Neutrons and Biology

by Maikel C. Rheinstädter

Neutron and x-ray scattering are indispensable tools in the life sciences. Their main impact is probably the determination of high-resolution protein structures using dedicated and high-throughput protein crystallography beamlines, where scattering of minuscule protein crystals is detected and analyzed to determine the structure of proteins and complexes to tell us for instance how receptors and enzymes work. While a large percentage of protein structures is still done by scattering techniques, nuclear magnetic resonance (NMR) and cryogenic electron microscopy (cryo-TEM) are catching up. While our current techniques often suffer from the fact that the corresponding structures are determined under non-physiological conditions, there is a strong effort to develop techniques to study protein structures in more physiological environments, such as in solution or in contact or embedded in membranes. These disordered and highly-dynamic structures pose particular challenges for the application of scattering techniques, which have been developed for the application in well-ordered and periodic systems. This challenge is being addressed by the development of new instrumentation and analysis techniques to get the most amount of structural information of this biological state of matter. The new Small Angle Neutron Scattering (SANS) for Nanostructured Materials instrument, which is currently being constructed at the McMaster Nuclear Reactor, is a good example for an instrument that will address this challenge.

Neutron and x-ray scattering are still one of our best microscopes for the determination of small structures and the global investment in neutron reactors and spallation sources and synchrotron facilities has reached an all-time high with far more than $20B spent in recent years. While there is a global network of these facilities, which provide access to users world-wide, the availability of local, national facilities, such as the Canadian Neutron Beam Centre at Chalk River Laboratories, the McMaster Nuclear Reactor and the Canadian Light Source, is a prerequisite for successful research programs and economic impact, and indispensable for the training of highly qualified personnels.

Summary

Neutron beams are indispensable tools to determine molecular structure and dynamics of proteins and membranes. We review properties and their use to investigate membrane rafts.

As distances between molecules are typically much larger than atomic distances in crystals, the corresponding scattering signals occur at small scattering angles ($\lambda/d = 2\sin(\theta)$, with λ the wavelength of neutrons or x-rays, d the molecular spacing and θ the scattering angle). Neutron beams can be optimized for large scale structures by preparing so-called cold neutrons with velocities between 100 and 2,000 m/s, only, and corresponding long de-Broglie wavelengths ($\lambda = h/(m \cdot v)$) of 2-40 Å. In combination with long flight tubes, which allow the measurements of signals at very small scattering angles, these instruments are optimized to detect molecular distances of up to 10,000 Å.

Because of their relatively low speed, neutron beams can also be used to determine dynamical processes. By measuring the velocity of neutrons before and after the scattering process, their energy transfer during the scattering process can be calculated. The corresponding dynamical processes are related to well-defined excitation frequencies of rotations and vibrations of molecules, or protein or lipid diffusion. Energy transfers of as small as nano electron Volts can be measured, approaching slow, almost microsecond time scales. Changes in energy are detected at different values of the scattering vector, Q. In contrast to other spectroscopic techniques, inelastic neutron scattering thus results in wave vector resolved access to molecular dynamics. A typical dynamic scattering experiment measures $(Q,\hbar\omega)$ pairs, resulting in a frequency along with a corresponding length scale, and possibly a corresponding direction such as parallel or perpendicular to a protein's axis. This additional information is of paramount importance when it comes to relating dynamical information to structure. In short, the suite of inelastic instruments used to study soft and biologically relevant materials comprises of time-of-flight, backscattering, triple-axis and spin-echo spectrometers.

As shown in Fig. 1, neutron spectroscopy fills an important gap in the suite of spectroscopic techniques [1]. The relevant length scale for dielectric spectroscopy is in the order of an elementary molecular electric dipole, which

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can be estimated by the bond length of a C--O bond (about 140 picometers), and frequencies from kiloHertz to GigaHertz can be measured.

Because the wavelength of the probe is usually around λ = 500 nm, light scattering techniques are limited to small momentum transfers of about 10^{-4} Å^{-1} to 10^{-3} Å^{-1}, and corresponding to a length scale of about 100 nanometers. Inelastic neutron and x-ray scattering access length scales from smaller than Angstrom to more than 100 nanometres, and time scales from picoseconds to almost one microsecond. High speed AFM has combined a high spatial resolution of about 5 Å with a time resolution of milliseconds, just outside the time range in the figure. Molecular Dynamics computer simulations have become an invaluable tool in developing models for molecular structure and dynamics in membranes and proteins. Because of the ever-increasing computing power and optimized algorithms, large complex systems (i.e., hundred thousands of molecules) and long simulation times of µs can now routinely be addressed. The dashed rectangle in Figure 1 marks the dynamic range currently accessed by computer simulations – the elementary time scale for simulations is in the order of femtoseconds.

SELECTIVE DEUTERATION

While x-rays are electromagnetic waves and scattered by the electrons in the electron shell, neutrons scatter off the nuclei of atoms. The peculiarity of neutrons is that they may scatter incoherently or coherently, and give access to local or collective dynamics (see [2] and references therein). The scattering length, b, depends on the spin of the nucleus-neutron system. If the spin of the nucleus is I, then every nucleus with non-zero spin has two values of b, namely for $I+½$ and $I-½$, depending on the orientation of neutron and nucleus spin. The scattering length is thus not simply a monotonic function of the atomic number, as it is for x-rays, but depends on the spin configurations of the nuclei. In addition, different isotopes of the same element can have different scattering lengths as they have nuclei with the same number of protons (the same atomic number) but different numbers of neutrons and also different nuclear spins. So while for x-rays, all atoms of the same element look the same, they may look different for neutrons because of (1) different orientations of the nuclear spin, and (2) different isotopes (nuclides). The most pronounced difference between the x-ray and the neutron probe is, therefore, that x-ray scattering is always coherent, while neutron scattering may contain contributions from coherent and incoherent scattering.

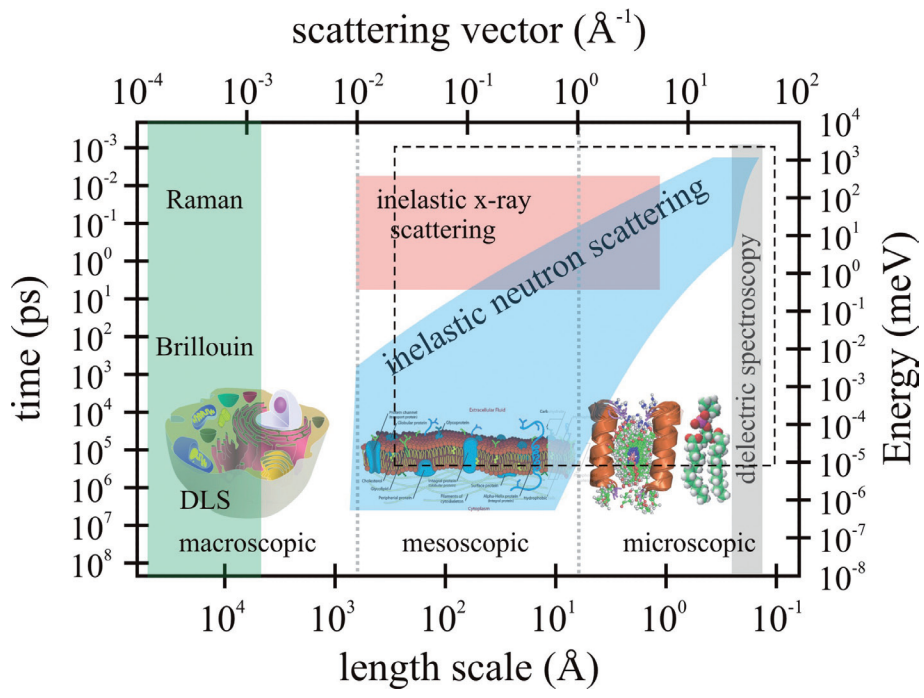

Fig. 1 Accessible length and time scales, and corresponding energy and momentum transfer, for some spectroscopic techniques covering microscopic to macroscopic dynamics. Light scattering techniques include Raman, Brillouin, and Dynamic Light Scattering (DLS). Inelastic x-ray and neutron scattering access dynamics on Angstrom and nanometer length scales. Dielectric spectroscopy probes the length scale of an elementary molecular electric dipole, which can be estimated by the bond length of a C—O bond (about 140 picometers). The area marked by the dashed box is the dynamical range accessible by Molecular Dynamics simulations (Adapted from [1]).

The coherent and incoherent neutron cross sections of an element can be illustrated by two extreme cases. If all the nuclei in a sample have different cross sections, there can be no interference between the waves scattered by different atoms. This incoherent scattering can only depend on the correlation between the positions of the same nucleus at different times and measures the auto or self-correlation function and diffusive processes. If all scattering lengths are the same, i.e., all nuclei are identical for the neutron probe, the coherent scattering still depends on the correlation between the positions of the same nucleus at different times, but more so on the correlation between the positions of different nuclei at different times. It therefore gives interference effects and allows measuring collective dynamics and interaction forces.

The fraction of coherent and incoherent scattering depends on the atomic composition and the respective scattering lengths. Substitution of certain elements in

a compound by their isotopes may increase contributions of certain molecules or functional groups to the coherent or incoherent scattering contribution. Because the incoherent cross section of hydrogen atoms is about 40 times larger than that of deuterium and of all other atoms present in biological macromolecules, hydrogen atoms dominate the incoherent scattering signal. The hydrogen atoms reflect the movements of larger groups to which they are attached, such as amino acid side chains. Deuteration, i.e., the substitution of protons by deuterium (^{2}H), is often used to suppress the incoherent scattering contribution of certain functional groups to the total scattering or increase their coherent contribution.

The effect of deuteration labeling is sketched in Fig. 2. While in protonated samples the incoherent scattering is usually dominant and the time self-correlation function of individual scatterers is accessible, (partial) deuteration emphasizes the coherent scattering and gives access to collective motions by probing the pair correlation function. In a membrane sample with protonated proteins, the experiments would be sensitive to the diffusive motions of the proteins. Deuteration of the proteins increases the coherent scattering and allows measuring the interaction forces between the embedded proteins. In Fig. 2(a), self-correlated, diffusive motions of membranes and embedded proteins can be accessed when the membrane-protein system is labeled, and deuterated solvent is used. Diffusive dynamics of the proteins is highlighted when solvent and membrane are deuterated, and hydrogenated proteins are used. The effect of protein insertion on membrane dynamics can be studied in Fig. 2(c), with deuterated solvent and proteins. Note that at the same time, the interfaces between protonated and deuterated areas scatter coherently. The preparation in Fig. 2(b) can therefore be used to study possible protein-protein interactions. Selective deuteration is in particular very powerful for the investigation of functional domains in membranes, so-called rafts.

THE RAFT CONCEPT

Biological membranes are the most important biological interface. Composed mainly of lipid molecules and proteins, they serve a number of functions, which include acting as a barrier to the external environment for the contents of the cell. Over 20-30% of genes encode membrane embedded proteins, and these proteins play important roles in cell signalling and cell adhesion. The cellular plasma membrane contains over 100 lipid species. Lipids are amphiphilic molecules with a hydrophilic head group and hydrophobic lipid tails. Lipid membranes are bilayers of lipid molecules, which form to minimize water contact with the lipid tails. This bilayer may then wrap to form a closed surface and a passive barrier. In early research of membranes and membrane embedded proteins, lipid molecules were not considered active participants in membrane processes. In 1972, shortly after it was determined that proteins may embed within the lipid membrane, Singer and Nicholson published their "Fluid Mosaic Model" of lipid membranes. In this model, the membrane serves as a passive, unstructured, two-dimensional liquid within which embedded proteins float and the lipid molecules act as a solvent. The model quickly became popular as it allowed for the lateral diffusion of protein molecules, as well as the transverse diffusion of small molecules, such as oxygen or carbon dioxide.

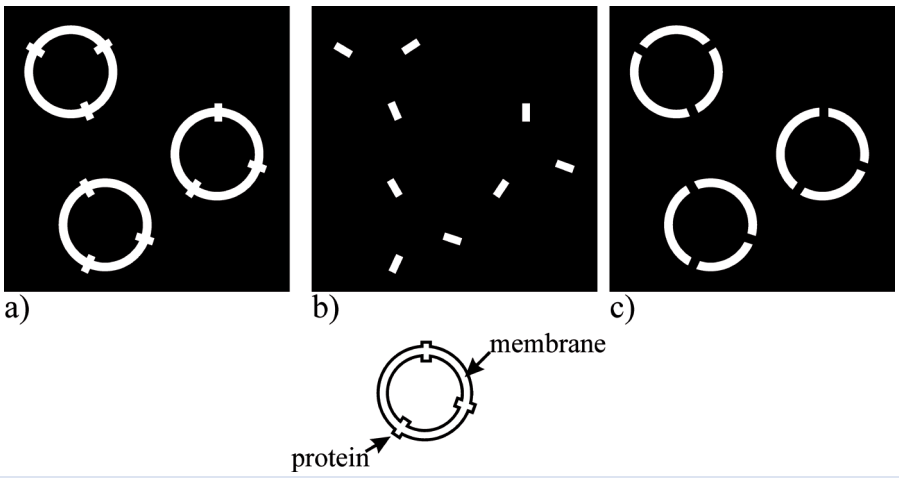

Fig. 2 Selective deuteration can be used to highlight molecules or functional groups in neutron scattering experiments. Depicted are vesicles with embedded proteins in a solvent: (a) membrane and embedded proteins are visible in incoherent scattering experiments for neutrons when the solvent is deuterated. (b) When solvent and membrane are deuterated, local dynamics of the embedded proteins can be investigated. (c) Deuterated proteins allow investigating membrane dynamics (Adapted from [2]).

In order to increase the amount of structural information, scattering experiments in membranes are often performed on oriented, stacked bilayers applied on a substrate. The membranes are typically prepared by dissolving the component molecules in solvent, then depositing the solution on flat, hydrophobic silicon wafers. Stacked bilayers are formed upon solvent drying and subsequent annealing in high humidity and temperature. The structure of the membrane perpendicular to the flat substrate (the out-of-plane axis, q_z) can then be determined independently of the lateral structure (in-plane axis, $q_{||}$), as shown in Fig. 3.

As research on membranes progressed it appeared that not all membranes are created equal

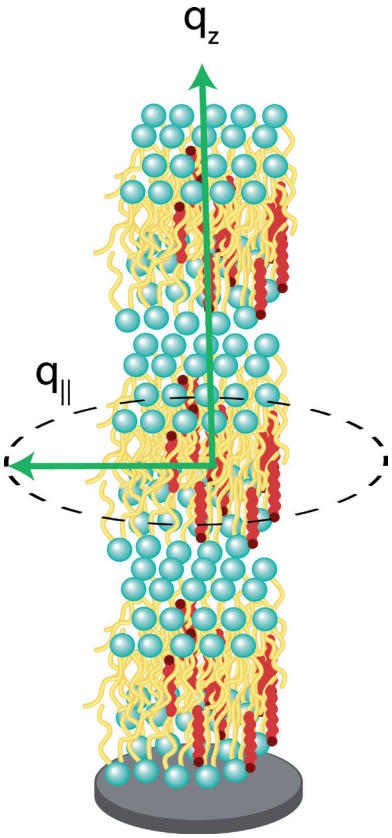

Fig. 3 Schematic of oriented, stacked lipid bilayers, which defines the principle axis used in scattering analysis. The q_z axis describes scattering along the direction of the oriented stack, while the $q_{||}$ axis describes scattering from the in-plane organization of membrane molecules (From [3]).

(see [3] and references therein). The preferential sorting of proteins in the plasma membrane was attributed to local differences in composition. Experiments on plasma membranes found that certain fractions were resistant to detergents, and that certain proteins are found in the detergent resistant fractions. In addition, the proteins in these detergent resistant fractions were often observed to be organized in specific membrane locations. The evidence led to in the proposal of "lipid rafts": isolated membrane fractions enriched in cholesterol and saturated lipids, which serve as membrane compartments, as pictured in Fig. 4. Proteins may interact with the raft region or non-raft regions, depending on hydrophobic thickness or specific lipid/cholesterol interactions. In the raft model, the lipids are no longer a passive solvent. Instead, their physico-chemical properties impact the structure and function of specific proteins. With rafts most likely being small and transient structures, understanding their structure, function, and even proving their existence is an experimental challenge.

There are two properties of neutrons scattering experiments, which make them amenable to identifying rafts. By selectively deuterating lipids, the experiment can be made primarily sensitive to lipid organization while cholesterol molecules are basically invisible or vice-versa (see [4] and references therein). Lipid structure can be determined unambiguously and independently from cholesterol structure. The second important property of neutrons is the ability to increase the experiment's sensitivity to smaller structures by controlling the (longitudinal) coherence length of neutron beams in situ. The coherence length ξ of the neutron is the spatial extent of the neutron particle and is given by the neutron wavelength, λ, and the uncertainty in the wavelength of the particle, $\Delta\lambda$, by $\xi = \lambda^2/\Delta\lambda$. In a scattering experiment, the structure of a domain smaller than the coherence length of the neutron will be

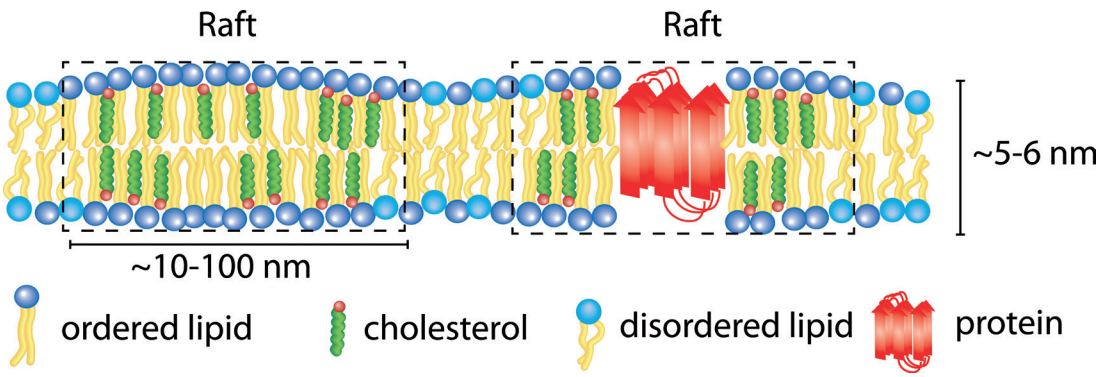

Fig. 4 Schematic of rafts in a lipid membrane. The raft region contains high concentrations of ordered lipids and cholesterol. Lipid motions are constrained in the presence of cholesterol and lipid molecules are found as well-ordered as in the gel state but with lateral and rotational diffusion similar to that of the fluid phase; in that respect, rafts are a manifestation of the liquid-ordered, l_o, phase. These rafts have different material properties, such as decreased elasticity and permeability, and may serve as anchor points for certain proteins (From [3]).

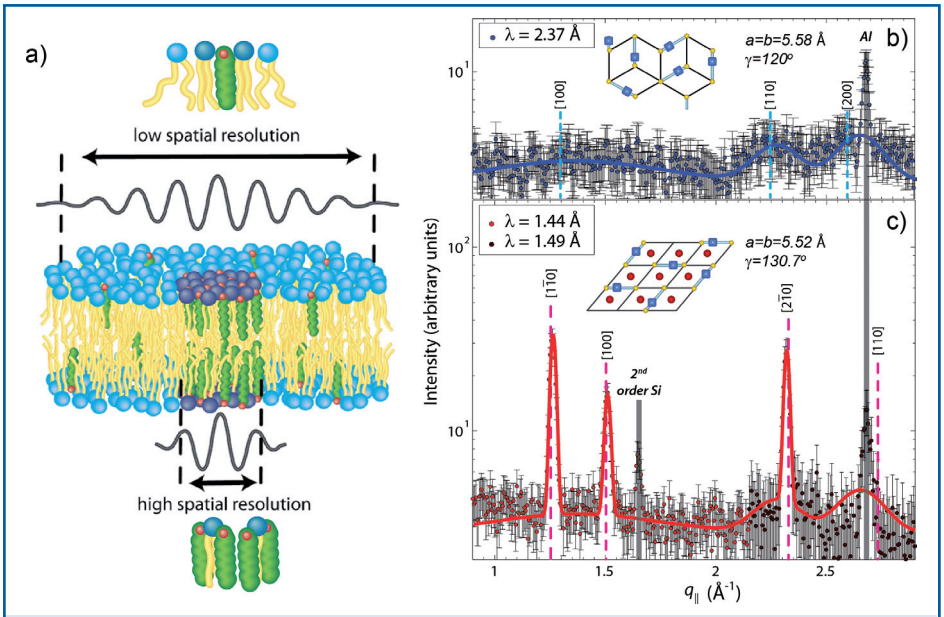

Fig. 5 Neutron diffraction of a deuterated lipid bilayer with 32.5 mol% (protonated) cholesterol. a) The "spatial resolution" of neutrons can be altered. At high spatial resolution, small scale structures are more visible in the diffraction signal. b) An in-plane measurement at low spatial resolution. Only disordered membrane features are observed. c) At high spatial resolution, sharper peaks are observed which are indicative of a more ordered, small scale structure (Adapted from [4]).

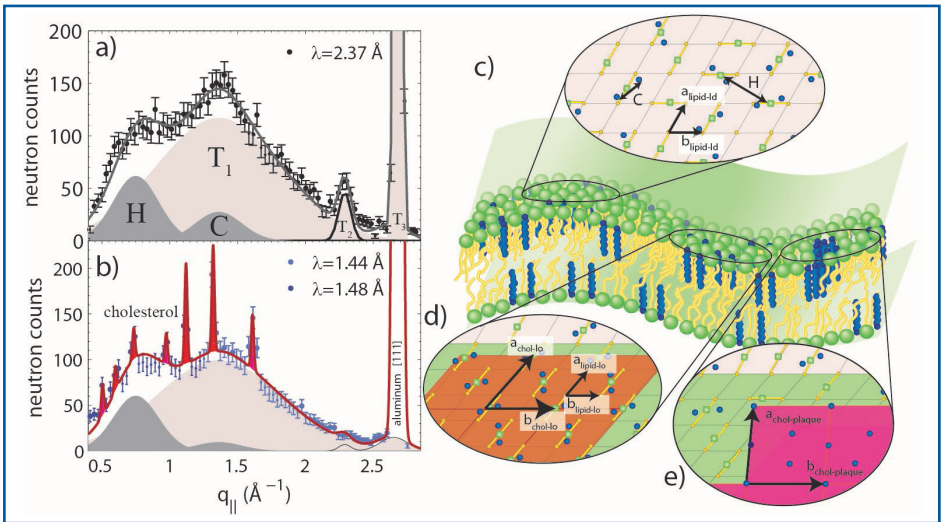

Fig. 6 Neutron diffraction of a protonated lipid membrane with 32.5 mol% deuterated cholesterol. This experiment is sensitive to the structure of cholesterol. a) A diffraction measurement at low spatial resolution, revealing a disordered membrane structure. Features describe scattering from lipid head-head interactions, tail-tail interactions, as well as direct cholesterol-cholesterol interactions. b) In addition to the broad peaks appearing in a), additional sharper peaks appear which describe small scale, ordered structures. c) A depiction of the disordered structures observed in the scattering. d) A monoclinic cholesterol lattice appears which agrees well with the structure observed in Fig. 5. e) Cholesterol plaques consisting of bilayers of cholesterol molecules (Adapted from [5]).

averaged with the surrounding membrane. In a membrane, raft regions will be averaged with the more abundant non-raft regions and the signal will be lost. However, by shrinking the coherence length of the probe, the scattering signal becomes an incoherent sum of many smaller coherent averages, giving more weight to the smaller scale structure, as depicted in Fig. 5. At high coherence length, non-raft regions dominate in a diffraction measurement. At low coherence length, both raft and non-raft regions are visible indicated by the well-developed scattering signals.

The results for (protonated) membranes containing 32.5 mol% deuterated cholesterol are shown in Fig. 6 [5]. At low spatial resolution, a diffraction pattern corresponding to a disordered structure was observed (Fig. 6(a)). The observed scattering is a result of lipid tail and head group scattering as well as cholesterol-cholesterol scattering (although the lipids were not deuterated, they still have some scattering power). These correlations are short ranged. At high spatial resolution, peaks appear to be significantly sharper (Fig. 6(b)). These peaks can be described by three different structures: Broad peaks correspond to a fluid membrane phase, where cholesterol molecules form complexes with lipids according to the umbrella model. Sharper peaks can be indexed by small, well-ordered lipid patches, where lipid and cholesterol molecules form l_o domains (rafts). The third set of peaks agrees well with cholesterol plaques, bilayers made entirely of cholesterol molecules, which have been observed at high cholesterol concentrations. By combining selective deuteration and the ability to control the neutrons' coherence length, these neutron diffraction experiments unambiguously identify the

presence of highly ordered, fluctuating domains in binary lipid cholesterol mixtures.

CONCLUSION

For decades, elastic and inelastic neutron scattering have provided essential information for the life sciences, such as molecular biology, biochemistry and molecular medicine and in particular computational biology. The neutron probe allows the determination of molecular structure and dynamics in biological systems, such as proteins and membranes, in a window of length and time scales not easily accessible by other techniques. By selective deuteration, different components of complex, multi-component systems can be highlighted and their structure, dynamics and interactions can be studied unambiguously and independently. The next generation of neutron instrumentation at the latest and most powerful neutron sources will likely give unprecedented insight into molecular processes to address important questions, such as infectious diseases and novel antibiotics, Alzheimer's disease and cancer.

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