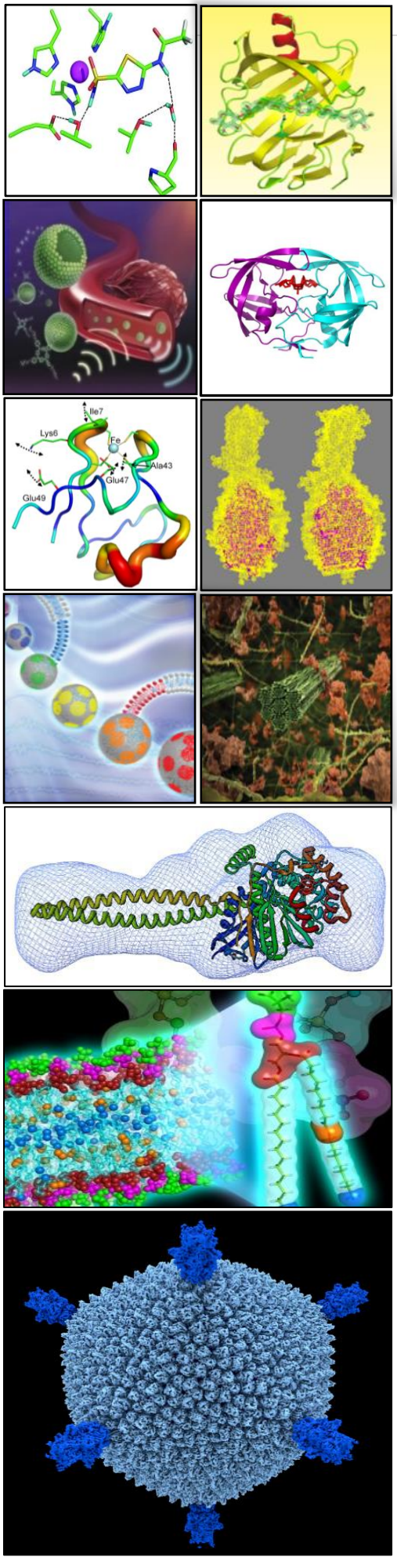

Grand Challenges in Biological Neutron Scattering

Workshop Report University of California San Diego, January 17th – 18th, 2014

Organizers: Susan Taylor (University of California San Diego) and Heidi Hamm (Vanderbilt University)

Grand Challenges in Biological Sciences for Neutron Scattering

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Executive Summary

Gaining a predictive understanding of the behavior of complex biological systems is one of the greatest scientific challenges that we will face over the next decade. This understanding will guide us in protecting and repairing physiological systems, it will allow us to mimic the architectures and processes of living systems to create new biomaterials and bio-inspired technologies, and it will provide the information necessary to manipulate micro-organisms and their ecosystems to create new biotechnology and biorefinery solutions to emerging energy and environmental challenges. A workshop was held to engage the scientific research community in further identifying grand challenges in biological sciences that neutron user facilities at ORNL can help to address. The major outcome was that neutrons can provide several types of unique information that will be important in addressing the identified challenges. Areas where neutron scattering is poised to have a major potential impact include membrane associated biological processes and the dynamic assembly and regulation of large biological complexes.

Neutron scattering will be most powerfully applied if combined with advanced deuterium labeling techniques and high performance computing. Neutrons are highly sensitive to hydrogen (H), the most abundant element in biological systems, and are sensitive in a different way to its isotope deuterium (D). This sensitivity to H and D allows for enhancing the visibility of specific parts of complex biological systems through isotopic substitution. Computer simulations using high performance computing allow for prediction and interpretation of neutron scattering data from systems that are too complex for analytical theory. Neutrons will also be most powerfully applied when combined with complementary experimental techniques that use photons and electrons. Photons and electrons interact with the atomic electric field and are most sensitive to heavy atoms; with just one electron H is all but invisible. Neutrons interact with nuclei; light atoms such as H are highly visible. Neutrons are also complementary to photons and electrons because they cause little radiation damage and are highly penetrating, enabling use of complex sample environments. These properties allow neutron scattering to be used to obtain precise information on the location and dynamics of H at the atomic level, as well as truly unique information on large, dynamic, multi-domain complexes at longer length and time scales using contrast variation.

Despite the advantages in using neutron scattering, significant technical gaps must be bridged not only in neutron scattering instrumentation but also in molecular biology, deuterium labeling and computational technologies. These gaps are discussed in the body of this report. They include the need for more advanced deuterium labeling techniques, better access to neutron beam-lines, increased neutron flux on available beam-lines, neutron beam-lines optimized for membrane diffraction, the development of innovative techniques for polarizing neutron beams and H atoms in samples to enhance scattering power and to dynamically control scattering contrast, the development of new instrumentation that allows simultaneous access to broad regions of time and space, better integration of high performance computing techniques with neutron scattering experiments, and the development of computational tools that allow the combination of experimental data from multiple complementary techniques to generate more complete models of complex biological systems.

Further, a major effort must be made to recruit a larger segment of the biology community to the new neutron resources that are becoming available at ORNL. Bridging those gaps will allow neutrons to be used in a transformative way to unify the structural and dynamical description of biological systems across length and time scales. This will transition the concept of a predictive understanding of biological systems to a reality. Emerging grand challenges at the interface of biology and neutron science over the next 10 years, and our recommendations to ORNL to help address them are given below:

Ten Grand Challenges

- **Bioengineering:** Understand and redesign plants, organisms and enzymes with new or improved properties and functions

- **Drugs:** Design multi-billion dollar drugs for human disease targets that have better target specificity, improved binding, and that are not susceptible to drug resistance.
- **Integration of structure and dynamics:** Unify the structural and dynamical description of biological systems.
- **Living cells and micro-organisms:** Understand how multi-domain and multicomponent molecular complexes are assembled and regulated during life processes, by dynamically visualizing and (computer) simulating complete living cells and micro-organisms.
- **Complex biological systems:** Seamlessly integrate information from different experimental techniques across length and time scales, and across different information types, using computational methods to obtain a predictive understanding of complex biological systems such as plant cell walls, complete living microorganisms, and multicellular systems such as microbial communities and the brain; from DNA sequences to systems biology.
- **Biomaterials:** Understanding the basic scientific principles that underpin photosynthesis, and other biological processes, as a basis for new manmade systems.
- **Biotechnology:** Transition from a biorefinery concept to its realization.
- **Membranes:** Understand natural bilayer membrane structure and dynamics, fusion, and vesicle, pore, and domain formation. Understand the structural and functional interplay between membranes and other molecules; membrane protein folding, structure and function; perturbation of membranes in disease.
- **Disorder and Flexibility:** Understand the roles of disorder and flexibility in biological systems; molecular communication mediating signaling and regulatory functions.
- **Kinetic Processes:** Determine the kinetics of signaling events within multi-protein assemblies

These grand challenges are a summary of several discussed in the body of the report. Also discussed is how the advanced neutron user facilities at ORNL can provide unique information that can help address these grand challenges. The new knowledge afforded will complement and extend information obtained from other user facilities utilizing techniques employing, for example, photons or electrons. Neutrons can probe large ranges of length and time scales; from Ångstroms to microns and picoseconds to microseconds; ideal for studying multi-scale phenomena intrinsic to biological processes.

Recommendations

- **Cold neutron flux:** Radically increase the flux of neutron beam-lines at long wavelengths in particular for small angle scattering, crystallography and spin echo.
- **Deuteration:** Establish advanced deuteration and sample preparation expertise and facilities
- **Access to facilities:** Improve access to neutron beam-lines and high performance computing.
- **New beam lines:** Build an improved spin echo beam-line and a second beam-line for studying aligned samples of membranes and biological fibers.
- **Computational tools:** Develop improved computational methods and tools that exploit high performance computing and that can integrate several/diverse experimental techniques with models and calculations.
- **Living cells:** Develop new cell and molecular biology techniques that will allow biological processes to be followed within living cells, model protocell platforms, and micro-organisms using neutron scattering in combination with deuterium labelling.
- **Time-resolved studies:** Develop technologies that allow more rapid data collection for time-resolved studies of kinetic processes.
- **Experiment and theory:** Develop a rigorous link from the theory of atomic systems to experimentally observable quantities.

- **Data sharing:** Develop integrated repositories for sharing data and computational tools for seamless access to complementary data for model building and systems analysis.
- **Community outreach:** Expand community awareness of the advantages of neutron scattering and recruit the biology community to the neutron sources that are available. At present the number of experiments that can be accommodated on available neutron beam lines is limited by the relatively weak flux of neutron beam lines. Accommodating more investigators will therefore require enhancing the efficiency of neutron beam lines to increase access.
- **Flagship experiments:** A small number of systems should be targeted for programs that can drive the technological developments, as discussed in the body of the report.
- **Improve integration of neutron and X-ray scattering**

Introduction

This report summarizes the outcome of a "biology grand challenges" workshop held at the University California San Diego (UCSD) over the weekend of January 18-19th 2014. The workshop is one of a series designed to engage the scientific research community in identifying grand challenges over the next 10 years that neutron user facilities at ORNL can help to address. At the workshop 37 invited researchers from over 20 different universities and institutes joined 5 participants from the Neutron Science Directorate of ORNL. The charge to participants was given in letters of invitation and was also read out at the start of the workshop:

"We have put together a small group of about 25-30 leading scientists to take part in a thought process to identify the needs of the scientific community in the area of Neutron Science as applied to biology. In particular we would like to understand possible areas of cooperation and overlap with other probes such, as photons and electrons, and how these orthogonal experimental techniques can best be integrated through modeling and computational methods. The key outcomes of this workshop will be presented to the Department of Energy to help in defining the future large-scale research user facilities. The outcome will include a list of 10 crucial problems that we face in biological research over the next 10 years that neutrons can help address."

The scientific program and agenda was organized by Susan Taylor (UCSD), Heidi Hamm (Vanderbilt University, VU) and Paul Langan (ORNL) and consisted of 5 topical sessions in which the areas of macromolecules, complexes, integrated techniques, signaling complexes, and membranes and biomaterials were discussed. Participants were given 15 minutes to speak followed by 5 minutes of discussion, and were asked to emphasize a 10 year outlook in their area of expertise. Each session was followed by time for discussion. The workshop finished with breakout sessions led by Jeremy Smith (University of Tennessee Knoxville, UTK, and ORNL), Robert McKenna (University of Florida, UF) and Susan Taylor (UCSD) to identify 10 crucial problems, or grand challenges, for the next 10 years. In the body of this report a brief summary is given of the presentations and discussions that took place in each session, followed by the full reports from the breakout sessions.

Report of presentations and Discussions

Macromolecules

Irene Weber (Georgia State University, GSU) discussed the potential application of macromolecular neutron crystallography (MNC) in drug design to combat HIV/AIDS by targeting the HIV protease enzyme (PR). Over the next 10 years there will be a need to determine structures of several drug resistant PR variants with drugs to explore molecular mechanisms of resistance. Drugs such as Darunavir were designed to increase the number of hydrogen (H) bonds to main chain PR atoms and therefore retain potency on resistant mutants, but H atoms are almost invisible to X-rays. MNC can visualize H atoms and represents a new way to guide the design of inhibitors with improved H-bond interactions. The ability to locate H atoms, and the scattering signal from biological crystals, can be further enhanced by deuteration; substituting H with its isoptope deuterium (D). An important future technology development will be combining MNC with quantum mechanics (QM) and molecular mechanics (MM) calculations to better understand reaction mechanisms of enzymes. Better knowledge of physical properties will improve QM/MM calculations and molecular dynamics (MD) simulations for predicting and simulating protein-ligand complexes, enzyme mechanisms and drug design. A major new

future direction will be the fusion of computational models/simulations with experimental structural data from neutron/ X-ray/ electron probes and dynamic analysis from NMR and EPR. That will require new computer software to integrate techniques and models.

Rob McKenna (UF) took us on a personal journey through the coming of age of MNC. Over the next 10 years MNC will be applied to design drugs that target different isoforms of carbonic anhydrase (CA) that are associated with diseases such as cancer and glaucoma. CA will also be bioengineered for various industrial and medical applications involving CO_2 conversion. Based on past experience, several challenges remain to be addressed before MNC can be more broadly used; there is a need for a national deuteration facility with focused resources; better access to MNC beam-lines; the development of cryogenic capabilities and crystallization kits for large crystals; better software for automatic location of H atoms from neutron data; new beam-lines with higher flux that can be used to study larger complexes.

Dagmar Ringe (Brandeis University and the National Science Foundation) gave an authoritative account of the past success of applying X-ray crystallography in structural enzymology and the unanswered challenges that remain. Visualizing H atoms in order to determine enzyme function, pKa values, improve ligand binding, and bioengineer new enzymatic functions not seen in nature, is one of the biggest remaining challenges and MNC can be developed and applied to address this challenge over the next 10 years. Although X-rays can be used to locate H atoms at ultrahigh resolution and cryogenic temperatures, there is evidence that pKa values within biomacromolecules can change as a function of temperature. The ability to collect MNC data at room temperature, because neutrons do not cause direct radiation damage, is therefore a major advantage for studying pKa values.

As we look forward to high performance computers having ever-increasing power, and the development of advanced computer simulation and quantum chemical calculation methods, it seems increasingly likely that computational methods will play a larger part in using experimental results to generate predictive models of biological systems. Jerry Parks (ORNL) described some of the first small steps taken to integrate information from MNC on H positions with computational methods to greatly enhance the accuracy of enzyme redesign. Specific examples included bioengineering diisopropyl flourophosphatase and xylose isomerase using MD/FEP and QM/MM methods with structures provided by joint neutron and X-ray crystallography. Over the next 10 years, there is an opportunity to combine ORNL's powerful neutron scattering and high performance computing capabilities into a unique integrated tool for bioengineer enzymes.

Neutron data are highly complementary to X-ray data, and the 3D structures of complex biological molecules are more complete and more accurately determined when these data are combined. However, BC Wang (University of Georgia Athens, UGA, and the SER-CAT beam-line at the Advanced Photon Source, Argonne National Laboratory) suggested that neutrons and X-rays can be used to provide additional information in a 4^{th} dimension on the chemical species of atoms. As a scientific grand challenge, BC proposed characterizing the metal components of the ribosome particles. He quoted one of his colleagues, Loren Williams (Georgia Institute of Technology), as saying *"…The origins and early development of the ribosome, billions of years ago, remain firmly imprinted in the biochemistry of extant life. The ribosome tells us part of the story of the origin of life and of the earliest biochemistry. The information contained within the ribosome … recapitulating critical chemical and biochemical steps in the origin and early evolution of life…"*

Grand Challenges

- Understand and redesign plants, organisms and enzymes with new or improved properties and functions.
- Design multi-billion dollar drugs for human disease targets that have better target specificity, improved binding, and that are not susceptible to drug resistance.

Capability Gaps

- Advanced deuteration and sample preparation expertise and facilities

- Restricted access to neutron beam-lines and their relatively low flux.
- Computational methods and tools that can integrate experimental techniques with models and calculations.

Opportunities

- Neutron crystallography is a new tool for drug design.
- The ability to collect neutron data at room temperature is a major advantage for studying pKa values and therefore enzyme mechanism.
- ORNL's powerful neutron scattering and high performance computing capabilities could be combined into a unique integrated tool for bioengineering enzymes.
- Combining neutrons and other experimental techniques with QM/MM calculations and MD computer simulations will provide better understand of reaction mechanism and ligand binding.
- The 3D structures of complex biological molecules are more complete and more accurately determined when neutron and X-ray data are combined. What about identifying metals (BC)?

Complexes

Susan Krueger (National Institute of Standards and Technology) explained why small angle neutron scattering (SANS), when combined with D-labeling and H_2O/D_2O contrast variation, is such a powerful technique for understanding the structure and arrangement of biological molecules and their complexes at the nanometer scale. Systems studied include proteins, nucleic acids, protein-nucleic acid complexes, multi-subunit protein complexes, protein-lipid complexes and model membranes. The science grand challenges for the next 10 years will be solved more easily by also solving the challenges to user accessibility to SANS techniques. In particular, better computational tools are required to help plan SANS contrast variation experiments, and to build model of complexes to fit the data. There has to be a strong internal program to develop sample deuteration and preparation methods to support short term users. There is also a need for improved computational methods for modeling complete sets of SANS data collected at different contrasts, and also for the incorporation of data from other experimental techniques such as X-ray crystallography, SAXS, EM, NMR and hydrodynamics. EM in particular is a technique that is seeing rapid development and provides images of complex systems that are highly complementary to the models derived from SANS. EM is underexploited at the moment in complementing SAXS and SANS.

Joseph Parello (VU) was a key figure in the earlier development and application of neutron scattering techniques to biology. Neutron scattering provides information not only on the conformation of biological systems but also on their internal dynamics. Neutrons can probe a large range of lengths from Ångstroms to microns, but they also have energies similar to atomic motions, allowing the study of dynamic processes over picosecond to microsecond time scales. As with elastic scattering, selective D labeling is important for best exploiting inelastic neutron scattering. An important point is that neutron spin echo techniques allow access to milliseconds dynamics, which are characteristic of correlated domain motions in proteins, and hard to access using other experimental techniques. In fact neutron spin echo techniques represent a unique method to verify molecular dynamics simulations which are increasingly providing new insights into the role of dynamics in biological function. Over the next 10 years, neutrons have a strong future in applications to study the internal dynamics and conformations of biological complexes, in particular those that involve membranes. However neutron techniques must be complemented by electron and photon approaches.

During the discussion sessions, Joseph also pointed out the potential of using spin polarization of protons in biological samples in combination with polarized neutron beams in order to enhance sensitivity. This approach was developed by Professor Stuhrmann in Berlin several years ago when the ribosome was under investigation by neutron scattering. However, it also has potential to be applied to crystal samples in order to greatly enhance the signal to noise ratio of neutron diffraction data.

Zimei Bu (City College of New York) has been using neutron scattering extensively to study the structure and dynamics of adapter proteins at the interface of the cell membrane and actin cytoskeleton, in particular the NHERF1 and Ezrin proteins that regulate membrane receptor and ion channel assembly, trafficking and signaling, and which influence cell adhesion and migration. These are multi-domain complex systems with intramolecular interactions that regulate protein-protein assembly. Although SAXS provides information on the conformation of the individual NHERF1 and Ezrin proteins, SANS in combination with contrast variation has provided additional information on structural changes that occur during their interactions. The application of neutron spin echo techniques has provided unique information on long range domain motions at the nanoscale and how they change as proteins bind. Over the next 10 years, one of the grand challenges will be to extend SANS to the study of signaling protein-adapter signaling complexes and other large multidomain, multicomponent complexes. In-cell neutron scattering to study signaling complexes would be truly revolutionary, but would require innovative new D labeling approaches. There is a need to systematically quantify the effects of deuteration if we are to go down this path. Finally neutron spin echo is intensity limited and to be broadly applied in biology that limitation has to be addressed.

Jack Johnson (Scripps Institute) discussed what SANS could tell us about virus particles and their maturation intermediates. After initial assembly viruses such as Papilloma virus, HIV, Herpesvirus, alpha viruses and flaviviruses gain stability and infectivity through programmed maturation. Both SAXS and SANS have been used to follow this process. The higher flux of X-ray beams allows higher spatial and time-resolution, whereas combining contrast variation with SANS provides unique information about the relative arrangement of different components of the virus, in particular proteins and nucleic acids. D/H exchange (HDX) mass spectrometry (MS) is also used to provide structural information on virus particles at the nanometer scale. When viruses are exposed to D_2O, H_2O in their interior is gradually exchanged. Regions that lacking secondary structure exchange more rapidly than regions that do. SANS could be used to provide information on gross structural changes to complement HDX MS, by following this exchange process as a function of viral maturation state. Over the next 10 years it is likely the cryo-EM will continue to change the game for studies of virus structure and function. Virus structure can now be determined from heterogeneous populations with “digital purification”, and transient states can now be studied with time resolved cryoEM. For SANS to contribute, technological advances will be required that promote rapid data collection and sample handling. More sophisticated computational analysis is required to interpret the experimentally determined accessibilities. Finally, repeating an emerging theme from the workshop, there is a need to computationally blend multiple types of data from HDX-MS, CryoEM and to tie the result to biological function.

Walter Chazin’s (VU) research on DNA replication involves developing an understanding of changing collections of proteins in multicomponent complexes as they are assembled and disassembly during their sequence of activities. Addressing this challenge requires not only information about the structure of these multi-protein machines but also on how they move. The approach that Walter has taken involves combining SAXS, SANS and NMR experimental techniques with computational modeling. The SAXS and SANS provide information about shape and NMR provides information about dynamics. The advantage of SANS for this system is that it can be combined with contrast variation to highlight buried single-stranded DNA, in a way that is impossible with SAXS. However for SANS to make a larger impact over the next 10 years, we will have to focus on improving three things: sensitivity, sensitivity and sensitivity, by increasing neutron flux. If this is possible there are important opportunities to integrate dynamics into molecular structure. One specific grand challenge would be revealing the structural dynamics of a replication fork at atomic resolution. Martin Egli (VU) discussed the molecular basis for the circadian clock in cyanobacteria. SAXS, SANS and cryoEM studies have begun to elucidate key complexes formed to rhythmically regulate the phosphorylated and dephosphorylation of key proteins in the cycle.

In the next 10 years we must meet the grand challenges from sequences, complexes and systems biology. That was the message that John Tainer (Scripps Institute and SIBYLS beam-line at the Advanced Light Source, Lawrence Berkeley National Laboratory) delivered, and emphasized by comparing the

doubling of DNA sequencing output every 9 months (over 3 billion years of evolution) compared to the current rate of doubling of magnetic disk storage and microprocessors MIPS which crawls along following Moore's Law (~2 years). John is helping to address this challenge by developing SIBYLS as a high throughput SAXS beam-line that enables protein/protein interactions and pathways to be determined, advancing us towards an interactome map controlling biological outcomes. Over the next 10 years, rapid data collection plus low sample requirements would make connecting dynamic protein structures to pathways robustly possible. Further, John described how SAXS can be used to obtain information on both structure and flexibility, both of which are required to understand processes such as complex assembly and disassembly, conformational or dynamic switching, ligand binding and order-disorder transitions. SAXS can better leverage X-rays for biology by providing shape, conformations and assemblies for most samples. Further, when it is combined with crystallography and NMR, SAXS enables structural systems biology for flexible complexes (pathways, networks).

Another synchrotron beam-line, this time BL4-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) at the SLAC National Accelerator Laboratory, was the subject of Thomas Weiss' (Stanford University) talk. BL4-2 has a broad structural biology program that uses SAXS, time-resolved SAXS, fiber diffraction, lipid diffraction and GiSAXS with an emphasis on developing high throughput automated approaches. Thomas also described some of the cutting edge new methods being developed at the Linear Coherent Light Source (LCLS) at SLAC, which will clearly revolutionize our ability to study biological systems. In particular, the extreme peak brightness of the LCLS enables diffraction patterns to be recorded on micron or sub-micron sized crystals, opening opportunities for systems where obtaining large enough crystals for synchrotron studies are challenging (*e.g*. membrane proteins). Further, the synchronization of fsec pulses with optical pump laser opens opportunity to study structural dynamics in sub-psec time domain and on the molecular-to-atomic scale. At future planned free electron x-ray lasers, it will be possible to collect molecular transforms from single molecules, removing the need for crystallization all together. Over the next 10 years neutron sources can exploit some of the technical advances being made at current synchrotron facilities. In particular, some of the sample environment developments pioneered on Bl4-2 and SIBYLS for in situ time-resolved studies have clear potential to be adopted by SANS beam-lines. Advances in the use of nanodiscs or special detergents to study membrane proteins may in fact be better exploited using contrast variation with neutrons.

Grand Challenges

- Extending neutron scattering and computer simulations to the dynamical visualization of large multidomain, multicomponent complexes within living cells and micro-organisms.
- Revealing the structural dynamics of a DNA replication fork at atomic resolution.
- Integrated information across DNA sequences, biological complexes, and systems biology.

Capability Gaps

- Advanced deuteration and sample preparation expertise and facilities
- Restricted access to neutron beam-lines and their relatively low flux.
- Computational methods and tools that can integrate data from multiple experimental techniques with models and calculations
- Computational tools to help plan SANS contrast variation experiments, and to build models of complexes to fit the data.
- Improved computational methods for modeling complete sets of SANS data collected at different contrasts, and also for the incorporation of data from other experimental techniques such as X-ray crystallography, SAXS, EM, NMR and hydrodynamics.
- In-cell neutron scattering to study signaling complexes would be truly revolutionary, but would require innovative new D labeling approaches.
- There is a need to systematically quantify the effects of deuteration.

- Neutron spin echo is intensity limited and to be broadly applied in biology that limitation has to be addressed.
- Technological advances will be required that promote rapid SANS data collection and sample handling.
- There is a need to computationally blend multiple types of data from SANS, HDX-MS and CryoEM and to tie the result to biological function.
- For SANS to make a larger impact neutron flux must be increased.

Opportunities

- SANS, when combined with D-labeling and H_2O/D_2O contrast variation is such a powerful technique for understanding the structure and arrangement of biological molecules and their complexes at the nanometer scale.
- EM in particular is a technique that is seeing rapid development and provides images of complex systems that are highly complementary to the models derived from SANS.
- Neutron scattering provides information not only on the conformation of biological systems but also on their internal dynamics.
- Neutron spin echo techniques allow access to milliseconds dynamics, which are characteristic of correlated domain motions in proteins, which are hard to access using other experimental techniques.
- Neutron spin echo techniques represent a unique method to verify molecular dynamics simulations which are increasingly providing new insights into the role of dynamics in biological function.
- Neutrons have a strong future in applications to study the internal dynamics and conformations of biological complexes, in particular those that involve membranes.
- SANS is highly complementary to HDX-MS, and combining these techniques would be powerful.
- Neutron sources can exploit some of the technical advances in sample environment being made at current synchrotron facilities.
- Advances in the use of nanodiscs or special detergents to study membrane proteins with X-rays may be better exploited using contrast variation with neutrons.

Integrated Techniques

One of Jeremy Smith's (UTK and ORNL) goals is to enable real time exascale neutron experimental design and interpretation. ORNL is developing the neutron source and supercomputer facilities required to realize this goal, but further development of high performance simulations optimized for supercomputers and algorithms for fast calculation of scattering factors are needed. With the current trend in advances in computers and computational methods it is entirely possible that within 10 years Jeremy will be simulating entire microorganisms. However, new concepts will be required to extend these simulations to biologically meaningful time scales. In the shorter term, the integration of neutrons with high performance computing will allow us to model disorder, complex conformational transitions, inter- and intra-domain dynamics in multicomponent complexes and drug binding.

Benoit Roux discussed his studies on the selectivity of the Na^+/K^+ ion pump and drug binding in Src kinase. Benoit uses several different experimental approaches, including crystallography, SAXS, SANS and EPR, to inform MD simulations and quantum calculations that provide functional insights. Remaining grand challenges over the next 10 years involve answering detailed questions about the presence of protons, hydrogen bonds and gating charges, improving the accuracy of force-fields and sampling issues, improving the accuracy and reliability of free energy calculations of conformational transitions, making the best use of mixed information at multiple resolutions, and developing a rigorous link from atomic systems to experimentally observable quantities.

Richard Kriwicki (Saint Jude Children's Research Hospital) discussed intrinsically disordered proteins (IDPs), which account for over 30% of human proteins. IDPs perform regulatory and signaling functions and are often disrupted in disease. Further, IDPs often fold upon binding their functional partners and are dynamic ensembles that exhibit varying degrees of compaction and structural heterogeneity. Over the next 10 years grand challenges will be in understanding how disordered regions within multi-domain and multi-subunit systems mediate molecular communication. That will require defining i) the structure of dynamic, multicomponent systems, and ii) the time- and length-scales of their interdomain interactions, *in solution.* SAXS/SANS have the potential to play an important role in revealing information on ensemble averaged conformations of individual components of dynamic systems (selective & segmental deuteration will be critical). HP computing and new computational methodologies will be needed to interpret SAXS/SANS data for these complex systems. Structural data from X-ray, NMR & modeling must be integrated with those from SAXS/SANS. A strategy to start addressing these grand challenges is to develop methodologies with currently tractable systems (e.g., p27) and extend them in the future to more complex systems (e.g., CBP/p300).

Grand Challenges

- Simulation of entire organisms.
- Developing a rigorous link from atomic systems to experimentally observable quantities.
- Answering detailed questions about the function of membrane proteins
- Understanding how disordered regions within multi-domain and multi-subunit systems mediate molecular communication.

Capability Gaps

- Development of high performance simulations optimized for supercomputers and algorithms for fast calculation of scattering factors are needed.
- New concepts will be required to extend simulations to biologically meaningful time scales
- Making the best use of mixed information at multiple resolutions.
- Improving the accuracy of force-fields and sampling issues
- Improving the accuracy and reliability of free energy calculations of conformational transitions.
- Selective & segmental deuteration.
- High performance computing and new computational methodologies are needed to interpret SAXS/SANS data for complex systems.
- Structural data from X-ray, NMR & modeling must be integrated with those from SAXS/SANS.

Opportunities

- Real time exascale neutron experimental design and interpretation.
- Simulation of entire organisms.
- The integration of neutrons with high performance computing will allow us to model disorder, complex conformational transitions, inter- and intra-domain dynamics in multicomponent complexes and drug binding.
- SAXS/SANS have the potential to play an important role in revealing information on ensemble averaged conformations of individual components of dynamic systems

Signaling Complexes

Heidi Hamm (VU) is studying signaling complexes, including G-proteins and their coupled receptors. G-proteins are heterotrimers (α,β,γ) and the receptors they couple to, which are studied by Heidi include rhodopsin, the β-adrenergic (AR) receptor, and various presynaptic receptors. Some of the most important remaining questions in the field concern i) Kinetics of conformational changes ii) How does GTP binding lead to reassociation of helical and GTPase domains? iii) Are the receptor-mediated G

protein activation mechanisms conserved? iv) What are the mechanisms of selectivity? and v) Many rich and biologically important protein interactions.[these items are not all phrased as questions!] Heidi is addressing these challenges by combining DEER, crystallography, SAXS, cryo EM, and modeling. However, an ideal technique has not yet been found to address the conformational changes and dynamics that occur at various stages during signaling, in particular when G proteins bind to their receptor. Neutrons could provide the solution. Over the next 10 years grand challenges will be in i) Topological aspects of membrane complexes, which could be addressed with SANS by matching out lipids with 15% D2O ii) Membrane-associated complexes, which could be addressed by deuterating one member of complex iii) Conformational changes and kinetics of domain motions, which could be addressed by neutron spin echo, and the association/dissociation of complexes, which could be addressed by time-resolved SANS. This will require significant enhancement of neutron scattering and deuteration techniques. Roger Sunahara (University of Michigan) presented his interest in the elucidation of the molecular mechanism by which hormones activate G protein-Coupled Receptors (GPCRs).

After activation by their receptors, G-proteins can interact with Adenylyl Cyclase to produce cAMP and initiate a cascade of cAMP dependent signaling processes. Giuseppe Melacini (McMaster University) discussed the molecular basis for selectivity in cAMP dependent signaling, as studied by NMR. Multiple cAMP dependent receptors are present in eukaryotes, and the fact that they share conserved binding domains (e.g. PKA, EPAC, HCN) presents a challenge in understanding their selectivity. One hypothesis is that dynamics plays a role in selectivity. Over the next 10 years challenges will involve studying a full thermodynamic cycle of binding and allostery to gain an understanding of how a highly conserved fold results in surprisingly different, selective binding domain targeting, how allosteric effectors control dynamics of hinges at domain boundaries and inter-domain re-orientations, and how to bring domain studies into the context of full-length systems with integral tertiary and ternary structures. Both NMR and SANS could play an important part in stitching all this structural and dynamic information together.

One signaling complex activated by cAMP binding is PKA. Susan Taylor (UCSD) discussed challenges for the next decade in understanding the structure, function and dynamics of the cAMP depended PKA signaling complex. PKA has served as an extensively studied model for investigations of the mechanism of all kinases. *In vivo*, the inactive PKA holoenzyme consists of two catalytic (C) and two regulatory (R) subunits. An increase in cAMP concentration, due to β-adrenergic stimulation of cells, activates PKA by providing four cAMP molecules that bind to the R subunits that in turn dissociate from the C subunits that had been bound in an inactive holoenzyme state. Susan uses several experimental techniques to study the PKA including microscopy, spectroscopy, X-ray and neutron small angle scattering and crystallography and computational simulations. Important questions to be addressed include how PKA is inhibited by the R-subunit and how cAMP activation is achieved. These questions are complicated by the existence of isoforms of the R-subunit that anchor the complex to other signaling proteins and to different regions within cells and are associated with different signaling processes, phenotypes, and diseases. Interestingly, PKA can be colocalized with the NHERF1 and Ezrin regulatory proteins studied by Zemi Bu, an opportunity for collaboration. SANS, neutron spin echo, and MNC all offer important new opportunities for studying the structure and dynamics of PKA and their extended and dynamic association with other signaling complexes. Another signaling protein that PKA can be colocated with is SERCA, which is involved in PKA-Mediated β-adrenergic signaling in the heart. Gianluigi Veglia (University of Minnesota) has developed oriented and MAS NMR spectroscopy techniques to study this and other membrane protein complexes.

Don Blumenthal (University of Utah) presented results from his collaborations with Susan on applying SANS to study PKA signaling complexes. Don pointed out that the R-subunit has an intrinsically disordered N-linker which is the most diverse sequence amongst the R isoforms. As the holoenzyme is assembled and dissembled there can be large conformation and dynamic changes, particularly in the R-subunit. There are significant advantages for using SANS in combination with contrast variation deuteration to study the changes to the individual subunits within the PKA complex. Over the next 10 years a grand challenge will be to develop multi-scale model systems (atomistic to

cellular) of multi-enzyme AKAP signaling complexes with membranes and integral membrane proteins (e.g., GPCRs and other receptors, ion channels, etc.). This could involve artificial/synthetic "cells" with the ability to simulate cAMP transients and the readout of PKA activation (e.g., ion channel fluxes, contraction, etc.) using biophysical and biochemical methods. It could result in computational models of complex multi-enzyme and cellular PKA signaling systems capable of predicting the effects of disease-causing mutations and drugs. Areas for collaborative development over the next 10 years include integrated data repositories for sharing of data and computational tools for seamless access to complementary data for model-building and systems analysis.

Geoff Chang (UCSD) finished this session on Complexes with a personal story of his passion for studying the structure, function and application of transporters in biomedicine. For Geoff an important point to keep in mind over the next 10 years is that structural biology is mostly a tool, and although tool development has obvious value, a strong biological basis is required. Neutron scattering is a tool that can be developed to look at complexes and conformations important to those biological challenges.

Grand Challenges

- Molecular basis for selectivity in cAMP dependent signaling.
- Understanding the structure, function and dynamics of the cAMP depended PKA signaling complex
- Topological aspects of membrane complexes, which could be addressed with SANS by matching out lipids with 15% D2O
- Membrane-associated complexes, which could be addressed by deuterating one member of the complex
- Conformational changes and kinetics of domain motions, which could be addressed by neutron spin echo, and the association/dissociation of complexes, which could be addressed by time-resolved SANS.
- Experimental systems (PKA signaling complexes and artificial cells) that can be studied using orthogonal methods.
- Multi-scale model systems (atomistic to cellular) of multi-enzyme AKAP signaling complexes with membranes and integral membrane proteins (e.g., GPCRs and other receptors, ion channels, etc.) that predict the effects of drugs and mutations.

Capability Gaps

- Integrated data repositories for sharing of data and computational tools for seamless access to complementary data for model-building and systems analysis.

Opportunities

- Neutrons could be an ideal technique to address the conformational changes and dynamics that occur at various stages during signaling, in particular when G proteins bind to their receptors.
- SANS, neutron spin echo, and MNC all offer important new opportunities for studying the structure and dynamics of PKA and their extended and dynamic association with other signaling complexes.

Membranes and Biomaterials

Bob Blankenship (Washington University in St. Louis) discussed SANS and MS studies of photosynthetic systems (PSs) that are designed to provide an understanding of the basic scientific principles that underpin the efficient functioning of the natural photosynthetic antenna system as a basis for manmade systems to convert sunlight into fuels. All PS organisms, including 6 known phyla of bacteria, contain light-gathering antenna systems such as giant chlorosomes or phycobilisome complexes. The reversible organization of pigments into oligomers in the PS chromosome from green sulfur bacteria is thought to be important for function at very low light intensity. Large SANS studies of optically

functioning chlorosome isolated from these bacteria and then fixed in a sol gel matrix have allowed structural characterization. The possibility of fast measurement times and the use of live cells mean that SANS can provide high resolution data on dynamic structural changes in membranes in live cells. In particular it has been demonstrated that SANS can be used to study rearrangements in the thylakoid membrane as it functions, or changes in membrane stacking due to truncations to the phycobilisome.

Loukas Petridis (ORNL) discussed how SANS is being combined with MD computer simulations and deuterium labeling to provide a dynamic visualization of lignocellulosic biomass and the morphological changes it undergoes during pretreatment processes involved in its conversion to biofuels and other bioproducts. This integrated approach has been used to characterize and provide new understanding of the behavior of lignin and also cellulose during pretreatment. The challenge over the next 10 years will be to transition from the concept of a biorefinery to an actual realization. This will require developing a more detailed understanding of cell wall structure and its response to chemical, physical and biological perturbation. Developing this understanding will require high-throughput experiments to provide the wealth of experimental data required to tailor pretreatment and to gain a predictive understand through computer simulations.

Steve White (University of California Irving, UCI) has been a leader for many years in developing the application of neutron diffraction to the study of membrane systems. Although the number of membrane protein structures being determined is going through a period of rapid growth, these structures must be considered in the context of a functioning bilayer, and this context can be provided by MD computer simulations or lamellar neutron diffraction. As with SANS, neutron diffraction from membrane systems is most powerful when combined with deuterium labeling and contrast variation, and also when neutron data are combined with X-ray data in joint refinement. Steve presented examples of this approach to locate melittin and the voltage sensor domain of KvAP channels. The biggest challenge in this area is the lack of a suitable beamline for neutron membrane diffraction measurements.

Alfredo Freites (UCI) further expanded on using MD computer simulations as a structural refinement tool for studying the structure and dynamics of proteins in membrane systems. Membrane diffraction and reflectivity experiments typically provide 1D information about scattering density in the direction perpendicular to the plane of the membrane. MD simulations of models of the proteins and bilayers, constrained by the 1D experimental scattering profiles, then provide a 3D interpretation of the system. A future challenge is to use atomistic MD simulations in concert with (incomplete or indirect) structural data to generate quantitative structure/function relationships. However, this will require bringing the structural analysis workflow to the 21st century, with high performance computing. Also selective deuteration of proteins, is not as straightforward as first thought, and requires significant technical development.

Huey Huang (Rice University) described the destructive interaction of two classes of proteins with membranes; amyloid forming peptides and pore forming peptides such as melittin. These interactions can be studied using small angle neutron diffraction, but current small angle scattering beam lines do not have sufficient resolution. There is a need for a small angle neutron diffractometer for partially aligned biology systems such as membranes and fibers. This instrument could be important for investigating the structure of functional β-amyloids.

Grand Challenges

- Understanding of the basic scientific principles that underpin the efficient functioning of the natural photosynthetic antenna system as a basis for manmade systems to convert sunlight into fuels.
- To transition from the biomass biorefinery concept to its realization.
- Understanding of cell wall structure and its response to chemical, physical and biological perturbation.
- Use atomistic MD simulations in concert with (incomplete or indirect) structural data to generate quantitative structure/function relationships of membrane proteins

- Bring the structural analysis workflow to the 21st century, with high performance computing.
-

Capability Gaps

- Lack of a suitable beamline for neutron membrane diffraction measurements.
- Lack of understanding of cell wall structure and its response to chemical, physical and biological perturbation.
- High-throughput (high intensity) SANS
- Access to high performance computing
- Selective deuteration of proteins requires significant technical development.
- A neutron small angle diffraction beam line needs to be developed with energy resolution better than 15%.

Opportunities

- Fast measurement times and the use of live cells mean that SANS can provide high resolution data on dynamic structural changes in membranes in live cells.
- There is an opportunity to engage a community of membrane and fiber diffraction researchers by building an appropriate beam line.

Reports from breakout sessions

Complexes

(Working Group: Blumenthal, Hamm, Kriwacki, Melacini, Parello, Sunahara, Taylor, & Veglia)

1) **Goal: To understand the structural and functional interplay between natural bilayer membranes and membrane proteins embedded within them.**

   Strategy: Apply SANS techniques to study the structural and dynamic features of lipids and membrane proteins in model bilayer systems that closely mimic the compositional, structural, electrostatic and dynamic features of natural membranes. In particular, probe the features of the lipid/protein interface using SANS methods that provide structural and dynamic information. Leverage structural data from other methods (X-ray, EM, NMR, DEER, etc.) as starting points for interpreting data from neutron experiments. Utilize computational methods to refine starting models to achieve the most accurate representation of lipid and protein structural features by maximizing agreement with neutron data. Apply neutron techniques that probe both the structural and dynamic features of the lipid and protein components of systems, with special attention to the lipid/protein interface. This builds a framework for thinking about the ways in which associated proteins can influence and interact with the membrane protein. Oligomerization state of the receptors is another major question that can be addressed by SAXS/SANS. Integration of computation with experimental [what?] information is critical.

   Target systems: GPCRs (Hamm,Sunahara,Handel), ion channels (Roux), transporters (Chang), pore forming proteins (Veglia).

2) **To understand how multi-domain complexes are assembled and regulated.** It is increasingly recognized that biological processes are mediated by macromolecular complexes that are assembled at specific sites such as the outer membrane of the mitochondria or the tail of an ion channel. How the components of these macromolecular complexes are assembled is often difficult to trap in a high resolution structure by crystallography and solving the structures of

these complexes can be challenging because the dynamic properties are such an important part of their function. By coupling low resolutions information from SANS to the higher resolution crystal structures should become an essential tool not only to understand the dynamic properties of the system but also to validate that the crystal structure reflects a biologically relevant state. In the case of multi-protein complexes, such as the scaffolded complexes associated with PKA, one can simply deuterated different proteins in the complex. For large multi-domain proteins one can use intein-mediated protein ligation to label domains. Taking advantage of the flexible linkers should facilitate this. Having access to the technologies for expressing and purifying deuterated proteins or labeled side chains should be routine and could be a major asset to the NMR community as well as to the structural biology community overall. How such complexes are then assembled at membranes and linked to membrane proteins is a challenge that merges with challenge 1. There is also a major opportunity to integrate with the cryoEM community and their ability to get higher resolution complexes. This is a frontier, like membrane crystallography, and a clear challenge for the next few years. By the end of the next decade this will be routine.

Target systems: PKA/AKAP scaffolds, GPCR/G-Protein scaffolds, kinase complexes – PKA isoforms, CDK complexes, Chemokines/chemokine receptors/intracellular scaffolds.

3) **Goal: To understand the roles of disordered regions within proteins in the molecular communications mediating their signaling and regulatory functions.**

Strategy: Apply SAXS and SANS in solution to determine the shapes of the dynamic ensembles that represent the structural features of proteins with disordered regions. Target studies on multi-domain proteins, and multi-protein complexes, in which disordered regions are experimentally known or implicated in signaling/regulatory function. Utilize subunit-specific and segment-specific deuteration to reveal the neutron scattering and thus shape features of individual structured domains and disordered regions within multi-domain proteins and multi-subunit protein complexes. Utilize SAXS data to complement those from SANS to define the shape features of entire molecular systems. Represent structured domains using data from other methods (X-ray, NMR & computational modeling). Use structural parameters from NMR (chemical shift values, PRE data, RDC data, relaxation data, etc.) to define the conformational and dynamic features of disordered regions; utilize selective/segmental labeling to obtain these data for select subunits/regions. Use neutron spin-echo methods to characterize dynamics on the ns to μ[?]sec time-scale; utilize selective/segmental deuteration to obtain these data for select subunits/regions. Use computational methods to generate ensembles of atomistic models consistent with all input data (SAXS & SANS, atomic coordinates for structured domains, and NMR data for disordered regions). Validate and refine ensemble models using distance and/or distance distribution data from complementary techniques (DEER & single-molecule fluorescence); utilize mutagenesis within specific subunits or segments to introduce the probes required for these experiments. Develop quantitative in vitro assays of the molecular communication processes that mediate signaling and regulatory function. Utilize emerging theories on the influence of the sequence features of disordered protein regions on their compaction and dynamics to guide sequence manipulations (termed "sequence permutation") within these regions to predictably alter their structural features. Determine the influence of sequence permutation on system structure and function using the methods described above to establish relationships between protein disorder and signaling/regulatory function.

Target systems: protein kinase regulatory systems (p27/Cdk/cyclin- Kriwacki) (PKA R&C – Taylor- and scaffolds), acetyltransferase systems (CBP/p300 – Kriwaki/Wright), cell adhesion regulators (NHERF1, Ezrin, Merlin – Bu/Taylor), transcriptional regulators (p53), other systems.

4) **Goal: To determine the kinetics of molecular signaling events within multi-protein assemblies.**

Strategy: Utilize photo-labile reagents to trap multi-domain and multi-protein signaling systems in pre-signaling states. Use pulsed laser methods to trigger signaling events and use SANS to monitor signaling-associated structural changes. Vary the time between photo-induction of signaling and initiation of SANS measurements to characterize the kinetics of structural changes. Time-resolved SAXS to explore dynamics of conformational changes. Introduce intein-mediated protein ligation as routine tool for exploring domain motions in multi-domain proteins. Use complementary structural methods and functional assays to measure the kinetics of the same photo-induced signaling events (fluorescence methods, biochemical assays with fast read-outs). Utilize structural data and computational methods to generate molecular models that describe the structural trajectories associated with signaling events. Correlate the kinetics of structural changes with the kinetics of signaling.

Target systems: Apply protein these kinetic approaches to the membrane/membrane protein and disordered protein systems described above, as well as other multi-protein signaling systems.

ADDITIONAL COMMENTS. A major goal is to recruit large segments of the biology community to the new neutron resources that are available to them. There are grand challenge opportunities for understanding new chemistry and for integrating rigorous computational tools with experimental data. Understanding dynamics is essential and is especially important as we now begin to look at large macromolecular complexes. How do we integrate high and low resolution data? How do we learn new chemistry? How do we learn new biology? To make the neutron resources widely used by the biological community one will need to expand awareness of the power of SANS and also the power of integrating computational and experimental resources. Neutron diffraction will provide extremely interesting new chemistry while back scattering and spin echo will provide essential information to bridge chemistry and physics, but these techniques are very expensive and costly and will not be used by a large segment of the biological community. However, SANS should be used routinely and can become an integral tool for many biological systems as we move to higher levels of complexity. Deuterated proteins and introducing deuterated domains or specific side chains are resources that could be highlighted at ORNL and their importance should be widely appreciated as an opportunity for enhanced understanding. It is important for the NMR community, where labeled proteins are essential but very expensive, for the IDP (intrinsically disordered protein) community, where the disordered proteins and regions have such rich biological function, the membrane protein crystallography community, where the next frontier will be understanding how the integral membrane protein is regulated by the ligands, proteins and proteins complexes that it associates with , the cryoEM community, where the resolutions at 3-4 angstroms will soon be routine, and lipid protein interfaces, where we are just beginning to appreciate that lipids serve as co-factors as well as a dynamic hydrophobic platform for proteins and protein complexes to function.

Communities to recruit to ORNL:

Intrinsically Disordered Proteins
NMR
Hybrid Methods
CryoEM
Membrane Protein Structure
Lipid:protein interfaces

Participation in national meetings. We could have special sessions at larger meetings or even organize a dedicated meeting to build on the workshop that we just held. However, there should

be sessions or workshops on neutron resources at the big national meetings such as the Protein Society and the Biophysics Society.

Gordon Research Conference. Could we establish a regular meeting?
Keystone meeting.
Hybrid Methods meetings
Protein Society meeting.
Biophysics Society meeting.

Workshops and Team Building

Workshops at ORNL and perhaps SDSC. West coast/East coast sites. This is an excellent way to educate users and to build a knowledgeable users community.

Building teams of biophysics and biology users. Bridging this interface is essential. This could be the theme of the Gordon Conference. You do not want to just bring in the biophysicists who know the power of neutrons already. You want to educate the large biological community and work with them as a team from the beginning. HOW CAN NEUTRONS ENHANCE OR UNDERSTANDING OF BIOLOGY? This is a driving question that should be emphasized.

BUILDING A USER FRIENDLY COMMUNITY IS ESSENTIAL.

Additional Resources for community

Expression and purification of deuterated proteins.
Intein-mediated protein ligation.
Site-specific residues.
Unnatural amino acids.

Program Project Grants.

We should definitely explore this immediately with the NIH staff. They will be looking for ways to creatively use this mechanism now that the big PSI grants will be discontinued. Several would already be obvious.

IDPs – Kriwacki
GPCRs – Hamm, Sunaharo, Handel
Scaffolds – Bu, Taylor

GRAND CHALLENGE CONCEPTS. Areas where we are poised to have a major potential impact.

Correlation of computational simulations, dynamics and experimental data.
Impact on membrane proteins and lipid:protein dynamics.

Dynamic regulation of large macromolecular complexes.

Macromolecules

(Working Group: McKenna, Parks, Ringe, Wang & Weber)

1) One data set per day per instrument*
Meeting this challenge will:

Permit the ability to obtain novel macromolecular structures and the sampling of multiple variants of a system to enable structure activity analysis (SAR) for: the development of inhibitors to human disease targets, engineering enzyme active sites for novel chemistry, and re-engineering enzymes for design properties such as thermal and acid stability for industrial uses.

Goals:

a) Drive neutron science technology development in: Focusing optics, source intensity, detector sensitivity etc., as well as in deuteration methods and refinement software.

b) Enable neutron crystallographic studies of diverse macromolecules including proteins, nucleic acids, and complex macromolecular machines.

c) Identify H/D atoms, water orientation, protonation states in diverse macromolecules, which is important for drug design and engineering of macromolecules with novel functions.

d) Provide the experimental basis to further develop computational approaches by creating a significant macromolecular neutron structure database - that would feed into computation methods and systems development.

*Data set defined as 2 angstrom resolution, with greater than 90% completeness, redundancy greater than 3, and Rpim less than 20.

Notes: Will require the movement of the neutron detector (angle and distance).

2) 4D neutron diffraction

Meeting this challenge will:

Permit the correct identification of metals in macromolecular assemblages using neutron anomalous scattering approaches. Metals are critical components of many macromolecular and molecular machines, being utilized in transport and storage, enzymes, signal-transduction, transcription factors, and the ribosome.

Goals:

a) Drive the development of anomalous scattering-like approaches for the identification of metals in biology such that atoms and atomic properties can be distinguished by differences in neutron elastic, inelastic and anomalous scattering.

b) Remove the ambiguity in metal identification in protein data base (PDB), by extending the usable wavelength of X-rays which is limited to between 1-2 angstroms.

c) Correcting errors in the PDB with metal identification will directly feed into aiding force field developments for computation methods.

Notes: MacPhail, MR. Phys. Rev. 57, 669–676 (1940) Anomalous Scattering of Fast Neutrons. Mg can be identified by neutron anomalous measurements. Mg ions are important in nucleic acids, protein-nucleic acid complexes (including ribosomes) and many enzymes. Need to check which metals could be identified from neutron scattering.

3) National deuteration facility

Meeting this challenge will:

Provide a center of expertise and production of deuterated biological and small molecules. There is a national need for this resource as many areas of neutron structural biology require deuterated biological materials (proteins, lipids, carbohydrates, and nucleic acids) and enzyme substrates and inhibitors.

Goals:

a) Drive neutron science technology development in large scale sample deuteration for both expression and purification of macromolecules and synthetic chemistry to produce small molecules (inhibitors, substrates) to probe these systems.

b) The use of deuterated samples in scattering studies would remove experimental ambiguity in exchangeable H/D within macromolecules and allow for unambiguous data interpretation. It would also reduce the sample size (crystals) requirements for neutron structural analysis.

c) Provide a resource of deuterated biologically important small molecules (cholesterol, nucleotides etc) that would directly aid in neutron structural biology in the understanding macromolecular mechanisms.

d) Provide a facility with the chemistry expertise to aid in synthesis of small molecule inhibitors directed against disease/pathogen targeted proteins, this would directly aid in neutron structural biology towards rational drug design.

Notes: Need understanding of H_2O/D_2O physical properties and possible effect on biology and mechanism interpretation. Need to cross talk with already established NMR discipline.

4) Integration/cross fertilization of experimental and computational techniques
Meeting this challenge will:
Permit the effortless exchange of information and software for data mining across multiple structural disciplines, allowing a coalescence of NMR, crystallography, EM, SAX, SAN, and computation structural information for use in the biomedical field.
Goals:
a) Develop data formatting and storage of all the neutron structure based methods to be unified and accessible across disciplines. This will also require the involvement of the PDB.
b) Cross discipline will create the flow of ideas, method developments, and better integration of combined structural knowledge.

5) Utilization of polarized neutrons as a source
Meeting this challenge will:
Permit the elimination of background scattering to significantly improve the signal to noise ratio of neutron scatter data acquisition
Goals:
a) Drive neutron science technology development and polarization of neutron sources.
b) Will improve the quality of neutron structures and aid in the development for new scattering information such as using neutron anomalous scattering approaches for metal identification.
c) Initiate the development of low temperature data collection.

6) Incorporation of hybrid quantum mechanical/molecular mechanical methods
Meeting this challenge will:
Permit accurate refinement of metalloenzymes with complex active-site electronic structures. Metalloproteins are essential to much of biology, being used in transport and storage, enzymes, signal-transduction, transcription factors, etc.
Goals:
a) Improved molecular mechanics force fields with or without polarization, efficient algorithms for computing relative and absolute binding free energies, enhanced sampling and multi-scale techniques for investigating protein dynamics on long time scales.
b) Improve computationally efficient density functional theory and *ab initio* quantum chemical methods with better descriptions of dispersion effects, metal-ligand binding energetics, pKas, and other properties and improved scalability on supercomputers. Free energy computation approaches such as finite-temperature string methods and swarms-of-trajectories approaches.

Biomaterials and integrated techniques

(Working Group: Smith, Petridis, Roux, Huang, White, Freites & Blankenship)

1) Be able to perform measurements and simulations revealing weak functional associations in very large membrane protein complexes. Examples: photosystem antenna systems, respiratory complexes in mitochondria; mitochondrial translocons.
2) Understanding complex biological tissues in a multiscale fashion e.g., plant cell wall.

3) Find a unified theoretical description and experimental protocol to join NSE with SANS to derive complete structural and dynamical information on domain communication in macromolecules.
4) Molecular basis for amyloid perturbation of cell membranes in the disease context.
5) Extending experiment and simulation to understand membrane protein folding and assembly so as to improve the prediction of the three dimensional structure of membrane proteins from amino-acid sequence.
6) Understanding disorder and flexibility in biological systems. (Determining the correct ensemble from many that would fit experimental profiles)
7) Design a multi-billion-dollar drug using techniques including neutrons (against, for example HIV, carbonic anhydrase or IDPs).
8) Neutron crystal structure of membrane proteins permitting higher definition of function (such as ion conduction).
9) Membrane fusion: hemifusion to full fusion in the context of vesicle formation.
10) Organizing a workshop that does not take up a holiday weekend.

Agenda

Structural Biology, Biomaterials and Bioengineering Workshop
Identification of Science Grand Challenges
University of California San Diego
Leichtag Building 107
January 18–19, 2014

Saturday, January 18th

8:30 - 9:00 a.m. **Welcome and charge**

- Paul Langan, Oak Ridge National Laboratory
- Susan Taylor, University of California San Diego

9:00 - 10:20 a.m. **Macromolecules 10-year outlook**
Chair: Paul Langan

- Irene Weber, Georgia State University
- Dagmar Ringe, Brandeis University
- Bi-Cheng Wang, University of Georgia
- Palmer Taylor, University of California San Diego

10:20 - 10:40 a.m. **Coffee**

10:40 - 11:40 a.m. **Macromolecules 10-year outlook (con't)**

- Robert McKenna, University of Florida
- Jack Johnson, Scripps Research Institutes
- Jerry Parks, Oak Ridge National Laboratory

11:40 a.m. - 12:00 p.m. **Discussion**

- Chairperson Paul Langan

12:00 - 1:00 p.m. **Lunch with continued discussion**

1:00 - 2:00 p.m. **Complexes 10-year outlook**
Chair: Susan Taylor

- Susan Krueger, National Institute of Standards and Technology
- John Tainer, Scripps Research Institutes
- Joseph Parello, Vanderbilt University

2:00 - 2:20 p.m. **Coffee**

2:20 - 3:40 p.m. **Complexes 10-year outlook (con't)**

- Walter Chazin, Vanderbilt University
- Zimei Bu, City College of New York
- Thomas Weiss, Stanford Synchrotron Radiation Laboratory
- Martin Egli, Vanderbilt University

3:40 - 4:00 p.m. **Discussion**

- Chairperson Susan Taylor

4:00 - 5:00 p.m. **Integrated techniques 10-year outlook**
Chair: Jeremy Smith

- Jeremy Smith, University of Tennessee/Oak Ridge National Laboratory
- Richard Kriwacki, St. Jude Children's Research Hospital
- Benoit Roux, University of Chicago

5:00 – 5:20 p.m. **Discussion**

- Chairperson Jeremy Smith

5:20 p.m. **Adjourn**
Dinner at Susan Taylor's house

Sunday, January 19th

9:00 - 10:20 a.m. **Signaling complexes 10-year outlook**
Chair: Walter Chazin

- Heidi Hamm, Vanderbilt University
- Giuseppe Melacini, McMaster University
- Tracy Handel, University of California San Diego
- Roger Sunahara, University of Michigan

10:20 - 10:40 a.m. **Coffee**

10:40 a.m. - 12:00 p.m. **Signaling complexes 10-year outlook (con't)**

- Susan Taylor, University of California San Diego
- Gianluigi Veglia, University of Minnesota
- Donald Blumenthal, University of Utah
- Geoff Chang, University of California San Diego

12:00 - 12:20 p.m. **Discussion**

- Chairperson Walter Chazin

12:20 - 1:00 p.m. **Lunch with continued discussion**

1:00 - 2:40 p.m. **Membranes and Biomaterials 10-year outlook**
Chair: Dean Myles

- Robert Blankenship, Washington University in St. Louis
- Stephen White, University of California Irvine
- Loukas Petridis, Oak Ridge National Laboratory
- Alfredo Freites, University of California Irvine
- Huey Huang, Rice University

2:40 - 3:00 p.m. **Discussion**

- Chairperson Dean Myles

3:00 - 4:40 p.m.	**Coffee & Breakout sessions** **Identify 10 Crucial Problems for Next 10 years – Role of Neutrons and Other Techniques**
4:40 - 5:50 p.m.	**Reports and Discussion**
5:50 p.m.	**Close**

List of Participants

Bethea, Katherine	Oak Ridge National Laboratory
Blankenship, Robert	Washington University in St. Louis
Blumenthal, Donald	University of Utah
Bu, Zimei	City College of New York
Chang, Geof	University of California, San Diego
Changeux, Pierre	University of California, San Diego
Chazin, Walter	Vanderbilt University
Egli, Martin	Vanderbilt University
Freites, Alfredo	University of California, Irvine
Hamm, Heidi	Vanderbilt University
Handel, Tracy	University of California, San Diego
Huang, Huey	Rice University
Johnson, Jack	Scripps Research Institutes
Kriwacki, Richard	St. Jude Children's Research Hospital
Krueger, Susan	National Institute of Standards and Technology
Langan, Paul	Oak Ridge National Laboratory
McKenna, Robert	University of Florida
McQueeney, Robert	Oak Ridge National Laboratory
Melacini, Giuseppe	McMaster University
Myles, Dean	Oak Ridge National Laboratory
Parello, Joseph	Vanderbilt University
Parks, Jerry	Oak Ridge National Laboratory
Petridis, Loukas	Oak Ridge National Laboratory
Ringe, Dagmar	Brandeis University
Roux, Benoit	University of Chicago
Smith, Jeremy	Oak Ridge National Laboratory
Sunahara, Roger	University of Michigan
Tainer, John	Scripps Institute
Taylor, Palmer	University of California, San Diego
Taylor, Susan	University of California, San Diego
Tennant, Alan	Oak Ridge National Laboratory
Urban, Volker	Oak Ridge National Laboratory
Veglia, Gianluigi	University of Minnesota
Walker, Ross	University of California, San Diego
Wang, Bi-Cheng	University of Georgia
Weber, Irene	Georgia State University
Weiss, Thomas	Stanford Synchrotron Radiation Laboratory
White, Stephen	University of California, Irvine

First Letter of Invitation to Participants

Dear Colleagues:

As part of the thought process to identify the needs of the scientific community in the areas of Neutron Science and possible areas of cooperation with Photon Science, we are organizing workshops to identify the Science Grand Challenges for the next decade. Workshops are being organized in three complementary topics: Quantum Condensed Matter (@ LBNL), Energy Science and Technology (@Chicago) and Biological Systems (@UCSD). One of the goals of these workshops is to better understand how neutron and other experimental scattering probes complement each other and, where they overlap, to understand their relative strengths. The key outcomes of all these workshops will be presented by the workshop leads to DOE HQ to help in defining the future course of these user facilities. In order to facilitate deeper interactions, these workshops are limited to about 40 participants and are by invitation only.

With this letter, we are inviting you to join us in defining the needs in Neutron Science as relevant to Biological Systems. We are planning to hold the workshop on 18th and 19th of January at University of California San Diego. There will be no registration fee for the workshop and local arrangements will be covered by the workshop. Limited travel funds will be available. In order to facilitate the logistics of organizing the workshop, we would appreciate it if you could let us know by return email if you are able to join us, by the 15th of December.

We look forward to a vigorous and thought-provoking workshop.

Best wishes,

Susan Taylor and Heidi Hamm
Workshop Conveners

Paul Langan
Workshop facilitator

R. Ramesh
Workshop facilitator

Second Letter of Confirmed Participants

Dear Colleagues:

Thank you for agreeing to participate in our workshop at University of California San Diego in January. We have put together a small group of about 25-30 leading scientists to take part in a thought process to identify the needs of the scientific community in the area of Neutron Science as applied to biology. The number of participants has been limited in order to facilitate deeper interactions. In particular we would like to understand possible areas of cooperation and overlap with other probes such, as photons and electrons, and how these orthogonal experimental techniques can best be integrated through modeling and computational methods.

The workshop will begin at 8:30am on the 18th and close at 5:30pm on Sunday 19th. The format of the workshop will be a mixture of short talks with an emphasis on a 10-year outlook in different scientific and technical fields, and discussion sessions. An agenda will be sent by December 13th with a requested title for your contribution. The key outcomes of this workshop will be presented to the Department of Energy to help in defining the future large-scale research user facilities. The outcome will include a list of 10 crucial problems that we face in biological research over the next 10 years that neutrons can help address.

There is no registration fee for the workshop and local arrangements will be covered by the workshop. A block of rooms is being reserved at the Del Mar Inn in La Jolla. Limited travel funds will be available. For travel assistance please contact

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We look forward to a vigorous and thought-provoking workshop.

Best wishes,

Susan Taylor and Heidi Hamm
Workshop Conveners

Paul Langan
Workshop facilitator

R. Ramesh
Workshop facilitator

Acknowledgements

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