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SAXS Combined with Crystallography and Computation: Defining Accurate Dynamic Macromolecular Assemblies in Solution. Michal Hammel[a], Greg Hura[a], John Tainer[a]. [a]*Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.*
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Crystallography supplies unparalleled structural detail for mechanistic analyses; however, it is restricted to describing low energy conformations of macromolecules within crystal lattices. Small angle X-ray scattering (SAXS) offers complementary information about macromolecular folding, unfolding, aggregation, extended conformations, flexibly linked domains, shape, conformation, and assembly state in solution, albeit at the lower resolution range of about 50 to 10 Å resolution, but without the size limitations inherent in NMR and electron microscopy studies. Examples from data collected at SIBYLS, a dual SAXS and protein crystallography synchrotron beamline, will be drawn upon to demonstrate the complimentary use of SAXS with protein crystallography. I will also describe the recent implementation of a sample loading automation tool for high throughput SAXS data collection. A particular emphasis will be placed on the need for computational development in light of the high throughput nature of SAXS data collection. The utility of high throughput SAXS will discussed in the context of program project SBDR (Structural Cell Biology of DNA Repair Machines) and its potential to contribute to Structural Genomics efforts.

Keywords: SAXS; protein conformational analysis; high throughput

FA5-MS05-O2

Coexisting Lipid Domains. Georg Pabst[a], Beate Boulgaropoulos[a], Bibhu R. Sarangi[b], Peter Laggner[a], Velayudhan A. Raghunathan[b]. [a]*Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, A-8010 Graz, Austria.* [b]*Raman Research Institute, Bangalore 560 080, India.*
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Sorting of membrane lipids and proteins into domains of particular composition (rafts) is supposed to be one of the most fundamental processes in cellular functioning. Although domains are known to exist in phospholipid model systems for more than 30 years, there is still plenty to learn from their biophysical properties which could be of physiological relevance. We have, therefore, probed the temperature and composition dependent properties of various coexisting phases using small- and wide-angle x-ray diffraction (SWAXD) in combination with several other complementary techniques. In particular, we have focused on structure and interactions of coexisting fluid-gel domains, found in lipid mixtures containing ceramide, a second messenger for apoptosis (programmed cell death). Additionally, I will also report on the partitioning of cholesterol in coexisting liquid-ordered (L_o) and liquid-disordered (L_d) domains of raft-like mixtures. Our results reveal a preferential partitioning of cholesterol into L_o domains. However, unlike previously assumed also L_d domains contain significant amounts of cholesterol.

Keywords: biological model membranes; phase separation; X-ray diffraction

FA5-MS05-O3

Laboratory SWAXS for Applications in Pharmaceutical Technology. Aden Hodzic[a,b], Manfred Kriechbaum[b,c], Peter Laggner[b,c]. [a]*Research Center Pharmaceutical Engineering GmbH, Inffeldgasse 21/A, 8010 Graz, Austria.* [b]*Hecus X-ray Systems GmbH, Reininghausstrasse 13a, A-8020 Graz, Austria.* [c]*IBN - Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, Schmiedlstrasse 6, Graz, Austria.*
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Combined small- and wide-angle X-ray scattering (SWAXS) is becoming an increasingly important technique in pharmaceutical solid-state characterization1). Highly relevant questions of polymorphism in crystalline materials, stability and nanostructure of amorphous states, inner surface in controlled-release formulations, and stability and ageing of controlled release formulations can be addressed by this technique. The information to be gained by SAXS expands largely the scope of conventional powder diffraction techniques. A particular advantage lies in the simultaneous observation of nano-scale (SAXS) and atomic scale (WAXS). With the development of high-brilliance laboratory SWAXS systems (Hecus S3MICROpix) the times for analysis have been greatly reduced, and hence the method can be applied to quality screening and process analytical technology (PAT). Examples will be presented for technologically relevant systems, such as polymorphic forms of active ingredients (carbamazepine), lactose-based inhaler powders, controlled-release microsopheres (EDLA), and amorphous fomulations. The results show, that an analysis in terms of robust SAXS parameters, such as inner surface, total absolute scattering power, and Porod exponent, can provide highly valuable technological information.

[1] Laggner, P., M. Kriechbaum, M. Rappolt, G. Pabst, H. Amenitsch, A. Johs, K. Lohner, D. Zweytick, R. Koschuch, and P. Abuja. **2005**, *Solid State Characterization of Pharmaceuticals*. Eds. A. and M. Zakrzewski. Assa International, Danbury, chapter 12.

FA5-MS05-O4

GISAXS for Single-Crystal-Like Silicate Films Formed at the Air-Water Interface. U-Ser Jeng[a], Ying-Huang Lai[a,b], Je-Wei Chang[b], Yi-Jiun Chen [b], Hsiang-Wei Cheng[b], Wei-Ting Hsu[b], Chih-Chang Weng[b], Chun-Jen Su[a], Chiu-Hun Su[a], Kuei-Fen Liao[a]. [a]*National Synchrotron Radiation Research Center,*

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At the National Synchrotron Radiation Research Center (NSRRC) with a 1.5 GeV storage ring, a small-angle X-ray scattering (SAXS) beamline has been installed. The X-ray beam can be selectively monochromatized by a double Si(111) crystal monochromator (DCM) with high energy resolution ($\Delta E/E \approx 2\times10^{-4}$) in the energy range of 5-23 keV or by a double Mo/B_4C multilayer monochromator (DMM) for 10-30 times higher flux (~10^{11} photons/s) in the 6-15 keV range. A plane mirror is especially installed to the beamline to selectively deflect the beam downwards with high precision for grazing-incidence SAXS (GISAXS) with liquid surfaces. Using a grazing incident angle near the critical angle of water with 10 keV X-rays, we have monitored in-situ the growth process of silicate films at the air-water interface. Cetyltrimethylammooium bromide (CTAB) is used as the surfactant template for the tetraethyl orthosilicate (TEOS) in the solution in forming mesoporous silicated films, with the film growth temple controlled by temperature (in the range 25-55 °C) and pH value. The TESO molar ratio is varied from 0.04 ro 0.7 with respect to the molar ratio of H_2O:HCl:CTAB = 100:2-0.5:0.11. At 25 °C, after an induction period the formation of a lamellar phase and its transformation to a hexagonal mesophase of single-crystal-like reflections can be clearly observed. The layering process of the silicate rods, however, can be suppressed at higher temperatures above 45 °C; namely, the hexagonally-packed silicate rods can be formed near the air-water interface without going through the layering process of the rods, which is due presumably to larger thermal fluctuations.[1] Furthermore, transmission SAXS is used to monitor the evolution of the aggregation structure of the silicate/surfactant complex in the bulk solution. During the induction period, there are mainly complex CTAB/TESO rod-like micelles. Later, randomly oriented domains of lamellarly and hexagonally packed rods appear sequentially in the bulk solution. These results imply that the lamellar-to-hexagonal phase transformation can occur both in the bulk and near the air-water interface. Instead of layer-by-layer formation, it is possible that the silicate films may grow via reorientation-and-attachment of ordered silicate domains that adsorb to the air-water interface, while there is a thin surface layer of one or two micelle thickness stabilized at the air-water interface.

[1] S. A. Holt; J. L. Ruggles; J. W. White; R. F. Garrett, *J.Phys. Chem.* **2002**, 106, 2330.

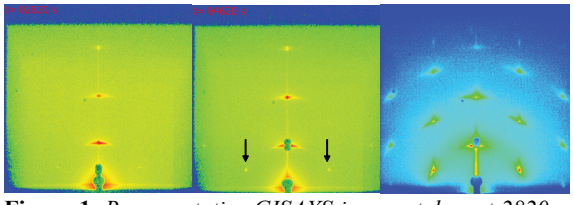

Figure 1. *Representative GISAXS images taken at 2820 s, 4620s, and 4 h, for a highly ordered silicate film in situ formed at the air-water interface at 25 °C.*

Keywords: GISAXS; air-water interface; silicate films

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New bio-SAXS Beamline ID14-3 at the ESRF. Petra Pernot[a], Adam Round[b]. [a]*ESRF, BP220, Grenoble, France.* [b]*EMBL Grenoble Outstation, France.*
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The new small-angle scattering beamline ID14-3 at the ESRF, Grenoble, France, dedicated exclusively to experiments of biological macromolecules in solution, is under user operation from November 2008 (bio-SAXS beamline). Originally running as a protein crystallography beamline, ID14-3 was refurbished, still as a part of the ESRF Macromolecular group (MX), with the main aim to provide a facility with 'quick and easy' access to rapidly growing demands from crystallographers, biochemists and structural biologists. The beamline provide manual and automatic sample loading/unloading, data collection, processing (conversion of a 2-D image to a normalized 1D X-ray scattering profile) and analysis. The users obtain on-line standard data concerning the size (radius of gyration, maximum dimension and volume) and molecular weight of samples which allow on-the fly ab-inito shape reconstruction in order to provide feedback enabling the data collection strategies to be optimized. Automation of sample loading is incorporated on the beamline using a device constructed in a collaboration between the EMBL (Grenoble and Hamburg outstations) and the ESRF. Semi/automated data analysis is implemented following the model of the SAXS facility at X-33, EMBL Hamburg. Future plans extend to allowing remote access, based on the system currently in use on the ESRF MX end-stations.
The photon source consists of three high power undulators shared with three other end-stations: ID14-3 is a fix energy beamline (E = 13.3 keV) as two others and the third end-station is tunable. The flux can consequently vary according to the tunable end-station request on undulator settings. The beamline optics consists of first diamond (111) monochromator in Laue geometry, second germanium (220) monochromator in Bragg geometry and a torodial mirror with the fixed focus spot close to the sample location. The beam defining slit just after the mirror reduces parasitic scattering downstream. An aperture slit is implemented after the 10 m long tube between optics and experimental hutches. A set of guard slits in the experimental hutch defines the illuminated region on the sample, being 0.7 × 0.7 mm^2 with the maximal flux 10^{12}ph/s. The spot on the detector is 1 × 1 mm^2 if 2 m from the sample. The sample station is mounted onto an marble table together with modulable lenght flight tube and Vantec-2000 detector (2D gaz filled detector from Bruker). The premise of the new bio-SAXS facility is to take advantage of having an optimized but simple experimental setup. The representative user experiments and inhouse research examples will be presented. An additional benefit of building this new facility at the ESRF is the collaboration with the already established small angle neutron scattering facility at the ILL, Grenoble. Users will have the possibility to access both facilities for appropriate experiments. This will allow the complementary information provided by neutron and X-ray scattering to be obtained in a single visit to the Grenoble site.

Keywords: proteins in solution; small-angle scattering; synchrotron X-rays