

Protein and water dynamics at the atomic level

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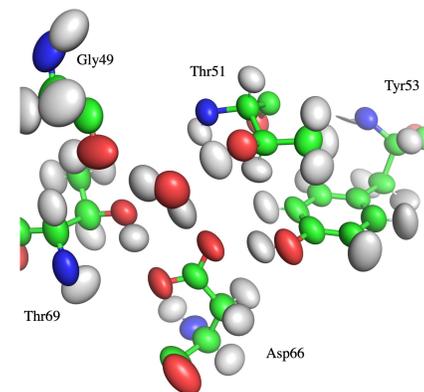
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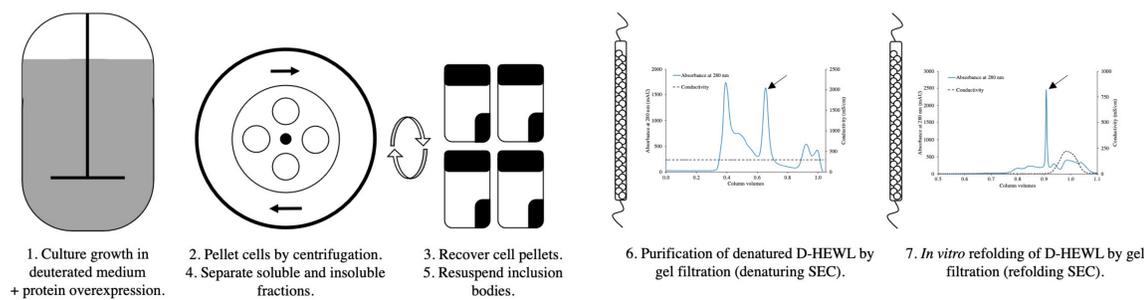
Introduction

Protein biological function is intimately related to protein structure as well as dynamics, at the molecular and atomic scales. While X-ray diffraction has been the most prominent technique in deciphering protein structure, atomic thermal motion has been somewhat overlooked due to limitations inherent to X-ray models, which do not account for deformations of valence electron-densities [1]. On the other hand, neutron diffraction has been the technique of choice to study atomic motion in small-molecule crystallography [2]. Our work aims at pushing the boundaries of neutron macromolecular crystallography to obtain anisotropic atomic displacement parameters (ADPs) from perdeuterated hen egg-white (D-HEWL) crystals. Complete atomic resolution neutron diffraction data allows a comparative analysis between neutron and X-ray derived ADPs, highlighting potential discrepancies.

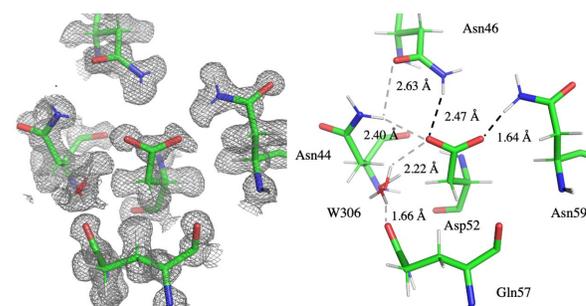


Ellipsoidal representation of anisotropic ADPs from the neutron room temperature model of perdeuterated hen egg-white lysozyme.

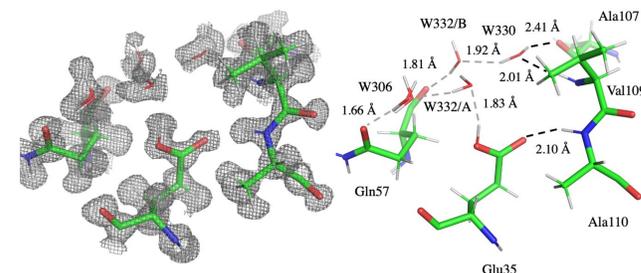
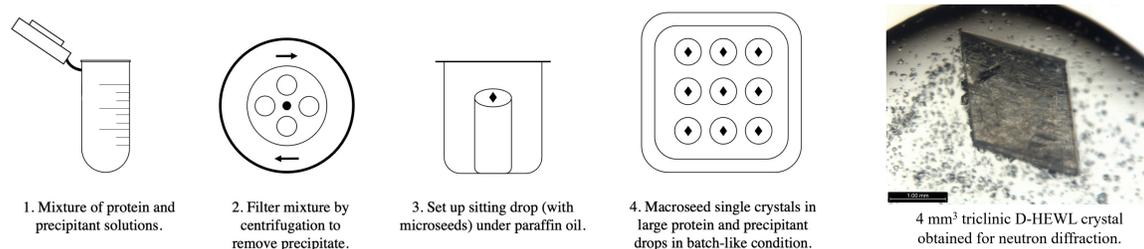
Production of D-HEWL in *E. coli* inclusion bodies and *in vitro* refolding [3]



The complete picture of HEWL active site

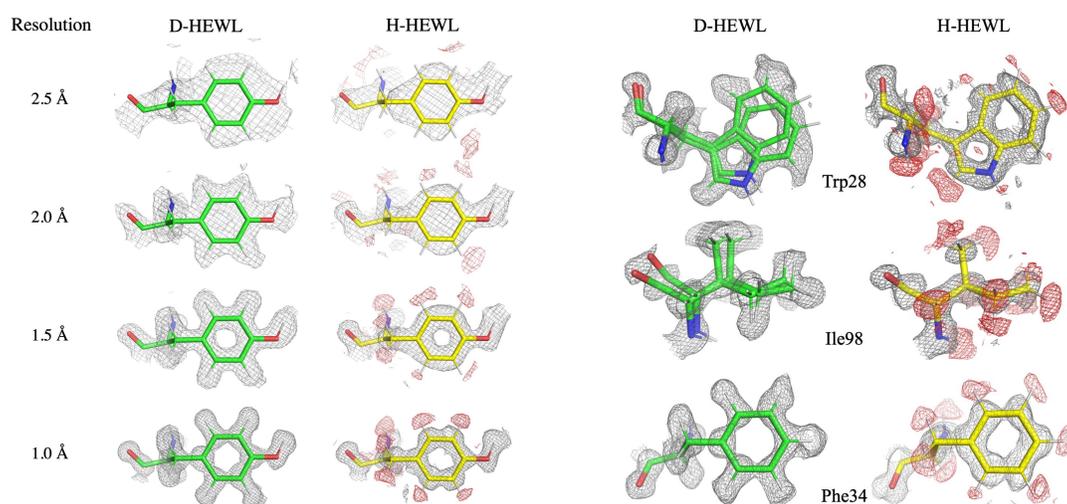


Crystallization of refolded D-HEWL for neutron diffraction



Neutron data at room temperature was recorded from a crystal grown at a pH close to optimal for enzymatic activity. The Glu35 and Asp52 catalytic residues were found to be protonated and deprotonated, respectively. Oδ1 from Asp52 side-chain carboxylate group is restrained by a short H-bond with Asn59, while Oδ2 participates in dynamical H-bonds with Asn46, Asn44 and W306. Glu35 forms an H-bond with the catalytic water W332/A, which is transient.

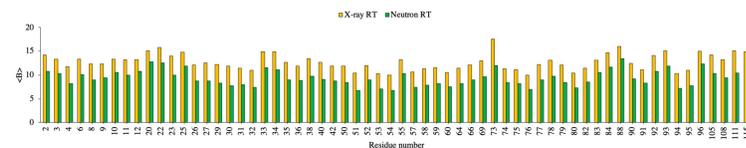
Atomic resolution neutron macromolecular crystallography



Different levels of structural information can be obtained from neutron diffraction depending on data resolution and sample deuteration.

Perdeuteration significantly improves neutron data quality, even from smaller crystals. Cancellation effects from H neutron scattering are also evident at atomic resolution, since protein disorder is precluded in neutron maps of hydrogenated sample.

ADPs from X-ray and neutron models are different



	X-ray/neutron ADP comparison	
	ΔB_{eq}^{X-N} (\AA^2)	
	Mean	σ
All atoms	3.3	0.5
Main-chain atoms	3.3	0.4
Side-chain atoms	3.4	0.8

	Anisotropy (A)			
	X-ray model		Neutron model	
	Mean	σ	Mean	σ
Protein	0.484	0.168	0.426	0.159
N	0.453	0.183	0.426	0.174
C	0.498	0.165	0.442	0.151
O	0.464	0.157	0.371	0.158
S	0.642	0.135	0.451	0.107

ADPs derived from room temperature X-ray diffraction data appear to be systematically larger than those obtained from equivalent neutron data. Additionally, X-ray ADPs are consistently more isotropic (anisotropy closer to 1) than those in the neutron model. Preliminary results suggest that neutron maps, due to the ability to locate deuterium atoms, allow a better interpretation of protein disorder. Therefore, the neutron ADPs have greater physical and biological meaning compared to those from the X-ray model.

Conclusions

- Production of perdeuterated protein in significant amounts was achieved by using the *E. coli* system and posterior *in vitro* refolding [3].
- Triclinic D-HEWL crystals of 4-5 mm³ were obtained by seeding techniques.
- Complete atomic resolution neutron diffraction data were recorded in D19, at Institute Laue-Langevin.
- The room temperature neutron structure enabled the unambiguous assignment of H positions, clarifying the configuration and dynamics of the enzyme's active site.
- Preliminary results show that X-ray and neutron derived ADPs are systematically different.

References

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2. Munshi, P., Madsen, A. Ø., Spackman, M. A., Larsen, S. & Destro, R. Estimated H-atom anisotropic displacement parameters: a comparison between different methods and with neutron diffraction results. *Acta Crystallogr.* **A64**, 465–475 (2008).
3. Ramos, J. *et al.* Structural insights into protein folding, stability and activity using *in vivo* perdeuteration of hen egg-white lysozyme. *IUCrJ* **8**, 372–386 (2021).