'substitution' model of core organisation. Cryo-EM reconstructions of rE2/E3BP and rE2/E3BP:E3 directly confirm that the core has open pentagonal faces, agree with the scattering-derived models and show density extending outwards from their surfaces which is much more structurally ordered in the presence of E3. Additionally, AUC characterisation of rE2/E3BP, rE2 and tE2/E3BP cores supports the substitution model. Superimposition of the SANS tE2/E3BP and truncated bacterial E2 crystal structures demonstrates conservation of the overall pentagonal dodecahedral morphology, despite evolutionary diversity. In addition, unfolding studies using circular dichroism (CD) and tryptophan fluorescence spectroscopy show that the rE2/E3BP core is less stable than its rE2 counterpart, indicative of a role for E3BP in core destabilisation. The architectural complexity and lower stability of the E2/E3BP core may be of benefit to mammals where sophisticated fine-tuning is required for cores with optimal catalytic and regulatory efficiencies.

Mathematical modeling predicts that an 'average' 48E2+12E3BP core arrangement allows maximum flexibility in assembly while also providing the most appropriate balance of bound E1 and E3 enzymes for optimal catalytic efficiency and regulatory fine-tuning. We also show that the rhE2/E3BP and bovine E2/E3BP cores bind E3s with a 2:1 stoichiometry and propose that hPDC comprises a heterogeneous population of assemblies incorporating a network of E3 (and possibly E1) cross-bridges above the core surface.

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Keywords: SANS, SAXS, protein

MS.82.3

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Neutron high resolution crystallographic study of perdeuterated *P.f.* rubredoxin

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The availability of perdeuterated protein has had a major impact on the scope of biological neutron scattering has occurred for a wide range of areas including neutron macromolecular crystallography, small angle neutron scattering (SANS), neutron fibre diffraction, and elastic incoherent neutron scattering (EINS), and the result has been a resurgence of interest in a wide range of biological applications. The ILL monochromatic D19 neutron diffractometer which was commissioned recently yielded major gains in terms of detector solid angle of a factor of approximately 25. D19 was originally focused on smaller molecular systems and is typically used to study crystals and fibres having relatively small unit cells. However, it is becoming increasing apparent that the instrument is capable of studying much larger systems. The degree to which monochromatic instruments benefit from sample perdeuteration has been questioned [1] but there exists no strong empirical base of data to draw on in evaluating this issue. This will supplement the information available on the gains to be expected by eliminating hydrogen incoherent scattering via *in-vivo* perdeuteration.

We use a well-known model protein for which large crystals are available [2]. Here we present the results obtained on a perdeuterated crystal of *Pyrococcus furiosus* rubredoxin, building on the information available from other crystallographic perdeuteration work [3], i.e. Xylose isomerase (XI) [4] and seeking consensus results that can be used to quantify this issue and provide a rational basis for the development of ILL-D19's biological user base.

Neutron data collection was performed successfully on a 7 mm^3 perdeuterated crystal at ambient temperature on D19. Full neutron datasets were recorded using 2.42Å and 1.46Å wavelenght, yielding excellent data to a resolution of 1.25Å. The high resolution structure is presented here. Additionally, the results are of biological interest. Rubredoxin is an electron transfer protein. Electron transfer is suspected to occur between rubredoxin and its redox partners (i.e. ferritin) and the protonation states of the amino acid residues are of central interest to this.

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Keywords: neutron, perdeuteration, rubredoxin

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Active site protonation states of perdeuterated Toho-1 beta lactamase

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Purified perdeuterated Toho-1 beta lactamase was obtained in high quantities (600mg/L) at the center for structural biology (CSMB) at Oak Ridge National Laboratory (ORNL). Mass spectrometry analysis showed a hydrogen/deuterium exchange of greater than 99% and large protein crystals (~8mm^3) where grown on site using the batch method.

Room temperature neutron diffraction data of the fully perdeuterated Toho-1 R274N/R276N double mutant beta lactamase in the apo form was collected on LADI III and used to determine the positions of deuterium atoms within the active site of the enzyme. This perdeuterated neutron structure of the Toho-1 R274N/R276N reveals the clearest picture yet of the ground-state active site protonation states and the complete hydrogen-bonding network in a β-lactamase enzyme [1,2]. The ground-state active site protonation states detailed in this neutron diffraction study are consistent with previous high-resolution X-ray studies that support the role of Glu166 as the general base during the acylation reaction in the class A beta lactamase reaction pathway. The current status of further structural studies aimed at determining the protonation states of active site residues when an acylation transition state inhibitor is bound will be given. These are some of the first protein structures to come from the new single crystal diffraction instruments (TOPAZ, MaNDi, IMAGINE) all of which are on site at ORNL.

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Keywords: beta lactamase, neutron diffraction, toho-1

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Deuteration of oleic acid, lipids and other molecules for neutron studies

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In this paper, the synthesis and application of a range of deuterated organic molecules for the investigation of complex systems in the fields of structural biology, biotechnology and nanotechnology will be discussed. In chemical reactions, deuterium (^{2}H) behaves similarly to hydrogen (^{1}H); however, the different physical properties of the hydrogen and deuterium nuclei mean that they scatter neutrons quite differently. Techniques such as Small Angle Neutron Scattering, Neutron Reflectometry and Neutron Crystallography exploit this difference in scattering length densities to obtain data for properties such as: atomic and molecular structure, the precise location of hydrogen atoms in organometallic systems, and highlighting molecular components in complex nanostructured systems.

In studies where one may wish to observe the interaction between hydrocarbon-based chemicals or biological molecules with lipids or surfactants, there is often a lack of neutron scattering contrast in the system which can be overcome by deuteration of the tail component of these lipids or fatty acids. Oleic acid forms an unsaturated tail component in many phospholipid molecules that are fundamental to the structure and functioning of cellular membranes. We have recently produced deuterated oleic acid on a gram scale in our laboratories from deuterated fatty acid molecules with the appropriate mono- and bi-functional terminal groups; prepared using a multi-step reaction scheme. This involved hydrothermal heterogeneous catalytic H/D exchange reactions in D_2O followed by synthetic reactions. Conjugating the two deuterated alkyl chains using the Wittig reaction afforded purely a *cis*-conformation around the carbon-carbon double bond, essentially producing deuterated oleic acid. This facilitated the synthesis of deuterated glycerol monooleate through esterification of the prepared d-oleic acid with a glycerol fragment. Similar reactions were also used to prepare lipids with hydrophobic (different alkyl chain lengths) and hydrophilic (ethylene oxide) moieties where the head group is conjugated to a sulfur- or alkene-containing anchoring ligands for surface modification and self-assembly (i.e., on gold or silicon surfaces).

The strategies used for deuteration and synthesis of oleic acid, lipids, surfactants, sugars, bioactive small organic molecules, heterocyclic and aromatic compounds will be presented. We will also demonstrate how the availability of these deuterated compounds greatly increases the scope of some neutron scattering, reflectometry and diffraction experiments. For example, deuterated trehalose was used to determine the localisation of sugar molecules with respect to lipid head groups using neutron diffraction, to provide insight into the molecular mechanisms of cryoprotection by sugar molecules. Using neutron scattering, deuterated oleic acid and glycerol monooleate allowed investigation of surfactant interaction with cubosome and hexosome liquid crystal nanoparticles forming 3D structures with intertwining aqueous channels. Deuterated organic light emitting diode (OLED) molecules facilitated investigation of the morphology of thin-film multilayer organic light emitting devices using neutron reflectometry [1].

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Cyber-enabled learning and practice in crystallography: educating the next generation

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This talk will describe the current state of crystallographic science and new pedagogy made possible by Web 3.0. In the last fifteen years, academic crystallography has largely migrated from a research specialty to a technique employed by a broad user community. Yet, the knowledge gained from analysis of its structures is a key underpinning of modern science and technology. Crystallography has gained importance for researchers in disciplines where it has not previously appeared, such as engineering and solar energy technology. Technical advances, however, now enable users with little or no training, or deeper understanding, to often but not always produce quality results, as revealed by recent high profile and embarrassing retractions in the peer reviewed literature, many the result of pathological science or inadequate review. The absence of crystallography in many curricula has led to growth of and dependence on independently funded workshops and summer schools, as well as other, non-traditional curricular resources for crystallography instruction, such as Web pages and online courses, which allow crystallography to be self-taught. Implementing modern Web technologies with sound pedagogy requires skilful integration of relevant, often disparate resources into useful and usable frameworks, enabling learners to interact, explore new situations, and use scientific reasoning skills such as hypothesis testing and model-based reasoning. The evident disproportion in implementing contemporary technologies into our global crystallography education resources requires that we shift our focus from simply imparting content knowledge to empowering students with the fundamental processes and skills needed for on-demand learning and practice in crystallography.

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Promoting crystallography: using crystal structures in chemical education

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Crystallography is truly interdisciplinary. It has its roots in physics, mathematics and computer science, and has practitioners and beneficiaries from chemistry, biology, materials science and many other disciplines. Despite its pre-eminence as *the* preferred method for