent of whole genome sequencing moving into a clinical tool, discovery of genetic variants within these membrane proteins are beginning to arise; yet, characterizing these understudied protein variants lags behind. The ability to model membrane proteins, particularly multiple pass members, is limited by structural targets and by the complexities of lipid membranes the proteins are present in. With proteins such as single pass receptors, the GPCRs, and the well-studied CFTR we have applied our deep Sequence-to-Structure-to-Function analysis (dSSFa) that compiles in silico data for gene evolution throughout vertebrate genomes, protein modeling, multiple species model statistics, post-translational modification predictions, Eukaryotic Linear Motif (ELM) analysis, and molecular dynamic simulations all combined to define protein function and activation. For example, we have been able to map the activation pathway of Leptin on the Leptin Receptor and various ligands (ANG peptides / melanocortins) on GPCRs, resulting in the growth of a signaling evolution database initiative. Expanding these capabilities, we have analyzed known variants of the CFTR gene using our dSSFa, and have begun to apply our pipeline to human and animal model variants from the HudsonAlpha sequencing initiatives for proteins such as VLDLR. The use and growth of in silico tools to understand the folding and function of membrane proteins will continue to grow and will be critical to helping the membrane protein community.

Real-Time Scoring of Protein Crystallization Trial Images

Ramazan S. Aygün
Computer Science Department
University of Alabama in Huntsville, Huntsville, AL 35899

Protein crystallization may require setting up thousands of combinations of conditions to determine the relevant conditions for a successful crystalline outcome of difficult crystallizing proteins such as membrane proteins that are in the presence of lipids and detergents. As crystalline outcomes may be rare for some proteins, this results in many unsuccessful trials that require tedious examining of plate wells using a microscope by experts. Development of high-throughput systems on protein crystallization analysis reduced the burden on experts by automatically capturing and analyzing trial images. The high cost of such systems as well as possibility of missing rare crystalline outcomes reveals the need of development of low-cost but reliable protein crystallization systems. We have analyzed hierarchical classification of crystallization trial images with respect to Hampton scoring for the accuracy of classification. The unbalanced distribution of images to crystallization categories may affect the performance of some classifiers. Thorough analysis of classification is essential for proper automation of these systems. In the past, the high complexity of image handling was handled through high-performance systems such as cluster computing, cloud or grid computing. To reduce the complexity of analysis, we have looked into the complexity of feature extraction and classification. We show our experiments on images collected through trace-fluorescence labeled microscopy and provide overview of feature categories and classifiers for effective real-time crystallization trial image scoring.

The role of protein dynamics in structure-based drug discovery: the case of membrane proteins.

Jerome Baudry
The University of Tennessee, Knoxville, TN 37996

Recent methodological advances in supercomputing approaches allow the modeling of complex interactions between proteins and drug candidates, greatly benefiting the drug discovery process. I will present how simulating proteins' dynamics led to the discovery of novel drug candidates in several pharmaceutical projects. I will present also how these approaches are extended to membrane proteins, with the goal of not only discovering new drug candidates but also to address problems of specificity and potency that have plagued the drug discovery process.

Clinical and translational use of genomic data

Elizabeth Worthey
HudsonAlpha Institute for Biotechnology, 601 Genome Way, Huntsville AL 35806

Despite significant advances in our understanding of the basis of human disease, the cause underpinning the majority of human diseases remains fully or partially unknown. Identification of molecular changes and their impact on the pathways and complexes found within cells provides an opportunity to understand their role in health and disease, and in a clinical setting to apply that understanding to prevention, diagnosis, and treatment. The advent of genome wide sequencing has altered how molecular changes are identified and has transformed both Translational Research and the practice of Medicine. It has uncovered disease associated molecular changes that would not otherwise have been identified and has dramatically increased the diagnostic success rate in patients with rare genetic diseases. In order to get to this point, our ability to accurately and rapidly predict the impact of molecular changes has had to improve dramatically, but much work still remains to define the impact on structure and function of molecular changes even within protein coding regions. This talk will focus on how genomics data is being used in a clinical setting, the impact its application has had, and the challenges that remain when attempting to define how a molecular change within a genomic region alters both the underlying molecule and its interactions with other molecules resulting in human disease.

Friday March 11, 2016

Session III. Methods for purification, preparations and study of transmembrane proteins

Soft x-ray Diffraction for Macromolecules Including Membrane Proteins

Bi-Cheng Wang, John Rose, John Chrzas, Lirong Chen, Palani Kandavelu, Dayong Zhou, Unmesh Chinte, Zheng-Qing Fu, Zhongmin Jin, James Fait, Gerold Rosenbaum, and Dennis Mills
University of Georgia, Athens, GA 30602, USA and
Advanced Photon Source, Argonne, IL, 60439, USA

There exists a growing demand for Native-SAD data since selenomethionine-labeled proteins of many eukaryotic complexes, membrane proteins and drug targets cannot be easily expressed in E. coli or yeast. Advances in technology and methodology during the past five years, show great promise in making Native-SAD phasing a routine approach for macromolecular structure determination (Rose, Wang & Weiss, 2015; doi:10.1107/S2052252515008337). Diffraction from soft X-rays region (wavelength > 1.5Å) has played an important role in the success of Native-SAD phasing. In addition, other unique applications of soft X-rays for structural biology research, which may not be well recognized, are also possible and currently under development/implementation at SER-CAT, Advanced Photon Source (APS), Argonne National Laboratory.

This talk will focus on the use of the two unique strengths of synchrotron X-rays: a) extremely high X-ray intensity for Native-SAD phasing and b) the ability to easily tune the X-ray wavelength for the exploration of the biophysical/biochemical aspects of metals/ions in protein crystals using wavelength-dependent data.

A Pilot Program was initiated at the APS in January 2016 for General Users to participate in the use of these soft X-ray tools. Theoretical and practical aspects of the above concepts, as well as the procedures for accessing the new Pilot Program through the General User portal, will be introduced.

High-throughput methods for detergents selection and screening

Edward E. Pryor
Anatrace, Inc. , 434 W. Dussel Drive, Maumee, OH 43537

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 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.

We are excited to present new tools to support extraction, purification, and detergent selection of many classes of membrane proteins. Data will presented from our Analytic Extractor and Analytic Selector kits. These two kits are designed to facilitate the choice of detergent for protein extraction (Extractor) and downstream applications including crystallization, NMR, EM, and binding studies (Selector). Lastly, we will introduce new detergent offerings, including a DDM/CHS premixed solution, with demonstrated success in membrane protein stabilization and crystallization.

Detection and imaging of microcrystalline membrane proteins using SONICC and other techniques

Tom Friedlander
Formulatrix, Inc., 10 Deangelo Dr, Bedford, MA 01730

Crystallization screening processes can often be a time consuming and frustrating process. When viewing images of crystallization plates, it is not often apparent if protein crystals have formed, especially if they are very small or obscured by a turbid matrix like lipidic cubic phase (LCP). Second Order Nonlinear Imaging of Chiral Crystals (SONICC) was developed to facilitate the detection of microcrystals even when buried in precipitate and to differentiate between salt crystals and protein crystals. SONICC is now routinely used at over 25 pharmaceutical and academic laboratories worldwide finding crystallization hits that would never be investigated with conventional techniques. SONICC has proven to be especially useful in detecting membrane protein crystals formed in LCP, with one study showing SONICC finding two times more hits than conventional imaging techniques. The use of SONICC for characterizing samples prior to diffraction using the Free Electron Lasers has also proven to be extremely useful in optimizing beam time on relevant samples. This talk will cover the nonlinear optical technology utilized in SONICC and how it has aided in the imaging and detection of membrane proteins. We will also discuss other integrated imaging technologies and how they and SONICC have been leveraged worldwide for screening membrane proteins both for macroscopic and nanocrystal formation.

Session IV. Channel and pump proteins

Determination of the mechanical properties of realistic bacterial inner and outer membranes

Hyea Hwang and James C. Gumbart
Georgia Institute of Technology, North Ave NW, Atlanta, GA 30332

The cell envelope in Gram-negative bacteria is made of two distinct membranes and a cell wall between them. From a mechanics point of view, the cell maintains a higher concentration of solutes in the cytoplasm than in the external environment and the difference in osmotic pressure, known as the turgor pressure, places a great stress on the envelope, although which element bears the most is unclear. Now, we have used molecular dynamics (MD) simulations of membranes to resolve how lipid membranes respond to changes in lateral tension resulting from the turgor pressure. In this study, we used two models of the inner, cytoplasmic membrane; the first membrane is modeled as a mixed 75%POPE/25% POPG bilayer while the second membrane model consists of saturated, unsaturated, and cycle-containing lipids that more accurately reflect the diverse population of lipids within the E. coli cytoplasmic membrane. Additionally, we looked at the bacterial outer membrane, which has an outer leaflet of lipopolysaccharides that stiffen it. We applied surface tensions of values from 10 to 100 dynes/cm and measured a variety of properties of membranes (area per lipid, thickness, etc.), thus providing a quantitative description of the membrane response. More general mechanical properties of membranes were also characterized, namely the elastic area compressibility modulus and Youngâ€™s modulus, in order to describe the elasticity of membrane. Our work demonstrates that differences in lipid composition result in a differential response to lateral tension.

Mechanism and function of integral membrane proteins

Christina Le, Stephen G. Aller, PhD
Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294

P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) superfamily, is one of the most promiscuous drug transporters in nature and capable of binding and expelling hundreds of drugs and toxins from the cell. P-gp is also up-regulated in tumor cells. Thus, the Multidrug Resistance (MDR) transporter P-gp represents a major barrier to effective cancer chemotherapy, since it enables cancer cells to develop a resistance to cytotoxic drugs. The American Cancer Society predicts 1.7 million new cancer cases and 590,000 deaths in the US for 2016.Of these cases, approximately 90% of the patients with metastatic cancer are expected to develop multidrug resistance. Plausible mechanisms for this resistance are not clear for P-gp and a better understanding of the molecular mechanisms P-gp is urgently needed. Atomic structures of P-gp in multiple conformational states would help reveal drug transport mechanisms and potentially allow the design of inhibitors to overcome MDR. The refined crystal structure of mouse P-gp reveals an abundance of aromatic residues in the drug-binding pocket in the transmembrane domains, which suggests aromaticity may play a key role in drug recognition and transport. It is our fundamental hypothesis that aromatic interactions are key for providing substantial polyspecific drug recognition by P-glycoprotein. We will explore this hypothesis by cataloging a broad series of aromatic-containing drug-like compounds with respect to effects on P-gp ATPase activity. Effects on ATPase activity with then be correlated to drug-binding shifts in mutants of P-gp by x-ray crystallography. A major goal is to provide a structural basis for polyspecific by P-gp and insights into the mechanism of cancer MDR.

Thursday March 10, 2016

Session V. Frontier Crystallography

Experiments on the Diffusion of Dyes and Ions into Protein Crystals

Alexander McPherson, Steven B. Larson and Yuri G. Kuznetsov
Department of Molecular Biology and Biochemistry
University of California, Irvine
Irvine, CA , 92697

Experiments have been carried out on 15 different protein crystals, most in the range of 0.5 to 1.5 mm dimensions, to measure the rates of diffusion of dye molecules into crystal interiors. Measurements have also been made of the diffusion of dyes through protein solutions of 50 mg/ml to 400 mg/ml concentration for comparison. We found in the course of our study that for most protein crystals, once saturated with dye (as indicated by their rich colors), the dye is retained in the crystals for at least six months, perhaps indefinitely, after the crystals are submerged in large volumes of clear, dye free mother liquor. This indicates strong association of the dyes with the interior of the crystals. Dialysis experiments further indicate a strong association between the dyes and the protein molecules even in solution. X-ray diffraction experiments on over 25 dye-saturated lysozyme, thaumatin and trypsin crystals, however, failed to reveal (with a few noteworthy exceptions) any difference electron density indicative of ordered binding. This raises the question of how high affinity between proteins and small molecules can arise from completely disordered interactions.

Some of the dyes we use are pH sensitive (pH indicator dyes) and change color as a function of H+ concentration. We exploited the color change of numerous dye-saturated crystals to measure the rates of H+ movement into and out of crystals as the pH of the mother liquor was changed. Some other dyes are sensitive to reduction by, among others, bisulfite or dithionite. Again, color changes within the dye-saturated crystals were used to measure the rates of flow of reductants into the crystals and the rates of subsequent reoxidation of the crystal bound dyes by ambient oxygen. We were further able to saturate protein crystals simultaneously with pH sensitive dyes and redox sensitive dyes and then produce a sequence of color changes in protein crystals by addition of reductants followed by changes in pH of the mother liquor.

Finally, we made the observation that, in general, crystals grown from PEG or other polymers of similar characteristics, unlike those grown from salt, MPD, low ionic strength, etc., can not be stained using any of the more than 30 dyes we investigated. Dyes appear to be barred from entering these crystals. We will discuss possible implications of this observation for crystals grown from PEG, and the mechanism by which PEG promotes crystallization.

Data Mining Crystallization Screening Plates

Marc Pusey, Qunying Yuan, Crissy Tarver, and Joseph Ng
iXpressGenes, Inc., 601 Genome Way, Huntsville, AL 35806
University of Alabama in Huntsville, Huntsville, AL

Macromolecule crystallization screening plates are digitally evaluated. Either a crystal is there, yes (1), or it is not, no (0). However, screening experiments typically involve exposing the target macromolecule to a wide range of solution conditions, with the targets response to those conditions reflected in the observed results. Whether a crystal is obtained or not this data is overlooked, not made use of, although it can be a powerful aid in arriving at crystallization conditions. We have pioneered the use of trace fluorescent labeling as an aid to crystallization plate results visualization. This has also greatly facilitated the scoring of those results, which scores are used in a subsequent analysis stage. The software, currently under development for carrying out this analysis, is known as Associated Experimental Design (AED). The software evaluates different combinations of the components present in the screens employed to arrive at new combinations, not present in the source screening conditions, which are output. It is not necessary for the screens to be balanced, any combination of commercial and/or in house screens can be used. The only requirement is that the results be scored. New screen conditions are generated on the basis of the input data. An early version of the software showed considerable promise, leading to further development and testing. To date 23 proteins have been tested, 5 of which did not give crystals in the original 4 screens. Two of these subsequently gave crystals using a screen based upon the AED analysis. Of the remainder, 72% gave as many or more crystals in the single AED derived output screen than found in the original four screens used for the input data. Three of the proteins were RrP41 and RrP42 of the archaeal exosome catalytic core and a 1:1 complex formed by these two proteins.

Macromolecular Femtosecond Crystallography at LCLS

A.E. Cohen representing the SSRL SMB and LCLS XPP/MFX team.
Stanford Synchrotron Research Lightsource, 2575 Sand Hill Road, MS 69
Menlo Park, CA 94025

A new experimental station, the Macromolecular Femtosecond Crystallography (MFX) instrument is under development at LCLS with first experiments planned for July 2106. MFX will provide a permanent location for a stable and flexible endstation supporting X-ray diffraction experiments in atmosphere. MFX will allow for the optimization of the operations of LCLS in general but also specifically as it relates to structural biology by relocating many of the experiments from the XPP and XCS instruments. Goniometer-based diffraction experiments will be supported at MFX using robotically mounted samples at cryogenic temperatures, amenable to longer term storage and mounting at a moment's notice. The experimental front-end is based on developments at SSRL and LCLS XPP to provide an efficient flexible framework to carry out goniometer-based FX experiments using automated strategies tailored to handle a variety of sample requirements, crystal sizes and experimental goals. These developments coupled with improvements in data processing algorithms make it possible to derive high resolution crystal structures using only 100 to 1000 still diffraction images. The MFX project and recent results using radiation sensitive crystals in limited supply at LCLS-XPP will be described. The MFX endstation will be very flexible and support a variety of experimental setups including crystal injectors. Also supported will be a new sample delivery device that uses a mesh to mount crystals directly from a vial of mother liquor. Initial experiments demonstrate the efficiency of this device for structure determination using delicate crystals at physiological temperatures.

Overcoming Antibiotic Resistance: Neutron and X-ray studies of a class A Beta lactamase

Leighton Coates
Biology and Soft Matter Division, ORNL, 1 Bethel Valley Rd, Oak Ridge, TN 37831

The catalytic mechanism of class A β-lactamases is often debated due in part to the large number of amino acids that interact with bound β-lactam substrates. The role and function of the conserved residue Lys 73 in the catalytic mechanism of class A type β-lactamase enzymes is still not well understood after decades of scientific research. To better elucidate the functions of this vital residue we used both neutron and high-resolution X-ray diffraction to examine both the structures of the ligand free protein and the acyl-enzyme complex of perdeuterated E166A Toho-1 β-lactamase with the antibiotic cefotaxime.

Neutron crystallography provides a powerful complement even to high resolution X-ray crystallography as it can provide the locations of hydrogen atoms directly, rather than by inference. Because X-rays are scattered by electrons, hydrogen atoms scatter X-rays weakly even at high resolution and are usually not detected. In our neutron and X-ray ligand free structures Lys 73 is present in a single conformation, however in all of our acyl-enzyme structures Lys 73 is present in two different conformations. In which one conformer is closer to Ser 70 while the other conformer is positioned closer to Ser 130. This evidence supports the existence of a pathway by which a proton is transferred from Lys 73 to Ser 130 and then to the β-lactam nitrogen atom to complete ring opening. This and further clarifications of the role of Lys 73 in the acylation mechanism may facilitate the design of inhibitors that capitalize on the enzyme's native machinery

Structure-based insight into the ligand orientation process in Arabidopsis dUTPase

Noriko Inoguchi and Hideaki Moriyama
School of Biological Sciences, University of Nebraska-Lincoln, Lincoln NE 68588

Deoxyuridine triphosphate hydrolase (dUTPase) is a homo-trimeric enzyme hydrolyzing dUTP. This enzyme activity helps to keep proper thymine / uracil ratio in a cell to prevent uracil misincorporation during DNA replication.
Structural analyses of apo and holo Arabidopsis dUTPase show differences in interpretable C-terminal lengths, and that seems to associate with variation in hydration states of ligands bound to each of three active sites. Interestingly, active site Tryptophan in one of the three active sites had different coordinate which bridges interaction with active site serine by water molecule. Sequence comparisons of dUTPases from other species show that low Km value species have positively charged or polar amino acid substitutions at Arabidopsis Tryptophan position while high Km value species have non-polar residues. These suggest that amino acid substitutions which alter water coordinates at the active site could regulate enzyme functions.

NSLS-II biomedical beamlines for micro-crystallography, FMX, and for highly automated crystallography, AMX: new opportunities for advanced data collections

Jean Jakoncic, Martin R. Fuchs, Wuxian Shi, Edwin Lazo, Dileep K. Bhogadi, Herbert Bernstein, Stuart Myers, Robert M. Sweet, Lonny E. Berman, John Skinner, Dieter K. Schneider and Sean McSweeney.

The two new beamlines at the National Synchrotron Light Source-II, for Frontier Macromolecular Crystallography (FMX) and for highly Automated Macromolecular Crystallography (AMX), will begin user operation in 2016. The low emittance of the NSLS-II storage ring is the basis for providing previously unattainable beam parameters to address current and new challenges in crystallography. With a flux of $0.5 – 1\times10^{13}$ ph/s at 1 Å, and beam sizes from 1 – 50 µm (FMX) and 4 – 100 µm (AMX), the new beamlines' dose rates are up to two orders of magnitude higher than those of the current brightest MX beamlines. They will cover a wavelength range from 0.4 Å (FMX) and 0.7 Å (AMX) to 2.5 Å.

A focus in designing the beamlines lay on supporting a broad range of structure determination methods. The highly flexible design of the experimental stations and of the beam control will support serial crystallography on micron- and sub-micron sized crystals, structure determination of complexes in large unit cells, rapid sample screening and room temperature data collection. The associated wide variety of samples includes frozen crystals in standard loops and meshes, single crystals or up to 20 acoustically deposited crystals, crystals in SBS trays, specialized sample holders such as silicon nitride membranes, micro-fluidic- or LCP-plates and jets.

Session VI. Crystallography Education

Crystal growing contests – a legacy of the International Year of Crystallography 2014

Ilia A. Guzei
Department of Chemistry, University of Wisconsin-Madison, 1101 University Ave, Madison, WI 53706

In honor of the 2014 International Year of Crystallography several Crystal Growing Contests took place around the globe in 2014. Since then several such contests have been conducted on an annual basis: the international video contest, the US national contest, and the Wisconsin Crystal Growing Competition. I will briefly highlight the international and national contests but will concentrate on the organizational details of a state-wide contest in Wisconsin and its educational impact. High school students from 26 schools across the state competed for prizes by growing large crystals of cupric sulfate. The contest organization was challenging: it was necessary to select the target audience, compound to crystallize, prize categories and criteria, to find sponsors, crystal judges, and plan contest promotion and award ceremony. All challenges were successfully resolved. The scientific and educational impact of the contest at the participating schools has been inspirational as reflected in the science teacher testimonials.

Education from 815,527 Crystal Structures

Amy A. Sarjeant[1], Peter A. Wood[2], Suzanna C. Ward[2], Colin R. Groom
The Cambridge Crystallographic Data Centre, Piscataway NJ 08854
The Cambridge Crystallographic Data Centre, Cambridge CB1 2EZ.

The Cambridge Structural Database (CSD) comprises over 800,000 small molecule crystal structures. Curated and maintained by the Cambridge Crystallographic Data Centre (CCDC), the CSD has become the go-to resource for structural chemists, worldwide. In addition to providing three dimensional images of each structure, the CSD contains a wealth of statistical information about symmetry, packing, coordination environments, bond distributions, and intermolecular interactions. By choosing our examples well, we can illustrate a variety of chemical and crystallographic principles using the information contained in the CSD. This talk will focus on several of the ways educators can use crystal structure data in the classroom to augment the learning experiences of students from high school to grad school.

Promoting a structural view of biology and medicine: An overview of RCSB PDB resources and experiences

Shuchismita Dutta
RCSB Protein Bank

The RCSB Protein Data Bank (RCSB PDB) is an essential bio-molecular data resource that supports scientific research and education worldwide. It provides free access to PDB data, along with tools/resources for visualizing and analyzing them. PDB-101 (http://pdb101.rcsb.org/), a dedicated education/outreach portal, was developed for students and educators to access a simplified view of PDB data. In the past decade, RCSB PDB has developed and offered courses, curricula, and workshops to teach a variety of users about topics ranging from protein crystallography methodology to structural views of biology and medicine. Examples of these educational experiences and resources will be presented.

Session VII. Crystallography crystallographic methods

Trace Fluorescent Labeling reveals significantly more crystal leads

AJ Singhal, Joseph Ng, Marc Pusey
iXpressGenes Inc., Huntsville, Alabama 35806

Efficient crystallization is an elusive achievement in structural biology labs. In order to minimize time and cost to obtaining a crystal and subsequent structure, we have pioneered Trace Fluorescent Labeling (TFL) technology. A trace amount of covalently bound probe (0.1-0.5%) dramatically decreases (>40%) our time to grow quality crystals. While we typically follow our screening experiments for up to 8 weeks, this enables us to determine initial leads, obvious or otherwise, for optimization within one week. We have proven with over 5 years and 50 proteins of proof-of-concept study, that TFL has no negative impact on crystal growth or diffraction data. Furthermore, we have validated TFL using differential tags, as a powerful tool for crystal complex confirmation (CCC) by visualization of multiple colors in a crystal. CCC gives absolute assurance whether the crystal has a complex of different macromolecules or not, before valuable time is spent on collecting diffraction data. Another efficiency feature of TFL is salt crystal confirmation (SCC), which is possible because salts are not tagged and thus do not fluoresce with our technology. SCC eliminates the wasted time of shooting salt crystals. TFL has been proven on a variety of proteins and complexes to consistently and significantly improve crystallization efficiency.

A Genetic Algorithm for the Optimization of Protein Crystallization Screening

Samyam Acharya, Marc L Pusey, Ramazan S Aygun

DataMedia Research Lab, Computer Science Department,
University of Alabama in Huntsville,
Huntsville, Alabama 35899, United States

Protein crystallization screening focuses on determining the factors crucial for successful protein crystallization. The protein crystallization may require large number of parameters to be considered for setting up of cocktails that would yield suitable large crystals for X-ray data collection. These parameters include types of reagents, ionic strengths, types of salts, pH value of buffers, temperature, etc. Our goal is to implement a genetic algorithm which isolates combinations of reagents and concentrations that have a higher degree of synergy and potentially offer better crystalline outcome.

Combinations of reagents along with their concentrations are mapped into binary strings called chromosomes (not to be confused with biological chromosome). Each chromosome represents a certain set of buffer, pH, salts, etc. The length of chromosome depends on the number of reagents we take into consideration for a particular experiment. Using expert score from previously conducted experiments, we identify new conditions generated by the algorithm in successive iterations. Undesired conditions, such as those that are known to cause phase separation and precipitates, are removed and favorable conditions are paired to produce the next generation of conditions. The top ranked conditions produced by the algorithm will be evaluated with respect to experiments conducted based on our associative experimental design [4].

The advantage of using genetic algorithm for protein crystallization screening lies in the ability of the algorithm to handle large number of parameters in an uneven search space environment. With this approach, we can employ selective pairing of conditions (chromosomes), which could be useful in identifying precipitant synergy for obtaining crystals and antergy (pairs that produce no crystals) and thus narrow down the screening process. The output conditions will be evaluated using the Bin – Recall Metric.