Workshop on Macromolecular Single Crystal Diffraction at The Spallation Neutron Source

Executive Summary

On December 18th and 19th of 2000 a workshop, co-sponsored by the Spallation Neutron Source (SNS) project and the National Aeronautics and Space Administration (NASA), was held in Knoxville to discuss the future use of the SNS for macromolecular single crystal neutron diffraction. This workshop brought together for the first time in the United States representatives of both the biological neutron diffraction and microgravity crystal growth communities. At the conclusion of the workshop, the following recommendations were made: 1) single crystal biological instrumentation should form an integral part of the SNS instrument suite; 2) all funding options should be pursued and supported to facilitate the development of two instruments for macromolecular crystallographic neutron diffraction studies at SNS; and 3) the workshop participants support efforts to fund the Long Wavelength Target Station (LWTS) and the proposed complex biological system diffraction station; in addition on behalf of the group, the workshop organizers should submit a letter of intent to the Experimental Facilities Advisory Committee (EFAC), proposing a high-resolution macromolecular diffraction instrument at the High Power Target Station (HPTS).

Introduction

Neutron diffraction provides important and unique information for macromolecular structure–function studies. Hydrogens comprise roughly half the atoms of biological materials such as proteins and DNA, and hydrogen ions supply the primary motive force in the molecular actions of such fundamental biological processes as metabolism and reproduction. The ability of neutrons to reveal the positions of hydrogens even at moderate resolution (2.5 Å) is the foundation of the scientific justification for neutron diffraction of biological samples. Although other techniques may provide complementary information, none do it at such moderate resolution levels nor as conclusively as neutrons. In order to reassert the value of neutron crystallography and the justification for macromolecular neutron diffraction experimentation and to explore the role of the SNS in future neutron diffraction studies a two-day workshop was organized.

In addition to presentations on the scientific role and justification for biological neutron crystallography, other presentations on the first day included an overview of the Spallation Neutron Source project, scientific advances with macromolecular neutron diffraction, recent developments in neutron detectors and beam lines world wide, and the role of microgravity in the growth of crystals suitable for neutron diffraction experiments.

The potential role of macromolecular neutron diffraction at SNS has not been recognized, as illustrated even in local news media reports. Therefore, an important mission of the workshop was to inform both crystallographers and other biologists interested in

structure-function studies of biomolecules on the merits of neutron diffraction studies, as a preamble to more widespread dissemination of information. There is a gathering confluence of events that will provide opportunities to grow large crystals on the International Space Station (ISS), and to collect data at high flux neutron sources. These include the Institut Laue Langevin (ILL), the Rutherford Appleton Laboratory's spallation source (ISIS), the SNS, and the proposed European Spallation Source (ESS). This potentially exciting new era in biological neutron diffraction studies is capturing the attention of governments and funding organizations worldwide.

Recommendations

Three primary conclusions were reached from the workshop presentations and discussions:

1.) The landscape of macromolecular neutron diffraction has undergone a significant positive alteration in the past three years. The consequence of this alteration is a significant shift in the conditions that existed at the time of the Biological and Environmental Research Advisory Committee (BERAC) Report of 1998. That committee suggested a de-emphasis of neutron diffraction in part because of the probability of more readily obtaining ultra-high resolution structural information from X-ray data collected at 3rd generation synchrotron sources; the advent of GHz and higher frequency NMR devices capable of generating interpretable spectra from larger proteins; the absence of large crystals suitable for neutron diffraction and the lack of reliable means to grow such crystals; and the less than optimal combination of neutron source flux and detector sensitivity that results in multi-week data collection times even from proteins with small unit cells. However, it is the consensus of this workshop that the 1998 report recommendations for neutron diffraction studies have been superseded by new scientific findings and instrumentation developments.

a.) In contrast to the views cited in the BERAC report, the outlook for using non-neutron sources to determine positions of hydrogens is less apparent. Recent evidence suggest that NMR spectra contain contributions from intermolecular interactions that are much more complex and potentially chaotic than previously thought [Lin *et al.*, 2000]. These "bizarre spin dynamics" will make interpretation of spectra from larger molecules much more problematic and less definitive. With X-ray studies, evidence now suggests that crystals of sufficient quality to diffract to > 0.9 Å resolution occur only in ~1% of proteins sampled [Langan, Myles, Timmins]. A further complication of high resolution 3rd generation synchrotron data collection is radiation damage to crystals, necessitating attenuation of high brilliance sources, thus reducing the ability to collect ultra-high resolution X-ray diffraction data needed to establish positional information on the hydrogen atoms in these molecules. In crystals that do diffract to ultra-high resolution, significantly more information on solvent structure and hydrogen positions can be derived from moderate resolution neutron data (2.3-2.5 Å) than from X-ray diffraction data (0.9 Å) [Myles and Langan]. A more conservative view suggests that static hydrogen positions will come in increasing numbers from ultra-high resolution X-ray data, but that X-ray data will never match neutrons in the positioning of partially mobile hydrogens [Helliwell].

Support for both positions is seen in crystals of concanavalin A where deuterium atom positions and orientations can be seen in a greater number of water molecules with neutrons (60 D_2O's) than with synchrotron X-rays (10 H_2O) [Habash *et al.,* 1999]. Thus, the neutron approach is more efficient even with the data collection occurring at room temperature [Helliwell. Positions of non-exchanged hydrogens can be determined in crystals that have been soaked in D_2O to replace labile hydrogens and solvent. The positions of these hydrogen atoms can be established from moderate resolution data (2.3 Å) by examining maps of negative density (corresponding to the negative scattering length of hydrogen) [Niimura *et al*., 1997]. The positions of deuterium atoms appear as positive density in these same maps. Neutron diffraction also presents the possibility of determining meaningful thermal parameters for H and D atoms, for high-resolution structures. For example, it may be possible to correlate these thermal parameters with hydrogen-bonding strengths. [Koetzle]

b.) As a result of research supported by both NASA and the European Space Agency (ESA), the production of crystals sufficiently large for neutron diffraction studies is considered an attainable goal. Growth of crystals with 2 mm x 1.5 mm x 1 mm (or larger) volume is now common for an increasing number of proteins [Carter]. With the commissioning of the International Space Station a permanent venue now exists that should be available to crystallographers interested in using microgravity to enhance crystal growth [Carter and Snell]. Based on previous microgravity crystal growth experiments, and the availability of a controlled environment for extended duration missions, it is estimated that ~90% of proteins crystallized on orbit will have the potential to reach the 1 mm x 1mm x 1mm size range needed for experiments on current and future neutron sources.

c.) Significant improvements have been realized in neutron detectors. Increased sensitivity of detectors has resulted in decreased data collection times. Detector and beamline developments in Japan, at Grenoble (ILL), and at Los Alamos (LANSCE), have resulted in full data sets being collected within 10 days, even 24 hrs, rather than months [Niimura, Myles, Langan].

d.) Thus, although neutron data collection will never be described as high-throughput, based on flux projections and detector developments, it should be possible at SNS to collect a complete neutron diffraction data set from a crystal of 0.5 mm x 0.5 mm x 0.5 mm with a longest unit cell dimension of 100 Å within 1 week of beam time. This is comparable to the size of crystal and time period needed to complete data collection with typical in-house X-ray systems.

Because of these advances and predictions, and in the absence of dedicated equipment planned for macromolecular crystallography, it was the consensus of the participants that single crystal biological instrumentation must form an integral part of SNS instrument station planning.

2.) Future planning for the SNS should include two different protein crystal diffraction instruments. The first instrument, capable of collecting high-resolution data from crystals with a maximum unit cell length of 100 Å, should be built at the High Power Target

Station (HPTS). A second instrument capable of collecting medium-high resolution data from crystals with unit cell axes of up to 250 Å should be built at the Long Wavelength Target Station (LWTS).

a.) These two instruments will address different crystallographic research. The HPTS instrument will provide essential high-resolution (1-1.5 Å) proton (deuteron) and water structure data for moderate sized proteins that cannot be determined using other diffraction or resonance techniques. This device will provide a means for determining mechanisms and biochemistry of novel enzymes and other proteins discovered in functional/structural genomic studies [Helliwell, McRee, Stallings]. The LWTS instrument will provide a venue to answer fundamental questions about large proteins and complex assemblages at medium-high resolution. The LWTS instrument will be able to determine the positions of the protons/deuterons in complex biomolecules like the nucleosome core particle, the fundamental building block of the chromosome, but may fall short of being able to pinpoint the position of the catalytic proton related to protein synthesis in the ribosome (unit cell dimensions exceeding 300 Å).

b.) Results from questionnaires distributed by the macromolecular beamline group at the spallation source at Los Alamos suggest that when fully commissioned the instrument there will be capable of meeting the needs of about 20% of the potential users in the US [Langan & Schoenborn, 1999]. The questionnaire results are based on present interest and do not reflect potential increased usage as a new generation becomes aware of the value of neutron diffraction data. Workshop participants suggested that similar levels of interest in synchrotron data collection existed prior to widespread recognition of their utility [Helliwell, Minor].

c.) Shared instrumentation with the small molecule crystallographic community at HPTS is not a viable option. The geometry of this device will not allow the resolution of data from large unit cell crystals. In addition, ancillary equipment at the small molecule instrument will restrict the sample and detector geometry required to collect macromolecular data [Zhao, Schultz]. These devices include furnaces and refrigerators for high and low temperature studies, magnets, and high-pressure chambers.

Based on the proposed mission and potential demand for instrumentation (and concomitant absence of similar instrumentation elsewhere) it was the sense of the workshop participants that all funding options be pursued and supported to facilitate the development of two instruments for macromolecular crystallographic neutron diffraction studies at SNS.

3.) Current proposals submitted to NSF for funding a LWTS at SNS include the second macromolecular device described earlier [Mason]. All efforts should continue to ensure that the LWTS is funded and constructed. For the proposed HPTS instrument, it is possible that an Instrument Development Team (IDT) will be needed to ensure its inclusion at SNS. The workshop participants charged the organizers with the task of submitting a letter of intent (LOI) for the HPTS instrument to the SNS Experimental

Facilities Advisory Committee (EFAC), and the identification of potential funding agencies. The next opportunity to submit the LOI is March 2001.

In addition to these conclusions, it was felt that educational and outreach activities should to be undertaken to acquaint a new generation of crystallographers with the scientific merits of neutron diffraction and with the advances that have been made in recent years. To this end, the workshop participants will seek to make presentations at various national societies, including the American Crystallographic Association and the Protein Society. Plans for the ACA 2001 annual meeting will include a special interest group session on macromolecular neutron crystallography.

Summary of Workshop Presentations

SNS and Advances in Neutron Crystallography

The SNS will have a 2 megawatt power output: this is about half the integrated neutron flux of ILL, but 50 times greater peak flux and significantly greater (12X) than that of the current highest-flux spallation source, ISIS, in England. The design of the SNS should permit power upgrades without significant future expenditures. Site excavation has been completed, foundations are being poured, and the first neutrons are expected in June of 2006. One target station, the high power target station (HPTS) is funded integral to the project, and funding is being sought from NSF for the construction of a second target station, the Long Wavelength Target Station (LWTS). Further information is available at the SNS website (www.sns.gov).

At present, no instrumentation for macromolecular crystallography is in development. The process for externally funded instrument selection at SNS starts with the formation of an instrument development team (IDT), the submission of a letter of intent from the IDT, followed by a decision from the Experimental Facilities Advisory Committee (EFAC) on recommending a conceptual design study of the proposed instrument. Alternatively, acquisition of a macromolecular instrument at the HPTS of SNS could be included in the competition for the initial suite of instruments. Instrumentation for a macromolecular crystallography beam line is currently included in proposals for funding the LWTS.

The scientific case for neutron diffraction was presented by John Helliwell whose research into the basis of sugar recognition by concanavalin A has encompassed extensive synchrotron X-ray and neutron data collection. Europeans view the SNS as becoming the state of the art facility for neutron users, and as a justification for the construction of the European Spallation Source (ESS). As Europe watches developments with SNS, continued upgrades of existing neutron facilities are underway both in Britain and on the continent. These upgrades include a new area detector for the D19 beam line at ILL, and upgrades of neutron Laue time-of-flight instruments at ISIS, indicating a movement toward more protein crystallography for detailed analysis of hydrogen bonding and catalytic site structure.

The traditional basis for neutron diffraction is the ease with which accurate positions of hydrogens/deuterons can be determined in crystals of macromolecules. Both deuterium

and oxygen scatter similarly in neutron diffraction experiments. Thus, solvent position and proton exchange can be readily identified from neutron diffraction data. A more recent rationale for neutron diffraction can be seen in the comparative diffraction data for concanavalin A between an ultra-high resolution X-ray cryostructure and a medium resolution neutron room temperature structure. The neutron data were collected at ILL from a D_2O soaked crystal of 3 mm x 2 mm x 1 mm. Data were collected in 10 days and were 89% complete to 2.4 Å. The neutron structure provided six times the number of well-determined waters (position and orientation) compared with the ultra-high resolution X-ray data (0.9 Å). Thus, neutron diffraction determines bound waters more efficiently, and it also should provide the primary means of identification of the positions of somewhat mobile waters in a protein structure [Habash *et al.,* 1999].

Neutron diffraction data should have a pre-eminent role in assisting future modeling studies and computational biology, by providing a structural data base for understanding the role of solvent in ligand interaction, and providing further needed information to understand the thermodynamics of ligand recognition from structural data. Such studies will have a direct impact on rational drug design, providing more accurate and complete molecular structures. Thermal parameter values for H and D atoms may also be assigned based on neutron diffraction data, for proteins that diffract to high resolution. To fully understand the water structure and proton exchange rates of native molecules low temperature X-ray data must be compared with room temperature neutron data. Even at ultra-high resolution, X-rays are not good at distinguishing water molecules from monovalent cations (sodium, ammonium, potassium). It will not be possible to understand things like RNA folding and catalysis, or DNA bending unless cation positions are identifiable.

Peter Timmins reported that unique information on the location of H atoms and water has been obtained by neutron fiber diffraction of biological polymers, including cellulose, hyaluronic acid, filamentous viruses, and DNA. For example, individual water positions along the DNA strand have been refined even at the low resolution of 3 Å. Other significant diffraction information can be obtained at low resolution including the localization of surfactants added to proteins. The detergent structure in integral membrane proteins has not been possible to determine in X-ray diffraction studies owing to the disorder of the surfactants in the unit cell. It has now been possible to resolve the detergent structure in crystals of OmpF porin of *E. coli* using neutron contrast matching studies [Pebay-Peyroula *et al.*, 1995]. Given that 40% of the genome is membrane-bound proteins, which are extremely difficult to crystallize, the strategic importance of such neutron studies in revealing the interactions of proteins and detergents cannot be emphasized enough.

Improvements in neutron sources, detector design and interpretation of multi-wavelength diffraction have improved the speed with which data can be collected. Detector improvements include the neutron image plate, which is currently in use in both Japan and Europe, a neutron area detector with 1mm pixel size being developed at Oak Ridge, and detector research at Brookhaven National Laboratory for the SNS project. Nobuo Niimura presented rubredoxin data (unit cell axes 34 Å x 35 Å x 44 Å; crystal size 2.6 mm x 1.7 mm x 1.0 mm) collected with a neutron image plate in 11 hrs. The resolution of the data is 1.5 Å and the refinement of the hydrogen positions at 1.5 Å is currently

underway. At the Japanese Atomic Energy Research Institute (JAERI), the rule of thumb for data collection from macromolecular crystals is that each axis must be less than 100 Å. However, longer unit cell lengths can be accommodated if the other axes are smaller (i.e., 50 Å x 50 Å x 200 Å). The volume of the crystal must be ≥ 2 mm^3, so 1.5 mm x 1.5 mm x 1.0 mm is around the current minimum acceptable crystal volume. Construction has started on the new BIX-4, the performance of which will be at least 3 times better than the present BIX-3. The necessary crystal volume should be ~1/3 that of BIX-3.

In addition to steady state neutron sources, several spallation sources are planning to contribute to biological neutron diffraction research. ISIS, at the Rutherford Appleton Laboratory, does not have a protein crystallography instrument in place, but the facility is currently undergoing upgrades consisting of a second target station, which will include such an instrument. In this country, the Protein Crystallography Station (PCS) at the Lujan Center source at the Los Alamos Neutron Sciences Center (LANSCE) produced its first neutron beam on a sample in the week prior to the conference. The PCS is a new diffraction instrument for protein crystallography, fiber, and membrane diffraction. This instrument, with a partially coupled water moderator, uses a large cylindrical position sensitive detector for collecting data. The detector has an active area of 3000 cm^2 and a resolution of 1.3 mm with a counting rate over 1 million counts/sec. A call for proposals at the LANSCE PCS will be issued in 2001. An advisory team of structural biologists is in place for the LANSCE PCS, and contacts with this group will be pursued.

As a result of microgravity studies supported by both NASA and the ESA, production of crystals sufficiently large for neutron diffraction studies could become commonplace. Dan Carter reported reliable growth of crystals with the 1-mm^3 volume needed for current neutron diffraction experiments for even such problematic proteins as bacteriorhodopsin. With the commissioning of the International Space Station a permanent venue now exists that should be available to crystallographers interested in using microgravity for crystal growth. It is estimated that with the extended duration missions on the ISS, ~90% of proteins crystallized on orbit will reach the 1-mm^3 size range needed for neutron diffraction experiments. For example, on flight STS-89, a crystal of ferritin was grown with a volume of 10 mm^3. Neutron diffraction data to 2.7 Å has been collected at ILL from this crystal, 1 Å higher resolution than the diffraction limit of ground grown crystals. In studies reported by Eddie Snell, microgravity grown crystals are consistently larger and more physically perfect than those grown terrestrially. This physical perfection takes place on both short and long-range scales, and has been quantified using X-ray diffraction rocking-curve studies. In contrast, despite early optimistic claims cited in the BERAC report, the evidence suggests that crystals of sufficient quality to diffract to > 0.9 Å for determination of hydrogen positions occur only in ~1% of proteins sampled.

One significant application for proteins structures determined by neutron diffraction will come in the area of rational drug design. Chris Dealwis pointed out the practicality of this approach, discussing the problems associated with finding an agonist of angiotensin to bind with rennin, and how this study carried over to drug discovery of anti-HIV proteases. Both rennin and HIV protease belong to the general class of aspartic proteases, so named for the aspartic acid moieties, which are an integral part of the catalytic site. A solvent molecule bound tightly to both aspartate carboxyl groups is presumed to take part in the catalytic mechanism. Currently proposed mechanisms are

largely based on X-ray inhibitor structures, but the assignment of protonation states to the catalytic groups during the reaction differ. Since the active-site H atoms cannot be located by current X-ray analyses, their putative positions have so far been inferred from the local geometry of surrounding polar atoms. Thus, locating the crucial protons at the active site will provide important information to firmly establish the catalytic mechanism.

Enzyme structure and mechanism continue to be fertile ground for neutron crystallography, from the seminal study of the catalytic triad of trypsin [Kosiakoff & Spencer, 1981], to new studies of aspartic proteases. A recent report of the neutron diffraction structure of the fungal aspartic protease endothiapepsin by Cooper and Myles (2000) is a research milestone for several reasons. It represents the largest protein solved by neutron diffraction methods (33 kDa), and, by establishing the positions of the catalytic protons, represents a route to the development of more effective inhibitors to aspartic proteases. The endothiapepsin structure is the beginning of what may be one of the more significant roles played by neutron diffraction studies.

Discussion of Neutron Instrumentation at SNS

Because important information will be provided by studies of both smaller and larger proteins the development of two instruments were proposed, one that would be capable of resolving atomic positions for protein crystals with maximum unit cell axes of ~100 Å, and another device to resolve medium-high resolution data from crystals with maximum unit cell axes of ~250 Å. Projected flux and wavelength models for SNS suggest that an instrument at HPTS coupled to a chopper to utilize neutron wavelengths of 0.9-2.1 Å will meet the parameters of the first device. When fully commissioned, this instrument should be capable of collecting a complete data set of atomic resolution data from a crystal 0.5 mm x 0.5 mm x 0.5 mm in less than a week. This translates into ~30-50 new neutron structures a year, or nearly fivefold greater than the neutron structures cataloged in the Protein Data Bank. The HPTS device could augment the role of a variety of high throughput programs, producing more readily interpretable molecular mechanism data for new enzymes discovered by Structural Genomics.

The instrument for larger unit cells should provide diffraction data to elucidate atomic position models of large proteins, protein-protein complexes and protein-nucleic acid assemblages. Plans for this instrument are included in the proposal for the LWTS submitted to NSF (www.sns.anl.gov/LWTS/NSF_LWTS_Proposal.pdf). The inclusion of both instruments at SNS represents the minimum fulfillment of potential needs of the macromolecular diffraction community. Further instruments may be needed if the use of SNS for neutron diffraction studies follows a similar trajectory as the use of X-rays at synchrotrons.

The second day was dedicated to detailed discussions of the needs to be met for successful macromolecular crystallography at SNS. Discussions of detector and sample position geometry made it clear that a dual use (small-molecule and macro-molecule) crystallography instrument will not fit the needs of either community. The needs of the small-molecule community include furnaces and refrigerators, as well as magnets and high-pressure devices that will interfere with the positioning of neutron detectors for macromolecular data collection. Because of the large differences in reciprocal lattice spacing of small molecules and macromolecules, the optimization and placement of

detectors for small and macromolecular data collection are quite different. Continuing research and development in both neutron moderators and detectors were encouraged by the group. The workshop participants noted that the budget for neutron detectors is under great stress at SNS. Developments in moderators and detectors over the next several years will provide the most beneficial and cost effective approaches for realizing additional improvements in data collection times, especially for the instrument at the LWTS.

Recommendation Summary

1) Single crystal biological instrumentation should form an integral part of the SNS instrument suite.

2) All funding options should be pursued and supported to facilitate the development of two distinct instruments for macromolecular crystallographic neutron diffraction studies at SNS.

3) The workshop participants support efforts to fund the Long Wavelength Target Station (LWTS) and the proposed complex biological system diffraction station.

4) The workshop organizers should submit a letter of intent to the Experimental Facilities Advisory Committee (EFAC), proposing a high-resolution macromolecular diffraction instrument at the High Power Target Station (HPTS). Planning should begin now to identify agencies for funding the development of this instrument.

5) Educational and outreach activities should be undertaken to acquaint a new generation of crystallographers of the scientific merits of neutron diffraction.

6) Develop plans for the ACA 2001 annual meeting to include either a workshop or a special interest group session on macromolecular neutron crystallography, if feasible.

7) Inform LANSCE PCS advisory board members of the SNS Macromolecular Crystallography Workshop and invite their participation.

Submitted on behalf of the workshop by:

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B. Leif Hanson, The University of Tennessee/ORNL Graduate School in Genome Science and Technology

John R. Helliwell, The University of Manchester, Department of Chemistry

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Appendix

SNS Macromolecular Crystallography Workshop Participants

SNS/LWTS Neutron Protein Crystallography
December 18-19, 2000 UT Conference Center

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Agenda

S N S Macromolecular Crystallography Workshop

Monday, December 18

Morning Session Chair: Chris Dealwis

8:00-9:00 a.m.	Registration and Continental Breakfast
9:00-9:10	Welcome / Opening Remarks (Gerry Bunick)
9:10-9:40	Overview of SNS Project (Thom Mason)
9:40-10:30	Neutron Macromolecular Diffraction (John Helliwell)
10:30-11:10	Biological Applications of Neutron Diffraction at the ILL High Resolution Diffraction (Dean Myles) Fiber and Low-Resolution Diffraction (Peter Timmins)
11:10-11:30	**Coffee Break**
11:30-12:30	Neutron Diffraction in Japan/ Neutron Area Detectors (Nobuo Niimura and Ichiro Tanaka)
12:30-1:00	Neutron Instrumentation and Biological Research at ISIS (Charles Wilson)
1:00-2:00	**Lunch Break**

Afternoon Session Chair: Gerry Bunick

2:00-2:40	The NeutronProtein Crystallography Station at LANSCE (Paul Langan)
2:40-3:10	SNS Detector Development at ORNL (Donald Hutchinson)
3:10-3:40	Microgravity Crystal Growth and Commercial Neutron Macromolecular Crystallography (Dan Carter)
3:40-4:00	**Coffee Break**
4:00-4:30	Exploiting Neutrons for Drug Design (Chris Dealwis)
4:30-5:00	Microgravity and Neutron Crystallography (Eddie Snell)
5:00-5:30	Options for Macromolecular Diffraction Beamlines at the SNS (Jinkui Zhao)

<u>Tuesday, December 19</u>

Session Chair: Jinkui Zhao

8:00-9:00	Continental Breakfast
9:00-11:00	Discussion: Instrument Selection and Design (Organizing Committee and Participants)
11:00-11:20	**Coffee Break**
11:20-1:00	Discussion: Instrument Selection and Design (Organizing Committee and Participants)
1:00-2:00	**Lunch**
2:00-3:30	Discussion: Instrument Administration and Funding (Organizing Committee and Participants)

References and Recent Publications

Bon C., Lehmann M.S. and Wilkinson C. (1999) Quasi-Laue neutron-diffraction study of the water arrangement in crystals of triclinic hen egg-white lysozyme. *Acta Cryst.* D**55**, 978-987.

Cooper J.B. & Myles D.A. (2000) A preliminary neutron Laue diffraction study of the aspartic proteinase endothiapepsin. *Acta Cryst*. D**56**:246-248.

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