4.6 Biology and Biotechnology

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Abstract
Neutrons have a unique role to play in determining the structure and dynamics of biological macromolecules and their complexes. The similar scattering signal from deuterium, carbon, nitrogen and oxygen allows the full determination of the positions and dynamics of the atoms “of life”. In addition the negative scattering length of hydrogen allows the well-known contrast variation method to be applied to dissect the component parts of multimacromolecular complexes. In the post genomic era structure determining techniques are reaching towards high throughput and a high number of proteins to be investigated. Thus, the ESS will offer major gains in neutron capability over the current technical frontier with reactor source technology whereby smaller samples, smaller quantities and lower concentrations all become viable. Thus, the major structure and dynamics techniques of protein crystallography, small angle neutron scattering, inelastic scattering and membrane reflectometry will all benefit in a major way. The considerably reduced measuring times will allow native rather than artificial membranes to be probed by reflectometry, including membrane bound proteins at the surface of actual cells. From the membrane biophysical studies via such native state reflectometry new nano-composites can be envisaged and designed. Structural biology, as well as biotechnology, will benefit from the powerful ability of neutrons to contribute to the location of hydrogen atoms and water molecules in biological systems. Thus it will contribute to the production of missing complementary data relevant for molecular modelling and to the strategy of rational drug design, in synergy with other biophysical approaches.

I. Introduction

We are in the post genomic era (Figure 1).

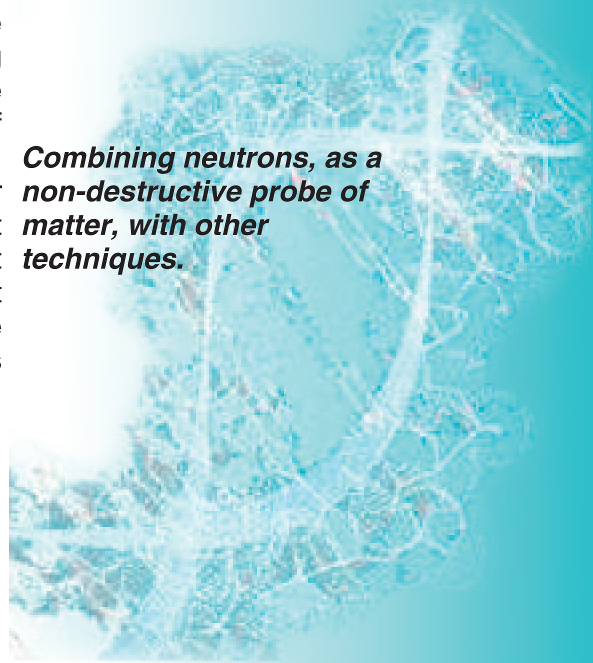
The present major driving forces of life science at the molecular and cellular scale are functional genomics and proteomics. Information on the specific functions of most if not all proteins encoded in human and other genomes is desirable. Major obstacles are the vast complexity of the individual proteins and the even more delicate interaction of different proteins and other biomolecules to form (transient) functional complexes. Neutrons are a unique non-destructive tool for probing precious biological molecular samples [e.g. see ref. 1].

Biology strives to understand molecular recognition.

Neutrons provide physiologically relevant, and yet precise, structural data.

Crystallography, SANS and inelastic scattering of biological molecules constitute major components of the future needs at ESS for 3-D structure analysis. In addition reflectometry on native systems will allow 2-D systems to be studied. Hence in all these methods the role of neutrons as a non-destructive and non-absorbing probe, with contrast variation involving H/D exchange, place neutron scattering as a unique approach. X-ray scattering and, in a different range of accuracy as well as time scale, NMR, provide, of course, important results in structure-function biology. However neutron studies more efficiently locate functional important water molecules and hydrogen atoms, especially at temperatures which are near physiological, rather than at cryo-temperature by SR X-ray protein crystallography. The structural precision of neutron room temperature data is routinely better than NMR atomic position data.

Combining neutrons, as a non-destructive probe of matter, with other techniques.



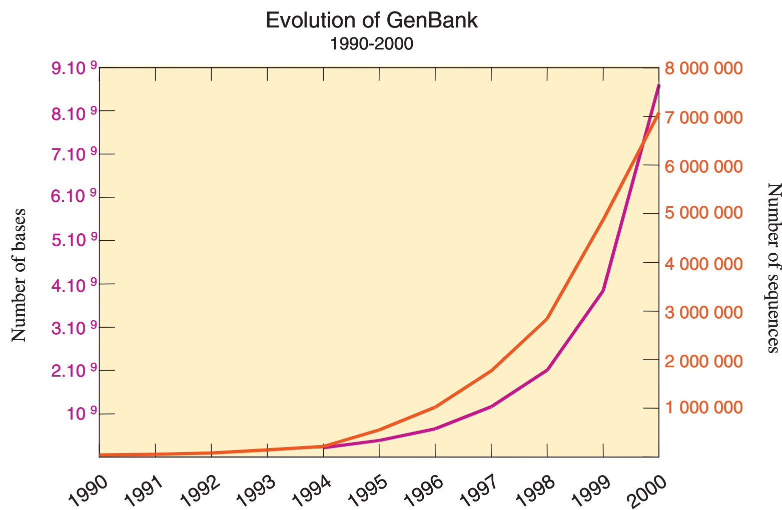


Figure 1: We are in the post genomic age of vast quantities of gene sequence information being available.

II. The role of neutrons

Neutrons are well suited to reveal water and hydrogen.

Hydrogen and water are involved in all the molecular processes of life. Very seldom is information on these aspects taken into account and in any case this experimental information is mostly incomplete. *If only neutron capabilities in protein crystallography could reach the throughput of X-ray work then the impact of neutrons would radically alter this situation.* In fact the research and development has largely been done with some very fine scientific examples e.g. on the detailed dissection of enzyme mechanism involving hydrogen location. Since many enzyme reactions involve hydrogen there is great potential for wide application if the technical capability can be found. Moreover the role of water in molecular recognition is pivotal as, for example, the lubricant of protein ligand interactions or the bonding mediator. Structural definition of bound water by X-ray diffraction is very sensitive to mobility especially at room temperature. Neutrons have the advantage that the scattering of deuterium is equal to that of the water oxygen. The incorporation then of the full structural detail of bound water can radically alter the modelling of proteins *in silico* for the improved discovery of new compounds, for example as leads in drug design. There are two major hurdles for wide application of neutron protein crystallography; firstly the size of crystals routinely available and secondly a molecular weight ceiling of about 30 kDa; the molecular weight histogram in the yeast genome for example peaks at 30 kDa and so at least half of all proteins in the genome are out of range of current neutron protein crystallography capabilities even if big crystals can be grown. This situation should change radically with ESS.

Molecular recognition involves water

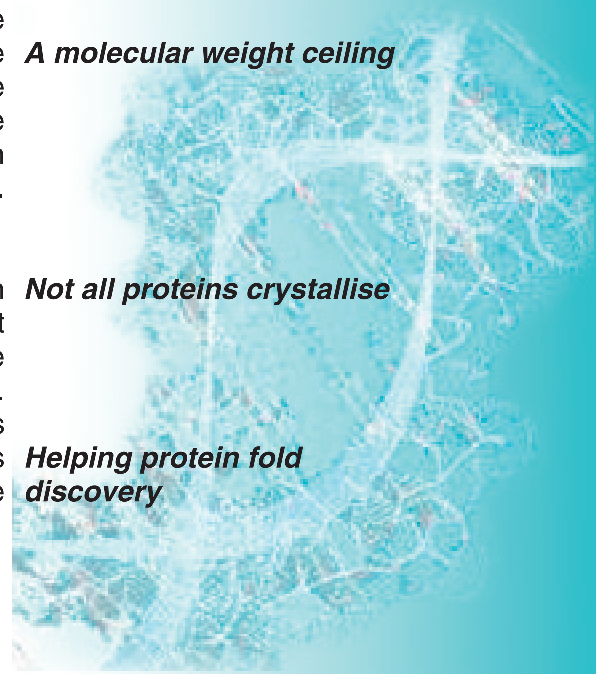
The small crystal challenge

A molecular weight ceiling

In the modern trend towards high throughput, as well as high resolution structure determination, many proteins will not readily crystallise. The field of solution scattering can offer the chance to help determine the fold of a protein in solution. Efforts have started in this direction and show promise but it is strongly felt that the use of deuterated specific amino acids can, with small angle neutron scattering (SANS), provide the

Not all proteins crystallise

Helping protein fold discovery



much needed breakthrough in this field. Major gains in neutron flux to reduce especially the quantity and concentration of protein required will be of interest for higher throughput fold discovery. SANS at ESS could do this.

Dissecting complexes via contrast variation.

Neutrons provide unique possibilities in solution scattering studies of biological macromolecules, primarily thanks to contrast variation by H/D exchange. For multi-component systems (e.g. the ribosome (Figure 2), nucleoproteins or lipoproteins) information about the distribution of the components has been obtained by variation of the D_2O content in solution. Moreover, specific deuteration permits to highlight and analyse selected parts of macromolecular structures *in-situ*.

Neutrons are crucial to biophysics.

The results provided by neutron scattering are highly complementary to other analysis techniques (small angle X-ray scattering (SAXS) and diffraction, electron microscopy (EM) and analytical ultra-centrifugation (AUC)). Moreover, structural models built on the basis of neutron scattering data are able to incorporate information from different high and low resolution techniques and possibly also reconcile concurrent models.

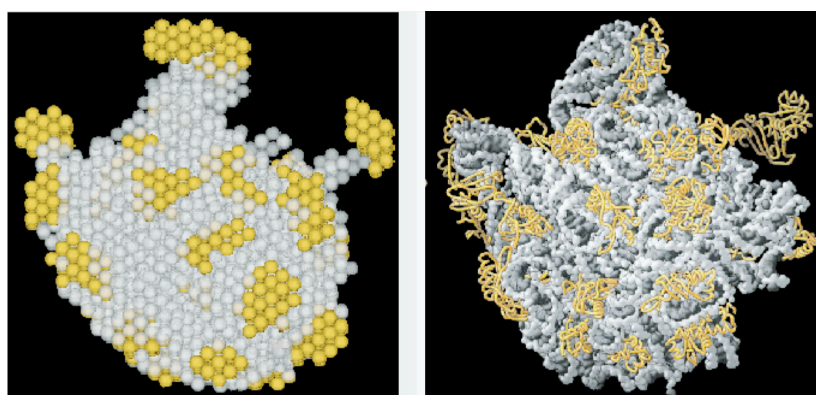
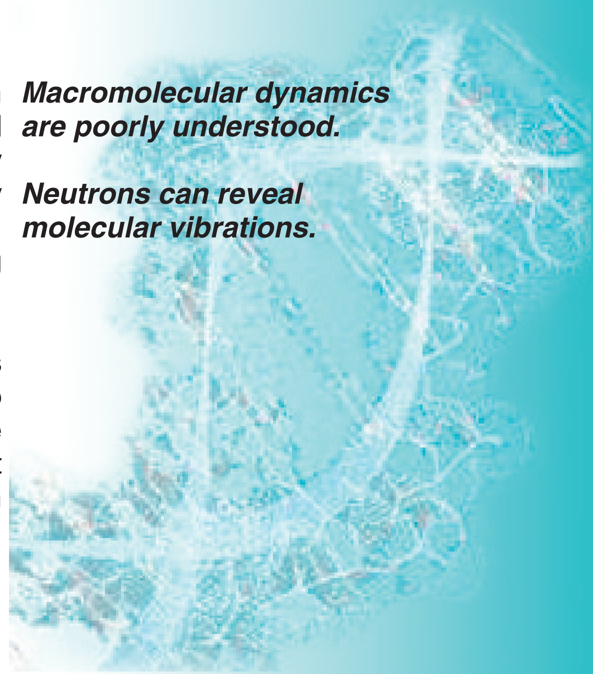


Figure 2: Comparison between the 50S subunit in the map of the 70S *E.coli* ribosome obtained from solution scattering [2,3] (left, resolution 3 nm), and the crystallographic model of the 50S ribosomal subunit *H.marismortui* (right, resolution 0.24 nm, Figure adapted from [4]. Yellow, ribosomal proteins, grey, ribosomal RNA.

Macromolecular dynamics are poorly understood.

Neutrons can reveal molecular vibrations.

Despite its acknowledged importance for biological function and activity, the dynamics dimension in molecular structural biology remains difficult to characterise and poorly understood. Neutron scattering is perfectly and uniquely suited to the space-time window of biological macromolecules. Protein function and activity, including enzyme catalysis, ligand binding, receptor action, electron and proton transfer, are strongly dependent on internal dynamics and conformational fluctuations or rearrangements such as postulated in the induced-fit hypothesis that long ago replaced the lock and key view for enzyme substrate interactions. Dynamics experiments probe the forces that underpin a molecular structure and its fluctuations, and in



chemistry they provide a stringent test for a structural or interaction model. Static or time-averaged structural information alone is not sufficient for a full understanding of specific drug binding to a protein or DNA, for example, and the molecular dynamics dimension (at the quantum chemistry and molecular mechanics levels) has to be taken into account in a "docking" experiment or calculation. Although here too it will be essential for our understanding of their function, there is practically no information available on internal dynamics at a higher level of molecular organisation in cells, protein-protein assemblies (e.g. large chaperones, multi-subunit proteases complexes, multi-enzyme complexes) or protein-nucleic acid assemblies (e.g. ribosomes, chromatin, transcription factor DNA complexes). In an original approach bridging molecular and cell biology, neutron experiments have recently provided data on the dynamics of proteins, *in vivo*, within their cellular environment (Zaccai pers. Comm.). Neutron scattering in fact provides unique opportunities to probe the natural cellular environment, which because of its molecular crowding properties, is very different from the usual conditions of laboratory biochemistry. The field of neutron applications to study biological molecular dynamics is wide open, with hydrogen-deuterium labelling allowing to focus on the dynamics of amino acid groups within a protein, or protein domains within a complex. Sample requirements, however, are at present unrealistic for the technique to accomplish a definitive impact. The ESS should provide a factor of at least 100 gain due to the source and optimised instrumentation.

The dynamics of molecular complexes remains understudied.

The cell is a crowded environment

ESS is a route to the real cell

Membrane biophysics studies on the molecular scale are crucial for understanding the self-organisation processes which underlie many functional aspects of the membrane, in particular membrane transport, molecular recognition on surfaces and adhesion between cells and substrates. While these problems have been studied in the past, largely in model systems, there is now a strong tendency towards studying far more complex *native* membranes. New preparation techniques allow native membranes (e.g. plasma membranes from eukaryotic cells) to be deposited onto solid substrates while maintaining their functional integrity. With faster data acquisition ESS should allow, via neutron reflectometry (NR), the study of membrane behaviour *in-situ* including membrane transport and membrane damage by invasive toxins.

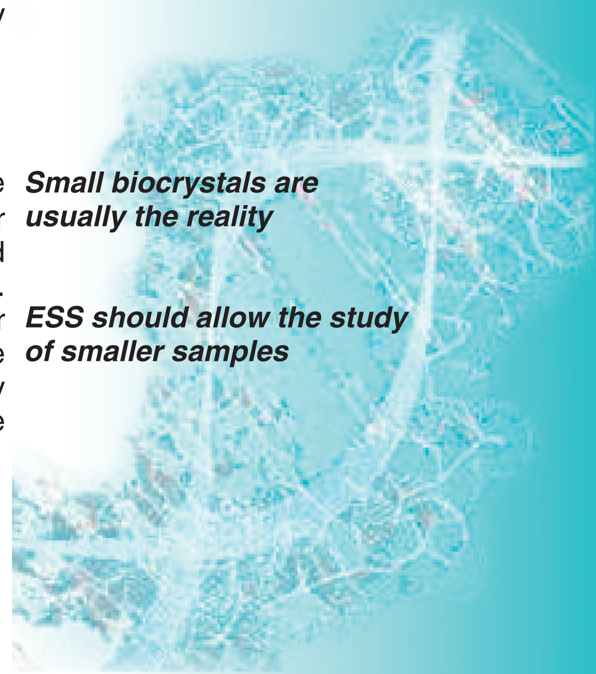
Functional membranes will be studied

III. Future opportunities

In high throughput structural biology research the best sample size is rarely above 100 x 100 x 100 μm. It is essential for neutron protein crystallography to find source, instrument and sample (deuteration) combinations to face this challenge. Since we know from genomics that the average molecular weight of, for example, yeast gene products is 30 kDa, there are many projects that would become amenable for study by neutron protein crystallography methods if the sample size requirement could be relaxed.

Small biocrystals are usually the reality

ESS should allow the study of smaller samples



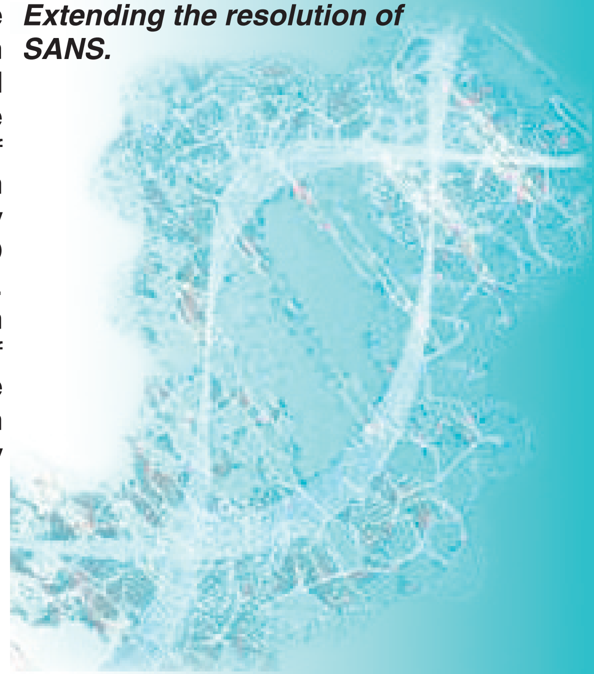
There is a barrier to the application of high resolution neutron structural study posed by molecular weight, which determines the unit cell volume, of large biological complexes. Such weakly scattering crystals cannot be studied currently (Figure 3). If, however, we combine the ESS source and instrument improvements, and improved knowledge of the protein preparation and crystallogenesis for the growth of large crystals, the unit cell size capability could reach 250 Å. Thus, for example, the small subunit of the ribosome could be amenable to study by neutron protein crystallography methods.

Large complex neutron crystallography at high resolution is a new frontier.

Figure 3: The determination of the hydrogen atoms for this tetrameric complex of concanavalin A with glucose is out of range of both SR X-ray and neutron techniques. The crystals are not stable in cryoprotectant and room temperature SR undulator diffraction (ESRF Quadrigia) is only at 1.9 Å resolution. On the LADI ILL instrument with a D_2O soaked crystal and a crystal size of 4x3x2mm the neutron diffraction resolution is 3.5 Å, insufficient for atomic analysis. An optimised ESS 'Large Molecular Weight' nPX instrument could bring the 50 kDa in the asymmetric unit crystal in range to find the sugar recognition hydrogen atoms (as deuteriums) [5,6].

Recently, a new method has been developed to analyse wide angle X-ray solution scattering data up to 5 Å resolution in terms of the approximate positions of dummy amino acid residues. With neutron scattering, this approach can be extended to gain additional information about the positions of *individual* residues, either by the preparation of proteins with specifically deuterated residues or, for native samples, by making use of the change in contrast of residues during H/D exchange (e.g. after placing a hydrogenated protein in D_2O). This requires a neutron source with high flux and high dynamic range. This should contribute to the determination of the protein fold in solution from experiments on native samples and it would be an approach relevant for high throughput fold definition for proteins which do not easily crystallise e.g. detergent solubilised membrane proteins.

Extending the resolution of SANS.



SANS is also used to determine the kinetics, stoichiometry and organisation of large macromolecular complexes (viruses, molecular machines such as chaperones, etc). According to its flux specification, ESS will reduce the quantities needed for these studies by at least ten. SANS will be used to study complexes identified in ever greater numbers by proteomic analysis. The products of structural genomics (i.e. atomic resolution structures) will be combined with the SANS results to deconvolute the complexes into their functional components (Figure 4).

SANS can help to define the make-up of molecular machines.

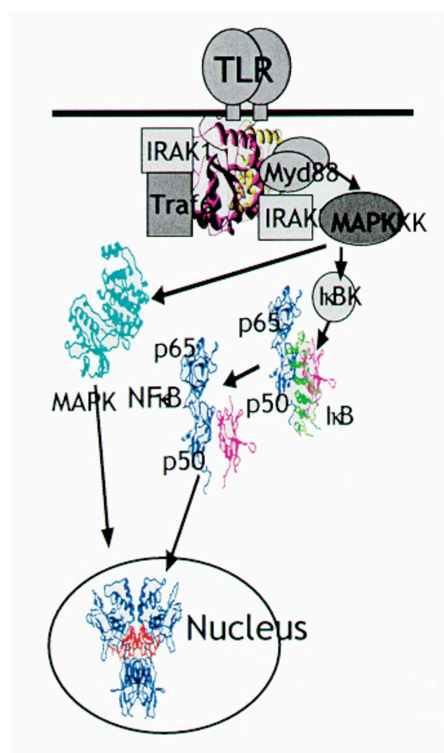
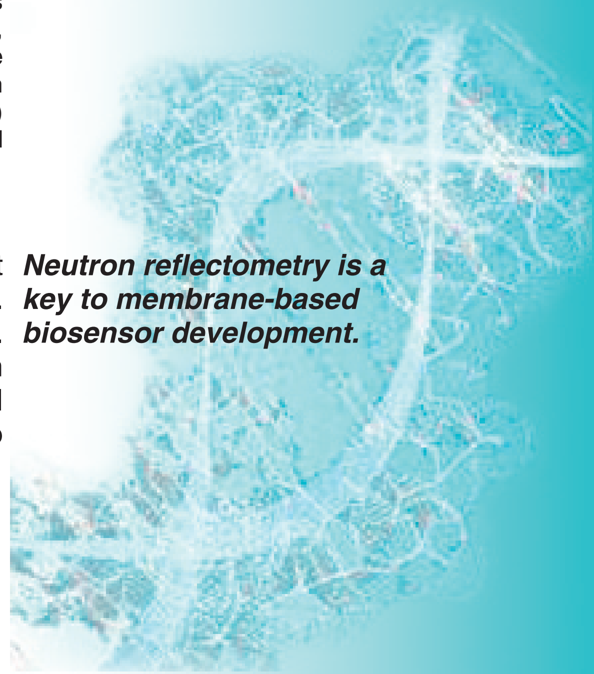


Figure 4: Schematic diagram of a typical eukaryotic cell signalling pathway. When the B cell receptor TLR2 binds its ligand it recruits a cascade of proteins in the cell cytoplasm: Myd88, IRAKs 1 and 2 and Traf6. This leads to the activation of MAPKKK and the phosphorylation and activation of the MAPkinase cascade and the IkBK signalsome. The resulting phosphorylation of IkB induces its ubiquination and degradation. Degradation of IkB results in the release of active p50/p65 components of NF-kB. NF-kB then translocates to the nucleus and transactivates immunomodulatory genes. Similarly, MAPkinase (MAPK) can translocate to the nucleus and activate transcription factors such as Elk, AP-1 and ATF-2. The resolution of structures for the constituent molecules (and their complexes) varies from zero to atomic. Neutron scattering and crystallography can help to fill in the gaps in this knowledge.

The long measuring times at current source flux levels restrict reflectometry and scattering studies to non-labile systems. Thus native membranes are not generally amenable to study. Major gains in peak and average fluxes open up such techniques to native, biologically relevant systems. This will widen the applicability of the knowledge gained to biotechnologies such as biosensors and nano biostructures.

Neutron reflectometry is a key to membrane-based biosensor development.



Taken together the above methods help create a cell model in silico; a virtual cell. The seamlessness and speed with which modern biologists can interrogate vast databases of genomic, proteomic and structural data was inconceivable to most in the field 15 years ago. How will we continue to improve the computer-user interface so that life scientists can maximise their use of these expanding reserves of knowledge? How can we ensure that all experimental data are being used, not just published? The integration of all existing databases into a virtual cell framework may accomplish this. The virtual cell will be a graphical, interactive, "functional" representation of a given cell (be it bacterial or even perhaps mammalian). The biologist will design investigations based on the response of the cell to imposed stimuli; the response will be the result of calculations made by the cell on the basis of literature data accessed from existing databanks. Neutrons should be significant contributors to the knowledge data base not only at the atomic level but more generally at the cellular and thus functional level.

The virtual cell

The role of neutrons in the virtual cell project.

Current and developing global research missions

How will the opportunities with neutrons at ESS map onto internationally agreed tasks and trends in the biosciences as well as open up new fields? In biology the post-genomic era, created by the speed and efficiency of gene sequencing, provides a huge stimulus to and the radical need for development of the structure determining techniques.

The 'business case' for ESS

New fields

It is a paradigm that structure is based on function. However this paradigm has been recast largely by neutron inelastic scattering techniques to include dynamics. Thus structure and dynamics determines function. The major gains in capability of ESS for inelastic scattering should greatly widen the applicability to many more systems.

Concerted action projects exist in structural genomics at world level. Notable cases include projects (i) in USA e.g. the Structural Genomic Program supported by the National Institute of Health (NIH) for high throughput structure determination of proteins from complete genomes (pathogens, arcahebacteria, …) and from human, mouse and other higher organisms (ii) in Europe on human proteins and also on pathogens and yeast and (iii) in Japan on various genomes including human and mouse proteins supported in particular by the Riken, the MITI and the Ministry of Education. Greatly expanded provision of synchrotron radiation beamlines for structural biology in the USA, Europe and Japan have been made for these projects and a general expansion of the field. Japan has a large new NMR park for structural genomics in Yokohama. The unique role of neutrons as a non-destructive probe of the structure and dynamics of biological macromolecules have been assessed by the USA and Japan who are now building state-of-the-art spallation neutron sources at the megawatt power level. The ESS reaches beyond even those power levels thus making a technical and scientific capability that is compelling.

Global concerted actions map well onto ESS frontier capabilities.

The USA and Japan have approved the construction of state-of-the-art megawatt level spallation neutron sources.

Finally the structure and dynamics results obtained will be applicable for industrial and biotechnology exploitation (see below).

Biological membranes are worthy of a special mention. The extreme sensitivity of neutron reflection makes it uniquely suitable for the study of labile biological structures [7,8,9]. The internal reflection at the solid/liquid interface combined with contrast variation allows the exact determination of the membrane structure and of the crucial polymer layer separating the biological membrane and the solid support. This will provide deep insight into the role of the soft polymer cushion for maintaining membrane integrity and function, crucial knowledge for the design of advanced biosensors. Moreover, using 2-D detection and in-plane Bragg scattering, there is the chance to study at molecular resolution the association and self-assembly of functional clusters in the plane of the membrane. This knowledge is crucial for the understanding of how proteins and lipids temporarily associate in a functional membrane. As a flagship experiment in this field we propose the study of native membranes and whole cultured cell layers on polymer cushioned solid substrates (Figure 5). This will permit the measurement of the cellular membrane response on the action of external stimuli (drugs, stress, pressure ...) at molecular resolution. It will also shed light on the extremely poorly understood interaction between different membrane constituents under conditions of membrane transport, ligand receptor binding and cell adhesion.

Advanced biosensors

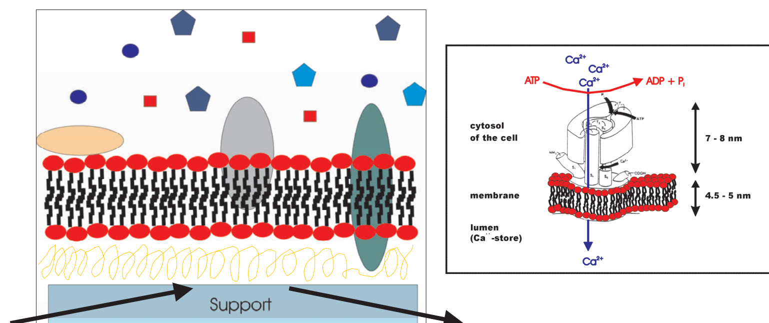
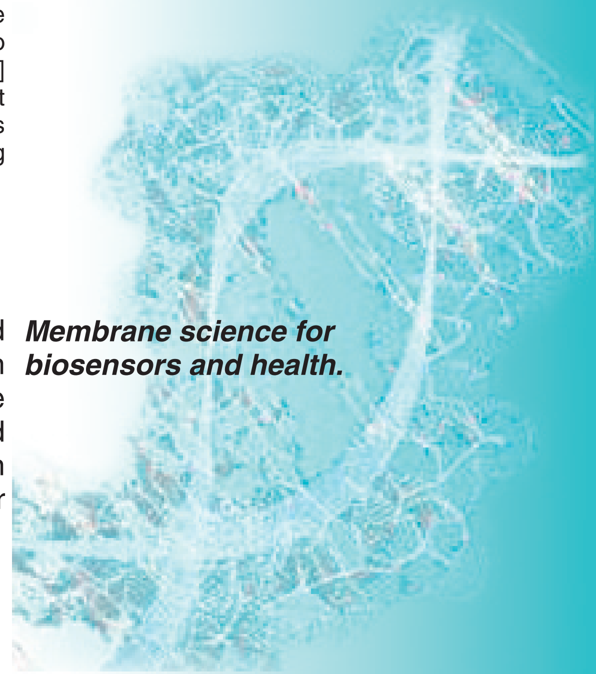


Figure 5: Neutron reflectometry. ESS fluxes will allow the study of native plasma membranes extracted from living cells deposited to planar substrates. A specially designed polymer cushion [9] between solid substrate and membrane will provide the soft interface required for keeping the membrane spanning proteins (ion channels, receptors, transporters) in their active state during the experiments.

Industry: implications in biotechnology and industry

The major advances in the fields of cell culture, softening and biocompatibilisation of solid surfaces, protein reconstitution techniques and nanostructuring methods can now provide native membrane samples of different protein and lipid composition in geometries that are uniquely suited for neutron studies. Membrane based biochips will become the key for

Membrane science for biosensors and health.



advanced biosensors, as well as screening and bioseparation devices. In particular, the combination of nanostructured semiconductor surfaces with native membranes via a soft polymer cushion represents a crucial technology for many applications in diagnostics and also in proteomics. Research and development in these fields amounts presently to $20 billion world-wide with an annual growth of 25 %.The enabling technologies that will emerge in the next years from this research will provide the tools for finding molecular markers for the early stage detection of illness and for the unravelling of the human proteome. Neutron data, if neutron developments cope with the need of the methods, on such complex systems can become a prerequisite in the design of even more advanced combinations of biological matter with solid surfaces for biochips including biosensors.

Rational drug design

The role of structural data in drug discovery in the pharmaceutical industry will increase when it is much more routine that hydrogen atoms, bound water deuteriums and the dynamics information can be incorporated. Thus the discovery of new pharmaceuticals and of enhanced efficiency compounds will accelerate. Also, because neutrons are non-destructive, unlike X-rays, room temperature structure and dynamics data can be provided.

Neutrons provide structure and dynamics data at physiologically relevant temperature.

IV. Instrument requirements at ESS

Target stations

50 Hz target is a popular choice for biology.

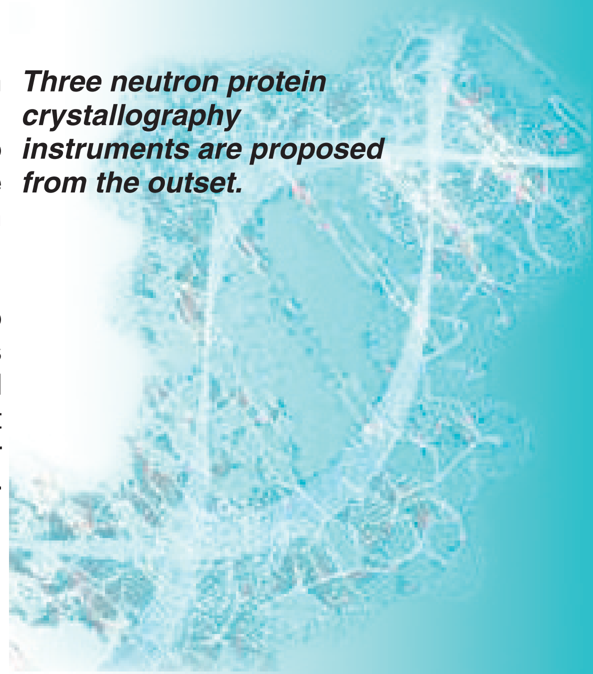
The 5 MW ESS targets are world beating. The 50 Hz short pulse target station is considered to be the most favourable choice for protein crystallography, dynamics, neutron reflection and in-plane Bragg scattering.

A SANS station on the long pulse target station should provide high flux and high dynamic range which would permit the use of smaller amounts and concentrations of material. The values of Q_{min} down to $5x10^{-4}$ and Q_{max} up to 1 $Å^{-1}$ are needed for detailing large complexes in solution and for protein fold definition, respectively.

Instruments

Three neutron protein crystallography instruments are proposed from the outset.

In neutron protein crystallography we forecast two main frontiers. The first is to considerably reduce the crystal size, including to reduce the data collection time from weeks to days, and the second frontier is to considerably extend the molecular weight capability to study complexes at high resolution (Figure 3, [5,6]). In the first case a brighter neutron source, well-focussed beams and smaller-pixel detectors will be in the design. To meet the second challenge we have to continue to harness the expertise of the crystallogenesis community to produce big crystals. Thus larger beams and bigger-pixel detectors are needed. This is a different instrument altogether. Also we should harness longer wavelengths to enhance the scattering-efficiency-with-



wavelength-effect as well. A methane moderator tailored to wavelengths 1.5 to 5 Å is being investigated. A third instrument should be developed at ESS for contrast variation at low resolution to dissect the intermolecular interactions in membrane crystals so as to better understand such crystallogenesis cases. There is a need here because the best current source, ILL, still has long measuring times (months). ESS will bring a benefit but perhaps demand will be low. Perhaps this third instrument could be shared with a high resolution SANS beamline.

High throughput SANS needs high flux.

In SANS we forecast an increase in the number of experiments that will be undertaken, not least because of genomics and initiatives in high throughput samples production, but also where experiments in solution will be at a premium (over techniques like crystallography where crystallisation is a recognised bottleneck to high throughput). The range of SANS experiments, tailored to molecular weight ranges that will be encountered, will be wide. Also the harnessing of as high an intensity as possible, to reduce the quantity of sample and the concentration needed, will be very important. Thus the long pulse target station in particular offers extra opportunities for realising such intensity gains for SANS.

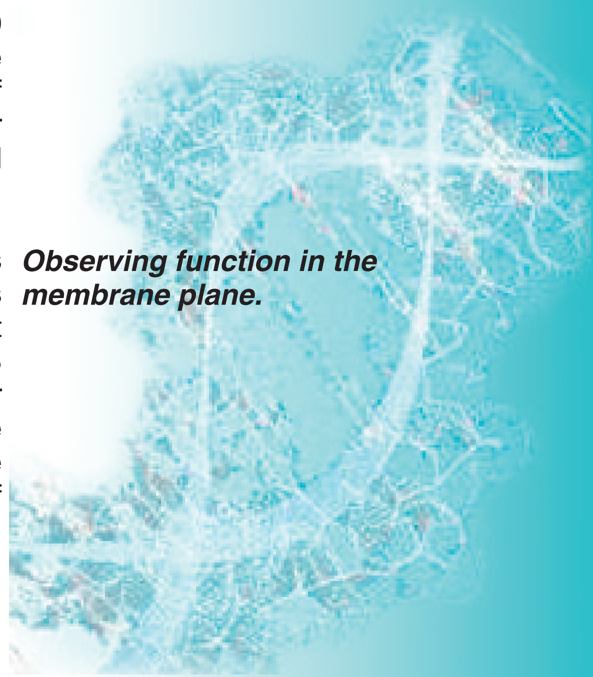
Molecular dynamics served by the 50 Hz target station.

In dynamics experiments the instrument and target options need to match the time-space windows of interest. These are from the picosecond to the nanosecond or longer timescales, and from 1 to 10 Å length scales. Gains with respect to present ILL instruments will be > 100 at ESS. The 50 Hz target station is appropriate for these instruments. Instrumental priorities are for the variable resolution cold neutron chopper, multichopper spectrometers, the 1.5 μeV backscattering instrument and an 8 μeV back scattering instrument with large Q range (up to 5 $Å^{-1}$, similar to IN13). As a benchmark the ESS 8 μeV instrument will be > 500 x IN13 performance at ILL. These instruments would cover the elastic, quasi-elastic and inelastic resolutions implicated in the dynamics of biological systems of various sizes, from diffusing water molecules to domain motions in large complexes. Neutron spin echo (NSE) may in principle present interesting applications for studying slow coherent diffusive motions, but an optimistic estimate is that a gain of a factor of at least 100 on present ESS instrument conceptual designs would be required. The potential to use NSE to study the dynamics of small drug molecules or hormones in the context of their biological interactions should nevertheless be studied and assessed.

Matching the energies of life.

Observing function in the membrane plane.

The membrane and cell surface projects identified in sections I-III are largely not feasible with today's reflectometers because of the flux which requires accumulation times that are long for labile biological structures. The planned ESS reflectometer with its more than an order of magnitude higher flux will for the first time enable such studies. The desirable Q-range for these types of studies is up to 0.5 $Å^{-1}$ while resolution is not essential. Crucial will be the availability of



2-D detection and the option to measure Bragg scattering in the membrane plane.

Samples production is very important.

Sample production is as important as the source and the instrument for biology, especially in order to properly exploit the unique ability of neutrons to distinguish between major biological components. We must be able to produce and to label the constituents of our systems, be this individual atoms within a protein, proteins within a complex or complexes within a cell. Current, commonly-used, overexpression systems must be optimised for growth in deuterated media or in minimal media supplemented with deuterated amino acids (or sugars, co-factors etc). This must include not just the work-horse of protein production, *E. coli*, but also yeast, insect and mammalian cell systems. The future of molecular life sciences demands that we are able to successfully overexpress, purify and characterise fully post-translationally modified proteins. Further, if we are to achieve broad coverage of the cellular world in the virtual cell program, identified in section II, we must be able to characterise these cells in their entirety.

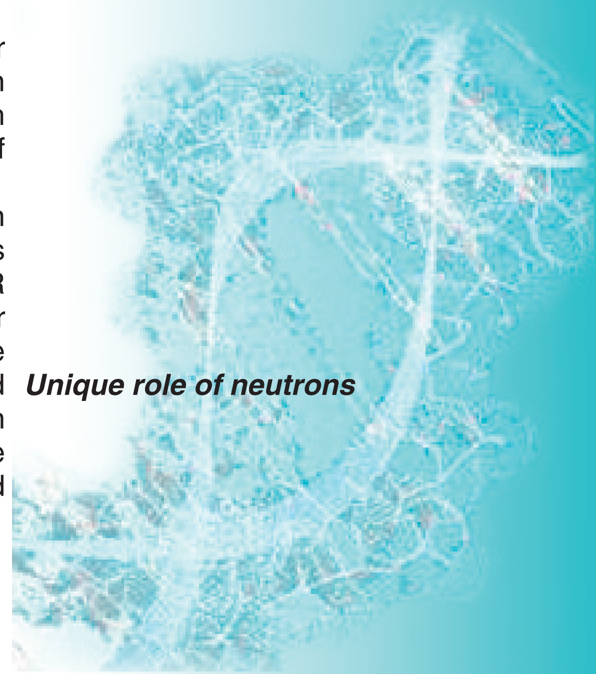
Labelling biomolecules

Multi-dimensional studies

Successful characterisation of biological systems depends on data from several orthogonal, complimentary studies. Increasingly, in order to ensure the reproducibility of conditions under which a system is studied and correspondence of the data so obtained, these studies are being carried out simultaneously. This is also efficient in terms of experimental time - a key factor in high-throughput post-genomic studies. Thus, in order to make a significant and rapid contribution to the databanks of the virtual cell (or even those in use currently) SANS can be coupled to capillary electrophoresis. Proteins within a cell extract will be separated electrophoretically in quartz capillaries as they flow slowly past an orthogonal, non-destructive neutron beam. Their meso-resolution structures will be restored automatically (using programs which will have evolved from DAMMIN, GASBOR etc [2,3]) and related directly to the electrophoretic band (and the identity of the protein obtained from MALDI-TOF analysis of digestion fragments thereof).

The combination of neutron reflectometry with either fluorescence microscopy (single molecule), plasmon spectroscopy or infrared spectroscopy can provide much additional information, in particular for the assessment of biomolecule interaction from the bulk with the membrane.
Neutron reflectometry is a natural partner for surface plasmon resonance (SPR) studies, although currently the timescales for these experiments are rather different. Nonetheless, SPR gives crucial information on the on- and off-rates of molecular interactions (even within deposited model membranes). The coupling of NR and SPR would permit the through-bilayer and in-plane structural characterisation of the equilibrium system (once binding saturation or de-saturation is reached). The design of a new reflectometer on a high intensity ESS should

Unique role of neutrons



be integrated with other biologically relevant and complementary techniques in a single setup.

Complementary aspects

There are of course other structure definition techniques such as X-ray crystallography and scattering, NMR, electron microscopy, mass spectroscopy, atomic force microscopy and light scattering. Data obtained with these techniques present key data to describe biological molecules and their complexes at different levels of detail. Neutrons provide a unique role in diffraction and inelastic scattering as a probe of biological structures because of the near equal scattering lengths of deuterium, carbon, oxygen and nitrogen and for dynamic measurements because the momenta of neutrons are matched to atomic vibrations. The additional possibility for harnessing the negative scattering length of hydrogen of course makes the unique contrast variation approach feasible. Thus the results provided by small angle neutron scattering are highly complementary to other analysis techniques (X-ray scattering and diffraction, electron microscopy, analytical ultracentrifugation).

Adding movement to static structures.

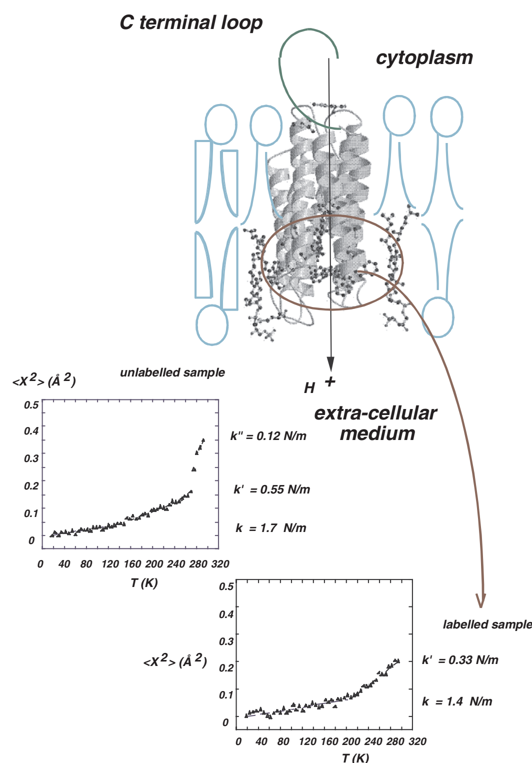


Figure 6: Dynamics-function relation in bacteriorhodopsin, the light driven proton pump in purple membranes, characterised by neutron scattering and specific isotope labelling of amino acids in the active core of the protein. An effective force constant, <k'>, for each part of the structure was calculated from the mean square fluctuations measured as a function of temperature. The protein, globally, is quite soft at physiological temperature with a <k'> value of 0.12 N/m. The core, however, is significantly stiffer with a <k'> value of 0.33 N/m, suggesting it ensures a valve function in the pump mechanism [10]. For a review see [11].

The structural models built on the basis of neutron scattering data and all other presently available methods allow to incorporate information from different high and low resolution techniques and possibly also reconcile divergent models. As a demonstrative example we can cite the determination by inelastic neutron scattering of the role of vibrational states in the function of the bacteriorhodopsin membrane proton pump (Figure 6), for which a high resolution structure is known.

V. Summary

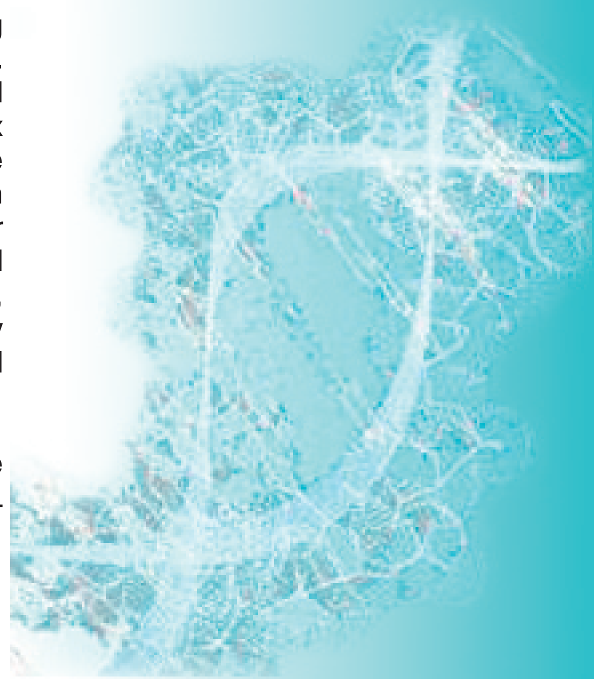
Biology research at the structural level is expanding enormously. Vast numbers of structures will bring encyclopeadic power to help understand biological function. The 'toolbox' of methods involves complementary structural probes (X-rays, neutrons, electrons and NMR).

Many biological mechanisms involve hydrogen transport or transfer and/or the role of bound water molecules.

Neutrons are pre-eminent in their capabilities to yield a complete structure of a biological macromolecule i.e. including **all** of the ordered atoms in a sample, including hydrogens. This is without radiation damage and thus can be at physiologically relevant temperatures. Small but significant differences in structure at the cryotemperatures routinely employed with SR X-ray crystallography are seen versus room temperature. Multi-temperature structure results are also then a necessity. Could neutrons yield these results routinely? The low flux of neutron research reactors, even the ILL, and the very low flux of the currently most powerful spallation source ISIS prevents this. At ISIS the flux is so low that very little biological macromolecular structure work is undertaken. In Europe biology use at all neutron sources is a mere 4 % of the total whereas at ILL it is 12 % - 14 %. There is therefore a flux threshold effect. A 5 MW ESS offers substantive gains in measured signal to background via time-of–flight methods. Biology's share of neutron facility provision would get a big boost from a 5 MW ESS with gains of up to 30 (depending on unit cell size) for neutron protein crystallography over the ILL.

A further unique opportunity of neutrons is selective labelling and contrast variation using hydrogen/deuterium exchange. These techniques are extremely effective in SANS and reflectometry to localise specific fragments in complex structures (e.g. nucleoproteins and protein-membrane complexes). A 5 MW ESS, yielding a gain of more than an order of magnitude for reflectometry and up to two orders for SANS, would allow one to perform qualitatively novel experiments (e.g. labelling of individual residues in proteins, analysis of macromolecules, which are only soluble at very low concentrations, and detailed structure of native biological membranes from off-specular reflectivity).

A special place for neutron capability is in its exquisite sensitivity to dynamics of atoms. The pioneering works at ILL

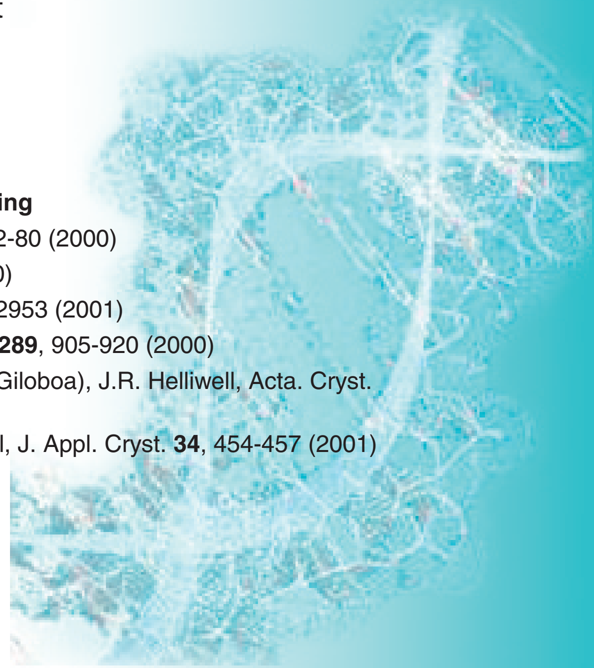


on bacteriorhodopsin for example show that it is structure and dynamics that determines function. The 5MW ESS instrument gain factors are large. Thus such gain factors offer extension to larger time domain vibrations. Hence, as biosimulation methods are extending their simulation times towards the microsecond domain, the reality checks' of experimental data would come only from ESS inelastic neutron scattering results.

Overall, if biology, and biotechnology, is to fulfill its promise of being the subject of the 21st century then Facilities provision for the determination of the structure and dynamics of biological macromolecules must be extended. Neutrons offer a unique role and the R&D preparation for ESS is very mature. ISIS, building on the Japanese source work, provides the platform technically. Biology at ILL is showing what can be accomplished with a regular stream of examples: enzyme crystal structures with key hydrogen positions, bound water structure in protein ligand recognition studies, first time-resolved SANS of the chaperonin structural intermediates, the dynamical basis of the proton transfer mechanism in bacteriorhodopsin, reflectometry studies of biomembranes, to name a few. ESS would greatly extend this output.

Because it will provide drastically improved specifications, ESS can and should be an actor in the development of structural proteomics in Europe. In this intensive context, it will bring, with SANS, complementarity and unique tools for the characterisation of samples. Furthermore, it will contribute to the comprehension of the dynamic of biological systems and to the determination of structures at all levels of resolution including atomic resolution, to the identification and location of light ions, water and hydrogens atoms, in the case of molecules and integrated complexes (proteomes, transcriptomes, viruses, with a special mention to membrane proteins). This will be achieved by taking advantage of the progress realised in the field of recombinant proteins in bacteria, eukaryotes and cell free systems for expression of labelled molecules.

We are grateful to Dr. Patricia Bassereau and Dr. Jon Cooper for their careful reading and helpful suggestions on the text at the ESS Dourdan Workshop March 2002.



Specific references for the figures and a few examples for further reading

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Achievements of neutrons in biology and biotechnology

- The positioning of all the 21 proteins in the 30S subunit of the ribosome of E. coli, as well as a map of protein-RNA distribution in the entire 70S ribosome using small angle neutron scattering (SANS).
- The organisation of nucleic acid and protein in spherical viruses using SANS and low resolution neutron protein crystallography.
- Characterisation of nucleic acid protein interactions fundamental for gene regulation and expression i.e. in nucleosomes, aminoacyl-tRNA synthetase, DNA dependent RNA polymerase using SANS.
- Characterisation of intermediates in the functional cycle of the chaperone protein complex GroEL/GroES using SANS.
- Determination of function-critical hydrogen positions in enzymes, for example aspartic proteinase using crystallography.
- Determination of solvent organisation in proteins using crystallography (such as concanavalinA and myoglobin) and other biomolecular systems such as DNA and cellulose using fibre diffraction. Dynamics of labile hydrogens in different parts of lysozyme and other proteins. Orientations of ring systems and methyl rotors.
- Characterisation of single and multilayers (adsorbed proteins) at the air/water and oil/water interface using reflectometry.
- Detailed structure of the lipid bilayer using reflectometry and biological membranes using diffraction.
- Determination of the protein-detergent distribution in membrane protein crystals, and the localisation of lipid in a lipoprotein using low resolution crystallography.
- Characterisation of a dynamical transition associated with the solvent environment in myoglobin and other proteins using inelastic scattering.
- Characterisation of dynamics correlated with function in purple membrane using inelastic scattering. Structural information on this photoactive membrane, including hydration properties, localisation of the retinal chromophore in bacteriorhodopsin (the membrane protein), the positions of glycolipids and the structure of trapped intermediates in the photocycle using diffraction and selective deuteration.
- Characterisation of dynamics-stability relations in proteins from extremophile organisms (adaptation to extreme environments) using inelastic scattering.