Synchrotron Radiation News
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t716100695

New Trends in High-pressure Molecular Biophysics

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To cite this Article Fourme, Roger , Ascone, Isabella and Kneller, Gerald(2009) 'New Trends in High-pressure Molecular Biophysics', Synchrotron Radiation News, 22: 5, 39 — 41
To link to this Article: DOI: 10.1080/08940880903256866
URL: http://dx.doi.org/10.1080/08940880903256866

The strong turnout for the school reflects the central role of modern synchrotron radiation (SR)-based X-ray absorption spectroscopy (XAS) techniques for studying the molecular-scale physical and electronic structure that govern the chemical properties of complex materials and molecular complexes. The high collimation, intensity, and tunability of synchrotron radiation allow the investigation of a wide range of materials, including interfaces, nanoparticles, amorphous materials, hydrated and disordered biogenic minerals, soils, and dissolved species.

The first day of the school featured classroom lectures. Seven speakers presented lectures, starting with a deep introduction to EXAFS (Corwin Booth, LBNL) and XANES theory (Ritmukta Sarangi, SSRL), followed by talks on X-ray sources and optics (Apurva Mehta, SSRL), and practical aspects of data acquisition (John Bargar, SSRL). Afternoon talks focused on cross-cutting themes and advanced spectroscopic techniques, including Resonant Inelastic X-ray Scattering and X-ray Emission (Uwe Bergmann, SSRL), polarized and grazing-incidence XAS (Glenn Waychunas, LBNL), and X-ray microprobe techniques (Sam Webb, SSRL). We thank the lecturers for their generous efforts.

Laura Wasylenki from Arizona State University assists a fellow student during the data analysis exercises on day three.

The second day of the school involved "hands-on-the-experiment" morning and afternoon training sessions at three of SSRL's EXAFS beam lines (BL4-1, BL4-3, and BL11-2) and at the hard X-ray microprobe (BL 2–3). Practical sessions were heavily attended. Participants overwhelmingly commented that the sessions were very useful. The third and fourth days of the school were devoted to hands-on data processing and analysis teaching/working sessions, expanded from one day in previous years in response to participant requests. Topics covered included: a detailed introduction to the SIXPACK data processing software package, data inspection, rejection, averaging, and dead-time correction, background subtraction, principal component analysis, linear combination fitting, FEFF fitting, examples of fitting environmental and materials samples, and a wrap-up session.

Another X-ray spectroscopy school at SSRL dedicated to X-ray scattering techniques in environmental and materials sciences is being planned for May/June 2011. The organizers thank the DoE, Office of Basic Energy Sciences, Office of Biological and Environmental Research, and NIH National Center for Research Resources for supporting this multidisciplinary school. ■

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New Trends in High-pressure Molecular Biophysics

Fundamental studies on the effects of pressure on biosystems emerged in the 1960s and 1970s while remaining confined to a small community. This situation has changed gradually in the past few years. First, specific interests in high pressure (HP) for the study of macromolecules have been clarified. Second, instrumentation has made progress, extending the range of measurements and relaxing constraints of the HP environment. Third, high-resolution structural methods, NMR, and macromolecular crystallography (MX) have been adapted to HP. These powerful newcomers complete low-resolution methods such as small-angle neutron and X-ray scattering (SANS and SAXS) and spectroscopic techniques such as infrared (IR) and Raman. Accordingly, there is now a toolbox for integrated pressure-perturbed biophysics, including structural and dynamical aspects. It was an opportune moment for HPMB 2008, an international meeting

focused on fundamental research in HP molecular biophysics, held near Paris from December 10–12, 2008. As SR is crucial, in particular for MX, SAXS and IR, HPMB2008 was held at a third-generation SR facility, SOLEIL. Organizing labs were SOLEIL, IBS (Grenoble) and CBM (Orléans). More than 70 participants, including a number of PhD students and postdocs, from nine countries including European Union countries, Japan, Russia, and the USA, attended the meeting. The format of the conference allowed extensive formal and informal discussions.

Unique potential of pressure perturbation

The Le Chatelier–Braun principle can be used to predict the effect of a change in conditions of a chemical equilibrium: the equilibrium shifts to counteract the imposed change. Accordingly, pressure displaces equilibrium toward more compact phases. Let us assume a simple case where a system is in equilibrium between a state N and a more compact state U. Pressure will shift the equilibrium toward U. The fractional ratio at pressure p is $[U]/[N] = \exp(-\Delta G/RT)$, with $\Delta G = G_U - G_N \approx \Delta G_0 + \Delta V_0 (p - p_0)$ . . . (the subscript zero is related to values at ambient pressure). These considerations apply to proteins, which have been designed by natural selection not just for the ground or "native" state but also for higher-energy, "non-native" states that are involved in function, folding, and unfolding. These conformers are distributed into a wide conformational space, from the folded state to the totally unfolded state(s). As a general semi-empirical rule, the higher-energy conformers are more compact and more disordered than the native state. In normal conditions, the fraction of higher-energy states in a solution of protein is generally too small for measurements. Increasing pressure will increase the fraction of low-lying conformers on the basis of decreasing specific volume. It is then possible to promote and stabilize non-native phases, which may be of high biological interest. Exploration of the energy landscape of a macromolecule is indeed one of the most exciting potentials of pressure perturbation, in addition to more traditional fields of interest such as the molecular basis of life adaptation to extreme conditions.

The useful range of pressure for all aspects of molecular biophysics extends up to about 2.5 GPa (SI unit of pressure: 1 Pascal = 1 $N.m^{-2}$ = 9.8692 atm; 1 GPa = 10^9 Pa = 1 kbar).

Conference sessions

Kazuyuki Akasaka (U. Kinki, Japan) gave in a plenary lecture a general introduction to HP molecular biophysics. He recalled the dynamism of the structures of proteins, which span enormously wide conformational space and time ranges. He described the effort of excavating protein dynamism by pressure perturbation (on the basis of free energy modifications as explained previously), which is rewarding, particularly when combined with atomic-detail information. The rest of his talk was centered on NMR. Heteronuclear two- or multi-dimensional NMR capability can now be performed up to ≈ 0.3 GPa on proteins and nucleic acids with the usual limitations in molecular weight.

HPMX is another high-resolution method that is now a full-fledged technique. A session dedicated to HPMX was one important issue of the conference. The keynote lecture of Eric Girard (IBS, Grenoble) presented the status of instrumentation and methodology developed on the ID27 beamline at ESRF since 2001, using X-rays of ultra-short wavelength (0.03–04 nm) and diamond-anvil cells with a large useful aperture (about 82°). Hydrostatic compression is required, which precludes sample cryocooling; radiation damage at room temperature is accommodated by irradiating successively different portions of the crystal with the tightly (40 µm) collimated X-ray beam. As crystals loaded in the diamond cell often take a single orientation with respect to diamond culets, a broader range of orientations is obtained by depositing the sample on a tiny diamond splinter introduced in the compression cavity. These procedures allow one to collect high completeness data from a small number of crystals.

Three examples of HPMX studies performed with this instrumentation were presented. Richard Kahn (IBS, Grenoble) gave a report on the study up to 2 GPa of an octanucleotide in the A-DNA form, showing that double-helix behaves as a molecular spring. Isabella Ascone (SOLEIL) described the 0.2 nm structure at 0.57 GPa of a dimeric protein, bovine superoxide dismutase. Nathalie Colloc'h (CYCERON, Caen, France) presented a comprehensive study of the tetrameric enzyme urate oxidase, combining information from HPMX, HPSAXS, fluorescence and activity measurements under pressure and after decompression. Marcus D. Collins (U. Washington, Seattle, USA) presented examples of studies performed at CHESS (U. Cornell, USA) and by collaborating groups. HPMX studies, such as how water interacts with the interior cavity of T4 lysozyme, were performed with a beryllium cell. A different technique presented by Marcus was HP cryocooling, where diffraction data are collected at ambient pressure on a crystal cryocooled in liquid He while compressed. Elena Boldyreva (U. Novosibirsk, Russia) reported HP diffraction and spectroscopic studies on crystals of amino-acids which mimic properties of peptide chains in larger biomolecules. HPSAXS is another important method, building on technology well mastered up to 0.5–0.7 GPa. Experimental data can be accurately corrected for effects related to HP environment; accordingly, the extensive developments of SAXS in extracting information from scattering curves and model fitting remain fully applicable. This potential was demonstrated by Javier Perez (SOLEIL), Stephanie Finet (U. Paris 6, France), and Eva Rosenbaum (IBS, Grenoble). Roland Winter (U. Dortmund, Germany) presented studies of lipid bilayers, combining time-resolved SR X-ray diffraction and the pressure-jump relaxation technique. The complementarity of neutrons and SR for the study of structure and dynamics of biomolecules under HP was illustrated by several communications. Recent developments and applications of well-established spectroscopic methods were also covered in other sessions.

Another important issue was the coupling of experiment and molecular simulation in HP studies of biomolecular systems, with a session

devoted to this topic. Dietmar Paschek (U. Dortmund, Germany) gave a keynote lecture on replica exchange molecular dynamics (REMD) simulations of liquid water and of the Trp-cage "mini-protein" in aqueous solution. Lars Meinhold (Caltech, Pasadena, USA) gave a talk on pressure-induced changes of the protein energy landscape, studied by molecular simulation. Vania Calandrini (CBM, Orléans) reported on a combined neutron scattering and molecular dynamics simulation study of lysozyme in solution under hydrostatic pressure. Paolo Calligari (ILL Grenoble/LLB Saclay and CBM, Orléans) presented his PhD work on the signature of adaptation of proteins to extreme conditions in the dynamics of the extremophile and mesophile variants of the ribosome anti-association factor IF6. Related to the simulation session was a tutorial, "Molecular Simulations at the Interface with Spectroscopic and Imaging Experiments," which was organized in the form of a satellite event. Here the participants worked on toy examples illustrating the applications of the simulation and analysis tools that are developed in Gerald Kneller's team (CBM, Orléans).

The last part of the conference was devoted to a symposium, "High-pressure Instrumentation for Macromolecules," organized by Isabella Ascone (SOLEIL) and Serge Pin (CEA, Saclay, France). After the presentation of the HP facilities at SOLEIL by Jean Paul Itié, topics included: instrumentation for UV/visible, fluorescence and flash-photolysis; XANES spectroscopy; HP crystallization; pulse radiolysis; and microscopic investigations of living cells. The conclusion of the symposium was a round-table in which ways to foster collaborations between different communities were discussed. In effect, HP studies are not yet in the mainstream of structural biology, so that exciting opportunities are lost. A coordination and support at the European level would be most welcome. HP platforms planned at several synchrotron radiation facilities, in particular SOLEIL and the ESRF, will be very useful for a wider access. Overbooking of general-purpose HP diffraction beamlines is a very serious limitation. The need for at least one beamline, similar to ESRF ID27 but dedicated to HPMX data collection as well as HP-style data collection at ambient pressure, was strongly advocated. ■

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MX Frontiers at the One Micron Scale: Making the Case for Micro-Beams

Macromolecular crystallographic (MX) structure determination at synchrotron radiation sources has the potential to advance significantly through use of X-ray beams of one micron or smaller cross-sections. Recently, the MX Frontiers at the One Micron Scale Workshop explored structural biology scientific opportunities made possible through the use of micro-beams, and anticipated technical challenges for developers of MX beamlines at the National Synchrotron Light Source II (NSLS-II). More than 100 attendees participated in the workshop, which included one-and-a-half days of lectures, discussions, and a semi-formal poster session on July 23–24, 2009, at Brookhaven National Laboratory (BNL).

The workshop was particularly relevant given the development of NSLS-II, a brilliant new synchrotron facility under construction at BNL. As he welcomed participants on the first morning of the workshop, BNL Laboratory Director Samuel Aronson highlighted the essential role played by the crystallography community at the National Synchrotron Light Source (NSLS) and expressed his hopes for its continued involvement in leveraging the unique capabilities of NSLS-II.

Wayne Hendrickson (Columbia University, BNL), the recently appointed Associate Project Director for Life Sciences at NSLS-II, launched the first scientific session by outlining concepts and opportunities for life sciences at NSLS-II. He reported the key milestones