C01

Title: What is the limit for doing it at home?

Presenter: **Cheng Yang**, Rigaku Americas Corporation

Abstract

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X-ray technologies have been advancing rapidly in recent years since we stepped into the era of high throughput crystallography and started to determine more structures of human and membrane proteins, large complexes. Rigaku as the leading innovator continuously partners with scientific community, pushes the limits of X-ray technologies and provide scientific community with the most suitable research tools. In the past, most people often pictured an in-house X-ray system is less powerful, high energy consumption and useful only for large and well diffracting crystal. Today, it has migrated into a new look which has microfocus (70 μm), synchrotron-like X-ray intensity, low energy cost, good to handle a small crystal or large unit cell crystal. Many samples labeled as synchrotron use only in past can be finished or tested or phased in the laboratories. These changes have greatly improved the productivity of research. In this report, we like to reveal new advances of X-ray technologies, new instrumentation including not only X-ray source and detector, but also crystallization viewing and imaging system, automation of data collection, crystal recovery system and so on. We hope it can answer this question – where X-ray technologies stand today and what is the limit for doing it at home?

C02

Title: Intelligent Purification for Structural Analysis

Presenter: **Ping Shen** (GE-HealthCare)

Abstract

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ÄKTAxpress, an intelligent way of multi-dimensional purification, is introduced to save the time of structural genomic scientist for sophisticated research work. Researcher can get the highest possible purity of proteins and monoclonal antibodies shortly. The system, software and media are designed and optimized to work together.

Multidimension

Single, two, three or four dimension purification can be performed seamlessly and continuously. No matter to start with affinity, ion exchange or gel filtration.

High capacity

50 to hundreds of milligrams of proteins can be obtained depends on the gel and column size.

Intelligent

All protocols are optimized with supported columns. Even inexperienced operator can obtain the highest possible purity shortly by the help of programming wizard.

High purity

Since different, complimentary techniques combined with intelligence as well as proven cleaning-in-place (CIP) protocols are used in multi-dimensional purification, operator can easily achieve >95% purity. Almost in all situations, multidimensional purification achieves higher purity of target protein than single step or double step affinity purification.

Affinity chromatography can not remove polymers and aggregates effectively. Only gel filtration can ensure the homogeneity.

Simple

AKTAxpress is controlled by UNICORN software which does not require chromatography expertise to operate. Users only need to program the method through wizard by ticking appropriate option.

C03

Title: Mechanisms of Protein-Protein Binding: Double-Mutant Cycle Analysis Using the ProteOn™ XPR36 System)

Presenter: **Andrew Wong** (Bio-Rad Pacific Ltd.)

Abstract

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Complex life processes require proteins to be able to transfer specific signals, build multiprotein complexes, control the function of enzymes, and regulate all other cellular activities. Many of these tasks are performed through specific protein-protein interactions. This is feasible due to the almost unlimited potential for the generation of protein binding sites, unique sites characterized by their shape and surface chemistry. A major research area today is the investigation of the structure, mechanisms, and dynamics of protein-protein interactions.

Surface plasmon resonance (SPR) technology is a central, widely used tool for kinetic studies of interactions between unlabeled biomolecules in real time. However, this analysis is usually in the bottleneck due to a lack of SPR platform that monitors multiple kinetic interactions in parallel. The ProteOn™ XPR36 protein interaction array system possesses all the qualities of high-level SPR biosensing technology combined with high-throughput and multiplexing capabilities. To demonstrate the power of multiplexing SPR analysis, single and multiple mutations of TEM1 β-lactamase enzyme (TEM1) and its inhibitor, β-lactamase inhibitor protein (BLIP) were prepared. Five mutants of TEM1 were used as ligands. The referencing surface (negative control) is immobilized with an irrelevant protein. One BLIP mutant was used as analyte in the mobile phase and six different concentrations were flown through the TEM1 mutant surface in parallel. In this approach, kinetic profiles were generated in five TEM1-BLIP pairs in a single experiment. The results suggest that two independent binding unit (cluster) show intracluster cooperativity as well as intercluster additivity between the protein residues.

Title: Higher Signal, Lower Noise: How to Get the Best Data from Your Crystals

Presenter: **Martin Adam**, Bruker AXS B.V.

Abstract

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In a macromolecular crystallography, where crystals are small and often weakly diffracting, it is imperative to obtain data with as high a signal-to-noise ratio as possible. Improving the signal usually involves using a more powerful source, since obtaining samples with larger diffraction volume is often not possible.

The contribution of the noise should not be underestimated. Especially when looking at weak reflections or small differences in anomalous signal (e.g. doing S SAD phasing on native proteins) one has to take great care to minimize the experimental noise as much as possible. This can be done by careful sample handling, minimizing the effect of background scatter from the cryo protectant and loop as well as in setting up the data collection experiment. Examples of more efficient data collection methods will be presented which can lead to a dramatic increase in signal to noise ratio, with the same amount of X-ray photons falling on the crystal. If all precautions have been taken to lower the noise of the experiment, then there is only one way to get a higher a signal-to-noise ratio: increasing the amount of X-rays on your sample. The MICROSTAR ULTRA, the next generation of the MICROSTAR series of generators, is substantially brighter than any other X-ray home source available today. With its revolutionary HyperCool™ anode cooling technology the MICROSTAR ULTRA is more intense and yet easier to maintain than other rotating anode generators.

Measurements show that this source compares with many second generation beam lines. Results of crystal data will be presented.