

MS.81.5

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Control of thermal expansion behavior by Zn deficiency in $Mn_3Zn_{1-x}N$
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We report the controllable zero thermal expansion (ZTE) behavior with a larger temperature range in the antiperovskite $Mn_3Zn_{1-x}N$ ($x=0-0.07$) system. The system has a cubic lattice and two magnetic order states. One magnetic order occurs at 185 K where the paramagnetic state (PM) at high temperature transforms to a non-collinear antiferromagnetic state (M1), accompanying with $\sim 0.5\%$ of the cubic lattice increasing in M1 state. Another non-collinear antiferromagnetic order (M2) occurs at low temperature (below 180 K). The M2 has no magnetic lattice affect compared to the PM phase and its magnetic ordering temperature depends on the vacancy x at Zn site. The M1 phase has ZTE behavior and the ZTE temperature can be adjusted by the vacancy x at the Zn site. The coefficient of thermal expansion (CTE) was achieved to $5.83 \times 10^{-7} \text{ K}^{-1}$ in the wide temperature range from 5 K to 180 K in $Mn_3Zn_{0.93}N$. The quantitative analysis of neutron diffraction data gives a quantitative description about the “spin – lattice” correlation that the change of the magnetic moment, due to the spin re-arrangement induces the lattice contraction, and coincidentally counteracts the normal positive thermal expansion, which is the origin to introduce near zero thermal expansion effect. It is suggested that the zero thermal expansion can be designed by adjusting the spin moment and its changing process with temperature.

Keywords: antiperovskite, magnetostriction, zero thermal expansion

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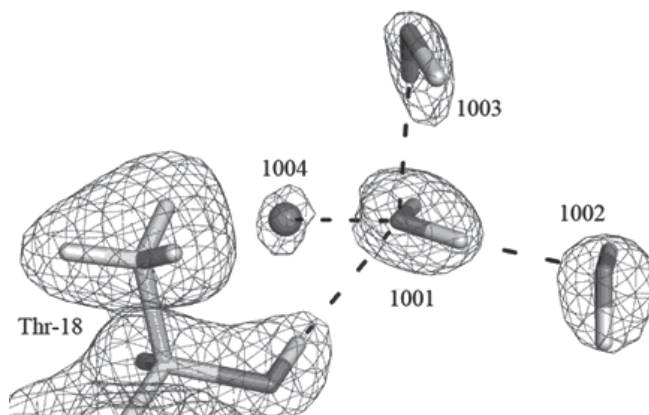
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Neutron structure of type-III antifreeze protein leads to ice interface model

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Antifreeze proteins (AFPs) inhibit ice growth at sub-zero temperatures. The prototypical type-III AFPs have been extensively studied, notably by X-ray crystallography, solid-state and solution NMR, and mutagenesis, leading to the identification of a compound ice-binding surface (IBS) composed of two adjacent ice-binding sections, each which binds to particular lattice planes of ice crystals, poisoning their growth. This surface, including many hydrophobic and some hydrophilic residues, has been extensively used to model the interaction of AFP with ice. Experimentally observed water molecules facing the IBS have been used in an attempt to validate these models. However, these trials have been hindered by the limited capability of X-ray crystallography to reliably identify all water molecules of the hydration layer. Due to the strong diffraction signal from both the oxygen and deuterium atoms, neutron diffraction provides a more effective way to determine the water molecule positions (as D_2O). Here we report the successful structure determination at 293K of fully perdeuterated type-

III AFP by joint X-ray and neutron diffraction providing a very detailed description of the protein and its solvent structure. X-ray data were collected to a resolution of 1.05 Å, and neutron Laue data to a resolution of 1.85 Å with a “radically small” crystal volume of 0.13 mm³. The identification of a tetrahedral water cluster in nuclear scattering density maps (see figure) has allowed the reconstruction of the IBS-bound ice crystal primary prismatic face. Analysis of the interactions between the IBS and the bound ice crystal primary prismatic face indicates the role of the hydrophobic residues, which are found to bind inside the holes of the ice surface, thus explaining the specificity of AFPs for ice versus water.



Keywords: neutron diffraction, antifreeze protein, protein crystallography

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Neutron scattering reveals human pyruvate dehydrogenase complex organisation

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Mammalian pyruvate dehydrogenase complex (PDC), a key multi-enzyme assembly responsible for glucose homeostasis maintenance and conversion of pyruvate to acetyl CoA, comprises a central pentagonal dodecahedral core consisting of two subunit types (E2 and E3BP) to which peripheral enzymes (E1 and E3) bind tightly but non-covalently. Neutron scattering, deuteration and a range of molecular-biological, protein-chemical and biophysical techniques were used to resolve a conflict between two extant models of PDC core organisation (the ‘addition’ (60+12) and ‘substitution’ (48+12) models) and to demonstrate clear differences in subunit content and organisation between a recombinant hPDC core (40E2+20E3BP), generated under defined conditions where E3BP is produced in excess, and its native bovine (48E2+12E3BP) counterpart.

The first ever low-resolution structures of human recombinant full-length (rE2/E3BP), truncated (tE2/E3BP) and native bovine heart (bE2/E3BP) PDC cores obtained by SAXS and SANS will be presented. These structures, corroborated by negative stain- and cryo-EM data, clearly reveal open pentagonal core faces, favouring the

'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

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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 increasingly 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 mm³ perdeuterated crystal at ambient temperature on D19. Full neutron datasets were recorded using 2.42Å and 1.46Å wavelength, 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 (~8mm³) 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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