Poster Presentation Abstracts

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

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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 – 1×10^{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.

Synchrotron X-Ray Diffraction Study of the Products and Reactants associated with the Photocatalytic Decomposition of [Ru(biq)2dpb](PF6) into [Ru(biq)2(CH3CN)2](PF6)2

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The sterically hindered Ru (II) complex, $[Ru(biq)_2dpb](PF_6)$ was synthesized using a building block approach. The resulting complex displayed photoinduced exchange of dpb ligand upon radiation with visible light to yield $[Ru(biq)_2(CH_3CN)_2](PF_6)_2$. Due to the instability of the designed complex and the very small size of the crystals grown by vapor diffusion techniques (less than 0.04 mm in the long direction) made data collection difficult with a conventional x-ray diffractometer. Therefore, data collection occurred through the SCrALS program (Service Crystallography at the Advanced Light Source). The wavelength of the synchrotron radiation was similar to that of molybdenum radiation with λ=0.7749 Å. All data were corrected for absorption using SADAB. Structure solution was accomplished with the Bruker Shelxtl program package; all refinements were done using the Olex[2] program package.

The complex, $[Ru(biq)_2dpb](PF_6)$ crystallized in the monoclinic crystal system $P2_1/c$. 12,908 unique reflections were evaluated. The refinement converged with R= 3.91% and GOOF= 1.017. The Ru atom possesses a distorted octahedral geometry and was bound to three bidentate ligands this resulted in significant steric strain that was responsible for the photolysis. The overall complex is roughly spherical with no observed π-stacking in the packing diagram.

Upon exposure to visible light $[Ru(biq)_2dpb](PF_6)$ decays into the complex $[Ru(biq)_2(CH_3CN)_2](PF_6)_2$. This compound crystallized in the triclinic space group $P\bar{1}$. 11,213 unique reflections were evaluated. The refinement converged with R= 3.68% and GOOF= 1.046. The Ru atom still adopts a distorted octahedral geometry surrounded by two bidentate ligands and two acetonitrile solvent molecules. This compound shows significant disorder in the binaphthyl ligands. This may result from a twisting of the carbon-carbon bond tethering the two naphthyl groups together or it may be the result of the super position of slightly twinned crystals. The small size of the crystals makes it difficult to choose one explanation over the other. The resolution of the study is not sufficient to completely resolve the position of the atoms in the crystal structure. The binaphthyl rings show a roughly parallel arrangement with π-stacking observed in the packing of the crystal structure.

Crystal structure of Salmonella typhi Inorganic Pyrophosphatase at 1.95 Å

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Family I Inorganic pyrophosphatase (IPPase) is a soluble, metal dependent enzyme that hydrolyzes inorganic pyrophosphate into two phosphate molecules. They are essential for life and growth in all domains of life and they are part of several major processes including DNA replication, transcription and translation. Here we present the molecular structure of IPPase from *Salmonella typhi*, a gram negative pathogenic bacteria, determined by X-ray crystallography. The codon optimized open reading frame DNA sequence, with N-terminal His-tag, was synthesized using the PCR-based SeqTBIO method. Recombinant IPPase was expressed in pET-*E. coli* Rosetta system and purified. Crystal hits were identified using trace fluorescent labelling and grown using sitting drop vapor diffusion. The crystals diffracted X-ray to a maximum resolution of 1.95 Å and they belong to space group I23 with unit cell parameters a = 142.076 Å. Its structure has been determined by molecular replacement using *E. coli* IPPase as the target model.

Honors Thesis Proposal

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Abstract
Inorganic pyrophosphatase (iPPase) is an enzyme ubiquitous to all organisms and is responsible for cleaving single molecules of inorganic pyrophosphate (iPP) into two molecules of inorganic phosphate (iP). The reaction that the enzyme catalyzes is highly exergonic and helps other energetically unfavorable reactions within the cell to proceed. Inhibition of iPP catalysis to iP causes a cell to die. Character analysis of the human sequence of the enzyme in comparison of a sequence of the enzyme from a pathogenic organism can be used to target iPPase for drug development. The purpose of this research project was to characterize a human sequence of the iPPase enzyme for optimal conditions and kinetic analysis. The enzyme showed optimal activity at 37* C at a pH of 7; other optimal conditions to be reported include optimal divalent cation, optimal concentration of optimal divalent cation, optimal concentration of substrate, and a kinetic analysis. Kinetic analysis of the enzyme's activity is still undergoing further research and results will be included in the final paper.

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

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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.

X-ray and Magnetic Analysis of Mineral Columbite in the Search for Natural Superconducting Materials

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Three natural samples of the mineral Columbite (Mn, Fe)$(Nb,Ta)_2O_6$ were evaluated as possible natural semiconducting materials. These types of materials are of interest, for they can be easily mined and would be more cost effective as compared to synthetically produced semiconducting materials. The samples evaluated were collected from the gem and mineral collection at the Carnegie Museum of Natural History with permission from Curator Marc Wilson. Minerals crystalized in nature have a very high probability of possessing solid solutions therefore, XRF measurements were critical to determine the exact purity and elemental composition of the samples. All samples proved to possess solid solutions at the 2+ and 5+ sites. Magnetic measurements revealed that all samples exhibited paramagnetic to antiferromagnetic interchanges at less than 6 K. This low temperature removes any chance for technological applications. The crystal structure of all three columbite mineral samples were investigated using X-ray powder diffraction data collected with a Rigaku Americas MiniFlex II Diffractometer. Phase purity was investigated by comparing the x-ray diffraction data to the ICDD PDF-4+ database. Rietveld refinements were accomplished using the GSAS/EXPGUI program package. The XRF measurements were used to determine starting occupancies and acted as a final check to the phase compositions of the converged crystal structure. The relationship of the unit cell parameters in the solid solution was examined. While these three samples did not demonstrate superconducting behavior, many other minerals are to be investigated in the search for abundant natural superconducting materials.

Visual-X2: Scoring and Symbolic Visualization Tool for Analysis of Protein Crystallization Trial Images

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In protein crystallization experiments, by the help of robotic systems, the images of thousands of drops can be captured in a very short time. The scoring and evaluation of the experimental results are quite time consuming processes for the experts since it requires some manual labor. To reduce this burden, we developed a tool called Visual-X2, which allows visualizing the protein images with an interface for fast scoring and comparison. Visual-X2 uses visually distinctive symbols to represent the score of protein images and it allows evaluating multiple scans of the same drop. The features of Visual-X2 include symbolic plate visualization with respect to scoring of plate wells, multiple scan analysis of wells captured using different light sources, and prioritized sequential view of plate wells with respect to scoring.

Segmentation of Protein Crystal Regions using Supervised Thresholding for Trace Fluorescence Labeled Protein Crystallization Trial Images

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Image thresholding is a challenging problem when the image is captured under varying illumination conditions or the foreground objects do not have a clear shape. There is not an optimal solution that works for all cases; however, it is usually possible to find a suitable thresholding method for different types of images. The varying depth of protein crystals when images are captured may cause blurriness and low intensity for crystal regions out of focus and clear and high intensity regions for crystals in focus. The traditional global and local thresholding techniques may generate proper segmentation for some images while failing for the rest. We introduce supervised thresholding by training a classifier to choose the best thresholding technique for a specific image. A classifier model is built using features extracted from either the original image only (priori) or the outputs of thresholding methods and the original image (posteriori). We applied our method for trace fluorescence labeled protein crystallization images, and compared our results with 6 thresholding techniques. Experimental results show that our proposed super-thresholding method outperforms stand-alone thresholding methods yielding the best performance for our protein crystallization dataset.

Focal Stacking of Trace Fluorescence Labeled Protein Crystallization Images using FocusALL

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Microscopy imaging is a critical module of scientific analysis systems in biochemistry, physics and space sciences. Traditional image acquisition systems usually capture objects of interest at a specific depth-of-field. If objects appear at varying depths from the camera, multiple images are captured to make sure that each object can be covered in focus in at least one of the images. This enables covering a wide range of depth-of-field. While an expert may go over a stack of images to observe different objects in focus, this adds another level of burden on the expert and prohibits full automation of such systems. It is important to be able to generate a single in-focus image that can identify in focus regions in each image and merge them into a single image. Traditional focal stacking methods have two important assumptions that do not work for trace fluorescence labeled protein crystallization trial images: 1) in focus areas have higher contrast then out of focus areas and 2) in focus areas have higher intensity than out of focus areas. We proposed a FocusALL technique using modified Harris corner measure to detect regions in focus. FocusALL can be applied to images captured at high resolution and varying illumination. FocusALL outperforms other methods on protein crystallization images especially around crystal region borders and performs comparably well on other real and simulated datasets.

Protein Crystal Growth on Time-Series Images of Crystallization Trials

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Protein crystallization is a complex phenomenon requiring thousands of trials for successful crystallization. Crystallization trials should be observed periodically to assess the evolving progress of crystal. Analysis of temporal images of crystallization trials can be useful for several reasons. No change in the crystallization trial image suggests that the crystallization droplet does not lead to crystallization. Because of the large proportion of unsuccessful trials, human labeling is significantly reduced. Crystallization droplets that change over time are of potential interest for a crystallographer as they might contain growing or newly formed crystals. CrystPro is an automated system to determine changes such as new crystal formation and crystal growth over time on trace flourescence labeled crystallization trials. CrystPro consists of three major steps—identification of crystallization trials proper for spatiotemporal analysis, spatiotemporal analysis of identified trials, and crystal growth analysis. Our system has been evaluated on three crystallization image data sets (PCP-ILopt-11, PCP-ILopt-12, and PCP-ILopt-13). To identify crystals for spatio-temporal analysis, our system yielded 98.3% accuracy and 0.896 sensitivity on identification of trials. For identifying pairs with new crystal formation, our system yielded 77.4% accuracy and 0.986 sensitivity. For protein crystal growth in size detection, our system generated 85.8% accuracy and 0.667 sensitivity. The results show that our method can be effectively used for tracking growth of crystals and identifying image sequences for further review by the crystallographers.

Associative Experimental Design for Protein Crystallization Screening

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Determining the main factors of protein crystallization experiments is a critical stage to obtain successful outcome. However, it is quite challenging task to predict those factors since the screening is often expanded to thousands of conditions to cover all possible combinations for a crystalline outcome especially for difficult crystallizing proteins such as membrane proteins that are in the presence of lipids and detergents. We have developed an experimental design technique called “Associative Experimental Design (AED)”, which generates novel candidate conditions that have higher possibility to yield a crystal. Also, an optimization method is included in our system to eliminate prohibited combinations and prioritize reagents of AED results.

AED analyzes scored experimental data to determine the screening factors that are most likely to lead to crystalline or high scoring outcomes and then generates candidate cocktails. AED has been tested on three proteins derived from the hyperthermophile Thermococcus thioreducens. The results show that AED generated novel cocktails (count provided in parentheses) leading to crystals for three proteins as follows: Nucleoside diphosphate kinase (4), HAD superfamily hydrolase (2), Nucleoside kinase (1). Moreover, the optimization of AED results yielded 4, 3, and 20 crystalline conditions for holo Human Transferrin, archaeal exosome protein, and Nucleoside diphosphate kinase, respectively.