Report on

NEUTRONS IN BIOLOGY WORKSHOP

The Scientific and Technical Requirements for Biology at Australia's Replacement Research Reactor

School of Biochemistry & Molecular Biology
University of Melbourne
10-11 July 2001

Australian Institute for Nuclear Science and Engineering
School of Biochemistry & Molecular Biology, University of Melbourne
Australian Nuclear Science and Technology Organisation

Executive Summary

A Symposium and Workshop on Neutrons for Biology was held in the School of Biochemistry and Molecular Biology at the U. of Melbourne, under the auspices of AINSE, U. of Melbourne and ANSTO on 10-11 July 2001. 38 participants attended, from 6 Australian Universities, New Zealand, 3 ANSTO Divisions, CSIRO and DSTO, and 3 other overseas institutes. Invited talks were given on the subjects of "Bringing the Genome to Life; Small-angle Neutron Scattering Provides a Critical Framework for Understanding Bio-Molecular Machines and Signalling Networks" (Jill Trewhella, Los Alamos, USA), "Neutron Diffraction at High and Low Resolution: From Catalytic Protons to Virus Structure" (Peter Timmins, ILL Grenoble, France) and "The Investigation of Large-scale Biological Structures using Neutrons" (Dick Wettenhall, U. of Melbourne). There were also talks from prominent NMR practitioners and X-ray protein crystallographers, with substantial discussion about how the various methods might fit together in the future. Significant progress was made on defining Australia's needs, which include a strong push to use SANS and reflectometry for the study of macromolecular complexes and model membranes, and a modest network of supporting infrastructure in Brisbane, Melbourne and the Sydney Basin. A watching brief should be kept on the technology of protein crystallography with neutrons, though this should not be an initial emphasis, and there was also some discussion of the dynamics of water in biological systems.

Specific Recommendations were that:

1. the small-angle neutron scattering and reflectometry instruments in the RRR be pursued with high priority
2. there be **no** specific effort to provide high-resolution protein-crystallography facilities at the RRR, but that a watching brief be kept on instrumentation and sample-preparation technologies elsewhere.
3. that a watch be kept on inelastic and quasielastic neutron scattering capabilities elsewhere, although these methods will not initially be pursued at the RRR.
4. there be input from this community into the design of the biochemistry/chemistry laboratories at the Replacement Research Reactor.
5. a small number of regional facilities be established (or enhanced) to allow users to perform deuteration of biomolecules. These facilities would be of significant value to the NMR and neutron scattering communities

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Appendix I. Workshop program

(i) Appendix II. Workshop participants

Front row: Trevor Hicks, Peter Holden, Geoff Jameson, Peter Timmins, Paul Curmi, Jill Trewhella, Tony Klein, Horst Schirra, Jichen Li, John Ferris

Middle Row: Kerie Hammerton, ?, Ross Piltz, Dennis Mather, Stuart Carr, Chris Garvey, Bipin Dhal, Shane Kennedy

Back Row: Rob Robinson, ?, Phil McMahon, Ron Cooper, Wim Klooster, Robert Knott, Patrick Hartley

1. Science background

Determining the nature of macromolecule-macromolecule interactions represents one of the greatest challenges in the post-genomic era. The importance of such interactions has been emphasized by the findings from recent genomics programs, which have in general shown that the number of genes making up higher organisms is significantly lower than had previously been anticipated. In other words, the complexity of higher organisms is determined not so much by the total number of genes and associated proteins, but more by the extent of interactions between the organism's complement of macromolecules.

We are thus entering a new era in biology in which it is becoming increasingly important to delineate the nature of macromolecule-macromolecule interactions. Australia already has significant strengths in the structural biology of individual macromolecules. The two major techniques are X-ray crystallography and NMR spectroscopy. Both techniques have provided valuable information on the structures of the individual macromolecules, but have significant limitations for the study of complex supramolecular assemblies. Neutron scattering studies offer unique opportunities to provide new information in this field.

The distinguishing feature of neutron scattering is the ability to exploit the difference between proton and deuteron neutron scattering length. This allows contrast variation techniques that can obtain structural information from individual members of multicomponent systems. It also allows for the accurately location of hydrogen atoms in ordered systems. When used in combination with other structural probes, neutron scattering can provide unique and complementary information.

3. Comparison with other techniques

Introduction

Neutron scattering is one of several experimental techniques that can be used to determine the structural parameters of biological macromolecules. It is important to compare the various strengths of neutron scattering techniques with the strength of complementary structural techniques such as NMR and X-ray crystallography.

NMR. NMR is the technique of choice for studying the solution structures of relatively small biological molecules (<30-40kDa). It has the particular advantage of being able to locate individual hydrogens, and hence determining the protonation states of chemical groups. NMR also provides information on the inherent flexibility of biomolecules. NMR is of very limited use for protein assemblies.

X-ray crystallography. X-ray crystallography provides high-resolution structures of much larger molecules and assemblies, providing diffraction quality crystals can be obtained. When ultra-high resolution diffraction data can be obtained it is possible to locate individual hydrogen atoms. In some cases radiation damage to crystals has been a complication in data collection and interpretation of these experiments.

Cryo-electron microscopy. Cryo-electron microscopy provides medium resolution structural information on very large macromolecular assemblies. It is possible to distinguish between components in these assemblies with exogenous labelling.

Small-angle neutron scattering from solutions. Neutron scattering can be used to study proteins, protein complexes, and protein/DNA complexes in solution. Endogenous deuterium labeling and contrast variation allows one to map the shapes and dispositions of individual components in complexes and assemblies. In cases where the component structures are available from NMR or crystallography, the neutron solution scattering data can be interpreted in terms of conformational changes and detailed quaternary structures.

In recent times, combined neutron scattering and high-resolution structural information on complexes has been extended to develop high-resolution models of molecular interfaces.

Figure 1. The crystal structure of calmodulin (left) and the NMR structure of calmodulin-MLCK-I (right). Small-angle neutron scattering provided the first evidence that the interconnecting helix in calmodulin is flexible, and that this enables the dramatic conformational collapse upon binding to its target sequence in myosin light chain kinase, MLCK-I (after Heidorn & Trewhella (1988) *Biochem. 27*, 909; Heidorn et al. (1989) *Biochem. 28*, 9316.)

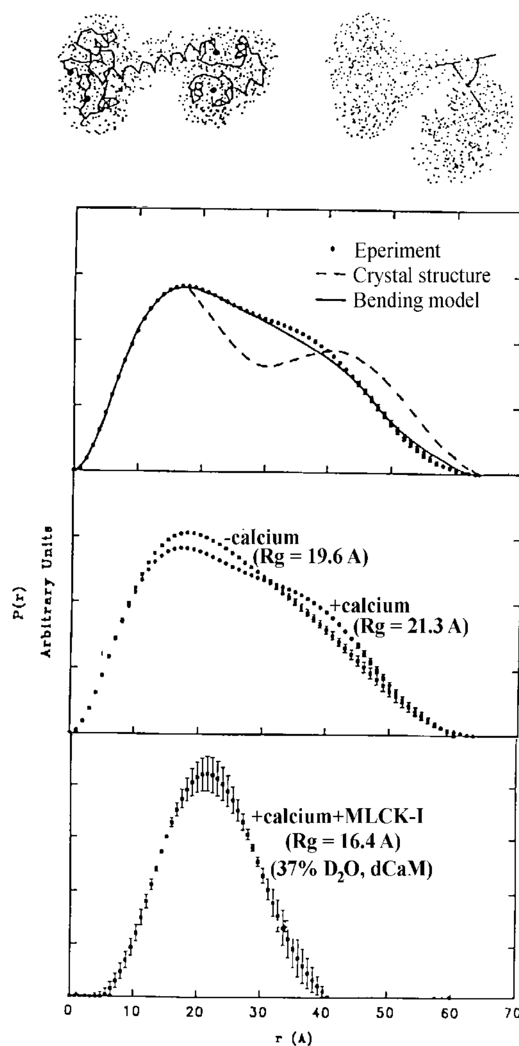

Figure 2. SANS data from calmodulin (upper panel) and calmodulin bound to myosin light chain kinase (bottom panel) (after Heidorn & Trewhella (1988) *Biochem. 27*, 909; Heidorn et al. (1989) *Biochem. 28*, 9316.)

Neutron reflectometry. The technique of neutron reflectometry can be used to study model biological membranes and bio-mimetic structures that are of interest in drug delivery, biomineralisation, biosensors and so on. Such studies can be made on solid substrates or at the air-liquid interface, often benefit from complementary X-ray reflectivity measurements, and are well suited to the contrast-variation method. Indications are that this field will grow enormously by 2005.

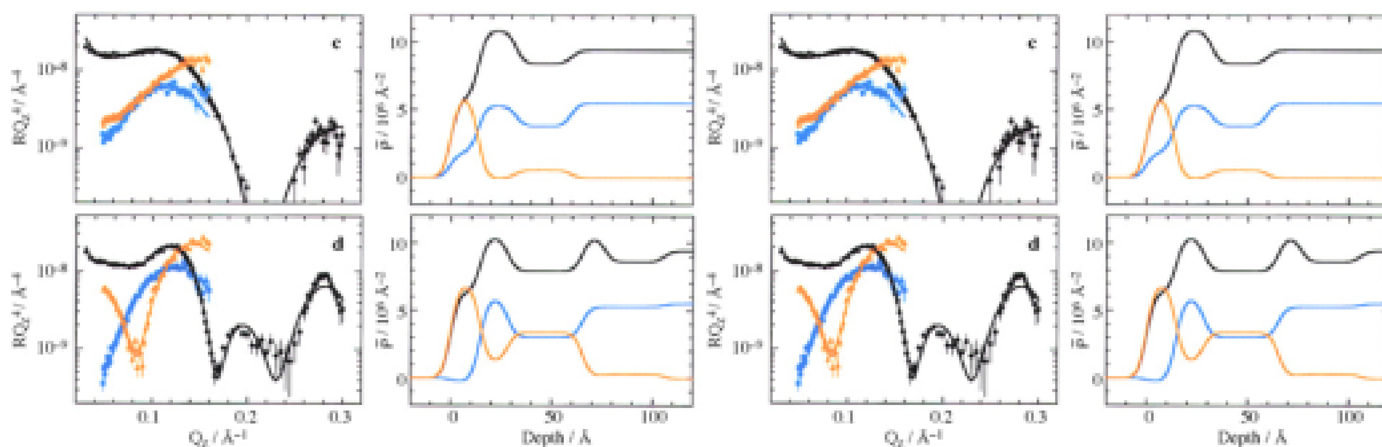

Figure 4. Reflectivity (left) and real-space model profiles (right) for (a) surfactant-only solutions. (b),(c) and (d) surfactant-silicate solutions after 25%, 75% and 90% of the time required for diffraction peaks to appear. Black lines refer to h_{33}-C_{16}TAC in H_2O x-ray data, orange lines to d_{33}-C_{16}TAB in ACMW neutron data, and blue lines to h_{33}-C_{16}TAC in D_2O neutron data. (from A. S. Brown, S. A. Holt, P. A. Reynolds, J. Penfold, and J. W. White, *Langmuir,* 14, 5532 (1998).)

High-resolution neutron crystallography. A particular strength of neutron crystallography is that it can directly locate the positions of hydrogen/deuterium atoms including ordered water molecules and in hydrogen-bonded networks, providing large crystals can be grown (size > 1 mm^3). This information is essential for understanding the function and mechanism of many bio-molecules. In a neutron crystallography experiment, hydrogen atoms can be accurately located using only modest-resolution data (~2Å), and they can also be accurately located in the vicinity of paramagnetic metal ions that can prevent the use of NMR techniques.

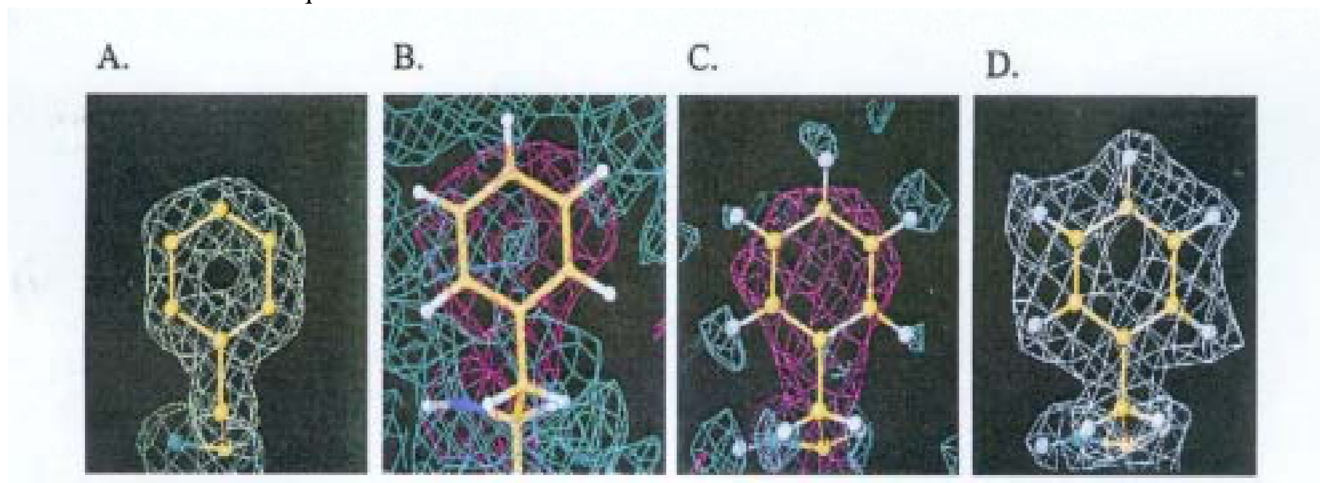

Figure 3. Locating hydrogen positions in a residue of myoglobin, (A) using X-rays, (B) using neutrons and an unlabelled sample, (C) the calculated map equivalent to (B), and (D) using neutrons and a fully deuterated sample. (from Shu, Ramakrishnan and Schoenborn, Proceedings of the National Acadenmy of Sciences, 97(8), 3872-3877, (2000)).

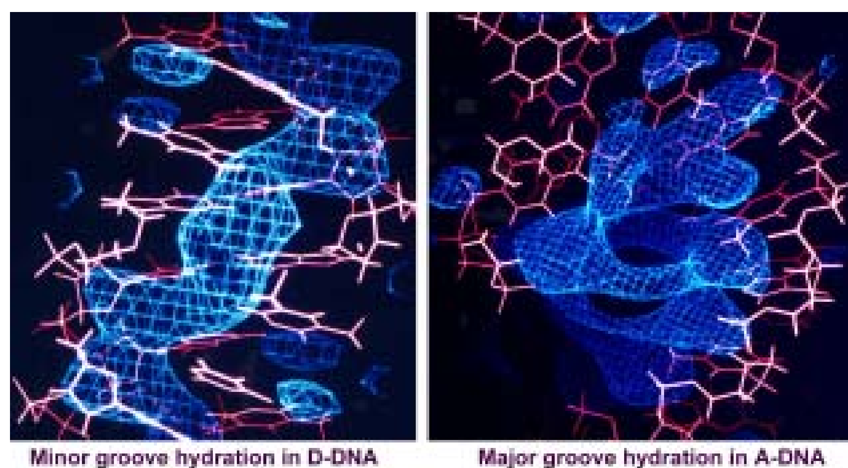

Figure 4. Low-resolution neutron diffraction data showing the minor-groove deuteration in A-DNA and major-groove deuteration in B-DNA (Courtesy of P. A. Timmins)

	Crystallography			NMR	Cryo-EM	Solution scattering		Reflectometry
	X-rays	Electrons	Neutrons			SAXS	SANS	
Sample state	Small crystals (10^{-3} mm^3)	2D crystals (10^{-6} mm^2)	Large crystals ($1mm^3$)	Solution (0.5 ml, ~1mM)	Frozen in vitreous ice	Solution (0.02 ml, 0.1 mM)	Solution (0.2 ml, 0.1 mM)	Monolayer, few cm^2
Observed atoms	Heavy atoms, C,N,O,S,P, metals	Potential function	All atoms, incl. H/D	protons	No atomic resolution	No atomic resolution	No atomic resolution	No atomic resolution
Resolution	0.5 – 3 Å, typically 2.2 Å	2 – 10 Å	1.5 – 10 Å	~1Å RMSD	>20 - 30Å	Shape and distances	Shape and distances	Shape and distances
Structures in data bases*	12,796	~5	9	2,394	n.a.	n.a.	n.a.	n.a.
Size limit	None	None	<30kDa	<30-40kD	None	None	None	None
Strengths	High resolution, large assemblies	Direct phase information, 2D-crystals, membrane proteins	H-bonding, hydration, hydrogens in metal sites	Dynamics, binding, hydrogens	Large assemblies	Shape, some orientation	Orientation, shape, multi-component analysis, contrast variation	Membrane systems, contrast variation

*Protein Database, 25th July 2001

3. Potential study areas

There are a number of areas of biology with biomedical and agricultural significance.

Multimeric macromolecular complexes:

There are numerous biological systems in which multimeric macromolecular complexes are of fundamental importance to cellular processes and, in the context of multicellular systems, extracellular tissue functions. A number of Australian research groups working in biomedical and agricultural areas, have major interests in such complexes. With the advent of contemporary genomics, proteomics, and associated biotechnology, including recombinant protein and RNA expression systems, there are major opportunities to develop macromolecular complex reconstitution systems for investigation by both small-angle neutron scattering and neutron reflectometry. Specific examples identified are the following:

Protein-protein interactions**:** Such complexes are of fundamental importance in cellular cytoskelatal structure and cellular regulation. Technological innovation is needed because of the limitations of protein crystallography and NMR in the analysis of complex structures. Biomedical areas that are in particular need of innovation and would benefit in the short term from the application of neutron scattering analysis (and which are likely to attract funding support form the major national funding bodies), include spatial arrangements of partners in the complexes and conformational changes induced by interactions with regulators.

- heteromeric enzyme complexes: for example, the interactions between protein kinases, phosphatases and their protein regulatory protein and small-molecule ligands (eg, calmodulin- and p21 protein-regulated kinases);

- cell-signalling complexes: for example, scaffold-anchored signal-transduction mediators containing several distinct protein regulators of intracellular metabolic and gene expression events.
- Cytoskeletal-regulatory enzyme complexes: eg GTP'ases and protein kinase complexes.

Protein-nucleic acid interactions: The successful application of neutron-scattering technology to the investigation of the conformation of higher order chromosomal structures and spatial relationships between individual proteins in ribosomes, illustrates the potential for the technology in the general area of investigation of protein nucleic acid complexes. Areas where there is already considerable critical mass in a number of Australian universities and research institutes likely to be of particular interest to funding bodies include:

- complexes between gene regulatory sequences and transcription factors where the DNA target sequences (usually 5'upstream regulatory sequences) contain multiple enhancer/silencer elements and have the capacity to bind a variety of distinct transcription factors:
- RNA-protein particles, for example, messenger ribonuclear protein complexes: this area is likely to become a major focus of attention in future investigations of forms of cellular regulation dependent on protein-mRNA interactions within untranslated regions of the mRNAs, particularly, the 3'UTR.

Protein-lipid interactions: The emerging interest in protein-membrane and intracellular protein phospholipid interactions in cell function, particularly cellular regulation, has created the need for new approaches to the investigation of the shapes and spatial arrangements of lipid/membrane bound proteins. Both neutron scattering and neutron reflectometry methodologies have considerable potential in areas which are either not suitable for NMR and X-ray crystallographic analysis, or where the neutron methodologies provide complementary information.

Specific needs for studies in the Australian context occur in the fields of:

- Pharmaceuticals/drug design
- Agriculture
- Food technology/processing
- Fundamental protein science
- Bioremediation

Pharmaceuticals

Protein-based therapeutics represent a new generation of drugs that have the potential to be more potent and have fewer side-effects than existing small-molecule drugs. The world market for protein-based therapeutics exceeded US$20 billion in 1999. The development of new molecules requires increasing efforts in the study of protein-protein interactions.

Agriculture

Insect predation accounts for substantial pre-harvest crop losses. Classical chemical insecticides place a significant economic and environmental burden on Australia. A new generation of insect control agents is under development in which the genes encoding novel insecticidal proteins can be inserted into commercial transgenic crops to provide in-

built protection against insect pathogens. One example concerns a multidomain protein that is made up of six protease inhibitor domains. In the past NMR has been used to determine the structures of the individual domains (~6 kDa) but so far it has not proved possible to crystallise the intact precursor, nor to determine its structure by NMR. Knowledge of the intact precursor structure is important because it will allow the design of new proteins with a different complement of individual domains. Since the structures of the individual domains have already been determined, small angle scattering experiments can be used to reconstruct the structure of the intact precursor to high resolution.

Food Technology/processing

The contrast variation provided by neutron scattering techniques allows the study of protein/lipid mixtures. This has potential in all sorts of areas, from milk, ice cream, chocolate, fats and their substitutes, to the digestion process itself.

4. Immediate Strategy

Biological systems are particularly suitable for study by neutron scattering due to their high hydrogen content. The neutron scattering power of hydrogen (b = -3.741 fm) and its heavy isotope deuterium (b = 6.674 fm) are very different and isotope substitution can therefore provide a very simple form of labelling. Several different neutron scattering techniques were considered in the workshop and it was concluded that small-angle neutron scattering (SANS) and reflectometry should be given the highest immediate priority for the Replacement Research Reactor, as they are currently of highest interest to the biological community. The workshop noted that two specialised instruments are foreseen in the initial instrument suite.

SANS is a powerful technique for studying the shape and conformation of macromolecules and macromolecular complexes in solution. It is particularly useful when used in conjunction with the contrast variation method, which allows one to visualize specific parts of a complex via selective deuteration of the molecules or of their aqueous environment. For example, a protein-nucleic acid complex, such as a virus, can be dissolved in a mixture of 40% D_2O:60% H_2O and this will render the nucleic acid invisible to neutrons and allow the protein alone to be seen. In a multi-protein complex one or more members of the complex can be deuterated and rendered invisible in a similar way. In this way the relative dispositions of components of a complex can be determined. As small angle neutron scattering is a solution technique, changes in conformation as a function of environment (ligand binding, pH, ionic strength etc) can also be monitored. Discussions at the workshop revealed a significant interest in such applications for example in the role of lipids in the regulation of protein conformation.

Another area of strong demand is the characterization of the nature and behaviour of membranes. Neutron reflectometry provides a unique analytical tool, which facilitates the visualization of interfacial structures and therefore lends itself to the study of membranes. Although applications to biological systems are in their infancy, major advances in the study of model lipid systems have been made recently using facilities both in the US and in Europe. Most biological applications will require the use of horizontal surfaces and the reflectometer design must take this into account.

In order to prepare the user community, it is essential that due consideration be given to the gaining of experience in biological applications of these techniques using present facilities at HIFAR and overseas, as well as to the future instrumentation required for the Replacement Research Reactor. This experience encompasses sample presentation preparation, deuteration of molecules, experience with the actual instruments, interpretation of data, and generation of successful applications to showcase biological applications.

Recommendation: *The community agreed that a number of projects using the SANS and reflectometry techniques should be developed to expand the present range of activities. The projects will use existing facilities at ANSTO plus access to overseas facilities in order to make a significant contribution to the field. We anticipate major use of both SANS and Reflectometry at the Replacement Research Reactor.*

5. For the Longer Term

The movement of hydrogen nuclei (with or without electrons) is fundamental to many biological processes. Neutron diffraction, in principle, is an ideal technique for revealing the location of hydrogen nuclei.

Ultra-high resolution X-ray structures (resolution better than 1.0A) do not provide the signal to noise ratio to unequivocally locate all hydrogen atoms. This is especially true for those attached to electronegative atoms, such as oxygen or nitrogen of enzymatically active side chains or water molecules, or water-derived species coordinated to transition metal ions (Z>20). This is largely because the X-ray experiment reveals electron density and electron density on polar X-H bonds is substantially withdrawn onto the electronegative atom, X. Another severe disadvantage in ultra-high resolution X-ray structures is the unpredictable effect of radiation damage on the sample, which can lead to unwanted change of metal oxidation state and loss of hydrogen atoms.

NMR protein structures also are limited in their ability to reveal hydrogen nuclei, especially in the vicinity of paramagnetic metal ions, those rapidly exchanging with water molecules and those protons attached to electronegative atoms.

In practice, however, high-resolution neutron crystallography has severe limitations. These ultimately derive from the low flux and brilliance intrinsic to all current and planned neutron sources. To date, the number of high-resolution protein structures determined by neutron diffraction is low (less that 10). Crystals must be large in size (>1 mm^3 volume), unit cell volumes must be relatively small, effectively limiting the technique to proteins less than around 30 kDa in size. Data collection times are on the order of weeks. A number of significant developments in the recording of diffraction patterns have led to a noticeable increase in productivity in the last several years. In particular, quasi-Laue methods or small crystal oscillations, coupled with area detectors now allow the recording of many reflections simultaneously. Also the techniques of molecular biology allow the preparation of large quantities of highly pure, highly active protein amenable to the growth of large crystals. Perdeuterated samples also greatly improve the quality of diffraction patterns. Thus data collection times have been substantially shortened. However, the order of magnitude improvements that would allow much smaller crystals, much larger proteins and much quicker data collection times have not yet eventuated. There are real prospects of significant further developments.

That high-resolution neutron crystallography can, in principle, inform in a detail not possible with any other technique the presence and precise location of hydrogen atoms, the movement of hydrogen nuclei on change of metal oxidation state and on ligand binding is indisputable. However, current technology has not allowed the promise of neutron diffraction to be fulfilled even on the world’s best facilities. There is a fundamental physics problem with the flux of neutrons incident on a crystal. However, given the potential importance of neutron diffraction, it would be a mistake to close the shutter on this technique.

Recommendation: *The community expressed interest in the science possible using high-resolution single-crystal protein diffraction but does not believe this*

capability will be possible in 2005 at the RRR. However, any future developments in this field should be monitored for possible instrument enhancement past 2005. The community did not express any need for small molecule capability beyond that required by the general small molecule community.

6. Other matters - Dynamics

There was some discussion at the workshop of needs in inelastic and quasielatic neutron scattering, particularly to study macroscopic motions of biomolecules and the motion of water around proteins and other biological molecules. There are no immediate plans to provide such a capability at the RRR, but the excellent cold-neutron source would provide an ideal location for high-resolution spectrometers capable of quasielastic scattering studies.

Recommendation: *It was clearly identified that a triple-axis spectrometer even of the most advanced design would not be suitable for studies of biomolecules. Specialist input into the selection process for the 'inelastic spectrometer' on the Replacement Research Reactor was recommended.*

7. Support infrastructure

Sample preparation requirements. NMR, neutron crystallography and solution scattering techniques all require highly purified samples that are monodisperse at relatively high concentrations (0.1 – 1mM). Current biotechnology advances being promoted by initiatives such as structural genomics promise to make sample preparation more routine and feasible on the scale needed to be able to apply the full suite of structural tools to a plethora on new systems.

On-site sample preparation facilities, close to the neutron instruments, are essential for the success of biological experiments. Users must be able to arrive at the facility with purified, perhaps deeply frozen, samples that require a minimum of manipulation before being placed in the neutron beam. This may involve storage at -70°C, solubilisation of protein, clarification via centrifugation, dialysis, fume hoods, … etc. Small standard lab equipment such as pipettes, analytical balances, pH meters, and a range of standard biochemicals should also be provided.

The provision of complementary techniques would also make the facility particularly attractive. For SANS the preparation of monodisperse samples is of the utmost importance. A dynamic light scattering instrument is required to characterise the aggregation state of samples. Likewise small-angle X-ray scattering is a complementary technique that should be applied on the same samples as used for SANS.

Recommendation: *Participants requested input to the design of the biochemistry/chemistry laboratories at the Replacement Research Reactor since there are a number of important support facilities that must be in close proximity to the neutron scattering instruments. These facilities include sample preparation (eg centrifuges) and characterisation (eg dynamic light scattering, spectrophotometers).*

All biological applications can be strongly enhanced by partial or complete deuteration. The techniques for the *in vivo* production of deuterated macromolecules are fairly well known and are but an extension of standard molecular biology techniques. However, the

effort required to adapt bacterial strains to produce the required deuterated proteins is not always easily available. We strongly suggest that a small number of regional facilities be established to provide the expertise to allow users to perform deuteration of their own proteins (or lipids, nucleic acids, etc.). These facilities should also operate for NMR users. Existing protein characterisation facilities such as the Institute for Molecular Bioscience at the University of Queensland and the Bio21 Molecular Science and Biotechnology Institute at the University of Melbourne are potential locations for such regional facilities remote from the Replacement Research Reactor instrumentation.

Recommendation*: That a small number of regional facilities be established (or enhanced) to allow users to perform deuteration of biomolecules. These facilities would be of significant value to the NMR and neutron scattering communities.*

Community development. The multidisciplinary approach to significant problems in biology was stressed. In general, no single technique will provide all the necessary information and a well-coordinated program integrating a number of techniques is essential. SANS is particularly powerful when the molecular envelopes revealed by SANS are complemented by molecular structures (as determined by X-ray crystallographic or NMR methods) of components of macromolecular assemblies.

Recommendation*: A* ***Neutrons in Biology*** *webpage be developed as a valuable source of information plus an important link in the biosciences community network.*

8. References and Resources

Structure and Dynamics of Biomolecules: Neutron and Synchrotron Radiation for Condensed Matter Studies, E. Fanchon, E. Geissler, J-L. Hodeau, J-R. Regnard, P. Timmins (editors) Oxford University Press (2000).

Neutrons and Life – A Review of Biological Research at the ILL
http://www.ill.fr/pages/menu_g/docs/biology2001.pdf

Useful Websites:

ANSTO – http://www.ansto.gov.au

ILL – http://www.ill.fr

Appendix I. Workshop program

Tuesday, 10th of July 2001

Purpose: To highlight the present and potential future uses of neutron scattering for the study of biological systems

Time	Presentation	Presenter	Chair
8.30	Arrival at U. of Melbourne		
9.00	Opening and Welcome	F. Larkins, Deputy Vice-Chancellor for Research, U. of Melbourne	A. Klein, U. of Melbourne
9.05	Opening and Welcome	S. Carr, Director Radiopharmaceuticals Division, ANSTO	
9:10 – 9.30	An Overview of Instrument Opportunities at the Australian Replacement Research Reactor	R. A. Robinson, ANSTO	
9:35 – 10.30	Bringing the Genome to Life; Small-angle Neutron Scattering Provides a Critical Framework for Understanding Bio-Molecular Machines and Signalling Networks	J. Trewhella, Los Alamos National Laboratory, USA	
10:40 – 11.00	Coffee		
11.00 – 11.30	The Study of Molecular Self-Assembly using Neutron and X-ray Reflectometry	J. White, ANU	R. Cooper, AINSE and U. of Melbourne
11:35 – 12.05	How NMR and Neutron Methods Might Fit Together for the Solution of Biological Problems	H. Schirra, U. of Queensland	
12.10 – 12.30	X-ray Structure of Manganese Superoxide Dismutase at 0.9 A Resolution: Subjectivity of Hydrogen Atom Positions	G. Jameson, Massey University, New Zealand	
12.40	Lunch		
13.50 – 14.50	Neutron Diffraction at High and Low Resolution: from Catalytic Protons to Virus Structure	P. Timmins, Institut Laue Langevin, Grenoble, France	P. Curmi, UNSW
15.00	Workshop Photo		
15:15	Afternoon Tea		
15.45 – 16.15	Inelastic Neutron Scattering Studies of Water in DNA, Proteins and Biopolymers	J. Li, UMIST, United Kingdom	G. Jameson, Massey U.
16.20 – 16.50	Haemoglobin Ordering in Erythrocytes Studied by Pulsed Gradient Field NMR and Small-Angle Neutron Scattering	C. Garvey, Sydney U.	

16.55 – 17.25	Summary of Biological Applications of Neutron Reflectometry from Workshop held at ANSTO on 8-9 May 2001. Charge to Workshop	R. A. Robinson, ANSTO	
18:30 for 19.00	Dinner		

Wednesday, 11th of July 2001
Purpose: to draft Report on the Science and Technical requirements for Biology at Australia's Replacement Research Reactor.

Time	Presentation	Presenter	Chair
9:00	Welcome Back		R. Knott, ANSTO
9:05 – 10.05	The Investigation of Large-scale Biological Structures using Neutrons	R. Wettenhall, U. of Melbourne	
10:10	Coffee		
10:30 – 11:00	Characteristics of the Cold Neutron Source and Neutron Guides at the Australian Replacement Research Reactor	S. J. Kennedy, ANSTO	D. Craik, U. of Queensland
11:00	Workshop Session		
12:30	Lunch		
13.30	Workshop Session & Write Report		A. Klein & R. Robinson
16.30	Workshop Summary and Close		

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