Probing Molecular Structures Using Neutrons to Answer Biological Questions

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Neutrons offer a range of analytical techniques to study biological processes and neutron research is making strides in cellular biology, virology, nanoscience and medicine. Giovanna Fragneto, Senior Fellow in Soft Matter at ILL, Europe's flagship centre for neutron science, looks at the techniques behind some recent findings.

Neutrons, subatomic particles found in the nuclei of atoms, have properties which provide biologists with unique analytical techniques. Neutrons beams fired at biological materials act like waves and are scattered when they interact with the nuclei of atoms. Analysis of the scattering pattern allows the structure and composition of the material to be determined.

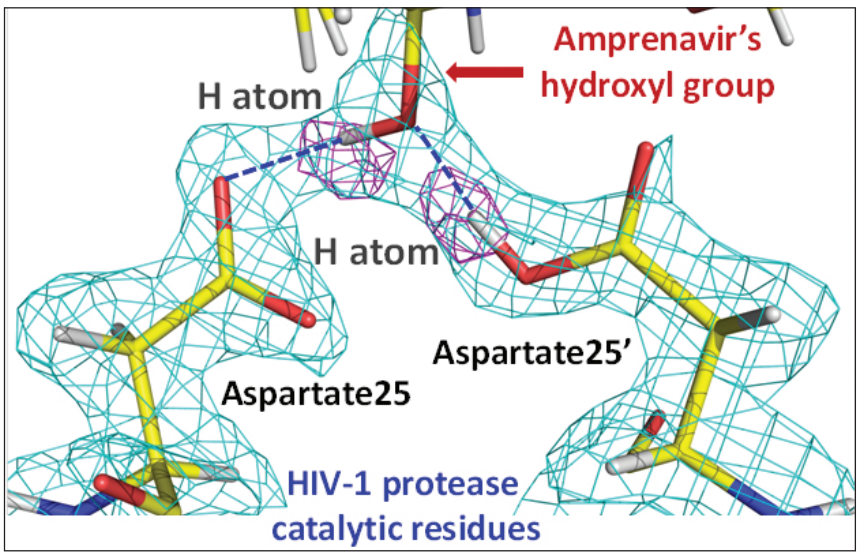
Neutron wavelengths range from fractions of nanometres to hundreds of nanometres - going from hot to thermal and cold to ultracold energies. Thermal and cold neutrons are most commonly used in biological studies as their wavelengths, approximately a thousand times shorter than that of visible light, makes them particularly suitable for the study of biological structures, from small molecules and monomers, such as lipids or peptides, to larger molecules including viruses and proteins. Being electrically neutral, neutrons are able to penetrate deep into materials and are non-destructive.

A number of neutron techniques are being deployed at Institut Laue-Langevin, a publically funded international research facility in Grenoble and one of the world's most intense neutron sources. Neutrons are being used to study a range of biological structures and processes, from proteins critical to virus replication, to ribosome assembly and molecular drug delivery systems. A number of these techniques are explored here in more detail.

Neutron Crystallography – New Insights in to Structure-Guided Drug Design

Traditionally, X-ray crystallography has been used to determine the structures of biological macromolecules such as proteins. Rosalind Franklin's infamous 'photo 51', an X-ray diffraction pattern, led to the discovery of the structure of DNA and has been described as the most important photos ever taken.

X-ray crystallography has had a huge impact over the last century. Nevertheless, a limitation of the technique is that hydrogen atoms are virtually invisible in electron density maps as hydrogen has only one electron and thus scatters X-rays very weakly. This has meant that scientists have been left to speculate on their positions in molecules, despite their importance in a vast array of biological processes such as enzyme-catalysed reactions and drug binding.



Picture: Andrey Kovalevsky

Where X-rays fail to locate hydrogen atoms, neutrons provide the answers. Rather than being scattered by the electron clouds of an atom, neutrons are scattered by the atomic nuclei. Additionally, the scattering from hydrogen is of a similar magnitude to the other common elements found in biological macromolecules – such as carbon, nitrogen, oxygen and sulphur - allowing the positions of all the atoms to be determined.

The ability of neutrons to provide the positions of hydrogen atoms in biological molecules is elegantly illustrated in a recent study of how HIV-1 protease, an enzyme critical for viral replication, binds to amprenavir, a commonly used inhibitor.

Previous X-ray studies of the binding of amprenavir to HIV-1 protease have speculated that a large number of hydrogen bonds were important. However, neutron studies on the quasi-Laue neutron diffractometer (LADI-III) at ILL indicated that in fact there are only two strong hydrogen bonds that contribute significantly to the binding between the enzyme and inhibitor.

These results provide new information on the binding that can be used to improve the drug's performance. For example, by modifying certain functional groups of the drug, stronger hydrogen bonds can be formed with the enzyme, improving its performance and potentially reducing dosages.

This unique sensitivity to hydrogen atoms using neutrons provides the pharma industry with a new and powerful tool for structure-guided drug design. Whilst the value of X-ray crystallography will continue for many years, combining the use of X-rays with neutrons increases the clarity of how drugs interact with their protein targets and will no doubt be used to improve the efficacy of other pharmaceuticals in the future.

Neutron Reflectometry – Probing Thin Films in Physiological Environments

Although neutron crystallography is complementary and in certain situations has clear advantages over X-ray crystallography, the technique still requires the molecule being studied to be dehydrated, kept in a crystallised rigid form and held at a low temperature.

However, neutrons can also be used to study biological molecules in fluids with similar properties to cytoplasm or blood, allowing analysis in environments that mimic physiological conditions. The diffraction technique, known as neutron reflectometry, is used to investigate the properties and behaviour of surfaces and thin films.

A recent study using the FIGARO instrument at ILL looked at the binding of silica nanoparticles to human serum albumin, the most abundant protein in the blood. The study examined the protein corona hypothesis, which suggests that nanoparticles are able to cross cell membranes because they bind to and become encased by proteins and are thus disguised from membrane receptors. It was not clear if the corona structure was prevalent at surfaces or not so neutrons were used to analyse human serum albumin behaviour at the air/fluid interface to mimic hydrophobic surfaces that nanoparticles may encounter in the body.

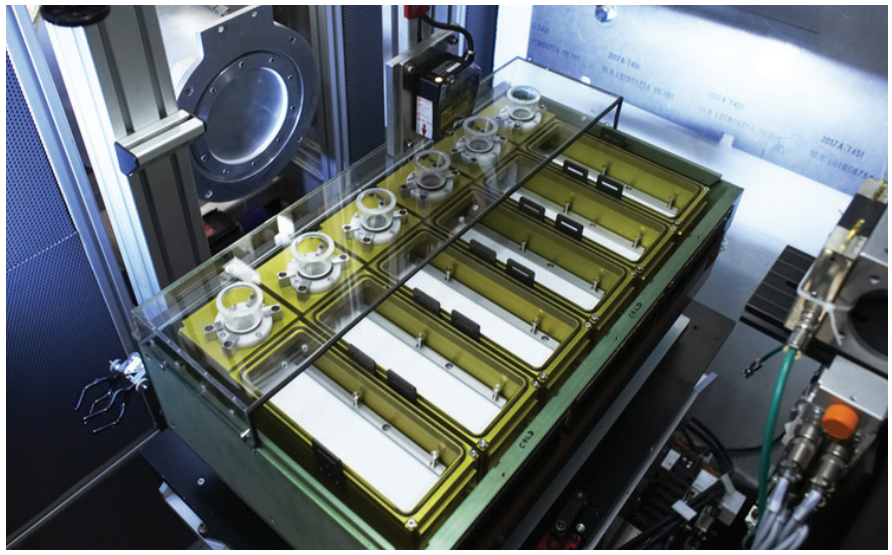
The FIGARO instrument allowed the proteins and nanoparticles to be studied in a buffer solution which had a pH similar to that of blood plasma. Neutron reflectometry determined how proteins transported the nanoparticles to the interface. A strong collimated neutron beam was fired at the surface, and the angle and wavelength of the reflected beam were measured. These data provided structural information about the different molecules at the surface of the film and showed how protein molecules decorated the silica nanoparticles at the interface.

The equipment also allowed the protein:nanoparticle ratio at the surface to be measured with respect to various properties of the fluid, allowing the study of the binding under a number of different conditions.

The FIGARO instrument is unique in that it can collect data from top and bottom surfaces of a sample during the same experiment in a time-of-flight mode suited to dynamic interaction studies, without disturbance of the sample. This technique is particularly useful when analysing the interaction of biological membranes with formulations which are sensitive to changes in orientation as a result of gravity effects from phase separation. An international team of scientists at ILL recently used this technique to investigate the binding of liquid crystalline reservoirs of phospholipids and biomedically-relevant macromolecules with a model cellular membrane.

It was shown that the 'parcels' packed full of functional molecules not only attached to model membranes of sufficient negative charge, but the macromolecules also transferred across to the other side. Even so, this mechanism was active only when the membrane was located above the formulation. This level of structural information is unique to neutron reflectometry and gave two key messages.

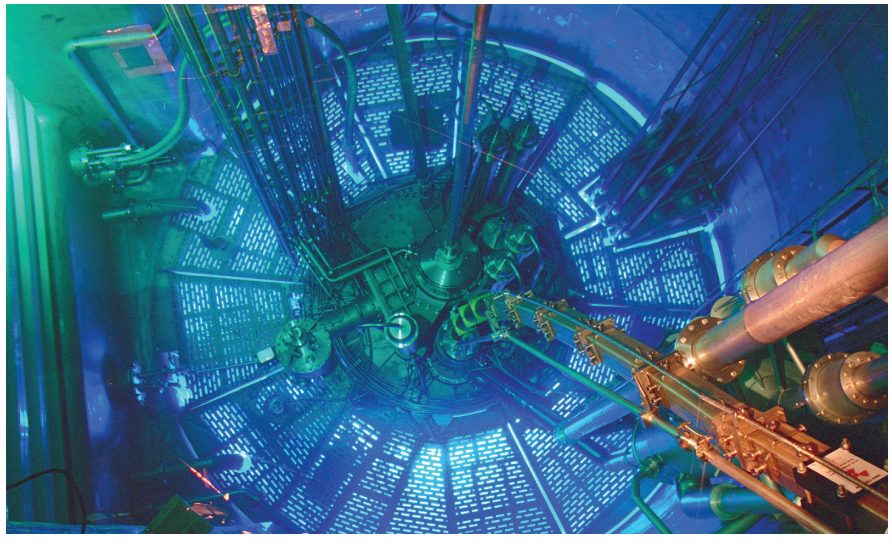
First, there is scope in the future to develop the investigated system of liquid crystalline reservoirs to target specific cell types given that, for example, diseased cells are generally believed to have more negative surface charge than healthy ones. Furthermore, researchers of complex drug formulations would be advised to consider effects of gravity in relation to their experimental tests.



SANS - Small Angle Neutron Scattering

Another popular neutron technique, small angle neutron scattering, is used to look at bulk samples. Beams of neutrons are deflected by an angle of only a degree or so, and the combination of scattered angles and wavelength are analysed to characterise structures of one to several hundred nanometres within a solution, dispersion or solid sample.

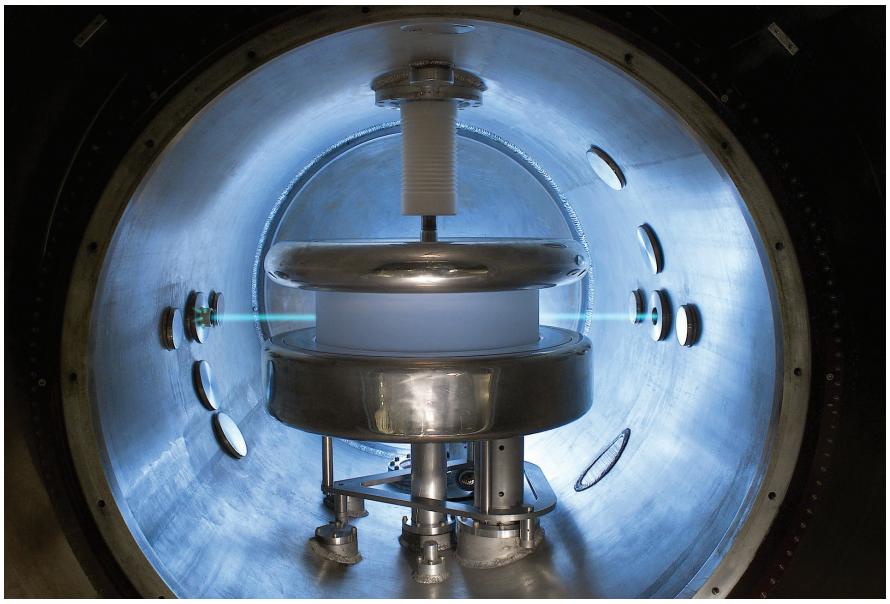
SANS is particularly useful in biology, where it allows the study of interactions in physiological environments and relevant thermodynamic conditions. In a recent study, this technique has been used to study the water purification properties of seeds from *Moringa* trees. Neutrons were directed at water containing small particles - a model system for waste water - mixed with protein extracted from the seeds. The amount of protein bound to the particles, as well as the formation of aggregates, was observed by measuring the scattering from the samples. The aggregates, known as flocs, can be separated from the water and the neutron data show that the aggregates formed with the proteins were more densely packed than those produced by conventional chemicals and could be separated more readily.



Contrast Variation and Isotopic Substitution – Enhancing the Signal

An additional trick used in neutron studies takes advantage of a unique feature - neutrons are scattered differently by different isotopes of the same element and thus can distinguish between hydrogen and deuterium atoms due to their different scattering power. This allows the scattered signal from specific molecules to be enhanced through isotopic contrast variation by modifying the ratio of H_2O to D_2O in the buffer solution.

A similar technique - isotopic substitution - replaces specific hydrogen-containing monomers in a biological oligomer with deuterium copies. The label means that the desired region 'stands out' during neutron scattering. The technique was recently used to elucidate the 3D structure of the box C/D Box ribonucleoprotein enzyme, responsible for methylation of RNA during ribosome assembly. Individual protein subunits were tagged in turn, allowing the determination of their position within the C/D box complex.



Ultracold Neutrons – Studying the Movement of Particles on Surfaces

Recent research has hinted that ultracold neutrons may be able to detect the movement of nanoparticles on surfaces. Ultracold neutrons are studied in 'traps', where properties such as their lifetime are determined. Studies at ILL revealed that neutrons collided with moving nanoparticles on surfaces and the interaction gave the neutrons sufficient energy to overcome gravity and escape from the chamber.

The results provide a possible mechanism to track the movement of particles on surfaces, such as the movement of virus particles on cell membranes. Although at a very early stage, the possible applications of the technique are diverse and exciting.

About ILL

Institut Laue-Langevin (ILL) is an international research centre based in Grenoble, France. It has led the world in neutron-scattering science and technology for almost 40 years, since experiments began in 1972. ILL operates one of the most intense neutron sources in the world, feeding beams of neutrons to a suite of 40 high-performance instruments that are constantly upgraded. Each year 1,200 researchers from over 40 countries visit ILL to conduct research into condensed matter physics, chemistry, biology, nuclear physics, and materials science. The UK, along with France and Germany is an associate and major funder of ILL.

For more information contact: welcome@ill.eu or visit: www.ill.eu