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Biomembranes research using thermal and cold neutrons

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ABSTRACT

In 1932 James Chadwick discovered the neutron using a polonium source and a beryllium target (Chadwick, 1932). In a letter to Niels Bohr dated February 24, 1932, Chadwick wrote: "whatever the radiation from Be may be, it has most remarkable properties." Where it concerns hydrogen-rich biological materials, the "most remarkable" property is the neutron's differential sensitivity for hydrogen and its isotope deuterium. Such differential sensitivity is unique to neutron scattering, which unlike X-ray scattering, arises from nuclear forces. Consequently, the coherent neutron scattering length can experience a dramatic change in magnitude and phase as a result of resonance scattering, imparting sensitivity to both light and heavy atoms, and in favorable cases to their isotopic variants. This article describes recent biomembranes research using a variety of neutron scattering techniques.

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1. Introduction

Achieving a predictive understanding of cells requires moving beyond individual genes and proteins, toward functional, interacting systems compartmentalized in space and time. The various cellular compartments are defined by lipid bilayer-based membranes, composed of hundreds of distinct lipid species, and densely packed with a host of embedded and peripherally associated proteins. Far from being a passive barrier, membranes provide functional interfaces that mediate and control critical cellular processes, including fusion, trafficking, signaling and communication. A comprehensive understanding of cellular membranes – their structure, dynamic behavior and function – remains a grand challenge, primarily due to their intrinsically multi-molecular and mesoscopic scale. As two-dimensional fluid mixtures stabilized by weak interactions, membrane structure and function emerges only at a minimum length scale of approximately ten nanometers, corresponding to ensembles comprising hundreds of cooperating molecules. Furthermore, their inherent disorder makes membranes unsuitable for study by traditional structural biology methods such as X-ray crystallography that have revolutionized the study of proteins and nucleic acids (Strandberg et al., 2009; Garman, 2014).

Among probes amenable to the study of membranes, neutrons stand out both for their non-destructive nature, and their multi-scale spatial and temporal information content. For example, neutron scattering techniques probe length scales ranging from angstroms to microns, dynamics occurring over picosecond to millisecond time scales, and kinetics spanning sub-seconds to hours. The value of neutrons in biology derives from the nature of their interaction with matter. Unlike X-rays, which interact with electrons, neutrons are scattered by atomic nuclei and can distinguish between elements of similar atomic and/or mass numbers. While X-ray interaction strength increases in proportion to atomic number, neutron scattering lengths show little variation across the periodic table (Table 1). Moreover, a distinct advantage of neutron scattering methods is their sensitivity to hydrogen, coupled with their ability to distinguish between the stable isotopes of hydrogen, protium and deuterium (i.e., ^{1}H and ^{2}H, referred to subsequently as H and D, respectively). Of the major classes of biomolecules (lipids, proteins, nucleic acids and carbohydrates), membrane lipids are the richest in hydrogen, and are thus readily detected and easily distinguished from other classes in a neutron scattering experiment. H/D isotopic labeling enhances selectivity and obviates the need for chemical tags and their associated artifacts (Morales-Penningston et al., 2010). As mentioned, thermal and cold neutrons are for the most part non-destructive, making them especially suitable for studying easily damaged unsaturated lipids, which are abundant in biology and the subject of much current interest. Important scattering techniques include small-angle neutron scattering (SANS), neutron diffraction (ND), neutron reflectometry (NR), neutron spin-echo

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Table 1
Neutron coherent scattering lengths and incoherent cross-sections in biological materials.

Isotope	Atomic number	Neutron incoherent cross section (10^{-24} cm^2)	Neutron coherent scattering length (10^{-12} cm)
^{1}H	1	80.27	-0.374
^{2}H (D)	1	2.05	0.667
^{12}C	6	0.00	0.665
^{14}N	7	0.49	0.937
^{16}O	8	0.00	0.580
^{24}Mg	12	0.00	0.549
^{32}S	16	0.00	0.280

spectroscopy (NSE) and quasi-elastic neutron scattering (QENS) (see Ankner et al., 2013 for introduction and review). Applications of these techniques range from probing the local environment of detergent solubilized proteins in single crystals, to solution scattering studies of membrane proteins incorporated in micelles, bicelles and vesicles, to scattering and diffraction from planar interfaces and surfaces in single layer or stacked lamellar systems.

Neutron scattering data alone do not provide a description of structures and events at the atomic level. However, simulations can often supply a critical link connecting scattering data to structure and dynamics, providing both interpretation and insights that guide subsequent experiments. In this regard, membrane systems have proven enormously challenging for simulation because of the number of atoms involved, the nature of their interactions, and the broad range of timescales relevant to biological membranes (nanoseconds to milliseconds). Molecular dynamics (MD) simulations have proven to be an effective approach for investigating these complex biological systems. In MD, the forces between atoms are described by a simplified empirical potential that attempts to mimic the 'real' underlying interaction potential. The primary output of an MD simulation is a trajectory of atomic coordinates through time, from which dynamic, structural and thermodynamic properties can be calculated. In favorable cases, MD simulations constitute a bridge between macroscopic (experimental) observations and microscopic details (Pan et al., 2012, 2014a,b). This review article highlights current research where neutron scattering, in conjunction with other techniques, including simulation, has played a significant role in addressing questions of current interest in molecular biology.

2. Neutron scattering basics

2.1. Neutron production

Soon after the discovery of the neutron by Chadwick (1932), the first demonstrations of neutron diffraction were performed using a Rn–Be source (Halban and Preiswerk, 1936; Mitchell and Powers, 1936). The first neutron scattering experiments using nuclear reactors came later, using the CP-3 reactor at Argonne (Zinn, 1947) and the X-10 pile at Oak Ridge (Wollan and Shull, 1948) (for a historical review, see (Mason et al., 2013)). These developments inspired the construction of facilities for the large-scale production of neutrons, subsequently creating new areas of research. Large quantities of neutrons suitable for scattering experiments are produced either by the fission of uranium-235 (*e.g.*, ORNL's 85 MW High Flux Isotope Reactor HFIR) or by spallation, where high velocity particles (*e.g.*, protons) produced by an accelerator impinge on a heavy metal target (liquid mercury in the case of ORNL's Spallation Neutron Source, SNS) (Harroun et al., 2009). The resulting fast neutrons are slowed ('thermalized') by passing through and interacting with a moderator (*e.g.*, H_2O, D_2O, graphite, Be). They can be further slowed through interactions with a cold moderator, for example liquid hydrogen at ~20 K. Neutrons for scattering experiments generally have wavelengths ranging from 1–20 Å, making them ideal probes for the study of a wide range of soft and hard materials.

2.2. Neutron scattering techniques

Neutron scattering is capable of probing material structural properties ranging from nanometers (10^{-9} m) to micrometers (10^{-6} m). Structure on these length scales is of interest to a wide range of scientific disciplines, including biology (Fitzsimmons et al., 2004; Shin et al., 2010; Breyton et al., 2013; Mehan et al., 2013). As a general rule, the neutron wavelength λ and range of detectable scattering angles $[\theta_{min},\theta_{max}]$ determine the accessible experimental length scale d, through the relationship $d = \lambda/2\sin\theta$. A number of different scattering techniques, each with different geometries and sample requirements, have been developed to probe different structural aspects of a sample.

2.2.1. Small-angle neutron scattering

In a SANS experiment, the large-scale structure (10–10^4 Å) of an unoriented sample (*e.g.*, a protein in solution, or a vesicle

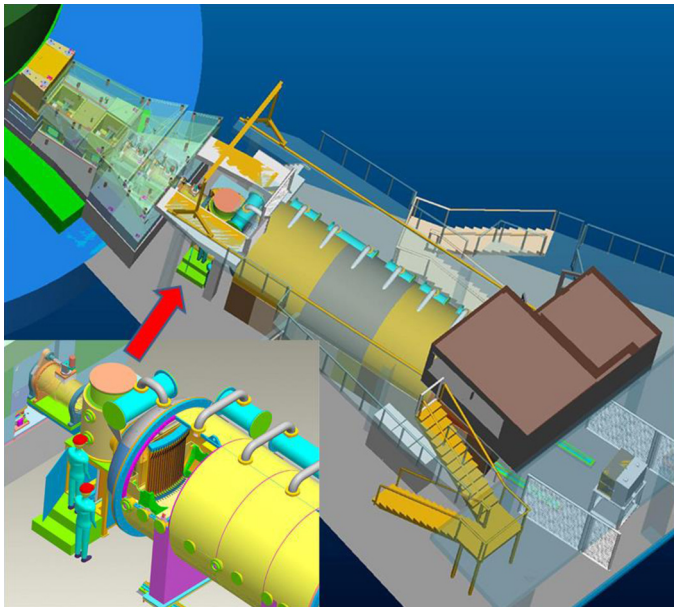

Fig. 1. Schematic of the Extended Q-Range Small Angle Neutron Scattering (EQ-SANS) instrument at the Spallation Neutron Source (SNS, ORNL). The instrument is capable of interrogating samples ranging from 0.1 to 100 nm. The two-dimensional detector is 1 m^2 and the sample to detector distance can be varied, in the evacuated detector tank (green with gray stripe), from 1.3 to 9 m. The inset shows the sample chamber, which is located at one end of the detector tank. Neutrons are produced at the mercury target and transported to the sample chamber, located 14 m from the target. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)