'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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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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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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