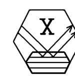

We used six single-tryptophan mutants to identify the binding site in FRET experiments. They revealed that RH421 binds directly into the ATP-binding site. This conclusion was further supported by results from molecular docking and by competitive experiments using ATP. Experiments with protein/DPPC mixture revealed that RH421 can bind to both protein and lipids, but only the former interaction was influenced by the presence of ATP.

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Friday, March 18, morning, Session IV

L15

AFFINITY. STABILITY. CONFORMATION.

Piotr Wardega

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MicroScale Thermophoresis (MST) is a powerful technique to quantify biomolecular interactions. It is based on thermophoresis, the directed movement of molecules in a temperature gradient, which strongly depends on a variety of molecular properties such as size, charge, hydration shell or conformation. Thus, this technique is highly sensitive to virtually any change in molecular properties, allowing for a precise quantification of molecular events independent of the size or nature of the investigated specimen. When performing a MST experiment, a temperature gradient is induced by an infrared laser. The directed movement of molecules through the temperature gradient is detected and quantified using either covalently attached or intrinsic fluorophores. By combining the precision of fluorescence detection with the variability and sensitivity of thermophoresis, MST provides a flexible, robust and fast way to dissect molecular interactions.

NanoDSF is our 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 its 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 250 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.

The surface acoustic wave technology (SAW) allows for an in-depth analysis of molecular interactions in real time. Binding kinetics can be precisely determined by detecting mass and binding-induced conformational changes. In addition to standard interactions, viscous, colored and turbid samples can be analyzed, and also complex samples including membrane preparations can be investigated. The SAW technology measures changes in mass and conformation separately, thus providing new insights to mechanisms of binding in addition to the binding kinetics (kon, koff and the dissociation constant Kd) and stoichiometry. SAW is based on the precise detection of the properties of surface acoustic waves that travel along the biosensor. Upon interaction with molecules on the sensor surface, distinct characteristics of the acoustic waves are altered; changes in total mass on the biosensor result in a shift of the wave's phase providing information about the on- and off-rates, as well as the stoichiometry of the interaction. Simultaneously a change in flexibility of the molecules alters the wave's amplitude. This directly reflects changes in the conformation of the molecules, e.g. after binding to compounds.Both signal types are detected and quantified separately, and can be used to comprehensively characterize the interaction mechanism of the molecules on a kinetic and structural level.

L16

ULTRAFILTRATION MASTER CLASS

Stanislav Kukla

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Sample preparation of macromolecular solutions, such as proteins, enzymes, antibodies and viruses, often yields in large volumes of diluted macrosolutes in buffers that are incompatible with downstream processes, detection or structural analysis. Various ultrafiltration devices are regularly used to concentrate and buffer-exchange these types of macrosolutes. When using ultrafiltration for sample concentration, particular attention has to be paid to choosing the correct membrane molecular weight cut-off, membrane material as well as the ultrafiltration device appropriate design. During the presentation a general overview of the Amicon© product family will be given with emphasis on some of the recently introduced, new family members – Amicon© Pro purification system and improved, newly designed Amicon© stirred cells. The presenter will also discuss some typical troubleshooting topics and will give useful tips & tricks for ultrafiltration experiments.

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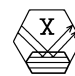

L17

MICROCALORIMETRY: A VERSATILE TOOL FOR THE CHARACTERIZATION OF BIOMOLECULAR INTERACTIONS

Raúl Pacheco Gómez

Product Technical Specialist - Malvern Instruments, UK

Higher-order biomolecular structures and their dynamic interactions with various ligands drive and regulate all biological processes; studies of biomolecular interactions are fundamentally important in all areas of life sciences. Isothermal titration calorimetry (ITC) is the ideal technique for the measurement of biological binding interactions since the data provided does not rely on the presence of chromophores or fluorophores, nor requires an enzymatic assay. ITC relies only on the detection of a heat effect upon binding and it is label-free, enabling scientists in academia and industry to better understand the conformational stability of their biomolecules and their binding to biologically relevant interactants.

This presentation covers the principles of Differential Scanning and Isothermal Titration Calorimetry (DSC and ITC) and exemplifies a broad range of applications enabled by the direct nature of the technique. The presentation will also cover examples of troubleshooting and how to obtain good data using the MicroCal ITC and DSC systems.

A special focus will be given to the benefits of PEAQ-ITC, the latest generation of MicroCal ITC instrumentation, and the solutions it offers for addressing current bottlenecks associated with the interaction analysis. Among the most recognized challenges is the need to adequately address a broad range of binding affinities and to reliably interpret the binding data, complicated by the presence of inactive protein or inherent uncertainty in the concentration of the ligand.

We will discuss the improvements in PEAQ-ITC data quality which enables increased confidence and data resolution when measuring low heats at low or uncertain sample concentrations and complex binding modes.

L18

STATE-OF-THE-ART PIXEL ARRAY DETECTORS FOR CRYSTALLOGRAPHY

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Hybrid Pixel Array Detectors (HPAD) were developed at the beginning of this millennium[1] and have since replaced imaging plates and charge-coupled device (CCD) detectors at synchrotrons. These HPAD detectors offer a number of advantages such as fast data read-out and low visible noise but are not without their limitations. One of the most prominent is that HPAD detectors suffer from charge sharing noise. This occurs when a X-ray photon gets absorbed within more than one pixel of an HPAD detector. In many of today's detectors 20% of the pixel area are affected by this charge sharing noise. In addition, HPAD detectors suffer from other shortcomings, such as count rate limitation and parallax effects for high energy radiation (e.g. Mo-K and Ag-K radiation).

These shortcomings in combination with the availability of X-ray free-electron lasers (XFEL) have triggered the search for more promising technology and led to the development of next generation detectors such as the Jungfrau[2], the Adaptive Gain Integrating Pixel Detector and the Cornell-SLAC Pixel Array Detector. All these detectors are Charge-integrating Pixel Array Detectors (CPAD), eliminating the disadvantages of a HPAD detector, like count rate capability, pixel size or the low energy limit[3].

The recent introduction of the PHOTON II CPAD brings the technology developed for XFEL sources into the home lab. The PHOTON II features the largest monolithic active area of 10×14 cm^2, the highest detective quantum efficiency and the highest frame rate of any home laboratory detector. By design, the PHOTON II also completely eliminates the charge sharing noise and parallax issues.

This new CPAD technology takes Pixel Array Detectors to the next level and it is anticipated that we will see a subsequent change of detector technology in the near future not only at the synchrotron beamlines but also within the home lab.

1. C. Brönnimann, et. al. J. Sync. Rad. 2000, 7, 301-306.
2. B. Schmitt, et. al., J. Sync. Rad. 2014, 21, 1006-1010.
3. G. Hülsen, et. al., J. Apply Cryst 2006, 39, 550-557.