1.5.7 JRA 7: D-LAB

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Project objectives

The main objective of the DLAB JRA (JRA7) was to develop, produce and evaluate systems and protocols for deuterium labelling of biological macromolecules with the aim of enhancing the scope and interdisciplinarity of biological neutron scattering at large scale facilities throughout Europe. This initiative has focused on a number of key issues that have, during the period of the JRA, had a decisive impact on the application of neutron methods for the study of biological systems. Central to this has been the exploitation, throughout Europe, of the capabilities of the ILL-EMBL Deuteration Laboratory in Grenoble to enable more efficient production of selectively and non-selectively H/D-labelled cells, proteins, nucleic acids and other bio molecules. A measure of the importance and impact of this area has been demonstrated (i) by a very clear increase in the requests for facility beam time for studies involving deuterated macromolecules (ii) by the fact that the concept of a Deuteration Laboratory has been duplicated widely at neutron scattering facilities throughout the world (iii) through the results that are now coming into press that would not have been possible without the developments that have occurred as a result of this JRA. A further key element has been training of young scientists in this area – here the impact of this whole initiative is again demonstrable at an international level.

Methods

A number of important methodologies have been developed during the course of this JRA work. These include selective labelling of DNA, selective labelling of DNA-protein complexes, selective deuteration of specific amino acids within proteins, methods for selective hydrogenation of otherwise perdeuterated systems – providing information on dynamics. Other method development has occurred to improve the efficiency and cost effectiveness of bacterial growth, and also for the cheaper production of bacterial feedstock. All of these methods, both directly and indirectly, have related strongly to the impact and exploitable results, contributing to a widened access to neutron scattering by biologists, and promoting mature and lasting exchange of expertise within Europe. In summary, the methods that have been developed are:

• Improved cost effectiveness of biological isotope labelling

• Development of methods for selectively deuterating specific amino acids in proteins

• Development of methods for deuteration of specific nucleotides in nucleic acids

• Development of methods for reverse labelling: hydrogen labelling in deuterated protein

• Development of novel organisms for deuteration

Impact

The impact of this JRA has occurred within a growing sector of the biological sciences sector where it has for a long time been recognised that the ability to label or selectively label biological molecules can have a huge effect on the scope of the work possible by neutron scattering, providing information that is outside the scope of X-ray diffraction methods. For **low resolution**

studies, the strength and uniqueness of this approach relies on the application of the contrast variation methods. In the absence of any specific isotope labelling, the large range of contrasts available through the use of D_2O/H_2O can and has allowed a great deal of useful work to be done. However the only way to distinguish between the components of a system in which the scattering length densities are all similar (e.g. a multi-subunit protein system) is through the use of selective labelling of specific components - normally with deuterium. Methods that have been developed at the start of this JRA are now showing an impact at the neutron instruments and within the scientific press, with in the last year, a variety of publications coming out that will stimulate other scientific endeavours requiring the same methodology. This methodological progress demonstrates the major opportunities available to SANS work by selective labelling of particular domains/subunits.

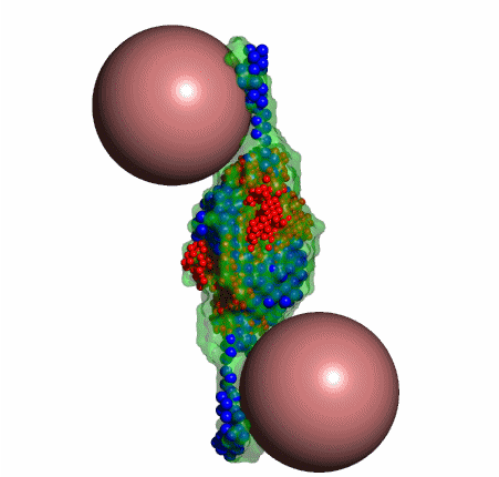

Figure J7.1 Molecular model determined from SANS data recorded from selectively deuterated preparations of a restriction-modification (RM) system. The analysis shows where different function parts of the protein are located within the complex and provides further information on its relationship to the DNA (Callow et al, J. Mol. Biol. **69**, 177-185 (2007)).

At **higher resolution**, the ability to distinguish H/D or solvent molecules in crystalline or fibrous samples provides unique information and insight into hydration, catalysis, and protein-ligand interactions. Furthermore, perdeuteration of macromolecular systems provides a major advantage through the elimination of hydrogen incoherent scattering from both solvent and macromolecule, relieving severe limitations on sample size and data collection times. These approaches for deuteration are allowing the use of much smaller samples – a very significant development given that such sample are extremely difficult to prepare. The same types of opportunity are being exploited in studies of partially ordered systems (e.g. fibres), and neutron reflection. Specific *hydrogenation* of deuterated proteins has also been powerful in elastic incoherent neutron scattering experiments for the of study protein dynamics. Here the dynamic information is contained in the incoherent scattering from hydrogen.

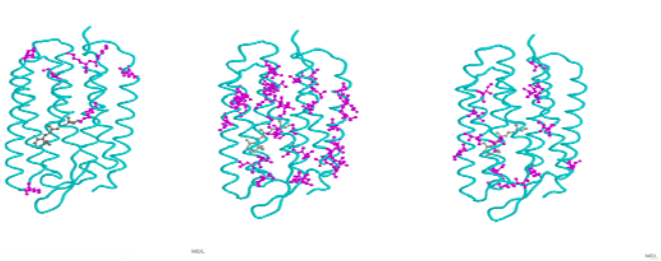

Figure J7.2 Molecular ribbon diagrams showing the way in which an otherwise perdeuterated trans-membrane protein (bacteriorhodopsin) has been selectively hydrogenated – yielding specific information about the dynamics of the labelled regions.

In each of these areas the JRA has had a decisive impact. Up until now the ability to produce such deuterated samples has been outside the scope of typical user laboratories. The methods developed as part of this JRA have brought sample deuteration capabilities closer to individual facilities and thereby closer to individual users.