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NEB (Nudged Elastic Band)

► Carboxypeptidase A – Computational Studies

(Negative) Lateral Tension Profile

► Lipid Bilayer Lateral Pressure Profile

Nerve Growth Factor (NGF)

► Molecular Simulations of NGF Protein

Neural Networks

► Computational Modeling of Neuronal Networks

Neuraminidase

► Influenza Neuraminidase – Computational Studies

Neutron Diffraction

► Diffraction Methods for Studying Transmembrane Pore Formation and Membrane Fusion
► Macromolecular Neutron Diffraction

Neutron Scattering of Membranes

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Synonyms

Lipid bilayer

Definition

We discuss the use of neutron scattering as a tool for revealing the internal structure of biological membranes.

Basic Characteristics

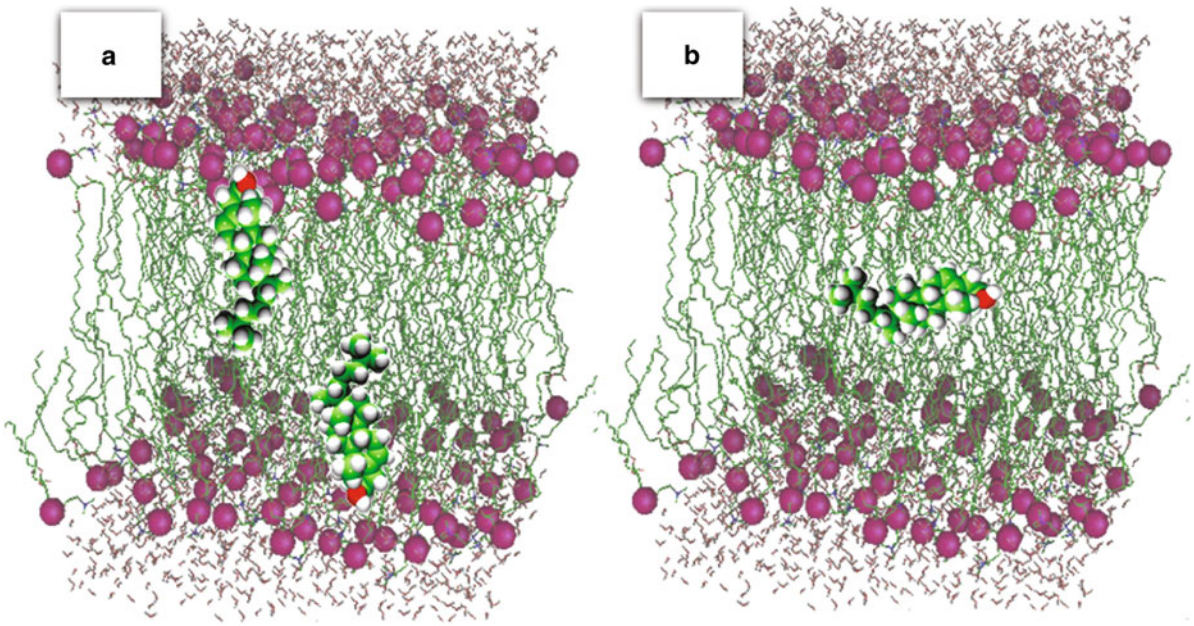
Clifford Shull and Bertram N. Brockhouse were awarded the 1994 Nobel Prize in Physics "*for pioneering contributions to the development of neutron scattering techniques*." Today, neutron scattering offers a powerful suite of techniques for determining atomic and spin structures, and dynamics (i.e., local and collective motions). These techniques are widely used in condensed matter physics, materials science, materials chemistry, polymer science, the biological sciences, and engineering. Because of their intrinsic properties, thermal and cold neutrons are ideally suited for studying a wide range of systems, including biological materials. For example, the interaction with matter of neutrons with energies less than 1 eV reveals atomic resolution detail, as their wavelengths ($\lambda > 0.03$ nm) are of the order of interatomic distances. Similarly, because their energies are comparable to chemical bond energies, and thermal motions, in crystals and liquids, neutrons are extensively used in dynamical studies. Neutron scattering techniques are also complementary to those of X-rays and electrons, especially in studies of biological materials inherently rich in hydrogen and low atomic number elements. In fact, neutron scattering takes advantage of such materials, where contrast variation methods – the selective substitution of hydrogens for deuterons – can either amplify or nullify the scattering from selected parts of a biomolecule (Fitter et al. 2006).

The technique of elastic neutron scattering has been successfully applied to four general areas of biophysics: high-resolution single crystal diffraction; a) low-resolution diffraction from samples with long-range order in one or two dimensions; b) reflectometry from thin films; c) and d) small-angle scattering from randomly oriented structures. The detailed analysis of protein interactions mediated by hydrogen atoms is usually non trivial to determine by X-ray crystallographic studies, as it is difficult to locate hydrogen atoms even when high-resolution X-ray data are available – i.e., X-ray scattering increases with increasing atomic number. On the other hand, high-resolution neutron diffraction studies have accurately revealed the positions of hydrogen atoms, and distinguished nitrogen atoms from similar atomic number elements, such as carbon and oxygen (Schoenborn and Knott 1996).

Neutron Diffraction

With regard to structural biology, the various scattering techniques complement crystallographic studies that, in many cases, require hard-to-obtain, high-quality crystals of macromolecules. Due to the intrinsic disorder present in biomimetic systems – disorder is considered important for the proper function of biological systems – the vast majority of membrane samples do not form perfect, or even near perfect crystals, that are needed to solve structure to atomic resolution. The limited amount of attainable data from such samples is then best described by broad statistical distributions and a membrane's overall characteristics. For example, in the case of model membrane systems (i.e., positionally correlated structures), the position and amplitude of Bragg reflections reveal the membrane's lamellar periodicity and one-dimensional scattering density profile. Further, by changing the systems "contrast" through the exchange of H_2O for D_2O, it is possible to determine the effect of membrane additives, such as cholesterol, on the lipid bilayer and its associated water layer, and the extent that water penetrates into the bilayer. For example, bilayers made of short- and long-chain phospholipids thickened in the presence of cholesterol. This manifestation by cholesterol on bilayer thickness was interpreted by Kučerka et al. as a disorder-order transition induced by cholesterol on the lipid's acyl chains. This effect dominates over the other option of rectifying the hydrophobic mismatch (Kučerka et al. 2009).

Another set of experiments that take advantage of the H/D substitution is selective labeling. The use of bulky labeling probes is ubiquitous in fluorescence light microscopy – and other techniques (e.g., electron spin resonance) – but they can affect a membrane's structural and dynamical properties. For the most part, substituting H for D does not alter the system's chemicophysical properties but does profoundly change the system's contrast. Importantly, neutron data from a system with and without the label differ only in the region of the label. A Fourier difference profile can then directly localize the labeled portion of a molecule (Schoenborn and Knott 1996). Over the years, this technique of isotopic substitution has been extensively used to study the structure of model membranes, proteins, DNA/RNA, and viruses



Neutron Scattering of Membranes, Fig. 1 Schematic of cholesterol's orientation in (**a**) commonly studied lipid bilayers and (**b**) PUFA lipid bilayers (Harroun et al. 2006)

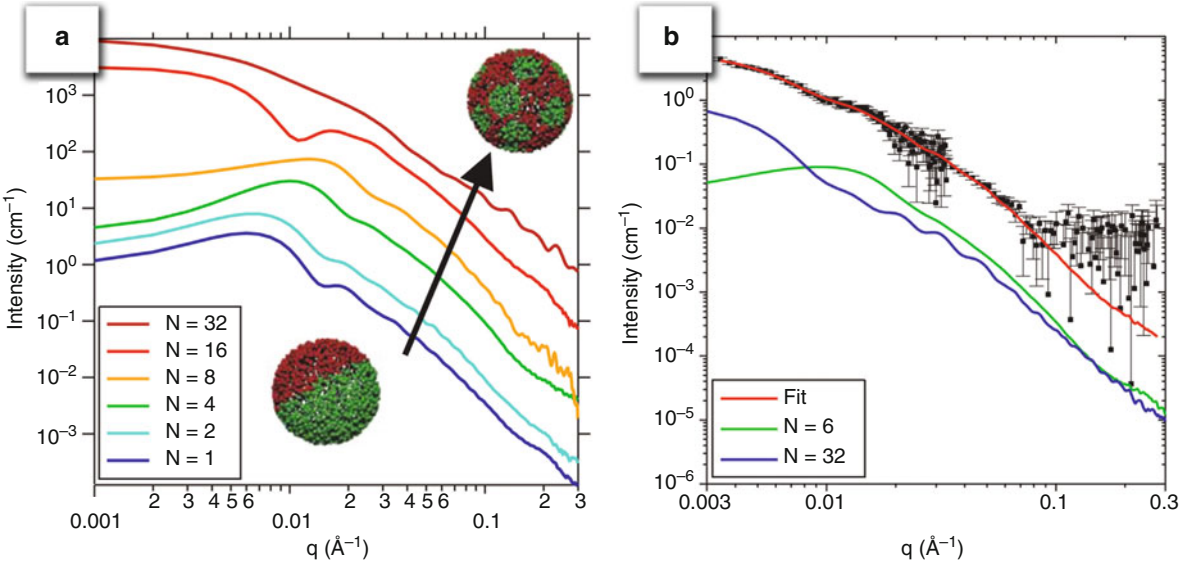
(Pabst et al. 2010). Using this technique, the location of cholesterol in lipids with polyunsaturated fatty acid (PUFA) chains was determined.

Cholesterol is understood to orient parallel to the lipid hydrocarbon chains. In 2006 using deuterated cholesterol, Harroun et al. demonstrated this to be the case in a number of different lipid bilayers. In contrast, however, cholesterol was found to sequester in the center of PUFA lipid bilayers (Fig. 1). Follow-up studies demonstrated the modulated preference of cholesterol for different lipids, clearly highlighting the need for the great diversity of lipids found in biology (Kučerka et al. 2010). For example, in plasma membranes sphingolipids are primarily located in the outer monolayer, whereas unsaturated phospholipids are more abundant in the inner leaflet. It is thus possible that the presence of PUFA in the inner bilayer leaflet may serve to enhance the transfer of cholesterol to the outer layer, potentially modifying membrane function. This mechanism then invokes the necessity for rafts – (i.e., functionalized domains) that could facilitate the biosynthetic pathways of cholesterol and its transport to and from cells.

In order to reconstruct the real-space structure of a membrane, both amplitude and phase are needed. However, in the majority of scattering experiments, only the amplitude of the structure factor can be obtained by experiment – the phase information is lost. In order to recover the phase component of the structure factor, various methods have been developed. The cholesterol example presented above used contrast variation to obtain the phase component of each individual Bragg reflection. In reflectometry and small-angle scattering experiments, real-space solutions rely on fitting the data to a realistic model. In doing so, one must keep in mind the possibility that the data can be fitted equally well by more than one model. In the case of neutron reflectometry studies, Majkrzak et al. (2000) have made good use of polarized neutrons and reference substrates made of Cu, Ni, and Mo to obtain both the amplitude (real part) and phase (imaginary) components of the complex reflection, allowing for the data (reciprocal space) to be directly inverted into a unique scattering density profile (real space).

Small-Angle Neutron Scattering

Small-angle neutron scattering (SANS) is probably the technique most commonly applied to biological materials as it can probe length scales ranging from nanometers to fractions of a micron when ultra SANS is used. In the case of unilamellar vesicles (ULVs)



Neutron Scattering of Membranes, Fig. 2 (**a**) Predicted scattering from polydisperse ULV containing multiple domains (N = 1–32). The number of domains increases on going from the bottom to the top curves. *Curves* are shifted on the vertical axis to facilitate viewing. (**b**) Fits to a 1:1:1 DOPC to DPPC to cholesterol ULV system corresponding to the superposition of signals from ULVs with single and multiple domains (Adapted from Pencer et al. 2005)

(hollow spherical structures consisting of single lipid bilayer), SANS can evaluate both the ULV's size, and the thickness of the bilayer that forms the ULV. This technique thus allows for the accurate structural determination of ULVs that have been fabricated for delivering drugs and imaging diseased tissue, introducing genetic material to living cells, and enhancing the efficacy of various medical imaging techniques (Nejat 2009). Although ULVs exist in solution, and their isotropic structural dimensions are typically characterized by low-resolution data, much insight into a system can be achieved through the previously discussed contrast variation techniques. For example, Pencer et al. (2005), using coarse-grained models of heterogeneous ULVs, demonstrated SANS' potential to detect and distinguish between lateral segregation in membranes. This was done by exploiting the unique sensitivity of neutron scattering to differences between H and D atoms (Fig. 2), as was discussed previously. Most recently, a new and innovative advance in scattering theory and experiment enables neutron data to be analyzed in such a manner as to provide not only structural information in the direction perpendicular to the plane of the bilayer, but also information regarding a membrane's in-plane organization, e.g., lateral lipid segregation (Iglič 2010).

Cross-References

- Chemical Diversity of Lipids
- Fluorescence and FRET in Membranes
- Lipid Bilayer Asymmetry
- Lipid Trafficking in Cells
- Membrane Proteins: Structure and Organization
- Protein Fluorescent Dye Labeling
- Small Angle Neutron Scattering (SANS) Software
- Supported Lipid Bilayers
- Vibrational Spectroscopy with Neutrons

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New Protein Folds

▶ Protein Structure: Potential Folds

N-Glycan Analysis

▶ Mass Spectrometry of *N*-Linked Carbohydrates and Glycoproteins

Nicotinamide Adenine Dinucleotide (NAD)

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Synonym

Diphosphopyridine nucleotide (DPN)

Definition

NAD is a dinucleotide composed of two mononucleotides (adenosine 5′-monophosphate, AMP, and nicotinamide mononucleotide, NMN), which are joined through their 5′ phosphate groups (Fig. 1). The molecule is present in all living cells and reversibly converted between its oxidized (NAD^+) and reduced (NADH) forms acting as a coenzyme in many metabolic hydride transfer (redox) reactions (Fig. 2). NAD^+ serves also important functions in signal transduction pathways. A phosphorylated derivative of NAD, NADP, is also present in all organisms, exhibits similar physicochemical properties, but apparently does not have a prominent role in signaling.

Physicochemical Characteristics of NAD

In its naturally occurring, biologically active form, the nicotinamide moiety of NAD is attached to the anomeric carbon of the ADP-ribosyl moiety in the β-conformation (Fig. 1). Both NAD^+ and NADH exhibit an absorption maximum at 259 nm owing to the presence of the adenine base ($\varepsilon = \sim 16{,}200\ M^{-1}cm^{-1}$ for NAD^+). Due to the reduced nicotinamide ring, NADH has an additional absorption maximum at 340 nm ($\varepsilon = \sim 6{,}200\ M^{-1}cm^{-1}$). This property is widely used to measure enzymatic reactions which require NAD as coenzyme. NADH is a strong reducing agent, the midpoint ▶ redox potential of the NAD+/NADH redox pair being –0.32 V.

Unlike NAD^+, NADH is fluorescent with an emission maximum at ~460 nm (excitation at 340 nm). The fluorescence lifetime (~0.4 ns in aqueous solution) increases significantly, when the molecule binds to proteins. This property has been exploited to estimate concentrations of free vs. protein-bound NADH or the redox state (NADH/NAD^+ ratio) in living cells by means of fluorescence microscopy, but can also be used to determine binding constants for isolated proteins.

Biological Functions of NAD

NAD plays two distinct biological roles. Similar to ATP, it is a key molecule in both energy transduction and signaling pathways. In bioenergetics, NAD is a coenzyme which serves as reversible electron carrier in a multitude of redox reactions. In signaling processes, however, only NAD^+ is involved. NAD is regarded as an intracellular molecule. However, in higher organisms low amounts are present in extracellular fluids (e.g., blood plasma), thereby providing substrate for extracellular NAD^+-mediated signaling events.