HIGHLIGHTS OF
ILL RESEARCH

NEUTRONS AND HEALTH

2

A review of recent ILL research
with applications in medicine

NEUTRONS
FOR SOCIETY

FOREWORD

Improving the health of the global population has far-reaching consequences for human progress and is a major societal challenge. It requires understanding the highly complex molecular machinery which Nature has developed, at the relevant length- and time-scales – that is, in terms of the equilibrium and transient molecular structures that form, and the dynamics underpinning their biological function.

The complexity of biological molecules and complexes has dictated the pace of growth of this field in neutron-scattering centres around the world. As we identify the areas in which neutrons can provide key insights, complementary techniques and methods have been in place and experimental protocols elaborated. In this context, the ILL benefits considerably from the rich infrastructure on the EPN (European Photon and Neutron) campus in Grenoble, which includes the ESRF (the European Synchrotron Radiation Source), the EMBL (the European Molecular Biology Laboratory) and the IBS (Institut de Biologie Structurale), as well as the ILL.

Two very successful partnerships, the Partnership for Structural Biology (PSB) and the Partnership for Soft Condensed Matter (PSCM), formalise and provide the framework for cooperation and collaboration in biological research at the ILL. The PCSM and PSB are both flourishing with, for example, a new agreement and new partners for the PSCM and a federated activity around emerging cryo-electron microscopy techniques in the PSB. There is also an externally funded (Human Frontier Science Programme) link-up between the study of biological problems using neutrons and those making use of the new free-electron laser (FEL) facilities: FLASH at DESY in Hamburg, Germany and SwissFEL at the Paul Scherrer Institute in Villigen, Switzerland. These partnerships highlight the need for complementary, inter-disciplinary approaches when probing such complex systems at the molecular level.

OUR STATE-OF-THE-ART INSTRUMENTS

At the ILL, a range of instruments is required to explore the structure and dynamics of biological systems. High-resolution crystallography provides unique information on the hydrogen-bond networks and surrounding water structure that mediate the behaviour of complex molecular assemblies. This complements the detailed crystal structures of biomolecules determined with synchrotron X-ray techniques. Our instrument capacity in this area is in the process of being doubled. Furthermore, the use of neutron reflectometry for surfaces, and small-angle neutron scattering for liquids, often enables structural information to be elucidated under real, physiological conditions (though at lower resolution). Dynamics studies tend to require high-resolution spectrometers, which can access information at the nanosecond-to-microsecond timescales. These include instruments that exploit neutron backscattering and spin-echo techniques, which have been developed significantly in our recent and ongoing upgrade programmes.

A major strength in the use of neutrons to probe biological systems is the technique of selective deuteration to modify the scattering contrast from different molecular components. Supporting our users in the production of deuterated samples has been a longstanding commitment provided through the ILL's Deuteration Laboratory (D-Lab), which is now being extended to lipids through an EU-funded project.

Finally, the neutron, being a key component of the atomic nucleus, plays a major role in nuclear physics studies. This is sometimes overlooked in the context of biology and health. Nuclear medicine is growing in importance and new radiotherapies are being developed. Neutron-capture by boron-10 can generate highly localised irradiation that can destroy unhealthy cells – and this is exploited in boron neutron capture therapy. The very high neutron flux at the ILL also enables novel radioisotopes to be produced for therapeutic applications. By exploiting cancer-specific target vectors such as peptides, short-lived isotopes can be transported to damaged cells and deliver targeted, short-range radiation that kills them. This is an emerging, highly promising cancer treatment, and each year, several thousand patients are treated with isotopes like lutetium-177 produced at the ILL.

In this latest ILL brochure, *Neutrons and health 2*, all of the above developments are brought together and illustrated via a range of highlights that demonstrate our state-of-the-art research. They reveal how probing exquisite biomolecular structures, their detailed dynamics and subtle interactions explains biological function. This gives us key insights into the causes of major diseases that affect millions of people – like Alzheimer's, hepatitis C, diabetes and obesity – and so allows us to develop effective drugs and therapies to combat them.

I hope you enjoy the brochure and look forward to exciting developments and research in the future.

Mark Johnson
Associate Director and
Head of Science Division

CONTENTS

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 NATURAL 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 over 30 countries visit the ILL. More than 800 experiments, which have been selected by a scientific review committee, are carried out 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 a wide range of complex structures and systems at the atomic and molecular scale – from biological and soft-condensed matter and new chemicals such as drugs and polymers, 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
The 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 the ILL: Spain, Switzerland, Austria, Italy, the Czech Republic, and more recently Sweden, Belgium, Slovakia, Denmark and Poland.

NEUTRONS FOR SOCIETY

INTRODUCTION

EXPERIMENTS USING NEUTRONS ARE PLAYING AN INCREASINGLY IMPORTANT PART IN IMPROVING OUR HEALTH

Giovanna Fragneto and Trevor Forsyth

Maintaining good health is probably the most important aspect in our quality of life. However, ensuring good health for all 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 still 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, major challenges are emerging. One growing concern is the expected increase in age-related disorders, particularly various dementias, as more people live into their 90s and even beyond. In western countries, the increase in so-called lifestyle conditions, such as obesity, type 2 diabetes, and heart disease, is also becoming a concern. Awareness of mental health issues is becoming more focused, as well as the need to alleviate problems associated with disability and trauma.

Meeting these challenges requires a profound understanding of living processes – not just at the level of organs, tissues and cells, but right down at the molecular level. Most illnesses result from biomolecular processes not working correctly. This may be due to viral or bacterial infection. Viruses invade cells and take over their molecular machinery, eventually killing the cells. Bacteria may release toxins that block a vital molecular interaction. Genetics is also extremely important; a small chemical alteration, or mutation, in the genetic code can result in the expression of a protein that does not function correctly, causing, for example, a problem with the body's metabolism or with cognitive function.

The ambitious aim of biologists today is thus to observe not only the structure and behaviour of individual biological molecules at atomic resolution in their natural, physiological environment but also to follow their often complex interactions with other molecules, so that the molecular networks and cascades underlying an essential function can be fully elucidated. In this way, medical researchers can decide on the best strategy to treat a disease. By uncovering the effects of subtle genetic mutations on these systems, drug designers and health providers are moving towards more effective and personalised medication.

The Titan Krios electron microscope at the ESRF
This is a crucial part of the PSB platform for electron microscopy and links all the institutes on the campus. Cryo-electron microscopy (Cryo-EM) is now widely used to image the structure of biological macromolecules at high resolution. The technique uses an electron beam in combination with advanced detector and software technologies to study samples made by 'flash freezing'. This rapid freezing avoids the formation of ice crystals and allows biological molecules to be studied in a stable non-crystalline environment. Keele University in the UK, through its partnership with the ILL, has just recruited a scientist who is now working in the ILL's Life Sciences group – exploiting the powerful synergies with neutron-scattering approaches on behalf of the ILL's users.

Cande/ESRF

PROTEINS: THE MACHINES OF LIFE

Crucial to living processes are the thousands of different proteins encoded for by the human genome. Analysing the detailed structure and behaviour of these proteins is key to medical advance. Each protein molecule consists of a chain of molecular units, amino acids, folded into a distinctive three-dimensional 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 transporting energy or communicating between cells.

A major subject of study is how proteins fold into their characteristic shape. Subtle changes in the local physiological environment can trigger misfolding to create compact structures called amyloid aggregates. These structures play a part in a number of pathologies including diseases such as Alzheimer's and Parkinson's. Neutron diffraction methods are ideal for studying these three-dimensional structures and the way in which misfolding can occur.

CELL MEMBRANES

Very often, 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 ever more aware of the importance of research into the structure and behaviour of membranes, and how the lipids and embedded proteins interact to mediate the membrane's complex functions. Membrane proteins, for example receptors, are particularly difficult to study in their native environment. However, neutron methods, combined with novel sample-preparation techniques, have been developed to elucidate their structures.

NOTHING IS STATIC IN BIOLOGY

While it is clearly understood that the three-dimensional structure of biomolecules is directly related to their behaviour and function, it is also understood that they are not rigid; they are typically 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, for example, in a cell membrane, but move around as they are buffeted by the random jostling of their molecular neighbours.

THIS BOOKLET DESCRIBES NEUTRON RESEARCH RELATING TO:

- Protein folding and unfolding
- Enzyme activity
- Protein regulation
- Protein biosynthesis
- Cell signalling
- Cell membrane structure and interactions
- Membrane proteins
- Diagnosis and treatment of amyloid diseases
- Genetic diseases such as cystic fibrosis
- Neurological diseases such as multiple sclerosis and schizophrenia
- Metabolic diseases such as type 2 diabetes
- Identification of new drug targets such as antiviral drugs to treat hepatitis
- Cancer diagnosis and therapy

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 and rapidly developing role. The various neutron techniques now available can reveal aspects of 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 dedicated research reactor employs nuclear 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 one ten-billionth and a millionth of a metre. Neutrons have enough energy to penetrate a structure and scatter from the nuclei of individual atoms.

APPLICATIONS TO BIOLOGY

Neutron beams are particularly suitable for biological research. They can be generated with energies and wavelengths appropriate for probing a wide range of biological structures, from small molecules such as lipids or peptides, to larger molecules and molecular assemblies such as the ribosome. 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 (protons), so can pick out all the hydrogen atoms in a structure, including those in water molecules, as well as hydrogen ions. This is in contrast to X-ray analysis, in which the X-rays interact only by the electrons in an atom, and thus do not easily see hydrogen nuclei, which have no electrons. Determining the precise locations and orientations of hydrogens in a macromolecule is often crucial for an 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 heavy hydrogen isotope, deuterium, has a different scattering power from that of normal hydrogen and its scattering is more clearly seen. This property offers a excellent tool for 'highlighting' hydrogen atoms 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 are 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 parts of the structure 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. This pattern is recorded by a detector, and can be used to determine the detailed molecular structure of a biological macromolecule using a well-established technique called crystallography. Modern molecular biology, rooted in the seminal DNA structure determination, has had a crucial impact on the field of protein production and subsequent applications of X-ray crystallography. While this approach, now increasingly being used alongside cryo-electron microscopy, has become a routine method for the structure determination of large biomolecules at atomic resolution, neutron crystallography is used to provide strongly complementary and essential information about the individual positions of light atoms – notably hydrogen. The use of neutron crystallography is becoming ever more widespread and will grow steadily, as deuteration and crystal preparation techniques improve (see right).

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.
- **Small-angle neutron scattering:** the ILL has three dedicated SANS instruments, D11, D22 and D33.
- **D16** is a diffractometer used to study of a wide range of systems in biology including membranes.
- **D19** is a monochromatic diffractometer that can record diffraction data with subatomic resolution from small protein crystals and fibrous systems.
- **Reflectometry:** the ILL has three reflectometers, D17, FIGARO and SuperADAM. FIGARO is optimised for horizontal surfaces, and can reflect neutrons from above and below a sample surface.
- **Inelastic and quasi-elastic scattering:** the IN5, IN6, IN13 and IN16B spectrometers can look at the rapid molecular motions in biological molecules in the picoscond scale.
- **Spin-echo:** IN11 and IN15 spectrometers are used to analyse the motion of the macromolecular objects on the nanometre scale.

SMALL-ANGLE NEUTRON SCATTERING

In diffraction studies, the range of scattering is inversely related to the scale (length-scale) of the structure producing the diffraction pattern. Beams of neutrons deflected by an angle of only a degree or so, provide structural information at length-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 sophisticated deuteration approaches 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 two-dimensional 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.

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 (p31) 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. Even subtle effects such as the stiffness of a molecular component may be significant. Neutron scattering experiments are providing 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 radioisotopes routinely available to the clinic.

THE FUTURE

Neutron-based research has 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. 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 instruments and supporting technologies, and in providing experimental support and knowledge, to researchers in the life sciences, who wish to exploit the huge analytical potential of neutrons.

Key to the full exploitation of these remarkable resources are the powerful synergies that exist with the capabilities of the other institutes on the ILL campus - which overall contains some 300 scientists working in life sciences and structural biology. These synergies are reflected by the formalised partnerships that have been formed – for example, the Partnership for Structural Biology (PSB) and the Partnership for Soft Condensed Matter (PSCM). As an example – a powerful emerging trend that will play a key role in the future is the combined use of neutron methods alongside cryo-electron microscopy. The ILL, in partnership with Keele University, is now fully committed to this joint initiative.

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 to prepare membrane-protein samples suitable for structural studies are consistently developing.

- The Partnership for Structural Biology (PSB), which is located on the same site, is a collaboration between the ILL, the European Molecular Biology Laboratory (EMBL), the European Synchrotron Radiation Facility (ESRF), and the Institut de Biologie Structurale (IBS). It provides a comprehensive suite of facilities for life-science scientists, including preparing samples for neutron experiments (https://www.psb-grenoble.eu/).
- A vitally important part of sample preparation is the deuteration process. The ILL operates the Deuteration Laboratory (D-Lab) platform as part of its Life Sciences Group in the PSB. Researchers can obtain deuterated proteins and other bio-macromolecules that are of crucial benefit to neutron studies. Samples can be prepared using genetic techniques, for example, by expressing proteins in a deuterated medium using bacteria, yeast, or even in advanced cell-free systems. Since deuteration results in greatly enhanced neutron scattering signals, smaller crystals can be used in crystallography and better-quality data produced. In SANS, deuteration enables greatly improved contrast of components within large complex systems (https://www.ill.eu/users/support-labs-infrastructure/deuteration-laboratory/).
- The importance of *in-situ* preparation and a good characterisation of the samples before neutron scattering experiments has become ever 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. A platform for providing model membrane systems to users includes the extraction purification and analysis of deuterated lipids from cell extracts produced in the D-Lab. This initiative is part of the Partnership for Soft Condensed Matter, which is an ILL/ESRF programme hosted in the shared facilities of the Science Building, providing several complementary techniques and sample preparation tools made available to soft-matter and biophysics users (www.epn-campus.eu/users/partnership-for-soft-condensed-matter-pccm).

MEMBRANE PROTEINS TARGETED

A NOVEL METHOD, PIONEERED BY THE ILL, ENABLES SUBTLE STRUCTURAL CHANGES IN DIFFICULT-TO-STUDY MEMBRANE PROTEINS TO BE VISUALISED AND WILL SIGNIFICANTLY HELP IN THE DEVELOPMENT OF NEW DRUGS

Inokentijs Josts, Sylvain Prévost and Trevor Forsyth

Although cell membranes consist largely of a phospholipid bilayer (p31), they also contain many different proteins. These membrane proteins play key roles in living processes such as providing transport gateways into and out of cells, facilitating signalling between cells, as well as behaving as enzymes in mediating molecular transformations. Such functions make them particularly important drug targets; indeed, the majority of current therapeutics target membrane proteins.

However, structural studies of proteins that are permanently bound in a membrane (integral membrane proteins, IMPs) have proved extremely challenging. In their native lipid environment, IMPs cannot be crystallised – as traditionally required to analyse a protein's three-dimensional structure (and thus function) using X-ray or neutron diffraction (p31) – and many are insoluble or inactive in the absence of the surrounding membrane. We therefore need other approaches to study these proteins in their native membranous environment.

Preparing samples in the ILL's Deuteration Facility

NANODISC MEMBRANE MIMICS

A few years ago, in collaboration with Copenhagen University, the ILL's Life Sciences Group successfully pioneered the development of 'stealth carrier nanodiscs' (see references 2 and 3 below). These nano-carriers consist of a phospholipid bilayer held together by a protein scaffold. The disc-shaped protein–lipid complexes can provide a suitable membrane 'mimic' to host an IMP. Using selective deuterium labelling and contrast matching (p8), the nanodisc can be made invisible to low-resolution neutron diffraction while highlighting the structure of an IMP embedded within a lipid environment.

More recently, we completed the first structural study of an IMP exploiting this strategy in combination with SANS (p8) and complementary X-ray studies (SAXS) carried out at the nearby European Synchrotron Radiation Facility, making full use of the PSB SAXS/SANS platform (p9). The protein chosen was one that plays an important role in lipid transport in bacteria – the ATP-binding cassette (ABC) transporter protein, MsbA. The protein translocates lipids from the inner to the outer layers of the membrane in an energy-dependent process. We were able to observe directly the signal from the incorporated membrane protein without any contribution from the surrounding lipid. The SAXS data provided a clear reference for the external shape of the MsbA-nanodisc complex, inclusive of the lipid bilayer.

In addition, we could study subtle conformational changes in MsbA, which has a flexible two-lobed structure, in different functional states, and compare the results with theoretical predictions.

The results have successfully demonstrated the sensitivity of the method and its general applicability to structural studies of IMPs. This approach is likely to become increasingly important in future studies of these difficult, but critically important, biological macromolecules, in turn supporting a better understanding for the development of drugs aimed at membrane proteins.

Figure left: The integral membrane protein MsbA (blue) embedded in a 'semi-transparent' membranous nanodisc (grey). The nanodisc itself, through sophisticated selective deuteration, is rendered invisible in SANS experiments that focus on the conformation of the protein in its native context

RESEARCH TEAM: Henning Tidow and Inokentijs Josts (Hamburg University, Germany), Selma Maric (Malmo University, Sweden), Sylvain Prévost and Trevor Forsyth (ILL)

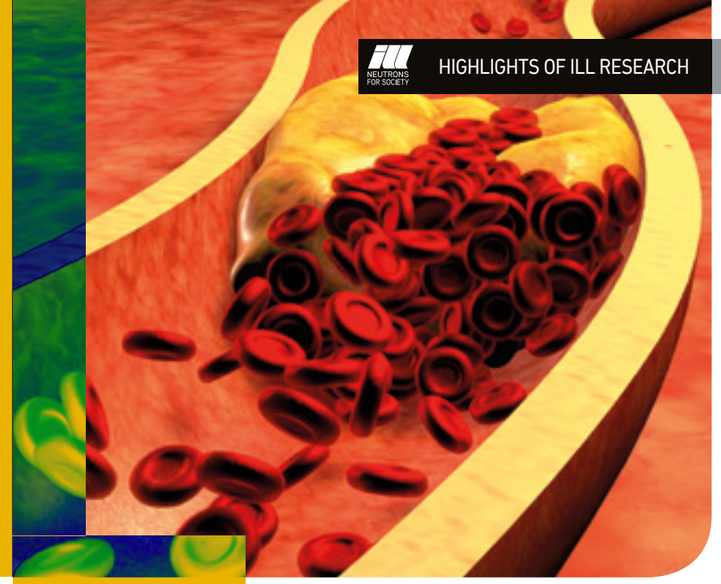
ILL FACILITIES AND INSTRUMENT USED: D-Lab, PSB and small-angle scattering diffractometer D11

REFERENCES: 1. J. Josts *et al., Structure*, 2018, **26**, 1072. 2. J. Nitsche *et al., Communications Biology,* 2018, **1**, 206, 3. Maric *et al., Acta Crystallogr. D Biol Crystallogr.*, 2014, **70**, 317.

HIGHLIGHTING CHOLESTEROL

DEUTERATED CHOLESTEROL, NOW PRODUCED BIOSYNTHETICALLY IN THE ILL'S DEUTERATION LABORATORY, OFFERS A VALUABLE TOOL FOR STUDYING VARIOUS ASPECTS OF LIPID MEMBRANES IMPORTANT IN COMBATTING DISEASE

Martine Moulin

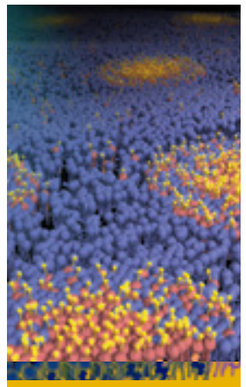


Cholesterol plaques blocking a blood vessel in atherosclerosis

Most people are familiar with the significance of cholesterol for human health and disease – in particular, its role in the formation of atherosclerotic particles or 'plaques', which block blood vessels resulting in heart disease and strokes. The risk factors underlying these major causes of death worldwide have become well-known to us through routine blood tests measuring cholesterol levels as part of low density lipoprotein (LDL, so-called 'bad cholesterol') and high density lipoprotein (HDL, known as 'good cholesterol'). Unsurprisingly, there is considerable interest in trying to understand the formation of LDL versus HDL and their dynamic relationship in the development of the plaques.

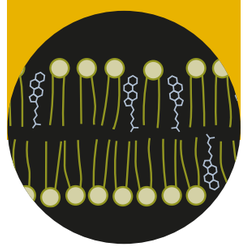
It may not be realised that the cholesterol molecule is also a vital constituent of mammalian cell membranes. Its presence in lipid bilayers (p19) affects the ordering of their structure and the diffusion of materials across the membranes. The chemical composition of the lipids involved (and in particular that of the lipid 'tail') is thought to influence where cholesterol sits in the membrane. This may be towards the head of the lipid molecule (in the case of saturated lipid membranes) or towards the inner interface between the two bilayer leaflets (polyunsaturated lipids). Cholesterol is also centrally involved in the structure and behaviour of areas in the membrane known as lipid rafts (p30), which are believed to be important in physiological events such as signal transduction.

The neutron techniques of diffraction and reflection (p8) are ideal probes for investigating interactions of molecules such as cholesterol in lipid membranes. However, the scope of these methods has been limited by the fact the visibility of cholesterol amongst the other lipids is low, making it extremely difficult to image and distinguish each component independently.



lipid raft

The location of deuterated cholesterol in a membrane bilayer as visualised by neutron reflectometry



BIO-ENGINEERING A YEAST

One way of highlighting the scattering contribution of cholesterol, so that it stands out, is to make a version with all the constituent hydrogen atoms replaced by deuterium (p8). However, this rather complex lipid is a challenging molecule to synthesise chemically. In collaboration with colleagues at the University of Graz in Austria, we therefore embarked on developing an alternative, biosynthetic route using the yeast *Pichia pastoris*. Natural ('wild type') yeast does not actually produce cholesterol but a related molecule called ergosterol. However, we have used a bio-engineered a strain of *Pichia* to make cholesterol instead. We were able to produce cholesterol in which the hydrogen was replaced by deuterium to a level greater than 98 per cent.

There has been a great deal of interest from the scientific community in exploiting the availability of the fully deuterated cholesterol for a wide variety of applications. Researchers have used it to carry out various structural characterisations, including identifying the location of the molecule within lipid bilayers that are composed of a natural mixture of phosphatidylcholine, and demonstrating that it sits closer to the lipid headgroup–tail interface rather than the core of the bilayer (see figure opposite). Ongoing research includes investigations into lung surfactants at the air–water interface, and the migration and distribution of cholesterol in mammalian cells.

This biosynthetic approach for the production of deuterated cholesterol has now been taken further such that its scattering strength can be matched to that of pure deuterated water; this will be invaluable in SANS and reflection studies of membrane proteins studied in nanodiscs (p10), allowing molecular models to be built of protein systems in a native habit and free of detergents.

RESEARCH TEAM: Martine Moulin, Sarah Waldie, Trevor Forsyth, Michael Haertlein (ILL), Harald Pichler and Gernot Strohmeier (Graz, Austria), Selma Maric and Marité Cardenas (Malmo, Sweden)

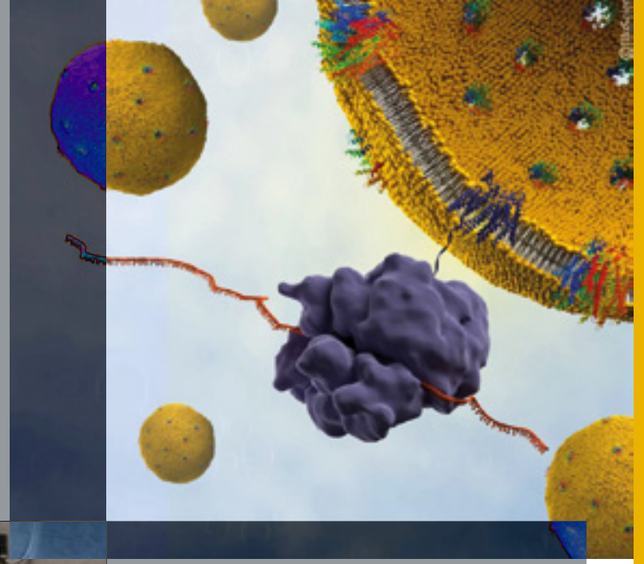
ILL FACILITIES AND INSTRUMENT USED: D-Lab, PSB and horizontal reflectometer FIGARO

REFERENCES: 1. M. Moulin *et al., Chem. Phys. Lip.*, 2018, **212**, 80. 2. S. Waldie *et al., Langmuir*, 2018, **34**, 472. 3. A. Luchini *et al., Colloids Surf. B Biointerfaces*, 2018, **168**, 126. 4. S. Waldie *et al., Scientific Reports* 2019, **9**, 7591.

A NEW TOOL TO IDENTIFY A HEPATITIS C DRUG

WE HAVE DEVELOPED A NEW TECHNIQUE FOR ANALYSING VIRAL MEMBRANE PROTEINS IN THEIR NATURAL LIPID ENVIRONMENT

Thomas Soranzo

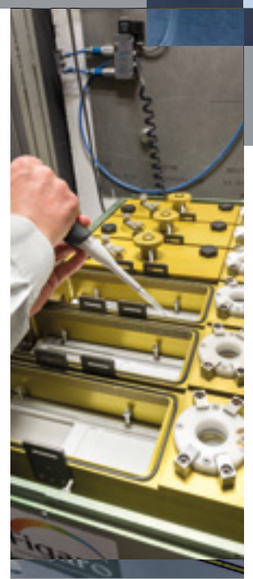


Cell-free expression of membrane proteins in a lipid bilayer of liposomes'
Synthelis/Illuscencia

Cell membranes host a multitude of proteins, which play vital roles in many biological processes. It is estimated that around 20 to 30 per cent of all genes encode membrane proteins. Mutations, lack of expression, over-expression or misfolding of these proteins can cause diseases such as cystic fibrosis, heart disease, obesity and cancer. Membrane proteins also play a key role in the pathological action of viruses.

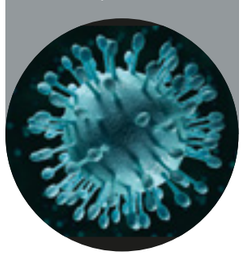
Some viruses possess an outer envelope, which typically contains membrane proteins with unique structural and functional characteristics that are crucial for all stages of its cycle. This is true for the hepatitis C virus (HCV). It is considered to be the cause of a major public health problem, as 150 million people are currently infected, resulting in more than 300,000 deaths annually. Chronic infection can lead to liver diseases such as fibrosis, cirrhosis or liver cancer. While there are vaccines to prevent hepatitis A and B; no vaccine for hepatitis C is yet available to prevent the spread of infection, and potential new treatments are very expensive.

Another therapeutic approach is to identify an HCV membrane protein whose action could be inhibited by a suitable drug molecule. HCV viroporin p7 is such a candidate; it is crucially involved in the life-cycle of the virus including its assembly and ability to infect a host. Most viroporins are small molecules that stretch across the membrane of an infected cell, creating an ion channel (through which selected ions can pass) that encourages the virus to be released to the environment. However, until recently, the structural data on HCV p7 have been rather sparse, hampered by the fact that the production of such proteins in enough quantity for in-depth study has proved a challenge.



The FIGARO instrument

The hepatitis C virus



CELL-FREE EXPRESSION

Proteins are conventionally made in the laboratory by expressing them in suitably genetically engineered bacteria or other cells. However, viroporins cannot be produced in this way because they destabilise the host cell membrane, so the yield is low. Fortunately, there is now an alternative, cell-free method, which relies on a simplified combination of selected biological constituents in an open system. In this way, the membrane protein like HCV viroporin p7 can be prepared in its natural three-dimensional folded state in a lipid bilayer that mimics its biological environment.

Using this method, we were able to insert viroporin p7 into a model membrane and study its structure using neutron reflectometry. By deuterating (p8) the protein's amino acids so as to highlight them, we could observe that the p7 protein complex forms a funnel shape in the lipid bilayer. Using a technique called electro-impedance spectroscopy (EIS), we could also show that the protein acted as an ion channel by measuring its conductivity as it sat in a lipid bilayer placed on a gold electrode.

This study demonstrated for the first time the use of a cell-free expression system in such as study. This new approach is simple and time-efficient, and it is complementary to other structural – and more complex – techniques, such as NMR (p31) and crystallography. These results pave the way not only to the potential development of hepatitis C drugs, but also, more generally, to the structural characterisation of a wider variety of difficult-to-prepare membrane proteins in lipid bilayers – as well as the development of novel biosensors that exploit membrane proteins.

RESEARCH TEAM: Thomas Soranzo (Université Grenoble Alpes, formerly of Synthelis, France), Jean-Luc Lenormand and Donald Martin (Université Grenoble Alpes, France), and Erik Watkins (Los Alamos National Laboratory, US), and supported by Synthelis (https://www.synthelis.com)

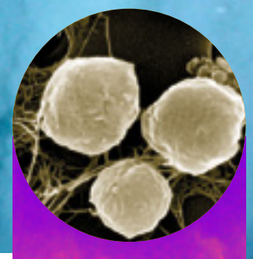
ILL INSTRUMENT USED: Horizontal reflectometer FIGARO

REFERENCES: 1. T. Soranzo *et al., Sci. Rep., 2017*, **7**, 3399. 2. ILL press release: https://www.ill.eu/news-press-events/press-corner/press-releases/neutrons-reveal-hidden-secrets-of-the-hepatitis-c-virus

WATCHING A PROTEIN UNFOLD

A TIME-RESOLVED SMALL-ANGLE NEUTRON SCATTERING STUDY HIGHLIGHTS A PROTEIN WRECKING MACHINE AT WORK

Frank Gabel



Methanocaldococcus jannaschii lives near hydrothermal vents

Living cells depend upon a vast number of proteins to carry out various essential biological functions. Their ability to work correctly depends on maintaining and controlling a specific three-dimensional shape (conformation), developed via a characteristic folding of their long-chain amino-acid structure. If a protein becomes misfolded, then it can no longer do its job correctly, and that may lead to severe health issues such as cancer or neurodegenerative diseases.

Fortunately, cells have a mechanism for destroying misfolded proteins by chopping them up into their amino-acid building blocks (proteolysis) in special chambers constructed from a large assembly of macromolecules. These chambers are well-protected – and to avoid the proteolysis of healthy proteins, access to them is tightly regulated. Proteins destined for destruction are recognised by 'gatekeeper' enzymes, which unfold the proteins and direct them into the proteolytic chambers.

The gatekeepers, known as 'unfoldases', belong to the large family of enzymes called AAA+ ATPases, which can do mechanical work on proteins by consuming the energy-rich molecule ATP. Unfoldases form ring-shaped pores consisting of six subunits, through which the protein substrate is pulled as it is bound to the enzyme and unfolded. An important feature of these ATP-powered enzymes is that they are flexible and can change conformation as required by the unfolding process.

Elucidating this process is extremely challenging because it is fast and transient. Fortunately, neutron scattering offers a technique – time-resolved small-angle neutron scattering (TR-SANS) – whereby measurements can be taken over time to capture rapid structural changes in molecules.

A NOVEL COMBINED APPROACH

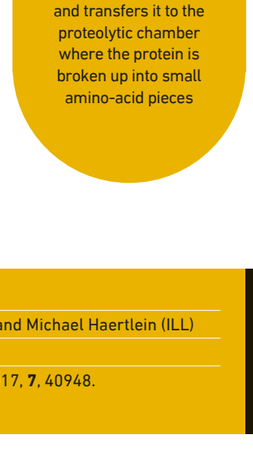
In a collaboration between the ILL and the IBS (Institut de Biologie Structurale), we developed a novel approach to study the unfolding of a protein by an unfoldase. It combines TR-SANS with a spectroscopic technique which exploits a protein that fluoresces green when exposed to light. In the experiment, this specially tagged Green Fluorescent Protein (GFP) was the substrate to be unfolded. Continually measuring the fluorescence then allows the unfolding to be followed, while the TR-SANS provides parallel structural information.

For the unfoldase, we chose the PAN system from a single-celled microbe, *Methanocaldococcus jannaschii* – an extremophile that lives near hydrothermal vents at temperatures approaching 100 °C, in ocean abysses, several thousand metres deep. The advantage of working with this unfoldase assembly is that it is very robust. We could activate it with heat and then control the speed of the unfolding process by adjusting the temperature.

By using the neutron techniques of deuteration (collaboration with ILL D-Lab) and solvent contrast variation (p8) to highlight the substrate and the unfoldase separately, and by controlling the enzymatic activity of the PAN system through temperature activation at between 55 and 60°C, we could follow conformational changes of PAN and GFP individually during unfolding at a time-resolution of 30 seconds.

The TR-SANS experiments revealed the progressive unfolding and aggregation of GFP, as well as a reversible contraction of the PAN unfoldase during the active reaction. The PAN complex underwent an energy-dependent transition from a relaxed to a contracted conformation, followed by a slower expansion to its initial state at the end of the reaction.

The results confirmed our current understanding of the way unfoldases and other related ATP-driven enzymes work. They also demonstrate the general usefulness of this time-resolved neutron approach for studying the structural molecular kinetics of complex protein assemblies and their substrates on the timescale of seconds at the nanometre scale. We expect to be able to apply this methodology to a wide range of biological macromolecular complexes.



The PAN unfoldase system unfolds the substrate protein GFP and transfers it to the proteolytic chamber where the protein is broken up into small amino-acid pieces

RESEARCH TEAM: Frank Gabel and Emilie Mahieu (IBS, France), Ziad Ibrahim (IBS/ILL) and Anne Martel, Martine Moulin and Michael Haertlein (ILL)

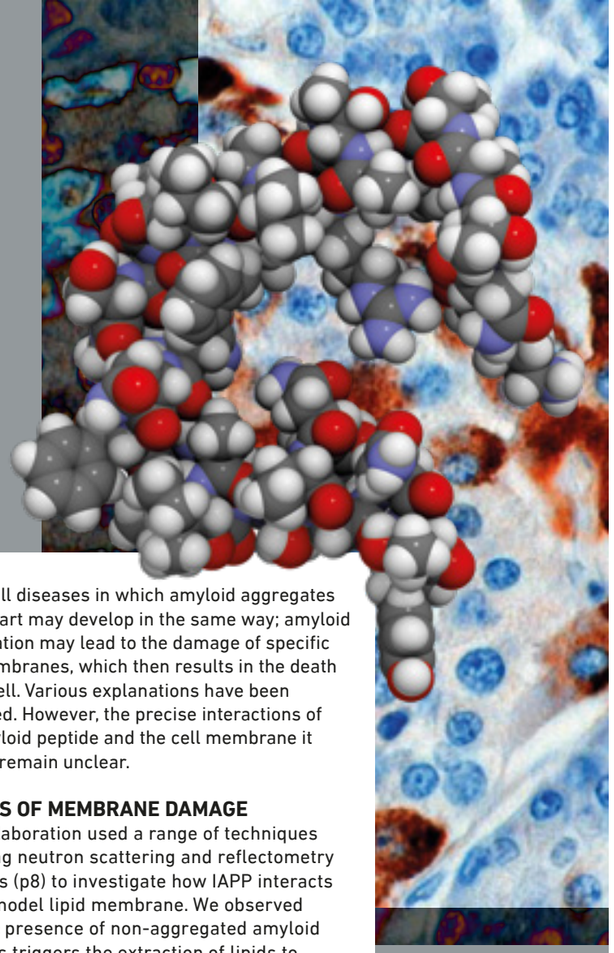
ILL FACILITY AND INSTRUMENT USED: D-Lab and small-angle diffractometer D22

REFERENCES: 1. R.T. Sauer and T. A. Baker, *Annu. Rev. Biochem.*, 2011, **80**, 587. 2. Z. Ibrahim *et al., Scientific Reports*, 2017, **7**, 40948.

A BETTER UNDERSTANDING OF TYPE 2 DIABETES

NEUTRONS UNCOVER IMPORTANT INTERACTIONS BETWEEN DISEASE-CAUSING PEPTIDES AND CELL MEMBRANES

Anne Martel



Aggregates of islet amyloid polypeptide (IAPP) forming in pancreatic cells are thought to affect insulin secretion

In 2014, the number of people worldwide with diabetes reached 422 million, which is 8.5 per cent of adults over the age of 18. A key pathological consequence of type 2 diabetes is the progressive failure and depletion of the cells that produce and secrete insulin, subsequently causing a loss of control over the regulation of glucose. These 'beta-cells' are found in the pancreas, located within clusters of cells, the islets of Langerhans. There is increasing evidence to suggest that this depletion is correlated with the formation of amyloid aggregates (p30) in the islets, which is toxic to beta-cells. These aggregates – large insoluble protein fibres – are primarily composed of islet amyloid polypeptide (IAPP), a hormone co-secreted with insulin by the beta-cells. While in the healthy situation, a lower amount of IAPP is produced than insulin, the hormone's rate of production rises dramatically during the development of type 2 diabetes.

Although we know something about IAPP's role in the development of type 2 diabetes, the precise biological mechanisms underpinning the metabolic disorder's pathology remain unclear, including how IAPP induces the depletion of beta-cells. With the prevalence of type 2 diabetes expected to continue to rise, gaining a thorough understanding of the underlying biology is crucial for developing better drugs and improving current therapeutic approaches. A collaboration between the ILL, the Institute for Molecular Engineering at the University of Chicago and Institut de Biologie Structurale recently investigated the mechanisms of the IAPP interaction with a model cell membrane (p30), and its role in the pathology of this type of diabetes.

Because the amyloid aggregates seem to be associated with beta-cell depletion, it is possible that aggregation triggers processes which lead to the death of the beta-cells, so causing the diabetes symptoms. In fact, the current thinking is that all diseases in which amyloid aggregates play a part may develop in the same way; amyloid aggregation may lead to the damage of specific cell membranes, which then results in the death of the cell. Various explanations have been proposed. However, the precise interactions of the amyloid peptide and the cell membrane it targets remain unclear.

CAUSES OF MEMBRANE DAMAGE

Our collaboration used a range of techniques including neutron scattering and reflectometry methods (p8) to investigate how IAPP interacts with a model lipid membrane. We observed that the presence of non-aggregated amyloid peptides triggers the extraction of lipids to produce permeable regions in the membrane (membrane damage). Testing different fragments of rat and human IAPP peptides that had a range of propensities for aggregation and membrane-damage activity, we showed that the widely-held belief that membrane damage is a consequence of amyloid aggregation was not true. Rather, these two processes happen independently and in competition – a low propensity for aggregation, in fact, correlates with strong membrane damage.

This finding pushed us to come up with a new explanation for the relationship between the aggregation-prone behaviour of amyloid peptides and their involvement in many human diseases. We propose that amyloid aggregation could be a defence mechanism employed by the human body in order to 'silence' the cytotoxic peptides, thus lowering the toxicity of IAPP to pancreatic beta-cells, and delaying the onset of disease. Indeed, many amyloid diseases are related to ageing. The tendency to produce the amyloid version of a protein or peptide could therefore have been selected via evolution to delay the onset of disease, once the fertile period of a mammal's life is over.

The D22 instrument

RESEARCH TEAM: Anne Martel, Yuri Gerelli, Lionel Porcar, Irena Kiesel and Giovanna Fragneto (ILL), Lucas Antony, Aaron Fluitt, Kyle Hoffmann and Juan J. de Pablo (University of Chicago, US), and Michel Vivaudou (IBS, University Grenoble Alpes, France)

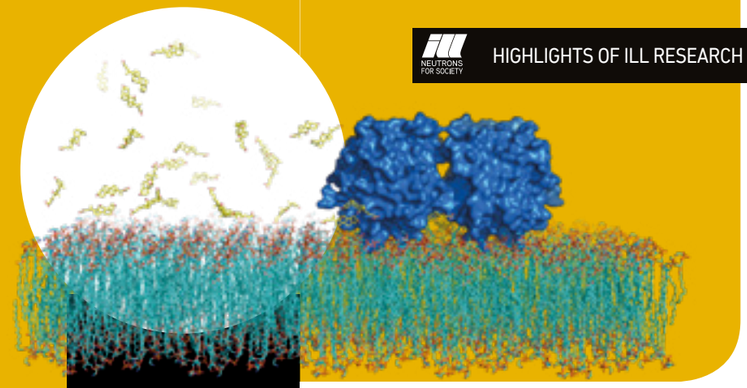
ILL FACILITIES AND INSTRUMENTS USED: PSCM, Chemistry lab, reflectometer FIGARO and small-angle scattering instrument D22

REFERENCES: A. Martel *et al., J. Am. Chem. Soc.*, 2016, **1**, 139.

INSIGHTS INTO METABOLIC DISORDERS

UNDERSTANDING THE ENDOCRINE FUNCTIONS OF BILE ACIDS IS HELPING TO COMBAT TODAY'S MAJOR HEALTH PROBLEMS, FROM TYPE 2 DIABETES TO OBESITY

Gianpiero Garau and Francesca Natali



The structure of the human NAPE-PLD protein (blue) at the membrane interface (cyan); the bile acid molecules are depicted in yellow

Maintaining a healthy body depends upon the optimum functioning of a complex network of interactions between cells, mediated by 'signalling' molecules that interact with specific proteins – receptors – of the cell membrane. These networks usually involve multiple nodes of interaction, feedback and cross-regulation. Deciphering the precise function of each node provides essential clues to the signalling mechanisms. It thus helps to identify their role in diseases, and unveils promising novel therapeutic interventions.

One important class of signalling molecules are the bile acids. These natural detergents are secreted by the liver to aid the digestion and absorption of dietary fats in the intestine. They also act as versatile signalling molecules within the endocrine system, regulating their own circulation in the gut, and the amounts of lipids and glucose in the body, which affect the concentrations of cholesterol and glucose in the blood.

As a result, understanding the role of bile acids in metabolism and signalling is an important goal in developing new drugs to manage a variety of metabolic diseases. These include conditions that are ravaging many communities around the world: obesity, type 2 diabetes, hyperlipidaemia (too high levels of lipids in the blood) and atherosclerosis (loss of arterial elasticity by lipid accumulation) which can have dramatic influences on the cardiac function.

NEW DRUG TARGETS

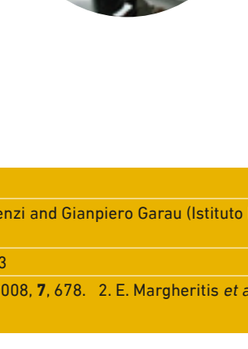
Interestingly, most of the drugs used for modulating these diseases are bile-acid derivatives. For example, the bile-acid derivative, ursodeoxycholic acid, is approved for treating gallstones and also primary biliary cirrhosis (which results in the destruction of the bile ducts in the liver). It also increases a patient's sensitivity to insulin, which is important for the management of diabetes. Other bile-acid analogues also look promising for treating metabolic diseases.

These findings thus strongly encourage the investigation of new bile-acid binding targets. One such target is the human membrane protein, NAPE-PLD (N-acyl phosphatidylethanolamine phospholipase D). This is a zinc-containing enzyme that generates important bioactive lipid signalling molecules, which have essential protective roles in many aspects of health, including stress and pain response, appetite, and even lifespan.

Recently, we solved its molecular structure using X-ray crystallography (top graphic). Unexpectedly, we discovered that NAPE-PLD has specific binding sites for the bile acid, deoxycholic acid (a secondary bile acid produced by the bacterial flora in the gut). We then asked whether the interaction between NAPE-PLD and bile acids might affect the membrane protein's flexibility and enzymatic activity. Changes in the mobility of membrane proteins observed under different conditions can provide essential insights into how these biomolecular machines work and promote their key processes in cells.

To investigate, we carried out a range of elastic incoherent neutron scattering (EINS) experiments (p30) on NAPE-PLD bound to bile acids at different concentrations. This method allowed us to probe the dynamics associated with structural changes of protein complexes. We noted that when the bile acid molecules are bound to their specific sites, they contribute to the stiffness of the enzyme. As a result of the interaction, the membrane protein motion is restricted, allowing the phospholipid substrate (NAPE) to bind into the active catalytic site and the generation of the protective lipid-signalling molecules (middle figure).

Our studies have shed light on a key physiological event linked to our fat digestion process. Overall, the results have shown key structural elements for the design of small-molecule modulators with potential applications in several bile acid-related metabolic disorders, including obesity, metabolic syndromes and type 2 diabetes.



The interaction of bile acids restricting NAPE-PLD structure fluctuations, as revealed by neutron scattering

The IN13 backscattering instrument

RESEARCH TEAM: Eleonora Margheritis, Valentina De Lorenzi and Gianpiero Garau (Istituto Italiano di Tecnologia, Italy) and Francesca Natali (ILL/CNR-IOM')

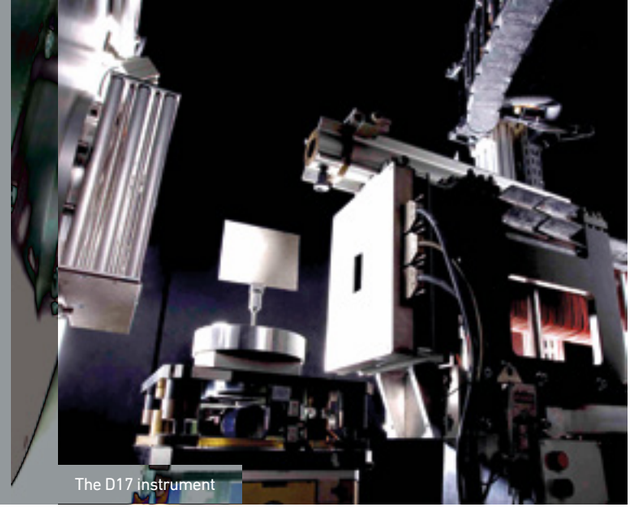
ILL INSTRUMENT USED: backscattering spectrometer IN13

REFERENCES: 1. C. Thomas et *al., Nat. Rev. Drug Discov.*, 2008, **7**, 678. 2. E. Margheritis *et al., ACS Chem. Biol.*, 2016, **11**, 2908.

UNCOVERING THE CAUSES OF ALZHEIMER'S

WITH AN ESTIMATED 131.5 MILLION ALZHEIMER'S SUFFERERS BY 2050, UNDERSTANDING THIS TERRIBLE DEGENERATIVE DISEASE IS MORE IMPORTANT THAN EVER

Valeria Rondelli



The D17 instrument

By 2050, more than 20 per cent of the world's population will be 65 or older – medical advances and the increase in global living standards means that the human race is living longer than ever before. As a result, more of us are being diagnosed with age-related illnesses such as Alzheimer's disease. In 2015, there were 46.8 million people living with dementia, costing the world economy more than $800 billion.

The mechanisms underlying Alzheimer's are not completely understood, but studies have indicated that the progressive production and accumulation of protein units called beta-amyloid peptides (Aβ) in the brain, play a pivotal role. These fragments come from the break-up of the membrane-associated amyloid precursor protein (APP), and are released by neurons in a soluble form (p17). They then gradually aggregate into a range of oligomers (p31) – from small, soluble structures to much larger ones – ending up as insoluble fibrils. In particular, it is the soluble oligomers that are thought to be largely responsible for the onset and progression of the cognitive dysfunction characterising the disease.

One of the peculiar properties of soluble Aβ oligomers is that they are 'membrane-active' in that they can promote the puncturing of a cell membrane and increase its permeability. They localise in the lipid membrane, in structured domains called rafts (p11), which are enriched in cholesterol and a lipid–carbohydrate complex molecule, GM1-ganglioside. It may be that GM1acts as a templating spot for Aβ aggregation in the membrane, thus promoting the disease.

This suggestion is one of several processes that might lead to Alzheimer's, so we set out to investigate the existence and extent of the interaction between Aβ peptides in progressive aggregation states and a lipid raft in the lipid bilayer (p8). Neutron reflectometry offers the ideal technique for this because it can reveal the transverse structural details of a bilayer (p8), discriminating between regions at different depths within the membrane. Moreover, the deuteration and contrast variation strategy (p8) allows us to enhance the visibility of the hydrogen-containing peptides when they interact with the deuterated phospholipid membrane.

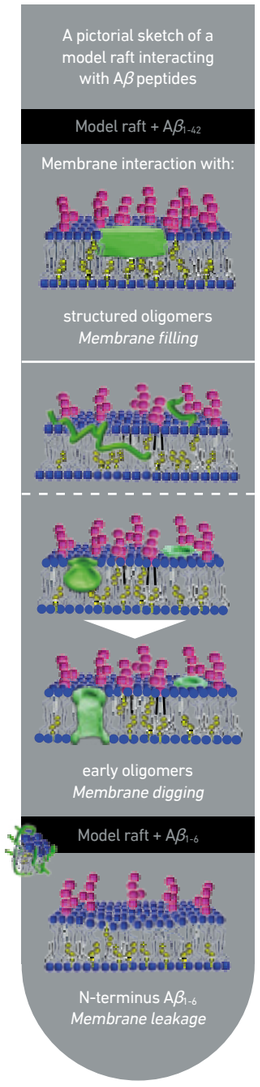
A MODEL LIPID RAFT

First, we developed a model lipid raft suitable for structural investigation using reflectometry. We prepared and characterised single bilayer membranes with a composition as close as possible to a natural raft – that is, with phospholipids, GM1 and cholesterol, and, notably, with an asymmetric distribution of components, which is a distinctive property of cell-membrane rafts.

We focused on two conditions: the first was when the Aβ oligomer had already started to aggregate into a structured state that was expected to interact with the membrane surface of the raft; the second was when the peptide was in an early soluble, unstructured state and the initial formation of oligomers might take place at the membrane.

We found that the structured version of Aβ – its most membrane-active state – embeds into the external layer of the membrane. However, surprisingly, the unstructured early oligomers penetrate deeply into the membrane. We also tested the membrane-interaction ability of the small Aβ fragment that terminates in a free amine group (N-terminus). It was seen to remove lipids from the bilayer, thus suggesting its role, in the whole peptide, in causing membrane leakage, which would encourage more peptides to interact with the membrane.

These results push us towards developing drug compounds that mimic the peptide but inhibit amyloid aggregation and interaction with cell membranes.



A pictorial sketch of a model raft interacting with Aβ peptides

RESEARCH TEAM: V. Rondelli, Laura Cantù, Paola Brocca and Elena del Favero (Università degli Studi of Milano, Italy), Laura Colombo and Mario Salmona (Mario Negri Pharmacological Research Institute, Milano, Italy), and Giovanna Fragneto (ILL)

ILL INSTRUMENT USED: reflectometer D17

REFERENCES: 1. V. Rondelli, *Sci Rep.*, 2016, **6**, 20997. 2. D. H. Small and R. A. Cappai, *J. Neurochem.*, 2006, **99**, 708. 3. A. Kakio *et al.*, *Biochem.*, 2002, **41**, 7385. 4. V. Rondelli *et al.*, *BBA Biomembranes*, 2012, **1818**, 2860.

A NEW APPROACH FOR TREATING ALZHEIMER'S

NEUTRON DIFFRACTION UNCOVERS WHY ALZHEIMER'S DISEASE DEVELOPS DIFFERENTLY IN DIFFERENT INDIVIDUALS

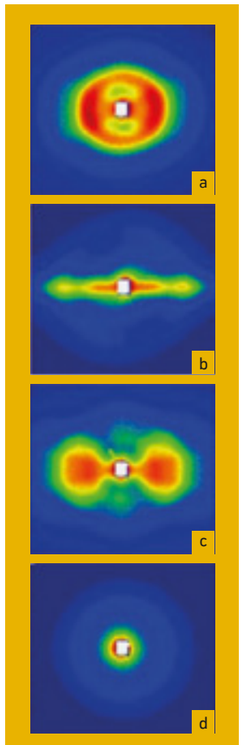
Daniel Kirschner and Bruno Demé

Aggregates of fibrous protein (amyloid plaques) forming on nerve cells (blue) are characteristic features of Alzheimer's disease

Alzheimer's disease is the most common form of dementia in the elderly. To date, all strategies to treat the disease have faced serious hurdles in clinical application: the tested drugs have proved ineffective or have caused severe adverse events. Furthermore, in clinical trials, different Alzheimer's patients can respond differently to the same drug – probably because of intrinsic genetic differences that might affect molecular pathways involved in triggering the disease. There is, therefore, an urgent need to find safer drug molecules with a more targeted action to treat this devastating disease.

Alzheimer's is thought to be caused by the formation in the brain of fibrous protein aggregates (called amyloid plaques) between nerve cells, and fibrillary tangles within nerve cells. The principal pathological hallmark is the accumulation of amyloid-beta peptides (p16) consisting of chains of between 36 and 43 amino acids, generated by the abnormal cleavage of a large transmembrane glycoprotein known as amyloid beta precursor protein (APP). The misfolding of these peptides promotes their aggregation into disorganised flexible oligomers (p31), as well as structurally-regular fibrils that condense into the fibrous plaques. Such amyloid-beta aggregates are postulated to trigger a cascade of degenerative events affecting the phosphoprotein, tau, which is an essential protein that stabilises neuronal structure and ensures their correct function. If the tau protein becomes hyperphosphorylated ('pTau'), it destabilises the neuron's cytoskeleton, self-assembles into abnormal fibrillary tangles, and leads to neuronal death.

One approach to treating Alzheimer's may be to design a drug based on a mutated version of the amyloid-beta peptide that inhibits the triggering of this cascade. To this end, we decided to study the effects of a novel mutation in the APP gene resulting in the substitution in the corresponding APP protein of the amino acid, alanine, with another, valine. This mutation is associated with the early onset of Alzheimer's in individuals having two copies of this gene, whereas it has a protective effect in those with only one copy of the mutation.



Neutron diffraction images of the natural and mutant beta peptide–amyloid assemblies: (a) natural peptide, (b) mutant peptide, (c) 1:1 mixture of both, and (d) a 4:1 mixture of the natural and the mutant hexapeptide fragment

CLUES FROM A PEPTIDE MUTATION

First, we synthesised short peptide sections with just six amino acids and containing the mutation, and tested its ability to bind preformed amyloid-beta fibrils. We found that it did not do so, whereas the non-mutated peptide did.

We then carried out a range of complementary structural analytical techniques including X-ray and neutron diffraction on samples of the natural (alanine-containing) and mutant (valine-substituted) versions of the amyloid-beta peptide, including a sample containing an equal mixture of them, and another one that contained a mixture of the normal peptide and the short six amino-acid mutant fragment. We found that the diffraction patterns for the four samples were distinctly different: that from the mutant peptide indicated well-formed fibres that were largely oriented, whereas that from the natural peptide indicated poorly-organised, less well-oriented fibres. The mixtures showed less clear diffraction patterns indicating the presence of structures that interfered with the progressive formation of fibres.

In summary, the change of a single amino acid can cause strikingly different features in the aggregated structures formed by this peptide. The mutant version promotes the formation of much more compact fibres, whereas exposing molecules of some of the shortened version of the mutant form to the natural form disrupts the interaction that drives the peptides to condense into fibres. These results suggest why the degeneration-fuelled dementia is so aggressive in people carrying both copies of the mutant gene, but paradoxically one copy of the mutant gene is sufficient actually to protect the individual. These findings should help to direct efforts toward finding more personalised Alzheimer's drugs.

RESEARCH TEAM: Laura Cantù, Laura Colombo, Tatiana Stoilova, Valeria Rondelli, Giuseppe Di Fede, Fabrizio Tagliavini, Elena Del Favero and Mario Salmona (Milan, Italy), Hideyo Inouye, Rachel Booth and Daniel Kirschner (Boston, US), and Bruno Demé (ILL)

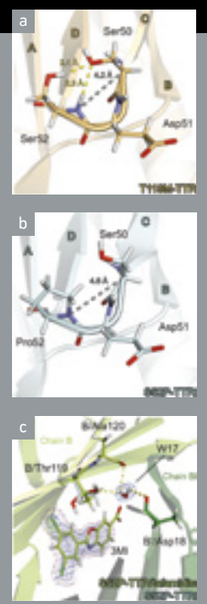
ILL INSTRUMENTS USED: diffractometer with variable vertical focusing D16, small-angle scattering diffractometers D11 and D22

REFERENCE: L. Cantu *et al.*, *Sci. Rep.*, 2017, **7**, 5510.

TARGETING FAMILIAL AMYLOID POLYNEUROPATHY

NEUTRON STUDIES ARE HELPING TO REVEAL THE MECHANISMS UNDERLYING AN INVARIABLY FATAL DISEASE

Alycia Yee and Trevor Forsyth



Neutron crystallography reveals how the stability of the 'C-D loop' segment in the transthyretin molecule is affected: (a) in the stable T119M mutant version (protective against amyloidosis), it is tight; (b) in the S52P mutant (associated with the most aggressive form of transthyretin amyloidosis), it is loosened, allowing the protein to misfold into amyloid fibrillary deposits; (c) when the drug tafamidis binds to transthyretin, it mimics the effects of the T119M mutant and stabilises it

Transthyretin amyloidosis is a progressive condition in which abnormally-folded forms of an important hormone transporter protein accumulate as amyloid fibrils/plaques (p30) in various parts of the body – resulting in the condition known as familial amyloid polyneuropathy (FAP). The most affected tissues include the peripheral nervous system (sensory perception – pain, touch, heat, sound), the autonomic nervous system (involuntary functions such as breathing, heart rate, digestion). Other regions such as the central nervous system, the heart, the kidneys and the gastrointestinal tract may also be afflicted.

In this study, we have investigated normal human transthyretin, alongside two variants of the protein ('mutants' that are found in different parts of the population). One of these (the T119M or 'stable' mutant) imparts remarkable stability to transthyretin and is strongly protective against the formation of amyloid fibrils. However, the other (the S52P or 'unstable' mutant) results in the most aggressive form of hereditary amyloidosis.

All of the transthyretin mutants were studied using neutron and X-ray crystallography, along with mass spectrometry (p31) and computer-modelling simulations. Crucially, the project was able to take advantage of deuteration methods (p30) available from the Deuteration Laboratory (D-Lab) platform in the ILL's Life Sciences Group. These investigations have resulted in the proposal of a molecular mechanism by which transthyretin forms amyloid fibrils through a parallel equilibrium of partially unfolded protein forms.

Transthyretin has a complex hierarchical structure of four subunits (monomers). The results suggest that unfolding events originate in a particular part of the protein, the C–D loop, that de-stabilises the structure in this region and renders the protein more susceptible to degradation, enhancing the rate of amyloid fibril formation. Furthermore, the study suggests that the binding of small-molecule drugs to transthyretin stabilises the folded state of transthyretin in the same way as happens in the protein mutants that remain stable.

A NEW NEUTRON METHOD

Additionally, this study has emphasised the potential exploitability of a new neutron approach that can be used to provide important information on the dynamics and stability of particular regions of a protein. Neutron crystallography is usually carried out with all of the 'normal' water (H_2O) replaced by 'heavy' water (D_2O) – this helps to optimise the quality of the data recorded. However, controlled 'back-exchange' of this D_2O by H_2O can be carried out. This solvent exchange occurs more readily in regions of the protein that have greater flexibility and movement – regions of a protein are more susceptible to denaturation, misfolding, and amyloid fibril formation. This type of approach has been successfully used in NMR (p31) and mass spectrometry but has never been seriously applied in neutron crystallographic studies.

In the case of transthyretin, the back-exchange data recorded on LADI from fully deuterated transthyretin crystals demonstrates very clear differences in the patterns of stability amongst the transthyretin mutants – with the unstable (S52P) mutant showing evidence of a highly unstable fold, consistent with the computer-modelling work. Intriguingly, recent research has implicated plasminogen – a precursor of plasmin, which breaks up blood clots – in transthyretin degradation and amyloidosis. This observation may be related to increased accessibility in or near the C–D loop that could allow the enzyme to cleave the protein at this location.

RESEARCH TEAM: Alycia Yee (Keele University, UK), Matteo Aldeghi (MPI, Göttingen, Germany), Matthew Blakeley, Martine Moulin, Michael Haertlein and Trevor Forsyth (ILL)

ILL FACILITIES AND INSTRUMENT USED: D-Lab, PSB and quasi-Laue diffractometer LADI

REFERENCES: 1. A. Yee *et al., Nature Communications.* 2019, **10**, 925. 2. "Uncovering the path to amyloidosis", *Highlights of ILL research, Neutrons and Health*, 2014, p18. 3. A. Yee *et al., J. Appl. Cryst.*, 2017, **50**, 660. 4. P. Mangione *et al., J. Biol. Chem.*, doi: 10.1074/jbc.RA118.003990 (2018).

WHAT MAKES LIPID MEMBRANES STICK TOGETHER?

NEUTRON DIFFRACTION COMBINED WITH COMPUTER SIMULATIONS REVEAL FUNDAMENTAL DIFFERENCES IN THE WAY GLYCOLIPID AND PHOSPHOLIPID MEMBRANES INTERACT

Matej Kanduč, Emanuel Schneck, Juliette Jouhet and Bruno Demé

Biological membranes are vital components of all living cells with multiple essential roles. They protect the cell contents, and act as gate-keepers in allowing the transfer of materials in and out of the cell and its constituent compartments; they also play a part in sticking cells together or to various extracellular scaffolds. Today, investigating how the detailed structure of cell membranes affects their behaviour is an active research area, underpinning the understanding of many diseases and the search for new therapeutic compounds.

The main building blocks of these membranes are lipids, which consist of long hydrocarbon chains that repel water molecules, attached to 'headgroups' that interact with water, particularly through the electrostatic forces associated with weak hydrogen bonding. The headgroup can be electrically charged, or neutral but with an internal distribution of electrical charges (dipoles). The lipids organise themselves into a double layer with the headgroups facing towards the watery environment. These bilayers host proteins and carbohydrates essential for cellular function.

The neutral lipids can be divided into two main classes: lipids with headgroups dominated by one large electric dipole, as in the most abundant phospholipid species, phosphatidylcholines (PCs), which consist of a negative phosphate group attached to a positive choline molecule; and lipids whose headgroups contain multiple small electric dipoles, typically polar hydroxyl (OH) groups, as exist in the sugar-containing glycolipids.

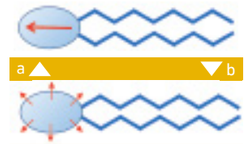
In Nature, membranes in different cell compartments have quite different lipid compositions. For instance, the so-called Golgi membranes, which are found inside cells and regulate protein activity, are highly dynamic and loosely packed membrane systems rich in PC lipids. In contrast, the structurally more stable and densely-packed multilayer membrane systems, such as the myelin sheaths (p31) of neurons and the photosynthetic membranes (thylakoids) found in plants, are high in glycolipids with multiple OH groups. This correlation suggests that headgroup architecture plays a significant role in determining the structural and dynamic characteristics of individual biological membrane systems.

MEMBRANE STACKING

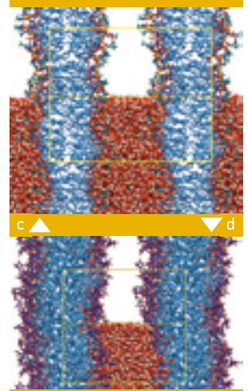
In the case of glycolipid membranes, the extent to which the glycolipids contribute to the stack formation and stabilisation is under debate. We know that membrane proteins play key functional roles in membrane stacking in thylakoids, but the tight layer cohesion suggests that the lipids on their own also contribute significantly.

Neutron diffraction – especially when combined with molecular dynamics (MD) computer simulations (p31) of what is happening at the atomic level – offers a particularly effective methodology for investing the structure and behaviour of membranes. Although challenging because of the need to take into account the complex interactions of surrounding water molecules, we have, during the past few years, established a successful methodology for determining the magnitude of molecular interactions from MD simulations with high precision. This enables us to investigate membrane interactions on a chemically detailed and mechanistic level. In the present work, we compared the interaction mechanisms of glycolipid and phospholipid membranes in water, showing that our MD simulations did indeed quantitatively reproduce the neutron-diffraction results.

The key interaction affecting the packing of the membranes is the water-uptake that results in the swelling of the bilayers. There is a slight repulsion between lipid membranes resulting from an interplay of the headgroup chemistry and water. We found that the degree of repulsion was different in the two lipid types. In the glycolipid membranes, the sugar–water interactions due to hydrogen bonding led to shorter-range repulsion, which accounts for the tighter stacking. In the PC lipid membranes, however, the stronger dipole of the headgroup induced broader structural effects and distinctly longer-range repulsion, leading to looser packing of the layers.



The two lipid types in living cells: (a) with a headgroup dominated by one large electric dipole, as for a phospholipid, and (b) with a headgroup containing multiple small electric dipoles, as in glycolipids. The dipoles are indicated by arrows



Computer simulations of two interacting hydrated membranes (blue): (c) consisting of phospholipids, and (d) glycolipids. The latter is revealed as more tightly stacked. For clarity, the water molecules (red and white) are shown only in the lower half of the graphics

RESEARCH TEAM: Céline Cataye, Maryse Block, Eric Maréchal, and Juliette Jouhet (Grenoble, France), Matej Kanduč, Alexander Schlaich, Roland Netz (Berlin, Germany), Alex de Vries (Groningen, The Netherlands), Emanuel Schneck (Potsdam, Germany) and Bruno Demé (ILL).

ILL INSTRUMENT USED: diffractometer with variable vertical focusing D16

REFERENCES: 1. https://www.ill.eu/news-press-events/press-corner/press-releases/ills-d16-instrument-enables-greater-understanding-of-the-unique-interaction-characteristics-of-glycolipid-membranes-april-2017 2. M. Kanduč *et al., Nature Communications*, **8** (2017); DOI: 10.1038/ncomms14899. 3. B. Demé *et al., FASEB Journal*, 2014, **28**, 3373.

AN UNEXPECTED ROLE FOR HEAVY PROTEINS

DEUTERATED PROTEINS MAKE BIGGER CRYSTALS FOR HIGH-RESOLUTION STRUCTURAL STUDIES

Saara Laulumaa and Petri Kursula

A crystal of the deuterated protein, CNPase

Enzymes are proteins catalysing a plethora of chemical reactions essential for life. Roughly one-half of all atoms in a protein are hydrogen, and those in the enzyme's active site and in the substrate are often central to the catalytic reaction mechanism (p30). Hydrogen atoms, and the weak hydrogen bonds (p30) that they participate in, are key determinants for both protein folding (p13), interactions and enzymatic activity. However, determining the presence and location of individual hydrogens with conventional techniques, such as X-ray crystallography or NMR (p31), is usually impossible.

Neutron crystallography (p31) can better detect hydrogen atoms, especially when they are replaced with the heavier deuterium isotope. This process of deuteration is used a great deal in biological studies with neutrons, as this booklet's research highlights describe. The ILL has a very successful deuteration facility, D-Lab. A drawback, however – compared to X-ray crystallography – is that much larger crystals are needed because the neutron beams used are weaker; generally, protein crystals are notoriously difficult to grow beyond a microscopic size.

Nevertheless, we recently made an interesting and useful discovery regarding the growth of protein crystals when working on the mammalian enzyme CNPase (2',3'-cyclic nucleotide 3'-phosphodiesterase). CNPase is highly abundant in the nervous system – in myelin, which is the fatty insulating layer around the axons that conduct the electrical impulses between nerve cells. It is implicated in various neurological and psychiatric diseases, such as multiple sclerosis and schizophrenia. The catalytic activity of CNPase had already been characterised in the test tube in the 1960s, but we still know only a little about its actual function.

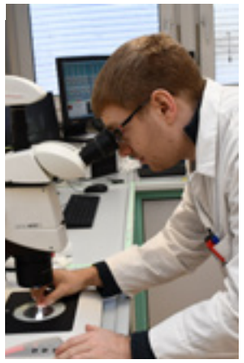
To understand the function of CNPase, we have been carrying out extensive X-ray crystallographic studies on its structure and function. Specifically, we wished to visualise the arrangement of hydrogen atoms in the enzyme active site. This is important, since several hydrogen atoms are expected to participate in the reaction mechanism. We therefore prepared samples of CNPase, in which all hydrogen atoms had been replaced with deuterium, in the hope of carrying out neutron crystallography.

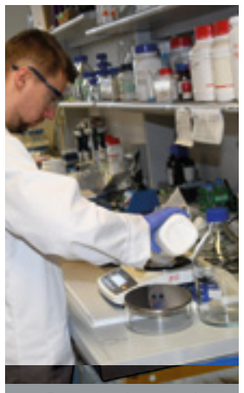
DEUTERATION BENEFITS X-RAY STUDIES

Surprisingly, we found that we obtained much larger crystals than from the normal hydrogenated enzyme in water. Unfortunately, they were still too small for neutron studies; however, we found that using X-rays from a synchrotron source (p31), we could record diffraction patterns to an amazing atomic resolution of one-tenth of a nanometre. The results confirmed the predicted mechanism of how the enzyme interacts with its substrate. Deuteration does not appear to affect the catalytic efficiency, although the reaction is slower in a deuterated solvent, and CNPase binds its substrate tighter. While we still do not know the exact physiological relevance of CNPase activity, we have now a very detailed view of its reaction mechanism, at the level of individual hydrogen atoms.

The study has indicated that deuterated proteins can also be of use in X-ray crystallographic work. Indeed, we have afterwards been able to grow large crystals for another deuterated protein, to provide even higher-resolution structural data.

We think that the reasons for the larger crystals, and the better diffraction properties, are the slightly different bond lengths and energies for deuterium *versus* hydrogen. Deuteration of the protein and the solvent thus provide useful factors to be screened during optimising protein crystallisation.





Preparing deuterated protein crystals in the D-Lab platform of the ILL's Life Science Group

RESEARCH TEAM: Saara Laulumaa (University of Oulu, Finland and ESS, Sweden), Arne Raasakka and Petri Kursula (University of Oulu, Finland and University of Bergen, Norway), and Michael Härtlein and Martine Moulin (ILL)

ILL FACILITY USED: D-Lab

REFERENCES: 1. A. Raasakka *et al., Sci. Rep.*, 2015, **5**, 16520. 2. S. Laulumaa *et al., Acta Cryst.* F, 2015, **71**, 1391.

TOWARDS UNDERSTANDING NEUROLOGICAL DISEASES

NEUTRON STUDIES REVEAL HOW A FLEXIBLE PROTEIN STICKS TOGETHER THE INSULATING LAYERS PROTECTING NERVE CELLS

Arne Raasakka and Petri Kursula

Our nervous system consists of a complex network of nerve cells, or neurons, which communicate with each other via electrical impulses that travel along neuronal extensions called axons. These axons are wrapped in an insulating sheath of myelin – a multilayered, tightly-packed assembly of stacked lipid bilayers (p19) held together by several myelin-specific proteins. The myelin sheath is not continuous but divided into units along the axon with a gap between each unit. The nerve impulse 'jumps' from one gap to another – a mechanism that speeds up nerve conduction, and has huge benefits for large animals like ourselves. Maintaining the myelin structure is thus extremely important.

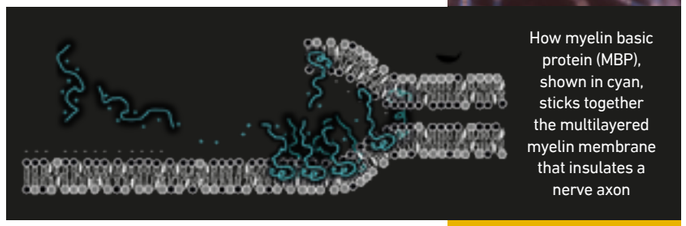
However, myelin is also a vulnerable point in our nervous system. Problems in myelin development (dysmyelination) or maintenance (demyelination) are causes of chronic neurological diseases in humans. Both genetic and environmental factors, including autoimmunity, can cause myelin abnormalities.

It is therefore important to develop a detailed understanding of the formation and structure of myelin as well as its behaviour. Fortunately, the diverse neutron techniques available – crystallography, reflectometry, diffraction, and inelastic and elastic scattering – are ideal for studying such a lipid–protein membrane multilayer at the molecular level.

PROBING MYELIN FORMATION

We were interested in one of the main myelin constituents, myelin basic protein (MBP) – a positively charged molecule, which is highly flexible and elongated in solution, but becomes more compact and sticky upon binding to a lipid membrane. We used neutrons, complemented by biophysical techniques, to probe the different stages of the myelin formation process involving MBP. We used an artificial setup consisting of highly purified MBP protein and synthetic lipid membranes, and detergent micelles (p31).

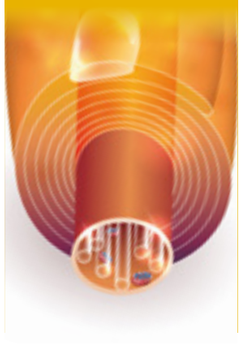
Two kinds of neutron experiments were carried out to follow MBP binding to synthetic membranes. SANS (p21) was performed to compare the molecular conformation of MBP in the presence and absence of detergent micelles. MBP remains elongated, but becomes more compact when bound to a micelle. Neutron reflectometry (p21) allowed us to observe the details of MBP binding to a membrane surface and embedding deep into the lipid bilayer. A time-resolved experiment showed that MBP becomes partly inserted into the upper half of the lipid bilayer, stays anchored to the membrane interior and forms a brush-like amorphous protein phase at the membrane surface. This structure has a high positive charge and can act as a glue to bind a second membrane surface.



How myelin basic protein (MBP), shown in cyan, sticks together the multilayered myelin membrane that insulates a nerve axon

A picture is thus now emerging of how MBP is instrumental in forming the myelin membrane multilayer. First, positively charged MBP binds to the negatively charged phospholipid surface of the membrane and inserts partially into the hydrophobic layer underneath. Simultaneously, the protein changes conformation and anchors to the membrane irreversibly. As MBP continues to accumulate on the surface, it creates a charged brush-like system. After reaching a critical concentration, this positively charged surface can attract another phospholipid bilayer, and MBP can insert into the second membrane as well. The structure compacts, and solvent is squeezed out, leaving a thin layer of protein between two lipid bilayers.

Recent work using neutrons, X-rays, and complementary methods has indicated that various myelin proteins share common properties in lipid membrane binding and stacking, and we are now on our way towards constructing more complex model systems, which include different myelin proteins and lipid compositions. This 'artificial myelin' will thus help us to understand normal myelin structure as well as the factors that may induce demyelinating diseases.



A cross-section of a neuron myelin sheath

RESEARCH TEAM: Arne Raasakka, Anne Baumann and Petri Kursula (University of Bergen, Norway), Salla Ruskamo (University of Oulu, Finland), Robert Barker (University of Kent, UK) and Anne Martel (ILL)

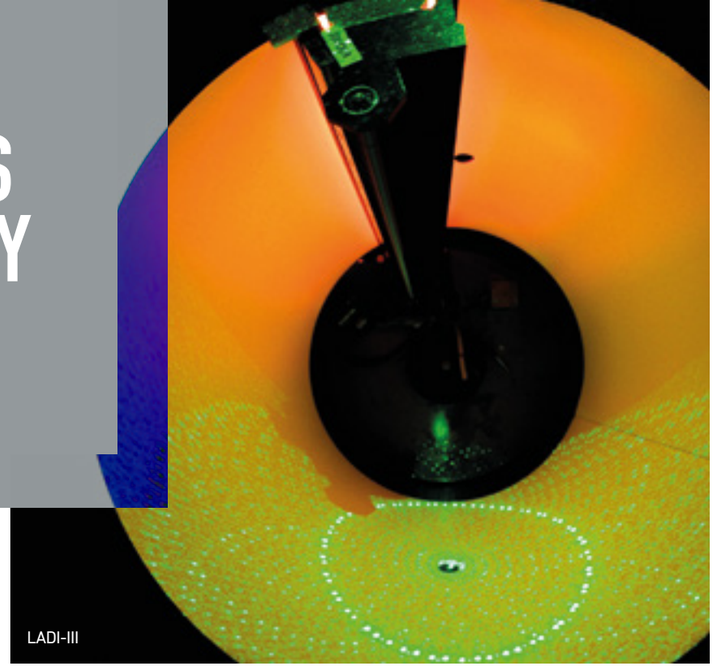
ILL INSTRUMENTS USED: small-angle scattering diffractometer D22 and reflectometer D17

REFERENCES: 1. A. Raasakka *et al., Sci. Rep.*, 2017, **7**, 4974. 2. S. Laulumaa *et al., PLoS ONE*, 2015, 10: e0128954.

IRON REVEALS ITS REACTIVITY IN PROTEINS

THE EXACT CHEMICAL NATURE OF THE IRON-CONTAINING ACTIVE SITES OF HAEM ENZYMES IS MADE CRYSTAL CLEAR

Emma Raven and Peter Moody

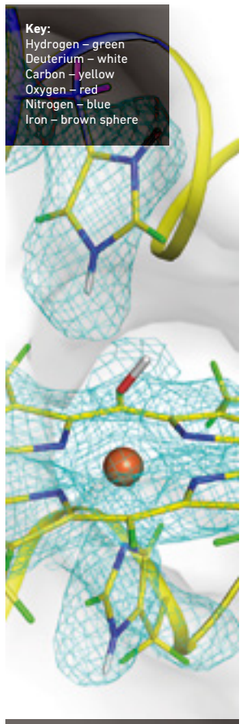


LADI-III

It is well known that we rely on iron-containing proteins to transport (haemoglobin) and store (myoglobin) oxygen. The iron atom sits at the centre of a flat cyclic molecular unit called haem, which forms a complex with the protein. Biology has also produced a wide range of haem-containing enzymes which take advantage of the fact that iron atoms can exist in variable oxidation states (by losing or gaining electrons) to catalyse a variety of processes involving electron transfer, notably the activation of oxygen (O_2). Such reactions have been well studied in two large classes of oxidising haem enzymes: the peroxidases and the cytochrome P450 family. The P450s transfer electrons via an O_2-dependent pathway, whereas the peroxidases utilise hydrogen peroxide (H_2O_2). The P450s fulfil many important physiological roles, including drug metabolism, whereas the haem peroxidases protect against hydrogen peroxide (H_2O_2) and other oxidative stresses in cells.

In both cases, the mechanism of oxygen activation involves the formation of two iconic intermediates: Compound I and Compound II, which contain an oxidised haem in which the iron is in the ferryl oxidation state, Fe(iv). When Compound I gains one electron (in the peroxidases) or abstracts a hydrogen atom from the substrate (in the P450s), it yields the closely related Compound II.

Nature uses both Compounds I and II for a large number of different (often difficult) oxidations in a diverse group of enzymes that also include the nitric oxide synthases, cytochrome *c* oxidases, and haem dioxygenases. Understanding the reactivity of these intermediates underpins many aspects of industrial biotechnology and drug design. All the major pharmaceutical and many biotechnology companies have a core interest in at least one, and often several, haem enzymes, as part of their drug-development programmes. For these reasons, understanding the precise nature and reactivity of these iron-containing intermediates is one of the most long-standing objectives in the field.



The neutron crystal structure of Compound II. The neutron scattering density is shown in cyan

A key question is the exact chemical state of the iron in the two oxidised intermediates. Is it simply bound to oxygen (Fe=O), or is the oxygen also bound to hydrogen (Fe–OH) – the protonated state? Answering this question has been the major objective of our work. Previous X-ray studies have not been able to differentiate unambiguously between the two forms, because they cannot directly visualise the positions of the hydrogens, and X-rays may, in fact, trigger a change in the oxidation state.

SEEING HYDROGENS CLEARLY

Our use of neutron crystallography overcomes these problems because neutrons can see hydrogens, particularly if they have been replaced with deuterium. We collected neutron crystallography data from Compound I (of cytochrome *c* peroxidase, CcP) and Compound II (of ascorbate peroxidase, APX). These intermediates had been 'frozen' in position by cooling them down to 100K. We could clearly see the positions of hydrogen atoms (as deuterium) in the active sites of Compound I and Compound II.

Compound I in CcP turned out to host the Fe=O species, while Compound II in APX it is Fe-OH. This, and the other details revealed regarding the immediate molecular environment in the two enzymes, has allowed us re-assess the enzymes' mechanism and has provoked further questions.

RESEARCH TEAM: Cecilia Casadei, Juliette Devos and Matthew Blakeley (ILL), Emma Raven (University of Bristol) and Peter Moody (University of Leicester), Andreas Ostermans and Tobias Schrader (Heinz Maier-Leibnitz Zentrum, Germany).

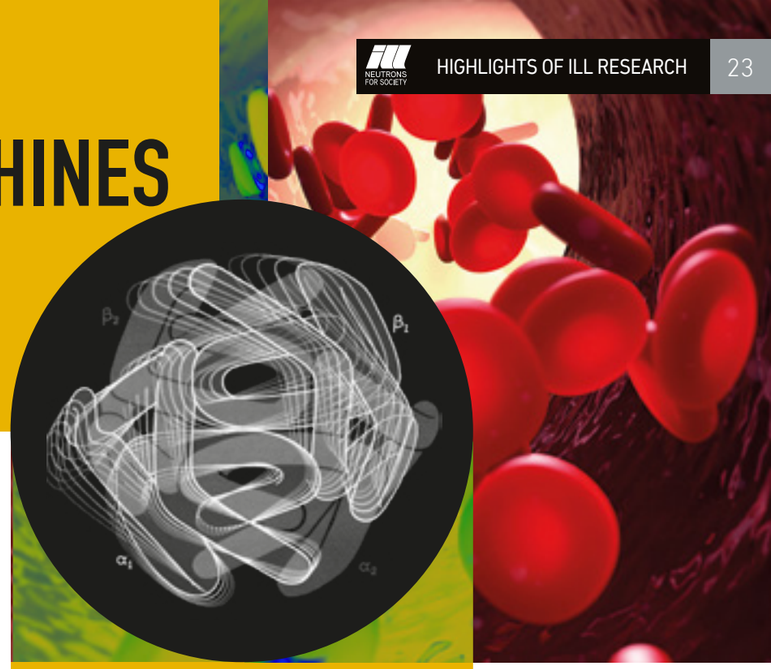
ILL FACILITY AND INSTRUMENT USED: D-Lab and quasi-Laue diffractometer LADI

REFERENCES: 1. C. Casadei *et al., Science,* 2014, **345**, 193. 2. H. Kwon *et al., Nature Communications,* 2016, **7**, 13445.

PROTEIN MACHINES IN MOTION

THE SUBTLE LARGE-SCALE MOVEMENTS WITHIN PROTEINS PLAY A MAJOR ROLE IN THEIR FUNCTIONS

Lee Makowski and Jyotsana Lal



A schematic representation of the structure and subdomains of the haemoglobin in motion

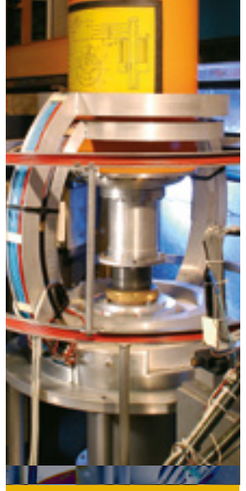
Proteins are the molecular machines of the cell, and to understand how they operate requires capturing the details of how they move. The dynamics of these large molecules involve both the rapid motions of the constituent amino acids and small chemical groups within or on the surface of the protein, and larger-scale, slower movements of particular domains within the protein. The movement of these domains is significant in that they most likely regulate the function of the protein, or interact with other proteins in carrying out an important biological function. Studying these slow, coordinated motions of a protein is much harder, and few biophysical tools are capable of doing it.

One of these tools is neutron spin echo, NSE (p31), which can probe slow correlated protein motions (including translational and rotational diffusion, and internal modes of motion) on the nano- to picosecond timescale. To test NSE's ability to follow internal protein motions, we decided to study the relative motions of the protein, haemoglobin. This is one of the best characterised of all proteins, and so is ideal for studying the relationships between the structure, dynamics, and function of a protein molecule.

Haemoglobin transports oxygen through the blood stream, with each molecule transporting up to four oxygen molecules. The efficiency of the process is aided by cooperation between the oxygen-binding sites in the protein. When a haemoglobin molecule binds one oxygen molecule, it becomes more likely to bind a second one, and on binding a second, it becomes even more likely to bind a third, and then a fourth.

HAEMOGLOBIN'S LITTLE HELPER

This cooperativity is aided by small molecules called allosteric effectors (p30) that regulate the affinity with which the binding happens. One such small molecule is inositol hexaphosphate (IHP), and we chose to investigate how it does its job so effectively. Since carbon monoxide binds haemoglobin more tightly than oxygen (which is why carbon monoxide can kill you quickly), it is easier to study the effect of IHP on carbon-monoxide binding. We therefore investigated the impact of IHP on the binding of carbon monoxide to haemoglobin, using a combination of NSE to measure the dynamics, and wide-angle X-ray solution scattering (WAXS) to analyse the structure.



IN11

The first thing we learnt was that IHP does not alter the structure of haemoglobin in any meaningful way. This was a shock, because if IHP does not change the structure of haemoglobin, how could it alter its binding affinity? We hypothesised that the critical variable must be the dynamics. We asked the question: "Can NSE detect a change in the motion of the subunits of haemoglobin in response to the binding of IHP?" The answer was a resounding "Yes". Interestingly, the binding of IHP lowered the affinity of haemoglobin for carbon monoxide while increasing the slow correlated motions of the subunits.

Why would an increase in dynamics lower the probability that carbon monoxide would bind to haemoglobin? One possibility is that when the protein is in motion, it will only occasionally be in a position to bind carbon monoxide, whereas when it is relatively stationary, it might take on a conformation that has a high affinity for carbon monoxide (or oxygen) much more often. This work not only illuminated our understanding of the regulatory power of allosteric effectors, it also provided support for the hypothesis that allosteric effectors might work by regulating the dynamic structural ensemble of a protein, as it appears to do when altering the affinity of oxygen for haemoglobin.

RESEARCH TEAM: Jyotsana Lal (Argonne National Laboratory, US), Marco Maccarini and Peter Fouquet (ILL), Nancy T. Ho and Chien Ho (Carnegie Mellon University, US), and Lee Makowski (Northeastern University, US)

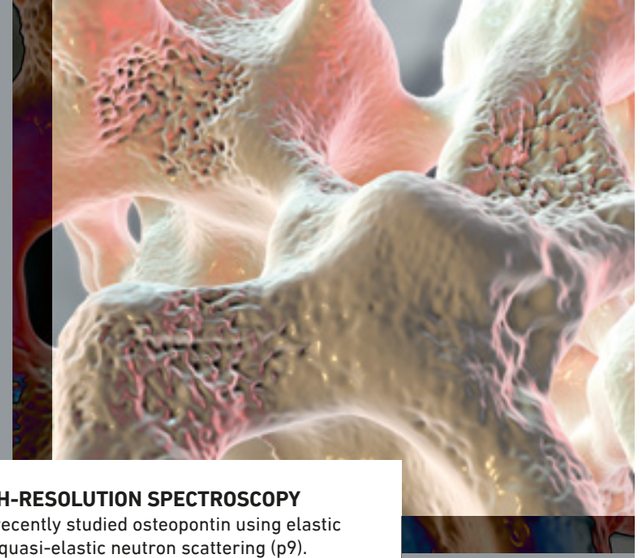
ILL INSTRUMENT USED: spin-echo spectrometer IN11

REFERENCE: J. Lal *et al., Protein Sci.*, 2017, **26**, 505.

THE DANCE OF DISORDERLY PROTEINS

THE CONFORMATIONAL DYNAMICS OF AN INTRINSICALLY DISORDERED PROTEIN REGULATES THE DEVELOPMENT OF BONE TISSUE

Tilo Seydel and Frank Schreiber



Bone tissue

The classical view of proteins is that it is their overall three-dimensional structure that determines their function. However, in recent decades, researchers have uncovered a large number of proteins that do not maintain a stable 3D form, but nevertheless contribute importantly to living processes and also may be significant in the development of disease. These so-called intrinsically disordered proteins (IDPs) fluctuate in conformation and often rely on so-called allosteric mechanisms – changes in their regulatory binding sites – for their function (p30). These dynamic mechanisms require long-range intramolecular communication, and so uncovering the associated dynamics may contribute to understanding this communication.

One class of IDPs include proteins that control the process of biomineralisation. A crucial part of this regulation involves the protein binding chemically to a phosphate unit (phosphorylation). In this way, for example, the protein, osteopontin (OPN), expressed in bones as well as in soft tissues, regulates the mineralisation of collagen and the build-up of calcium phosphate mineral via nucleation, growth, and precipitation. This IDP occurs as an extracellular matrix protein, lacking the secondary structure associated with protein folding (p31).

Osteopontin offers an ideal model for studying the dynamic mechanisms in an IDP, in particular the effects of phosphorylation, which modifies the protein's function, typically either activating or deactivating it in a fast and reversible process. Phosphorylation effects at various length- and timescales are paramount to IDPs. The majority of mammalian proteins are phosphorylated, with the phosphorylation sites frequently found in the disordered regions of the protein structure. Excess phosphorylation can cause subtle changes in conformational dynamics, leading to proteins misbehaving, as happens in diseases like Alzheimer's.

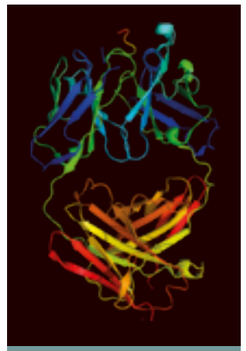
HIGH-RESOLUTION SPECTROSCOPY

We recently studied osteopontin using elastic and quasi-elastic neutron scattering (p9). Complementary small-angle X-ray scattering experiments did not have the power to discern any significant spatial changes between the protein's phosphorylated and unphosphorylated states. Thus, the structural study alone did not provide a conclusive picture of the effect of phosphorylation. In contrast, the neutron spectroscopy experiments revealed changes caused by phosphorylation, on the scale of pico- to nanoseconds, at the molecular level.

The data were recorded at different temperatures on both unphosphorylated and phosphorylated osteopontin in deuterated water. The presence of the water ensured that the proteins were dynamically active in their native state, and the deuteration ensured that the scattering signal from the water was negligible (through contrast matching, p8). This allowed the hydrogens in the protein to be clearly seen. We were thus able to identify the localised diffusive motions of the protein backbone and its side-chains, as well as obtain information on the geometry in which these motions were confined.

We found that the phosphorylation of OPN makes the side-chain motions on the picosecond scale faster, increasing their flexibility, but blocks the motions of some of the slower-moving side-chains occurring over the nanosecond scale. We assume that the faster picosecond motions could plausibly enhance the ability of the proteins to interact with surfaces of the bone material, and provide a potential advantage in interactions with so-called pre-nucleation calcium phosphate clusters. These clusters are picosecond-lived species present in supersaturated solutions of calcium phosphates and other salts.

This dynamic picture is important for the understanding of the function and efficiency of the OPN protein in contributing to bone formation. More generally, this study on the model intrinsically disordered protein, OPN, has established a framework for future studies of the entire class of IDPs.



The protein osteopontin regulates bone development

IN16B

RESEARCH TEAM: Samuel Lenton, Felix Roosen-Runge, and Tommy Nylander (Lund University, Sweden), Roger Clegg (Hannah Research Institute, UK), Carl Holt (University of Glasgow, UK), Victoria García Sakai (ISIS Neutron and Muon Source, UK), Marco Grimaldo, Michael Härtlein, and Tilo Seydel (ILL), Frank Schreiber (University of Tübingen, Germany), Susana C. Marujo Teixeira (NIST Center for Neutron Research, US)

ILL INSTRUMENT USED: backscattering spectrometer IN16B

REFERENCES: 1. S. Lenton *et al., Biophys. J.*, 2017, **112**, 1586. 2. Robin van der Lee *et al., Chem. Rev.*, 2014, **114**, 6589. 3. L. N. Johnson and D. Barford, *Annu. Rev. Biophys. Biomol. Struct.*, 1993, **22**, 199.

THE FLUCTUATING RIBOSOME

NEUTRON STUDIES REVEAL THE DIFFERENTIAL MOLECULAR DYNAMICS OF THE RIBOSOME'S TWO SUBUNITS, MOST LIKELY ASSOCIATED WITH THEIR RESPECTIVE ROLES IN MAKING PROTEINS

Joseph Zaccai

The 50S ribosome subunit from a salt-loving microorganism found in the Dead Sea

Ribosomes are molecular machines, found in the cells of all living organisms, which enable the genetic information (encoded in DNA and transcribed into messenger RNA) to be translated into the characteristic functional chain of amino acids constituting proteins. They consist of two subunits, made up of specific proteins and ribosomal RNA molecules that enable the formation of bonds between the amino acids, and protection of the growing protein chain. In bacteria, the subunits are labelled 50S and 30S. Understanding ribosomes is of paramount importance and urgency because of their fundamental role in living processes, and as favoured targets for the discovery of new antibiotic drugs – in the current context of increasing calamitous antibiotic resistance seen in pathogenic bacteria.

Electron microscopy and X-ray crystallography studies have determined that as the ribosome goes through its work cycle, it undergoes conformational changes, ranging from the fast reorientation of small groups of atoms to the slower movements of large domains in the structure.

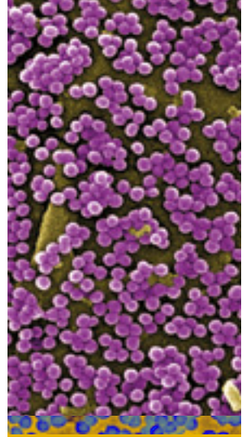
To gain a further detailed understanding of these dynamics requires unpicking the molecular forces that stabilise structures and allow motions across a range of timescales. Our work at the ILL has established neutron scattering as an appropriate tool for such studies. We found, for example, that proteins in bacteria from hot environments display the molecular motions necessary to function at high temperatures but are too stiff to move properly at lower temperatures. Similarly, proteins from bacteria living in our gut at 37°C display appropriate motions at around body temperature and become too soft to function at high temperatures, while proteins of bacteria from Arctic waters are already too soft to be active at 20°C! The study led to the proposition that it is through dynamics that evolution selects fitness to the environment.

NEUTRON BILLIARDS

The properties of neutron beams are perfectly adapted to provide information on molecular forces and the amount of motion amplitude. We can think of neutron scattering as a game of billiards; a ball of known velocity (the neutron) bounces off a group of balls held together by springs (atoms in a molecule). By repeating the experiment at different temperatures and noting the direction in which the neutron ball emerges from the fray and its final velocity, we calculate the strength of the springs holding the molecule together and the motion amplitudes of its atoms.

We applied neutron scattering to compare the dynamics in the 50S and 30S subunits of ribosomes from halophilic organisms (adapted to high salt) under their physiological conditions. Fast atomic motions were found to be similar in both subunits. On the slower timescale, however, the resilience (strength of the springs stabilising the structure) of the 30S was found to be significantly weaker than that of the 50S, with larger motion amplitudes, thus quantifying a softer, more flexible 30S structure.

These results throw further light on how the ribosome does its job to build proteins. In an oversimplified picture, messenger RNA coding for a specific gene binds to the 30S subunit, which subsequently links up to the 50S. A procession of transfer RNAs carries to the ribosome the specific amino acids coded on the messenger for their insertion into the protein sequence, which grows and exits the ribosome through a tunnel in the 50S subunit. When the protein chain is complete, the subunits fall apart ready to restart the process on another messenger RNA strand. The 30S and 50S subunits playing different roles during translation, the neutron results suggest that the higher flexibility of the 30S would facilitate the conformational adjustments required for binding of messenger and transfer RNA.



Antibiotic-resistant *Staphylococcus aureus* bacteria

RESEARCH TEAM: Joseph Zaccai (IBS and ILL), Francesca Natali (CNR and ILL), Jacques Ollivier (ILL) and Judith Peters (UGA and ILL)

ILL INSTRUMENTS USED: time-of-flight spectrometer IN5 and backscattering spectrometer IN16B

REFERENCE: G. Zaccai, F. Natali, J. Peters, M. Rihova, E. Zimmerman, J. Ollivier, J. Combet, M.C. Maurel, A. Bashan, A. Yonath, Sci Rep. 6 (2016)37138. doi:10.1038/srep37138

A MATCHED SET OF RADIOISOTOPES TO COMBAT CANCER

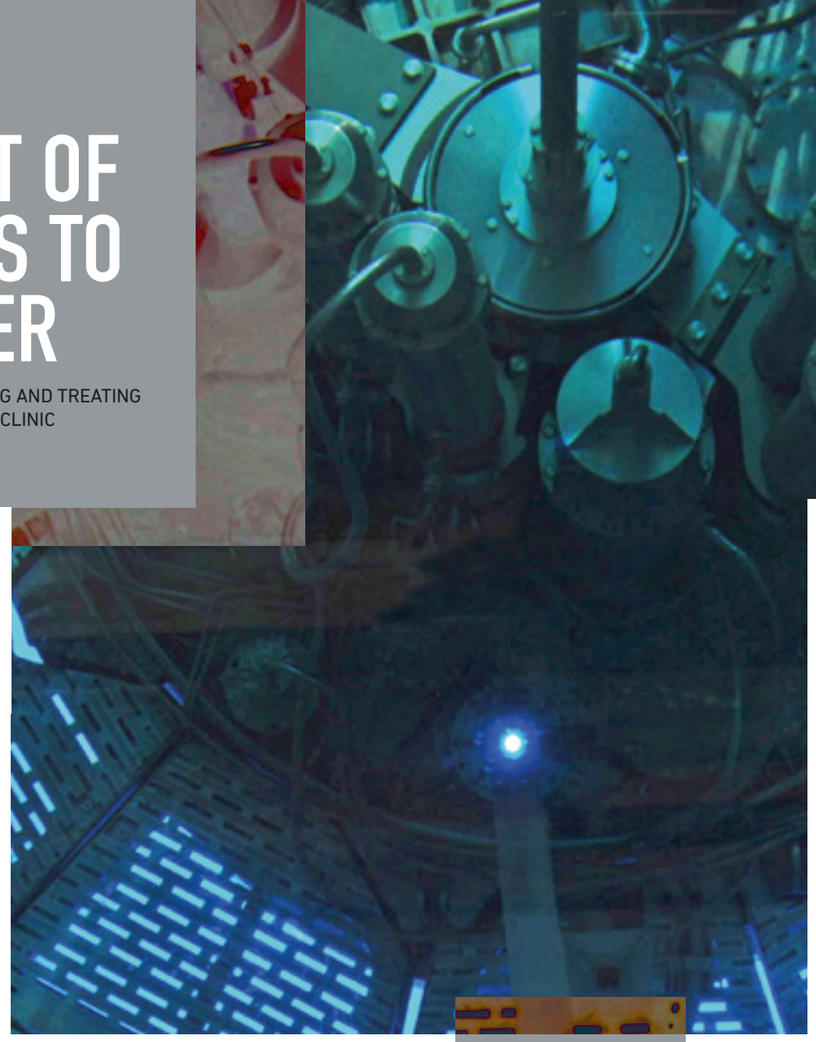
THE PRODUCTION OF TERBIUM ISOTOPES FOR IMAGING AND TREATING TUMOURS IS NOW BEING SCALED UP FOR USE IN THE CLINIC

Ulli Köster

Radioactive isotopes (p31) can be used 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 of the tumour or to kill the tumour cells.

Images of tumours are generated from the gamma-rays emitted by an isotope, which a 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 three-dimensional 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 tissue to release gamma-rays that are then detected. Both techniques are increasingly employed in the clinic.

Therapeutic applications depend on the targeted localisation of specific isotopes that emit electrons or 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 they 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 radioisotopes with more favourable characteristics – for example, causing less collateral damage to healthy tissue – are being developed. These include the radioisotope lutetium-177 – the present 'gold standard' for this kind of therapy.



A view of the ILL reactor from above: the bright blue spot below the centre marks the V4 beam tube where radioisotopes are produced by irradiation in a thermal neutron flux

THE TERBIUM FOUR

Radiotherapy increasingly involves directly combining imaging with therapy, so that the efficacy of the procedure can be monitored during the course of treatment. The element terbium offers a unique version of this approach because it has four isotopes – terbium-149, -152, -155 and -161, which together furnish a suite of radiation characteristics that provide excellent tumour visualisation and therapeutic efficacy.

Terbium-155 emits a spectrum of gamma-rays that can be used for SPECT at a relatively low radiation dose, while terbium-152 emits positrons ideal for PET. Terbium-161 emits low-energy electrons suitable for radiotherapy and also gamma-rays suitable for SPECT. Terbium-149 emits both alpha particles and positrons, and so could be used in so-called targeted alpha therapy while at the same time revealing where it is acting via PET.

Because the terbium isotopes all have the same chemical properties, they distribute in the body in the same way. This means that the same targeting vector can be used for all four isotopes. 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.

MAKING TERBIUM ISOTOPES

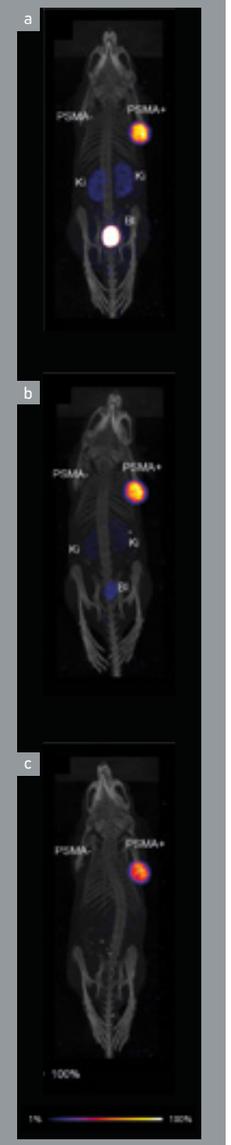
The ILL is collaborating in optimising the production of the terbium isotopes with the Paul Scherrer Institute (PSI) in Switzerland and ISOLDE, the nuclear physics accelerator facility at CERN in Geneva.

Terbium-161 is made by irradiating a target of gadolinium-160 with neutrons. This isotope then captures a neutron to produce gadolinium-161, which rapidly decays to terbium-161. Since natural gadolinium contains only 22 per cent of the target isotope, this has first to be enriched to more than 98 per cent abundance. After one to two weeks of irradiation, the resulting terbium-161 is transported to PSI, and 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 three lighter terbium isotopes are made at CERN-ISOLDE using a proton beam to break up nuclei in a tantalum-foil target, followed by so-called on-line mass separation of the isotopes of interest.

PRECLINICAL STUDIES

The combined radioisotope agents have now been tested in both tissue samples and in tumours (in mice). The tumours were imaged at different time points after injection, using PET (with terbium-152 and terbium-149) and SPECT (with terbium-155 and terbium-161). To test the efficacy of terbium-149 and -161 as tumour-killing agents, the tumour growth in cell cultures and mice was compared with that in untreated control groups. PET and SPECT provided clear images of the tumours for the terbium isotopes used in diagnostic mode. 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. In all experiments, both of the therapeutic isotopes caused a marked delay in tumour growth, or even complete remission.



SPECT images showing the uptake in a mouse tumour after: (a) 1 hour, (b) 4 hours, and (c) 24 hours after injection with the terbium-161–PSMA molecule

Reprinted from reference 6

Terbium-161, in particular, could offer an even better therapeutic alternative to lutetium-177, which is currently employed to treat tumours associated with the neuroendocrine system or metastasised prostate cancer. Each isotope is bound via a chelating agent called DOTA to a peptide molecule that recognises particular receptors on the cell surfaces of the corresponding tumours. To test the efficacy of the terbium radioisotopes, they were combined with different targeting vectors such as peptides, monoclonal antibodies and folate (vitamin B9) derivatives. Head-on comparisons were performed between terbium-161 and lutetium-177. These studies demonstrated indeed that terbium-161 showed higher efficacy against cancer cells than lutetium-177, without any additional damage to healthy tissue such as in the kidney.

These preclinical studies provide strong arguments for applications in the clinic. Terbium-152 produced at ISOLDE-CERN for PET imaging has already been trialled. After chemical separation at PSI, it was combined with a peptide that targets neuroendocrine tumour cells, and used to identify small metastases in a patient.

SCALING-UP PRODUCTION

Further development, however, requires the scaling-up of isotope production. The irradiation of gadolinium targets in high-flux reactors like the ILL, when combined with the optimised radiochemical separation process developed at PSI will ensure the regular availability of high-quality batches of reactor-produced terbium-161. The three lighter isotopes require an accelerator for production, and various studies are ongoing world-wide to make more of them. CERN has constructed a new facility called MEDICIS that uses 'waste protons' previously sent into a 'beam dump' but now serve to produce these valuable isotopes. With an improved supply of enriched gadolinium-155 and gadolinium-152 targets, they could be made routinely at selected accelerator centres. Both terbium-155 and -161 are long-lived enough to be shipped from a central facility to clinics worldwide. However, terbium-152 and -149 being shorter-lived, would require a network of facilities for regional supply.

RESEARCH TEAM: Cristina Müller and Nick van der Meulen (PSI, Switzerland), Ulli Köster (ILL) and Karl Johnston (ISOLDE/CERN, Switzerland).

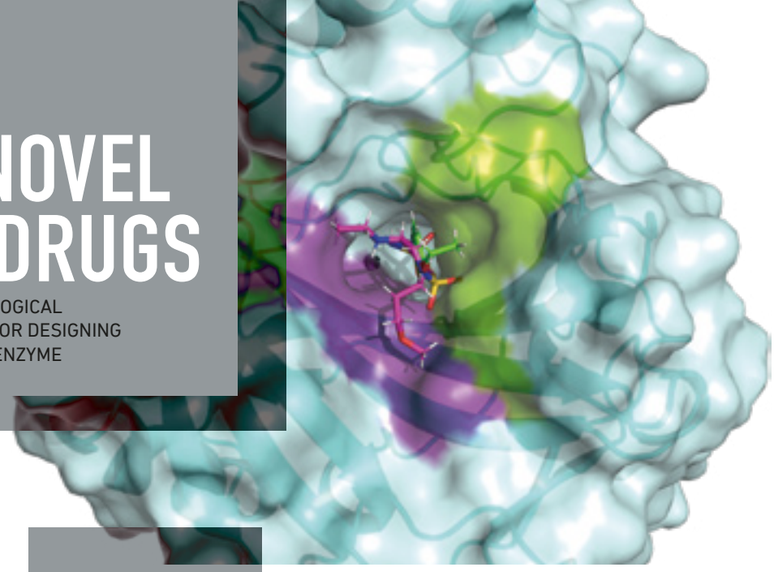
ILL FACILITY USED: V4 beam tube

REFERENCES: 1. C. Müller *et al., J. Nucl. Med.*, 2012, **53**, 1951. 2. R. P. Baum *et al., Dalton Trans.*, 2017, **46**, 14638. 3. C. Müller *et al., Nucl. Med. Biol.*, 2014, **41**, e58. 4. C. Müller *et al., EJNMMI Res.*, 2016, **6**, 35. 5. S. Haller *et al., EJNMMI Res.*, 2016, **6**, 13. 6. C. Müller *et al., Eur. J. Nucl. Med. Mol. Imaging* **46**, 1919, 2019, DOI: 10.1007/s00259-019-04345-0. 7. N. Gracheva *et al., EJNMMI Radiopharm. Chem.*, 2019, **4**, 12.

AIMING FOR NOVEL ANTICANCER DRUGS

NEUTRON CRYSTALLOGRAPHY AT NEAR-PHYSIOLOGICAL TEMPERATURES PROVIDES CRITICAL INSIGHTS FOR DESIGNING NEW DRUGS SPECIFIC FOR A CANCER-RELATED ENZYME

Andrey Kovalevsky



The 3D structure of human carbonic anhydrase (hCA II) and clinical inhibitors bound to the active site. The active site is flanked by hydrophilic (violet) and hydrophobic (green) binding pockets that can be used to design specific drugs targeting cancer-associated hCAs

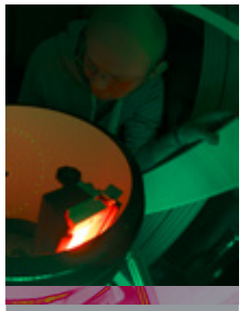
Credit: ORNL/Kovalevsky

Most therapeutic drugs are designed to bind as strongly and specifically as possible with a target biological macromolecule such as an enzyme, so that its biological function is inhibited and side-effects are kept to a minimum. The main workhorse of rational drug design has been X-ray crystallography, which is used to determine a drug target's overall three-dimensional structure; this is normally done at very low temperatures (–170 to –180°C). However, the crucial chemical and dynamical details of drug binding often remain hidden. For a complete picture of how drugs work, and to identify the optimum drug structure, requires not only revealing the positions of the hydrogen atoms in both target and drug structures but also how they behave at body temperature.

About half of all atoms in a biological macromolecule are hydrogens. They are key players in biochemical reactions and in intermolecular interactions, such as the weak hydrogen bonding (p30) that govern drug binding and enzyme function. The only technique capable of accurately determining the positions of hydrogen atoms, and visualising hydrogen bonds, is neutron crystallography (p8); it can be performed at room temperature (20°C), which is close to body temperature (37°C).

The power of neutron crystallography was demonstrated recently in a study that carefully examined the binding of three clinical drugs to the enzyme human carbonic anhydrase (hCA). Humans express 15 different versions, or isoforms, of this enzyme. Each isoform, though similar, has a specific biological function. Isoform II (hCA II) has long been a target for glaucoma, as well as diuretic, anti-epileptic and altitude-sickness drugs.

Recently, research efforts have shifted to the study of hCA IX and XII – the isoforms that can be targeted to treat various cancers with new, isoform-specific inhibitors. Nonetheless, hCA II is a prototypical carbonic anhydrase; accurate structures of this enzyme complexed with known drugs can inform the design of inhibitors aimed at hCA IX and XII.



LADI-III

ANOTHER HYDROGEN PLEASE

Using neutron diffraction at near-physiological temperatures, we obtained structures for hCA II complexed with sulfonamide drugs, brinzolamide, dorzolamide, and ethoxzolamide, which are clinically used to treat glaucoma. The data, collected at high resolution (0.18-0.20 nm), allowed us to locate the hydrogen atoms and visualise the critically important intermolecular interactions. Particular observations of where hydrogen atoms are located led us to conclude that introducing extra hydrogens into a drug's chemical structure could translate into the formation of additional hydrogen bonds that would improve the drug's binding characteristics.

Additionally, our research revealed the importance of studying drug binding at close to body temperatures. Previous low-temperature X-ray studies had shown that a long flexible 'tail' of the brinzolamide molecule binds in a pocket inside the hCA II enzyme, called the hydrophobic site. In contrast, the neutron structure at room temperature of the same complex revealed that the tail moves into the site opposite, called the hydrophilic pocket. These observations prompted us to carry out computer simulations in order to understand why the drug-binding is dependent on temperature. The simulations demonstrated that at room temperature the flexible tail is free enough to move into the hydrophilic pocket, but it remains frozen near the hydrophilic pocket at low temperatures. The hydrophobic pocket in hCA IX (and XII) is more dissimilar to that in hCA II than are their hydrophilic pockets, so it is therefore a good target for redesigning inhibitors and make them bind more specifically to hCA IX.

These results not only help improve the design of isoform-specific hCA drugs, but also revealed the importance of studying enzyme–drug interactions at physiological temperatures, rather than relying entirely on low-temperature structures.

RESEARCH TEAM: Andrey Kovalevsky, Mayank Aggarwal, Hector Velazquez, Matthew J. Cuneo and Kevin L. Weiss (Oak Ridge National Laboratory, US), Jeremy C. Smith (University of Tennessee, Knoxville), S. Zoe Fisher (European Spallation Source), Robert McKenna (University of Florida) and Matthew Blakeley (ILL)

ILL INSTRUMENT USED: quasi-Laue diffractometer LADI-III

REFERENCE: A. Kovalevsky *et al.*, *Structure*, 2018, **26**, 383.

COMMUNICATION UNIT
Institut Laue-Langevin
71 avenue des Martyres
BP 156
38042 Grenoble Cedex 9
France
Tel: 33 47620 7179
Email: communication@ill.eu
URL: www.ill.eu

CONTRIBUTORS

PAGE 10
MEMBRANE PROTEINS TARGETED
Sylvain Prévost
ILL
Email: prevost@ill.fr
Tel: 33 47620 7943

Inokentijs Josts
Universität Hamburg,
Luruper Chaussee 149
22761 Hamburg
Germany
Email: josts@chemie.uni-hamburg.de

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Martine Moulin
ILL
Email: moulinm@ill.fr
Tel: 33 47620 7168

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A NEW TOOL TO IDENTIFY A HEPATITIS C DRUG
Thomas Soranzo
Université Grenoble Alpes
621 avenue Centrale
38400 Saint-Martin-d'Hères
FRANCE
Email: thomas.soranzo@univ-grenoble-alpes.fr
Tel: 33 77805 1582

PAGE 13
WATCHING A PROTEIN UNFOLD
Frank Gabel
Institut de Biologie Structurale (IBS)
71 avenue des Martyrs
F - 38044 Grenoble Cedex 9
France
Email: frank.gabel@ibs.fr
Tel: 33 45742 8573

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A BETTER UNDERSTANDING OF TYPE 2 DIABETES
Anne Martel
ILL
Email: martela@ill.fr
Tel: 33 47620 7239

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Ecliptique/L. Thion (p2-8-12-14-24)

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INSIGHTS INTO METABOLIC DISORDERS
Gianpiero Garau
Center for Nanotechnology Innovation@NEST
Istituto Italiano di Tecnologia
Piazza San Silvestro 12, 56124 Pisa
Italy.
Email: gianpiero.garau@iit.it
Tel: 39 050 509379

Francesca Natali
ILL
Email: natali@ill.fr
Tel: 33 47620 7071

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UNCOVERING THE CAUSES OF ALZHEIMER'S
Valeria Rondelli
Università degli Studi di Milano
Via Fratelli Cervi, 93
20090 SEGRATE (MI)
Italy
Email: valeria.rondelli@unimi.it
Tel: 39 02503 30323

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Bruno Demé
ILL
Email: deme@ill.fr
Tel: 33 47620 7311

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Trevor Forsyth
ILL
Email: tforsyth@ill.fr
Tel: 33 47620 7158

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Bruno Demé
(as above)

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Petri Kursula
University of Bergen
Postboks 7804, N-5020 Bergen
Norway
Email: petri.kursula@uib.no
Tel: 47 5558 0000

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Petri Kursula
(as above)

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Emma Raven
University of Bristol,
Cantock's Close, Bristol BS8 1TS
UK
Email: emma.raven@bristol.ac.uk
Tel: 44 117 928 7657

Peter Moody
University of Leicester
Lancaster Rd LE1 7HB
UK
Email: peter.moody@le.ac.uk
Tel: 44 116 229 7097

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PROTEIN MACHINES IN MOTION
Lee Makowski
Northeastern University
206 ISEC (ISEC)
Boston, MA 02115
US
Email: l.makowski@northeastern.edu
Tel: 1 617 373 3006

Jyotsana Lal
Argonne National Laboratory
9700 Cass Avenue, Lemont IL 60439
US
Email: jlal@anl.gov
Tel: 1 630 252 2000

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THE DANCE OF DISORDERLY PROTEINS
Tilo Seydel
ILL
Email: seydel@ill.fr
Tel: 33 47620 7579

Frank Schreiber
University of Tübingen, Germany
Geschwister-Scholl-Platz
72074 Tübingen
Germany
Email: frank.schreiber@uni-tuebingen.de
Tel: 49 7071 2976058

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Joseph Zaccai
ILL
Email: zaccai@ill.fr

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Ulli Koester
ILL
Email: koester@ill.fr
Tel: 33 47620 7154

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Andrey Kovalevsky
Oak Ridge National Laboratory
1 Bethel Valley Rd
Oak Ridge, TN 37831
US
Email: kovalevskyay@ornl.gov
Tel: 1 505 310 4184

Active site/pocket A cleft or pocket in the three-dimensional structure of an enzyme where the chemical reaction that it catalyses is carried out.

Allosteric effector A molecule that controls the activity of an enzyme by binding at a site away from the enzyme's active site.

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

Amine group A chemical group consisting of a nitrogen atom bound to two hydrogen atoms. It is a key constituent of amino acids and proteins.

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 misfolded proteins called amyloid fibrils, which form plaque-like aggregates that damage body tissues.

Amyloid fibril/aggregate See above.

ATP (adenosine triphosphate) The molecule that captures and transfers chemical energy needed to drive processes in the living cell.

Axon The long thin projection of a nerve cell that conducts information via electrical impulses by connecting with the axon of another neuron, as part of a network.

Beta cell The cell that secretes insulin in the pancreas.

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

Biomineralisation The molecular process by which organisms synthesise minerals such as those constituting bones and shells.

Cell-free expression A laboratory method of producing a required protein as it would be made in a living cell, but not in a cellular environment. The method has many technical applications and may be used to produce deuterated proteins.

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

Cell signalling The essential processes by which information – chemical, electrical or mechanical – is transferred within a cell and to other cells.

Chemical group A group of chemically bound atoms that have a characteristic chemical behaviour (see 'Amine' above).

Cholesterol A type of sterol (a lipid) that is an essential component of cell membranes in vertebrates, and which is heavily implicated in atherosclerosis.

Conformation Different forms (or shapes) of a molecule, such as a protein, generated by constituent atomic groupings rotating about single bonds within the molecule. The specific conformation adopted may depend on the immediate environment.

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

Contrast variation A technique by which the visibility of specific molecular components is altered – usually by adjusting the light/heavy-water composition of the solvent. Contrast matching (above) occurs when the variation of contrast renders a specific component invisible. The component is then said to be matched out.

Crystal A solid material consisting of a regular three-dimensional array of atoms or (macro)molecules.

Detergent A water-soluble cleansing agent which combines with impurities and dirt to make them more soluble.

Deuteration The replacement of a hydrogen atom in a molecule by its isotope, deuterium (see below) – also called deuterium labelling.

Deuterium A stable heavy isotope of hydrogen having a neutron as well as a proton in the nucleus.

Dipole A pair of interconnected but separated positive and negative charges.

Elastic incoherent neutron scattering (EINS) A method for determining structure by measuring the change in direction of scattered neutrons. Neutrons are scattered by the atomic nuclei in the sample, the phase is not conserved and the neutrons retain all of their kinetic energy during collisions.

Electron An elementary particle, which is an important constituent of atoms. Unstable nuclei may emit electrons, and this is called beta radiation.

Endocrine system A complex set of interacting glands in the body which secrete hormones (see below) that play a crucial role in basic functions, including metabolism, reproduction, and development.

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

Extremophile An organism that lives in extreme environments such as at high or low temperatures, under high pressure, or in highly acidic or saline conditions.

Fluorescence The emission of visible light by a substance after it has absorbed higher-energy electromagnetic radiation.

Glycolipid A lipid molecule with a carbohydrate unit attached.

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

Heavy water (D2O) Water in which hydrogen has been replaced by its heavier isotope deuterium.

Hormone A class of signalling molecules that are transported in the circulatory systems (eg blood) of organisms, and that are central to the operation of the endocrine system.

Hydrogen atom The simplest atom consisting of one proton and one electron.

Hydrogen bonding A weak form of bonding in which the proton of a hydrogen atom is electrostatically attracted to atoms such as oxygen or 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, giving information about their motion and flexibility. (When a neutron is scattered elastically, there is no energy transfer.)

Hydroxyl group A chemical group consisting of an oxygen atom bound to a hydrogen atom (OH).

Inhibitor. In the context of biology, an inhibitor molecule binds to an enzyme and blocks its action. Many drugs are enzyme inhibitors.

Interface The boundary between two phases of matter.

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

Ion channel A protein sitting in a cell membrane that acts a pore controlling the flow of ions across the membrane.

Isotope Chemical elements characterised by the same number of protons but different number of neutrons in the nucleus. Hydrogen and deuterium are isotopes.

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.

Macromolecule A large molecule that may consist of smaller sub-units.

Mass spectrometry A technique for determining the mass of a molecule by breaking it up into charged fragments and then measuring their mass-to-charge ratios.

Micelle Small organised spherical structures of detergent or other amphiphilic molecules dispersed in a liquid.

Molecular dynamics (MD) A computer simulation technique that can follow the motions of atoms and molecules over a given time.

Myelin The insulating layer around nerve axons that speeds up the transmission of electrical impulses.

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

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

Neutron crystallography A neutron diffraction method for the study of crystals. A single crystal is oriented over a range of angles so that data can be recorded from different planes in the crystal It gives important information on molecular structure. The method is complementary to X-ray crystallography.

Neutron diffraction Neutrons can be reflected, or scattered, from a material in which the interatomic distances are similar to the neutron wavelength. The scattered waves interfere to produce a characteristic diffraction pattern.

Neutron reflectivity, neutron reflectometry (NR) A technique in which neutrons are reflected off a surface or interface. It is used to characterise the structure of surfaces and thin layers.

Neutron spin echo spectroscopy 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 magnetic resonance (NMR) An analytical technique that can be used to probe molecular conformation in solutions or in the solid state. It is complementary to X-ray and neutron diffraction in the information it provides.

Oligomer A molecule composed of a small number of repeating chemical units.

Oxidation state The numerical assignment indicating the number of electrons an atom has lost or gained, and thus becomes charged, when it is in a bound chemical state.

Peptide A chain of amino acids, each linked by an amide bond, –C(=O)–NH–.

Phospholipid A class of lipids containing a phosphate group.

Phosphorylation The addition of a phosphoryl group (PO_3–) to a molecule; it is an important biochemical process in cells.

Picosecond One-trillionth of a second (10^{-12}s).

Polar group A molecular group of atoms that has an inherent distribution of positive and negative charge.

Polymer A molecule comprising repeating molecular units (monomers) usually in long chains.

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

Protein One or more long chains of a specific sequence of amino acids. Generally, proteins fold into a unique three-dimensional structure having a particular biological function.

Protein folding The process by which most protein molecules assemble into a distinctive three-dimensional shape that relates directly to its biological function.

Protein mutant A specific protein that has been formed with one or more amino-acids changed.

Proteolysis The breakdown of proteins into smaller amino-acid chains – often by enzymes known as proteases.

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^+).

Protonation The addition of a proton (H^+) to a molecule.

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

Ribosome A large molecular machine unit made of protein and RNA, whose main function is to synthesise proteins according to the genetic information provided by messenger RNA (mRNA).

RNA A chain-like molecule similar to DNA, but generally single-stranded. Its main function is to pass on the genetic information from DNA such that the ribosome can synthesise the correct amino-acid sequence in a protein.

Saturated lipid A lipid that has no double carbon–carbon bonds in its molecular structure.

Small-angle neutron scattering (SANS) The measurement of neutron scattering at small angles. For the study of biological systems, it is usually used to investigate structures such as proteins at relatively low resolution and often, in conjunction with contrast variation and deuteration.

Small-angle X-ray scattering (SAXS) X-ray scattering carried out at small angles. It is complementary to SANS.

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

Substrate A molecule upon which an enzyme acts.

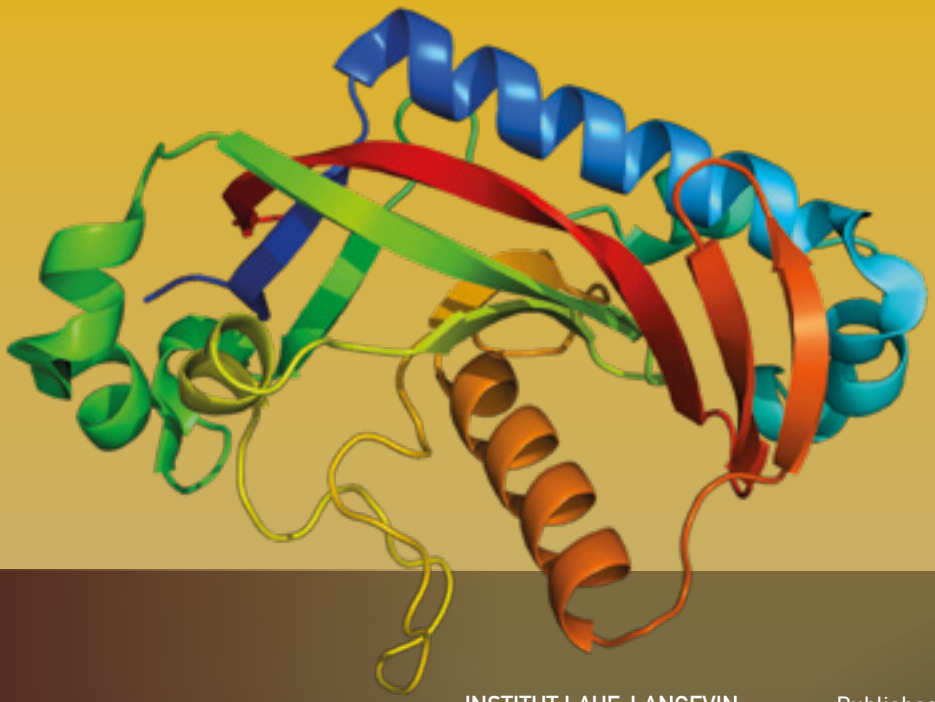
Surfactant A surface-active (surfactant) molecule containing both hydrophilic and hydrophobic regions

Synchrotron radiation The electromagnetic radiation emitted when charged particles such as electrons are accelerated in a path that changes direction. Accelerators called synchrotrons are often used to produce high-intensity X-ray beams for experiments that complement those performed using neutron beams.

Unsaturated lipid A lipid that contains double carbon–carbon bonds in its molecular structure.

Wide-angle neutron scattering The measurement of neutron scattering at large angles. It is used to investigate structures at the atomic and molecular levels.

X-ray diffraction A technique used to determine the structure of materials such as crystals, fibres, and solutions.



INSTITUT LAUE-LANGEVIN
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France

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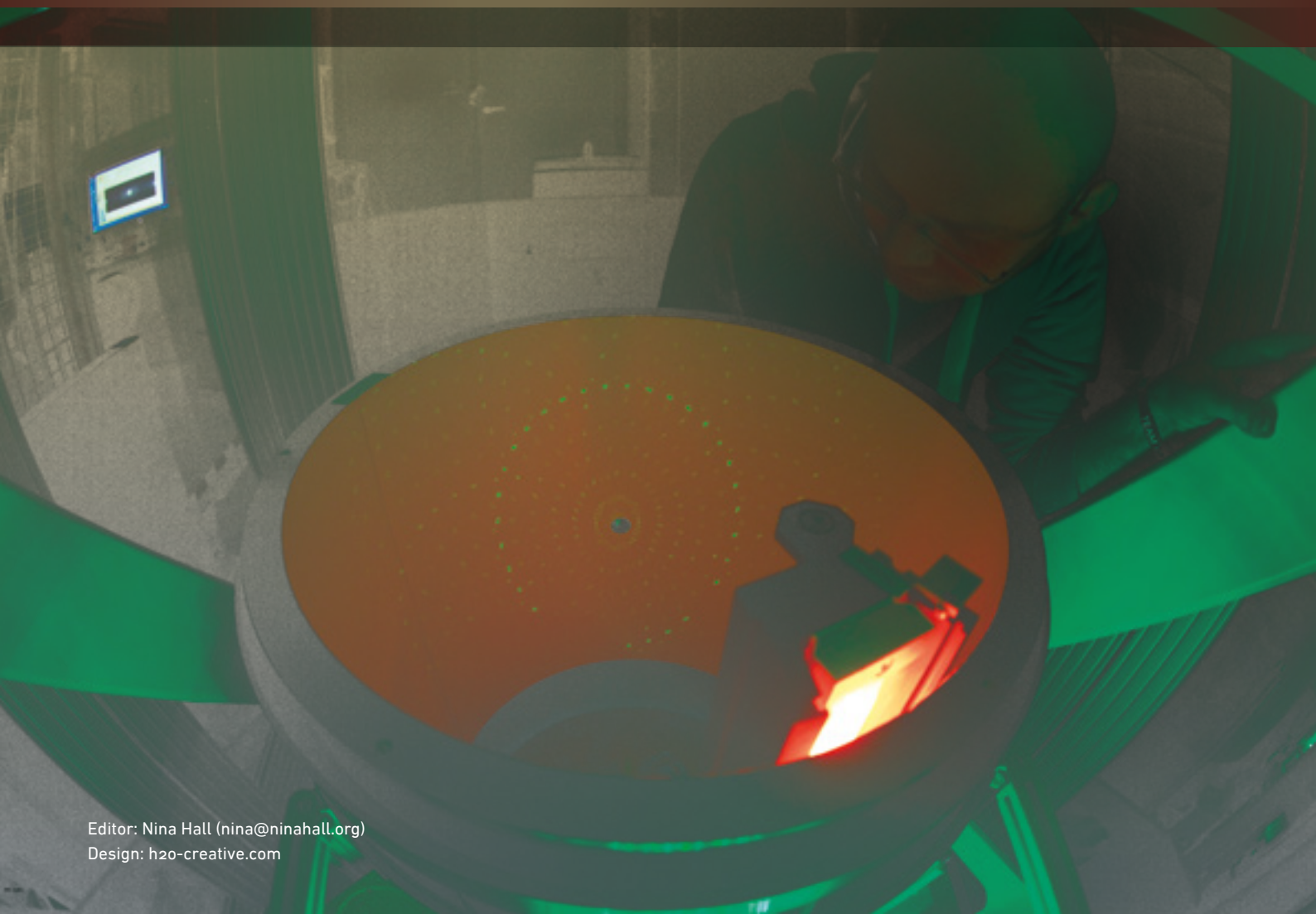

Editor: Nina Hall (nina@ninahall.org)
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