Neutron Scattering for Biological Research: Progress at the Bio-SANS Beam Line

S. Qian*, S.V. Pingali, K. L. Weiss, V. Urban, H. M. O'Neill, P. Langan

Center for Structural Molecular Biology, Oak Ridge National Laboratory, Oak Ridge, TN, USA 37831
*qians@ornl.gov

ABSTRACT

Structure-function relationships remain a critical theme in understanding many important biological processes regarding energy, disease and other applications. At Oak Ridge National Laboratory, home to two of the most powerful neutron sources for research, the High Flux Isotope Reactor (HFIR) and Spallation Neutron Source (SNS), we have developed an open-access user instrument, the Bio-SANS. As an instrument dedicated for biology-related research, it applies techniques of small-angle neutron scattering (SANS) to a broad range of research topics. The unique advantage of neutron scattering contrast, often naturally occurring between different types of biomolecules such as protein, lipids, RNA/DNA, etc., affords researchers the ability to study the structures of individual components in complex biological systems and under biologically relevant conditions. Furthermore, the high penetration power of neutrons and the lack of radiation damage make SANS well-suited for the study of large, multi-component biological complexes both *in situ* and *in vivo*.

Keywords: neutron scattering, structural biology, SANS, protein, membrane, RNA, DNA, hierarchical structure, kinetic processes

1 INTRODUCTION TO SMALL ANGLE NEUTRON SCATTERING

Small-angle scattering (SAS), developed with two probes X-ray photons (SAXS) and neutrons (SANS), respectively, is a structural technique that can obtain the size, shape, correlation, and other dimensional properties of bulk materials. While it is not an atomic resolution method like crystallography and nuclear magnetic resonance spectroscopy. It can be applied more broadly to materials in different states, with structure features from a few nanometers to a few hundred of nanometers.

In biological and medical research, the materials, including biological macromolecules, engineered polymers, etc., usually require solution conditions for them to carry out the desired function. SAS is able to obtain structural information on those systems that are not amenable to other techniques.

The fundamental principles governing SAS, irrespective of X-ray photons and neutrons are the same, with the differences in the interaction of the scattering particles, X-ray photons are sensitive to the distribution of electron density, while neutrons are sensitive to the nuclei of the atom. SAXS and SANS are complementary to each other. The difference in neutron scattering properties of different elements and their isotopes, especially hydrogen and deuterium, provide significant contrast variation that can be taken advantage of (Figure 1). The abundance of hydrogen in biomaterials and the development in deuterium-labeling techniques make neutron probe preferable in such biomaterial research. Neutrons are highly penetrating due to their neutral charge. The low energy neutrons used in typical SANS experiment are in the orders of mW, which causes little radiation damage to bio-active samples.

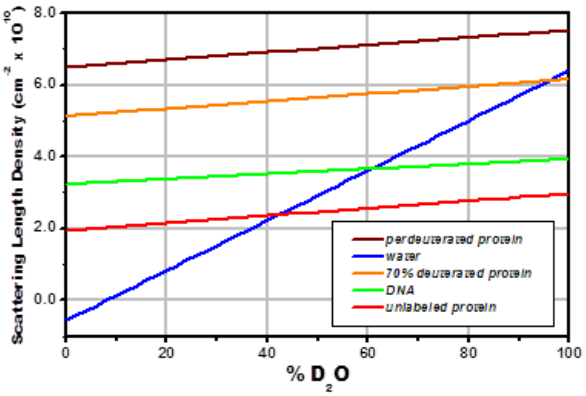

Figure 1. Scattering length densities of a few common molecules as a function of D_2O ratios. The differences in the scattering length density provide neutron contrast between different biomolecules that can be matched by different ratio of D_2O.

As a quick reference, here is a list of structural information can be obtained from SANS: particle size, size distribution, shape, correlation, fractal dimension, molecular weight, distance distribution, detailed structure by more sophisticated structural modeling such as *ab initio*,

rigid body modeling, molecular dynamic simulations and etc. Please note, not all approaches are applicable in a certain system; a careful design of the SANS experiment is crucial to the success, like any other experiment.

2 THE BIO-SANS INSTRUMENT

The Bio-SANS instrument (Figure 2), operated by the Center for Structural Molecular Biology (CSMB), is located at one of the most powerful research reactors, the High Flux Isotope Reactor (HFIR).

Figure 2. A view of the Bio-SANS instrument from the direction of neutron source.

With the high flux cold neutron source of HFIR, we have developed the Bio-SANS into a versatile tool for different types of biological research. Specifically, we have developed various sample environments tailored to biomaterial research and we are continuing to upgrade the instrument detector system for better dynamic range and enhanced productivity. The Bio-SANS instrument is supported by additional ORNL capabilities that include the advanced computational tools for analysis and modeling, as well as biophysical characterization and X-ray scattering infrastructure.

2.1 Instrument Specifications

A cold neutron sources situated in the HB4 beam tube of HFIR provides neutron for the Bio-SANS. The neutron wavelength λ is adjustable from 6-25 Å with a mechanical velocity selector of wavelength resolution from Δλ/λ=9-45%. Pin-hole apertures are used to collimate the neutron beam. Neutrons are delivered to the sample position by 8 sections of neutron collimator boxes equipped neutron guides, providing a variable source-to-sample distance for the different divergence on the sample. A sample aperture can further shape the beam size from 1 to 20 mm at the sample position. The detector, situated in a vacuum tank, is movable to provide sample-to-detector distance of 1.1 to 15.5 m, affording a q range ($q = \frac{4\pi\sin(\theta)}{\lambda}$, where 2θ is the scattering angle) of 0.001 to 0.7 $Å^{-1}$. A two-dimensional linear position-sensitive detector developed by ORNL provides count rate up to 1M Hz count rate. Although data acquisition time depends highly on the sample concentration and contrast, the typical exposure time ranges from a few minutes to a few hours.

Recently, we have upgraded data reduction software to a Python based framework called Mantid (mantidproject.org). It makes possible a one-click experience for data reduction and merging.

2.2 Sample Environment

Biological and biomaterials research encompass a range of physical states that include colloids, gels, liquids, fibrils and etc. Environmental factors can play a critical role in those studies due to the complex relationship between structure and properties. Therefore, a wide range of sample environments are available for the users of the Bio-SANS.

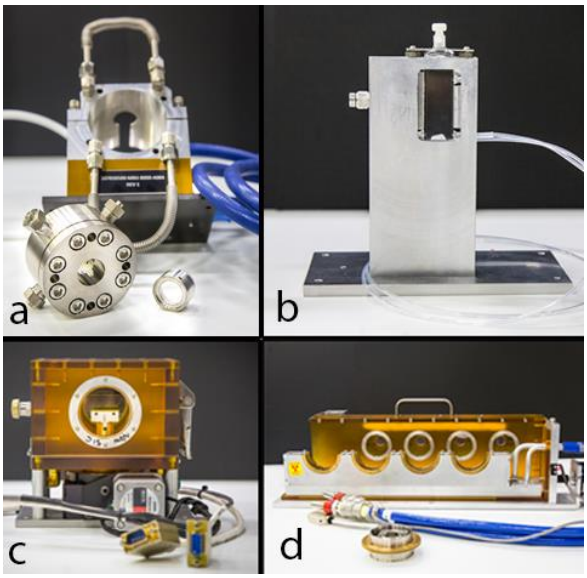

Figure 3. Specialized sample environments: (a) Enhanced-angle high pressure cell; (b) multi-phase flow cell; (c) relative humidity cell with rotational stage; (d) the 'tumbler' rotation cell

The typical sample holder at the instrument is a automated multi-position sample holder for cylindrical or rectangular liquid cell (Hellma quartz cell). Typically, a sample volume of ~100 micro liter to a few mL can be accommodated according to the requirements of the experiment. In addition, titanium sample cells with detachable quartz windows can be used for gels, slurries, and solid samples and as alternate to the cylindrical liquid cells. A circulating water bath controls sample temperature from 5 to 90 ^{o}C.

Recently we have commissioned a few sample environments. A high pressure cell with a maximum operating pressure of 0.5 kbar and temperature of 473K is

available. A flow cell was developed to study phase separation in a multi-phase water-oil system with the ability to perform spatial and temporal structure study. We have developed a humidity controlled chamber for samples sensitive to water content. A humidity generator delivers relative humidity from 3 to 95% (±1%). The chamber houses with a rotational stage which enables both grazing-incidence and transmission geometry, especially useful in examining film-like samples. A 'tumbler' rotating cell is available for sample suspensions that are prone to settling. By controlling the rotation rate of the cell, the sample can be kept in the part of the cell that is being illuminated by the neutron beam.

Furthermore, we have collaborated with users to develop additional novel sample environments to enable innovative experiments.

2.3 Detector Expansion

Currently, different sample-to-detector distances at the Bio-SANS, like other SANS instruments, are required to obtain a broad *q*-range to cover the length scales of the structure in many complex biological systems with hierarchical structure. To increase the dynamics range of the instrument, we are in the process of commissioning a 'wing' detector (Figure 4 and 5), on the instrument (available to general user after July 2016). With this upgrade, the Bio-SANS is able to cover a q range of 0.006 to 1 $Å^{-1}$ in a single measurement, increasing the dynamic *q*-range from ~x40 to x166. It gives the Bio-SANS world-leading simultaneous *q*-range coverage and to enable new types of experiments that track *in-situ* structural changes over time, temperature, humidity and other conditions.

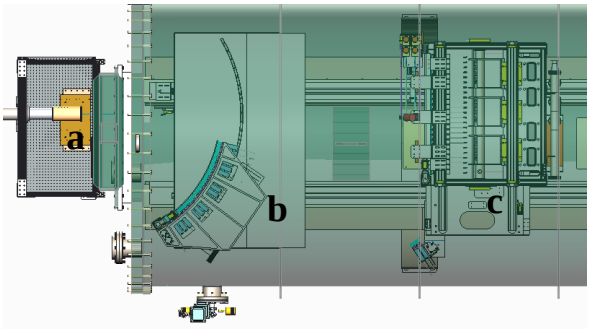

Figure 4. Top view of the Bio-SANS instrument with two detector arrays in the vacuum tank (green-shaded). The yellow region (labeled as *a*) is the sample area outside the tank. The main detector (labeled as *c)* shown on the far right can be moved along the rails inside the tank. The expanded wing detector (labeled as *b*) is curved and positioned near the sample area. Curved to reduce geometry distortion to the data, it can rotate around sample area on an arc rail.

2.4 The Bio-Deuteration Laboratory

One of the greatest advantages in SANS is the contrast tunability provided by the very different neutron scattering properties of hydrogen and deuterium. The abundance of hydrogen in biological materials makes it possible to replace hydrogen with deuterium and thus selected parts of a biomolecular complex. Such labeling has a minimal effect on structure and function. The deuteration capability becomes necessary to the success of many neutron scattering techniques. The CSMB operates the Bio-Deuteration Laboratory to develop techniques for expressing and purifying deuterium-labeled proteins and other biomolecules as part of the user program. General users access the expertise and resources through the same mechanism as the Bio-SANS.

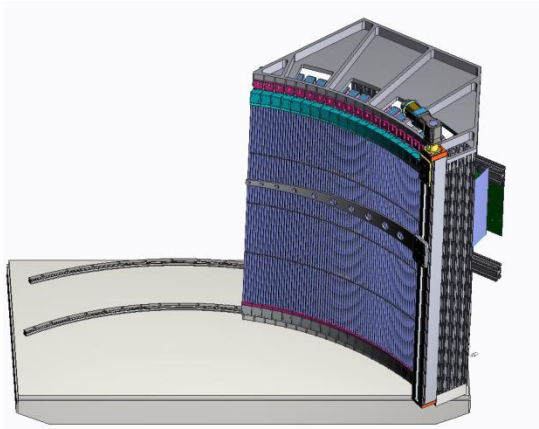

Figure 5. Front view of curved wing detector.

3 RECENT RESEARCH EXAMPLES

Over the years the Bio-SANS has enabled a user community from universities, research institutes and industry to study a range of complex biological systems including protein, protein/DNA complexes, lipid membranes, virus, bio-active gels, fiber and fibrils, detergent and microemulsions and hierarchical systems. Here are some recent highlights of user research. A complete list of publications can be found at the Bio-SANS webpage (http://neutrons.ornl.gov/biosans/publications).

3.1 Biomaterials and Biotechnology

Engineered bio-active or bio-compatible materials are important to many medical, biological or energy applications. For example, the Bio-SANS helped the understanding of the structure of the gelation of a helical N-substituted homopolypeptide poly(L-proline) (PLP) in water[1], an aqueous solution of poly(oligo(ethylene oxide monomethyl methacrylate)-grafted silica nanoparticles)[2], and diblock copolymer micelles for bio-inspired light-harvesting system[3]. SANS is widely applied in the study of polymers, the Bio-SANS is used to provide very basic understanding on those systems, such as scattering function

for branched wormlike chains [4], and coacervate micelles and hydrogels from ionic diblock and triblock copolymers[5].

In addition, a large part of the research at the Bio-SANS addressed understanding biomass structure and changes in nanoscale structure during biomass deconstruction using thermochemical pretreatments[6,7].

3.2 Biomolecular Complexes

The Bio-SANS has played an important role as an important tool for structural biology, especially in understanding of the structure of biological macromolecules in solution.

In protein research, for example, it revealed the trimeric structure of CESA catalytic domain of Arabidopsis cellulose synthesis complex[8], the assembly of alpha-Synuclein in lipid membrane environment[9], the oligomerization state of the Peridinin-Chl alpha-protein[10], protein kinase A[11], macromolecular crowding on intrinsically disordered protein[12], detergent interactions with photosystem I[13], Huntingtin's diesease[14], and the membrane protein ExbB-ExbD complex in a membrane-mimetic environment[15].

SANS is especially userful in understanding the complex lipid membrane organization with or without stimuli such as antimicrobial peptides[16,17], and drugs[18]. Recently SANS also helped to shed light on the size and morphology of lipid raft[19].

4 CONCLUSIONS

The Bio-SANS is a high-flux, low background instrument designed and optimized for analysis of the structure, function, and dynamics of complex biological systems. We are continuing to develop it as a leading instrument for biological research.

The Oak Ridge National Laboratory Center for Structural Molecular Biology (FWP ERKP291) is supported by the Office of Biological and Environmental Research of the US Department of Energy. Research at the High Flux Isotope Reactor and the Spallation Neutron Source of Oak Ridge National Laboratory was sponsored by the Scientific User Facilities Division, Office of Basic Energy Sciences, US Department of Energy.

REFERENCES

1. Gkikas, M., Avery, R. K. & Olsen, B. D. Thermoresponsive and Mechanical Properties of Poly(l-proline) Gels. *Biomacromolecules* **17,** 399–406 (2016).
2. Lorenzo, A. T., Ponnapati, R., Chatterjee, T. & Krishnamoorti, R. Structural characterization of aqueous solution poly(oligo(ethylene oxide) monomethyl methacrylate)-grafted silica nanoparticles. *Faraday Discuss.* (2016). doi:10.1039/C5FD00137D
3. Adams, P. G. *et al.* Diblock Copolymer Micelles and Supported Films with Noncovalently Incorporated Chromophores: A Modular Platform for Efficient Energy Transfer. *Nano Lett.* **15,** 2422–2428 (2015).
4. Vogtt, K., Beaucage, G., Weaver, M. & Jiang, H. Scattering Function for Branched Wormlike Chains. *Langmuir* **31,** 8228–8234 (2015).
5. Krogstad, D. V. *et al.* Small Angle Neutron Scattering Study of Complex Coacervate Micelles and Hydrogels Formed from Ionic Diblock and Triblock Copolymers. *J. Phys. Chem. B* **118,** 13011–13018 (2014).
6. Pingali, S. V. *et al.* Morphological changes in the cellulose and lignin components of biomass occur at different stages during steam pretreatment. *Cellulose* **21,** 873–878 (2014).
7. He, J. *et al.* Controlled incorporation of deuterium into bacterial cellulose. *Cellulose* **21,** 927–936 (2013).
8. Vandavasi, V. G. *et al.* A Structural Study of CESA1 Catalytic Domain of Arabidopsis Cellulose Synthesis Complex: Evidence for CESA Trimers. *Plant Physiol.* **170,** 123–135 (2016).
9. Anunciado, D., Rai, D. K., Qian, S., Urban, V. & O'Neill, H. Small-angle neutron scattering reveals the assembly of alpha-synuclein in lipid membranes. *Biochim. Biophys. Acta BBA - Proteins Proteomics* **1854,** 1881–1889 (2015).
10. Jiang, J. *et al.* Oligomerization state and pigment binding strength of the peridinin-Chla-protein. *FEBS Lett.* **589,** 2713–2719 (2015).
11. Blumenthal, D. K. *et al.* The Roles of the RIIβ Linker and N-terminal Cyclic Nucleotide-binding Domain in Determining the Unique Structures of the Type IIβ Protein Kinase A A SMALL ANGLE X-RAY AND NEUTRON SCATTERING STUDY. *J. Biol. Chem.* **289,** 28505–28512 (2014).
12. Goldenberg, D. P. & Argyle, B. Minimal Effects of Macromolecular Crowding on an Intrinsically Disordered Protein: A Small-Angle Neutron Scattering Study. *Biophys. J.* **106,** 905–914 (2014).
13. Le, R. K. *et al.* Analysis of the solution structure of Thermosynechococcus elongatus photosystem I in n-dodecyl-β-d-maltoside using small-angle neutron scattering and molecular dynamics simulation. *Arch. Biochem. Biophys.* **550–551,** 50–57 (2014).
14. Perevozchikova, T., Stanley, C. B., McWilliams-Koeppen, H. P., Rowe, E. L. & Berthelier, V. Investigating the Structural Impact of the Glutamine Repeat in Huntingtin Assembly. *Biophys. J.* **107,** 411–421 (2014).
15. Sverzhinsky, A. *et al.* Amphipol-Trapped ExbB–ExbD Membrane Protein Complex from Escherichia coli: A Biochemical and Structural Case Study. *J. Membr. Biol.* **247,** 1005–1018 (2014).
16. Qian, S. & Heller, W. T. Melittin-induced cholesterol reorganization in lipid bilayer membranes. *Biochim. Biophys. Acta BBA - Biomembr.* **1848,** 2253–2260 (2015).
17. Qian, S., Rai, D. & Heller, W. T. Alamethicin Disrupts the Cholesterol Distribution in Dimyristoyl Phosphatidylcholine–Cholesterol Lipid Bilayers. *J. Phys. Chem. B* **118,** 11200–11208 (2014).
18. Khadka, N. K., Cheng, X., Ho, C. S., Katsaras, J. & Pan, J. Interactions of the Anticancer Drug Tamoxifen with Lipid Membranes. *Biophys. J.* **108,** 2492–2501 (2015).
19. Heberle, F. A. *et al.* Hybrid and Nonhybrid Lipids Exert Common Effects on Membrane Raft Size and Morphology. *J. Am. Chem. Soc.* **135,** 14932–14935 (2013).