

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 ERSF, 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

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Speakers in the MX Frontiers at the One Micron Scale workshop.

and current status of the NSLS-II project, highlighting the facility's design features, new capabilities, and project scope. Regarding the future at NSLS-II, Hendrickson predicted that "what we think of as difficult today will become routine by 2015."

David Eisenberg (University of California, Los Angeles) then illustrated Hendrickson's assertions as he spoke about his adventures in microcrystallography of biological specimens, which have led to stunning breakthroughs in structure determination of proteins in the amyloid state from crystals that are 10,000 times smaller than conventional samples. Eisenberg revealed that it was the scientific problem of Alzheimer's and other amyloid-related diseases that first sparked his interest in micro-diffraction and he expects further developments to enable him to probe even smaller granules *in vivo*.

He was followed by Gebhard Schertler (Medical Research Council), who spoke about the structure of G protein coupled receptors and shared his work on studying the structures of rhodopsin and beta adrenergic receptors, all membrane proteins yielding small variable crystallites. He highlighted the crucial role microcrystallography played in his work, requiring extensive experimentation in close collaboration with beamline scientists "on equal terms." Weighing in on how recent developments in

crystallography may be used to inform the design of beamlines at NSLS-II, both Eisenberg and Schertler underscored the importance of incorporating full-automation and beamlines with the flexibility to deliver a stable one-micron beam as well as larger beams.

Tiny beams also drive the development and use of new crystallographic methods, such as that of serial crystallography pioneered by John Spence (Arizona State University). After summarizing the state of microdroplet delivery, he addressed the problem of solving protein structures from molecular aggregates containing only a few unit cells. Soft X-rays, as well as methods borrowed from electron diffraction, may make it feasible to construct the image of one particle from the scattering of many identical, randomly oriented ones. However, serial crystallography assumes mastery of powder diffraction analysis, a technique then reviewed by Irene Margiolaki (European Synchrotron Radiation Facility). In a lucid talk, she highlighted the experimental and analytical methods that now yield high-resolution structures of modest size proteins, such as the SH3 domain of ponsin. She demonstrated the efficient use of powder diffraction as a complementary tool to traditional approaches in the classification of insulin crystallites that now enable pharmacological companies to optimize formulations.

Thursday afternoon's session examined the problem of radiation damage and how the use of micro-beams could mitigate sample damage. With this in mind, Colin Nave (Diamond Light Source) showed how photoelectrons escape from micro-focused beams and, thus, reduce radiation damage in the probed volume. He drew attention to the largely unexplored potential of near parallel beams and truly high-resolution detectors as additional ways to improve signal-to-background ratios, pointing out that "we're not yet in the position where all of the possibilities have been explored."

Next, Robert Fischetti talked about microcrystallography developments at GM/CA-CAT, and reviewed his team's results on the experimental verification of the anisotropy and reduction of radiation damage around one-micron beams. He outlined plans for future experiments aimed at providing more conclusive evidence of damage export. James Holton (University of California, San Francisco) then spoke about simulating the complete diffraction experiment on an absolute scale as a means of experiment planning and beamline design evaluation. Finally, Elspeth Garman (University of Oxford) lectured about the parameters affecting crystal lifetime and possible radiation damage mitigation strategies, which provided a broad picture of the afternoon's deliberations.

That evening, following dinner, attendees heard a talk given by microcrystallography pioneer Christian Riekel. Riekel traced the origins of micro-diffraction at the European Synchrotron Radiation Facility's (ESRF) ID13 to small-angle X-ray scattering (SAXS) and documented its multi-disciplinary evolution. However, crystallography dominated his program as he demonstrated remarkable results including work with chromatin, spider silk, inorganic dusts, and Eisenberg's amyloid proteins. In a bold outlook, he touched on the many optical techniques and innovative instruments that now propel micro-diffraction toward the nanoscale, a development that will require exhaustive experimental studies as well as strong, renewed collaborations between scientists in the field.

The workshop resumed the next day with reviews and discussions of existing and planned micro-diffraction instruments, a

theme introduced by Clemens Schulze-Briese the day before. Schulze-Briese depicted the protein microcrystallography setup at the Swiss Light Source, which operates in tandem with a full-beam instrument and takes advantage of extraordinary beam stability and a sagittally focusing monochromator. Sean McSweeney began the second day's meeting by describing the wide-ranging and ongoing upgrades to the ESRF and its structural biology beamlines. He shared the facility's plan to link all of the Structural Biology Group beamlines into a highly automated cooperative of specialized instruments and emphasized the value of community development.

"To address issues that will come up in the next five to 10 years, we need to find better ways to integrate and share access to beamlines," said McSweeney, stressing the significance of scientific community building.

Next, Gwyndaf Evans spoke about micro-focusing beamlines at the Diamond Light Source, recommending that NSLS-II beamlines with the ability to deliver a stable one-micron beam should also have the flexibility to deliver larger beams. This capability is already in demand at Diamond, where users may move to secondary optics and thus, dial the focal spot size to suit experimental needs. Following Evans, Masaki Yamamoto talked about the targeted protein research program and the development of a micro-beam instrument at the SPring-8 facility in Japan. This very long RIKEN beamline expected by next year aims to achieve a one-micron focal spot and emphasizes beam stability by using a single-stage optic layout, which minimizes the number of components that interact with the X-ray beam.

Challenges in optics and instrumentation were addressed next by Kenneth Evans-Lutterodt (BNL), who explained the benefits of kinoform focusing optics for macromolecular crystallography. He showed that existing technologies suffice to produce beams of

suitably small convergence in ways that preserve spatial coherence. After this, Antonio Lanzirotti (University of Chicago) shared lessons learned from his work with K-B mirror based optics and expressed a desire for smaller beams to extend the performance of the geo-environmental scanning micro-diffraction and spectroscopy project beamline that he is designing for NSLS-II.

The workshop concluded with a final discussion that considered the key question of whether or not a solid case had been made in favor of micro-beams. Participants debated the matter with enthusiasm throughout, deliberating about the appropriateness of the one-micron scale, the role of submicrons, and the usefulness of beamlines with flexible capabilities. Ultimately, the sum of lectures and discussions clearly illustrated that tiny beams will enable new science, particularly so if the beam is of sub-micron size. At this scale, structural work will be characterized by experimentation rather than routine measurements and involve many pursuits in life sciences such as MX, SAXS, and fiber diffraction, potentially borrowing from electron microscopy as well. Finally, the workshop illuminated the benefits that micro-beams offer for the mitigation of radiation damage; however, more experimental verification and method development is needed.

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