MS33-04 **Neutrons on the Loose: Tracking Down Weaknesses in the Hydrogen-Network of Transthyretin.**
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Human Transthyretin (TTR) is a protein found in the plasma and cerebrospinal fluid, and is normally associated with the transport of thyroid hormones. Despite its high abundance (~20 mg/dL) and the reasonable stability of wild-type TTR, the molecule has an intrinsic tendency to form amyloid fibrils in individuals above a certain threshold of age, leading to the outbreak of diseases such as polyneuropathy, cardiomyopathy, liver failure, carpal tunnel syndrom, and vitreous amyloidosis. A distinction is made between senile systemic amyloidosis caused by the wild-type form and familial amyloidosis, provoked by specific point mutations in the genome.

We have overexpressed recombinant perdeuterated wild-type human TTR in *E. coli* in fermenters at the ILL Deuteration Laboratory and grown large crystals of up to 3 mm^3.

Two neutron data sets have been collected on the same crystal; firstly on a quasi-Laue diffractometer to 2.0 Å resolution (LADI-III, ILL), and secondly on a monochromatic thermal neutron diffractometer to 2.3 Å resolution (D19, ILL). A room temperature X-ray data set to 1.9 Å resolution collected at ID23-1 (ESRF) has been used for joint X-ray/neutron structural refinement with phenix.refine. Independently, a structural refinement of these data has been carried out [1].

The analysis yields important information on the orientation of buried water molecules and the proton orientation of central hydroxyl groups, as well as details of hydrogen bonds stabilising the native tetrameric structure.

[1] Haupt, M., Blakeley, M.P., Teixeira, S.C., Mason, S.A., Mitchell, E.P., Cooper, J.B. & Forsyth, V.T. (2011). *Acta Cryst. F* **67**, 1428-1431.

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MS33-05 **A joint neutron/X-ray crystallographic study on the mechanism of pectate lyase.**
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Pectate lyases such as those excreted by *Bacillus subtilis* (BsPel) are potent virulence factors of plant pathogenic bacteria that cause soft rot disease, a major problem for plants in the field and in storage. It is a result of the lyases breaking down the stable pectin network, a major component of plant cell walls, then allowing for invasion by other pathogens. BsPel belongs to a family of lyases that cleave α-1,4-linked galacturonic acid units of pectin via an *anti* β-elimination reaction. A proposed catalytic mechanism [1] features a conserved arginine acting as a base as low as pH 4.5. Where calcium ions are required for activity; the first is involved in substrate binding and the subsequent two in stabilisation of the intermediate. The major mechanistic question surrounds the protonation state of this proposed catalytic arginine, which at physiological pH (7.0) is fully protonated. Therefore, proton abstraction initiating the reaction is likely to result from a local shift of pK_a that has yet to be proven. We have over-expressed, purified and crystallised perdeuterated recombinant BsPel. Neutron and X-ray diffraction data were collected at room temperature on the same crystal sample. The results of a joint neutron/X-ray structure refinement coupled with the results of theoretical pK_a calculations of the active site residues will be presented and the results discussed.

[1] Seyedarabi, A., To, T.T., Ali, S., Hussain, S., Fries, M., Madsen, R., Clausen, M., Teixeira, S., Brocklehurst, K., Pickersgill R. (2010). *Biochemistry.* **49**, 539–546.

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