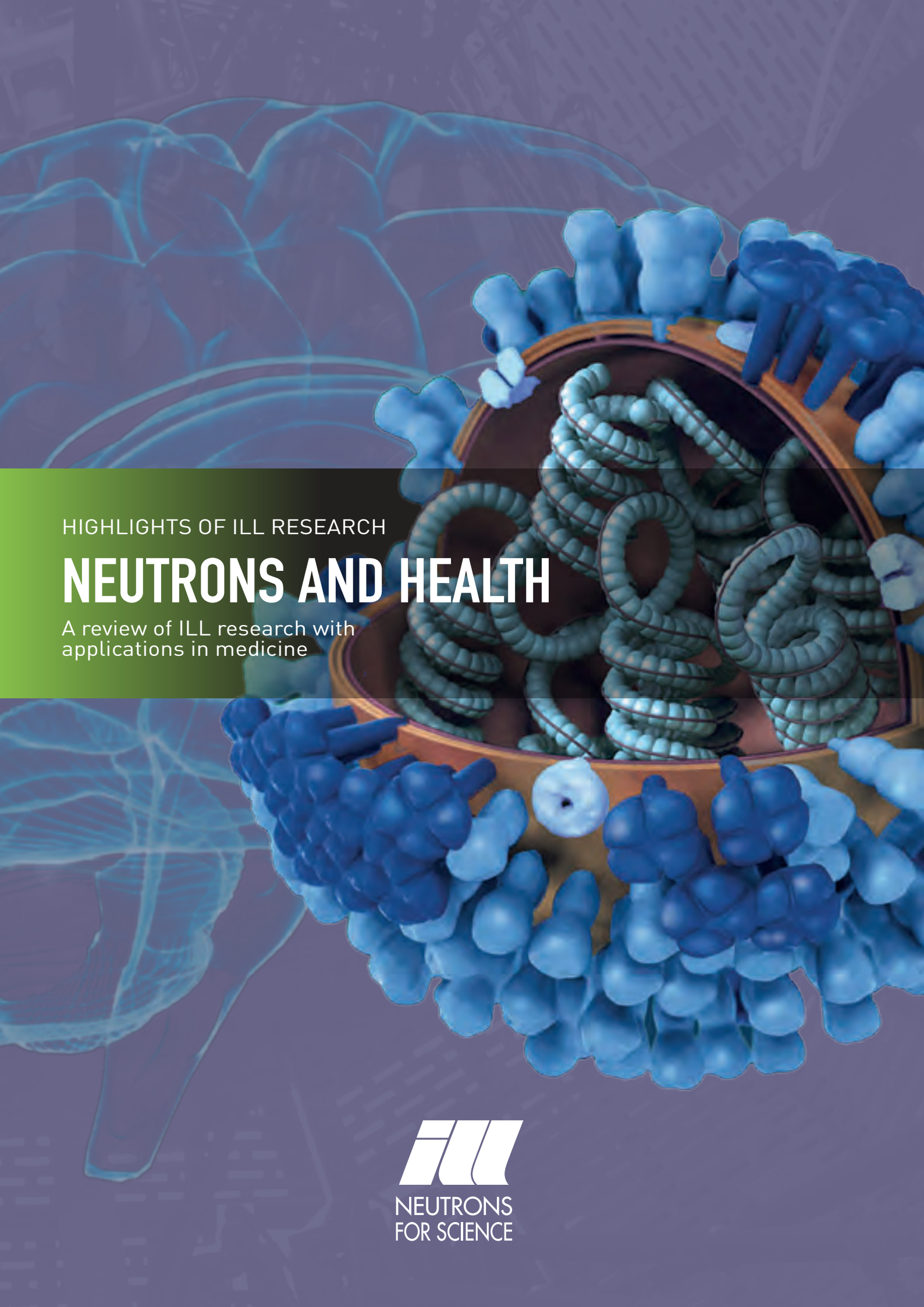


HIGHLIGHTS OF ILL RESEARCH

NEUTRONS AND HEALTH

A review of ILL research with applications in medicine

NEUTRONS FOR SCIENCE

FOREWORD

According to the German-American political philosopher, Hannah Arendt, it is our destiny to actively shape our world. Scientific activity, and its transcription into technological progress, is one of the royal ways of heeding that call. No other human endeavour has left more profound traces in the world surrounding us. One of the areas in which scientific and technological impact becomes most obvious is health. It is also one of the few areas where the benefit of progress is more or less undisputed. Modern medical care based on a thorough understanding of the biological mechanisms governing our bodies has improved both the life expectancy and quality of life for the major part of humanity. Apart from biological research being a goal in its own right, a society alleviated from health problems will have more freedom to design its future, thus closing a virtuous circle in the thinking of Hannah Arendt.

Understanding the complex biological processes that regulate our bodies requires the use of the full scientific armoury. Methods that offer insight into the structure and dynamics at the molecular scale are particularly potent analytical weapons. While X-ray-based methods spring immediately to everyone's mind, the application of neutron scattering to health-related topics is not common knowledge. This is certainly due to the fact that neutron scattering targets very specific information. Neutron crystallography reveals the position of hydrogen in biological structures with atomic resolution, provided these can be crystallised. Small-angle neutron scattering opens the door to structures on a slightly larger scale, but this time in the natural environment of liquid solutions. Neutron reflectometry characterises surfaces and interfaces, with particular attention given to membranes. Neutron spectroscopy provides access to the motion of atoms and molecules on microscopic timescales.

Given the societal relevance of biology, and sharing the conviction that neutron scattering can contribute important pieces of information to the huge biological puzzle, the ILL has deployed particular efforts in both instrumentation and auxiliary infrastructure. The European Photon and Neutron Campus (EPN) based in Grenoble offers ideal conditions for embedding the ILL's efforts into a broader collaborative activity including the European Synchrotron Radiation Source (ESRF), the Institute of Structural biology (IBS) and the European Molecular Biology Laboratory (EMBL). The Partnerships for Structural Biology (PSB) and Soft Condensed Matter (PSCM) were born out of the conviction that, by combining our methods, the EPN campus can offer unique, worldwide services to the scientific community.

The collection of articles in this brochure stems from recent results obtained on ILL neutron instruments – apart from one example that deals with isotope production employing neutrons directly generated in the Institute's reactor. The articles were carefully selected to give you an overview of the areas in which neutrons currently have an impact on health-related investigations. I hope that they convince you that the understanding of biological processes at the atomic level helps to rationalise how diseases affect our bodies. Once this first step is taken, we can go on to study how medication affects the pathological processes, which in turn will lead to novel or better-adapted therapeutic treatment.

I hope you enjoy the reading.

Helmut Schober
Associate Director
Head of Science Division

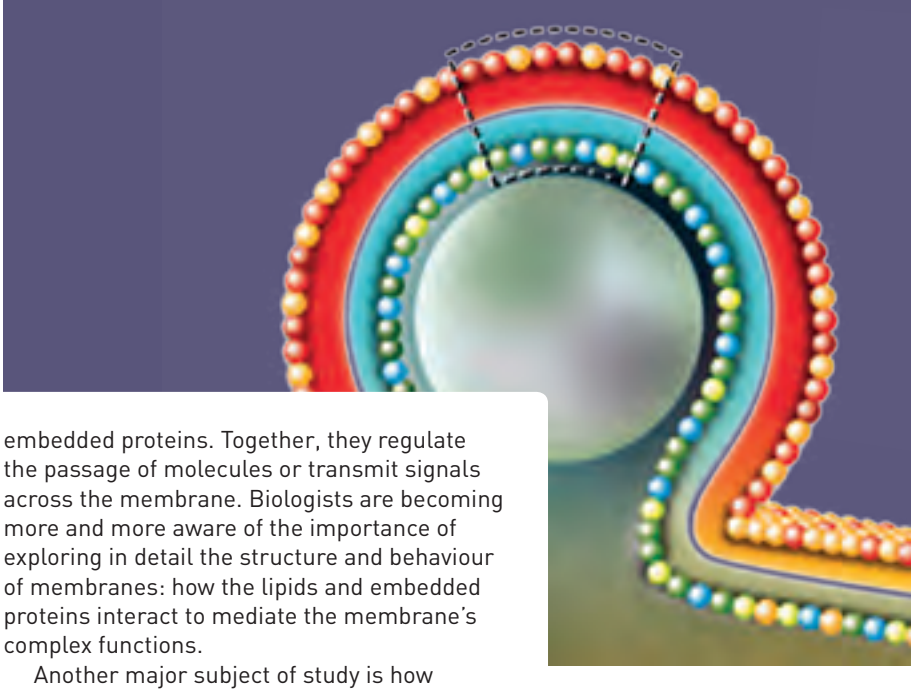
Front page image: The influenza virus
Centers for Disease Control and Prevention

CONTENTS

INTRODUCTION

EXPERIMENTS USING NEUTRONS ARE PLAYING AN INCREASINGLY VITAL PART IN BIOLOGICAL RESEARCH RELATED TO IMPROVING OUR HEALTH

Giovanna Fragneto and Matthew Blakeley



Good healthcare is an important component in our quality of life. However, it represents a significant socio-economic challenge in both the developed world, where rapidly ageing populations are expected to make increasing demands on medical resources, and the developing world, where transmitted diseases contribute to shortened life-expectancy.

The past few decades have seen major improvements in tackling a wide range of health problems, from cancer to viral infections. Nevertheless, future major challenges are emerging. One urgent concern is how to deal with the growing problem of microbial resistance to established drugs; another is the expected increase in age-related disorders, as more people live into their 90s and even beyond.

To meet such challenges requires a profound understanding of living processes – not just at the level of cells (the basic unit of life) but right down at the molecular level. Most diseases result from biomolecular processes going wrong. Viruses invade cells and take over their molecular machinery, eventually killing the cells. Bacteria may release toxins that block a vital molecular interaction. The ambitious aim of biologists today is thus to observe the structure and behaviour of biological molecules at atomic resolution, as they function in their natural, physiological environment.

THE MOLECULES OF LIFE

These molecules include the 40,000 to 100,000 different proteins specified by the human genome. Each protein structure consists of a chain of molecular units, amino acids, folded into a distinctive 3D shape that allows them to do a particular job. Some proteins, known as enzymes, mediate chemical reactions, others form important structures like skin and muscle, and yet others are involved in trapping energy, for instance by harvesting light in plants. Very often, the proteins do not act alone but as components of complex interactive molecular assemblies containing other proteins or large molecules such as DNA or RNA, sugars and fats. A significant example of one of these assemblies are cell membranes. They are composed predominantly of long-chain molecules, lipids, with water-loving and water-hating parts, which self-assemble into bilayers, and maintain the integrity of the cell and its various constituents. The lipid bilayer acts as a hydrophobic barrier that prevents the arbitrary exchange of solutes. However these bilayers, are not uniform; they contain not only different kinds of lipids but also embedded proteins. Together, they regulate the passage of molecules or transmit signals across the membrane. Biologists are becoming more and more aware of the importance of exploring in detail the structure and behaviour of membranes: how the lipids and embedded proteins interact to mediate the membrane's complex functions.

Another major subject of study is how proteins fold in cells. Subtle changes in the local physiological environment can trigger mis-folding to create compact structures associated with diseases such as Alzheimer's and Parkinson's. Protein structures and organisation are very much affected by water molecules and ions surrounding them.

NOTHING IS STATIC IN BIOLOGY

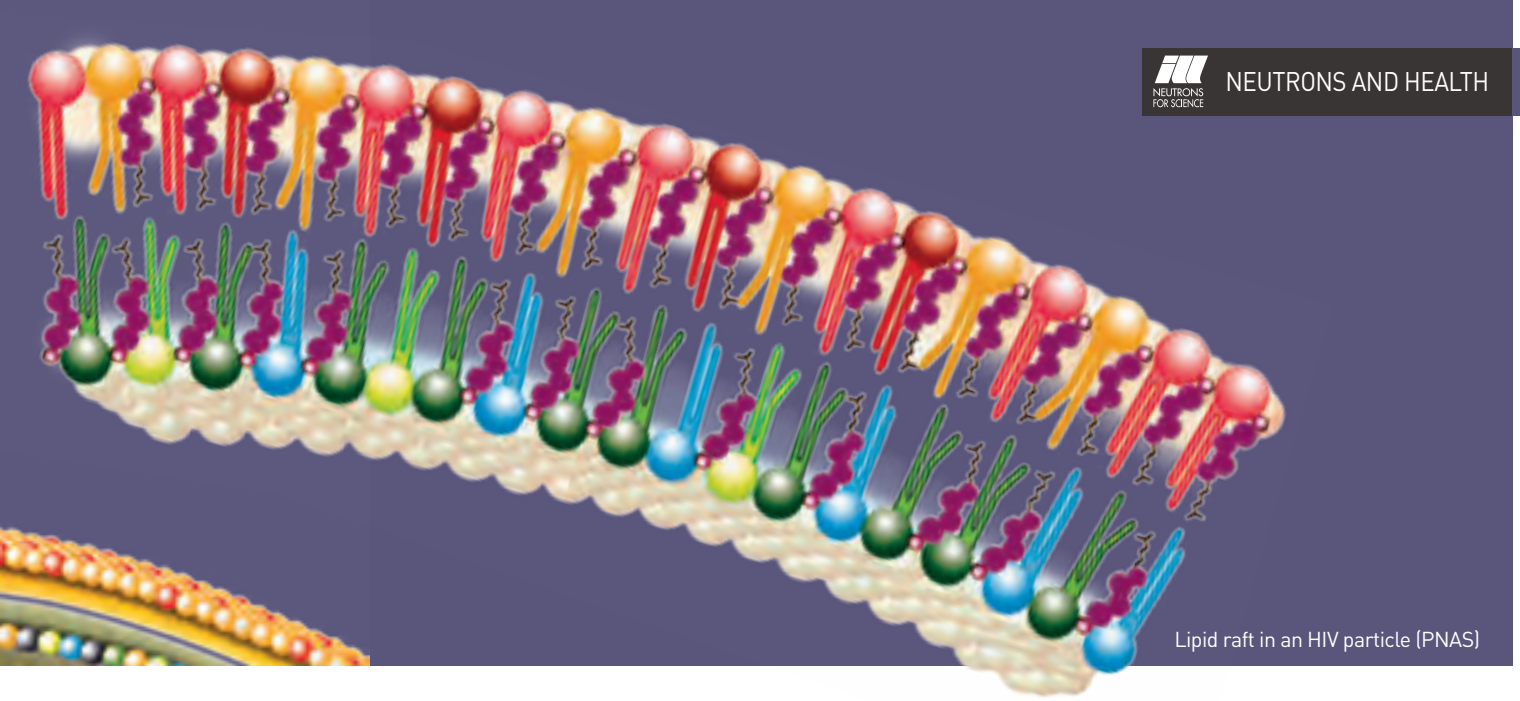
While it is clearly understood that the structure and 3D shape of biomolecules are directly related to their behaviour, it is well known that they are not rigid, but are soft and malleable, and dynamic changes within a molecular structure may play a significant part in correct functioning. Furthermore, the cellular environment is highly fluid; the molecules themselves do not remain in one location in, for example, a cell membrane, but move around as they are buffeted by the random jostling of their molecular neighbours.

Fortunately, we have a large number of chemical, biochemical and physical tools to study biological molecules and microcellular structures. In recent years, neutron scattering has played an increasing role, which will continue to grow. The various neutron techniques now available can reveal aspects of structure and dynamical behaviour not easily accessed by other methods.

ADVANTAGES OF NEUTRONS

Neutrons are one of the constituents of the atomic nucleus, and are produced by either breaking up nuclei (fission) or by knocking them out of nuclei (spallation). The ILL's nuclear reactor employs fission to produce the most intense beams of neutrons in the world.

Like X-rays, neutrons provide an invaluable analytical tool for studying the properties of matter at the atomic and molecular scales. Because neutrons are quantum particles, they have a characteristic wavelength, which is directly related to their energy. This wavelength is at least 1000 times shorter than that of visible light, so that, like X-rays, neutrons can 'see' matter at scales between tenths of nanometre and micrometres. Neutrons have enough energy to penetrate a structure and scatter off the nuclei of individual atoms.



Lipid raft in an HIV particle (PNAS)

APPLICATIONS TO BIOLOGY

Neutron beams are particularly suitable for biological research. They can be generated with energies and wavelengths appropriate for probing a range of biological structures, from small molecules such as lipids or peptides, to larger molecules and molecular assemblies including viruses. Being electrically neutral, neutrons can travel deep into materials and are non-destructive.

One of their most important advantages is that they interact quite strongly with hydrogen nuclei, so can pick out all the hydrogen atoms in a structure, including those in water molecules. This is in contrast to X-ray analysis, in which the X-rays are scattered only by the electrons in an atom, and thus do not easily see hydrogen. Determining the precise locations and orientations of all the hydrogens in a molecule is usually crucial to understanding its biological behaviour.

ENHANCING THE SIGNAL WITH DEUTERIUM

A unique feature of neutrons is that they are scattered differently by different isotopes of the same element. In particular, the heavier hydrogen isotope, deuterium, has a different scattering power from normal hydrogen, such that its scattering signal is more clearly seen. This property offers a fantastic tool for 'highlighting' hydrogens in a molecule by selectively replacing them with deuterium. It allows, for example, particular components of a large molecular structure to be picked out. This idea is taken further in the technique of 'contrast matching', in which a proportion of the hydrogens in the surrounding aqueous solvent is substituted with deuterium to a level such that the solvent's scattering power matches that of a selected component. The contrast between the solvent and the component is therefore zero; it is rendered invisible so that only the remaining structures are seen.

NEUTRON CRYSTALLOGRAPHY

In a crystal, the atoms are arranged in regular arrays, and as the neutron waves are reflected, they interfere with each other (a process called diffraction) – like ripples meeting on the surface of a pool – to produce a scattering pattern, recorded in a detector, that is characteristic of the atomic arrangement – a technique called crystallography. Modern molecular biology emerged through the application of X-ray crystallography of key biological structures such as DNA and proteins. While this remains the principal and routine method for solving the structure of large biomolecules at atomic resolution, neutron crystallography is used to provide complementary but essential information about the individual positions of light atoms like hydrogen. The use of the technique will expand as crystal preparation techniques improve (p7).

MEDICAL RESEARCH TOPICS WHERE NEUTRON EXPERIMENTS ARE CONTRIBUTING

- Investigating protein folding
- Details of enzyme behaviour
- Understanding of cell-membrane interactions
- Identification of new drug targets
- Advanced drug-delivery systems
- Solutions to antibiotic resistance
- Improved antiviral drugs
- Diagnosis and treatment of amyloid diseases
- Treatment of genetic diseases
- Orthopaedic implants and tissue regeneration
- Cancer diagnosis and therapy

SMALL-ANGLE NEUTRON SCATTERING

In diffraction studies, the angle of scattering is inversely proportional to the scale of the structure producing the diffraction pattern. Beams of neutrons deflected by an angle of only a degree or so, thus provide structural information at scales between 1 and several hundred nanometres, depending on the wavelengths used. Small-angle neutron scattering (SANS) is becoming ever more popular for probing larger cellular and other biological structures, especially in combination with isotopic substitution and contrast matching. SANS can be used to study bulk samples in solution as well as in solid form. The distance to the detector from the sample has to be very long (several metres) in order to be able to measure the tiny scattering angle.

NEUTRON REFLECTOMETRY

Neutrons have another property useful in biological research, and that is they can be reflected at grazing angles from surfaces, and the analysis of the signal after the interaction with the interface can reveal the 2D organisation of a surface layer. Neutron reflectivity offers an ideal tool for studying layered structures such as the cell membrane. Recent advances include the possibility of preparing model membrane systems in aqueous solution that mimic a real membrane in its physiological environment – that is, with a typically fluctuating complex composition involving different lipids and proteins.

ILL INSTRUMENTS USED IN BIOLOGICAL RESEARCH

- Neutron crystallography: the new LADI-III neutron diffractometer can work with sample crystals as small as 0.05 of a cubic millimetre. It is strongly complemented by the D19 diffractometer, which can obtain higher-resolution data from smaller proteins.
- Small-angle neutron scattering: the ILL has three dedicated SANS instruments, D11, D22 and D33.
- D16 is a diffractometer used to study a wide range of systems in biology including membranes.
- Reflectometry: the ILL has three reflectometers, D17, SuperADAM and FIGARO. The third instrument can reflect neutrons from above and below a horizontal sample surface.
- Inelastic and quasi-elastic scattering: the IN5, IN6, IN13 and IN16B spectrometers can look at the rapid molecular motions on biological molecules on the picosecond scale.
- Spin-echo: IN11 and IN15 spectrometers are used to analyse the motion of the macromolecular objects on the nanosecond scale.

PROBING DYNAMICS

Neutrons can be scattered in different ways. Instead of elastically bouncing off an atom like a tennis ball hitting a wall, they may lose or gain energy to or from the atom, such that the atom itself changes its motion. This is called inelastic or quasi-elastic scattering. We can use it to examine how a molecule moves and determine if a particular part of it is rigid or flexible. The way in which the molecule functions will often depend on this flexibility and the ability to adapt to the other molecules around it. Inelastic scattering techniques, including both spin-echo (p27) and quasi-elastic scattering, allows us to explore motions over periods from picoseconds to hundreds of nanoseconds, and so probe a wide range of dynamics, from the fast motions of small groups of atoms in proteins and other macromolecules to slower collective changes in large molecular assemblies and cellular structures. Dynamics studies are expected to become increasingly essential to understanding biological behaviour, and neutron-scattering experiments will provide a crucial tool here.

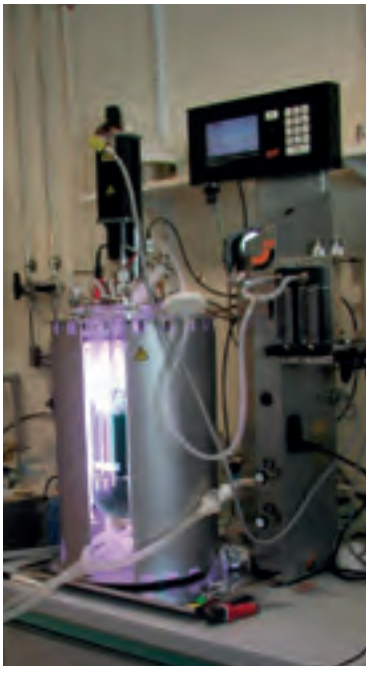
NEUTRONS AND RADIOLOGY

Neutrons have one more use in medicine. The neutrons generated in the ILL's nuclear reactor can be employed to make exotic isotopes with suitable radiological and pharmacological characteristics for scanning and diagnosing tumours, and also treating them. The ILL is developing techniques to make a new generation of more efficacious radio-isotopes routinely available to the clinic.

THE FUTURE

The future role for neutron experiments in understanding biological processes that will play a role in improving our health is thus set to expand. The ILL will continue developing the necessary techniques and instruments, and providing experimental support and knowledge, to researchers in the life sciences, who wish to exploit the huge analytical potential of neutrons.

Neutron-based research will have a special role in not only uncovering the extraordinary subtleties of living processes but also in contributing to the research of treatments that enable us to live long healthy lives.



FACILITIES FOR SAMPLE PREPARATION

Much of the progress in the application of neutron scattering to biological problems in recent years has been as a result of advances in optimising sample preparation and sample environment. The ILL participates in joint initiatives with its scientific neighbours in providing preparation and characterisation facilities for scientists carrying out biological research including neutron experiments.

One obstacle has been that sample crystals for neutron diffraction have to be quite large; however crystals of proteins of interest that can be grown are generally tiny. Samples of membrane proteins are particularly difficult to prepare, and yet they are one of key targets for drug designers. Nevertheless, techniques for preparing adequate membrane protein samples for structural studies have developed substantially.

- **The Partnership for Structural Biology (PSB)**, which is also located on the European Photon and Neutron (EPN) campus, is a collaboration between the ILL, the European Synchrotron Radiation Facility (ESRF), the European Molecular Biology Laboratory (EMBL), and the Institute of Structural Biology (IBS). It provides a comprehensive suite of technology platforms for life scientists, including advanced sample preparation and characterisation. Techniques for making suitable protein samples are continually being improved.
- A vitally important part of sample preparation is deuteration. The ILL operates a Deuteration Laboratory (D-Lab) as a PSB platform within the Life Sciences group. Researchers can obtain deuterated proteins and other bio-macromolecules using state-of-the-art molecular biology techniques. Macromolecular deuteration has a dramatic impact on sample needs and on the quality of the final molecular structure analysis.
- The importance of *in-situ* preparation and a good characterisation of the samples before neutron-scattering experiments has become more and more evident. An area where advances have been made is in the preparation of model lipid membranes that have the same kind of complex asymmetric structure found in real cell membranes. **The Partnership for Soft Condensed Matter (PSCM)**, which is an ILL/ESRF programme hosted in the newly built Science Building, provides a facility where several complementary techniques and sample-preparation tools are made available to soft-matter and biophysics users.

ABOUT THE ILL

The Institut Laue-Langevin (ILL) is an international research centre at the leading edge of neutron science and technology. It is located in a setting of outstanding beauty in the cosmopolitan city of Grenoble in south-east France.

The Institute operates the most intense neutron source in the world, feeding neutrons to a suite of 40 high-performance instruments that are constantly upgraded.

As a service institute, the ILL makes its facilities and expertise available to visiting scientists. Every year, about 2000 researchers from more than 30 countries visit the ILL. Over 800 experiments, which have been selected by a scientific review committee, are performed annually. Research focuses primarily on fundamental science in a variety of fields; these include condensed matter physics, chemistry, biology, materials science, engineering, nuclear physics and particle physics.

Neutron-scattering experiments have made significant contributions to our understanding of the structure and behaviour of biological and soft condensed matter, to the design of new chemicals such as drugs and polymers, and to materials used in electronics and structural engineering. Neutron studies also offer unique insights into the nature of complex systems at the most fundamental level.

NEUTRONS FOR EUROPE

ILL was founded in 1967 as a bi-national enterprise between France and Germany with the UK joining later in 1973. As well as these three Associate Members, 10 Scientific Members now participate in ILL: Spain, Switzerland, Austria, Italy, the Czech Republic, and more recently Sweden, Hungary, Belgium, Slovakia, Denmark and India.

The Partnership for Structural Biology on the ILL site

A REALISTIC MODEL CELL MEMBRANE

ADVANCED EXPERIMENTAL METHODOLOGIES DEVELOPED AT THE ILL WILL ENABLE BIOLOGISTS TO INVESTIGATE DETAILED STRUCTURES OF ASYMMETRIC MULTI-COMPONENT BIOLOGICAL MEMBRANES

Giovanna Fragneto and Valeria Rondelli

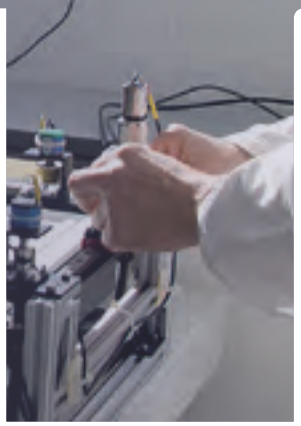
The cell membrane is extremely complex. Its phospholipid bilayer is host to many different components including specialised lipids and proteins. These constituents may be compartmentalised into spatially defined micro-domains called lipid rafts. These rafts, which host receptors involved in cell signalling, have a specific lipid composition that is high in cholesterol, and may also be enriched with more complex structures called gangliosides (sialic acid containing glycosphingolipids).

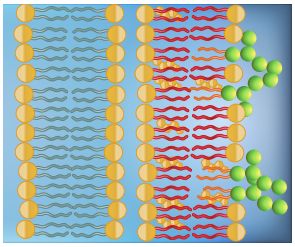
The gangliosides are found only in the outer layer of the membrane and have a special link with cholesterol, which largely resides in the inner membrane layer. The ganglioside-cholesterol coupling is thought to constitute a structural unit that traverses the membrane, regulating its structure, and mediating many biological processes dependent on cell communication, such as neural response and cell differentiation.

INVESTIGATING LIPID RAFTS

At the ILL, we recognised the need to validate an experimental model of a cell membrane that could be used to probe its structural, mechanical and biological complexities in a more realistic way than had been achieved previously. In particular, our aim was to prepare an artificial membrane that mimicked the asymmetric arrangement within the lipid raft. In collaboration with a team from the University of Milan, we therefore developed a protocol for preparing asymmetric floating lipid membranes containing the relevant amounts of cholesterol and glycosphingolipid, by building them up layer by layer on a silicon surface. We could then take neutron reflectivity measurements to ascertain the membrane's internal organisation and whether it remained stable.

We investigated two different model floating membranes – one with cholesterol, and one with both cholesterol and glycosphingolipid (GM1) – in order to see how the addition of GM1 affected the distribution of the cholesterol. By selectively deuterating the components and contrast-matching (p5) with the solvent (water) to highlight each component, we were able to show that we had indeed built, for the first time, a multi-component asymmetric system similar to that of a real biological membrane.





Work on a Langmuir trough (above) and floating asymmetric lipid membrane (below)

Our study also threw light on another aspect. Preparing model membranes usually involves annealing (heating and cooling the sample to melt and gel the lipid chains so that they arrange themselves evenly). We found that if we prepared a model membrane with an asymmetric layer of cholesterol only and then annealed it, the cholesterol was redistributed symmetrically – possibly indicating that annealing is not a suitable method for preserving some complex membranes. In the presence of GM1, however, annealing, does not lead to an even distribution of all components.

The presence of the glycosphingolipid appears to enforce the continued asymmetric distribution of cholesterol in the membrane at the same level, regardless of the initial distribution of the components. The proportions in the layers are very close to the proposed physiological distribution (roughly 80 per cent in the inner layer of the membrane and 20 per cent in the outer, together with GM1).

This is an important finding, as ganglioside–cholesterol structural coupling is considered by many to determine the membrane raft's structure, although this has never been experimentally confirmed.

These experiments have amply demonstrated that the combination of highly advanced neutron reflectivity techniques and the newly developed asymmetric membrane deposition method developed at the ILL promises to be a powerful tool in exploring the structure and function of biological membranes at the molecular level.

RESEARCH TEAM: Valeria Rondelli, Simona Motta, Elena Del Favero, Paola Brocca, Sandro Sonnino and Laura Cantù (University of Milan), and Giovanna Fragneto (ILL)

ILL INSTRUMENT USED: D17 reflectometer

REFERENCES: V. Rondelli *et al., Biochim. Biophys. Acta*, 2012, **1818**, 2860; V. Rondelli *et al. Eur. Phys. J. E*, 2013, **36**, 9888

STEALTH NANO-CARRIERS FOR ANALYSING MEMBRANE PROTEINS

TINY PARTICLES THAT MIMIC THE CELL MEMBRANE, AND CAN BE MADE INVISIBLE TO NEUTRONS, COULD PROVIDE A POWERFUL NEW TOOL FOR STUDIES OF MEMBRANE PROTEINS

Selma Maric and Trevor Forsyth

Most drugs in development today are targeted at proteins embedded in the cell's membranes. This is because membrane proteins are responsible for mediating many key cellular processes. Examples are the G-protein-coupled receptors, which play a central role in cell signalling and account for almost half of all drug targets today. Drug molecules that modify or obstruct the function of such membrane proteins can induce a therapeutic change – or even kill the cells, as in the case of invading bacteria. Obtaining structural information about membrane proteins is thus of key importance to developing more specific drugs.

While huge steps have been made in determining the 3D crystal structures of proteins using X-ray – and more recently neutron crystallography (p5) – membrane proteins remain difficult to crystallise, and only a very small number of their structures have been solved. Furthermore, the solid-crystal structure may not represent the protein's true arrangement in the cell membrane, where interactions with the constituent lipids and surrounding water molecules may be significant. Now, however, tiny self-organising systems that mimic the native environment are being investigated: liposomes – lipid vesicles about 120 nanometres across – and 'nanodiscs', consisting of a phospholipid bilayer (as found in cell membranes) bound by a stable protein 'belt' into a disc about 10 to 14 nanometres in diameter. Both systems can be used to host target membrane proteins for diffraction experiments.

These nano-carriers allow the study of membrane proteins that are usually only available in tiny quantities. However, the presence of the carrier introduces additional complexities in the data obtained, making it difficult to extract the relevant information from the sample about the protein alone.

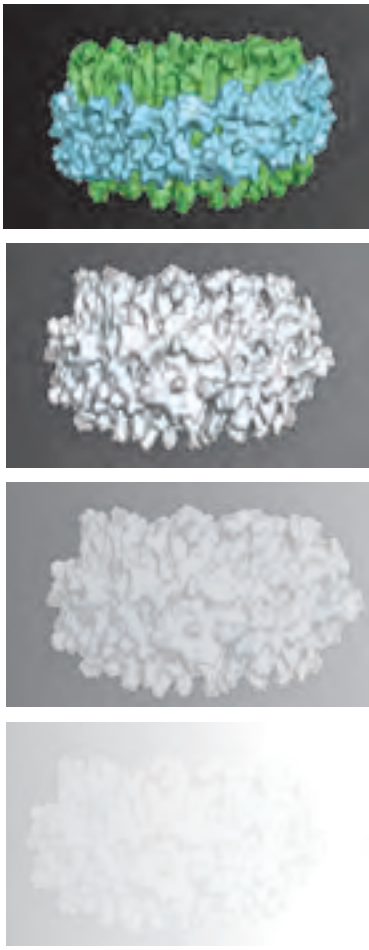
NOW YOU SEE IT; NOW YOU DON'T

This is where neutron methods can help. Using the well-established techniques of selective deuteration and contrast matching (p.5), we have been carrying out experiments that show it is possible virtually to eliminate the interfering scattering signal from the carrier. The lipid and/or protein components can be deuterated in such a way that their scattering signals match that of the solvent (deuterated water). They then become invisible, behaving like 'stealth' carriers so that only the sample protein is seen.

The lipid chosen was phosphatidylcholine as best matching the membrane lipids. Because deuterated versions are not commercially available, we obtained samples by producing the lipid in a specially engineered strain of the bacterium, *Escherichia coli*, which had been grown in selected deuterated nutrients. In this way, we were able to obtain samples with high levels of deuteration in the lipid component of the discs. The nanodisc protein belt – which gave an nanodisc about 10 nanometres across – was similarly deuterated.

Using pure heavy water (100 per cent D_2O) as the solvent to achieve the clearest scattering signal, we then looked at how close to invisibility we could get in the liposomes and the nanodiscs. While for both systems, a residual signal could be seen, in the case of the stealth nanodiscs – because of their smaller size – their scattering contribution could be considered negligible. We believe that the contrast matching could, in fact, be improved further through careful tuning of the deuteration protocols.

The studies demonstrate that these advanced stealth carriers have enormous potential. In particular, they offer a route to studying the very important class of membrane proteins in a close-to-native environment. Furthermore, the deuteration approach through biosynthesis allows for the production of different types of specifically deuterated physiologically-relevant lipids that can be exploited using other structural techniques.



The decreasing contrast in neutron-scattering signal of a nanodisc consisting of a deuterated phospholipid bilayer (green) and protein scaffold (blue) in increasing proportions of deuterated water to 100 per cent (top to bottom)

RESEARCH TEAM: Selma Maric, Nicholas Skar-Gislinge, Søren Midtgaard, Mikkel Thygesen, Thomas Günther Pomorski and Lise Arleth (University of Copenhagen), Jürgen Schiller (University of Leipzig), Henrich Frielinghaus (JCNS), Martine Moulin and Trevor Forsyth (ILL/Keele University), and Michael Haertlein (ILL)

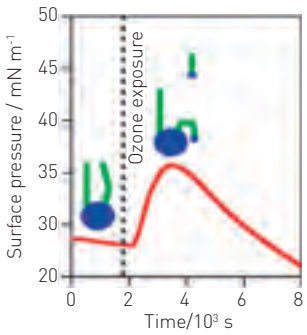
ILL INSTRUMENT USED: D11 SANS diffractometer, Deuteration Laboratory (D-Lab)

REFERENCES: Maric *et al.*, *Acta Cryst.*, 2014, D**70**, 317; doi:10.1107/S1399004713027466

UNDERSTANDING OZONE POISONING

NEUTRON STUDIES REVEAL HOW OZONE POLLUTION DAMAGES THE LINING OF THE LUNG

Katherine Thompson and Richard Campbell



The damaging effect of ozone on an unsaturated lipid monolayer showing how one hydrocarbon tail bends round and breaks off as the surface pressure of the monolayer increases

Anyone who lives in a traffic-congested city will know that ground-level pollutants triggered by hot sunny weather can cause serious respiratory problems, especially in the elderly or those suffering from asthma. Using neutrons, we have been investigating at the molecular level just how the pollutant ozone affects the lungs.

The lung surface is lined with a fluid mixture of phospholipids and different types of protein, which together act as a surfactant (p27) that operates under dynamic conditions. The mixture lowers the surface tension at the air–water interface such that oxygen and carbon dioxide can efficiently pass in and out as you breathe. A deficiency in the lung surfactant is known to be the cause of respiratory failure in premature babies. It is also believed that ozone, which is a highly active molecule made up of three oxygen atoms, attacks the lipid lining and diminishes surfactant function.

The phospholipids each have two long-chain hydrocarbon tails that protrude from the lung surface. The dominant component is a saturated lipid, dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), in which both tails are fully saturated, and is therefore not very chemically reactive. Unsaturated phospholipids such as 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) are also present, and are essential for the surfactant to function correctly. In POPC, one of the hydrocarbon tails has a reactive carbon–carbon double bond in the middle, which can react with oxidising molecules like ozone. Indeed, studies have indicated that the unsaturated POPC lipid – though not DPPC – does react with ozone at an air–water interface.

A BROKEN TAIL

To investigate in more detail what happens at a model lung surface, we employed neutron reflectometry (p6) to probe changes in the structure of a model lipid monolayer over time, when concentrations of ozone similar to those associated with air pollution were introduced.

At the same time, we also monitored the changing surface pressure (the difference in surface tension from that of pure water). Measurements were made both with POPC alone, and with POPC and DPPC together. We wondered if reactions between ozone and POPC could produce highly active chemical species that would also attack DPPC. We wanted to see if this happened and if the presence of the saturated lipids had any effect on the fate of the unsaturated lipid. The lipids were partially deuterated so as to highlight selectively each lipid and/or the different parts of their hydrocarbon chains (p5).

What we found was that in the pure POPC layer, the ozone rapidly reacted with the unsaturated tail so that its end broke off and disappeared at a rate that correlated with a reduction in surface tension (20 minutes). Only a small amount of the other, saturated tail was lost. We inferred that the remaining damaged portion of the hydrocarbon then bends around causing overcrowding in the surface layer in a way that increases surface pressure, thus lowering the surface tension. This is followed by a slower increase in surface tension over several hours. Our experiments on mixed POPC/DPPC monolayers reveal that DPPC remained unaffected by exposure to ozone, even in the presence of reactive lipids such as POPC.

Although we now have a better understanding of the processes involved, there are still many unknowns: how long the damaged lipids remain at the interface, how they affect lung function, and how ozone attacks the active proteins. We do not yet know what causes the final gradual increase in surface tension, which may be linked to the respiratory problems associated with ozone poisoning. Further experiments are underway on FIGARO to answer these questions.

RESEARCH TEAM: Katherine Thompson and Brian Hughes (Birkbeck, University of London), Stephanie Jones (Royal Holloway, University of London/CLF, STFC Rutherford Appleton Laboratory), Adrian Rennie (Uppsala University), Martin King (Royal Holloway, University of London), Andrew Ward (CLF, STFC Rutherford Appleton Laboratory), Claire Lucas (Royal Holloway, University of London/ISIS, STFC Rutherford Appleton Laboratory), Richard Campbell (ILL) and Arwel Hughes (ISIS/STFC Rutherford Appleton Laboratory)

ILL INSTRUMENT USED: FIGARO reflectometer

REFERENCES: Katherine C. Thompson *et al.*, *Langmuir*, 2013, **29**, 4594, dx.doi.org/10.1021/la304312y |

THE ONLY WAY IS UP

NEUTRONS HIGHLIGHT THE POTENTIAL OF ADVANCED DRUG-DELIVERY SYSTEMS

Richard Campbell and Marité Cárdenas

A key goal for drug designers is to find ways of delivering therapeutic agents effectively to sites of disease in the body. Ideal carriers must be able to bind selectively with target cells and mediate the passage of an optimised concentration of drug molecules across the cell membrane in a sustained and controlled way. An exciting new approach is to attach 'reservoirs' of the drug to the membrane in the form of liquid-crystalline particles. These particles form spontaneously from long-chain lipids and branched tree-like molecules called dendrimers. They are ideal candidates for transporting large amounts of small drug molecules for diffusion across cell membranes.

Delivery systems using similar principles are already being tested in clinical studies. However, the development of market products would benefit from a more detailed understanding of the interactions of the drug reservoirs with membranes. The composition of the particles will affect how they interact with the cell membrane, which is important in controlling the dosage over an extended time. Furthermore, the achievement of selective targeting of, for example, cancerous tissues – given that there is a higher electrostatic charge on cancer cells than on healthy ones – requires knowledge of how the charge on the membrane affects the binding process.

Neutron reflectometry is an excellent probe to help answer these questions. A neutron beam is obliquely bounced off a model cell membrane – a lipid bilayer prepared on a smooth silicon crystal – to reveal the membrane structure and that of any attached material. In particular, the ILL's FIGARO instrument can uniquely take measurements from both above and below a horizontal sample in kinetic mode, which proved to be essential in this study.

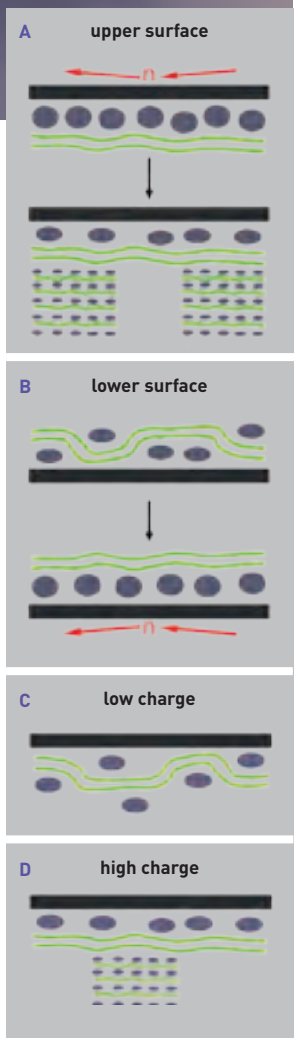
INTERACTIONS WITH MODEL CELL MEMBRANES

Recently, we carried out experiments, in which we looked at the interactions of a suspension of the lipid–dendrimer particles with model surfaces located both above and below it. Over time – as a result of their lower density – the particles separate out into a concentrated phase floating on a more dilute phase. We then regularly measured the reflectivities of the layers that formed on the model surfaces, and determined their evolving structures over a period of 30 hours.

We found that when the suspension interacted with a model surface positioned below, only a single lipid–dendrimer layer formed on it. However, when the model surface was in the above position, the liquid-crystalline particles in the concentrated phase attached themselves to it, to create the kind of reservoir–surface configuration that is of interest. Such surprisingly dramatic differences in the surface interactions provide an important message to formulation scientists: gravity can have a substantial effect, so the orientation of the surface during trials of new products can be critical to their success.

We then carried out similar experiments using silicon supports positioned above the suspension, which were coated with one of three model membranes, each a lipid bilayer with a different electrostatic charge. The liquid-crystalline particles quickly attached to the two bilayers with the greatest negative charge, and dendrimer molecules diffused across to the other side. However, the particles did not attach to the supported bilayer with the least charge, and the dendrimer diffusion was much slower. This result demonstrates the potential for tuning the charge within new lipid–dendrimer formulations to deliver drugs for targeting specific cell types.

Key questions remain concerning the mechanism that controls the diffusion of the dendrimer molecules across the membrane combined with the attachment of the reservoirs. Future research is planned, which includes encapsulating small drug molecules in the particles for controlled slow release.



The interactions of a lipid–dendrimer particle suspension (molecules in green and purple respectively) with model silicon supports, positioned (A) above and (B) below the sample, and with supported lipid bilayers of (C) low and (D) high electrostatic charge

RESEARCH TEAM: Research team: Richard Campbell and Erik Watkins (ILL), Vivien Jagalski, Anna Åkesson-Runnsjö and Marité Cárdenas (University of Copenhagen)

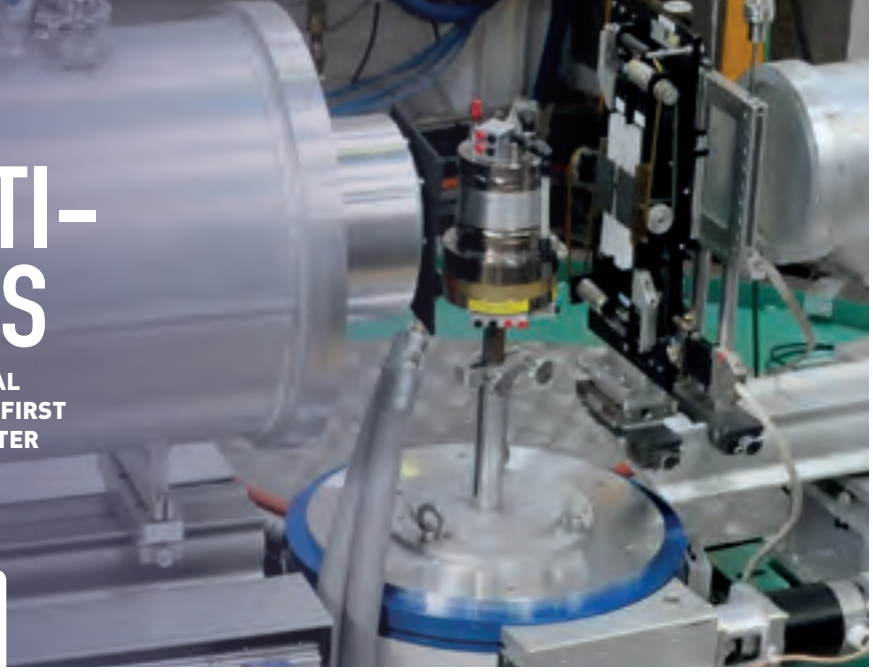
ILL INSTRUMENT USED: FIGARO reflectometer

REFERENCES: Richard Campbell *et al.*, *ACS Macro Lett.*, 2014, **3**, 121

IMPROVED ANTI-FUNGAL DRUGS

STRUCTURAL STUDIES OF HOW THE ANTI-FUNGAL AGENT, AMPHOTERICIN B, WORKS PROVIDE THE FIRST STEP IN THE DEVELOPMENT OF DRUGS TO COUNTER ANTIBIOTIC-RESISTANT INFECTIONS

David Barlow and Bruno Demé



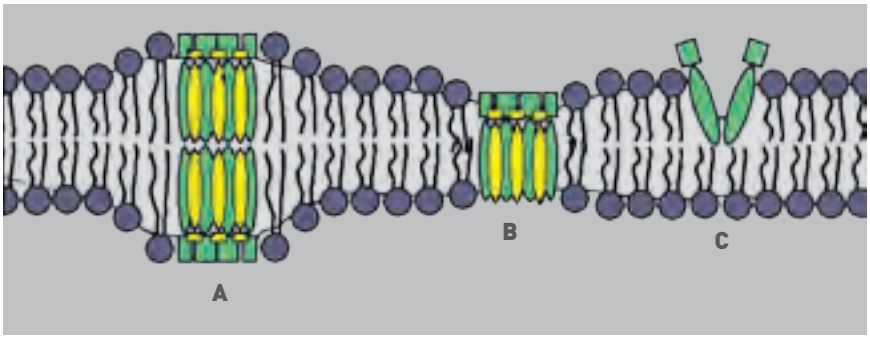
Amphotericin B (AmB), is widely used to treat life-threatening fungal infections that may arise in severely immuno-compromised patients, for example, those who have undergone chemotherapy or have contracted AIDS. Recently, however, fungal pathogens resistant to the drug have emerged. This has led to higher doses of amphotericin being prescribed, which can result in kidney damage. There is therefore an urgent need to find new drugs that work in the same way as AmB, but have a molecular structure that is different enough such that the fungi cannot easily evolve to overcome its toxic effects.

Despite the fact that the AmB has been in clinical use for more than 50 years, we still do not know exactly how it works at the molecular level. The most accepted idea is that the molecule alters the structure of the cell's lipid membrane – a double layer of phospholipids in which are inserted various other fatty molecules such as sterols (p27). We do know that fungal cell membranes are slightly different from human ones in that they contain the sterol, ergosterol, instead of cholesterol. AmB is thought to interact more strongly with ergosterol than with cholesterol – but exactly how remains unclear.

PINPOINTING THE DIFFERENCES

Several ideas for the mechanism have been proposed, and the most likely one is that the drug molecules insert themselves across the membrane so as to form pores. These facilitate the indiscriminate passage of ions in and out of the cell, so that it can no longer function correctly and therefore dies. Recent evidence, however, has suggested that this description is too simplistic. Our aim was to find out the precise difference between the interactions of AmB and cell membranes containing cholesterol and those with ergosterol. In this way, we hoped to pinpoint the critical chemical features needed in an effective anti-fungal agent. Neutron diffraction provided the methodology to do this.

We carried out experiments using model lipid membranes supported on silica, containing either cholesterol or ergosterol, and with and without AmB. The lipid layers were partially deuterated so that the position and orientation of AmB within them could be readily distinguished in the scattering patterns.



The various structures proposed for the pores formed by amphotericin in lipid bilayers: aligned half-pores/ion channels (A), half-width pores (B) and half-pores (C). Amphotericin molecules are shown in green, sterols, yellow, and phospholipids, red

The results showed that AmB combined with both sterols to form pores in the lipid bilayer. However, the ergosterol molecules were more extended across both lipid layers, and that may help to stabilise the formation of continuous channels right across the membrane. The cholesterol–AmB combination tended to form separate 'half-channels' for each lipid layer, which were less likely to be aligned. The insertion of AmB also caused the sterols to become tilted with respect to the membrane, with cholesterol tilting more than ergosterol. It may be that AmB is able to react more readily with the latter.

These experiments have provided a clear view of how AmB interacts with fungal and human cell membranes and we now plan more detailed studies, which we hope will uncover the exact chemical differences that we can then use to design new anti-fungal drugs with the same specificity as AmB, but lacking its damaging side-effects.

RESEARCH TEAM: Fabrizia Foglia, M. Jayne Lawrence and David Barlow (King's College London), and Bruno Demé and Giovanna Fragneto (ILL)

ILL INSTRUMENT USED: D16 small momentum-transfer diffractometer

REFERENCES: F. Foglia *et al., Sci. Rep. 2*, 778; DOI:10.1038/srep00778 (2012)

TOWARDS SELF-INJECTABLE TARGETED DRUGS

NEUTRON BEAMS REVEAL HOW ANTIBODIES CLUSTER IN SOLUTION

Peter Falus

Targeted treatment using monoclonal antibodies (mAbs) is an important tool in modern pharmacology; these large proteins provide the basis for a growing number of successful drugs for treating cancer, and also autoimmune disorders such as arthritis and multiple sclerosis. As agents for targeted therapy with a good safety profile, they are an alternative to harsher, traditional chemotherapy treatments.

The mAbs work by attaching themselves to specific protein targets (an antigen), for example, on cancerous cells or in a known biochemical pathway responsible for a disease. These treatments usually require high doses, which are fed through a series of intravenous drips in the clinic. Recently, there has been considerable interest in moving to a more convenient subcutaneous delivery (that is, via a shallow injection just below the skin, as routinely self-administered by diabetics). However, progress has been hampered by the fact that solutions containing large amounts of protein are very viscous for some mAbs. This makes them not only difficult to administer via a single injection with a thin needle, but also presents considerable challenges to their large-scale production and purification through filtration or freezing.

As a result, researchers have been trying to understand the root cause of this thickening so that systems can be devised, which contain concentrated amounts of the mAbs that are much less viscous.

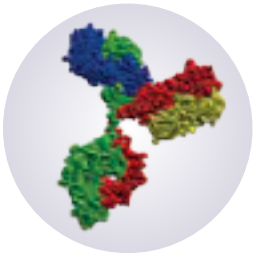
To investigate the structure and dynamics of the clustering at the molecular length-scale, a team led by the US NIST Center of Neutron Research (NCNR), and including researchers from the biotechnology company Genentech, the University of Delaware and the ILL, employed a combination of two neutron techniques – small-angle neutron scattering (SANS, p6) and neutron spin-echo (NSE, p27) – to study two types of antibody: one known to increase the viscosity of the solution and one that does not, so as to compare their behaviour at the microscopic scale. The two mAbs differed only in the short sequence of amino acids in their protein structures, which is responsible for binding with their antigens.

PROBING CLUSTER FORMATION

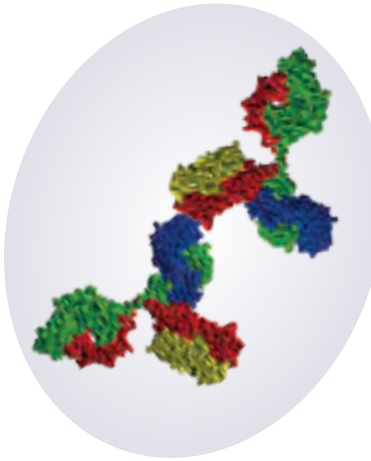
NSE is able to follow the individual movement of protein structures, as in the case of cluster formation of large biological molecules like mAbs. The unrivalled high resolution and neutron intensity provided by the ILL's NSE instrument, IN15, enabled many different mAb samples to be explored.

Previous light-scattering studies from a mAb solution had suggested that the high viscosity could be due to the formation of protein clusters. The results from the neutron-scattering experiment revealed that the mAb solutions with high viscosity are dominated by small mAb clusters. Once formed, the size of these clusters is almost constant over a wide range of concentrations.

This understanding provides the basis for designing an optimal device system for delivering injectable biopharmaceuticals at very high concentrations. In addition, the subject of protein clustering is an extremely interesting area in its own right. Many well-known phenomena, such as the cataracts in our eyes, or Alzheimer's disease, are the results of proteins clustering in our bodies, and neutron techniques provide a unique, high-resolution tool to investigate these complex interactions. This result also provides new physics insights into the phenomenon of protein clustering.



MONOMER



CLUSTER

Monoclonal antibody proteins form clusters in solution which thickens it, making them difficult to inject

RESEARCH TEAM: Yun Liu, Eric Yearley, Paul Godfrin and Tatiana Perevozchikova (NCNR/ University of Delaware), Hailiang Zhang (NCNR), Peter Falus and Lionel Porcar (ILL), Michihiro Nagao, Joseph Curtis, Pradad Gawande and Rosalynn Taing (Genetech), Isidro Zarraga (Genentech) and Norman Wagner (University of Delaware)

ILL INSTRUMENT USED: IN15 neutron spin-echo spectrometer, D22 SANS diffractometer

REFERENCES: E. J. Yearley *et al., Biophysical Journal*, doi:10.1016/j.bpj.2014.02.036, April 16, 2014

A GREEN ROUTE TO CLEAN WATER

PROTEIN FROM THE SEEDS OF THE MORINGA TREE OFFERS THE IDEAL SOLUTION TO WATER PURIFICATION

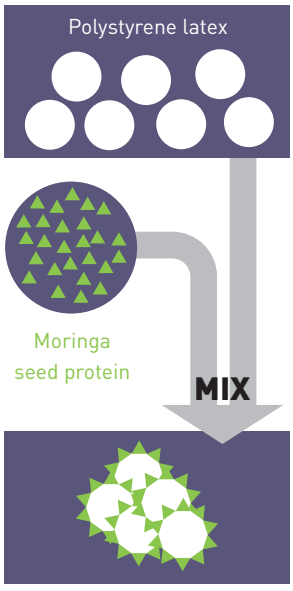
Lionel Porcar

Providing safe, clean drinking water is a major health issue in many developing countries, where local supplies can often be contaminated. One step in water purification is to add a 'flocculating' agent that causes any particulate impurities to clump together into an insoluble mass, which can then be filtered off – in much the same way as chicken broth can be clarified with egg white. A similar approach is used in many industrial processes to separate out waste products.

Until now, metal salts or synthetic polymers have been the main agents used. However, studies over recent years have uncovered a potentially cheaper, greener and more sustainable agent for water purification, based on protein obtained from the Moringa tree. Various species of this plant are native to Africa and Asia, and are already widely cultivated as a highly nutritious food source for both humans and livestock. The seeds, in particular, provide a concentrated protein source, and when ground into a paste, also act as a flocculating agent that appears to be more effective than many conventional agents.

INVESTIGATING FLOCCULATION

An international team from Africa, Europe and the US has been using neutron scattering to investigate the structure of the flocs that form with this protein in order to understand why it is so effective, and to ascertain the ideal concentration of protein needed to ensure maximum flocculation. The work was carried out at both the ILL and the NIST Center for Neutron Research in the US, using a suspension of polystyrene latex particles in water. It compared the aggregates formed by protein extracted from the seeds of two Moringa varieties: *Moringa oleifera* and *Moringa stenopetala*, at different concentrations of protein and latex particles.



At the ILL, small-angle neutron scattering (SANS, p6), combined with contrast matching (p5) was used to probe the bound protein on the latex particles. The latex particles were deuterated and contrast matched to the surrounding solvent, making them virtually invisible. This enabled the scattering signal from just the protein that adsorbed onto the particle surfaces to be distinguished. In this way, we could measure the amount of the protein around the particles. At NIST, the team used an ultra-small-angle neutron scattering (USANS) instrument with a new setup that rotated the samples to prevent sedimentation. A range of samples was studied.

The measurements showed that the proteins stick strongly to the latex particles and the flocs formed are very dense. The protein aggregates formed by *M. oleifera* were almost as compact as they could possibly be, while those from *M. stenopetala* were a little looser. The denser the flocculated material, the more easy it is remove.

The research group has already presented the results to government agencies, particularly in Namibia and Botswana. The aim is to replace conventional materials with the seed protein in both large water-treatment plants and in small-scale units.

RESEARCH TEAM: Maja Hellsing, Adrian Rennie and Ida Berts (Uppsala University), Habauka Kwaambwa (Polytechnic of Namibia), Fiona Nermark and Bonang Nkoane (University of Botswana), Andrew Jackson (ESS/Lund University), Matthew Wasbrough (NIST Center for Neutron Research/University of Delaware, US) and Lionel Porcar (ILL)

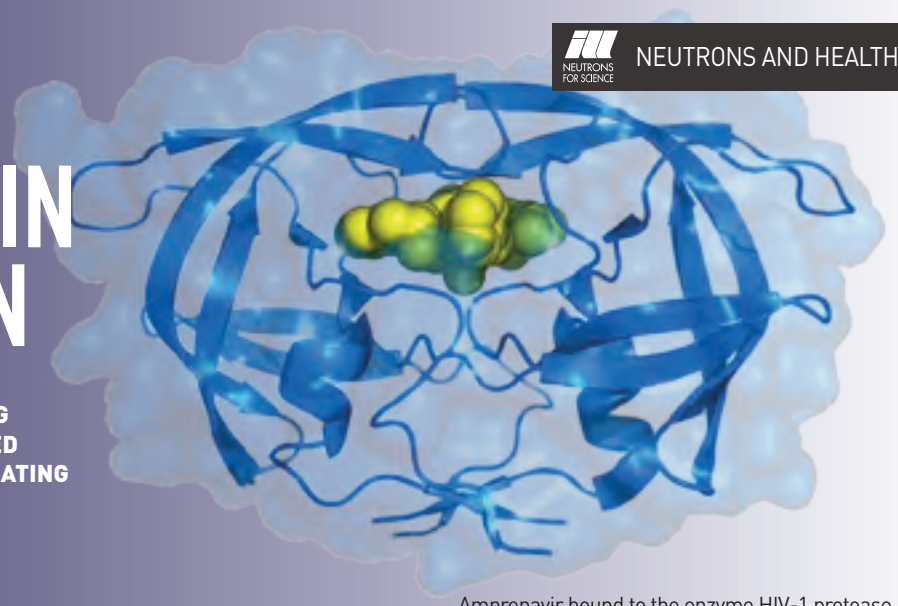
ILL INSTRUMENT USED: D22 SANS instrument

REFERENCES: M. S. Hellsing *et al., Colloids Surf. A: Physicochem. Eng. Aspects*, 2013, in press, http://dx.doi.org/10.1016/j.colsurfa.2013.11.038

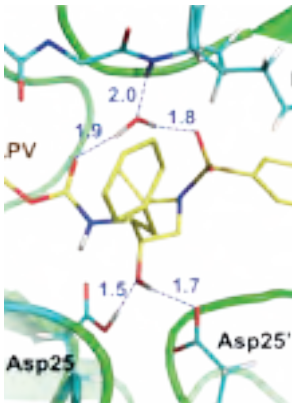
NEW VISTAS IN DRUG DESIGN

NEUTRON CRYSTALLOGRAPHY IS PROVIDING BREAKTHROUGHS IN DEVELOPING IMPROVED ANTIVIRAL DRUGS SUCH AS THOSE FOR TREATING HIV INFECTION

Andrey Kovalevsky and Matthew Blakeley



Amprenavir bound to the enzyme HIV-1 protease



The active site in HIV-1 protease

Most drugs work by binding to a specific enzyme involved in the proliferation of a disease, so that its function is inhibited. Modern drug design focuses on analysing and optimising the interactions between the drug and its target, and X-ray crystallography has been the routine method of choice to unravel these structural details. However, X-rays do not generally reveal the position of hydrogen atoms in a molecule, and yet their role in binding can be crucial, as it is often mediated by important weak interactions known as hydrogen bonds.

Neutrons, however, can locate the positions of all the atoms including hydrogen, and so they provide a powerful analytical tool for analysing drug-binding interactions. Until recently, neutron crystallography was hampered by the fact that large crystals were required and data took a long time to collect. However, thanks to advances in instrumentation and sample preparation at the ILL, crystals can now be much smaller (only 0.05 of a cubic millimetre), which opens up new avenues for structure-guided drug design using neutrons.

The potential has been acutely demonstrated in a recent study of the binding between an anti-retroviral HIV drug (amprenavir) and its target enzyme, HIV-1 protease. This is a key enzyme in the HIV life-cycle, breaking down viral polypeptides to create the proteins needed for the maturation and the production of new infectious virus particles.

Using a 0.2 cubic-millimetre crystal of HIV-1 protease (deuterated to improve the neutron signal, p5) bound to amprenavir, neutron diffraction data were collected at a resolution down to 0.2 nanometres. These data allowed the research team to locate the positions of the hydrogen atoms in the enzyme–drug complex – and, critically, to identify those participating in hydrogen bonding between the drug and the enzyme.

Previous X-ray studies had speculated that several hydrogen-bond interactions were important in the binding; however, the neutron study showed that, in fact, only two strong, direct hydrogen bonds exist between the drug and the enzyme. This finding presents drug designers with new ways to strengthen the binding through subtle modifications of the drug's molecular structure, thereby increasing the effectiveness of the drug and reducing the necessary dosage. For example, the two strong hydrogen bonds can be made yet stronger by creating so-called low-barrier hydrogen bonds via the introduction of a reactive atom such as fluorine. Or, alternatively, the weaker water-mediated hydrogen bonds could be replaced by stronger, direct hydrogen-bond interactions, by incorporating larger groups of atoms in the structure that would expel water molecules currently found in the binding site.

OVERCOMING DRUG RESISTANCE

Another important issue in combatting HIV infection is drug resistance. The evolution of the virus over time produces enzyme variants with weakened binding affinity – a process that is actually sped up by the introduction of the drugs themselves. One way around this would be to improve the binding of the drug with the atoms in the enzyme's main-chain rather than side-chain atoms, as the main-chain atoms cannot mutate. Before this latest study, researchers thought that the potential for advances in this area were limited, because the hydrogen bonds with the main-chain atoms were already strong. However, this has now been shown not to be the case, creating a new avenue for the development of HIV pharmaceuticals much less affected by virus evolution and resistance.

RESEARCH TEAM: Andrey Kovalevsky, Marat Mustyakimov and Paul Langan (Oak Ridge National Laboratory, US), Matthew Blakeley (ILL), Irene Weber (Georgia State University), Mary Jo Waltman (Los Alamos National Laboratory, US), David Keen (ISIS/Rutherford Appleton Laboratory, UK) and Arun Ghosh (Purdue University, US)

ILL INSTRUMENT USED: LADI-III quasi-Laue neutron diffractometer

REFERENCES: Weber *et al., J. Med. Chem.*, 2013, **56**, 5631

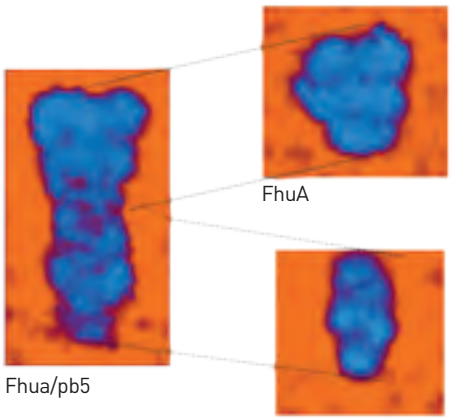
A VIRUS'S TALE

HOW DO BACTERIOPHAGES SENSE THE PRESENCE OF THEIR HOST?

Cécile Breyton, Frank Gabel and Christine Ebel

An electron micrograph of phage T5 showing its long flexible tail ending in fibres that bond to the bacterial membrane receptors

Bacteriophages, the viruses that infect bacteria, are the most abundant and diverse organisms on Earth. They play a crucial role in the ecology, evolution and pathogenicity of bacterial populations – whether in the oceans, or in the human intestine where they also contribute to our immunity. Phages consist of a capsid containing the phage genetic information, and a tail that allows it to recognise its host. Following the binding of the tail tip to the host receptor, the viral genome is injected into the cytoplasm of the bacterium. The biosynthetic host machinery is then hijacked to produce new virions, killing the cell. Phage therapy is becoming an alternative to treat antibiotic-resistant bacterial infections, so it is important to understand the mechanism of infection.



Electron microscopy class averages of the bound complex bacterial–phage complex, FhuA–pb5

At the molecular level, phage infection is triggered by the interaction of a receptor-binding protein to the bacterial receptor, which is either a sugar or a protein of the surface of the cell. In the case of the phage T5 (top), which infects the bacterium *Escherichia coli*, a receptor binding protein, pb5, irreversibly binds to a protein in the bacterial outer membrane, called FhuA. This interaction is sufficient to induce the opening of the capsid and the perforation of the cell wall by phage-tail proteins, but how is the binding information transmitted to the rest of the phage? To obtain insights into this question, we have purified both proteins and studied their structures both separately and within the complex.

PROTEINS IN SOAPY SOLUTIONS

Small-angle neutron scattering (SANS), combined with contrast variation (p5 and p6) and deuterium labelling of proteins, is ideal for monitoring conformational changes undergone by each protein within a complex; each component can be selectively highlighted via deuteration, by matching the neutron-scattering level of the other components with that of the solvent to make them invisible. However, it is more tricky in the case of membrane proteins because they are naturally embedded in a hydrophobic lipid bilayer. To manipulate them in aqueous solution, we replace the lipids with a surfactant (p27). This then also needs to be contrast-matched in order to obtain structural information from the non-matched protein partner. Fortunately, the fluorinated surfactant F6-DigluM, has the same contrast match-point as most proteins, so is an ideal surfactant for the SANS study of solubilised membrane-protein complexes.

We analysed the FhuA–pb5 complex solubilised in F6-DigluM using SANS. The low-resolution structure of FhuA in solution was determined and is in very good agreement with its crystal structure. FhuA remains unchanged upon formation of the complex. The pb5 protein is shown to have an elongated structure, which – at the resolution level of the technique – does not show significant conformational changes upon binding with FhuA. These results are in total agreement with electron microscopy images of the isolated proteins and of the complex (top left).

Using biochemical and biophysical methods, we had previously shown that, upon binding to FhuA, changes in the pb5 secondary structure occur – which could be sufficient to initiate the cascade of signalling events that lead to DNA release and cell-wall perforation. We therefore think that the mechanism of signal-transduction from the receptor binding protein at the tail-tip to the rest of the T5 is different from that in phages that bind to sugars in the bacterial cell wall – for which structural information is already available. This mechanism would be general to phages that bind protein receptors.

RESEARCH TEAM: Cécile Breyton, Frank Gabel, Ali Flayhan, Mathilde Lethier, and Christine Ebel (IBS, Grenoble), Grégory Durand (University of Avignon), Pascale Boulanger (IBBMC, Orsay) and Mohamed Chami (C-CINA, Basel)

ILL INSTRUMENT USED: D22 small-angle scattering diffractometer

REFERENCES: C. Breyton *et al., J. Biol. Chem.*, 2013, **288**, 30763

MOLECULAR INSIGHTS INTO PARKINSON'S DISEASE

THE INTERACTIONS OF A NERVE-CELL PROTEIN WITH CELL MEMBRANES MAY HOLD THE KEY TO UNDERSTANDING THE UNDERLYING CAUSES OF THE CONDITION

Sara Linse

Parkinson's disease is caused by the gradual degeneration of nerve tissue in the brain, causing tremors and other motor-related symptoms. About one in 500 people is affected by the disease.

There is thus a considerable research effort to explore the biomolecular changes that mark the onset and progression of the disease. A key marker is the development of so-called Lewy bodies inside the afflicted nerve cells, which consist largely of small clumps of a protein called alpha-synuclein, together with some lipids. This protein is an important component in nerve cells, being normally localised at the synaptic terminals, where it is thought to help regulate the release of neurotransmitters such as dopamine.

Unlike most proteins, alpha-synuclein does not fold into a stable 3D shape in solution; and there is some discussion as to its normal functional conformation. It does, however, sometimes aggregate into denser folded structures as seen in the Lewy bodies. In addition, it binds with phospholipids – the main components of cellular membranes – and it is possible that the mis-folded protein can transfer from one nerve cell to another, seeding further aggregation via small lipid vesicles budding off from the cell membrane.

Not much is known, however, about the details of interactions of alpha-synuclein with cellular lipid bilayers, except that the protein molecule readily binds to phospholipids with a negative electric charge. (Phospholipids consist of two molecular components: a long hydrocarbon tail attached to a charged head-group, (p26).

We decided to look more closely at this process in a systematic way, using a combination of neutron reflectivity and quartz microbalance measurements. The latter can be used to quantify the amount of protein attached to a membrane surface on a support. Reflectivity experiments provide information about structure and thickness of the protein–lipid layers. Using deuteration and contrast-matching with the solvent and silica support (p5), each component – the protein on its own, the bound lipid-protein and the lipid bilayer – could be highlighted, and its structure analysed.

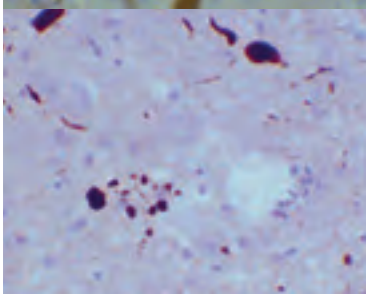
THE ROLE OF A MITOCHONDRIAL LIPID

Model lipid systems were chosen that are relevant to Parkinson's, in particular, cardiolipin a chemically complex, negatively-charged phospholipid found in the mitochondrial membrane. Mitochondrial dysfunction is thought to be associated with the disease. We compared the interactions between alpha-synuclein and this lipid, with those between the protein and the most common negatively charged lipid, phosphatidylserine, and also another lipid phosphatidylcholine, which is electrically neutral and the most abundant cell-membrane constituent.

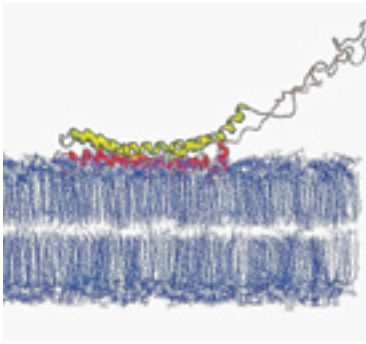
Important aims were to explore how electrostatic interactions affected the conformation of alpha-synuclein, its penetration into the lipid layers, and any subsequent changes in the lipid-layer thickness and density. The results showed that the protein does not bind to neutral phosphatidylcholine but binds equally to the two negatively charged phospholipids, cardiolipin and phosphatidylserine – in other words, the interaction is independent of the chemical nature of the head-group. The protein does not penetrate very deeply but causes some changes in the head-group area of the membrane.

The rest of the unfolded protein remains in the solvent, while the portion close to the membrane becomes more folded. A key factor here may be that the protein's long-chain structure is itself polar, with a prevalence of positive and negative terminating groups at each end. The positive end is attracted to the negative lipid head-group while the negative portion is repelled and spreads away from the lipid layer.

The results therefore indicate that the distribution of charge across the system is the key molecular determinant in the interactions of alpha-synuclein with cell membranes.



Lewy bodies inside nerve cells



Simulation of the binding of alpha-synuclein to a cell membrane (Igor Tsigelny/ Supercomputer Center, UC San Diego)

RESEARCH TEAM: Erik Hellstrand, Marie Grey, Hanna Nilsson, Sara Linse, Tommy Nylander and Emma Sparr (Lund University), Marie-Louise Ainalem (ESS), John Ankner (ORNL, US), Trevor Forsyth (ILL/Keele University), Giovanna Fragneto, Michael Haertlein and Marie-Thérèse Dauvergne (EMBL), and Patrik Brundin (Lund University and Van Andel Research Institute US)

ILL INSTRUMENT USED: D17 reflectometer

REFERENCES: E. Hellstrand *et al., ACS Chem. Neurosci.*, 2013, **4**, 1339; dx.doi.org/10.1021/cn400066t |

UNCOVERING THE PATH TO AMYLOIDOSIS

KEY ASPECTS OF THE TRANSTHYRETIN MOLECULAR STRUCTURE MAY BE CRUCIAL TO IMPORTANT DISEASE STATES

Melina Haupt and Trevor Forsyth

Hydrogen bond networks and water molecules in the transthyretin dimer as revealed by neutron analysis

Front and side views showing the binding channel in human transthyretin created by the four subunits of the dimeric structure

Amyloidosis is a serious health condition that occurs when specific proteins do not fold properly into their native functional forms and instead form insoluble fibrous deposits (amyloid deposits) in tissues. The consequences are often catastrophic in humans, and are associated with several diseases including Alzheimer's and Parkinson's.

Transthyretin is one such protein. In its natural context, it transports an important hormone in the blood and cerebrospinal fluid. It is implicated in senile systemic amyloidosis and familial amyloid polyneuropathy. For reasons that are not clear, transthyretin has an intrinsic tendency to form amyloid fibrils. Like all proteins, it consists of a long chain of amino acids that normally fold into a 3D shape with delicately balanced geometrical and chemical properties that enable it to do its job. It is thought that specific aspects of this structure are crucial in stabilising or destabilising the functional form of the protein.

Transthyretin's architecture is complicated. It is composed of an ensemble of two similar subunits (a dimer), which, in turn, pair up with another identical dimer, forming a tetramer. This hierarchical arrangement is stabilised by subtle molecular interactions. The monomers are largely composed of 'beta sheets' – twisted, pleated layers of chains held together by hydrogen bonding. These particular structures are characteristic of amyloids, and if the tetramer dissociates into its subunits, fibrillar aggregates may be formed.

A promising approach to the treatment of transthyretin-related amyloidosis is the development of drugs that stabilise the tetramer and prevent it from falling apart. Several drug candidates have been identified and tested. However, to design the best possible drug, a clear understanding is needed of the details underlying the tetramer instability and triggering amyloid formation.

CRUCIAL HYDROGEN ATOMS VISUALISED

This is where analysis with neutrons can add vital information, since they can detect not only the hydrogen atoms in the protein, but also those in water molecules buried in the structure, which may help to stabilise it. Substituting selected hydrogen atoms with deuterium provides even more detailed structural data. Using a combination of X-ray and neutron crystallography, the structure of transthyretin was refined, inclusive of water molecules and hydrogen atoms, and their orientations at key sites.

The tetramer subunits were not symmetrical; there were a number of differences relating to the orientation of hydrogen atoms and the number of buried water molecules present. The structure of the protein is shown opposite. Each half of the dimer consists of monomer units, A and B, each of which is basically a sandwich of beta sheets. Coupled together by six hydrogen bonds, A and B form a continuous surface at the interface between the dimers. The larger interface between the dimer units is held together by just eight hydrogen bonds, producing a double wedge-shaped channel between them. This opens out on each side of the dimer to create two active 'pockets' where other molecules can bind (as part of its transport function in the body).

The neutron analysis uniquely shows that the binding pockets have slightly different sizes due to the differing orientation of hydrogen atoms and the fact that the larger pocket has an extra water molecule attached. They are thus not equivalent, with different opening angles and probably different binding strengths. These differences may be significant in the dissociation process, which probably occurs between the weakly bound dimers and not between the monomers.

Current work is focused on structural differences between normal transthyretin and mutated versions that are known to either stabilise or destabilise the tetramer. This should provide a better understanding of the molecular changes leading to amyloid formation so that effective methods of countering this currently incurable disease can be found.

RESEARCH TEAM: Melina Haupt and Trevor Forsyth (ILL/Keele University/PSB Grenoble), Stuart Fisher (University of Salzburg/ILL), Matthew Blakeley (ILL/PSB Grenoble), Sax Mason (ILL), Alycia Yee (ILL/Keele University), Jon Cooper (UCL), and Edward Mitchell (ESRF/Keele University)

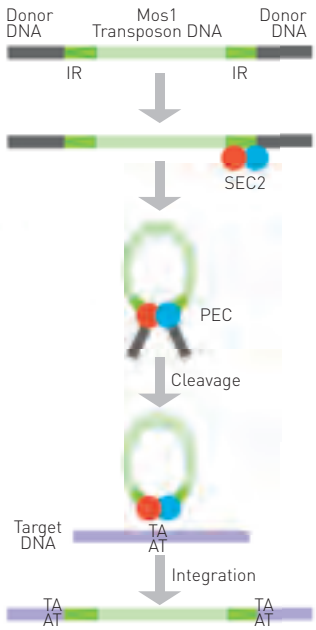
ILL INSTRUMENTS USED: LADI-III quasi-Laue diffractometer, D19 diffractometer, Deuteration Laboratory (D-Lab)

REFERENCES: Melina Haupt et al., *IUCrJ Biology and Medicine*, submitted

SNAPSHOTS OF A JUMPING GENE

HOW AN ENZYME CUTS AND PASTES DNA FRAGMENTS IS REVEALED

Julia Richardson and Trevor Forsyth



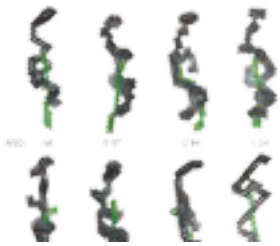
Cut-and-paste DNA transposition
A transposase dimer binds to one end forming a single-end complex (SEC2). The transposon is cut from donor DNA in the paired-end complex (PEC). Mariner/Tc1 transposons are pasted into TA sequences, resulting in signature duplications either side of the inserted transposon

DNA transposons are short pieces of DNA capable of migrating from one place to another in a genome, and causing shuffling of the genetic code. They make up a large proportion of many genomes (45 per cent of the human genome), and were originally thought to be 'junk DNA' with no purpose. However, it is now emerging that transposons are powerful drivers of genetic change. The resulting DNA rearrangement shuffles the genetic code, which can lead to beneficial changes in cells. For example, in the human genome, rearrangement of antibody genes enables the immune system to target infection more effectively.

DNA transposition is now being exploited to develop new biotechnology tools for genetic engineering and gene delivery. One family of transposons – the mariner/Tc1 family – has proved particularly useful because it is widespread in nature and can jump in a broad range of species, including mammals.

Our aim is to understand how the jumping happens, by focusing on the transposition pathway of the mariner/Tc1 transposon, Mos1. The transposon encodes a single enzyme, a protein called transposase, to promote its own cut-and-paste movement around the genome.

The first binds to one end of the transposon in a so-called single-end complex (SEC2). It then binds with the other end to form a paired-end complex (PEC), so that the DNA fragment is completely cut out (top). By characterising the structures of the enzyme and the protein–DNA complexes it forms, we have gained a deeper understanding of how Mos1 transposase does its job at the molecular level. These results will help us to engineer the transposase and speed up the development of biotechnology applications of transposons.



Conformations of SEC2 containing transposon DNA (green) and a transposase dimer (grey)

PINPOINTING SHAPE-SHIFTING

We used small-angle neutron scattering (SANS, p6) to determine the shape of the Mos1 transposase before it binds to the transposon, and then the shape of the resulting SEC2 complex. Using contrast variation (p5) to mask either the protein or the DNA, we could determine their spatial arrangements in the complex. Complementary small-angle X-ray scattering (SAXS) experiments were carried out at the neighbouring European Synchrotron Radiation Facility, ESRF (p7).

The results of the SAXS and SANS experiments revealed that unbound Mos1 transposase is an elongated assembly consisting of twin amino-acid chains (a dimer), with a maximum dimension of 18.5 nanometres. By comparison, the transposase in the PEC crystal structure has a compact crossed conformation with a dimension of 11 nanometres (obtained from earlier X-ray studies). From these data, we conclude that the transposase changes shape, either upon binding one transposon end (in SEC2) or after the transposon ends are paired (in the PEC). The results of our contrast-variation SANS experiments on SEC2 (opposite) revealed that it is more elongated than the DNA-free Mos1 transposase dimer, and has a maximum dimension of 22 nanometres. Furthermore, the DNA component is associated predominantly with just one transposase chain only, and this may endow the other transposase unit with the rotational freedom to drive a change in the shape of the complex.

The similar architectures of the DNA-free transposase and transposase in SEC2 suggest that binding of a pre-formed transposase dimer to the first transposon end occurs without major shape changes in either the protein or the transposon DNA. We propose that these changes occur in the SEC2-to-PEC transition, by rotation of one transposase monomer and its capture of the second transposon DNA end.

We are now working on structures of the second part of the pathway involving the integration step of transposition. Little is known about how the TA sequence (where the transposon is inserted) is recognised.

RESEARCH TEAM: Julia Richardson (University of Edinburgh), Maxime Cuypers and Trevor Forsyth (ILL/Keele University)

ILL INSTRUMENT USED: D22 small-angle diffractometer, Deuteration Laboratory (D-Lab)

REFERENCES: M.G. Cuypers *et al.*, *Nucleic Acids Res.*, 2013, **41(3)**, 2020

ORDERLY FOLDING IN RIBOSOMAL RNA

BUILDING THE CELL'S PROTEIN FACTORY – THE RIBOSOME – INVOLVES CAREFULLY CHOREOGRAPHED STEPS IN THE ASSEMBLY OF ITS RNA COMPONENTS

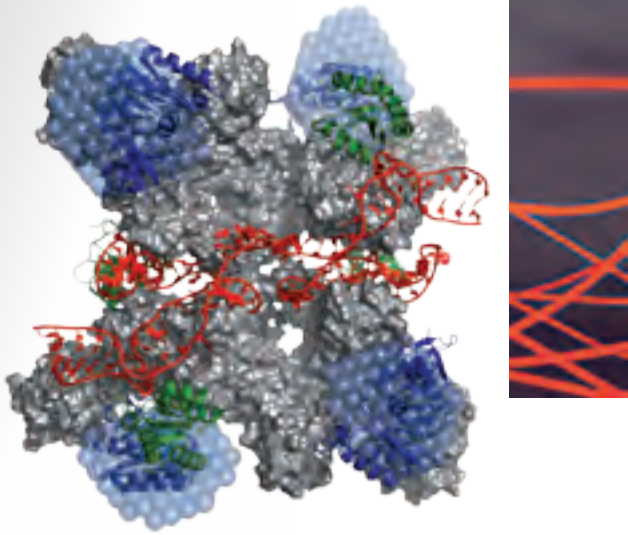
Frank Gabel and Teresa Carlomagno

The microbe *Pyrococcus furiosus*

The ribosome is a large, biomolecular machine, which is responsible for making the proteins essential for cellular function. It is made up of RNA (rRNA) and various proteins – the exact composition varying in the three branches of living organisms: eukaryotes (cells with nuclei, as in humans), and bacteria and archaea (microbial cells without nuclei). Some years ago, the structure of the ribosome was solved for the first time, with neutron scattering playing an important role in the research. Today, researchers are starting to uncover just how this complex assembly is put together.

THE ROLE OF METHYLATION

A key element in rRNA processing is the addition of chemical units called methyl groups (CH_3) to specific sites in its nucleotide sequence. Methylation is known to be an important regulator in the folding and stabilisation of rRNA prior to ribosome assembly. Indeed, if it is suppressed, the cell dies. A complicated enzyme complex consisting of protein units and a small 'guide' RNA, known as 'box C/D ribonucleoprotein' (RNP), carries out the selective methylation in two separate rRNA sequences. It does this by binding with the rRNA in such a way that a constituent guide RNA can pair up with two different rRNA sites; each pairing enables a methyl group to be incorporated at a designated point in the rRNA sequence. In humans, using a variety of guide RNAs, the complex can methylate more than 100 different rRNA sequences.



Model of the apo Box C/D complex (no substrate RNA bound) showing the guide RNA (red), the protein components (green, blue and grey). The structural information from the SANS data regarding the positions of some of the proteins is depicted as transparent spheres

We were interested in exploring the complex found in archaea, called box C/D sRNP, which consists of three proteins surrounding the guide RNA. Studies indicated that the complex is a flat square-shaped assembly of two RNPs (a dimer). To shed further light on the detailed structure and function of this important enzyme, we used a combination of advanced analytical methods – nuclear magnetic resonance (NMR), and small-angle X-ray and neutron scattering (SAXS and SANS) – to probe a slightly simplified version of the dimeric complex. This was reconstituted *in vitro* from recombinant proteins and RNAs belonging to *Pyrococcus furiosus* – a species of archaea able to withstand extreme temperatures. The experimental complex was prepared so as to be as close as possible to the real physiological functional structure. We were able to determine the locations and arrangements of the key structural elements in this complicated molecular assembly, and so infer how they might interact when bound to rRNA.

Using the same analytical methods, we then monitored the changes in structure of the complex as it binds to its substrate rRNA. SANS and SAXS showed that there was a large conformational re-arrangement on binding. The complex formed a more elongated shape that allowed various subunits to come together so as to orchestrate methylation at the two designated rRNA sites in a controlled and sequential way. The guide RNA first adds one methyl group at one site; the subsequent modification then allows another methyl group to be added at an entirely different site with different binding characteristics.

This sequential methylation was unexpected and may provide important clues into how the folding mechanism in rRNA is regulated, as it is built and then assembled into the elaborate machinery of the ribosome.

RESEARCH TEAM: Audrone Lapinaite, Bernd Simon, Lars Skjaerven, Magdalena Rakwalska-Bange and Teresa Carlomagno (EMBL Heidelberg), and Frank Gabel (University of Grenoble, CEA Grenoble, CNRS Grenoble and ILL)

ILL INSTRUMENT USED: SANS D22

REFERENCES: *A. Lapinaite et al., Nature*, 2013, **502**, 519

BETTER IMPLANTS FOR BONE REGENERATION

COATINGS THAT MIMIC THE NATURAL SCAFFOLDING HOLDING TISSUES TOGETHER COULD IMPROVE THE EFFECTIVENESS OF ORTHOPAEDIC IMPLANTS

Ida Berts and Giovanna Fragneto

Implants made of titanium are regularly used in orthopaedics, in the form of plates, pins, and rods, for example, to aid bone repair, and also to screw in dental prostheses into the jaw. Titanium is extremely strong and is compatible with biological tissues. However, one drawback is that the bare metal binds poorly to surrounding tissues such as bone and cartilage, which shortens its lifetime.

To improve the binding, current research is focusing on coating implants with suitable bioactive materials – in particular, biodegradable polymers combined to other biomolecules that enhance adhesion and bone growth. The ideal material should behave like a scaffold, mimicking the extracellular matrix that naturally provides the 'glue' holding cells together in body tissues. At the same time, the artificial coating should gradually degrade during the tissue regeneration associated with the healing process.

Hyaluronan (HA), a carbohydrate polymer that is one of the main components of the extracellular matrix, is a strong candidate coating. The HA molecules can be chemically modified so that they cross-link to form a 3D gel-like structure that mimics the physiological matrix. However to optimise adhesion with the titanium implant, as well as to induce the bone growth, extra chemical groups such as bisphosphonates (BPs) can be coupled to HA.

BPs have a natural affinity for calcium ions and a protein that naturally promotes bone growth – bone morphogenetic protein-2 (BMP-2). Due to this interaction, the protein BMP-2 can then be incorporated in the gel – so that it is delivered in the correct amounts at the correct rate to the site of regeneration. Concentrations that are too low will not have any effect, while those that are too high will lead to abnormal tissue development. It is thus essential that there is also a mechanism for removing the growth factor from the coated implant.

SMART COATINGS

BPs are used as drugs in the treatment of various bone diseases, such as osteoporosis, to inhibit bone loss. However, they have rarely been combined with polymers before. BPs also bind readily to titanium oxide, which always covers the titanium metal. We were particularly interested in the interaction between the growth protein BMP-2 and the HA coating – with and without bisphosphate groups.

Neutron reflectivity (p6), combined with delicate measurements (using a quartz crystal microbalance) of the mass of the surface coatings that formed, allowed us to explore in real time how well the HA gels – both with and without BP – bound to a titanium-oxide surface, and then compare the interactions of the incorporated protein in each case. We also compared the interactions with that of BMP-2 on a bare titanium-oxide surface. Of particular importance was the ease of adsorption of the protein onto the oxide surface and then its release. The latter was achieved by washing the samples with calcium-chloride solution.

The data gave us a detailed structural description of the bound polymer coatings and behaviour of the growth factor. We found that the coating containing BP anchors strongly to the titanium by chemically reacting with the oxide. It forms a thicker HA layer than that without BP. The adsorption of the protein onto the oxide surface was reversed by the presence of calcium ions when BP was present, but not from the uncoated surface. This is because the BP prefers to bind with calcium, which then weakens the binding of the BMP-2 to the coated surface.

These results, we believe, demonstrate that this HA–BP coating system is thus an effective option for ensuring the effectiveness of implants. The research group has now launched trials of similar materials for metal implants, as the next step towards transferring the results to clinical applications.

Amounts of BMP-2 introduced to a titanium surface coated with a chemically modified hyaluronan (red), when dilute (blue) and concentrated (pink), and after rinsing with calcium chloride solution (green)

RESEARCH TEAM: Ida Berts (Uppsala University/ILL), Dmitri Ossipov, Andreas Frisk and Adrian Rennie (Uppsala University) and Giovanna Fragneto (ILL)

ILL INSTRUMENT USED: D17 reflectometer

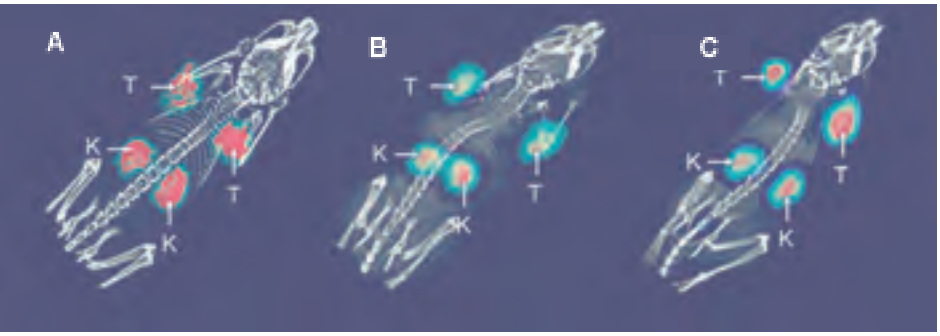
REFERENCES: I. Berts *et al., Advanced Engineering Materials*, 2014; DOI: 10.1002/adem.201400009

A MULTI-TASKING WEAPON TO COMBAT CANCER

RADIOACTIVE ISOTOPES OF THE ELEMENT TERBIUM COULD PROVIDE THE IDEAL MATCHED SET FOR IMAGING AND TREATING TUMOURS

Ulli Köster

Radioactive isotopes offer a set of powerful tools for diagnosing and treating malignant tumours in a targeted way. The principle involves attaching the isotope to a vector – a molecule that preferentially binds to receptors found on cancer cells – so that, when injected, the isotope–vector complex becomes localised at the tumour site. Depending on the particular isotope and radiation emitted, it can be used either to create an image or to kill the cells.



(A) A PET/CT image of a tumour–bearing mouse 24 hours after injection of terbium-152-cm09; (B and C) SPECT/CT images after injection of terbium-155-cm09 and terbium-161-cm09 (K = kidney, T = tumour)

Images of tumours are generated from the gamma-rays emitted by an isotope, which a special gamma camera detects. Today, so-called tomographic techniques are favoured, in which the patient is scanned to generate a sequence of image 'slices' through the tissue. The slices are then combined in a computer into a 3D image. One technique used is known as single-photon emission computed tomography (SPECT). Another imaging method – positron emitting tomography (PET) – relies on a radioactive isotope that emits positrons (positively-charged electrons). The positrons annihilate with the electrons in the surrounding tumour to release gamma-rays that are then detected. Both techniques are increasingly employed in the clinic.

Therapeutic applications rely on the targeted localisation of specific isotopes that emit electrons (either directly from the nucleus or knocked out from the surrounding electron shells), or emit alpha particles (helium nuclei). The emitted particles travel only a short distance in the tumour before they deposit their energy, disrupting the cells' DNA so that the cells die. The first radioisotopes used for cancer therapy, such as iodine-131 and yttrium-90, do not offer ideal radiation properties for all therapeutic applications. However, new radio-isotopes with more favourable characteristics, for example, causing less collateral damage to healthy tissue, are being developed. These include the radio-isotope, lutetium-177, now in use.

THE TERBIUM FOUR

Recently, the ILL participated in the pilot study of a new set of candidate radio-isotopes for nuclear medicine. The element terbium is unique in having four isotopes that are clinically interesting – terbium-149, -152, -155 and -161. They offer a suite of radiation characteristics that together produce excellent tumour visualisation and therapeutic efficacy. Their combined use could be optimised to provide a personalised treatment according to the type and size of tumour, and how well the patient is likely to respond to a particular isotope–vector combination.

Terbium-155 emits a spectrum of gamma-rays that could be used for SPECT at a relatively low radiation dose, while terbium-152 emits positrons and is suitable for PET. Terbium-161 emits low-energy electrons, which are suitable for radiotherapy, and also gamma-rays suitable for SPECT. Terbium-149 emits alpha particles, and so could be used in so-called targeted alpha therapy.

Radiotherapy increasingly involves closely combining imaging and therapy, so that the efficacy of the procedure can be monitored during the course of treatment. The terbium isotopes could be used in this mode because they all have the same chemical properties, so will also distribute in the body in the same way. This means that the same targeting vector can be used for all four isotopes.

The ILL research reactor

MAKING TERBIUM-161 AT THE ILL

Terbium-161 is made by irradiating a target of gadolinium-160 with neutrons from a research reactor such as that at the ILL. This isotope then captures a neutron to produce gadolinium-161, which rapidly decays to terbium-161. Since natural gadolinium contains only 21.86 per cent of the target isotope, it has first to be enriched to more than 99 per cent abundance. After one to two weeks of irradiation, the resulting terbium-161 is chemically separated from the starting material and further decay products, using an advanced technique of ion-exchange chromatography to separate compounds of chemically similar elements.

The ILL has a strong research base in nuclear physics, and is now developing an automated irradiation system for routinely making terbium-161, lutetium-177 and other novel radio-isotopes that would improve cancer treatments.

The aim of the study was to investigate the potential of this isotope quartet from both a medical and production point of view. The three lighter isotopes were made at ISOLDE, the nuclear physics facility at CERN in Geneva, using a proton beam to break up nuclei in a tantalum-foil target. Terbium-161 was created at the ILL, or at the Paul Scherrer Institute (PSI) in Switzerland, using neutrons, which were allowed to impinge on a target of gadolinium-160.

Terbium-161, in particular, could offer an even better therapeutic alternative to lutetium-177, which is currently considered the gold standard in targeted radio-therapy. It is employed, for example, to treat tumours associated with the neuroendocrine system. Each isotope is bound via a chelating agent called DOTA to a peptide molecule that recognises particular receptors on the cell surfaces of neuroendocrine tumours.

To test the efficacy of the terbium radio-isotopes, they were combined, via the DOTA chelator, with a new, specially-designed targeting vector. A number of aggressive tumours, including ovarian, breast, renal, lung, colorectal and brain cancers, are marked by having excess amounts of a receptor that binds folate (vitamin B9) compounds, while very few of these receptors are found in normal tissues and organs. A particular folate derivative, cm09, was designed that would circulate for longer in the body so that it could better reach the target tumours.

A SUCCESSFUL PARTNERSHIP

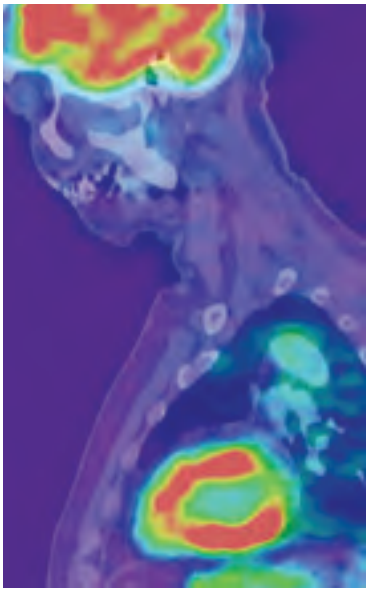
The combined radio-isotope agents were tested in both tissue samples and in tumours (carried by mice). The tumours were imaged 24 hours after injection, using PET in the case of terbium-152, and SPECT in the case of terbium-155 and terbium-161. To test the efficacy of terbium-149 and -161 as tumour-killing agents, the tumour growth in the same mice was compared with that in an untreated control group.

This is the first time that the four terbium isotopes have been tested in this way. We found that PET and SPECT provided clear images of the tumours for the three isotopes used in diagnostic mode. Both of the therapeutic isotopes caused a marked delay in tumour growth, or even complete remission. In particular, therapy with terbium-161 resulted in complete remission in 80 per cent of cases.

On the basis of our findings, it looks likely that terbium-152 and terbium-155 could be ideal diagnostic matches for terbium-149 and terbium-161 as therapeutic agents. We plan to conduct further, larger-scale tests, as well as carrying out comparative therapy studies between terbium-161 and lutetium-177.

Another important issue is the production of the isotopes in quantities suitable for medical use. In the case of terbium-161, radiochemists at the Technical University of Munich, Germany and PSI have developed a method for large-scale production using a neutron source. It will be possible to produce this isotope in the volume and quality needed for clinical applications. With an improved supply of enriched gadolinium-155 and gadolinium-152 targets, the three lighter isotopes could be made routinely at selected accelerator centres.

In terms of availability, terbium-155 and -161 are long-lived enough to be shipped to clinics worldwide, while the half-lives of terbium-152 and -149 are shorter-lived, and would require a network of accelerator facilities for regional supply.



RESEARCH TEAM: Cristina Müller and Konstantin Zhernosekov (PSI), Ulli Köster (ILL), Karl Johnston (ISOLDE/CERN), Holger Dorrer and Andreas Türler (PSI/University of Bern), and Roger Schibli (PSI/ETH Zurich)

REFERENCES: C Müller *et al., J. Nucl. Med.*, 2012, **53**, 1951. S. Lehenberger *et al., Nuclear Medicine and Biology*, 2011, **38**, 917

INSIGHTS INTO NEURAL TISSUE PRESERVATION

NEUTRON SCATTERING OPENS UP NEW PATHS IN BRAIN IMAGING AND THE DIAGNOSIS OF NEUROLOGICAL DISEASES

Francesca Natali

The IN5 instrument

The human brain contains about 80 per cent of water by weight. Mapping the content, motion and distribution of water in the brain can pinpoint physiological changes associated with a tumour or neurological conditions such as multiple sclerosis, thus providing a means of diagnosis. A technique called diffusional magnetic resonance imaging (dMRI) does exactly this, and is used routinely in the clinic. It relies on 'seeing' the diffusion of water through the tissue; the movement of water molecules is modified by the surrounding structure, enabling an image of the patient's brain to be constructed – with millimetre resolution.

The same imaging technique is also employed to analyse brain sections taken after death. However, decomposition must first be prevented either by chemical fixation using formalin (formaldehyde water solution) or by freezing. In recent years, however, concerns have grown about the reliability of this approach in accurately representing the true physiological situation *in vivo*. This is because the preservation treatments potentially alter the tissue micro-structure and permeability of cell membranes – and thus the movement and distribution of water. Formalin is readily absorbed by tissues, where it reacts with the constituent proteins, forming chemical cross-links that impart rigidity and cause shrinkage, while freezing requires special care in exposing the tissue to liquid nitrogen used for cooling, or the use of cryoprotectants, to prevent minute ice crystals forming in cells and rupturing them. In order to optimise these protocols and interpret the results, it is therefore essential to gain a better understanding of the preservation processes at the molecular level.

We decided to address these issues using neutron scattering. The measurement of low-energy exchanges (quasi-elastic neutron scattering, QENS) between the sample molecules and the incident neutrons (p27) provides information on fast, localised molecular dynamics such as rotation, re-orientation and translational diffusion. QENS, being particularly sensitive to the motion of hydrogen atoms, can probe the movement of water in tissues, in a way that is relevant to dMRI.

MOLECULAR MOTIONS REVEALED

We obtained fresh *post-mortem* bovine brains from a slaughter-house and prepared samples employing the two preservation techniques. Using QENS, we measured the associated changes in neutron energies after scattering, and showed that they did indeed primarily originate from the rotational and translational motions of water molecules both within cells and the spaces between them. We were also able to measure the rotational motions of carbon–hydrogen subunits in proteins and lipids.

The results so far reveal that, in tissues fixed with formalin, the water molecules are more confined than in cryopreserved tissues – as previously suspected, therefore altering their diffusion characteristics as compared with those in living tissue. Thus, when comparing dMRI data from *in-vivo* and *ex-vivo* tissues preserved with formalin, changes in dynamical and structural properties, as well as shrinkage effects, must be taken into account.

These experiments, moreover, demonstrate the potential of neutron scattering in detecting pathological changes in *post-mortem* tissues by mapping water movement, with relevant impact on diagnostic imaging techniques, such as dMRI. To explore the possibilities further, we have been investigating the movement and distribution of cellular water in brain tissue containing different amounts of myelin – the protective, electrically-insulating sheath around nerve axons. The aim is to determine whether QENS can reveal changes in water dynamics associated with the occurrence of neurodegenerative diseases, including multiple sclerosis, which are characterised by the degradation of myelin over time.

RESEARCH TEAM: Francesca Natali (CNR-IOM,OGG and ILL), Yuri Gerelli (ILL), Calogero Stelletta (University of Padua, Italy), Judith Peters (University Joseph Fourier and ILL) and Jacques Ollivier (ILL)

ILL INSTRUMENT USED: IN5 time-of-flight spectrometer

REFERENCES: F. Natali *et al.*, *AIP Conference Proceedings*, **1518**, 551 (2013); doi: 10.1063/1.4794632

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GLOSSARY

Active site/pocket A cleft or pocket in the 3D structure of an enzyme where the chemical transformation that it catalyses is carried out.

Alpha particle The nucleus of helium with two protons and two neutrons, which may be emitted by unstable nuclei.

Amino acid The basic building block from which proteins are made. There are 20 different natural amino acids.

Amyloid disease Diseases characterised by insoluble deposits of proteins called amyloid fibrils that have mis-folded into dense beta-sheet structures (see beta sheet).

Amyloid fibril See above.

Antigen A molecule that is recognised by and binds to an antibody.

Archaea A broad range of microbial cells without nuclei, which may have a biochemistry somewhat different from those of bacteria and eukaryotes (see below).

Bacterium Single cell in which the genetic material is not enclosed in a separate nucleus.

Bacteriophage A virus that infects and replicates in a bacterium.

Beta decay A type of radioactive decay in which a neutron converts into a proton, an electron and an antineutrino. Only the emitted electron (beta particle) is of practical relevance.

Beta sheet Proteins fold into various secondary 3D structures, one of which is a pleated beta sheet held together by hydrogen bonds. They are implicated in amyloid diseases.

Bilayer A term applied to double layers of lipid molecules, as found in cell membranes.

Cell membrane A self-organised structure that encapsulates the contents of a cell and controls the passage of molecules across it.

Chelating agent A molecule that binds to metals via several bonds.

Conformation A certain 3D structural arrangement of a group of atoms or a molecule.

Contrast matching A technique whereby a proportion of the water in which a sample is dissolved is replaced by heavy water, so that the scattering power of the solvent matches that of a designated component of the sample, rendering it invisible. Other components are then seen more clearly.

Contrast variation A technique in which certain atoms in a sample are substituted by another isotope with a different scattering strength in a way that preferentially enhances the scattering pattern of particular components of interest. In biological neutron experiments, hydrogen atoms are replaced by deuterium, which scatters differently.

Cryopreservation A process in which biological materials are preserved by cooling to sub-zero temperatures.

Crystal A solid material with a regular 3D array of atoms or molecules.

Cytoplasm The gel-like material inside a cell, which provides a supporting medium for the cell's internal structures and molecules involved in cellular processes.

Dendrimer A polymer with a highly branched structure.

Deuteration The (chemical) replacement of a hydrogen atom in a molecule with its isotope, deuterium.

Dimer A molecule composed of two similar subunits; proteins in their natural environment sometimes consist of dimers.

Electron An elementary particle, which is a significant constituent of atoms and is also emitted by unstable nuclei as beta radiation.

Enzyme A type of protein that mediates a specific chemical reaction in living systems.

Eukaryotes Cells with nuclei, as in humans.

Gamma-ray Very high-energy electro-magnetic radiation. Gamma-rays are emitted in nuclear decay.

Ganglioside A component of the cell membrane that modulates cell signalling and is concentrated in lipid rafts.

Gene A sequence of DNA bases, that provides the code to make a specific protein.

Genome The entire sequence of genes that characterises a particular organism.

Head-group The component of a surfactant or lipid molecule that is hydrophilic.

Heavy water (D_2O) Water in which hydrogen has been replaced by its heavier isotope deuterium.

Hydrocarbon chain A molecular chain composed of carbon–carbon links to which hydrogen atoms are attached. They form a major component of lipids. If the carbon–carbon bonds are double bonds, the hydrocarbon is said to be unsaturated and is more chemically active.

Hydrogen bonding A weak form of bonding in which the proton of a hydrogen atom is electrostatically attracted to pairs of electrons on atoms like oxygen and nitrogen.

Hydrophilic Water-loving.

Hydrophobic Water-hating.

Inelastic scattering A neutron technique in which there is an exchange of energy between the neutrons and the molecules being studied, thus giving information about their motion and flexibility. (When a neutron is scattered elastically, there is no energy transfer).

Ion An atom or molecule that has gained or lost electrons so that it has become electrically charged.

Isotope Chemical elements characterised by the same number of protons but different number of neutrons in the nucleus.

Lipid A molecule consisting of a long hydrocarbon chain, often with a hydrophilic group of atoms at one end (head-group). Lipids arrange themselves in layers and are the scaffold of biological membranes.

Lipid raft The cell membrane contains compact micro-domains consisting of proteins, specific lipids and sterols, and are thought to play a central part in cell signalling.

Lipid vesicle/liposome A microscopic sac-like structure consisting of a lipid or surfactant membrane, in which molecules, or even genes, can be inserted.

Liquid crystal A material in which all the molecules self-align into an organised arrangement, as a result of interacting electrostatic forces between the molecules.

Magnetic resonance imaging A version of NMR used in medical imaging. A radio-frequency field is applied in an extremely strong magnetic field, and changes in magnetic energy of hydrogen nuclei of water molecules in tissues are detected, and used to create a 3D image as a patient is scanned.

Methyl group (CH_3) A molecular unit consisting of a carbon atom linked to three hydrogen atoms.

Methylation The process of adding methyl groups to a molecule by replacing a hydrogen atom. It is an important chemical step in many biochemical processes.

Monoclonal antibody Specific proteins secreted by just one type of cell of the immune system, which may neutralise invading bacteria and viruses by binding to one particular target antigen on their surfaces.

Monomer A single molecular unit that can bind to similar units to form a larger structure.

Nanometre One billionth of a metre (10^{-9} metres).

Nano-particle A microscopic particle of matter with at least one dimension smaller than a hundred nanometres.

Nanotechnology The manipulation of materials with at least one dimension sized from 1 to 100 nanometres.

Neutron A neutral particle found in the atomic nucleus. The number of neutrons in the nucleus of an atom reveals the specific isotope of that atom.

Neutron crystallography The basic form of neutron diffraction that gives the positions of atoms in a crystal and thus provides information about the molecular structure.

Neutron diffraction Neutrons can be reflected, or scattered, off a material in which the interatomic distances are similar to the neutron wavelength. The scattered waves interfere to produce a characteristic diffraction pattern. In neutron crystallography, a single crystal is oriented over a range of angles to collect neutron beams diffracted from different planes of atoms in the crystal (see above).

Neutron reflectivity The process by which neutrons are reflected off a surface or interface.

Neutron reflectometry A technique in which neutrons are reflected off a surface or interface. It is used to characterise the structure and composition of surfaces and thin layers.

Neutron spin-echo A neutron technique that measures the changes in the spins of neutrons passing through a material caused by small changes in energy associated with molecular movements over relatively long timescales.

Nuclear fission The process by which heavy nuclei break up to produce smaller nuclei.

Nuclear magnetic resonance (NMR) An analytical technique in which powerful static and radiofrequency magnetic fields induce magnetic transitions, usually in hydrogen atoms. It is complementary to X-ray and neutron diffraction in the information it provides, and can be also used to reconstruct 3D images.

Peptide A small chain consisting of a few amino acids (formed when the carboxyl group of one amino acid reacts with the amino group of another to form an amide bond).

Phospholipid A class of lipids containing a phosphate group.

Positron The positively charged (antimatter) version of the electron.

Protein One or more long chains of a specific sequence of amino acids, which fold into a unique 3D structure with a particular biological function.

Protein folding Each type of protein molecule folds into a distinctive 3D shape that is essential for its function.

Proton A positively-charged subatomic particle found in the nucleus. An element is defined by the number of protons in its nucleus. In chemistry and biology, a hydrogen atom that has lost its electron to form a hydrogen ion is often simply referred to as a proton (H^+).

Quasi-inelastic scattering A type of inelastic scattering in which there is only a tiny energy transfer between the neutron and the atomic nuclei in the target sample.

Radio-isotope An isotope that is unstable and emits particles in its decay.

Receptor A protein that is embedded in cellular membranes and binds with specific molecules and ions to bring about a cellular response.

Ribosome A large molecular unit made of protein and RNA, which has multiple functions. Amongst others, it is responsible for transcribing the DNA genetic code into the sequence of amino acids that make up a protein.

RNA A chain-like molecule similar to DNA, which is responsible for transcribing the DNA genetic code into the amino-acid sequence of proteins.

Small-angle neutron scattering (SANS) The measurement of neutron scattering at small angles. It is used to investigate structures with large interatomic distances such as proteins and other large biological molecules.

Small-angle X-ray scattering (SAXS) X-ray scattering carried out at small angles and used to investigate structures with large interatomic distances, such as polymers or biological structures. It is complementary to SANS.

Sterol A class of organic molecules consisting of conjoined ring-like structures and containing a hydroxyl group. They play several important roles in living organisms.

Surface tension The elastic force produced by intermolecular attraction in a liquid at its surface, such that it generates resistance to an external force, for example, mechanical pressure from an object on a water surface.

Surfactant (**surface-active agent**) A class of molecule that adsorbs at surfaces or interfaces. Surfactants are usually molecules with a hydrophilic head-group and a hydrophobic tail. They can dissolve in both water and oil, forming organised structures such as micelles.

Tetramer An assembly of four similar molecular units, for example, in a protein.

Transposon (jumping gene) A DNA sequence that can change its position within the genome, to create a new mutation.

Ultra-small angle neutron scattering (USANS) An extension of SANS aimed at characterising the larger-scale microstructure of materials in the range from 0.1 to 50 micrometres. In biology, it is used to study molecular assemblies, viruses, and bacteria.

Virus A small infectious particle, consisting of either DNA or RNA covered in a protein coat, that replicates only inside the living cells of other organisms.

Virion A virus particle.

X-ray diffraction A technique used to determine the structure of materials. X-rays are reflected, or scattered, off a material in which the interatomic distances are similar to X-ray wavelengths such that the scattered waves interfere coherently to produce a characteristic diffraction pattern.

NEUTRONS FOR SCIENCE

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August 2014

Published by the
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