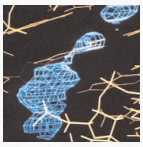
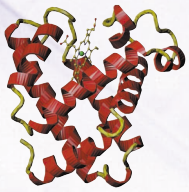
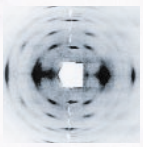
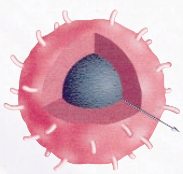


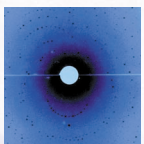
Neutrons and life

A review of biological research at the ILL









NEUTRONS FOR SCIENCE

This booklet is the first of a series devoted to the application of neutron techniques in different research areas. The next one, to be published in 2002, will focus on the use of neutrons to determine the structure of new materials.

Foreword

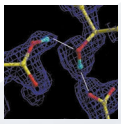
Our understanding of living processes at the molecular level continues to make extraordinary progress, thanks to a battery of complementary analytical techniques, of which neutron scattering methods developed at the ILL are a growing component. Today, biologists from laboratories across Europe are increasingly exploiting the capabilities offered by the ILL facilities to decipher the structure of large biological molecules such as proteins, how they are arranged in complex assemblies and how they carry out their functions in the cell. With the recent completion of the project to map the human genome, these investigations are expected to expand radically, and potentially will have an enormous impact on our daily lives – particularly health.

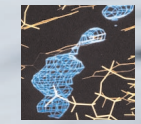
While it is important to emphasise that neutron scattering is only one of many techniques needed in the post-genome era, it has been recognised that ILL's participation in biological studies should be expanded for the benefit of the life-sciences research community. To realise this objective, the ILL management has launched a vigorous in-house programme of biological research, in parallel with an ambitious instrument and infrastructure renewal strategy.

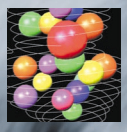
The success of these projects, however, will depend a great deal on how effectively we can demonstrate the wide impact of neutron techniques on the life sciences. With this in mind, we have decided to publicise more widely the successes of researchers exploiting ILL facilities. This booklet *Neutrons and life*, which is the first in a new series of publications highlighting work at the ILL, focuses on recent significant developments in biological research.

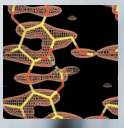
I am confident that this presentation of achievements at ILL will be persuasive in illustrating the essential role of neutron methods in the study of life. □

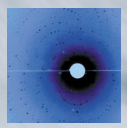
Dr Christian Vettier
Associate Director

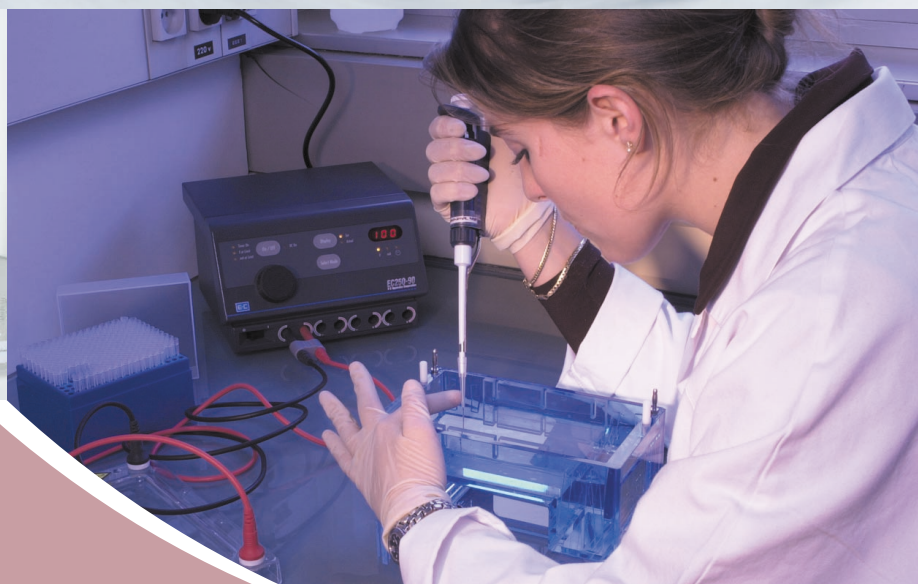












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Neutron scattering is a diverse technique which is becoming increasingly important in understanding living processes

Introduction

Peter Timmins
Head of the Large Scale Structures Group

All living organisms are made up of cells. Understanding how they work at the molecular level is one of the main aims of modern biology. One of the most dramatic advances over the past two decades has been the unravelling of the genetic code which controls the molecular machinery of cells. We now know the sequence of 'letters' that make up the genes in the human genome – as well as those of many other species, from the mouse to worms to bacteria.

Today, we are moving into an exciting 'post-genomic' era in which this genetic sequencing information is being harnessed to untangle the complex and diverse chemistry of cellular processes. The human genome is believed to specify between 40,000 and 100,000 different proteins. Each protein does a particular job – some, known as enzymes, mediate chemical reactions, others form important structures like skin and muscle, and yet others may be involved in trapping energy, for instance, harvesting light in plants. Very often the proteins do not act alone but as components of complex interactive molecular assemblies containing other proteins or other large molecules, such as DNA or RNA, sugars and fats. For example, cell membranes are made of fat (lipids) with protein molecules embedded in them.

The importance of structure

A real understanding of life's essential processes requires a knowledge of how all these proteins, and other large molecules, perform their roles. It is well-known that their structure – the exact arrangement of atoms – and the overall shape is directly related to their function. However, it is now becoming clear that another characteristic is also important. Biological molecules may not be rigid but soft and malleable, and dynamic changes within a molecular structure may play a significant part in correct functioning.

The molecular biology of the cell is thus very complex, and determining the structure and dynamics of all the molecules and molecular complexes that go to make it up is essential to understanding life's message. This also has clear practical applications – in the treatment of illness. Most diseases result from cellular processes going wrong at the molecular level. Viruses invade cells and take over their machinery, eventually killing the cells. Bacteria sometimes release toxins which are molecules that may block the action of our own enzymes.

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

The advantages of neutron scattering

Neutrons are subatomic particles, that along with protons, make up the nuclei of atoms. They are produced by either breaking up nuclei (fission) or by knocking them out of nuclei (spallation). The ILL's nuclear reactor employs fission to produce the most intense beams of neutrons in the world.

How can neutrons tell us anything about structure and biology? Well, neutrons also behave as waves like visible light or X-rays. Whereas light usually just scatters off the surface of an object to reveal a visual image, X-rays and neutrons have enough energy to penetrate the structure and scatter off the internal arrays of atoms. As the waves are reflected they interfere with each other (a process sometimes called diffraction), like ripples meeting on the surface of a pool, and when detected they produce a pattern characteristic of the arrangement of atoms that allows us to analyse molecular structure.

The scattering process for neutrons and X-rays is different, however. X-rays are scattered by the electrons in atoms whereas neutrons are scattered by the atomic nuclei. The magnitudes of the waves produced are very different and are therefore used to investigate different aspects of structure. The wavelength of neutrons makes them suitable for investigating molecules of sizes

ranging from about 1 to 100 nanometres (a nanometre is one-billionth of a metre).

In biology, the main objects of study are quite large molecules such as proteins, nucleic acids, lipids or polysaccharides (carbohydrates) which can be of the order of 1 to 10 nanometres in size. One variation of the neutron technique which involves scattering neutrons at very small angles (small angle neutron scattering, or SANS) can be used to resolve larger, intermolecular distances, say, in a complex of several molecules such as viruses, which contain proteins and nucleic acids, or cell membranes made of lipids and the occasional protein.

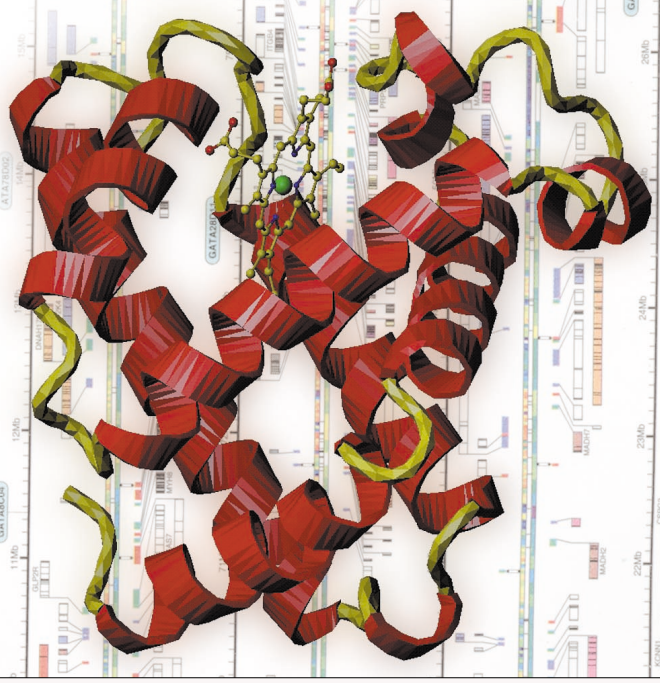
Another important characteristic of neutrons is their scattering power, which depends on the exact structure of the target atom. An oxygen atom, for example, scatters with about two-thirds the power of a nitrogen atom. A hydrogen atom scatters about half as much again but in an opposite sense, while deuterium, a heavy form of hydrogen, scatters as strongly as oxygen. We therefore often use biochemical tricks to exchange hydrogen in a molecule for deuterium in order to see it more clearly.

Substitution with deuterium

This is the basis of an ingenious technique called contrast variation where we can 'highlight' parts of a structure by replacing the constituent hydrogen atoms with the stronger-scattering deuterium. Since biological molecules are best studied in water (so as to mimic their natural environment), they are most easily seen if they scatter much more strongly than the surrounding water or vice versa. We can go even further – increasing the relative contrast between different structural components by judicious proportional mixing of ordinary and heavy water (in which the hydrogen atoms have been substituted with deuterium) such that the scattering power of the water matches that of one of the components which then is rendered invisible (see pages 7 and 18).

Another important characteristic of neutrons is that they can be scattered in different ways. Instead of elastically bouncing off an atom like a billiard ball, they may lose or gain energy to or from the atom, which will itself change its motion. This is called inelastic scattering and gives information about the motions of the atoms. 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 from biological molecules is a rather new technique requiring sophisticated neutron instruments and advanced computing facilities to understand the results.

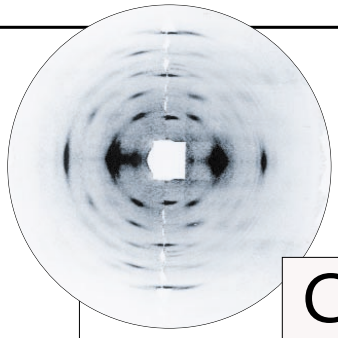
One of the beauties of neutron scattering is that there are so many variations on the technique which are continually being developed. There is, for example, crystallography for the study of molecules in atomic detail in crystalline form, and also reflectometry for looking at the structure of surfaces such as cell membranes. The research results described in the following articles have been obtained using these and other neutron methods, and illustrate the wide range of biological problems that are being tackled at the ILL. □



Neutron diffraction offers an excellent way to study the intricate bonding in natural polymers like cellulose

Why do trees stand up?

>> HENRI CHANZY, PAUL LANGAN and YOSHIHARU NISHIYAMA



A comparison of the two diffraction patterns of cellulose from normal and deuterated cellulose

PhotoDisc

Cellulose is probably the most abundant polymer on Earth. Occurring as slender crystalline microfibrils, it is certainly one of the most important structural elements in plants, and without it trees could not grow to the height they do.

One of the key features of this polysaccharide is that each of the constituent molecular units bears three hydroxyl (OH) groups. It is these hydroxyl groups and their ability to bond via weak 'hydrogen bonding' that not only play a major role in directing how the crystal structure of cellulose forms but also in governing important physical properties of cellulose materials such as strength.

Cellulose can exist in a number of different forms. Natural cellulose is known as cellulose I, but other forms such as cellulose II, cellulose III and cellulose IV have been described. We are involved in a long-term study at ILL to unravel the fine details of these different forms.

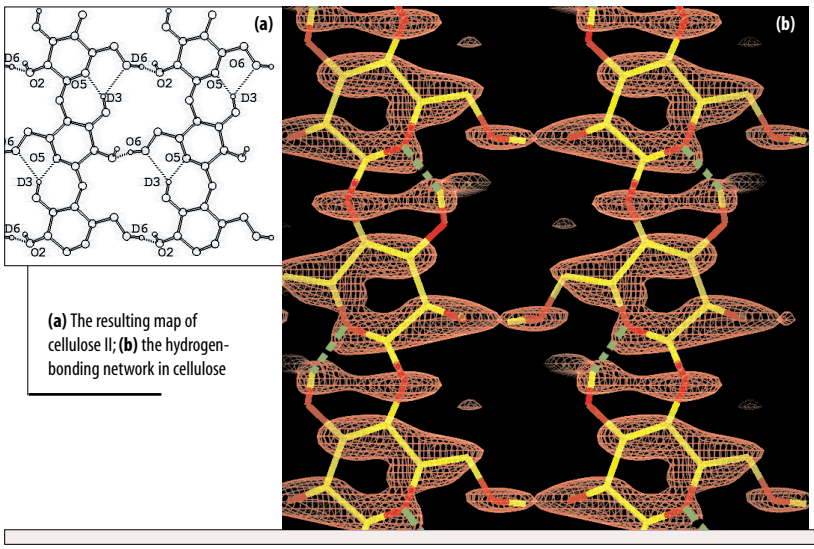
Importance of hydrogen bonding

Neutron diffraction is the only method that offers a detailed picture of the hydrogen bonding network. The experiments were carried out on an instrument called D19. This is the only instrument in the world that can carry out this sort of study (it has also been used to carry out work on other biological molecules such as other polysaccharides, DNA and filamentous viruses).

The idea behind the experiment is simple. Neutrons are fired at the sample of cellulose and the pattern of neutrons scattered by the sample is recorded. This pattern can then be used to determine the structure of the cellulose. Since hydrogen atoms cause significant scattering of neutrons, their position in the cellulose can be determined as well. To be absolutely sure of the process, crystalline fibres were also prepared in which the hydrogen atoms in all the OH groups were replaced by deuterium (which scatters neutrons even more strongly than hydrogen does) and the same diffraction experiment repeated.

Recently we studied cellulose II fibres that result from the swelling of fibres in concentrated sodium hydroxide. There were two suggested model structures. Studies employing X-ray crystallography could not distinguish between the two models, but using our neutron diffraction data we were able to decide which of the models was correct.

The picture (top left) shows the diffraction patterns recorded from cellulose II. The left half of the picture shows the diffraction pattern recorded from normal cellulose and the right half shows that from deuterated cellulose (each picture has been cut in half and they are shown side by side for comparison). The differences between these two patterns arise from the hydrogen/deuterium atoms alone. These patterns were used to calculate maps that show where the hydrogen atoms are located. The figure (a), left, shows the map, and figure (b) shows the hydrogen bonding network in cellulose that was determined from it. This study is the first three-dimensional description of a hydrogen bonding system in a fibrous polysaccharide. ■



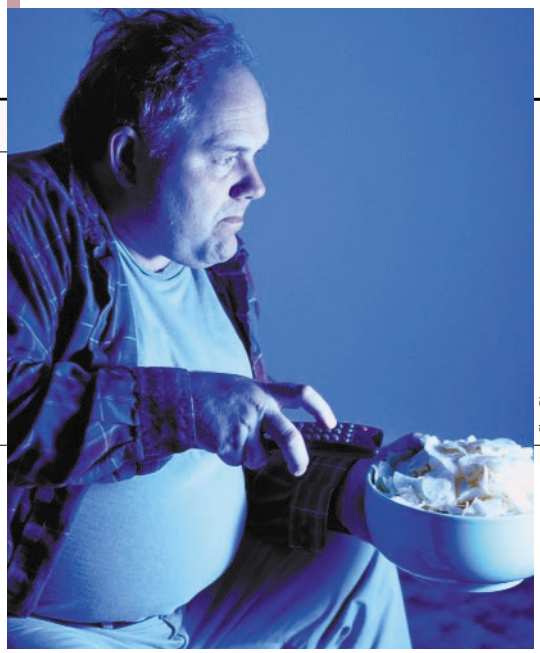
(a) The resulting map of cellulose II; **(b)** the hydrogen-bonding network in cellulose

Research team H. Chanzy *(CERMAV-CNRS, Grenoble)*, Y. Nishiyama *(Univ. Tokyo)*, P. Langan *(Los Alamos National Laboratory)*, T. Forsyth *(ILL)*

Neutrons help fight obesity

Studies revealing the shape and structure of a molecular complex involved in fat digestion could lead to new slimming drugs

>> DAVID PIGNOL and PETER TIMMINS



PhotoDisc

Mammals, including humans, digest food with secretions produced in several organs along the digestive tract – the salivary glands in the mouth, the stomach, the gall bladder, the pancreas and the intestine. The secretions contain enzymes which break down the complex food molecules – proteins, fats and carbohydrates – into simpler units that can then be absorbed into the bloodstream.

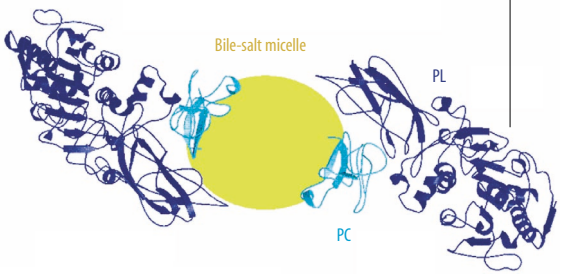
We are particularly interested in the way fats are broken down. The reason is that the process is quite complicated. Ninety-five per cent of fats in the Western diet consist of molecules known as triglycerides which, because they do not dissolve in water, exist in the form of oily droplets. To be digested, an enzyme produced by the pancreas called pancreatic lipase (PL) must first attach itself to the oil globules. However, PL cannot act alone. Its molecular shape is such that its so-called active site – the area in the enzyme structure where the triglyceride would interact and be broken up – is hidden away. Two other components are needed to unmask the site and help the enzyme adsorb onto the droplet surface. These are colipase, which forms a complex with PL, and bile salts which solubilise the fatty acids from the triglyceride breakdown to form minute spherical structures called micelles.

Analysing the active complex

We wanted to understand at a molecular level how these components come together to form an active complex. Small angle neutron scattering (SANS, see p.5) is ideal for investigating large molecular structures such as this one. By using a method called contrast variation, we could visualise specific parts of the complex whilst other parts remained invisible. The principle behind this is the same as the familiar school laboratory experiment whereby sugar is slowly added to water in which is immersed a glass rod. At a certain sugar concentration the glass rod becomes invisible because it has the same refractive index as the sucrose solution. In other words, light is scattered equally by the glass and the solution. We say that there is no 'contrast' between the rod and the solution. In the case of neutron scattering, heavy water is added (water in which hydrogen is substituted by its heavier isotope deuterium, D_2O). So, for example, when the water contains 10 per cent heavy water to 90 per cent ordinary water, the lipase and colipase are visible and the bile salt invisible.

Using these techniques we have been able to show that the complex contains two molecules of colipase attached to each pancreatic lipase molecule and they are connected and stabilised by a micelle of the bile salts. The model of the complex is shown below.

This knowledge could enable us to design drugs that can prevent the complex from forming in the first place and thus limit the amounts of fat assimilated by the body. Our colleagues in Marseille are currently conducting preliminary clinical studies using rats. ■



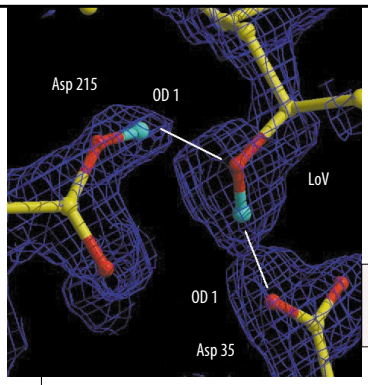
The calculated model built with two lipase/colipase molecules per micelle corresponding to the observed crystal structure

Research team D. Pignol, J. Hermoso and J. C. Fontecilla-Camps *(LCCP, CEA-CRNS Grenoble)*, L. Ayvazian, B. Kerfelec, I. Crenon and C. Chapus *(CNRS Marseille)*, P. Timmins *(ILL)*

Enzymes uncovered

Neutron studies highlighting hydrogen atoms in enzymes are helping to understand how they work

>> DEAN MYLES, LEIGHTON COATES and JONATHAN COOPER



A map of the neutron density near the catalytic aspartate amino acids in the active site of the aspartic proteinase, Endothia pepsin, complexed with a transition-state analogue, H261

Dean Myles with the LADI neutron image plate detector

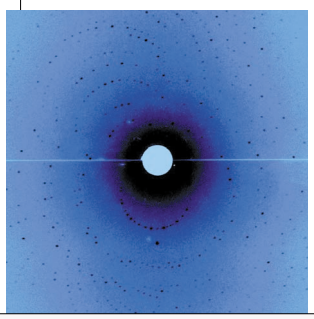
Virtually all chemical reactions in cells are catalysed by specialised proteins – the enzymes. Cells contain thousands of different enzymes, each capable of mediating a different kind of chemical reaction. The key to understanding the mechanism lies in determining the fine detail of their atomic structures.

Each protein consists of a sequence of hundreds, sometimes thousands, of amino acids, linked into long chains that twist and fold into a unique three-dimensional shape which determines its biological activity. In an enzyme, this folded structure creates a deep groove or pocket which is lined with exposed amino acids designed to bind a specific reacting molecule (substrate). The substrate is bound in an orientation that stabilises the intermediate structure – a 'transition state' – which forms as the substrate is transformed into a new molecule, and which favours the transformation process.

Knowing the structure of these intermediate states can help to reveal the catalytic reaction steps involved. This can have important clinical applications. Certain enzymes are involved in key stages of common disease states. In such cases, synthetic compounds – drugs – can be designed that so closely resemble the structure of the crucial intermediate state that enzymes are fooled into binding them – thus inhibiting or blocking their activity. Precise knowledge of these active sites can therefore help in designing better drugs.

Aspartic proteinases are a good example of enzymes targeted for drug development. These enzymes are associated with disorders such as hypertension (renin), gastric ulcers (pepsin), muscular dystrophy and neoplastic diseases (cathepsins D and E). Moreover, synthetic chemicals that block enzyme activity in the aspartic proteinase from human immunodeficiency virus (HIV) have a therapeutic role in the treatment of AIDS.

A neutron diffraction image collected from a crystal of aspartic proteinase/inhibitor complex



The standard method for studying the structure of proteins is X-ray diffraction. Detailed X-ray studies of aspartic proteinases with inhibitors bound at the active site, however, led to several different proposals for the catalytic mechanism. The descriptions differed in the positions of crucial hydrogen atoms near two catalytic aspartate amino acids in the active site.

LADI spots the difference

Hydrogen atoms are difficult to see with X-rays and they could not be resolved in the X-ray structures. Neutrons, on the other hand, are highly sensitive to hydrogen – especially the deuterium isotope – and can be used to locate precisely the position of even a single hydrogen atom in complex molecules like proteins. We therefore used neutron diffraction to shed new light on this problem.

The neutron structure of an aspartic proteinase/inhibitor complex was determined using the LADI neutron protein crystallography instrument at ILL/EMBL-Grenoble. LADI is a powerful new device in which a novel image plate detector (see above), which is sensitive to neutrons, completely surrounds the sample and provides 10 to 100-fold gains in efficiency compared with conventional neutron diffractometers.

The neutron structure of this aspartic proteinase/inhibitor complex provides a wealth of information on the hydrogen positions in the protein, and specifically reveals the positions of the vital hydrogen atoms at the key catalytic locations in the active site (see above left). The determination of this structure clarifies earlier models that sought to explain how these enzymes work and offers new information for drug development. ■

Research team D. Myles *(EMBL)*, L. Coates and J. Cooper *(University of Southampton)*

Some enzymes like it hot

Enzymes that work best at high temperatures have many biotechnological applications. Neutron scattering throws light on how they remain stable

>> JÖRG FITTER

Proteins are long-chain molecules which naturally fold into characteristic complex three-dimensional structures held together by weak interatomic forces. Any protein must remain in its unique folded state in order to carry out its biological function. However, the 'free energy' of the folded state is only slightly less than that of the unfolded state by the equivalent of the energy of a few hydrogen bonds. This means that the folded state is only marginally more stable, reflecting a compromise between the rigidity required for selectivity of action and a degree of flexibility needed for the protein to function.

We were interested in investigating how this balance relates to stability, particularly in the case of proteins that operate at high temperatures. While most enzymes (which are proteins) function at room temperature, those found in certain heat-loving microorganisms actually work best at temperatures ranging from 70°C to well above the boiling point of water! The biotechnology industry is extremely interested in these enzymes but the factors affecting their stability 'such as structural flexibility' are not well understood.

We therefore decided to compare the relative flexibilities of two closely related enzymes, heat-stable α-amylase and its room-temperature counterpart. These enzymes digest starch and related carbohydrates. Since starch is most soluble at high temperatures, it is the heat-stable version that plays a key role in industrial applications, for example, in the paper industry, in waste treatment, as well as in starch processing.

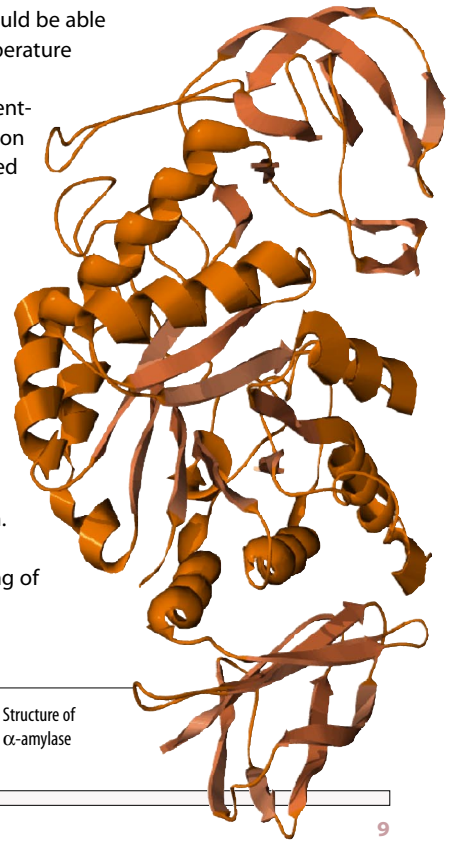
Monitoring fluctuations

The approach we took was to examine the rapid movements within the enzyme structures by recording the neutron scattering from the constituent hydrogen atoms. First, the enzymes were dissolved in heavy water (containing deuterium) so that any easily removed hydrogen atoms were exchanged with deuterium, which does not, in this experiment, scatter as strongly as hydrogen. Then, by comparing the velocity of the neutrons before and after scattering we could measure the energy transfer to the hydrogen atoms in the sample, and deduce the rapidity as well as the size of fluctuations in the enzyme structure – and therefore the flexibility.

Preparing samples for neutron experiments

The results were surprising. Previous experiments based on other techniques had suggested that the heat-stable proteins were more rigid than their low temperature counterparts – so that they would be able to resist any tendency to unfold as the temperature rose. We found, however, the thermostable α-amylase was more flexible than the ambient-temperature enzyme. We think that the reason is thermodynamic. The flexibility of the folded state increases its structural disorder, or entropy, reducing the difference in entropy between the folded and more disordered, unfolded states. Thermodynamics predicts that the smaller entropy change would have the effect of increasing the folded-to-unfolded free energy change – which would tend to stabilise the folded state at higher temperatures.

This work shows the particular usefulness of neutrons in investigating directly the dynamics of proteins in solution. Neutron scattering has much to offer in solving major problems in the understanding of living processes. ■



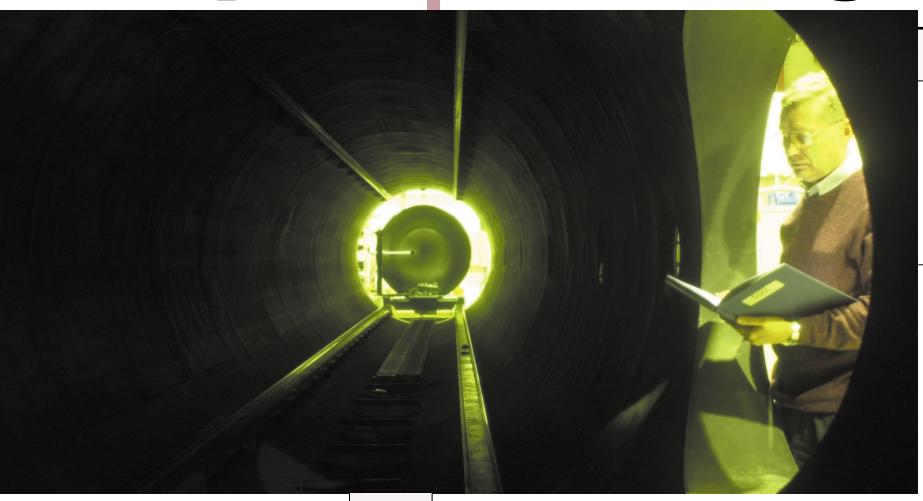
Structure of α-amylase

Research team N.A. Dencher and R. Herrmann (*Technische Universität Darmstadt*), M. Ferrand (*ILL*)

European researchers have been probing the workings of molecular assemblies responsible for protein folding in ancient hot-spring microorganisms

Inside protein folding machines

WOLFGANG BAUMEISTER and ROLAND MAY <<



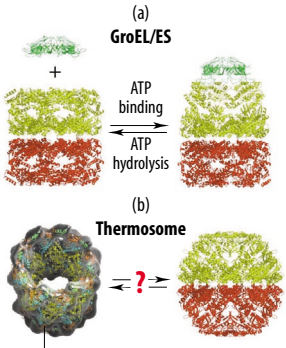
Roland May inspecting the detector vacuum tube of the instrument D22

For proteins to carry out their job, they must fold into the correct three-dimensional structure (see p.8). This process may be assisted by large proteins called chaperonins – double-ring structures with a central cavity which holds the protein as it folds up. Protein folding is extremely important – incorrect folding is, for example, implicated in diseases such as Alzheimers and BSE.

The folding process has been well-studied in a chaperonin found in bacteria, called GroEL (left, a). It is driven by a complex mechanism involving the binding of adenosine triphosphate (ATP) – a molecule which is an energy source for biological reactions. The binding and break-up (hydrolysis) of ATP into adenosine diphosphate (ADP) and a phosphate ion switch the chaperonin between an open state, which can receive the unfolded protein, and a closed state. In the latter, a helper molecule (cofactor), GroES, sits on top of the ATP-loaded chaperonin to seal off the folding compartment.

We were interested in investigating the same process for a group of microorganisms that survive extreme conditions, the archaea, in particular *Thermoplasma acidophilum* which lives in hot sulfur springs at temperatures between 45°C and 63°C. The chaperonin involved, which is similar to chaperonins in mammalian and human cells, is called the thermosome and functions without a cofactor. So how does protein folding by the thermosome work?

It was known that the thermosome has at least two different structures. It appeared cylindrical when viewed, trapped in ice, with an electron microscope but spherical in the crystalline state as observed with X-rays! The interpretation is that the thermosome has a built-in lid instead of a detachable cofactor, and that the cylindrical shape represents the open thermosome – ready for ATP-binding – while the spherical shape indicates the closed, activated folding state (left, b).



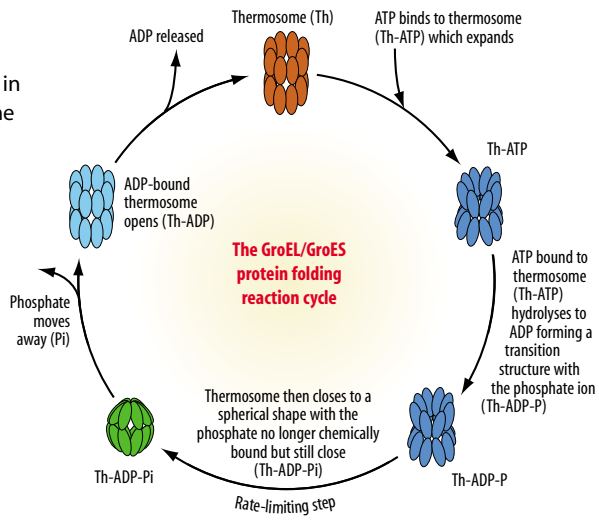
Two chaperonin structures which enable proteins to fold up correctly: (a) GroEL found in bacteria and (b) thermosome found in archaea

This finding raises two important questions. First, what prompts the closure of the thermosome? Would it be ATP-binding as in the case of GroEL/GroES? Secondly, if no, then why is the thermosome closed in the crystal?

Thermosome structure

We tested these ideas using SANS (see p.5) to examine the overall structure and behaviour of the thermosome. The results confirmed that the thermosome not bound to ATP or ADP was indeed an open cylinder. We found that the salts used for the crystallisation are responsible for the closed appearance of the thermosome in the crystal. We then examined the structural changes occurring in solution at room temperature. The resulting data enabled us to propose a model of the reaction cycle. Using specific analogues, we could mimic two intermediate states of ATP hydrolysis (Th-ADP-P and Th-ADP-Pi). Surprisingly, ATP binding did not induce closure of the chaperonin as in the case of the GroEL/GroES system at room temperature. We found that the cycle was quite complex as the sequence of events shows.

These neutron data have given us the first real insight into the changes happening during protein folding in archaeal chaperonins. The next step is to study these folding machines in action with their protein substrates. ■



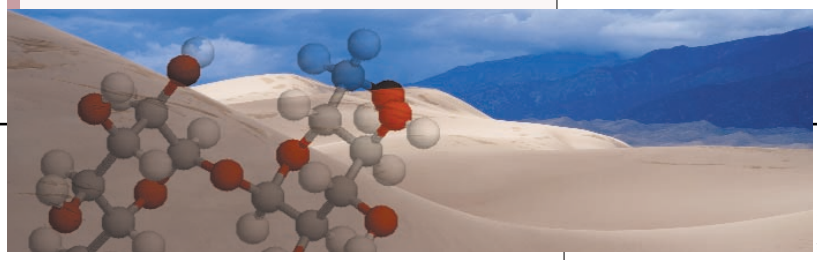
10 **Research team** I. Gutsche, M. Rössle, H. Heumann, W. Baumeister *(Max-Planck-Institut für Biochemie, Martinsried)*, J. Holzinger *(Max-Planck-Institut für Biochemie, Martinsried and ILL)*, R.P. May *(ILL)*

Soft proteins are hardened by sugar coats

Organisms that live in the desert have developed an ingenious molecular survival plan for their proteins

JOSEPH ZACCAI and LORENZO CORDONE <<

D. Bartels



PhotoDisc

The trihalose molecule (above) ensures that the resurrection plant, Craterostigma, can survive a drought as shown (far left) by the fresh, dried-out and resurrected specimens, left to right

A protein molecule consists of a chain of smaller units that folds upon itself to form a structure which has been selected by evolution to do a specific biological job. For example, proteins called enzymes have a structure with a 'pocket' – the active site – which selectively binds a given molecule – the substrate – so that it can undergo a particular chemical transformation. The classical description of this process is that the enzyme acts like a lock into which the substrate fits like a key.

Further insights have, however, supplanted this explanation with a more dynamic view: the active-site lock and substrate key are not rigid structures, but display a certain flexibility that allows them to adapt to each other.

This flexibility can be described in terms of the internal dynamics of the protein – the motions of its various components. It turns out that a neutron beam has just the right properties to investigate these dynamics. It can measure simultaneously the amplitude which tells us how far various parts of the protein move, and the frequency which tells us how often they move in a given time. This is significant because the forces that govern molecular motions in a protein are also the ones that hold it in its folded, active state. They are characterised by the 'softness' of the structure, which changes with temperature.

Neutron studies on protein dynamics can therefore be used to probe the relationships between softness, temperature and biological activity. At extremely low temperatures, experiments have shown that proteins behave like hard solids. When the temperature reaches about -70°C, however, they go through what has been called a dynamical transition, where their structures become much softer. It is in this soft regime that the protein has the right flexibility to accomplish its biological function.

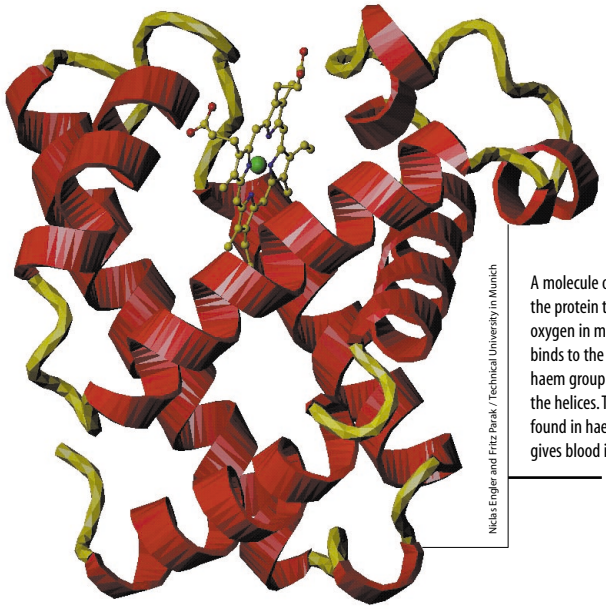
The trehalose protection racket

A recent neutron study investigating an unusual biological survival mechanism showed this relationship very nicely.

At high temperatures, or in absence of water, a protein loses its structure (denatures). However, some desert plants and animals can survive extremes of temperature and dehydration in which you would expect their constituent proteins to denature. How are they protected?

It seems that the organisms produce large quantities of a sugar called trehalose which helps them to retain their cellular and molecular structures. Neutron experiments revealed how: if myoglobin, the protein that binds oxygen in muscle, is encapsulated in a vitreous layer of trehalose, it does not undergo the dynamical transition to the soft regime but remains hard to high temperatures. The trehalose-coated protein can then be subjected to much higher temperatures than usual. Under these conditions, oxygen cannot diffuse in the protein, confirming that the soft form is required for biological function.

The protein structure, although inactive, is thus protected while the organism waits for more favourable times. When the rains come and there is again sufficient water to dissolve and dilute away the sugar, the protein becomes soft and full biological activity is recovered. ■



Niclas Engler and Fritz Parak / Technical University in Munich

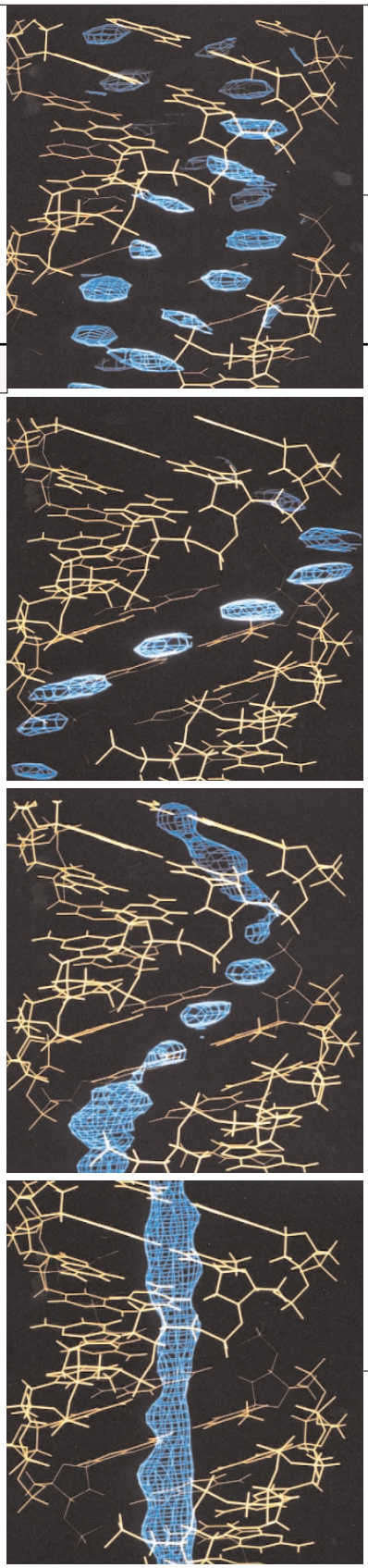
A molecule of myoglobin – the protein that stores oxygen in muscle. The oxygen binds to the iron atom on the haem group in the centre of the helices. The haem is also found in haemoglobin and gives blood its red colour

Research team J. Zaccai *(Institut de Biologie Structurale, CEA-CNRS, Grenoble)*, L. Cordone *(University of Palermo, Palermo)*

Water in your genes

Chains of water molecules play a crucial role when DNA changes form

>> TREVOR FORSYTH



Pictures showing hydration features observed in the A conformation of DNA

The double helical structure of DNA with its sequence of pairs of bases sitting on a sugar-phosphate backbone (left) is well known. What is less known is that this structure is capable of enormous variation – there are five major variants. Four of these have a right-handed helical twist and a fifth is left-handed. Depending on the sequence of base pairs in the DNA chain, it is possible to observe transitions between these structures, some of which even involve a complete reversal of handedness of the molecule. It would be remarkable if these dramatic changes in structure were not exploited biologically.

Water molecules play a critical role in these transitions, so there is considerable interest in determining their positions in the different DNA structures and how these positions change during transitions. In this respect neutron diffraction methods provide information that cannot be obtained by X-ray diffraction (which was the technique originally used to determine the structure of DNA).

The advantage of neutrons

To obtain the location of water molecules, we prepared DNA samples as fibres (the DNA molecules are aligned side by side). The water inside the sample was replaced by heavy water (D_2O), which makes it much more visible. We recorded the neutron diffraction patterns using the unique facilities available on the D19 diffractometer at ILL.

The pictures opposite show the images of water we obtained. These were recorded for DNA trapped in a conformation known as the A conformation. Because this conformation is adopted by the double helices of both DNA and RNA as well as DNA/RNA hybrid molecules, speculation has persisted over decades that it may be implicated in the process of transcription of genetic information (RNA acts as the messenger molecule in transcribing the DNA code for making proteins).

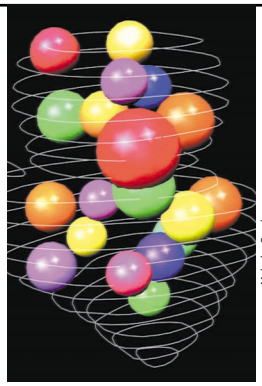
These experiments showed that there is a chain of water molecules along the outside of the DNA molecule, which links the oxygen atoms of the phosphate backbone. There are also two chains of water molecules in the middle of the large exposed 'major groove' of DNA (the helix is not completely regular but has features called major and minor grooves). Intriguingly, there is also a strong water feature along the centre of the molecule. This feature, which is close to the bases of the DNA, is believed to depend on the sequence of bases and is currently being investigated using novel synthetic versions of DNA. ■

12 **Research team** T. Forsyth *(ILL/ Keele University)*, M. Shotton, L. Pope and W. Fuller *(Keele University)*, P. Langan *(Los Alamos National Laboratory)*

Mapping the ribosome with neutrons

Neutron scattering played a significant role in tackling a central problem in molecular biology – solving the structure of the ribosome

>> VENKI RAMAKRISHNAN and ROLAND MAY



Location of the centres of mass of the 30S proteins as determined by neutron scattering

Malcolm Capel

The ribosome is an essential constituent of life. It is a large, complex molecular unit found in cells and is responsible for making all the proteins needed in living processes. It acts like a molecular assembly line, translating the messenger-RNA sequence of bases, which has been copied from the DNA genetic code, into the corresponding chains of amino acids that constitute a specific protein. A ribosome consists of two subunits, a large 50S subunit and a smaller 30S subunit, each with a specific role, though working together.

For the past 30 years, research groups have been working to analyse the molecular structure of the ribosome. It has been a major challenge. The 30S subunit alone consists of about 20 proteins and 1500 RNA bases, so – until the 1990s – solving the structure of such a complex molecular assembly by the traditional method of X-ray crystallography was out of reach.

As a result, researchers at Yale University began a project in the 1970s to determine the spatial arrangement of the proteins in the 30S using neutron scattering. Most the work was done at the Brookhaven National Laboratory in the US but some was also carried out at ILL.

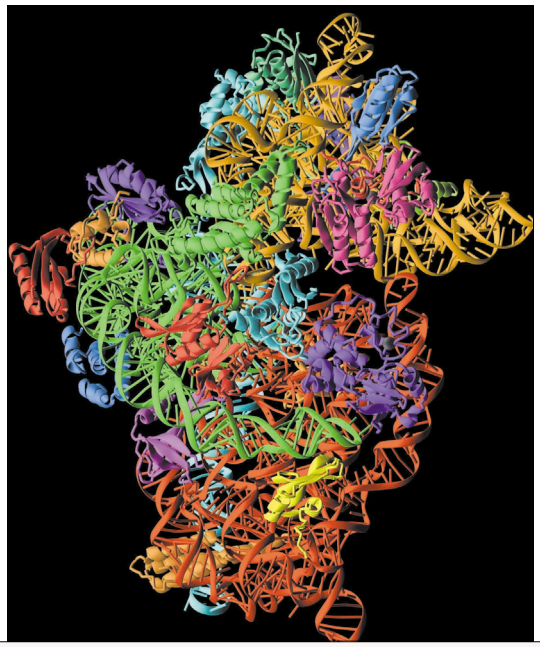
Measuring the distance between protein pairs

The technique used was both ingenious and laborious. First the 30S particle was separated into its RNA and the protein constituents. A selected pair of proteins was then deuterated, and the 30S was reassembled (which it does quite naturally!) incorporating the deuterated proteins. The 30S was placed in a solution whose neutron-scattering strength matched that of the non-deuterated sample so that the deuterated protein pair would be 'highlighted' in the scattering process. Using SANS (see p.5) the distance between the deuterated proteins could therefore be assessed. By repeating this process for every possible pair of proteins, the researchers could obtain a large number of pair-wise distances, and thus determine the three-dimensional arrangement of the proteins.

The final neutron map was published in 1987 (above) and had a huge impact on ribosome research. Since many of the proteins were known to bind specific regions of RNA, the map led to the first rough models for the structure of the 30S subunit.

More recently, our research group began studies on the 30S structure using X-ray crystallography. At a resolution of 5.5 angstroms, we could recognise the features of individual proteins. The neutron map was of significant help, because once we had located one or two proteins by their shape and various other structural elements, we could quickly locate the other proteins whose structure had been solved at the time.

We have now determined the complete X-ray structure of the 30S and we can now see how well it agrees with the neutron map. The position of the proteins that bind directly and most strongly to RNA, are generally in excellent agreement; less good are those that were difficult to purify, or were small and thus had a small neutron signal. ■



Ribbon diagram of the X-ray crystal structure of the 30S ribosomal subunit from *Thermus thermophilus*. The four domains of 16S RNA are coloured separately, and the 20 proteins in the structure are given contrasting colours

Research team V. Ramakrishnan, D. Brodersen, B. Clemons, A. Carter, R. Morgan-Warren and B. Wimberly *(MRC Laboratory of Molecular Biology, Cambridge)*, P. Moore and D. Engelman *(Yale University)*, B. Schoenborn and D. Schneider *(Brookhaven National Laboratory)*

Neutron scattering offers a unique probe of complex biological membranes

Highlighting reactions in membranes

BRUNO DEMÉ <<

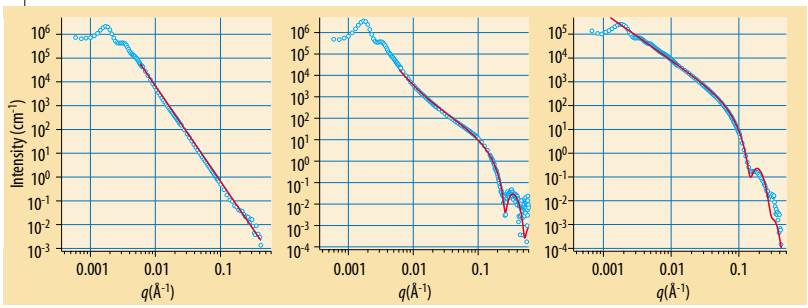
The instrument D11 used in the small-angle neutron scattering experiments

Biological membranes, which encapsulate cells and structures within cells, play a major role in living processes. They consist of a thin, hydrophobic (water-hating) and fluid layer made of self-organised lipids, sugars and proteins (see p.15), which selectively allows the transfer of molecules, and which also harbours molecular assemblies responsible for important biochemical processes – in particular, enzyme reactions. Enzymes are proteins which catalyse a huge variety of chemical reactions in living organisms.

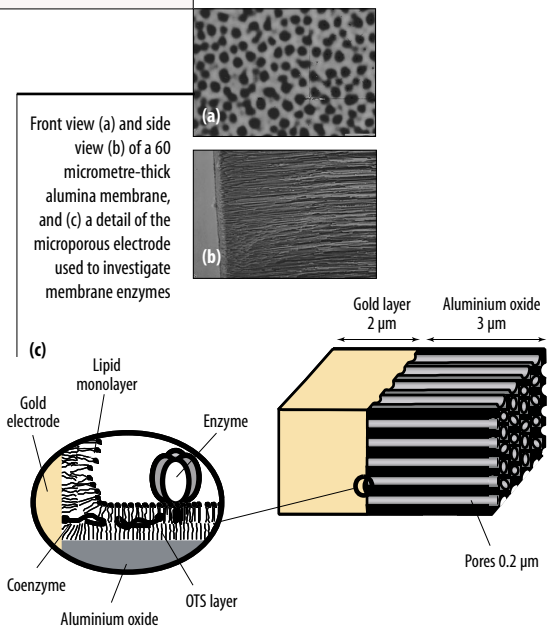
Because of the structural complexity of their environment, these membrane processes are not easy to study. Researchers therefore resort to making simple models of the membrane that specifically simulate the conditions of the reaction they wish to investigate. ILL has been involved in such a study carried out by French chemists.The reaction studied is involved in bacterial respiration, in which the enzyme (pyruvate oxidase) speeds up the transformation of its substrate (pyruvate) with the help of its coenzyme (quinone Q8). By definition, these 'redox' reactions involve the transfer of electrons, and the researchers wanted to unravel the precise reaction steps and how fast they go in a two-dimensional medium.

A hybrid bilayer

The model membrane used was a hybrid bilayer which is very stable. It consisted of a layer of a long-chain hydrophobic material (OTS), chemically bound to porous alumina (aluminium oxide), with a second, lipid layer was added on top of the OTS layer. The bilayer coats the interior of the alumina pores.



Small-angle neutron scattering by bare porous alumina (left), by the OTS-grafted alumina (centre) and by the full membrane (right). Useful information on the structure of the sample is extracted from the slopes of the curves, and from the positions of peaks and oscillations. The experimental curves (o) can be reproduced by calculating the scattering based on detailed models (solid red lines)



Front view (a) and side view (b) of a 60 micrometre-thick alumina membrane, and (c) a detail of the microporous electrode used to investigate membrane enzymes

To study the catalytic process by following the redox reaction, the porous alumina is placed in contact with a gold electrode which can supply electrons for the reaction. The enzyme is then attached to the lipid layer inside the pores, and the coenzyme commutes between the enzyme and the gold/alumina interface by lateral diffusion in the membrane (see above).

Our task at ILL was to characterise the shape of the supported membrane. SANS (see p.5) is the only technique that allows the study of this complex system step by step. We had already studied the porous alumina without the membrane and determined its exact honeycomb structure, its specific area, and its roughness, so we focused first on the single OTS layer. Then the whole hybrid bilayer was examined in mixtures of water and heavy water (D_2O) using the contrast matching technique (see p.7). This new approach to characterising a supported membrane in a porous material provides information on the homogeneity, the specific area, the roughness, the thickness and the density of the membrane.

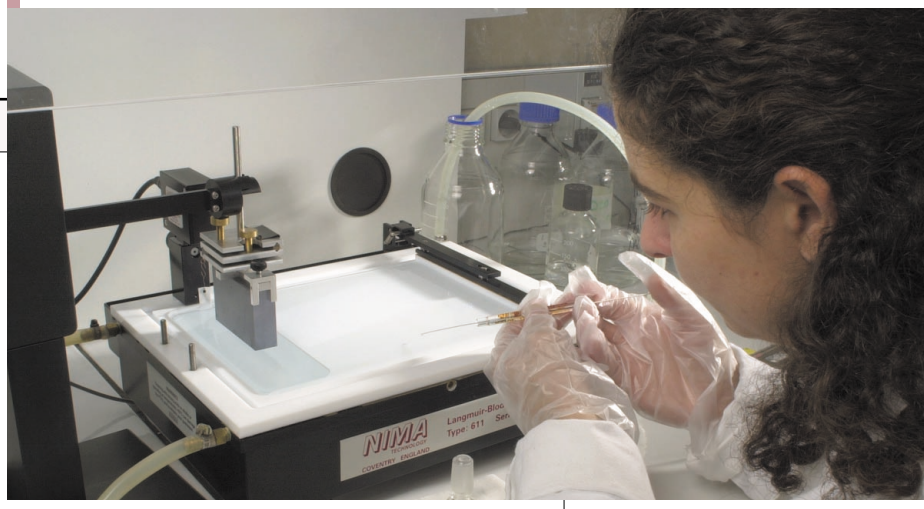
The next step is to carry out similar but more difficult experiments on a pure lipid bilayer which is more fluid and not directly attached to the alumina, but is more like a real biological membrane. Eventually we hope to incorporate a membrane enzyme and study the whole system with SANS. This will mean deuterating the enzyme – a difficult task that will require special preparation facilities but will be an exciting challenge for the future. ■

 Research team B. Demé *(ILL)*, D. Marchal *(University of Paris)*

Crossing the cell membrane

How does a peptide called penetratin slip through a cell membrane without perturbing it?

>> GIOVANNA FRAGNETO



Giovanna Fragneto preparing samples

Cells in all organisms have an outside wall called a membrane formed mainly by lipids – molecules consisting of a head which likes water and a tail that does not – and also proteins. The proteins act as guardians of the wall, controlling and continually regulating what goes in and out of the cell. The lipids provide a protective barrier permeable only to certain very small molecules. They do so by forming a bilayer – that is, two layers of molecules assembled one alongside the other. The two layers come in contact in such a fashion that the tails form an internal impermeable core with the heads arranged pointing outwards. The structure of the bilayer and its interaction with proteins play an important role in the activity of the cell. Real cell membranes are very complicated, so physicists often use simpler models for studying membrane structure and behaviour.

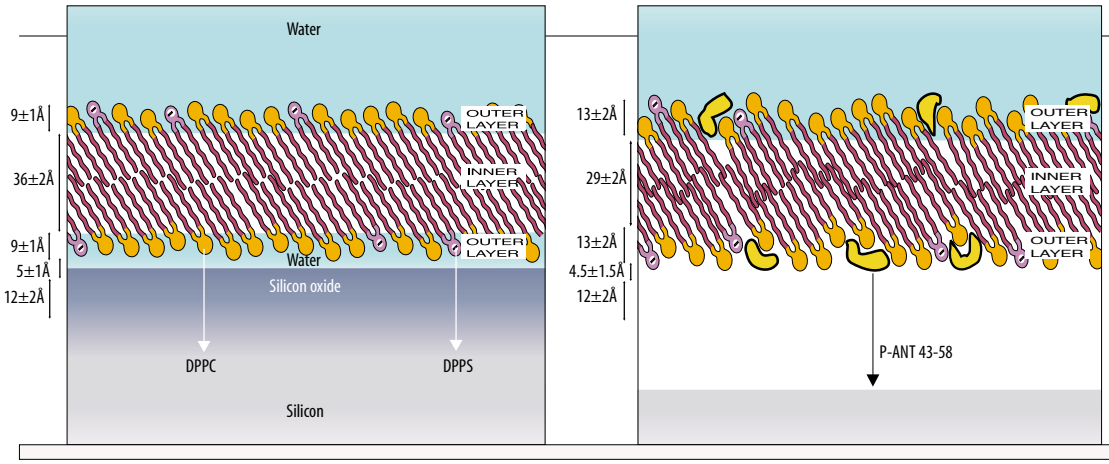
All membranes contain a substantial proportion of phospholipids (a class of membrane lipids), and since pure phospholipid bilayers show many of the properties of membrane bilayers, they are widely used as model systems. We have succeeded in preparing phospholipid bilayers on solid surfaces and in seeing how a peptide – a small part of a protein – influences the bilayer. This peptide, known as penetratin, has the special property of being able to cross the membrane without perturbing it. It is therefore used to transport drugs into cells. How the penetratin manages to cross the membrane without destroying it is unclear, but it does seem to depend on the way the peptide interacts with the membrane lipids.

We were able to 'see' the peptide using neutron reflection (neutrons are reflected off an interface just like light). This is the great advantage of this approach since the peptide is too small to be seen with most other techniques.

Reflecting neutrons at the interface

Our experiment consisted in bouncing a neutron beam off the bilayers at the solid/water interface and collecting the reflected neutrons at a detector while rotating the sample. The signal from the reflected neutrons at the different angles was analysed to obtain the structure at the interface. Measurements were made in pure water, in heavy water (D_2O) and in mixtures of the two. The reason we do this is that it allows us to obtain several sets of data from the same chemical system which then ensures that the subsequent analysis and interpretation are more precise.

The data led us to conclude that there was an affinity between penetratin and the lipid heads if they possessed a negative charge, as in real membranes, but there was no interaction with the tails. This is a first step towards understanding the mechanism by which the peptide penetrates the cell. ■



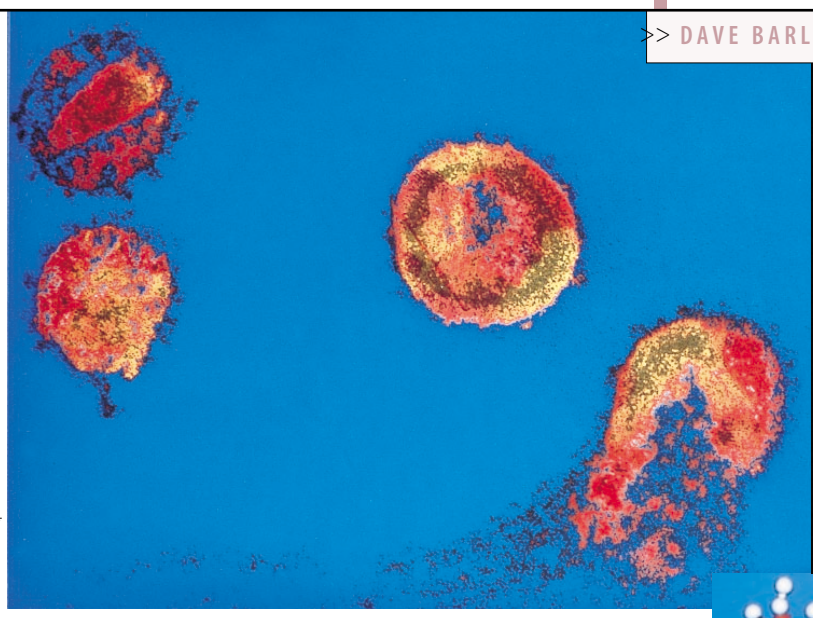
On the left is the phospholipid bilayer on a silicon substrate showing the arrangement of water-loving heads (orange and mauve) and water-hating tails (pink). The right-hand image shows how penetratin (yellow blobs) interacts with the phospholipid heads

Research team G. Fragneto *(ILL)*, F. Graner *(CNRS and University of Grenoble)*, T. Charitat *(CNRS and University of Grenoble)*, P. Dubos *(CNRS and University of Grenoble)*, E. Bellet-Amalric *(ILL)*

Neutrons help in gene therapy

Investigations into new methods of delivering genes to the body are aiding the search for cures to inherited diseases and AIDS

>> DAVE BARLOW, JAYNE LAWRENCE and PHILIP CALLOW



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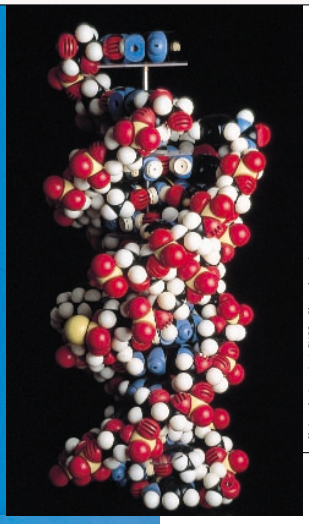
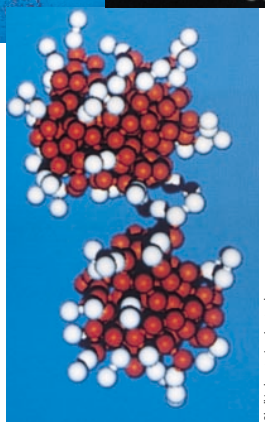


Christophe Lapetit, © CNRS – Phototheque



Shell Laboratories, Amsterdam

Attaching a gene (top) which makes a protein capable of killing the AIDS virus (top left) could be transferred into a cell via a liposome (left)

Gene therapy has the potential to treat many different diseases, even those previously impossible to cure. For inherited diseases like cystic fibrosis and muscular dystrophy, the idea is to transfer a gene which makes the crucial protein that is lacking into the patient's body so that the protein can then be produced and the patient returned to good health. With infectious diseases like AIDS, the proposed strategy is to deliver a gene making a protein that kills the invading organism directly or triggers the body's natural defence system to destroy it.

Now, although the range of clinical applications of gene therapy is potentially enormous, we do not yet have an effective method of gene delivery. Early efforts to transfer genes to a patient's cells by packaging them inside viruses have proved disappointing. More recently, therefore, researchers have focused on inserting the genes into particles known as liposomes. These are bubble-like structures made from detergents which, like viruses, can penetrate cell membranes.

Liposome-gene constructs

Exploiting these liposome-gene constructs successfully, however, depends upon knowing precisely how the liposomes interact with the added genes, and how they then behave when they encounter the body's cells. If the liposome-gene complex sticks to the cell surface and then collapses, the component gene will be rapidly digested and so will be ineffective; whereas if the complex sticks to the cell surface and remains intact, it is more likely to be taken inside the cell as required.

Surprisingly, there have been few studies looking at how the structures of different kinds of liposomes influence the way they interact with genes, and how in turn this affects their interactions with cells and thus their effectiveness as gene deliverers.

Our research group at King's College London, in collaboration with colleagues at the ILL, is doing precisely this. We are using SANS (see p.5) to study the structures of various liposome-gene systems when they are simply dissolved in water, and then employing neutron reflectance to look at what happens to these systems when they approach the surfaces of artificial cell membranes.

When the work is completed we will be far better placed to know what kind of liposome would be best to use for gene delivery, and this will take us another step forward in the fight against disease. ■

 Research team D. J. Barlow, M. J. Lawrence and P. Callow *(King's College London)*, P.Timmins and G. Fragneto *(ILL)*

Purple pumps in motion

A purple membrane converts light into chemical energy in a salt-loving bacterium. Neutron studies reveal the detailed structure and dynamics

JOSEPH ZACCAI and DIETER OESTERHELT <<

All cells are surrounded by membranes made up of lipid and protein molecules that do much more than separate the outside from the inside. Cell membranes are highly dynamic structures with a variety of important biological functions. They ensure, for example, the functioning of energy conversion processes essential for the life of the cell.

We have been investigating just such a mechanism in a microorganism, *Halobacterium salinarum*, which lives in very salty water like the Dead Sea. Its cellular membrane contains two-dimensional crystalline arrays of a purple-coloured protein called bacteriorhodopsin, and lipids. The membrane is able to convert light into chemical energy for powering molecular processes inside the bacterial cell.

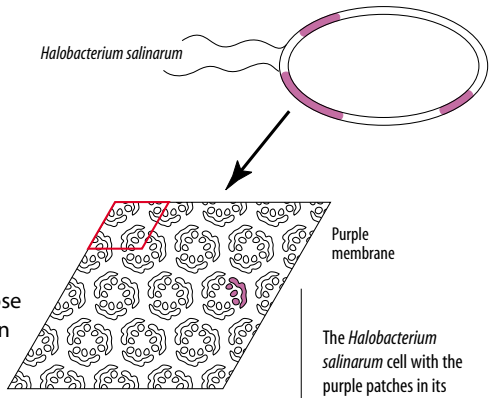
There is a great deal of interest in the structure of this purple membrane and its action, partly because the membrane is crystalline, providing easy-to-prepare samples for structure determination (biological membranes are notoriously difficult to extract for study) and partly because – in an interesting illustration of the unity of Nature – several related systems are found in the human nervous system, controlling sight, smell and other biochemical responses.

Bacteriorhodopsin consists of seven helical portions, which traverse the membrane to form a ring around a light-sensitive molecule, retinal (the same molecule that responds to light in human retinas). When retinal absorbs a photon of light it induces structural changes that allows a proton (the positively-charged nucleus of a hydrogen atom) to be pumped out of the cell. Just like in an electric battery, the charge imbalance across the membrane creates a voltage that is used as an energy source by the cell.

Relating structure and dynamics to activity

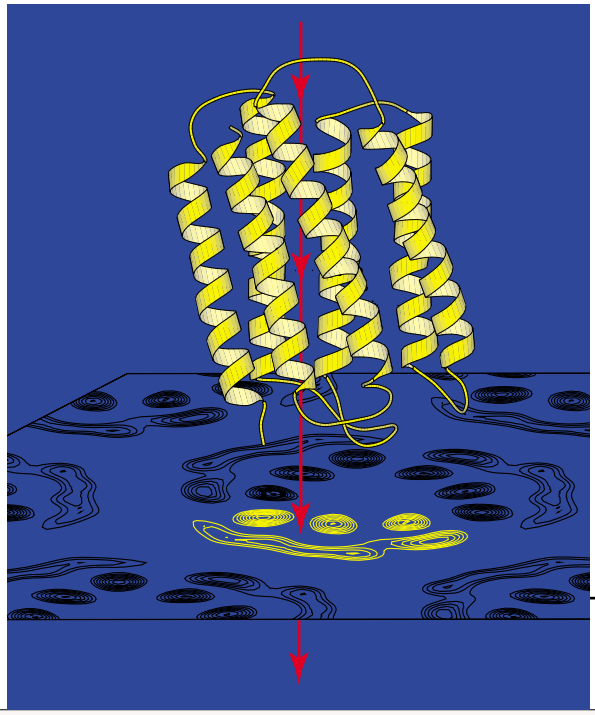
Neutron scattering offers a unique tool to study both the structure and dynamics of the membrane components. Labelling with deuterium (see p.5) allowed us to focus on selected structural parts.

Neutron diffraction established that water molecules essential to proton transfer were localised in the centre of the bacteriorhodopsin ring, while others allowing protein flexibility were found in the surrounding areas on each membrane face. The sites of sugar-binding lipid molecules, whose interactions with bacteriorhodopsin lead to the formation of the two-dimensional lattice in the membrane, were also discovered.



The *Halobacterium salinarum* cell with the purple patches in its surrounding membrane. The purple membrane is a two-dimensional crystal of the protein bacteriorhodopsin and lipids as shown

For the dynamics experiments, we obtained data on membrane flexibility and 'stiffness' by measuring scattered neutrons of well-defined energy. We found that the flexibility essential for function was obtained only in the presence of a minimum amount of water. Furthermore, the light-harnessing core of bacteriorhodopsin is relatively stiff – as you would expect for a valve mechanism in a pump – while the rest of the protein is soft enough to allow it to change shape so as to enhance proton transfer. These results provide a rare example in which the dynamics of a protein has been directly related to its function by experiment. We are now extending the study to characterise this complex system in greater detail. ■



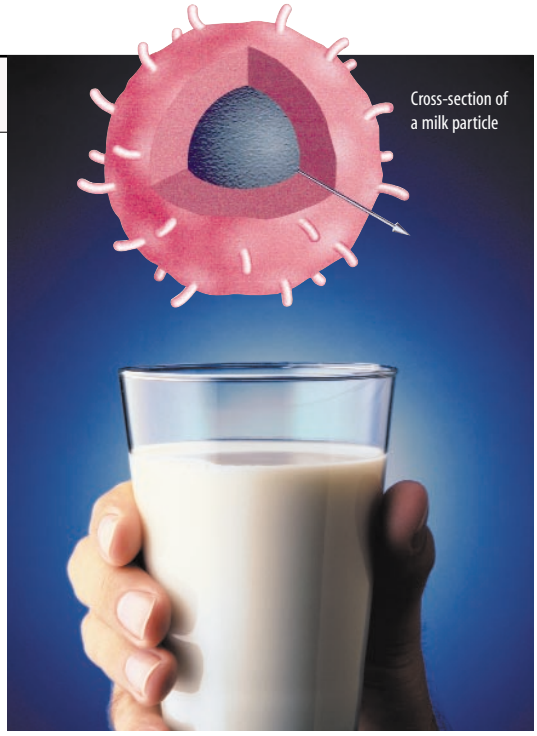
The seven helices of bacteriorhodopsin are shown as they project on the crystal lattice. The red arrow shows the path of a proton pumped from inside the cell to the outside when the protein is illuminated by light

Research team U. Lehnert *(ILL and MPI)*, D. Oesterhelt *(MPI)*, H. Patzelt *(MPI now at the University of Oman)*, C. Pfister *(IBS)*, V. Reat *(IBS, now at the CNRS, Toulouse)*, M. Weik *(IBS)*, J. Zaccai *(IBS)*

Milk contains large amounts of calcium and phosphate in the form of nanoclusters. Researchers at ILL have been probing their structure

Modelling the remarkable structure of milk

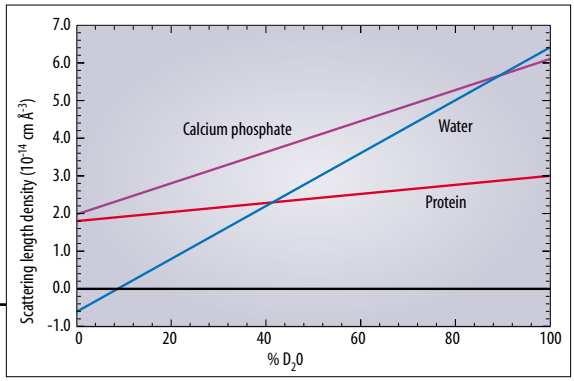
>> PETER TIMMINS and CARL HOLT



Milk is an amazing liquid. As well as proteins, fats, sugars and other nutrients needed to sustain life, it contains calcium and phosphate to allow bones to grow. In fact, milk contains so much calcium and phosphate that they should form an insoluble precipitate. This does not happen. Why not?

The essential factor is that the calcium and phosphate are in the form of clusters of ions embedded in a complex protein particle called the casein micelle. A typical casein micelle has a radius of 1000 angstroms and contains several hundred of these nanoclusters (diameter 25 angstroms). Using neutron scattering, we determined the structure of the casein micelle, first studying the individual nanoclusters and then showing how they are arranged in the micelle.

Artificial calcium phosphate nanoclusters can be prepared in the laboratory by carefully mixing the casein protein and various salts to give similar concentrations to those in milk. We can then determine their structure with a technique called contrast variation, also described on p.7. Since hydrogen and its isotope deuterium have very different scattering strengths for neutrons, it is possible to mix together ordinary water (H_2O) and deuterated, or heavy water (D_2O), so that it scatters neutrons with the same strength as the casein. Effectively, the protein becomes invisible when the proportion of D_2O is at 40 per cent. The properties of the calcium phosphate can then be measured. In a mixture containing 88 per cent of D_2O the calcium phosphate becomes invisible and the properties of the casein can be measured.



Scattering intensities of the nanocluster components as a function of D_2O/H_2O content

A complex structure

In this way, we could show that the nanoclusters consist of a core of calcium phosphate surrounded by a shell of casein. The size of the core, its mass, the thickness of the shell and details of the protein's structure were all determined. Using this information, we were able to predict the substructure – how the nanoclusters were arranged in the casein micelle. Again, we applied the contrast matching method to analysing the overall structure and substructure of the whole particle. The results showed that the nanocluster particles formed a kind of disordered lattice within the micelle with a spacing of 170 angstroms. This value is exactly what we had predicted from our previous knowledge of the ratio of calcium phosphate to protein in the micelle and the density of the particle.

Our findings strongly suggest that casein acts to control calcification of the mammary gland by transporting the calcium and phosphate in the form of small stable nanoclusters. Whereas we once thought of casein as only a nutritional protein, our work has shown that it has this additional, vital, biological function. ■

 Research team P.Timmins *(ILL)* and C.Holt *(Hannah Research Institute, Ayr)*

What switches you on (or off)?

Muscle contraction and relaxation involves some complex changes in muscle proteins regulated by calcium

>> ROBERT MENDELSON and PETER TIMMINS

PhotoDisc

Imagine we had no control over the muscles in our bodies. We would be permanently stiff with all our muscles blocked in a state called 'rigor' – hence *rigor mortis*, the term describing the condition that happens to the body after death. So in order for our muscle to relax and contract in a cyclic way the muscle must be regulated.

We have been studying one variation of the regulation process using neutron diffraction. All muscles are composed of two kinds of protein fibre, a thick filament composed of myosin, and a thin filament composed mainly of actin and tropomyosin. These are in contact with one another and the action of muscles comes from the sliding of these filaments over each other, as though along a track.

Changing shape

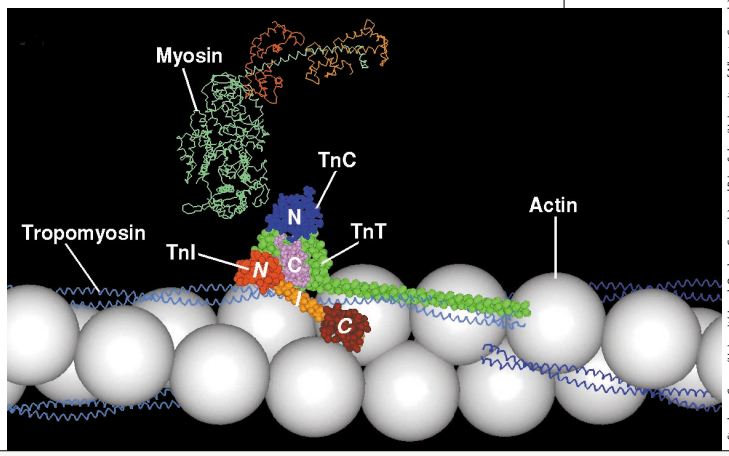
This action is regulated by calcium. In skeletal and cardiac muscle, a nerve impulse tells the sarcoplasmic reticulum, which surrounds the muscle fibres, to release calcium. This then binds to a protein called troponin forcing it to change shape. The thick filament can then attach to the thin filament in such a way as to provoke the sliding of the filaments. When the nerve signal stops, the sarcoplasmic reticulum re-absorbs the calcium, the troponin then reverts to its original shape and blocks the binding of the thick filament to the thin filament. In this way, contraction is turned on and off.

We are using SANS (see p.5) to study the details of how the troponin changes shape when calcium is added. Troponin is a large complex composed of three separate protein molecules, or subunits. These are known as troponin C (C for calcium binding), troponin T (T for tropomyosin binding) and troponin I (I for inhibiting).

Using the contrast variation technique (see p.18) we have been able to visualise each individual subunit while making the other two invisible, or to look at pairs of subunits whilst making the third subunit invisible. It requires rather sophisticated preparation. First, the genes that make each of the three proteins were identified. Then, using a standard genetic technique whereby the genes are put into bacterial cells which separately make the proteins, we could not only isolate any one of the subunits but also prepare them in either hydrogenated (normal) or deuterated forms.

The troponin molecule was then rebuilt with any one or two subunits deuterated and the other two or one normal. Mixtures of ordinary water and deuterated water were used that rendered the hydrogenated protein invisible so that the neutron scattering gave information only on the deuterated subunits. In this way, we could carry out experiments to see the effect on each subunit of adding or subtracting calcium to the troponin molecule. When studying the troponin with the C-subunit deuterated and the other two hydrogenated we observed no significant change in shape, indicating that the calcium has little or no effect on the C-subunit, even though this is the subunit to which the calcium is directly bound.

However, when measuring the scattering from samples with deuterated I (C and T invisible) or deuterated T (C and I invisible) subunits, there was a significant change in shape. Further studies are being carried out in order to make a model of the whole troponin molecule with and without the bound calcium. ■



Stephane Gagne (Université Laval, Quebec, Canada) and Brian Sykes (University of Alberta, Canada)

A schematic view of the muscle thin filament formed by actin molecules linked together in a chain with the tropomyosin molecule wrapped around the filament. The troponin molecules, which are arranged regularly along the filament, change shape when calcium is introduced, are composed of three separate units (I, C and T). Also shown is the myosin of the thick filament which attaches itself to the thin filament when calcium binds to the troponin

Research team D. Stone *(UCSF, San Francisco)*, R.Mendelson *(UCSF)*, P. Timmins *(ILL)*

Glossary

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

Amino acid
The basic unit of which proteins are made. There are 20 different natural amino acids.

Angstrom (Å)
10^{-10} metres (1Å = 0.1nm).

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

Cellulose
A natural carbohydrate consisting of a chain of simple sugar molecules in a complex configuration.

Chaperonin
A large ring-like protein which assists in the folding of proteins.

Contrast variation
A technique in which certain atoms in a sample are substituted by another isotope with different scattering strength in a way that preferentially enhances the scattering pattern of particular components of interest.

Deuteration
The process of substituting hydrogen atoms in a molecule with the heavier isotope deuterium.

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

DNA
The molecule responsible for storing and transmitting genetic information. It is composed of two strands of nucleosides twisted around each other in the form of a double helix.

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

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

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

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

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

Hydrophilic
Water-loving.

Inelastic scattering
A neutron technique in which there is an exchange of energy between the neutrons and the molecules being studied, thus giving information about their motion and flexibility.

Lipid
A molecule consisting of a long hydrocarbon chain with an electrically charged group of atoms at one end. Lipids arrange themselves in layers and are the basis of biological membranes.

Liposome
A microscopic sac-like structure consisting of a lipid or detergent membrane in which molecules or even genes can inserted.

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

Neutron
One of the two particles found in the atomic nucleus.

Neutron reflectivity or reflectometry
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 diffraction or scattering
Like other subatomic particles, neutrons have a characteristic wavelength depending on energy. When reflected, or scattered, off a material in which the interatomic distances are similar to the neutron wavelength, the scattered waves interfere to produce a characteristic diffraction pattern.

Nucleotide
Basic unit making up DNA. It is composed of a sugar attached to one of four different chemical bases and a phosphate group.

Peptide
A small section of a protein consisting of several amino acids.

Phospholipid
A class of lipid containing a phosphate group.

Polysaccharide
A polymer made up from sugar units.

Protein
A long chain of amino acids. Each protein has a specific amino-acid sequence which causes it to fold up into a unique three-dimensional structure with a unique biological function.

Ribosome
A large molecular complex made of protein and RNA found in all cells which is responsible for assembling proteins from their constituent amino acids.

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

Small angle neutron scattering (SANS)
Measurement of neutron scattering at small angles used to investigate structures with large interatomic distances such as polymers or biological structures.

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

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