

# *Workshop on Emerging Areas of Biological Crystallography: Summary and Recommendations July 26-28, 2004*

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Developments in synchrotron radiation sources over the past 20 years have revolutionized protein structure determination. The intense tunable undulator x-ray sources available at the third generation synchrotron facilities have enhanced the Multi-wavelength Anomalous Dispersion (MAD) technique for solving the phasing problem to obtain 3-D structures. This has enabled the collection of MAD data sets from favorable crystals in a few minutes. Much emphasis in recent years has been given to the effective use of synchrotron sources for protein structure studies. It consists of protein crystallization, storage, monitoring of crystal growth, harvesting and freezing crystals, robotic capabilities to mount and dismount the cryo-cooled crystals on a goniometer, improved 2-D detector systems and data acquisition. In addition, the interpretation of the x-ray diffraction data, and graphics display to enhance the quality of the molecular model have been given very high priority by the research community. The workshop on "Emerging Areas of Biological Crystallography," which was held from July 26 –28, 2004, addressed topics well beyond these issues to cover future grand challenges. This workshop was a part of a "Study on the Future Scientific Directions for the Advanced Photon Source" chaired by Gopal Shenoy and Sunil Sinha.

The workshop identified four grand challenge topics to define the emerging areas in the field of biological crystallography. They are:

## *1. Molecular Machines:*

Proteins combine to form multiprotein complexes that function as "molecular machines." The molecular machines form cells and are produced and controlled by the genome and the associated sensing and regulatory networks. The workshop addressed the methodology of mapping the structure of molecular machines by combing the results from electron cryo-microscopy (CryoEM) and x-ray diffraction using synchrotron radiation. The speakers at the workshop also addressed the structural changes in molecular machines associated with the dynamic interactions responsible for signaling, transport, motility, cell division, and virtually all other cell activities. This is an essential step before one can progress toward a systematic understanding of cell function. It was clear from the workshop discussions that this is a leading area of research being vigorously pursued by many of the principal research groups which were well represented at the workshop.

The workshop participants presented frontier work which showed the need for integration of tools, such as large-angle and small-angle x-ray scattering, electron cryo-microscopy, and circular dichroism (CD). As an example, we briefly mention the work of Jack Johnson's group (Scripps Research Institute) which used x-ray small-angle scattering, optical fluorescence spectroscopy, far-UV CD, and CryoEM to study the acid-induced maturation dynamics in virus particles. Bacteriophage HK97 is icosahedral virus capsids which has a massive size, large number of subunits, and highly symmetric architecture. It contains the primary machinery that packages, protects, and delivers the genome of icosahedral viruses. The changes in the virus due to acidification of the buffer are measured through various physical techniques, and reveal large scale structural changes involving reorganization in its gross morphology, surface charge, and hydrophobicity result. Understanding of the mechanisms and dynamics of these changes has provided considerable knowledge of virus function and insights into the function of macromolecular assemblies.

The trend of integrating various tools is a clear new direction which will help to understand the structure of complex macromolecular assemblies which form the important functional molecular machines (Wah Chiu, Baylor; Tom Steitz, Yale).

## *2. Membrane Proteins:*

The membranes in human body keeps a cell separated from the outside environment so as to protect the proteins from dissipating away and to safeguard from the entry of foreign molecules in to membrane structure. The membrane proteins are vital to many functions of human cellular control and growth and they act as transporters and receptor. Crystallization of membrane proteins is a major stumbling block en route to elucidating their structure and understanding their function. The novel concept of membrane protein crystallization from lipidic cubic phases has yielded well-ordered crystals and high resolution structures of several membrane proteins, yet progress has been slow due to the lack of understanding of the molecular mechanisms of protein transport, crystal nucleation, growth, and defect formation. Much anticipation existed among the workshop participants in reaching a rational understanding of membrane protein crystallization process and, as a result, in the development of new techniques to adequately improve the quality and purity of membrane proteins suitable for structural and dynamics studies.

## *3. Dynamics:*

The field of biological crystallography is astoundingly successful in the past at determining the static, ensemble-averaged structure of a vast range of proteins. What is increasingly important to biological understanding, however, are the questions: How do proteins move to affect their function? What is the role of dynamics in the microscopic mechanisms of enzymes? How can time-dependent structural changes be measured? How good are molecular dynamics theoretical models of these dynamics? Fundamental biology will drive dynamics to the forefront of many research programs.

The third generation synchrotron radiation sources have provided many time-domain tools which will form the core of future research programs to study the protein dynamics. They include large- and small-angle scattering, inelastic nuclear resonance scattering, x-ray microscopy, etc. For example, x-ray small-angle scattering has been successfully used to observe the unfolding of RNA (Lois Pollack, Cornell). The classic work of Keith Moffat's group on photobiology has opened new doors to study the reaction intermediates with sub-nano second resolution. The dynamics of select atoms such as iron in heme-proteins has been studied using the inelastic nuclear resonance scattering which complements the Raman scattering investigations (Steve Durbin, Purdue). In addition, all these dynamical measurements are now leading to a focused theoretical effort in performing molecular dynamics simulations. This new trend is robust both in terms of growing interest in the area of dynamics and successful development of a plethora of x-ray and optical tools for their study (Gerd Rosenbaum, UGA/ANL).

## *4. Physics of Biological Processes:*

It became apparent from the workshop presentations that there is a growing community of scientists which uses the tools of physics, both experimental and theoretical, in an innovative way to study biological problems, as well as research aimed at providing a better understanding of the physical principles underlying biological processes. In the post-genomic era, biology gets increasingly quantitative, and it is necessary to understand the biological processes at the molecular level. In order to address such problems, a whole set of complementary physical tools will be needed capable of monitoring, in real-time, the behavior of individual biological molecules and complexes, in vitro and in live cells. An awareness of the need to bring physical insight into solving problems in the entire field of biological crystallography existed among the workshop participants. Special focus was in the areas of:

### *a. Radiation Damage*

In almost all protein structure determination studies using synchrotron radiation beamlines, the samples are always cryocooled to reduce the radiation damage. However, there are limitations on the optimum use of high brilliance beams, although less intense beams inflict the same damage per absorbed photon as intense

undulator beam. In her well-received presentation, Elspeth Garman (Oxford) strongly argued for a systematic study of the effects of radiation damage on a structure and emphasized that wrong conclusions will be drawn on structure from damage induced data on biological mechanisms. The future directions in this field should focus on investigating radical scavengers to slow down the rate of radiation damage in cryo-cooled crystals, theoretical calculations and Monte-Carlo simulations on the x-ray absorption process in protein crystals as a function of x-ray wavelength and presence of heavy atoms (e.g., Se), and finally a study of unit cell and structural changes (phase transitions) in crystals as a function of temperature and radiation burden.

The subject of radiation-damage induced phasing (RIP) was also discussed in the workshop where in the damage is used to facilitate the structure determination. Further developments of the RIP method is expected to benefit the more traditional phasing techniques like S-, Se-, or Br-SAD/MAD (Zbigniew Dauter, NCI/NIH).

#### *b. Biomolecules Under High Pressure*

Investigating proteins subjected to extreme conditions is not only an important field of fundamental biology, but it also addresses the performance of life in deep oceans to high mountains. Significant proportion of the global biosphere is exposed to pressures of up to 120 MPa. The question is what happens to organisms when they are compressed, and how does life originate and survive at and below the deep sea floor? It is expected that in future the extreme conditions will become an important variable in microbiological and biochemical research, in particular to understand the structure/function relationship of proteins.

Early work by Richard Kahn (IBS Grenoble) and his collaborators presented at the workshop have paved the way to performing studies of biological structure under hydrostatic pressure using synchrotron radiation beams. They foresee growth of this area with the availability of high energy x-rays with sub-Angstrom wavelength to facilitate high resolution studies.

#### *c. Crystallography with Microfocused X-ray Beams*

Progress in the field of protein crystallography is often hindered by the size, shape and the quality of the available crystal. For example, crystals which are only a few tens of microns in dimension, or with a growth habit of a thin needle or a platelet, or highly mosaic or twinned will not produce quality diffraction patterns. It has been demonstrated that each of these difficulties can be overcome by the use of microfocused x-ray beam of the type routinely used in the materials research at all the third generation synchrotron radiation facilities (Gebhard Schertler, MRC Lab. Cambridge).

Micro-focused x-ray beams also offer the possibility to study partially ordered systems such as the membrane protein arrays, e.g. a class of membrane-bound proteins made up of microarrays of G-protein-coupled receptors (GPCRs). These have the potential use in medical diagnostics, biomarker discovery, and proteomics. Another example for the micro-focused x-ray beam investigation is the liposomes, made up of natural nontoxic phospholipids and cholesterol. They are used as drug carriers to cure inflammation to cancer. The challenge will be to identify such systems and to address the potential and the limitations of the technique in their structure determination.

From an experimental point of view the ability to provide  $10^{10}$  ph/s in a micron sized spot, and to align the micron sized sample in front of the beam over the duration of the experiment are quite challenging. But these requirements are becoming a common place at synchrotron facilities engaged in nanoscience research.

#### *d. Diffuse Scattering from Biomolecules*

The diffuse scattering has been used to study the nature of disorder in protein crystals and has been shown to be a useful experimental technique for characterizing the dynamics in crystalline proteins. It provides information regarding the directions of motions as well as the lengths and directions of correlations in

atomic displacements. Diffuse scattering is usually thrown away in most crystallographic studies, but the availability of synchrotron sources, advanced detectors, and sophisticated computing have made it easier to use it fruitfully.

Since the detailed work of Michael Wall (LANL) and his collaborators, not much attention has been paid to this important area of research. Measurement and analysis of diffuse features in scattering patterns would help to distinguish Bragg scattering from diffuse scattering, to improve models of dynamics for x-ray structures, and to improve the resolution of x-ray structures. Studies of x-ray diffraction from membrane phases, in which both the membrane structure and the bending and bulk elastic moduli were simultaneously modeled from diffuse scattering, hint at the major advance in development of protein structural models that might be achieved through integration of diffuse scattering data into crystallographic structure refinement.

It was pointed out by Richard Matyi (NIST) at the workshop that high resolution x-ray scattering methods can yield valuable insights into the nature of physical defects generated by radiation damage in protein crystals. In the semiconductor community, it has taken several years for these high resolution methods to elevate their status from that of laboratory curiosity to an established and important analytical technique. Hopefully, with continued progress (particularly in the modeling of the diffuse intensity) a similar progression will occur to the benefit of the protein crystal growth community.

#### *e. Powder Diffraction from Proteins*

While the powder diffraction is a routine tool in materials science, not a lot of attention has been given to this technique in biological structure determination. The pioneering work of Robert Von Dreele (ANL) leads to a new direction in this area with the potential of powder diffraction becoming an important complementary tool.

The reason that powder diffraction data from proteins and protein complexes have a high potential is fourfold: 1) powders are easily prepared under a wide range of conditions and not limited by the need for a suitable single crystals, 2) protein powders are inherently “perfect” for diffraction; they are of the right size (about 1 micron) and are almost completely strain free, 3) only very small samples sizes ( $\mu\text{g}$  quantities) are required, and 4) data can be collected rapidly, making the observation of many dynamic processes straightforward. For example, this method completely removes the problem of radiation damage in biological materials and allows study of *in situ* processes on minutes-to-hours time scales.

#### 5. Recommendations:

The workshop brought together internationally renowned specialists in their fields, not limited to x-ray expertise. To supplement the interdisciplinary format, there was a fruitful dialogue between biologists, chemists, and physicists to address the grand challenge topics in the field...

The workshop participants focused well beyond the current aggressive trend in protein structure determination. The emerging areas of biological crystallography were identified which are summarized above. The following recommendations support some of the identified grand challenges in the emerging areas of this field.

A new infrastructure may be called for, both in terms of physical facilities and in terms of interactions among organizations in the community. Some of the desired features of this new infrastructure are:

- A. Dedicated resident R&D expertise/personnel within APS
- B. Extended beamtime availability for suggested new programs, such as the radiation damage studies, diffuse scattering, microfocus studies, time-domain investigations, etc.
- C. Stations with flexible configurations to perform *in situ* studies using complementary tools
- D. Local availability of complementary facilities to develop integrated research programs, such as:
  - Cryo-electron microscopy
  - Associated biochemistry labs
  - In-line optical spectroscopies in experiment stations

- Microfocus beams for biological crystallography
- Dedicated protein SAXS, diffuse scattering and powder diffraction capabilities
- Resident detector development expertise
- Resident physicists with long term interest in molecular dynamics theory to perform radiation damage studies, diffuse scattering and small-angle scattering modeling, etc.