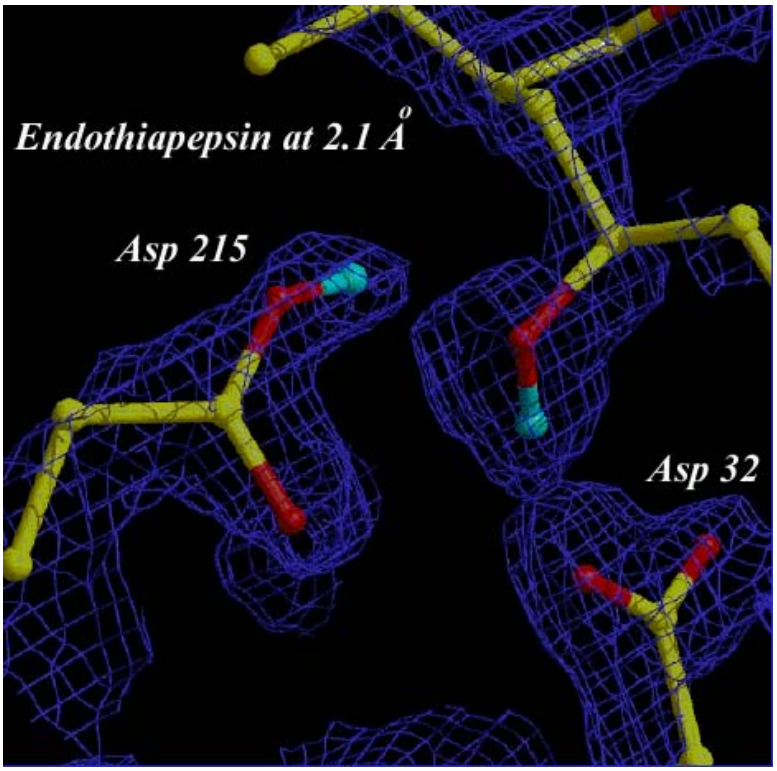
Conference on New Frontiers in Neutron Macromolecular Crystallography

July 12-13, 2005
Spallation Neutron Source
Oak Ridge National Laboratory, Oak Ridge, TN, USA



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Conference on New Frontiers in Neutron Macromolecular Crystallography

Conference Dates:
July 12-13, 2005

Location:
Oak Ridge National Laboratory, Oak Ridge, TN

Sponsors:
Intense Pulsed Neutron Source, Argonne National Laboratory
Spallation Neutron Source, Oak Ridge National Laboratory
Oak Ridge Associated Universities
University of Illinois at Chicago

Conference Organization:

Scientific Program Committee (scientific and technical leadership):
Prof. Andrew Mesecar, University of Illinois, Chicago
Dr. Dean Myles, Oak Ridge National Laboratory
Dr. Arthur Schultz, Argonne National Laboratory
Dr. P. Thiyagarajan, Argonne National Laboratory

Organizing Committee (logistics, administration, program committee assistance):
Dr. Al Ekkebus, Spallation Neutron Source/Oak Ridge National Laboratory
Ms. Peggy Anderson, Spallation Neutron Source/Oak Ridge National Laboratory

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ACRONYMS

ANL ..... Argonne National Laboratory
DOE ..... U.S. Department of Energy
ILL..... Institute Laue-Langevin (Grenoble, France)
IPNS ..... Intense Pulsed Neutron Source
LANL ..... Los Alamos National Laboratory
LANCSE ..... Los Alamos Neutron Center for
MaNDi..... macromolecular neutron diffractometer
MIR ..... multiple isomorphous replacement
NIH ..... National Institutes of Health
NIST ..... National Institute for Standards and Technology
NMC..... neutron macromolecular crystallography
NSnB ..... neutron SnB
ORNL ..... Oak Ridge National Laboratory
PCS..... Protein Crystallography Station (LANL)
SIR..... single isomorphous replacement
SnB ..... *Shake and Bake* direct methods procedure
SNS ..... Spallation Neutron Source
UHRMXC.....ultra-high resolution macromolecular x-ray crystallography

PHOTOGRAPH OF CONFERENCE PARTICIPANTS

1. EXECUTIVE SUMMARY

1.1 MOTIVATION AND OBJECTIVES

The conference "New Frontiers in Neutron Macromolecular Crystallography" was held at Oak Ridge National Laboratory, Oak Ridge, Tennessee during July 12-13, 2005 to highlight the impact of the proposed Macromolecular Neutron Diffractometer (MaNDi) at the Spallation Neutron Source (SNS), Oak Ridge, TN, that can potentially transform finding the location of hydrogen atoms in biological structures into a routine technique for structural biology research. This is due to the fact that Neutron Macromolecular Crystallography (NMC) can provide accurate hydrogen atom positions, protonation states and hydration states, as well as information on hydrogen/deuterium exchange in macromolecular crystal structures, even at a moderate 2 Å resolution. In contrast, to observe hydrogen atoms via ultra-high resolution X-ray crystallography requires diffraction data beyond 1.0 Å. This limit accounts for less than 1% of all protein systems being studied by X-ray diffraction.

The advent of the SNS, with over an order of magnitude increase in neutron flux, combined with advances in neutron optics and detectors, structural genomics, and protein deuteration, promises to revolutionize NMC. In order to realize this potential, a dedicated, best-in-class high-resolution time-of-flight single crystal macromolecular neutron diffractometer (MaNDi) has been proposed for the SNS. An optimized instrument design has been developed that will enable data collection rates over 50 times faster than at existing facilities. Furthermore, it will enable studies of crystals with lattice constants substantially larger than currently possible. The MaNDi Instrument Development Team secured beam line 11B at the SNS in October 2004. It is expected that the unprecedented speed and resolution limits achievable with MaNDi will greatly advance the fields of structural biology, enzymology, and computational chemistry. The project is now at an advanced stage. It is necessary to secure funding for the engineering design and construction of the diffractometer and the associated infrastructure at SNS. This will be possible only with the strong support of the broader structural biology community.

An outstanding scientific program attracted about 100 macromolecular X-ray crystallographers and structural biologists from research institutions across the US, Europe and Japan. Invited talks by Jenny Glusker, Herbert Hauptman, Wayne Hendrickson, Andrzej Joachimiak, Anthony Kossiakoff, Brian Mathews, Alberto Podjarny, Dagmar Ringe, Gerald Stubbs and B.C. Wang and posters on current research in high resolution neutron diffraction in structural biology as well as potential scientific problems for NMC were presented. In addition, the status of the SNS, MaNDi and of NMC programs worldwide was given by Thom Mason, P. Thiyagarajan and Dean Myles. A highlight of the conference was the tour of the SNS experimental hall containing the target and the instruments in various stages of construction.

1.2 MAIN OUTCOMES

The conference was highly successful in meeting its planned objectives:

1) To increase interest and awareness of the important new capabilities of neutron diffraction in structural biology
2) To engage and involve the community in defining challenge areas and problems that can be addressed by high resolution neutron macromolecular crystallography and
3) To showcase the novel and interesting macromolecular systems that will be amenable to

neutron diffraction studies upon completion of MaNDi.

In the final panel-led discussion, highly enthusiastic remarks and unanimous support were expressed both by the audience and all the leading scientists. With such endorsements and with the active participation and support of the wider structural biology community, the US now has an opportunity to obtain a world-leading new facility for NMC.

1.3 MAJOR RECOMMENDATIONS

Major recommendations from the conference attendees are:

- MaNDi will enable new science and will greatly enhance our understanding of the macromolecular structure and function. In particular, much quantitative information that is not currently available can be obtained on protonation states, lone pair electrons, solvation, hydrogen bonding, and H/D exchange in macromolecules.
- The structural biology community strongly supports MaNDi based on its unique capability to obtain data with small crystals in a reasonable time even for large macromolecules.
- In order to fully exploit MaNDi's capabilities, it is necessary to have extensive support facilities in place, including a protein deuteration laboratory.
- Based on the strong support of the community, the conference attendees strongly recommended that the Instrument Development Team (IDT) assemble a group of Principal Investigators to prepare a proposal to secure timely funding for the design and construction of MaNDi.

2. CONFERENCE AGENDA

2.1 AGENDA AND INVITED TALKS

Conference on New Frontiers in Neutron Macromolecular Crystallography

Research Support Center, Oak Ridge National Laboratory
July 12-13, 2005 (Agenda draft – July 1)

Monday, July 11

5 – 8 p.m. Registration & ORNL Site Access Badge Pick Up for ORNL (Comfort Inn)

Tuesday, July 12

7:30 a.m. Buses depart Comfort Inn

8:00 Coffee

8:30 Thom Mason, Oak Ridge National Laboratory /Spallation Neutron Source
"Overview of the SNS"

9:00 P. Thiyagarajan, Argonne National Laboratory /Intense Pulsed Neutron Source
"Design and Expected Performance of the next generation Macromolecular Neutron Diffractometer (MaNDi) for the Spallation Neutron Source"

9:30 Anthony Kossiakoff, University of Chicago
"Back to the basics, what neutrons can tell us about protein structure that X-rays can't"

10:00 Dean Myles, Oak Ridge National Laboratory
"Current status of macromolecular neutron crystallography"

10:30 Coffee break

11:00 Andrzej Joachimiak, Argonne National Laboratory
"Structural genomics – exploring protein structure and function"

11:30 Alberto Podjarny, University of Strasburg
"Complementary neutron and subatomic resolution X-ray studies of Aldose Reductase"

Noon Lunch; discussions

1:30 p.m. Wayne Hendrickson, Columbia University
"Impact of radiation damage in biological crystallography"

2:00 Dagmar Ringe, Brandeis University
"Pushing the limits of protein structure"

2:30 Brian Matthews, University of Oregon, Institute of Molecular Biology
"Flash-freezing: It's cool but is it kosher?"

3:00 Coffee break and poster session

4:00 SNS tour

6:00 Continuing discussions at Doubletree Hotel

7:00 Dinner with a Talk by Ian Anderson on *SNS as a Science Facility*

8:30 Return to Comfort Inn

Wednesday, July 13

7:30 a.m. Depart Comfort Inn via bus

8:00 Coffee

8:30 Herbert A. Hauptman, Hauptman-Woodward Medical Research Institute
"Neutrons break the low resolution barrier to direct methods"

9:00 B.C. Wang, University of Georgia
"If You Build It "We" Will Come: Past Experiences and Predictions"

9:30 Gerald Stubbs, Vanderbilt University
"Fiber diffraction in biology: new opportunities from neutron scattering"

10:00 Jenny Glusker, Fox Chase Cancer Center
"*Time-of-flight neutron diffraction as an aid to elucidating enzyme mechanisms: D-xylose isomerase."*

10:30 Coffee

11:00 Andrew Mesecar, University of Illinois at Chicago
*"The future of macromolecular neutron crystallography in enzymology & drug discovery***"**

11:15 Dean Myles, ORNL
"Infrastructure at ORNL"

11:30 Panel discussions

Scientific direction, infrastructure & funding strategies for MaNDi

12:30-1:30 pm Lunch and continuing discussions

1:30 -3:30 Full Instrument Development Team session

3:30 Depart ORNL

2.2 SUMMARY OF INVITED TALKS

The meeting began with an overview of the SNS project by Thom Mason (SNS Director). The SNS project is on schedule to provide users of the first suite of instruments in the fall of 2006. Nearly all of the beamlines are assigned to an instrument, including beam line 11B for MaNDi (Figure 1). However, MaNDi is the only instrument that does not have funding for construction.

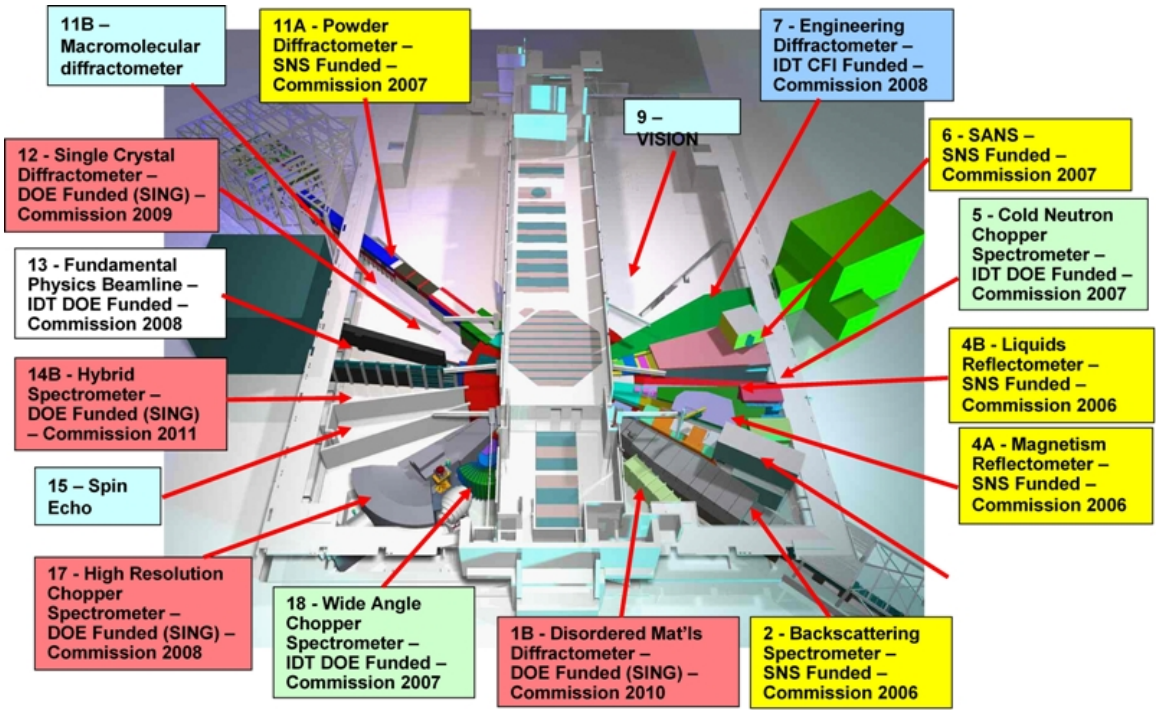


Figure 1. Instrument layout for the SNS. MaNDi has been allocated beam line 11B.

In a talk by P. Thiyagarajan (IPNS, ANL), the design and performance of the proposed MaNDi instrument were presented. MaNDi will be situated on a 24 m beamline with a curved guide so that the crystal will be out of line-of-sight of the moderator. The results of calculations and simulations carried out over a two year period show that a decoupled hydrogen moderator provides the best combination of intensity and resolution for neutron macromolecular crystallography. Figure 2 shows that for a fully deuterated 0.125

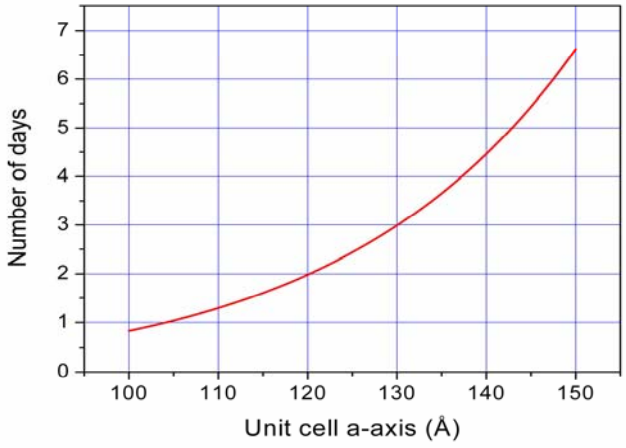


Figure 2. Data collection time vs. cubic unit cell size for a 0.125 mm^3 deuterated crystal to 2 Å resolution.

mm^3 crystal, with a primitive cubic lattice constant of a = 150 Å (V = 3,375,000 Å^3), data collection of a full hemisphere with average $I_{hkl}/\sigma(I_{hkl})$ = 3.0 at d = 2.0 Å will take a week of total beam time. For crystals with $a \leq 105$ Å, beam time will be less than 1 day. This performance is at least 10 to 50 fold better than existing instruments for neutron protein crystallography and will make NMC a routine technique.

Anthony Kossiakoff (University of Chicago) began his talk to reviewing some basic ideas regarding protein structure and neutron diffraction: structural details of protein packing, including the H atom positions, are a surprisingly fertile area; H/D exchange is an underutilized method to study protein dynamics and spatial organization of secondary structure; hydroxyl orientations (Figure 3) are the most valuable probe to assess electrostatic/van der Waals forces in protein packing. Kossiakoff then described how D_2O minus H_2O solvent difference maps provide a powerful and unbiased method to locate exchangeable H atoms, and therefore to determine protonation states, hydroxyl rotors, H/D exchange, water orientations, and deamidation. This technique is made easily feasible with perdeuteration of the protein, which will be available at the SNS.

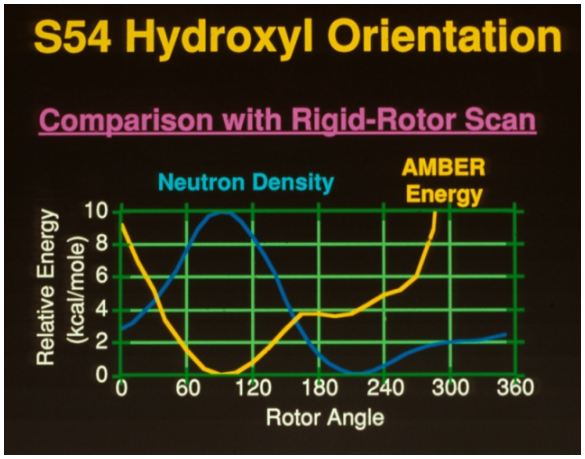


Figure 3. Plot of neutron density and energy from MD calculations of a hydroxyl rotor in trypsin.

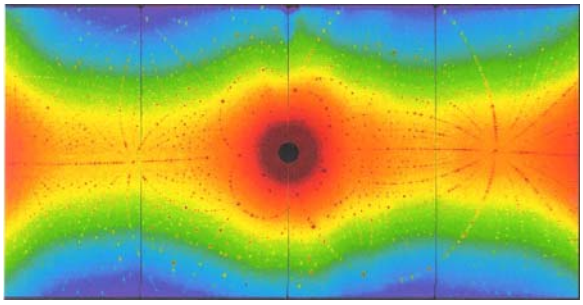


Figure 4. Neutron Laue diffraction from sperm whale myoglobin measured on the LADI instrument at the ILL reactor.

Both the exciting new accomplishments of existing neutron macromolecular instruments, and their limitations, were presented by Dean Myles (Oak Ridge National Laboratory). He described the quasi-Laue image plate instrument LADI at the ILL reactor in France (Figure 4), the monochromatic image plate instrument BIX3 at the JAERI reactor in Japan, and the time-of-flight Laue instrument PCS at the pulsed spallation neutron source at Los Alamos National Laboratory. Future instruments in the design or development stages include the LADI-3 instrument, BIXP1 at the Japanese J-PARC spallation source currently under construction, and of course, MaNDi at the SNS. The major advantages of neutron diffraction analyses are the ability to locate H or D atoms at > 1.5 Å resolution, and the ability to distinguish between H and D. Current limitations are the low flux of neutron beams, which then requires large crystals (often > 1 mm^3) and prohibitive time scales for many samples. Deuteration of the protein can provide a ten-fold improvement in signal-to-noise, and a bio-deuteration laboratory similar to the one at the ILL is planned for Oak Ridge.

The incredible advances in structural genomics which are being exploited at the Advanced Phonon Source at Argonne National Laboratory were presented to the audience in a talk by Andrzej Joachimiak (Argonne National Laboratory). Structural genomics (SG) will map protein folding space and provide insight into relationship between amino acid sequence and 3D structure. Structural information will provide new insight into protein function and we will gain complete structural understanding of many processes and pathways in the cell. From this, new hypothesis will be formulated and new functional proteins will be created through rational design. Libraries of genes, expression clones and proteins will be produced and will be available to public. With construction of the SNS there is an unique opportunity to build neutron crystallography beam line that will make an important contribution to biology and will be highly complementary to X-ray data. As shown in Figure 5, there is not shortage of potential systems to study. Protein neutron structures will reveal a number of important (and often unexpected) details: hydrogen atoms (~1% of the protein mass but critical for understanding protein packing, enzyme catalysis and interactions), protonation states and H-bonds, and solvent structure. In addition, there is no radiation damage – native structure can be observed at wide range of temperatures, and neutrons can elucidate hydrogen exchange with deuterium which provides information about solvent accessibility and dynamics. All these issues are critical to understand catalytic mechanism, interactions, solvent structure and potentially may have impact on structure-based drug design.

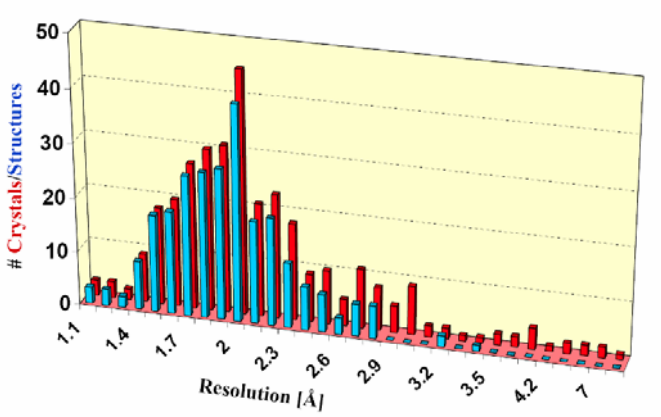


Figure 5. Histogram showing that 13% of the structures from the Midwest Center for Structural Genomics have been determined at 1.5 Å or better resolution.

Using both ultrahigh resolution X-ray diffraction and neutron diffraction, Alberto Podjarny (IGBMC, France) has been examining the enzyme aldose reductase in order to understand the mechanism of the aldose reduction to sorbitol, which is linked to diabetic complications. Since the enzymatic reaction involves a hydride donation from the NADPH coenzyme and a proton donation from the enzyme, the identification of hydrogen atoms in the active site is an important step to understanding the mechanism but even with 0.66 Å resolution X-ray data. In the active site, of the four H atoms interacting with the inhibitor head, two are seen at 3 σ contours, while the others appear only at 2 σ contours (Figure 6a). Preliminary analysis of neutron diffraction data obtained with the LADI instrument at the ILL at 2.2 Å resolution from a fully perdeuterated crystal shows more clearly the location of the hydrogen atoms in the active site. As an example, Figure 6b shows that one of the weak protons in the X-ray map appears strongly in the neutron map. This difference becomes very important when localizing mobile protons.

a) b)

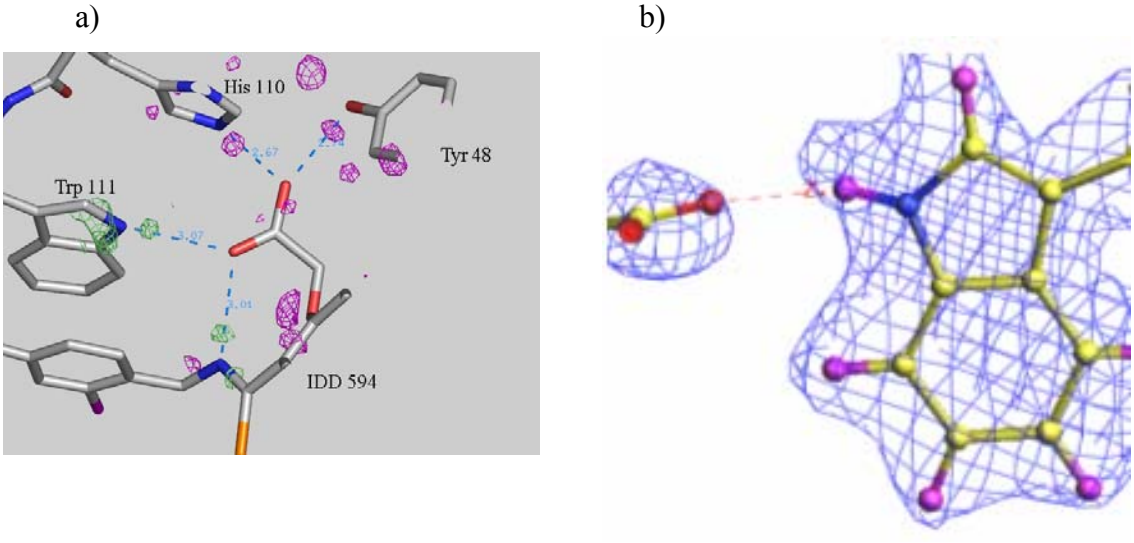


Figure 6. a) Fourier electron density difference map of aldose reductase using X-ray data at 0.66 Å resolution, contoured at 3σ (pink) and 2σ(green). b) Neutron scattering density showing the interaction of Trp 111 with the carboxylate head of the inhibitor.

An ongoing issue with X-ray protein crystallography is the problem of radiation damage, especially in the high flux beams available at synchrotron sources. Wayne Hendrickson (Columbia University) described that radiation damage occurs even in cryopreserved protein crystals, for which data must be corrected if possible. A main advantage of neutron diffraction data collection is the absence of radiation damage and the potential for collecting data at room temperature.

Brian Matthews (University of Oregon) presented the results of the effects of flash cooling on protein crystals. From a study of β-galactosidase, it was shown that flash cooling can increase the mosaicity of the unit cell, which could then reduce the signal-to-noise and the resolution (Figure 7). This might offset the effect of larger temperature factors for room temperature neutron diffraction data sets.

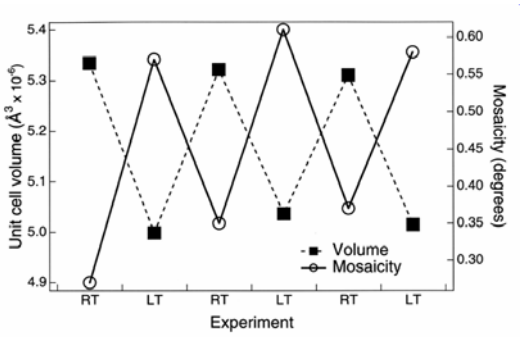


Figure 7. Plot showing the increase in mosaicity after flash cooling a crystal of β-galactosidase.

In a talk by Dagmar Ringe (Brandeis University), we heard about investigations of enzyme catalysis and mechanisms, particularly those related to hydrogen transfer. Clearly these would benefit from neutron diffraction data. We also heard about future designing new enzymes for carrying out non-biological reactions in industry based on knowledge of enzyme mechanisms.

Nobel laureate Herbert A. Hauptman (Hauptman-Woodward Medical Research Institute) noted in his talk and a poster that twenty years ago Kossiakoff reviewed the then recent advances in neutron diffraction technology as applied to macromolecules and predicted that "Clearly the time is close at hand when neutron diffraction will take its

place alongside X-ray diffraction as an equal partner in the structure determination of macromolecules." For many years most crystallographers believed that map "positivity" was a necessary condition for direct methods validity even though Hauptman had shown that direct methods could be rigorously extended to the case of unequal atoms, even to the extreme that some could have negative scattering density, such as H in neutron diffraction. With this in mind, the direct methods program SnB was modified to include the most negative peak positions as hydrogen atoms in structure factor calculations to recompute phases. Tests performed on experimental neutron data for cyclosporin A gave a 6% NSnB success rate for this 199 atom structure. In view of this evidence, one is forced to conclude that negative density is an asset rather than a hindrance when it comes to direct methods. Tests indicate that structures as large as lysozyme can be solved by NSnB if data < 0.90 Å are available. Larger structures, however, will require derivative SIR data to succeed at much lower resolution. One tremendous advantage afforded by neutron diffraction is that an H/D substitution strategy can provide an enormous number of perfect isomorphous replacement derivatives. The same can not be said of X-ray data.

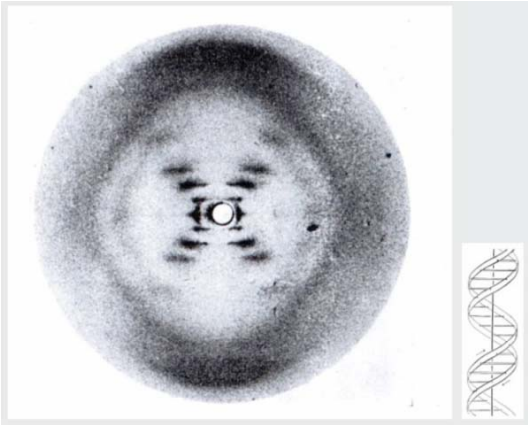


Figure 8. Diffraction from oriented DNA fibers obtained by Rosalind Franklin, from which the helical pitch and the axial rise was derived.

Macromolecules do not exist as single crystals in nature, but often as fibers. Gerald Stubbs (Vanderbilt University) explained the information obtainable about the structure of fibers from fiber diffraction. Diffraction from oriented fibers of materials from the classic example of DNA (Figure 8), to complex viruses, has been obtained. Techniques for orienting fibers were described. Neutron diffraction in combination with selective deuteration has been shown to provide unique information about the protein coat of a filamentous bacteriophage. These types of studies will be very useful in the future with the capabilities of a MaNDi instrument.

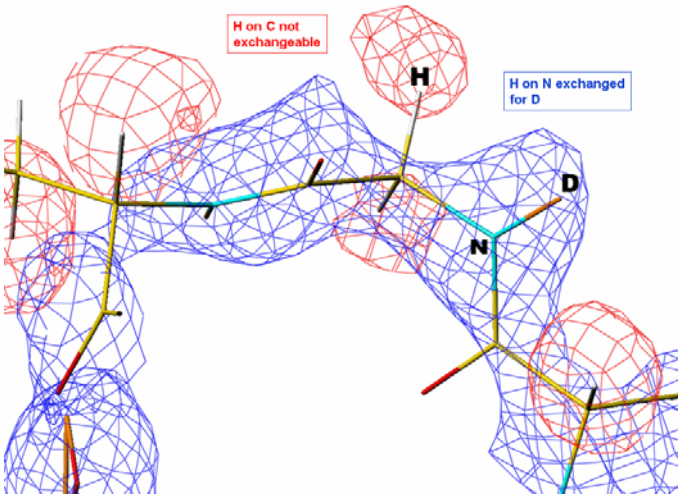


Figure 9. A 2 Å neutron map showing negative H contours and positive D contours.

Jenny Glusker (Fox Chase Cancer Center) re-emphasized how neutron diffraction data could aid in elucidating enzyme reaction mechanism by providing information not available even from high resolution X-ray data. With neutron data at modest resolution (~ 2 Å), the location of hydrogen atom positions in proteins, nucleic acids and water molecules are obtainable, which leads to: the protonation states of active-site residues, since these play critical roles in

enzyme mechanisms; information on labile and mobile hydrogen atoms using H/D substitution, as shown in Figure 9, since these indicate rigid or flexible interatomic interactions in the structure; the locations of hydrogen atoms in hydrogen bonds, particularly those connecting water with biological macromolecules or with other water molecules; estimation of the local pH, for example in the active site; and multiple conformations of proton-containing groups. The current status of the analysis of time-of-flight neutron diffraction data obtained using the Protein Crystallography Station at Los Alamos was presented.

To paraphrase B.C. Wang (University of Georgia), if MaNDi is built and if it functions as predicted, then "we" will come to use it, just as structural biologists came to use synchrotron sources. The ability to find accurate hydrogen positions is of fundamental importance in science! If we combine X-ray crystallography with neutron diffraction, this partnership will be the last link to effectively see all of the elements in the periodic table.

2.3 SUMMARY OF DISCUSSIONS

At the discussion following the invited talks and the poster session, over 70 participants, mostly consisting of X-ray protein crystallographers, were present. The participants were asked to address on three major themes:

1) Is neutron diffraction with MaNDi essential to the scientific community?
2) The important scientific issues that can be addressed by neutron macromolecular crystallography (NMC) in the areas of structural biology and enzymology.
3) How to secure timely funding for the realization of MaNDi and other associated facilities?

There was unanimous and enthusiastic support for the MaNDi project based on its ability to collect full datasets on reasonable-size crystals in a reasonable time. Wayne Hendrickson spoke about how his initial skepticism of NMC was converted into positive support based on the usefulness of neutron diffraction for structural biology research with small crystals and short experimental times. Alberto Podjarny, Wayne Hendrickson, Anthony Kossiakoff, B. C. Wang, Andrzej Joachimiak, Dagmar Ringe, Steve White, and several other scientists were highly supportive of MaNDi due to the new science that will be forthcoming based on the accurate positions of hydrogens in biomacromolecules that cannot be readily obtained with X-ray diffraction at synchrotrons. Wayne Hendrickson pointed out that the quantitative information on the protonation states and H/D exchange in proteins, that can only be obtained with NMC, will be of great interest, and when it can be obtained with smaller crystals he expects that the structural biology community will greatly benefit from MaNDi.

Given the enormous computational research on the design of inhibitor drugs and finding the enzyme mechanisms, both Dagmar Ringe and Steve White emphasized the importance of having accurate direct information on the protonation states at the active sites of enzymes. According to Anthony Kossiakoff, Andrzej Joachimiak and B. C. Wang, the new science with MaNDi in finding all the critical hydrogen atoms and hydration in proteins will transform the field of structural biology. A. Joachimiak expressed enthusiasm about NMC for two additional reason: ability of neutrons to measure the structure at physiological temperatures due to no radiation damage; and the synergy between the neutron diffraction and the ultra-high resolution macromolecular X-ray diffraction in shedding light on hydrogen positions.

Steve White pointed out lack of parity in the neutron and X-ray diffraction facilities for structural biology research. There are numerous synchrotron beamlines for X-ray diffraction and only one instrument (PCS) for neutron diffraction, which is highly oversubscribed.

Another feature of MaNDi that excited the community was its ability to collect data with smaller crystals in a reasonable time: Bob Bau pointed out that an order of magnitude decrease in crystal size will increase the community size by an order of

magnitude. Since perdeuteration increases the coherent neutron scattering cross-section and at the same time decrease the incoherent background, thus providing an order of magnitude increase in data rates (smaller crystals, shorter times) for the same signal-to-noise ratio, it was recommended by the community to develop a deuteration facility as an infrastructure associated with the MaNDi instrument.

Thus there was unanimous and enthusiastic support for the MaNDi instrument based on the new important scientific information on hydrogen positions, lone pair electrons and disordered water at the active sites.

The participants recognized the current tight budget situation with federal funding agencies. Hence, strong support for MaNDi from the large structural biology community has to be demonstrated for funding agencies to seriously look into funding this new facility. Wayne Hendrickson remarked that MaNDi has to be presented to the funding agencies as a facility that the whole community needs, not just specialists. To increase the awareness and support from the community, scientists familiar with the MaNDi project and with the unique information that neutrons will offer to structural biology and enzymology research should share this information with their scientific peers as well as the funding agencies. In order to increase the awareness of NMC, a transaction symposium at the ACA2006 meeting in Hawaii is being organized and scheduled so that there will not be parallel sessions on protein crystallography during the symposium. Anthony Kossiakoff observed that the realization of MaNDi can be achieved only through persistent leadership of a small group of active scientists with direct contacts with the funding agencies. Based on his proven leadership in building powerful facilities at APS, B. C. Wang was confident that once MaNDi is built it will benefit a large scientific community. He urged the scientific community to express strong support on the need for MaNDi to the funding agencies. He also mentioned that with wide support from the community it would be possible to approach other agencies, such as private foundations and state governments. Andrzej Joachimiak pointed out that the ability to collect a full hemisphere of neutron diffraction data in a reasonable time with MaNDi changes the landscape in structural biology research, and this opportunity has to be presented to the funding agency to increase their awareness and interest.

In summary, highly enthusiastic remarks and unanimous support were expressed both by the audience and all the leading scientists. With such endorsement and with the active participation and support of the wider structural biology community, the US now has an opportunity to obtain a world-leading new facility for NMC.

2.4 POSTERS

Successful neutron diffraction experiments conducted at two different sources on 0.3 mm^3 cocrystals of dihydrofolate reductase bound to methotrexate

Brad Bennett[1], Flora Meilleur[2], Leighton Coates[3], Dean Myles[2,4], Paul Langan[3], Elizabeth Howell[1], and Chris Dealwis[1]

[1] University of Tennessee- Knoxville, Knoxville, TN, USA; [2]Institut Laue-Langevin, Grenoble, France; [3]Los Alamos Neutron Scattering Center, Los Alamos, NM, USA; [4]Center for Structural Molecular Biology, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Studies toward the determination of catalytic mechanism in protein phosphatase by neutron diffraction.

Ewa Ciszak , Mark Swingle and Richard Honkanem

Laboratory for Structural Biology at the National Space Science and Technology Center, University of Alabama in Huntsville, 320 Sparkman Drive, Huntsville, AL 35805.

Protein crystallography with spallation neutrons: the PCS at Los Alamos

Leighton Coates, Paul Langan and Benno P. Schoenborn

M888 Bioscience Division Los Alamos National Laboratory, NM 87544

Proposed neutron diffraction studies to elucidate the proton transfer mechanism via hydrogen bonded networks in carbonic anhydrase and superoxide dismutase.

S. Zoë Fisher,[§] Patrick Quint,[§] Robbie Reutzel,[§] Deepa Bhatt,[¶] Kevin Weiss,[‡] Monika Budayova-Spano,[†] Flora Meilleur,[†] Chingkuang Tu,[¶] Lakshmanan Govindasamy,[§] Mavis Agbandje-McKenna,[§] David N. Silverman,[¶, §] Dean A.A. Myles,[‡] Robert McKenna[§]

[§]Department of Biochemistry and Molecular Biology, and [¶]Department of Pharmacology and Therapeutics, University of Florida, Gainesville, FL, USA; [‡]Oak Ridge National Laboratory, Oak Ridge, TN, USA; [†]EMBL - Grenoble Outstation, Grenoble, France.

A general acid-base mechanism for stabilization of tetrahedral adduct and intermediate in a serine-carboxyl peptidase

Hong Guo*, Haobo Guo and Alexander Wlodawer

Department of Biochemistry and Cellular and Molecular Biology and Center of Excellence in Structural Biology, University of Tennessee, Knoxville, TN 37996 and Protein Structure Section, Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, MD 21702, USA.

What can be learned about biochemical reactions from neutron diffraction studies?

Amy Katz[*#], Jenny P. Glusker[#], H. L. Carrell[#], Xinmin Li[*], B. Leif Hanson[%], Paul Langan[^], Benno P. Schoenborn[^], and Gerard J. Bunick[*$]

[#]Fox Chase Cancer Center, Philadelphia PA, [*]University of Tennessee-Genome Science & Technology, Knoxville TN, [%]Instrumentation Center, University of Toledo, Toledo OH, [^]Bioscience Division, Los Alamos National Laboratory, Los Alamos NM, [$]Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge TN

Study of allostery using dimeric hemoglobin
James Knapp,
University of Massachusetts Medical School

Neutron phasing methods applicable to D/H SIR replacement derivatives
David A. Langs, Hongliang Xu and Herbert A. Hauptman.
Hauptman-Woodward Medical Research Institute, 700 Ellicott St, Buffalo, NY 14203.

Use of X-ray and neutron diffraction to investigate the roles of functional groups in haloalkane dehalogenase
XUYING LIU and Ronald E. Viola
University of Toledo, Department of Chemistry, Toledo, Ohio

Neutron protein crystallography with LADI: recent results, technical advances and perspectives.
F. Meilleur
Institut Laue Langevin, BP156, 38042 Grenoble Cedex 9

Recent advances in Laue data processing
Zhong Ren
Renz Research, Inc, P. O. Box 605, Westmont, IL 60559

Hydrogen bonding in enzyme catalysis: dissecting the electrostatic complementarity of the ketosteroid isomerase active site
P. Sigala[1], D. Kraut[1], B. Pybus[2], G. Petsko[2] and D. Herschlag[1]
[1]Department of Biochemistry, Stanford University, Stanford, California 94305, USA;
[2]Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02454, USA

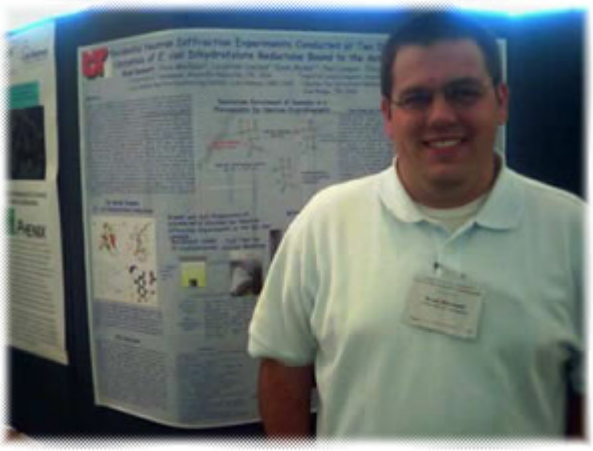
Ultra-high resolution studies of the R61 DD-peptidase
Nicholas R. Silvaggi* and Judith A. Kelly, Department of Molecular and Cell Biology,
*University of Connecticut, Storrs, CT 06269-3125 and *Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA 02118*

A neutron biological diffractometer in J-PARC
I. Tanaka(*1), N. Niimura(1), T. Ohhara(2), K. Kurihara(2), K. Kusaka(2), T. Ozeki(3)
(1)Ibaraki Univ., Hitachi, Ibaraki 316-8511, Japan; (2)JAERI, Tokai, Ibaraki 319-1195, Japan; (3)Tokyo Inst. of Tech., Meguro, Tokyo 152-8551, Japan

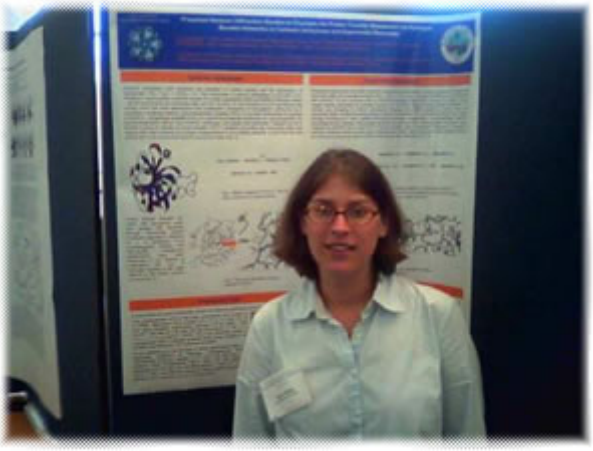
The molecular mechanism of DNA polymerases studied by small-angle X-ray scattering (SAXS)
Kuo-Hsiang Tang[1], William T. Heller[2], Gary W. Lynn[2], and Ming-Daw Tsai[1]
[1] Department of Chemistry and Biochemistry, the Ohio State University, Columbus, OH 43210, USA; [2] Chemical Sciences Division and Center for Structural Molecular Biology, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA

2.5 POSTER PRIZE WINNERS

A poster session was held on Tuesday evening of the meeting from 5:30 to 7:00 p.m. Poster winners were Brad Bennett for presenting results that utilized neutron diffraction and Zoe Fisher for the proposed concept of neutron use to further her research.



Poster Session Winner Brad Bennett



Poster Session Winner Zoe Fisher

APPENDIX: COMPLETE LIST OF ATTENDEES

Name	Organization Affiliation
Ian S. Anderson	Oak Ridge National Laboratory
Bryan Arendall	Duke University
Surajit Banerjee	Vanderbilt University
Robert Bau	University of Southern California
Brad Bennett	University of Tennessee
Hassina Bilheux	Oak Ridge National Laboratory
Robert Blessing	Hauptman-Woodward Medical Research Institute
Gerard Bunick	Oak Ridge National Laboratory/University of Tennessee
Christopher Bunick	Vanderbilt University
Chris Carrell	Washington University
Vincent Chen	Duke University
Ewa Ciszak	University of Alabama
Leighton Coates	Los Alamos National Laboratory
David Coe	University of Toledo
Cymbeline Culiat	Oak Ridge National Laboratory
Ian Davis	Duke University
Chris Dealwis	University of Tennessee
Juliette Devos	University of Toledo
Jill Dombrauckas	University of Illinois at Chicago
Stuart Endo-Streeter	Duke University
Christopher Faehnle	University of Toledo
Jim Fairman	University of Tennessee
Elias Fernandez	University of Tennessee
Zoë Fisher	University of Florida
Anna Gardberg	University of Tennessee
Nathaniel Ginder	Iowa State University
Jenny Glusker	Fox Chase Cancer Center
Hong Guo	University of Tennessee
Leif Hanson	University of Toledo
Joel Harp	Vanderbilt University
Herbert A. Hauptman	Hauptman-Woodward Medical Research Institute
William Heller	Oak Ridge National Laboratory
Wayne A. Hendrickson	Columbia University
Brian Hingerty	Oak Ridge National Laboratory
Roland F. Hirsch	U.S. Department of Energy
Christina Hoffmann	Oak Ridge National Laboratory
Andrzej Joachimiak	Argonne National Laboratory
Vinu Johnson	University of Toledo
Gary Kapral	Duke University
Amy Katz	Fox Chase Cancer Center/University of Tennessee
Glenn Keller	State of TN/TDEC/DOE-Oversight
Judith Kelly	University of Connecticut
Mitchell Klebig	Oak Ridge National Laboratory
James Knapp	University of Massachusetts
Thomas Koetzle	Argonne National Laboratory
Anthony Kossiakoff	University of Chicago

Paul Langan	Los Alamos National Laboratory
David A. Langs	Hauptman-Woodward Medical Research Institute
Johanne Le Coq	University of Toledo
Xinmin Li	University of Tennessee
J. S. Lin	Oak Ridge National Laboratory
Zhi-Jie Liu	University of Georgia
Xuying Liu	University of Toledo
Antonella Longo	North Carolina State University
Gary Lynn	Oak Ridge National Laboratory
Thom Mason	Oak Ridge National Laboratory
Brian Matthews	University of Oregon
John McCall	State of TN/TDEC/DOE-Oversight
Robert McKenna	University of Florida
Flora Meilleur	Institut Laue Langevin
Andrew Mesecar	University of Illinois at Chicago
Andre Mitschler	IGBMC CNRS
Dean Myles	Oak Ridge National Laboratory
Laura Murray	Duke University
M. Gary Newton	University of Georgia
Nobuo Niimura	Ibaraki University
John Norvell	National Institutes of Health
Alberto Podjarny	IGBMC CNRS
Linda Radcliffe	LSTPD Program
Zhong Ren	Renz Research, Inc.
Dave Riling	Thermo Electron Corp.
Dagmar Ringe	Brandeis University
Robert Rose	North Carolina State University
John Rose	University of Georgia
Arthur Schultz	Argonne National Laboratory
Paul Sigala	Stanford University
Nicholas Silvaggi	Boston University
Richard Simmerman	University of Tennessee
Harold Smith	Oak Ridge National Laboratory
Gerald Stubbs	Vanderbilt University
Lilyl Tam	Virginia Polytechnic Institute & State University
Ichiro Tanaka	Ibaraki University
Kuo-Hsiang (Joseph) Tang	Ohio State University
Raymond Teller	Argonne National Laboratory-IPNS
Kristina Thiagarajan	Oak Ridge National Laboratory
P Thiyagarajan	Argonne National Laboratory
Mark van der Woerd	BAE SYSTEMS
Ronald Viola	University of Toledo
Nancy Vogelaar	Virginia Tech
Bi-Cheng Wang	University of Georgia
Charles M. Weeks	Hauptman-Woodward Medical Research Institute
Stephen White	St. Jude Childrens Research Hospital
Sanath Wijerathna	University of Tennessee
David Worcester	University of Missouri, Columbia
Hongliang Xu	Hauptman-Woodward Medical Research Institute
Hai Xu	University of Tennessee
Charles Yard	State of Tennessee Oversight Office