NEUTRONS FOR SOCIETY

Seeing the chemistry in biology using neutron crystallography

Matthew Blakeley

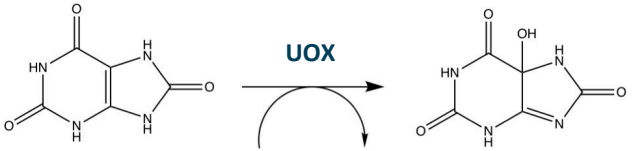
LADI-DALI instrument scientist

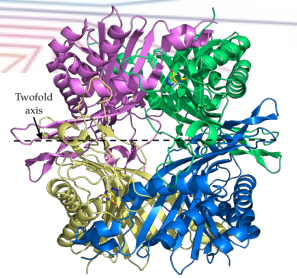
Large-Scale Structures Group, Institut Laue-Langevin

Urate oxidase enzyme mechanism studies

PhD project of Lindsay McGregor, ILL/King's College London (R. Steiner)

- **Urate oxidase (UOX)** is the archetypal co-factor free enzyme, catalyzing the reaction of uric acid to 5-hydroxyisourate.
- Neutron diffraction studies of UOX complexes can help determine the **exact catalytic pathway**.

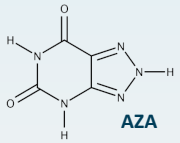


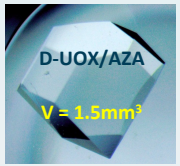


UOX tetramer (81, 96, 105 Å)

Perdeuterated UOX in complex with 8-azaxanthine (AZA).

McGregor *et al.*, (2021) *IUCrJ* **8**, 46-59.





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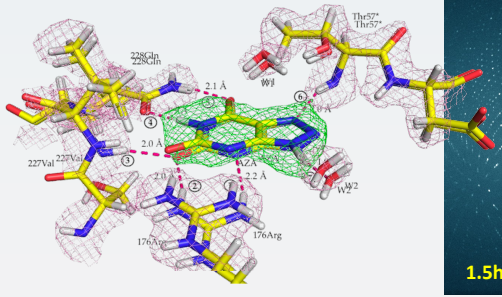
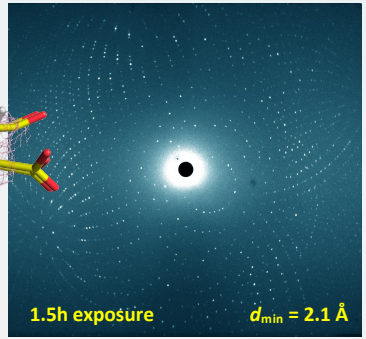
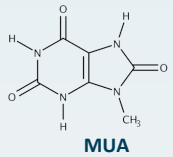


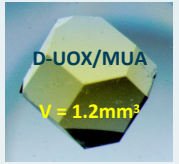


Identified the protonation states of active-site residues, and the W1 site as a neutral D_2O water molecule.

Perdeuterated UOX in complex with 9-methyl uric acid (MUA).

Peroxide intermediate formed in aerobic conditions.





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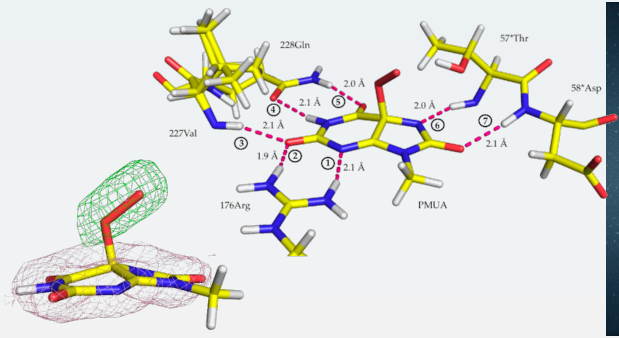

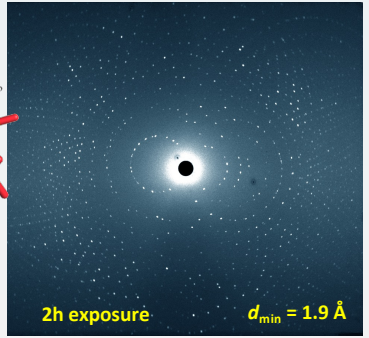

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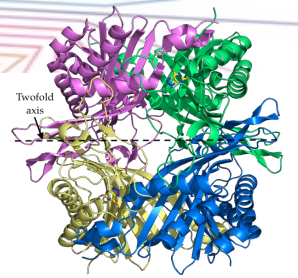

UOX tetramer (81, 96, 105 Å)

Neutron data from the UOX complexes was used in the development of new tools for neutron crystallographic refinement using REFMAC5, CCP4.

Catapano *et al.*, (2023) *Acta Cryst.*, D**79**, in press.

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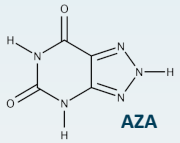

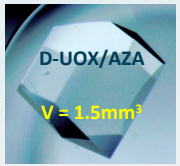

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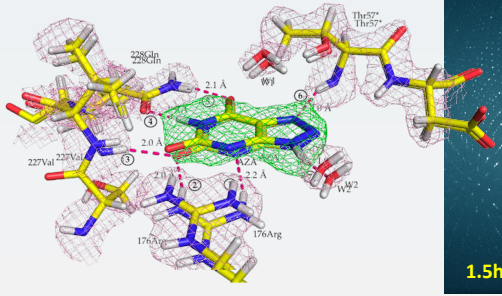

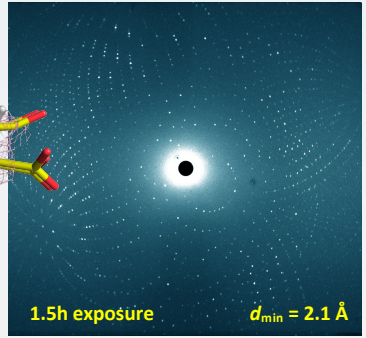

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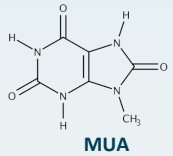

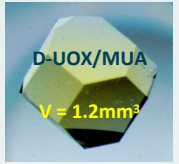

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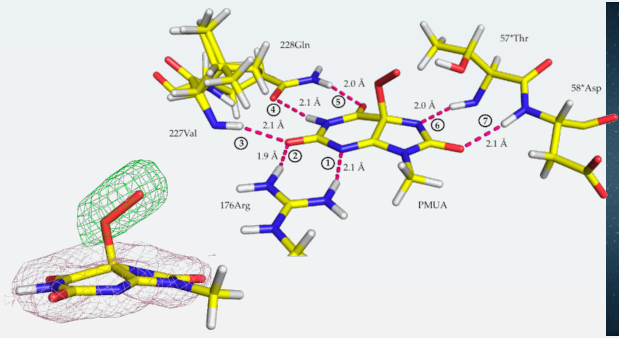

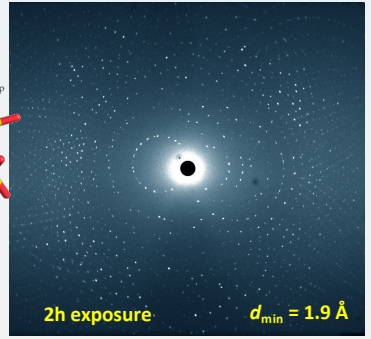

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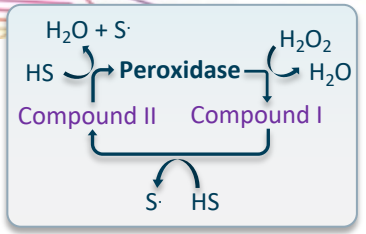
Heme peroxidases cryo-crystallography studies

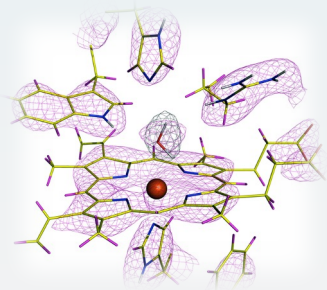
PhD project of Cecilia Casadei, ILL/University of Leicester (H. Kwon, P. Moody, E. Raven)



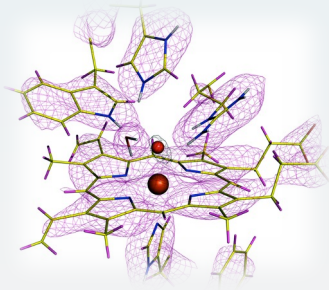
- **Heme peroxidases**, such as **cytochrome *c* peroxidase (C*c*P)** and **ascorbate peroxidase (APX)** carry out a wide range of oxidations using highly reactive ferryl intermediates. Reaction proceeds via **two transient intermediate states Compound I** (CI) **and Compound II** (CII). Longstanding debate regarding the actual chemical species of **CI** and **CII**.

Cytochrome *c* peroxidase CI and ascorbate peroxidase CII at 100K

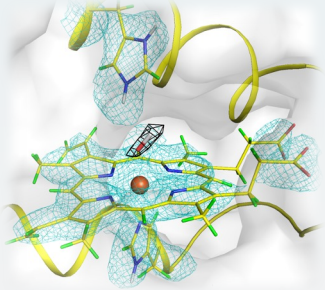
Cryo-trapped (at 100K) the transient reaction intermediates **CI in C*c*P** (*by reaction with H_2O_2*) and **CII in APX** (*by reaction of m-CPBA*), and collected neutron diffraction data at 100K. As a control, RT neutron data collected for **ferric C*c*P** (ground state).



Ferric C*c*P at RT (LADI, 2.4 Å)



C*c*P CI at 100K (BioDiff, 2.5 Å)



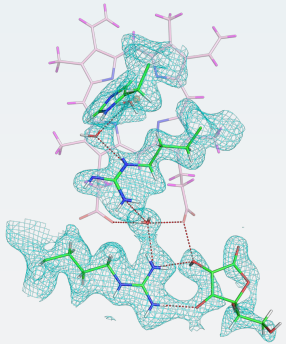
APX CII at 100K (LADI, 2.2 Å)

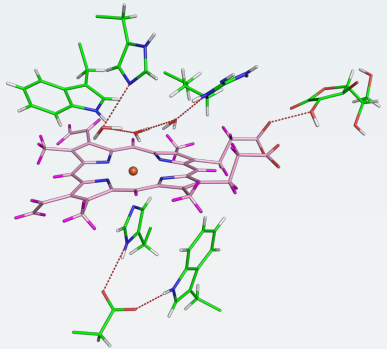
Revealed the chemical species of C*c*P CI as Fe(IV)=O and APX CII as Fe(IV)-OH.

Casadei *et al.*, (2014) *Science*, **345**, 193; Kwon *et al.*, (2016) *Nat. Commun.*, **7**, 13445.

Perdeuterated APX (Ferric and ascorbate complex)

Neutron data at 100K using **D-APX** for (i) **ferric APX** (LADI, 2.2 Å, V = **0.1mm³**, **7.5d**) and (ii) the **APX/ascorbate complex** (BioDiff, 2.1 Å). The ascorbate complex is stable and non-catalytic in absence of peroxide.





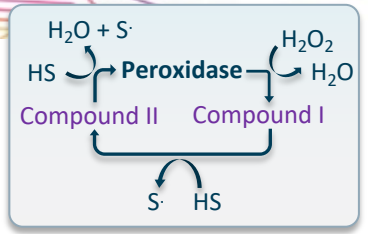
Revealed the protons in a PCET pathway, including an unexpected neutral Arg species in the APX/ascorbate complex.

Kwon *et al.*, (2020) *Proc. Natl. Acad. Sci., U.S.A.* **117**, 6484.

Heme peroxidases cryo-crystallography studies

PhD project of Cecilia Casadei, ILL/University of Leicester (H. Kwon, P. Moody, E. Raven)

This work led to the development of improved low-temperature sample environment capabilities for LADI and the results led to revision of the textbook enzyme mechanism. (Moody & Raven, (2018) *Acc. Chem. Res.*, **51**, 427.)



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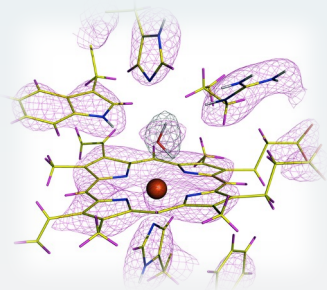

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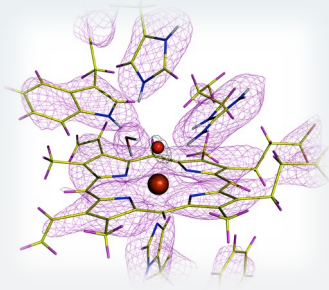
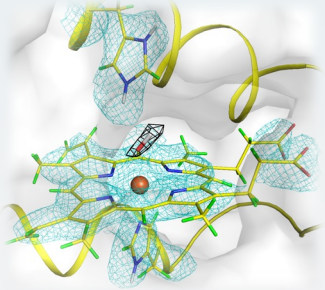


CcP CI at 100K (BioDiff, 2.5 Å)



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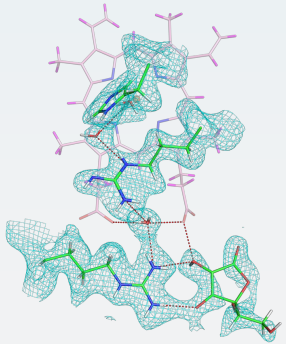

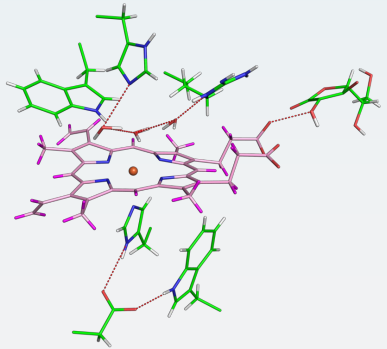

Revealed the protons in a PCET pathway, including an unexpected neutral Arg species in the APX/ascorbate complex.

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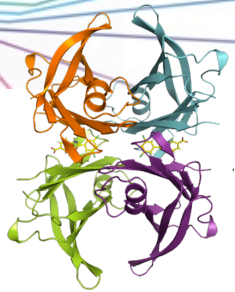
Transthyretin Amyloidosis

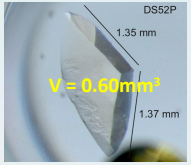
PhD project of Alycia Yee, ILL/University of Keele (T. Forsyth/E. Mitchell)

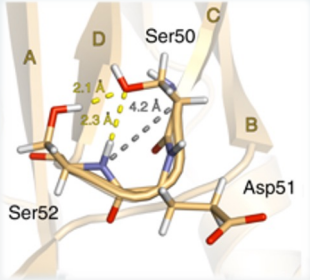


- **Transthyretin (TTR)** is a transport protein that carries thyroxine and retinol-binding protein bound to retinol.
- Wild-type TTR and certain mutants (*e.g.* **TTR_S52P**) are unstable, while other mutants (*e.g.* **TTR_T119M**) are stable. Unstable forms have a propensity to dissociate to form **amyloid fibrils** leading to a fatal hereditary disease, familial amyloid polyneuropathy (FAP).

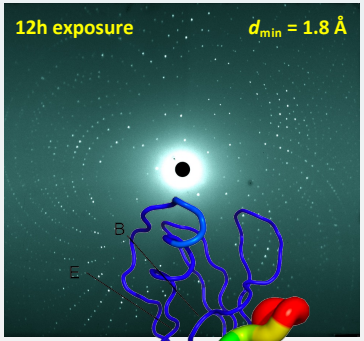
Perdeuterated WT TTR, TTR_S52P & TTR_T119M

Analysed the H-bond networks in wild-type and mutant forms of TTR. **LADI:** Neutron data to **1.8 Å** at RT using a **0.6mm³** crystal of D-TTR_S52P.





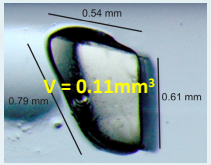
T119M

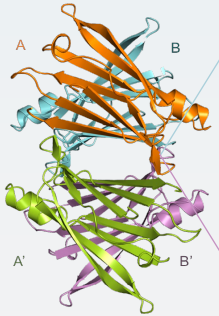


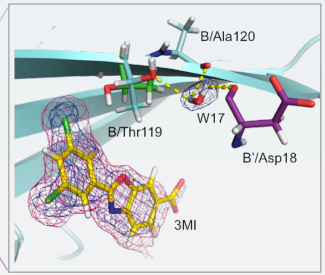
In T119M (and WT TTR), Ser52 forms two H-bonds with Ser50, while in S52P the absence of these H-bonds creates a looser CD loop.

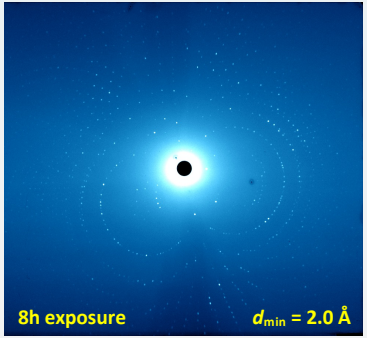
Perdeuterated TTR_S52P/tafamidis complex

One strategy to inhibit TTR dissociation is to stabilise the tetramer by small molecule drug binding to the thyroxine (T4) binding sites. **LADI:** Neutron data to 2.0 Å at RT in **4.5d** using a **0.11mm³** crystal of D-TTR_S52P/tafamidis complex.









Binding of tafamidis results in a change of orientation of Thr119, which creates additional water-mediated H-bonds between chains B and B'.

Yee *et al.*, (2019) *Nat. Commun.*, **10**, 925; Yee *et al.*, (2017) *J. Appl. Cryst.*, 50, 660