

## New sources and instrumentation for neutrons in biology

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### Abstract

Neutron radiation offers significant advantages for the study of biological molecular structure and dynamics. A broad and significant effort towards instrumental and methodological development to facilitate biology experiments at neutron sources worldwide is reviewed.

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## 1. Introduction

Biological applications at neutron research facilities are currently increasing significantly due to new developments in instrumentation, dedicated infrastructure and tailored samples. Neutrons penetrate deeply into biological material while distinguishing between isotopes, in particular hydrogen and deuterium. Neutron beams are unique in having wavelengths and energies that correspond, respectively, to atomic spacings or fluctuation amplitudes and excitation energies, and present negligible absorption even for relatively long wavelengths. Neutrons are therefore a unique non-destructive probe. The low flux of neutron sources compared to X-ray sources is compensated to some extent by much larger beam cross sections and wavelength spreads. However, neutron experiments still require correspondingly larger sample amounts and sizes, which may be prohibitive given the complexity of certain systems under study.

We describe in the review a number of recent and upcoming instrumental developments taking place at neutron research centres designed to facilitate biological applications. It is not a review of past neutron applications in biology (see for example [1]), and the examples of source and instrumental developments given here are only representative of current trends (for more complete information, see for example [2,3]).

## 2. Sources

In recent years, there have been major developments for neutron scattering facilities throughout the world. Existing sources are constantly being upgraded while others are being built and new ways to broaden the energy spectrum and the intensity of neutron beams are tested at a pilot scale to evaluate the effectiveness, safety requirements, costs and overall feasibility. Reactor-based fission sources have been providing neutron beams for more than 60 years and are still the majority among neutron sources. Despite the fact that the last 40 years have only seen a factor of 10 increase in the neutron source brightness [4], significant upgrades in neutron productivity have happened through the installation of hot and cold sources, neutron guides and various developments in instrumentation to match source technology. It is far beyond this review to detail these developments, but new sources in particular have benefited from these upgrades.

One of the newest neutron sources is the multi-purpose OPAL [5] reactor at the Australian Nuclear Science and Technology Organisation (ANSTO), where the low enrichment core is surrounded by a heavy water moderator/

reflector vessel with five neutron beam tube assemblies. The present facility has space for 18 instruments (4 at the reactor face and 14 in the neutron guide hall) and the initial suite will be 9 instruments, 3 of which will be useful for biology research – the reflectometer, the small angle neutron scattering (SANS) instrument and the single crystal diffractometer. The reactor and the neutron scattering instruments are in the final stages of commissioning and the user program will commence in 2008. For the future, the facility is designed to accommodate another thermal guide and another cold guide, a hot neutron source and a second similar neutron guide hall.

The FRM II research reactor in Munich, Germany, has been in full user operation since 2005 and includes at present 20 instruments available for users and distributed over two buildings: the experimental hall and the neutron guide hall west. FRM II is based on a compact core containing a single cylindrical fuel element installed in a heavy water moderator tank, equipped with several secondary sources [6]. These shift or convert the thermal neutron energy spectrum of the heavy water moderator into different energy regions. A hot neutron spectrum from 100 meV to 1 eV emerges from a block of graphite being heated by the gamma radiation of the core to a maximum temperature of about 2300 K. Of special interest for instruments with biological applications is the cold source, placed 40 cm from the reactor core axis; it provides a broad range of long wavelength output.

At the Oak Ridge National Laboratory (ORNL; USA), an upgrade of the high flux isotope reactor (HFIR) includes an installation of a new cold source, construction of a new guide hall, and the commissioning of two new SANS instruments. The new facilities will offer gains in performance, capacity and capability that will benefit not only traditional user communities in the physical and material sciences, but will also significantly extend the number, size and complexity of biological systems that are accessible to neutron scattering analysis.

Other neutron facilities, such as the Hahn-Meitner-Institute (HMI) in Berlin (Germany), the Laboratoire Leon Brillouin (LLB) in Saclay (France) or the NIST Center for Neutron Research in Gaithersburg (USA) also continuously develop and upgrade their sources and instrumentation.

Spallation neutron sources utilize a proton beam, generally pulsed, to knock out physically or ‘spall’ neutrons from a heavy metal target. Historically spallation sources like KEK (Japan), LANSCE (USA), and the recently closed IPNS (USA), have had lower integrated neutron fluxes than reactor sources and tend to generate shorter wavelength neutrons. However, they have much higher

peak fluxes and were at the origin of the development of very powerful time-of-flight instruments. The peak flux at the ISIS spallation facility at the Rutherford Appleton Laboratory in Oxfordshire (UK) has been comparable to the average neutron flux at the ILL.

Spallation sources are in continuous development. In 2006, for example, a liquid metal target with eutectic lead–bismuth target material was tested in an international Megawatt Pilot Experiment (MEGAPIE) collaboration, at the Paul Scherrer Institut (PSI) over four months to demonstrate the feasibility of such a target for spallation facilities at a beam power level of 1 MW. During this experiment [7], the SINQ continuous spallation source – the first of its kind in the world – experienced a significant increase of the cold and thermal neutron flux available for users. At present, SINQ [8] (PSI) is the only steady-state spallation source in operation.

With the reactor sources having reached their neutron flux limits (imposed by the amount of heat that can be removed from the reactor core) and the better sociological and environmental acceptance of spallation sources by the general public, it would seem that the future development of neutron scattering facilities lies with accelerator based sources.

ISIS is well established with a suite of high-performance instruments that exploit the pulsed nature of the neutron beam. A second Target Station [9] (TS-2) is being developed to generate a high flux pulsed neutron beam by taking one in five proton pulses from the existing ISIS synchrotron. The design of TS-2 will yield at least an order of magnitude increase in performance for large scale structure instruments, and low energy and high resolution spectrometers in comparison to current instruments at ISIS. The relatively low power of the beam allows highly efficient solid methane moderators to be used to generate a high flux of low energy, ‘cold’ neutrons of wavelength 1–20 Å. A low repetition rate means that a wide range of wavelengths can be used by time-of-flight (TOF) instruments providing either simultaneous access to unprecedented momentum transfer ( $Q$ ) ranges (2–4 times greater), very low energies, or very high energy resolution. For structural studies this makes it possible to probe a wide range of length scales simultaneously.

Very challenging projects are the new MW-class ORNL Spallation Neutron Source (SNS) at ORNL, designed to provide several orders of magnitude performance improvements across 24 new beamlines compared to most currently available instruments, and the Japan Proton Accelerator Research Complex (J-PARC). The latter includes an intense spallation neutron source facility (JSNS; for a more detailed description see [10]) that promises to deliver intensities at least an order of magnitude higher than those at traditional nuclear reactors such as JRR-3M (Japan Atomic Energy Agency). Both JSNS and SNS use liquid spallation targets, which are prompting further developments in the design and type of targets for neutron sources.

Table 1 shows a brief comparison of the characteristics of neutron sources (for more information on these and

other sources see for example [2] or [11]) where there are biological applications.

In terms of future projects, the European Spallation Source (ESS), for example, is designed to prolong the availability of high quality neutron beams to present and future generations of European scientists. The ESS will also profit from close contact with the SNS and J-PARC efforts in order to build a more powerful source, including two 5 MW target stations. More details of the ESS project are available on the internet [12].

Despite the clear bias towards spallation neutron sources in the next 5–10 years, there are other plausible directions for neutron sources. Preconceptual design studies [11] of next-generation-reactor-sources using particle fuel in a bed cooled by water at high pressure have indicated that the flux that could reach  $10^{19}$  neutrons  $\text{cm}^{-2} \text{s}^{-1}$ .

Laser inertial fusion has also been suggested [4] as a future pulsed neutron source, in which a sequence of very short laser-light pulses ignites a small pellet of D-T fuel, producing a short pulse of neutrons. The technical complexity has however been a strong argument against the expected factor of 2–3 potential gain over optimized third generation spallation sources [13].

Obviously, neutron research centres do not attain excellence based on the quality of the source alone, and this cannot be reflected by the numbers in Table 1 alone. Among a number of other factors determining the choice of source to use in biological studies, the efficiency of neutron transport is essential to reach a high flux of useful (in the desired wavelength range) neutrons for the individual instruments while eliminating undesired neutrons (fast neutrons that contribute to the background) as soon as possible along the flight path. Current developments in the construction of guide systems (such as the ones included in the ILL Millennium programme [14]) in terms of guide dimensions and supermirror coatings have played a major role in lowering background signal, vital for biological samples in which the amount of material is often limited and the scattering power is low.

Finally, regardless of the strengths and weaknesses of each new development, the complementarity of different neutron centres, as well as different instruments worldwide remains the most impressive development of all: the neutron research community as a whole is an indispensable asset in biological studies.

### 3. New instrument developments

In the current post-genomic era biologists are faced with an overwhelming number of new systems that need to be studied at the molecular level. There is an important deficit of structural and dynamic data to support a better understanding of the mechanisms and functions involved, often for molecules that are drug targets or have important pharmacological or technological applications. The pressure to improve the current structural biology techniques and methodologies is high. A number of developments are

ongoing at neutron research facilities, with new instruments being built for current sources and others already planned for sources under construction.

### 3.1. Biological macromolecular structures in solution and at low resolution: SANS, low and wide-angle diffraction

The SANS technique has been extensively used to characterize nanostructures and hierarchical structures of materials ranging from 1 to 100 nm, in the fields of materials and the life sciences. Protein–surfactant interactions, light-induced structural changes in pea thylakoids, the solution structure of human proliferating cell nuclear antigen (PCNA), biomineralization (exploring composite nanoparticles consisting of an inorganic mineral and a biological molecule as protein [15,16]), are amongst many examples at the limit of SANS studies. Recent progress in nanotechnology and research in complex multi-component, multi-phase and non-equilibrium systems require however the limitations of the technique to be pushed forward, with higher structural and time resolution.

Contrast variation [17] has had a major impact on the range of application of SANS techniques: the relative scattering power (and hence the contrast) is varied with respect to the structure being studied, making it possible to highlight internal features of the sample. The bulk neutron scat-

tering characteristics of proteins, nucleic acids, lipids and carbohydrates all differ significantly from one another [18]. This natural contrast difference between biomaterials is therefore exploited to locate individual components in functional biological structures or assemblies, such as in protein–nucleic acid complexes and membrane proteins (see for example [19–23]). The addition of specific isotope labelling of biological macromolecules further opened the technique to protein–protein complexes that would normally lack contrast between the subunits [24,25].

Traditional SANS instruments on reactor sources use a single wavelength of neutrons with a wavelength spread of about 10%, which is continuously scattered onto an area detector. Performance is then largely determined by absolute flux and the scattering wave vector modulus ( $Q$ -range) that can be collected in one shot which depends on the wavelength selected and the detector position. SANS instruments have been installed at most large and small neutron facilities all over the world. The two SANS instruments with highest flux at sample position are operated at ILL, named D11 and D22. Further instruments are used by biologists at HMI, LLB, the Budapest Neutron Center, at NIST, SINQ and elsewhere routinely.

At ILL, in addition to D11 – the paradigm SANS camera in operation since the beginning of the institute – the SANS instrument D22 went into operation in 1995. With

Table 1  
Brief comparison of some characteristics of the current and near-future neutron sources

Neutron source	Description	Peak thermal neutron flux (neutrons cm <sup>-2</sup> s <sup>-1</sup> )	Time-averaged neutron flux (neutrons cm <sup>-2</sup> s <sup>-1</sup> )	Frequency (Hz)	Thermal power (MW)
HFR/ILL	Fission Source. Enriched <sup>235</sup> U (93%); heavy water moderator; hot, thermal and cold sources.	150 × 10 <sup>13</sup>	150 × 10 <sup>13</sup>	continuous	58.3
HFIR/ORNL	Fission source. Enriched <sup>235</sup> U; light water moderator; thermal and cold sources.	130 × 10 <sup>13</sup>	130 × 10 <sup>13</sup>	continuous	85.0
FRM II	Fission source. Uranium silicide–aluminium dispersion with <sup>235</sup> U enrichment (93%); heavy water moderator; hot, thermal and cold sources.	80 × 10 <sup>13</sup>	80 × 10 <sup>13</sup>	continuous	20.0
OPAL/ ANSTO	Fission source. Low-enriched <sup>235</sup> U silicide plates; heavy water moderator; thermal and cold sources.	40 × 10 <sup>13</sup>	40 × 10 <sup>13</sup>	continuous	20.0
ORPHEE/ LLB	Fission source. Aluminium–uranium alloy, the latter enriched in <sup>235</sup> U; heavy water moderators; hot, thermal and cold sources.	30 × 10 <sup>13</sup>	30 × 10 <sup>13</sup>	continuous	14.0
BER II HMI	Fission source. Low-enriched Uranium; thermal and cold sources.	11 × 10 <sup>13</sup>	11 × 10 <sup>13</sup>	continuous	10.0
SINQ/PSI	Spallation source. Lead rods; liquid deuterium moderator; thermal and cold sources	12 × 10 <sup>13</sup>	12 × 10 <sup>13</sup>	continuous	0.83
TS-1/ISIS	Spallation source. Tungsten plates; light water moderator, 100 K methane moderator, 20 K hydrogen moderator; thermal sources.	4 × 10 <sup>15</sup>	1.0 × 10 <sup>13</sup>	50	0.16
TS-2/ISIS Under construction	Spallation source. Tungsten rod; coupled hydrogen/solid-methane moderator, decoupled solid methane moderator, full solid beryllium reflector; thermal and cold sources.	2 × 10 <sup>15</sup>	0.2 × 10 <sup>13</sup>	10	0.048
JSNS/J-PARC Under construction	Spallation source. Flowing mercury system; supercritical hydrogen moderator; thermal and cold sources.	1.2 × 10 <sup>17a</sup>	5 × 10 <sup>13a</sup>	25	1.0 <sup>a</sup>
SNS/ORNL	Spallation source. Flowing mercury system; cryogenic hydrogen moderator; thermal and cold sources.	1 × 10 <sup>17a</sup>	10 × 10 <sup>13a</sup>	60	2.0 <sup>a</sup>

<sup>a</sup> Projected values.

a maximum continuous flux at the sample position of over  $10^8 \text{ cm}^{-2} \text{ s}^{-1}$ , it is particularly suited to biological applications. With the planned construction of a third SANS instrument, D33, it is planned to identify specific subject areas for the 3 instruments. While all instruments will maintain a capability across a broad range of science, biology will be the main task for D22, while D11 and D33 will specialise in polymer and colloid science, and magnetism, respectively. In 2004, D22 was equipped with a new fast 2D detector, allowing one to count over 2 MHz of neutrons with only 10% dead-time losses. The allowed count rate no longer limits the acquisition. For this reason, samples containing a large amount of hydrogenated material giving very high background counting rates can be measured in reasonable times, and time-resolved measurements (e.g. of the kinetics of the reaction) of small biomolecules have become feasible. Although D22 uses reactor neutrons, the large size of the detector ( $1 \text{ m}^2$ ) together with the possibility of shifting it sideways allows D22 to cover a simultaneous  $Q_{\text{max}}/Q_{\text{min}}$  ratio of 20, which is usually sufficient for indirect Fourier transformation of the data and for model fitting, including by *ab initio* methods. The high flux of monochromatic neutrons on D22 also turns out to be of interest for the exploration of cancer treatments using neutron-induced secondary radiation from isotopes that can be administered in the form of biocompatible nanoparticles.

At SINQ, the small angle neutron instrument SANS-I [26] covers a  $Q$ -range up to  $10.5 \text{ nm}^{-1}$  for the detector displaced laterally by 50 cm (for structures ranging from about 1 to 400 nm). The instrument offers time resolutions down to a few milliseconds, achieved using a stroboscopic (cyclic) experimental set-up. Similar SANS instruments are under operation at HMI and NIST. After the shutdown of the Jülich research reactor FRJ-2 in May 2006, two SANS instruments were moved to the FRM II facility in Garching/Munich: KWS1 and KWS2. New elements were implemented on both instruments, such as a chopper in front of the collimator for adjusting a variable  $\Delta\lambda/\lambda$  wavelength distribution, in combination with a time-of-flight option between a few percent and that of the selector. This option allows an operation in the so-called pulsed beam technique, TISANE, to measure fast cyclic kinetic processes in the order of milliseconds or even less [27]. An additional high resolution detector in front of the conventional SANS detector permits high resolution experiments at finite  $Q$  (ordered structures of mesoscopic characteristic length) as well as an extension of the  $Q$  range to the order of  $10^{-4} \text{ \AA}^{-1}$ , when combined with an adjusted  $\Delta\lambda/\lambda$  and focusing achieved with the chopper and neutron lenses, respectively. KWS1 will have a velocity selector with  $\Delta\lambda/\lambda$  wavelength distribution of 10%, as well as polarised neutrons to avoid the high inherent background from incoherent scattering in aqueous solutions (while allowing for an intrinsic absolute calibration of the scattered intensity). To study kinetic processes, KWS2 was optimized for high neutron flux, using a velocity selector of 20%  $\Delta\lambda/\lambda$ . Up to two orders of magnitude increase of flux are expected by

comparison with the former conditions at FRJ-2. This gain will be achieved by the higher neutron flux of FRM II in combination with the implementation of neutron lenses, larger neutron guide area, and a properly adjusted wavelength distribution. A new small angle instrument, called SANS-1, is under construction at the FRM II as a joint venture between the Technische Universität München and the Geesthacht Neutron Facility (GENF). A vertical S-shaped neutron guide, a tower with two possible velocity selectors (one for medium resolution at high intensity and one for high resolution), two optimized transmission polarisers and a large  $1 \text{ m}^2$  detector will be the main advantages of this instrument [28].

At ORNL, the Centre for Structural Molecular Biology is also constructing a Bio-SANS instrument as part of the HFIR Scientific Facilities Upgrades Project. Bio-SANS and its sister instrument, the 40 m high resolution SANS, will use a horizontal beam located on a high-performance cold source that utilizes 505 mL of supercritical hydrogen, expected to provide usable neutron fluxes out to a maximum wavelength of 30 Å. Bio-SANS uses a fixed guide system designed to minimize fast neutron and  $\gamma$ -ray background at the sample and detector positions. A super-mirror arrangement removes neutrons with wavelengths shorter than 6 Å. A velocity selector provides a wavelength resolution of 8–45%. The sample area has a 2 m diameter footprint, making it suitable for a very broad range of sample environments ranging from traditional liquid cells to large-footprint high-field magnets. The instrument is capable of measuring momentum transfers of  $0.002\text{--}1.0 \text{ \AA}^{-1}$ . In addition to these instruments, the EQ-SANS instrument is currently being installed at the SNS. With an additional high-angle detector, this instrument is expected to become operational in 2008 and cover a  $Q$ -range of  $0.004\text{--}10 \text{ \AA}^{-1}$ .

A 40 m pin-hole geometry SANS instrument, QUOKKA [29] (named after a small Australian marsupial), is being commissioned on the OPAL reactor at ANSTO. The instrument design includes features of D22 at the ILL and the two 30 m SANS instruments at NIST Centre of Neutron Research (NCNR). The instrument is positioned at the end of a cold neutron guide, with a variable resolution velocity selector and a four position collimator (guide sections or apertures) to deliver a neutron beam at the sample position with maximum size  $50 \times 50 \text{ mm}^2$ . Neutron polarisation and focusing lens options can be inserted into the collimation system. The wavelength range is 4.5 Å to beyond 20 Å with a wavelength spread 8–21%  $\Delta\lambda/\lambda$ . The instrument is equipped with a  $1 \text{ m}^2$  detector which can be offset by 50 cm to increase  $Q_{\text{max}}$  to  $\sim 1 \text{ \AA}^{-1}$ . The  $Q_{\text{min}}$  is  $\sim 0.0008 \text{ \AA}^{-1}$  with focussing optics (at a specific wavelength) and  $\sim 0.0015 \text{ \AA}^{-1}$  for other wavelengths, with flux up to  $10^8 \text{ neutrons cm}^{-2} \text{ s}^{-1}$ . Sample environments include multiple sample changer with temperature control of 50–250 °C, a 5 T horizontal cryomagnet, a stopped flow cell, a high pressure cell (300 MPa) and a Couette geometry rheometer.

To cover a larger  $Q$ -range in particular towards smaller  $Q$ -values at FZ Jülich a novel focussing SANS instrument termed KWS-3 was developed which has been moved and installed recently at the FRM II facility. Using a toroidal mirror to focus the beam at the detector a  $Q_{\min}$  of down to  $10^{-4} \text{ \AA}^{-1}$  is achievable with exceptional  $Q$ -resolution. This allows measurements on large aggregates up to several hundred nanometers, overlapping with dynamic light scattering measurements. Similar focusing for SANS instrumentation is being deployed at HMI.

At ISIS, the key to the future SANS2d instrument performance at TS2 lies in having two independently movable  $1 \text{ m}^2$  detectors. In combination with the wide simultaneous  $Q$  range offered by the neutron spectrum, the two detectors mean the instrument can be configured to probe the entire  $Q$ -range of the instrument from  $\sim 0.001 \text{ \AA}^{-1}$  to more than  $2.5 \text{ \AA}^{-1}$  simultaneously. Conversely if the detectors are brought closer together a smaller  $Q$ -range is probed but with the detectors covering a greater solid angle data can be collected more rapidly. The strength of SANS2d will lie in this flexibility, making it possible to optimize the count rate for the required  $Q$ -range. Even with the detectors at the same distance from the sample the ratio of  $Q_{\max}$  to  $Q_{\min}$  will be  $\sim 200$ . SANS2d will therefore be particularly suited to experiments requiring large  $Q$ -ranges or involving unstable samples. These might include studies of large macromolecular complexes or other systems with widely differing length scales, such as membrane proteins within lipid vesicles. SANS2d will not compete with instruments like D22 at the ILL [30] for raw flux but for specific experiments, particularly where a wide  $Q$ -range is critical, it is expected to be world leading, offering lower counting times and better  $Q$ -resolution. It will be the first SANS instrument to be built with two small angle detectors, a configuration that is expected to become more common with future instruments such as the ILL planned D33 beam line.

Time-of-flight (TOF) instruments use a pulsed beam and the full range of beam wavelengths. The wider the range of wavelengths the wider the simultaneous  $Q$ -range that can be probed and the higher the count rate in the central region of the  $Q$ -range. On a TOF-type SANS instrument the range of useable neutron wavelengths is therefore a major contributor to the instruments performance. The applicability of TOF-type SANS instruments, however, has been limited due to the low neutron flux of kW-class spallation neutron sources. Recent projects of MW-class spallation neutron sources, such as J-PARC and SNS, bring new possibilities into this area and a number of instruments are currently being built accordingly. TOF-type SANS instruments essentially need broad wavelength bandwidths, covering a wide  $Q$ -range. The  $Q_{\min}$  is restricted by the short flight path of scattered neutrons. To overcome this restriction, without sacrificing the advantage of using a broad wavelength bandwidth, it is important to focus neutrons to the small-angle detector position by neutron focusing devices. According to the above idea, a high-intensity smaller-angle neutron scatter-

ing instrument (HI-SANS) is being built for the 1 MW spallation neutron source of J-PARC.

For more details on recent developments in SANS instruments see Table 2.

At the low frequency ISIS TS-2, the Near and Intermediate Range Order Diffractometer NIMROD will cover a wide range of length scales from sub-atomic up to distances characteristic of many small and medium sized macromolecular objects, such as proteins, nucleic acids and micellar objects. NIMROD will have a 20 m incident flight path and a scattered flight path (at the lowest angles) of up to 5.5 m. Compared to traditional disordered materials diffractometers this is a long flight path, but this is required for the instrument to reach a lowest scattering angle of approximately  $0.5^\circ$  ( $Q \sim 0.1 \text{ nm}^{-1}$ ) which is necessary to measure the large end of the desired length scale.

In a recent example of such work, performed on SANDALS at ISIS, it was shown that pressure induced unfolding of myoglobin in aqueous solution is associated with changes in the water structure. However, this work was frustrated by the upper length-scale accessible on that instrument, a limit that would be enhanced by more than one order of magnitude on NIMROD. Another example of biomolecular relevance that has recently captured the interest of the user community is the study of disaccharides, and their utilization as bioprotectants through effects such as glassification. NIMROD will be the first instrument worldwide, capable of tackling the interactions of biomacromolecules with their surroundings at the atomic level and the conformation of the molecule in solution. It will open the way for more detailed studies of protein–solvent and protein–ligand interactions, protein solution structure and protein folding.

### 3.2. Biomembranes and surface interactions

When biological interactions occur at planar surfaces, at interfaces, or in layered phases, neutron reflectometry (NR) can provide information on assembly, surface association and material penetration under a wide variety of experimental conditions [31–38]. When combined with synthetic and *in vivo* production of specifically D-labelled biomacromolecules or polymers, H/D-labelled components of complex systems and hybrid materials can then be selectively highlighted, located and analyzed *in situ*. This can be extremely powerful in the characterisation and analysis of molecular interactions with biomimetic materials and biological membranes, where neutron contrast variation techniques can allow the specific identification of marker, signalling or receptor proteins, peptides or nucleic acids to be discriminated from host substrates or supports that are composed of polymer or lipid matrices. This is an exciting and challenging area of development that promises to help bring understanding of the structure, function and dynamics involved in incorporation or assembly of ‘active’ biological agents or biosynthetic peptides into natural and synthetic substrates, matrices and membrane systems.

Table 2  
Developments at SANS instruments: a few characteristics

Instrument	$\lambda$ range (nm)	$Q$ -range (nm <sup>-1</sup> )	Status	Special options
D22 (ILL)	0.45–4	0.01–20	Operational	Large area multidetector (1 m <sup>2</sup> )
KWS2 (FRM II-JCNS)	0.45–2	0.001–3	Operational	High intensity, chopper option
SANS-I (PSI)	0.45–2	0.006–5.4	Operational	Polarisation (for 0.47–1 nm), <i>in situ</i> DLS, rheology, pressure sample environment, neutron lenses and stroboscopic (cyclic) detection mode, 11 T SANS cryomagnet
SANS-II (PSI)	0.45–2	0.02–3.5	Operational	Multi-position, circular sample exchanger, controller sample temperature and humidity conditions, 11 T SANS cryomagnet
QUOKKA (ANSTO)	0.45–2	0.008–1 0.015–1	Commissioning	Large area detector (1 m <sup>2</sup> ); comprehensive range of sample environments; polarised beam
KWS1 (FRM II-JCNS)	0.45–2	0.001–3	Final alignment	Polarisation, GISANS option for magnetic and non-magnetic problems, chopper option
SANS-1 (FRM II – TU München and GKSS)	0.45–2	0.001–20	Operational	Polarisation, two selectors, chopper option, laterally displaceable multidetector (1 m <sup>2</sup> ), second detector option and large area detector (1 m <sup>2</sup> )
BIO-SANS (ORNL)	0.6–3	0.002–1	Under construction	Broad range of sample environments
HI-SANS (J-PARC)	n.a.	0.01–150	Under construction	Broad wavelength bandwidth, large area detectors and neutron focusing devices
SANS2d (ISIS-TS2)	n.a.	0.001–25	Under construction	Two small angle detectors
KWS3 (FRM II – JCNS)	0.8–2	0.0005–0.04	Final Alignment	Reflectometry mode
VSANS (HMI)	0.35–3	0.001–8.5	Under construction	Optional high resolution mode using multi pinhole grid collimation
PACE (LLB)	0.4–2	0.02–5	Operational	Concentric rings detector $dR = 1$ cm TOF option
PAXY (LLB)	0.4–2	0.03–10	Operational	XY detector step = 0.5 cm
PAXE (LLB)	0.4–2	0.05–5	Operational	XY detector step = 1 cm TOF option
TPA (LLB)	0.7–1.6	0.002–0.2	Commissioning	Image plate detector, multibeam collimation

In bioscience, the samples are generally of high value and difficult to obtain in large quantities. The combination of membrane proteins that are challenging to produce and the need for deuterated lipids that are expensive is often the limiting factor for reflectivity experiments. Sample preparation is also a challenge, requiring the production of large areas of model membranes with high coverage. The ability to work with smaller or more dilute samples will make a significant difference.

The use of magnetic reference layers is an approach which is increasingly being used in the study of immobilised membranes and has no doubt had an impact in NR [39]. To carry out these experiments, a layer of magnetic material is included below the surface of the experimental substrate. In a magnetic field the scattering length density of this layer is different for opposite spin polarised neutrons. By measuring the reflectivity profile for both neutron spin states a data set is obtained with an additional contrast on precisely the same sample aiding greatly in data analysis, particularly for less well defined biological systems.

Although the structure of many enzymes that operate at the membrane surface is known with atomic resolution and even their mode of operation at the level of the catalytic site is understood, we do not have a clear picture of the infrastructure of their mode of action in the assembly (how or why they attach to the membrane surface, how their substrates or inhibitors are transported to them, what happens to the products, etc.). An understanding of this infrastructure is important for understanding their biological function, which, for example, is still unclear for the ubiquitous phospholipases. In the context of the development of gene therapy, recent focus has been on complexes involving plasmid DNA and cationic liposomes [40].

Time resolved measurements are usually restricted to increments of minutes to hours particularly where multiple detector positions are required or data is required to high  $Q$ . In cases where strong features in the reflection profile can be followed, or total reflection is a useful diagnostic quantity, it will be feasible to obtain useful data within ten seconds. This opens up a whole new area of studying surface binding via NR, an approach that has great

potential as a complementary technique to spectroscopic approaches such as surface plasmon resonance and ellipsometry. These techniques will continue to provide significantly better time resolution but provide less structural information than is available at neutron reflectometers.

For biomembrane studies instruments with high flux able to determine structures in the nanometer range are best suited and remarkable results have been obtained on instruments like NG1 or AND/R at NIST [41]; or the TOF instruments D17 at the ILL [42], or SURF at ISIS [43]. Reflectometers at other facilities as HMI, LLB, JINR or LANSCE have also been used regularly on biological systems. Both at existing and new sources a number of instruments are being upgraded or under development, such as AMOR [44], a TOF reflectometer at SINQ, that can handle any kind of solid–liquid sample cell (e.g. a pressure device developed by the University of Heidelberg or a flow cell by TU Delft), or N-REX<sup>+</sup> at FRM II which combines neutron and X-ray reflectometry within one experiment.

A dedicated reflectometer REFSANS [45] to measure also in grazing incidence small angle neutron scattering (GISANS) and diffraction (GID) has been installed at FRM II. REFSANS combines features of a TOF reflectometer and a TOF SANS instrument, to allow in particular the investigation of planar membranes and monolayers at liquid interfaces to study later aggregation and ordering in fluid self-assembled systems. The instrument will offer unique new possibilities to study organisation of proteins in membrane model systems and the adsorption and interaction of proteins and small biological molecules at interfaces.

Table 3 provides a list of current projects for reflectometers, most in either their installation or commissioning phases.

One of the first operational instruments at the SNS is the Liquids Reflectometer. The guide system supplies  $2 \text{ \AA} < \lambda < 16.5 \text{ \AA}$  neutrons at vertical incident angles ranging from  $0^\circ < \alpha_i < 5.5^\circ$  for free liquid surfaces and up to  $45^\circ$  for solid surfaces. Three bandwidth choppers, synchronized with the spallation source and operating at 15–60 Hz, provide neutrons in bandwidths ranging from 3.5 to 14  $\text{\AA}$  at a given  $\alpha_i$  onto a sample. The sample stage enables all of the motions necessary for positioning liquid and solid surfaces, while the detector arm allows position-sensitive ( $1.3 \times 1.3 \text{ mm}^2$  pixel resolution) or pencil  $^3\text{He}$  detectors (7-detector array) to view the sample at specular or off specular angles (up to  $90^\circ$ ) and can scan out of the specular plane by up to  $30^\circ$ . Instrument commissioning began in late 2006.

At J-PARC (BL16), a neutron reflectometer will use cold neutrons with wavelength ranging from 2.5 to 9  $\text{\AA}$  for an instrument with a total length of 17.5 m. The beam line design is optimized so as to satisfy the requirement that specular reflectivity can be measured for free interfaces at least up to 0.5  $\text{\AA}$  in neutron momentum transfer,  $Q_z$ , perpendicular to the sample surface. The maximum sample size is  $50 \times 50 \text{ mm}^2$ . This reflectometer is expected to have

at least a few hundred times higher intensity than the one at KENS (the first dedicated pulsed neutron facility in the world; it was shut-down in March 2006), which only had a proton accelerator power of 3 kW. The higher intensity makes it possible to perform a time-resolved reflectivity measurement in seconds for the sample systems varying their structures with time. More advanced measurements such as the use of focusing beam and neutron spin-echo options by a neutron resonance spin-echo method are under discussion.

At ISIS (TS-2), three reflectometers are being constructed in the first instrument suite: INTER, POLREF and OFFSPEC. INTER is optimized for the study of molecules at interfaces. It will provide faster counting times while providing simultaneous access to  $Q$ -ranges that would require at least two detector positions on the older instrument SURF. Sample areas as low as nine square centimetres will be routinely achievable. Alternatively it will be possible to use similar samples sizes to those currently used and increase the time resolution of measurements.

OFFSPEC is designed to provide enhanced access to a wide range of scattering from the plane of the sample in addition to the conventional reflectivity profile. OFFSPEC will have an area detector providing information on in-plane structures ranging from 0.1 to 40  $\mu\text{m}$  in scale. Thus, in addition to high resolution data on structures orthogonal to the plane of the sample provided by the conventional reflectivity profile, OFFSPEC will provide data on structures within the plane of the sample. A neutron spin-echo system, developed by the Technical University of Delft, uses neutron spin to encode the path of the neutron through the instrument. This “angular encoding” allows OFFSPEC to use a ribbon beam, as used for normal reflection experiments, but obtain resolution equivalent to a pin-hole geometry. By simply rotating the encoding plane (moving a magnet) it will also be possible to perform high resolution specular reflectivity measurements with relaxed collimation for high flux, or on samples that are not sufficiently flat for analysis on existing instruments. However, the main initial use of the system will be to obtain 5–200 nm structural data in the plane of the sample surface. The size range spans the micron sized domain structures formed by synthetic lipid mixtures often used as models of lipid raft formation to the  $\sim 10 \text{ nm}$  size that ‘real’ phase separated domains in natural lipids are thought to form. Along with advances in the preparation of model lipid membranes this offers the potential for the direct structural study of rafts formed in model membranes constructed from lipids isolated from natural membranes. In addition to lipid rafts the size range makes it possible to probe the aggregation state and possibly the orientation of proteins within a membrane. Structural interpretation of the orientation of proteins and peptides within membranes or at surfaces by NR currently extends only to a qualitative level; ‘standing up’ or ‘lying down’. With the additional data provided by OFFSPEC it will be possible to strengthen the arguments that have previously been made purely on the



Table 3  
New developments in neutron reflectometers

Instrument	Sample	Orientation of sample reflecting surface	Interesting options
REFSANS (FRM II)	Soft and liquid interfaces, surfaces	Horizontal	Grazing incidence small angle neutron scattering
PLATYPUS (ANSTO)	Liquid samples. Surfaces and thin film systems	Horizontal	Specular and off-specular scattering. Polarised neutrons
FIGARO (ILL)	Thin films at air/liquid and liquid/liquid interfaces	Horizontal	Simultaneous use of a Brewster Angle Microscope during measurements and the possibility to strike the interface from above and below in a wide $Q$ -range
MARIA (FRM II)	Soft and liquid interfaces, surfaces	Vertical	Specular and off-specular scattering. Polarised neutrons. Grazing incidence small angle neutron scattering
N-REX <sup>+</sup> (FRM II)	Solid and liquid surfaces, interfaces and thin film systems	Horizontal or vertical	Simultaneous <i>in situ</i> X-ray reflectometry; grazing incidence small and wide-angle neutron scattering, conventional or spin-echo resolved. Polarization analysis and large sample chambers
Liquids reflectometer (SNS)	Liquid and solid surfaces	Horizontal	Specular, off specular, near-surface small angle scattering, and crystalline diffraction geometries
BL16 Reflectometer (J-PARC)	Soft and liquid interfaces, surfaces	Horizontal	Specular, off specular, near-surface small angle scattering. Polarised neutrons
INTER (ISIS-TS2)	Interfaces	Horizontal	Small sample sizes
POLREF (ISIS-TS2)	Surfaces and thin film systems	Horizontal	Polarisation and Analysing systems
OFFSPEC (ISIS-TS2)	Interfaces, thin films and multilayers	Horizontal	Neutron spin-echo analysis system that will simultaneously provide data on in plane structures from 5 to 200 nm. Polarised neutrons

basis of the one dimensional density profile provided by specular reflectivity.

FIGARO [46] (Fluid Interfaces Grazing Angles Reflectometer) will be a high flux, flexible resolution reflectometer with a vertical scattering plane, to be commissioned at the ILL by mid 2008. Applications involve the study of the interaction of proteins with lipid monolayers, surface behaviour of surfactants, polymers and other amphiphiles at liquid/air and liquid/liquid interfaces. For liquid/liquid interfaces it is important that the incoming beam can approach the interface from above or below the horizon as one liquid phase may be far more easily penetrable than the other. With an incoming beam of wavelengths comprised between 2 Å and 30 Å, it will be possible to attain a  $Q$ -range of  $\sim 0.002\text{--}0.35 \text{ \AA}^{-1}$ . Four choppers, independently rotating in pairs, will allow the use of a beam at six different wavelength resolutions, ranging from 1.2% to 10%, by keeping the projected chopper openings equal to zero. A two dimensional multitube detector will be positioned at 3 m from the sample. This detector will allow measurements of specular and off-specular reflectivity.

The high flux and wide  $Q$ -range will allow the determination of structure and composition of layers in the fraction of nanometer range and to follow kinetics in the second timescale. Examples of applications of FIGARO include the adsorption of proteins at interfaces. As the

instrument flux and hence the sensitivity is increased, a greater extent of complexity can be explored.

A novel reflectometer called MARIA (magnetic reflectometer with high incident angle) is under construction by the Jülich Centre for Neutron Science at the FRM II to be in operation in 2009. With a focusing neutron guide and variable resolution at highest achievable flux the instrument will be used for time-resolved measurements of adsorption phenomena at interfaces. GISANS measurements will also be possible.

A TOF reflectometer, PLATYPUS [47] (named after an Australian semi-aquatic mammal), designed for soft matter studies, metallic multilayers and liquid surfaces, is being commissioned on the OPAL reactor at ANSTO. It is located at the end of a cold neutron guide with a variable resolution chopper system and a 2D detector system (500 mm horizontal and 250 mm vertical). The  $Q_{\min}$  is  $\sim 5 \times 10^{-3} \text{ \AA}^{-1}$  with  $Q_{\max}$  up to  $\sim 0.5 \text{ \AA}^{-1}$  for liquid–solid and air–solid interfaces, and  $\sim 0.35 \text{ \AA}^{-1}$  for air–liquid interfaces. The maximum flux is  $\sim 10^9$  neutrons  $\text{cm}^{-2} \text{ s}^{-1}$  and minimum reflectivity  $\sim 10^{-8}$ . It will have a polarisation analysis option and off-specular scattering will be possible. Sample environments which will assist studies in biology include a 5 T horizontal cryomagnet, gas mixer and controller, solid–liquid cell, Langmuir film balance, and sealed multiple troughs.

### 3.3. Dynamics in biological systems

The wide dynamical range in biological macromolecules is probed by an array of different types of neutron scattering spectrometers. The fastest dynamics are studied by thermal neutron time-of-flight (TOF), then comes the cold neutron TOF, which, although with a reduction in  $Q$ -range, can overlap with the backscattering instruments that are adapted to slower motions. The slowest motions are reached with the spin-echo technique.

Incoherent scattering from samples containing hydrogen and deuterium is strongly dominated by the motions of the hydrogen nuclei, which, in the time-space window examined by the neutron scattering experiments, essentially reflect the motions of the local structure to which they are bound. Inelastic neutron scattering experiments on dedicated TOF and filter-analyzer instruments provide high-quality data of dynamic structure factor  $S(Q, \omega)$  and vibrational densities of states in the energy domain from a few meV to a few hundred meV, which serve as input to, and constraints for, models of atomic bonding and even structural models of these complex compounds [48–50]. The ability to systematically highlight, isolate and probe the dynamics of specific H-labelled residues *in situ* within the natural environment of (deuterated) protein structures are of significant interest and value for the study of specific biological problems and also for the use of model proteins in biophysical/biotechnological applications [51].

For quasi-elastic spectroscopy the neutron instruments now available at sources worldwide make it possible to probe relaxations at very low energies with high resolution. Spectrometers are able to probe low energy dynamics including biomacromolecule breathing motions and water or molecular diffusion with at least a 10-fold reduction in collection time.

At and near biomolecular surfaces, significant structural and dynamical changes occur over narrow layers of hydration. Many important but as yet unresolved questions relate to the characterisation of the diffusive and rotational motions of water molecules in confined geometries and at hydrophilic/hydrophobic surfaces of complex structure [52–58]. It is only now becoming possible to simulate biomolecular hydration processes more accurately, and comparison of the resulting space-time correlation functions with experimental data requires instrumentation with sufficient resolution and dynamic range. With a variable resolution of between 7 and 30  $\mu\text{eV}$ , and excellent statistics, it will become possible to perform detailed analyses of quasi-elastic lineshapes due to multicomponent diffusional and rotational motions in selectively deuterated samples, and thus to gain information on hydrogen-bond connectivity and lifetimes, microdiffusivity and microviscosity.

#### 3.3.1. Triple axis spectrometers

Triple axis spectroscopy has not been used very much to probe dynamics in biological systems or of biological macromolecules. The triple-axis instrument IN8 has been used

to probe the collective dynamics in dry and hydrated C-phycoerythrin protein [59] and recently, with a better resolution of 300  $\mu\text{eV}$ , the IN12 instrument at ILL has been used to probe the collective dynamics in model membrane systems.

#### 3.3.2. TOF spectrometers

In principle, all TOF instruments could be suitable for the study of the fast motions in biological molecules. Amongst them, one can make the distinction between crystal TOF (e.g. IN4 and IN6 at the ILL, FOCUS at PSI) and disk-chopper TOF (e.g. MERLIN and LET at ISIS, CNCS at SNS, MIBEMOL at LLB, DCS at NIST, IN5 at ILL, TOFTOF in Munich, NEAT(V3) at HMI). The high resolution versatility of the disk-chopper instruments is well adapted to problems in biophysics. On very specific applications where a high flux is required, e.g. for time-resolved studies, in which a minute quantity of biological material is available, or when using a complex sample environment, one must rely on the high flux spectrometers that are found at high intensity neutron sources. Selected examples of TOF instruments, especially where there have been upgrades to enhance their performance for biophysics are given in what follows.

TOF spectrometers can cover both the inelastic and quasi-elastic scattering range, depending on their incident energy and energy resolution. Whereas thermal TOF spectrometers are applied to vibrational studies mainly, cold neutron TOF spectrometers work for both. It is generally necessary to carry out inelastic and quasi-elastic neutron scattering experiments on a wide range of instruments at a variety of facilities to gain a complete picture of the dynamics of a particular system.

FOCUS, for example, is a direct-geometry, hybrid TOF spectrometer for cold neutrons at SINQ [60,61] operated by PSI and Saarland University. It can be operated either in time or monochromatic focusing mode. FOCUS is highly flexible and can be optimized widely according to user demands. The instrument is suited for both quasi-elastic and inelastic scattering. FOCUS has been applied to study water dynamics in model membrane systems [62], dynamics of proteins at low temperatures [63] or molecular dynamics by cold neutron spectroscopy [64].

A setup for the investigation of the (light-induced) protein dynamics in real-time, using quasi-elastic neutron scattering (QENS), was recently developed for the first time at the HMI Berlin. In this configuration, laser pulses initiating the protein working cycle were synchronized with the neutron pulses of the TOF spectrometer NEAT [108]. The delay between the laser pulse and the neutron pulse can be optimally chosen in the  $\mu\text{s}$  to ms-range in order to probe the dynamics at time points of interest. The sampling time depends on the energy resolution of the neutron spectrometer but is typically in the order of a few tenths of microseconds. The repetition frequency of the laser flashes has to be adapted to the functional process under study. Therefore, a thorough characterisation of the sample system by optical

spectroscopy prior to the actual QENS experiment is an important prerequisite to achieve an optimal activation of the protein by the laser flash. A first successful series of QENS experiments has been performed with the integral membrane protein bacteriorhodopsin, a light-driven proton pump. Future applications of the new method include e.g. photosynthetic pigment–protein complexes or caged compounds for the light stimulation of non-photoactive proteins. Experiments on this system have continued on IN5 at ILL to profit from the higher flux.

The upgrade of the IN5 secondary spectrometer that is expected to be completed by Spring 2008 will see the replacement of the single detector bank to a 30 m<sup>2</sup> position sensitive detector array inside a 4 m flight path chamber under vacuum. The improvement of the instrument will increase detected neutron flux by a factor of 6 when compared to the present situation with its large gap-free solid angle offering scattering angles ranging from  $\sim 1^\circ$  to  $140^\circ$  by steps of  $0.4^\circ$ . The 800 mm diameter sample area allows accommodating a great variety of exotic sample environment. Efforts have been made to develop time-resolved and stroboscopic measurements with a flexible programmable electronics that can trigger any external parameter such as magnetic field [65], temperature jumps and [66] and light excitation (Pieper et al., unpublished) with the neutron pulses.

In July 2005 a multi-chopper time-of-flight spectrometer TOFTOF went into user operation at the FRM II. In the combination of high intensity (continuous white beam flux at the sample position  $\sim 10^{10}$  n cm<sup>-2</sup> s<sup>-1</sup>), good resolution and excellent signal to background ratio the spectrometer offers new scientific prospects in the field of inelastic and quasi-elastic neutron scattering [67]. For biologically relevant systems this could be demonstrated by investigations on the dynamics of phospholipids in the stabilizer layer of colloidal emulsion droplets with only 20 mg of phospholipids in the beam [68].

At ISIS TS-2, the LET spectrometer will be a versatile direct geometry instrument that aims to reduce the need for experiments on multiple instruments. It will operate over a wide 0–80 meV energy range, with a sophisticated chopper system that gives a variable energy resolution. The chopper system also allows multiple measurements to be made within a single time frame utilising the multiple repetition rate approach first introduced on IN5 [69]. LET will have position sensitive detectors covering a massive  $\pi$  steradians solid angle. This will make it possible to map a vast swathe of momentum transfer-energy ( $Q$ – $E$ ) space in a single measurement. The instrument is similar to IN5 (ILL), or NEAT (HMI), or TOFTOF (FRM II). It will have a large flux at the sample position, similar to the new improved IN5 at low energies but much larger at higher energies, greater than 20 meV. This is because such a spectrometer ideally requires a high ‘peak’ flux such as that from the coupled moderator of TS-2, and not a large time averaged flux produced by reactor sources. It will be both an excellent quasi-elastic spectrometer with a resolu-

tion that exceeds the 17  $\mu$ eV available on IRIS, and a low energy chopper spectrometer with count rates and resolution superior to that of any other existing machine.

### 3.3.3. Backscattering spectrometers

At the ILL, the IN13 backscattering instrument is characterized by the relatively high energy of the incident neutron beam (16 meV), which makes it possible to span an exceptionally wide range of momentum transfer (up to  $Q = 5.2 \text{ \AA}^{-1}$ ) with a high energy resolution ( $\sim 8 \mu$ eV). IN13 allows the investigation of a space-time window of about 1  $\text{\AA}$  and 0.1 ns. In the past years a major upgrade of the instrument has been performed leading to higher neutron flux and improvement of instrument versatility, especially in the domain of sample environment (see Table 4). Recent achievements include: oriented membrane investigations, experiments using high pressure, and molecular dynamics measurements in live cells [70,71] (Table 5).

Cold neutron reactor backscattering instruments like IN10 and IN16 at ILL, HFBS at NIST or SPHERES at FRMII offer the best possible energy resolution among neutron crystal spectrometers. They operate in exact backscattering which therefore allows, within the limits imposed by e.g. beam divergence or sample size, to push the energy resolution further and potentially considerably below the energy resolution of 0.19  $\mu$ eV, measured for silicon (111) [72]. For example with GaAs crystals this limit could be a factor 8 lower. Because any improvement in energy resolution is on expense of the count rate, the basis for such changes must at first be laid by increasing the neutron flux.

At the ILL the count rate is expected to improve by one order of magnitude with the new backscattering spectrometer IN16B due to better guide optics and a phase space transformation chopper. Together with new Doppler drives (at NIST, FRM II and ILL), which double the maximum energy transfer range, these spectrometers will offer a dynamic range of nearly 2 decades, quite close to those of spallation source backscattering instruments, but with its energy range shifted towards lower energy transfer. Today neutron backscattering and cold neutron chopper TOF spectrometers complement each other ideally to a wide dynamic range of three decades by applying Fourier transformation methods. In future combinations of reactor and spallation backscattering spectrometers and/or of TOF-spectrometers may allow extending the dynamic range to up to 4 decades. This is important for investigations of the dynamics of complex systems like in biology which display a wide distribution of relaxation times or for studies of confinement, where the high energy resolution is needed to decide on the presence of a strictly elastic component. Already today IN16 at ILL is used to a large part for biological studies (see e.g. two recent studies [73,74]) and these ongoing changes will render it even more promising.

The SNS backscattering silicon spectrometer (BASIS), the most advanced neutron spectrometer of this type, was designed to provide very high energy resolution at the elas-

Table 4  
Options available at some neutron spectrometers suitable for biological applications at neutron research facilities

Instrument	Energy resolution	$Q$ -range ( $\text{\AA}^{-1}$ )	Comments
NEAT (HMI)	6–5400 $\mu\text{eV}$	0.3–3.5	Studies of kinetically modulated protein dynamics
TOFTOF (FRM II)	4 $\mu\text{eV}$ –5 meV	$\leq 3.9$ (for $\lambda = 3 \text{\AA}$ )	Resolution can be doubled when using the small slit option (at a cost of $\sim 14$ times less intensity)
MIBEMOL (LLB)	15 $\mu\text{eV}$ –5000 $\mu\text{eV}$	0.5–2.5	Photo-induced dynamics of protein by synchronizing laser flashes and neutron pulses
IN5 (ILL)	5 $\mu\text{eV}$ –3 meV $\lambda_o = 1.8$ – 20 $\text{\AA}$	0.11/ $\lambda_o - 11.9/\lambda_o$	Full gapless position sensitive detectors. Flexible programmable electronics for time-resolved studies. Large sample chamber (800 mm diameter) allowing fitting a large variety of sample environment. Hyperfocusing guide for very small sample under study
IN13 (ILL)	8 $\mu\text{eV}$	0.3–4.9	High-pressure device for liquid samples able to reach 7000 bar and a TOF unit in order to develop real-time experiments. Large $Q$ -range
IN16 and IN16B (ILL)	0.35/0.85–32 $\mu\text{eV}$	0.1–1.9 0.5–3.8	IN16B will have an order of magnitude higher count rate and doubles the dynamic range; a side position will even improve the signal to background ratio compared to IN16
SPHERES (FRM II)	0.65 $\mu\text{eV}$	0.1–1.9	Doppler drive and phase space transformation chopper in operation
BASIS (SNS)	3.3 $\mu\text{eV}$	0.17–1.97	Flexibility to shift the incident neutron wavelength to cover (in separate settings) energy transfers from 18 meV to approximately $-0.8$ meV
MUSES (LLB)	6.6 meV–33 neV	0.005–4	NRSE technique
J-NSE (FRM II)	2 neV–0.66 meV	0.02–1.5	Very high energy resolution
RESEDA (FRM II)	65 neV–1.5 meV	$\leq 3.5$ (for $\lambda = 2.5 \text{\AA}$ )	For incoherently scattering samples, a modification of the NRSE technique can be used, where depolarization at the sample does not deteriorate the measured signal, which is time dependent in return
FOCUS (PSI)	10–2000 $\mu\text{eV}$	0.1–6	High-pressure INS studies to 1.2 GPa, sample environment for continuous T-range 10–650 K, magnetic field of 9 T.

Table 5  
A comparison of current and near-future neutron diffractometers for biological studies

Instrument	Type	Applications and Sample environment
TOPAZ (SNS), due online in 2008	Quasi-Laue	Smaller oligonucleotides and proteins (less than $\sim 25$ –30 kDa). Resolution up to 1 $\text{\AA}$
MaNDi (SNS), due online in 2008	$\lambda$ resolved Laue TOF	Crystals of macromolecules of $> 0.1 \text{ mm}^3$ , with lattice repeats up to 150 $\text{\AA}$ . Resolution up to 1.5 $\text{\AA}$
PCS (LANL)	$\lambda$ resolved Laue TOF	Organic or inorganic small molecule crystals (resolution up to 1 $\text{\AA}$ ). Crystals of macromolecules of $> 0.3 \text{ mm}^3$ , with lattice repeats up to 180 $\text{\AA}$ (resolution up to 1 $\text{\AA}$ )
LADI-III (ILL), operational	Quasi-Laue	Crystals of macromolecules of $> 0.1 \text{ mm}^3$ , with lattice repeats up to 150 $\text{\AA}$ . Resolution up to 1.5 $\text{\AA}$
IMAGINE (HFIR), planned	Quasi-Laue	Organic or inorganic small molecule crystals, and small protein single crystals
KOALA (ANSTO), commissioning	Quasi-Laue	Organic or inorganic small molecule or small protein crystals of $> 0.1 \text{ mm}^3$ or less, with lattice repeats up to 40 $\text{\AA}$ . Control of temperature (6–800 K; standard), gas-environment and applied electric fields
IBARAKI diffractometer (J-PARC), due online in 2008	Quasi-Laue	Crystals with lattice repeats up to 150 $\text{\AA}$ : organic or inorganic small molecules – resolution up to 1 $\text{\AA}$ – and biological macromolecules ( $> 2 \text{ mm}^3$ ) – resolution up to 1.2 $\text{\AA}$
(FRM II), planned	Monochromatic 2.4–4 $\text{\AA}$	Crystals of biological macromolecules. Resolution up to 1.5 $\text{\AA}$
D19 (ILL), operational	Monochromatic 0.7–3.9 $\text{\AA}$	Organic or inorganic small molecule, small protein and oligonucleotide single crystals, as well as fibre diffraction studies of biological and synthetic polymers with lattice repeats up to 50 $\text{\AA}$

tic line enabling quasi-elastic measurements of molecular dynamics over times ranging [75] from 1 to approximately

1000 ps. A long flight path coupled with the sharp time pulses of neutrons produced by the SNS decoupled, poi-

soned cryogenic hydrogen moderator provides the required high energy resolution of neutrons incident on the sample. At the primary operating frequency of the SNS (60 Hz), three neutron choppers select a useful incident neutron wavelength band of approximately 0.7 Å, corresponding to a range of energy transfers greater than  $\pm 0.25$  meV centered at the elastic line. The combination of dynamic range and high energy resolution makes the spectrometer ideally suited for the study of systems exhibiting dynamics over a wide range of time scales, such as exhibited in biological molecules or glassy systems. The dynamic range can also be extended by operating the chopper system at sub-harmonics of 60 Hz.

#### 3.3.4. Neutron spin-echo instruments

The neutron spin-echo (NSE) technique provides the highest energy resolution in inelastic neutron scattering and is suited for slow processes of large molecules, as for example proteins [76]. NSE spectrometers are in use at ILL, HMI, NIST and at FRM II.

The dynamic range of the J-NSE (Jülich Neutron Spin Echo) spectrometer at FRM II profits from the possibility of varying the neutron wavelength over a wide range between 4.5 and app. 17 Å (with no hard cut-off but rather a question of sufficient intensity for the actual experimental problem) and using a high magnetic field integral of 0.5 Tm. NSE can for example be used to study coherent scattering from proteins in a deuterated buffer solution in the  $Q$ -range comparable of that of small angle neutron scattering and complements the structural with dynamic information on mesoscopic length scales. Recently, internal domain motions of the protein Taq polymerase in water have been investigated by NSE [77].

At SNS a challenging NSE instrument is currently under construction by JCNS which will extend the current measurable Fourier times up to 1  $\mu$ s allowing probing extremely slow motions as in the movement of protein subunits.

At FRM II, the resonance spin-echo (NRSE) spectrometer RESEDA (Resonance Spin Echo for Diverse Applications) provides large time and scattering vector intervals for quasi-elastic measurements. Typical applications are dynamical studies in soft biological systems, for example protein diffusion. Furthermore, the dynamics of macromolecular and magnetic systems, as for example diffusion of polymers in bulk or confinement, and (spin) glass dynamics can be investigated at RESEDA. A main advantage of the NRSE technique lies therein, that the magnetic coils can be realized very compactly. This facilitates experiments at high scattering vectors, involving large scattering angles, and, finally, makes it possible to build a multi-detector wide-angle scattering spin echo spectrometer.

#### 3.4. Macromolecular crystallography

In neutron macromolecular crystallography, the enhanced visibility of hydrogen atoms on water molecules, substrates and proteins, allows direct determination of pro-

tonation states and helps provide a more complete picture of atomic and electronic structures in biomacromolecules. The difference in magnitude and phase between the hydrogen and deuterium isotopes also means that neutron diffraction can distinguish and determine the pattern and extent of H/D isotope substitution in proteins, providing unique information on the solvent accessibility of individual amino acids, on the mobility and flexibility of interesting domains and on the H/D exchange dynamics themselves. The ability to locate hydrogen atoms in macromolecular structures with much lower resolution data than X-rays can help bring fundamental understanding of the physics and chemistry of ligand binding interactions, of solvent structure, of electron and proton transport, and of reaction pathways in catalytic mechanisms.

After establishing the first Laue neutron diffractometer for protein crystallography LADI, at the ILL, an upgraded neutron Laue diffractometer LADI-III has been designed and installed. The new instrument includes a large cylindrical area detector made of neutron-sensitive image plates that completely surround the sample and allows many stimulated Bragg reflections to be recorded simultaneously. The quasi-Laue method is used to provide a rapid and efficient survey of reciprocal space, maximizing the flux on the sample by using all available neutrons within a selected wavelength range (using a Ni/Ti multilayer filter), while at the same time reducing the background on the detector compared to the full white beam Laue technique. The instrument has been fully operational since early 2007 and is producing high quality data and exciting results. An improved detector design and readout system has been incorporated so that a miniaturized reading head located inside the drum scans the image-plate. From comparisons of neutron detection efficiency (DQE) with the original LADI-I instrument, the transfer of the image-plates and readout system internally provides a 2–3-fold gain in neutron detection. Moreover, in order to help reduce the background and the number of spatially overlapped reflections, the dimensions of the drum have been increased and the entire detector height can be adjusted to allow for relocation to a higher intensity beam, providing yet further gains. Thus by harnessing the improved neutron detection of LADI-III data can be collected to higher resolution ( $\sim 1.5$  Å), using shorter exposure times and smaller crystal volumes. This is a critical advance for neutron protein crystallography. The technique has become more accessible to the structural biology community by extending the size and complexity of systems that can be studied (up to 150 Å on cell edge) while lowering the sample volumes required ( $\sim 0.1$  mm<sup>3</sup>). Moreover, at LADI-III, the ability to flash-cool and collect high resolution neutron data from protein crystals at cryogenic temperature (15 K) has opened the way for kinetic crystallography on freeze trapped systems and allows analysis of structure (and transitions) as a function of temperature.

A quasi-Laue single crystal instrument KOALA (named after an Australian herbivorous marsupial), with a design

based on the VIVALDI instrument at the ILL, will be available on the OPAL reactor at ANSTO. It will be initially located on a thermal neutron guide (end position with peak neutron wavelength at  $\sim 1.3$  Å) but it is reasonably portable and may be shifted to a cold guide as required. The solid angle for quasi-Laue diffractometer is at least  $2\pi$  and the  $Q$ -range on the thermal guide is  $\sim 10$  ( $\sin\theta/\lambda \sim 0.9$  Å $^{-1}$ ).

FRM II, in cooperation with the Jülich Centre for Neutron Science (JCNS), will construct a monochromatic single crystal diffractometer for biological macromolecules. To cover a large solid angle the detector of the diffractometer consists of a neutron imaging plate in a cylindrical geometry, similar to the LADI-III diffractometer. The big advantage of this instrument is the possibility to adapt the wavelength to the unit cell of the sample crystal.

Similarly, the ILL upgraded high-resolution monochromatic diffractometer D19 [78] is located on a thermal beam, providing a unique and flexible range of wavelengths corresponding to three possible takeoff-angle options ( $42^\circ$ ,  $70^\circ$ ,  $90^\circ$ ). The instrument is suited to crystallographic studies of smaller biological crystal systems such as DNA oligonucleotides and oligonucleotide-drug complexes [79]. The new diffractometer provides an efficiency gain of approximately 25 by comparison with the original instrument, opening up completely new areas of bioscience for crystalline and partially ordered systems. Key areas that will be addressed for biological crystallography include studies of hydrogen interactions/hydration, particularly for situations where these groups have high B factors ( $>10$  Å $^2$ ), or where radiation damage issues encountered in X-ray studies severely restrict the acquisition of this type of information. For biological fibre diffraction, it will be possible to study samples that have hitherto been far too small for neutron work. It will also mean that detailed measurements will be possible of continuous diffraction. This type of diffraction predominates in diffraction studies of many filamentous viruses, drug–DNA and protein–DNA complexes and is also a key aspect of changes in ordering that occur during structural transitions [80]. Major opportunities exist to exploit selective deuteration so that particular parts of a structure can be highlighted [81], as well as for the investigation of key problems in the study of amyloid fibres [82].

At spallation neutron sources, where the beam has a pulsed time structure, time-of-flight Laue techniques are most attractive to reduce the background and improve the signal-to-noise ratio of single crystal diffraction data. At the Los Alamos Neutron Science Center [83,84], TOF Laue techniques are used to increase the efficiency and speed of data collection from samples as small as a few tenths of a cubic millimetre at the Protein Crystallography Station (PCS) – the first neutron crystallography beam line to be built at a spallation source.

The SNS will provide two single crystal instruments that are suitable for the determination of supra-molecular and macromolecular structures. The first, TOPAZ, has been designed and optimized specifically for high resolution

chemical crystallography and will be able to resolve unit cell edges of up to 50 Å with ease. The second instrument, MaNDi, (MAcromolecular Neutron DIffractometer), has been specially designed for the determination of larger macromolecular structures and complexes, and is optimized for high resolution crystallography of large unit cell systems of 150 Å and beyond [85]. In addition, the MaNDi design allows for neutron data collection to medium resolution (2 Å) on more challenging macromolecular samples with unit cell dimensions up to 300 Å. Moreover, with projected data collection times of between just one and a few days for most applications, MaNDi can be expected to have significant scientific impact on many areas of structural biology, including enzymology, protein dynamics, drug design, and the study of membrane proteins.

The IBARAKI Biological Crystal Diffractometer is a single-crystal neutron diffractometer for biological and chemical crystallography, which will be completed at J-PARC in 2008 and will be using a wavelength range of 0.70–3.85 Å. The measurement efficiency will be more than 100 times larger than the neutron biological diffractometers BIX-3 and BIX-4 in JRR-3 reactor at JAEA. For the detector, a new wavelength-shifting-fibre type scintillation area detector system with high spatial (less than 1.0 mm) and time (1  $\mu$ s at the smallest) resolution is under development, along with software to deconvolute overlapped spots in data reduction.

In the field of detectors, a noteworthy development has been the neutron imaging plate (NIP). It was a breakthrough event in 1994 for neutron protein crystallography [86] and it is used on diffractometers such as LADI-III and KOALA. The NIP has also been used at the JEA in high-resolution neutron diffractometers dedicated to biological macromolecules (BIX-3, BIX-4). At LANSCE, a time-of-flight  $^3\text{He}$  detector for neutron protein crystallography has also been used successfully [87].

#### 4. Sample preparation and infrastructure

Sample preparation can be the bottleneck of a neutron scattering study, even more so for biological compounds. A well-engineered preparation will take into account the objectives of the study and the technical limitations of the sources and instruments being used. This requires expertise, infrastructure and adequate supporting instrumentation.

##### 4.1. Membrane preparation

As a highlight, an elegant sample preparation technique has been developed at the NIST Center for Neutron Research (NCNR) (for instrumental developments at NIST see [88]) allowing for studies of tethered single bilayer model membrane systems. The design of a sparsely-tethered bilayer lipid membrane (tBLM) was the result of collaboration with scientists from the NCNR, NIST's Biochemical Sciences, Optical Technology and Semicon-

ductor Electronics Divisions, as well as scientists from Carnegie Mellon University and the Institute of Biochemistry in Vilnius, Lithuania. The tBLM is robust and stable over several days when in contact with aqueous solution. This advance, along with improvements in sample cell design and data collection and fitting techniques, have now made measurements of membrane proteins in tethered model membranes more routine.

The newly-developed tBLM consists of a self-assembled monolayer (SAM) formed from a synthetic 1-thiahexa(ethylene oxide) lipid, WC14, bound to a gold surface and a phospholipid layer that is deposited on the SAM using the method of rapid solvent exchange, whereby the SAM is incubated with phospholipid in absolute ethanol before the solution is displaced within a few seconds by a large excess of aqueous buffer solution. If the SAM consists of pure WC14, the hexa(ethylene oxide) tether region was shown by NR to have low levels of hydration. However, if a mixed SAM is formed by coadsorption of WC14 with a short-chain “backfiller”,  $\beta$ -mercaptoethanol, the open spaces between the tBLM and the substrates were shown to contain up to 60% exchangeable solvent by volume [89]. This made possible the functional reconstitution of the *Staphylococcus aureus*  $\alpha$ -hemolysin channel protein in these model membranes [109]. Since the tBLMs are robust, one physical substrate consisting of a silicon wafer with oxide layer, a chromium adhesion layer, a gold film of  $\sim 100$  Å thickness and a tBLM can be used during an entire experiment in which the sample can be characterized sequentially at various stages of the preparation procedure under various solvent conditions. This process is simplified by the use of an improved sample cell designed so that solvents can be exchanged *in situ* at the reflectometer without the need for disassembly.

Since the same sample can be measured using multiple solvent contrasts, simultaneous fitting techniques can now be routinely used to obtain scattering length density profiles for tBLMs and tBLM/protein systems. Thus, the tBLM can be characterized, followed by protein reconstitution and further reflectometry measurements, and the structural changes due to protein incorporation can be determined in unprecedented detail. Phase-sensitive NR techniques, which allow unique structural depth profiles to be obtained by first-principles inversion of the reflectivity data [39], have been under development at the NCNR for some time. Now, in collaboration with NIST’s Advanced Measurement Laboratory, such methods are used routinely, employing substrates coated with a buried magnetic layer under the gold layer. Typically, silicon substrates are coated with a  $\sim 50$  Å layer of iron or magnetic alloy that also serves as the adhesion layer for a top layer of  $\sim 150$  Å of gold. tBLMs can then be formed on the iron/gold-coated substrates and measured using polarised neutrons.

Similarly, at the ILL, the infrastructure for model membrane preparation has been implemented for a few years. The current project of creation of soft matter laboratories

in the framework of a Partnership for Soft Condensed Matter with the neighbouring European Synchrotron Radiation Facility will no doubt lead to a better exploitation of beamtime while allowing for faster high quality sample preparation.

#### 4.2. Deuteration laboratories

The ability to deuterate or selectively deuterate biological macromolecules adds a powerful dimension to neutron scattering studies, often allowing experiments to be carried out that are outside the scope of X-ray scattering. At low resolution, the large range of contrasts available through the use of  $D_2O/H_2O$  solvent mixtures can be used in SANS and crystallography to distinguish between protein, nucleic acid, and lipid components of a multi-component system. For multi-component systems in which the scattering length densities are all similar (e.g. a multi-subunit protein system), selective deuteration allows effective modelling of individual components [17,19,20,24,90,91]. Similar approaches are equally powerful when studying biological membranes with NR [82]. At higher resolution, perdeuteration of macromolecular systems in crystals and fibres provides a major advantage through the enhanced coherent scattering power of substituted deuterium and the elimination of hydrogen incoherent scattering. One important consequence of this is that the serious limitations in sample size and data collection times required for hydrogenated samples are alleviated [92,93]. In inelastic neutron scattering experiments, the application of specific hydrogen–deuterium labelling patterns can be used to highlight specific dynamics within a system. Selective labelling of specific amino acids or nucleotides within a molecule can also allow novel approaches in crystallographic structure determination [85], and can in principle be used in SANS studies [94].

Despite the obvious advantages of deuterium labelling, its exploitation in biological neutron scattering has, until relatively recently, been rather less than might have been expected. The reasons for this are complex but one major factor is simply that support for the provision of deuterated biological molecules demands continuity of facilities and expertise that is typically not available within individual research groups. In recent years, neutron facilities throughout the world have taken decisive steps to address this problem. On the Grenoble site, the ILL, in collaboration with the EMBL, proposed the creation of a Deuteration Laboratory aimed at maximising the effectiveness of biological neutron scattering work [95]. The ILL-EMBL Deuteration Laboratory reached the final stage of its development when it moved into the Partnership for Structural Biology (PSB) in 2006. The laboratory now has a thriving peer-review user programme, extensive in-house activity, as well as a strong commitment to the development of new approaches that are steadily extending the scope of labelling methods [18,66,86], and has benefited from synergy with the solid state and solution NMR communities [96]. At LANCSE, a Biological Deuteration

Laboratory has existed for many years. The unique aspect of the LANCSE facility is that it has unique expertise in the use of photosynthetic algae, capable of autotrophic growth in an inorganic environment using CO<sub>2</sub> as the sole carbon source. The mass production of fully deuterated algae is achieved by using D<sub>2</sub>O as the only deuterium-containing compound required to support growth [97,98]. The Center for Structural Molecular Biology (CSMB), established at ORNL to support and develop the user research and science programs in neutron structural biology, has also established a Bio-Deuteration Laboratory to support and develop the facilities and expertise required for the expression and purification of deuterium-labelled proteins and macromolecules. ANSTO has set up a Biodeuteration Facility for the production, purification and characterisation of deuterated biomolecules. This has now been extended to a National Deuteration Facility (NDF) for both biodeuteration and chemical deuteration of a wider range of molecules. The main objective of this facility will be to support and extend the science undertaken at the recently opened OPAL reactor. At ISIS TS-2, new support facilities are being put in place with a fully equipped biological laboratory due to be up and running in mid 2008. Here, particular emphasis is being placed on the exploitation of developments to provide deuterated analogues of small biomolecules such as amino acids, nucleotides, and lipids, generating a capability that will be genuinely complementary to that available at the Deuteration Laboratory in Grenoble.

These initiatives are now starting to have a strong impact. At the ILL and other European neutron beam facilities, increasing numbers of beam time applications involving deuteration are passing through biology review committees. The impact is also evident in the scientific press. In SANS, two recent examples in which labelling of selected sub-units has allowed quaternary structure to be determined have been published by King et al. [99], and by Callow et al. [24]. In the former case, selective deuteration of a full set of ternary complexes of troponin has been used to study the large structural change that occurs upon calcium binding and to identify the sub-unit in which this change principally takes place. In the latter example, the deuteration of specific protein sub-units was used in conjunction with the *ab initio* modelling methods of Svergun [100] to study the subunits within a type I restriction modification system. The impact for crystallography is also becoming clear: the fact that radically smaller samples (~0.1 mm<sup>3</sup>) can be used is opening up the technique to a much wider range of problems. This has been demonstrated in recent work on aldose reductase [86], for which neutron data to 2.2 Å resolution were collected on LADI-I from a perdeuterated crystal with volume of only 0.15 mm<sup>3</sup>. Since the growth of very large single crystals is the major bottleneck in neutron protein crystallography, this result demonstrates the critical advance offered by perdeuteration. The smaller crystal volume required for fully deuterated samples aids the efficient cryo-cooling of the

crystals, and also reduces the number of spatially overlapped reflections on a fixed-radius detector due to the smaller spot size. Fully deuterated crystals also aid in the interpretation of the nuclear maps whereby the potential cancellation of nuclear density between a H atom and a positive scatterer does not occur and rather positive cooperation between two positive scatterers is observed. With hydrogenated crystals that have been pre-soaked in D<sub>2</sub>O there always exists the possibility of partially occupied H and D atoms being located at the same positions within the crystal, and this can lead to difficulties in interpretation of the neutron data. With fully deuterated crystals this ambiguity is removed.

Deuterium labelling has also had an important application in neutron spectroscopy to focus on dynamics of components of complex biological systems. The method is based on incoherent scattering and deuterium-labelled components are essentially masked compared to the strong incoherent scattering from the hydrogen nuclei [74].

#### 4.3. Computational tools

A major effort to provide a wide ranging user friendly suite of programs for SANS data analysis has been made by the Svergun group at EMBL Hamburg (available from the website [101]).

To support the users of the Bio-SANS, the CSMB is also developing methods for data reduction and analysis of SANS and SAXS data. The effort is an extension of previous efforts within the group that have produced *ab initio* approaches for modelling uniform density structures from small-angle scattering data [90] and rigid body methods for simultaneously fitting entire neutron contrast variation series of data [102,103]. The suite of tools has been further expanded to include simple shape modelling approaches that statistically characterize the structures that best fit the data [104] and custom modelling approaches for specific problems [21,105] that utilize existing knowledge to build models from scattering data. Modelling expertise within the CSMB is as critical as the toolset, particularly for the novice user. Future developments will be driven by the needs of the user community of the SNS and HFIR with the overarching goal of providing efficient and effective tools for the visualisation of complex biological systems using neutron scattering.

The science performed at neutron scattering facilities has benefited tremendously from the progress in computational tools. With the new instrument and source developments, improved software and hardware tools came to serve specific purposes and needs. Furthermore, there is also a ubiquitous effort towards an homogenisation of the computational tools to promote the movement of users and expertise between different neutron scattering facilities and instruments: the multi-platform GUI for X-ray and neutron scattering experiments, for example, was developed for instruments at OPAL but configurable for instruments anywhere else [106].



For crystallography, a Macromolecular Neutron Crystallography [107] consortium, between Los Alamos and Lawrence Berkeley National Laboratories, has developed tools (free and downloadable from the consortium website) to deal with the growing number of neutron macromolecular structures with increasing size and complexity, to be used alternatively or at the same time for X-ray and neutron crystallography.

## 5. Conclusion

The review highlights developments now occurring for biological neutron scattering throughout the world. New sources are being built in the USA, Europe, Japan, Australia, and elsewhere, where there are major developments in instrumentation and support facilities at existing sources. They occur alongside an explosive growth in biotechnology and seemingly relentless increases in available computing power – developments that are likely to affect dramatically the scope and analysis of future neutron work. It can be anticipated that these major investments for neutron-related infrastructure will have an important impact on studies of the structure and dynamics of biomolecular systems, and that they will play a critical role as trends develop towards increasingly interdisciplinary approaches for the study of complex and interacting systems. In ensuring that the most is made of these developments, it is of course important that researchers and facility operators learn the lessons of the past – it makes no sense for facilities to operate excellent neutron beam sources, at considerable recurrent cost, without considering the ancillary capabilities that are needed to maximise the impact of the work. One encouraging aspect emerging from this review is clear evidence that this issue has been recognised.

Finally we would like to end with a disclaimer! The authors realise the review may not be an extensive, complete list of new developments, and apologise in advance for those not mentioned.

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