INSTITUT MAX VON LAUE - PAUL LANGEVIN

NEUTRONS FOR SCIENCE ®

TARGETING TRANSTHYRETIN AMYLOIDOSIS: NEUTRON AND X-RAY DIFFRACTION ANALYSIS OF A PATHOGENIC PROTEIN

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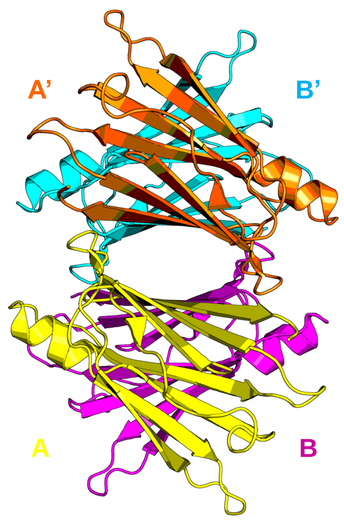
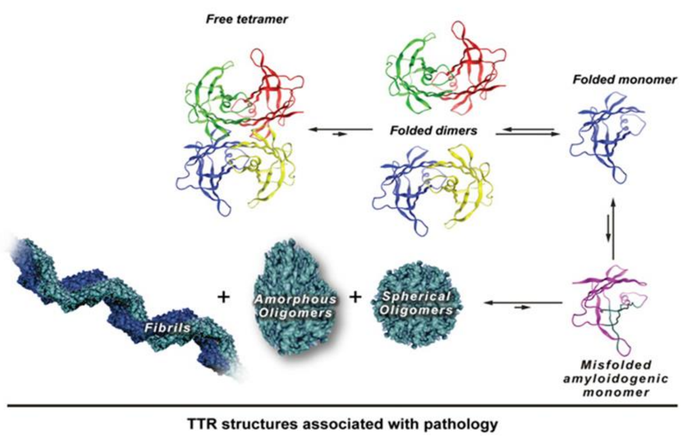
STRUCTURAL RELEVANCE TO PATHOGENESIS

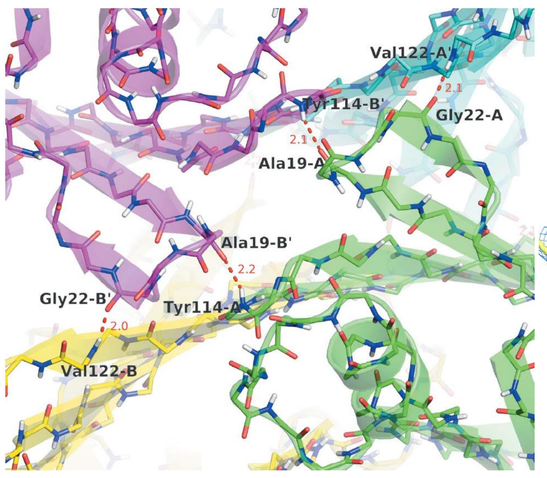


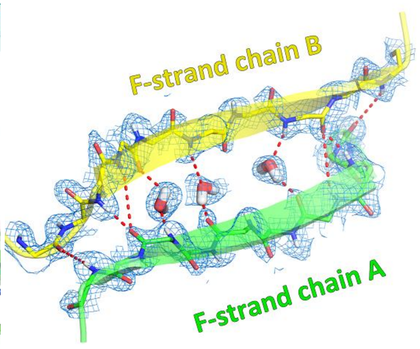
Human transthyretin (TTR) is a 55kDa plasma protein composed of 4 identical subunits. Each subunit is formed from two four-stranded β-sheets linked by a short α-helix loop.

Wild-type TTR has an intrinsic tendency to form amyloid fibrils in all elderly individuals over 70-year-old and causes senile systemic amyloidosis (SSA), predominantly affecting the heart. Mutations in the TTR gene result in early onset familial amyloid polyneuropathy (FAP) diseases, which affects the peripheral and autonomic nervous systems. Up to date, more than 80 pathogenic mutations have been described.[1]



Adapted from Bulawa et al. (2012); PNAS





Haupt et. Al. (2014); IUCrJ

Dissociation of tetramer is believed to be highly related to the hydrogen bonding interactions in the protein. Several neutron crystallographic studies[2,3] have been carried out on the wild-type TTR, revealing the importance of water molecules at the protein monomer-monomer (*right*) and dimer-dimer (*left*) interfaces.

PROJECT AIM

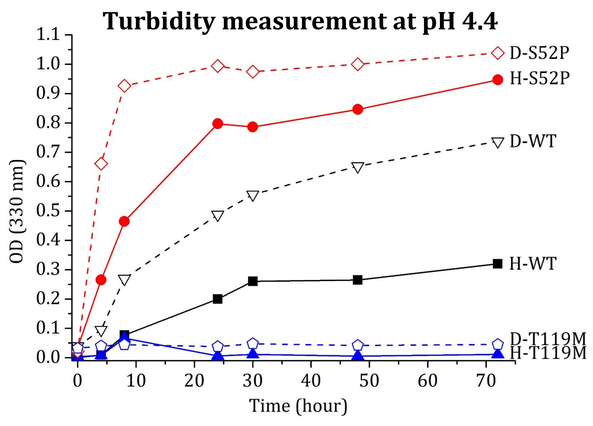
This project aims to study the structural differences between the S52P and the T119M mutants comparing to that of the wild-type (WT), specifically looking at hydrogen bonding, amino-acid protonation states and hydration. The S52P mutant is highly unstable and forms fibres very quickly[4]; the T119M, on the other hand, represents the superstable form of TTR mutant and is resistant to amyloid formation[5].

DEUTERATION EFFECTS

High resolution X-ray data have been collected on the WT, S52P, and T119M protein crystals, and in both hydrogenated (H) and deuterated (D) forms at ID23-1, ESRF. All six variants belong to the same space group ($P2_12_12$) and showed no major differences in the structures.

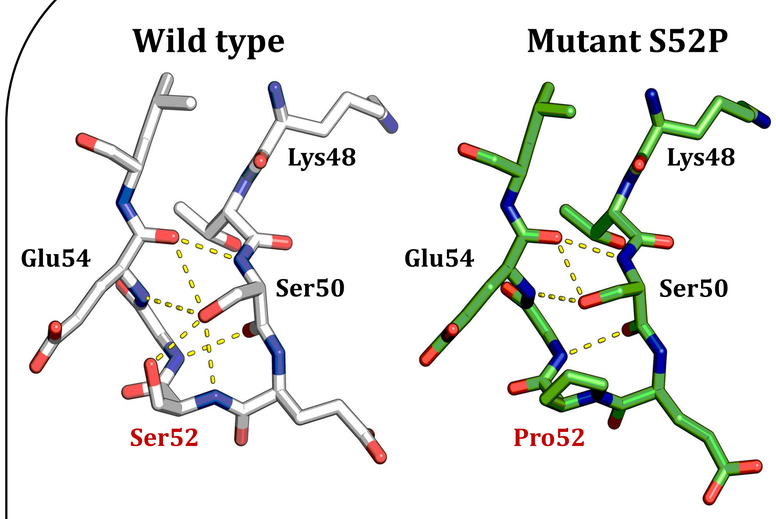
	HWT	DWT	HS52P	DS52P	HT119M	DT119M
Resolution (Å)	1.30	1.42	1.23	1.28	1.22	1.22
R-factor	0.145	0.141	0.150	0.144	0.162	0.164
R-free	0.180	0.199	0.185	0.175	0.198	0.199

Despite having similar structures, the WT protein and the other two variants showed very different rates in fibril formation. When triggered with acidic buffer, the S52P mutant aggregates at a higher rate than that of the WT; whereas the T119M mutant remained stable even in fibril-stimulating condition. The D-protein showed higher rate of fibril formation comparing to that of the H-protein in all cases.

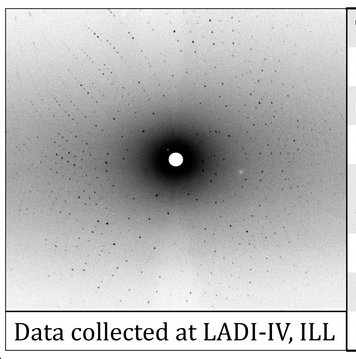


It is a well known premise that deuteration does affect kinetics. However, it was shown that the magnitude of structural changes occur due to deuteration is not relevant to the diffraction resolutions commonly encountered with protein crystallography[6].

THE NASTY S52P MUTANT

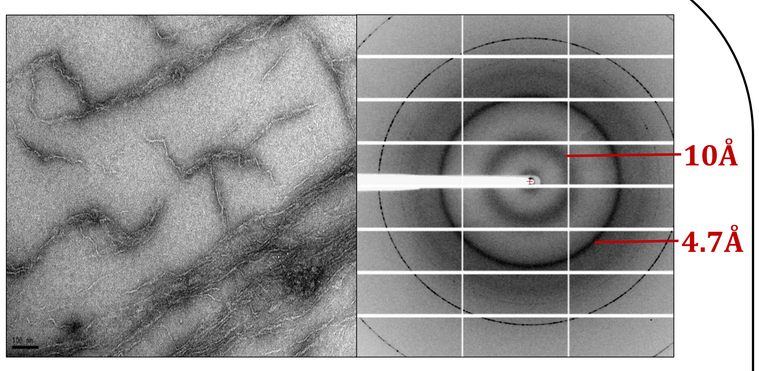


As shown in the X-ray crystal structures above, two hydrogen bonds with Ser50 are lost in the S52P mutant. The lost interactions may destabilise the β-turn and increase the susceptibility of the protein to dissociation into monomers. The serine to proline substitution may also abolish a water-mediated hydrogen-bonding network. This work is being further investigated with neutron crystallography. Quasi-Laue neutron diffraction data from a 0.5 mm^3 perdeuterated crystal has been collected and detailed analysis is being carried out.

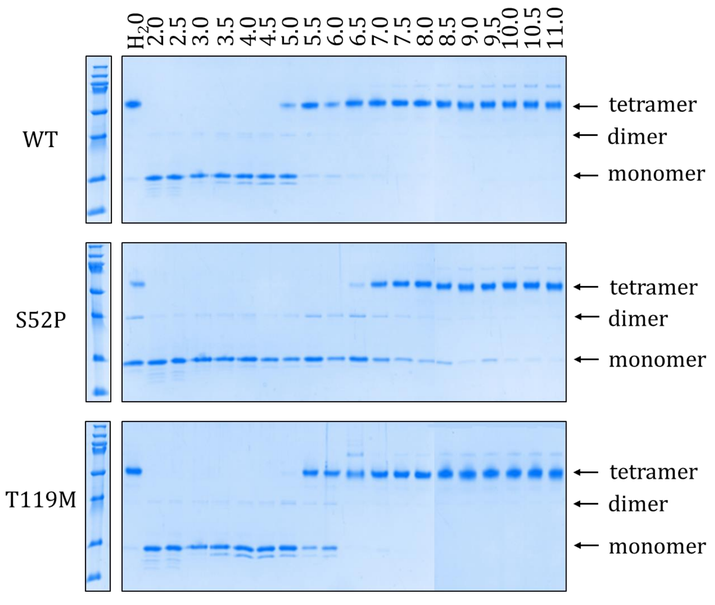


Temperature	293 K
Resolution	43.69 – 1.8 Å
Completeness	70.4%
No. of images	20
Exposure time per image	10 hours
Oscillation	Step angle = 7°
Mean I/σ(I)	8.1
Multiplicity	4.6

Data collected at LADI-IV, ILL



S52P amyloid fibrils. *Left:* Negative-stain EM image after 72 hours incubation in fibril-stimulating condition. *Right:* X-ray fibre diffraction pattern shows characteristic features associated with the cross-β amyloid motif.



Analysis of tetramer stability. The propensity of amyloid formation is coupled to tetramer dissociation. A fraction of tetrameric S52P dissociates into monomeric state at all pH values, showing its decreased stability as compared to WT and T119M.

References:

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