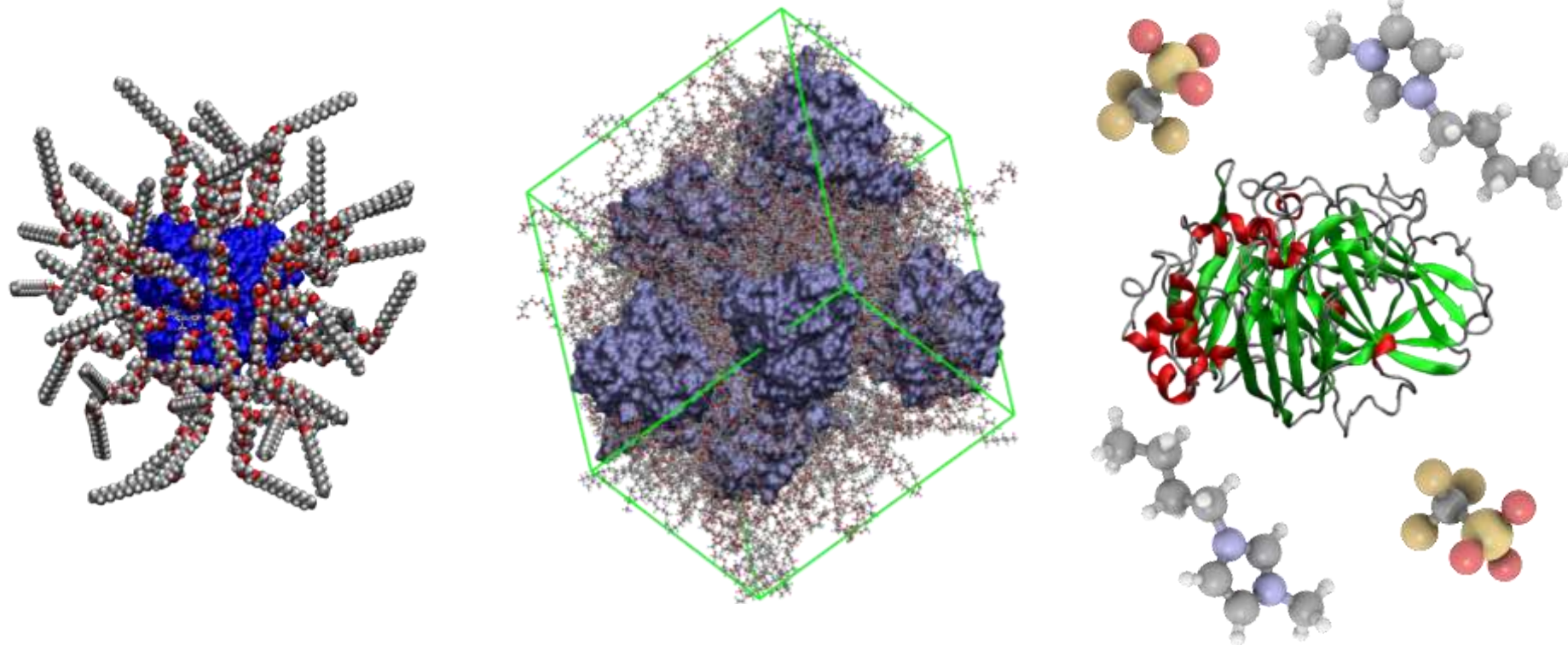


Solvent-Free Functional Biofluids as a Route Towards Highly Stable Biocatalysts in Non-Aqueous Environments



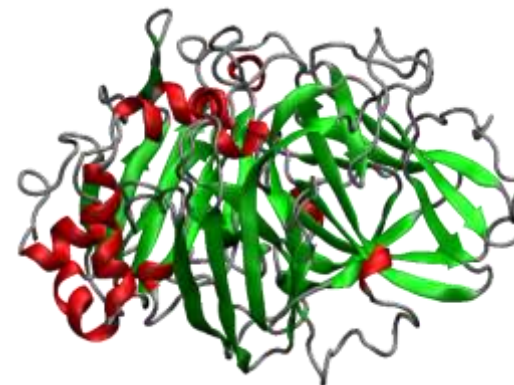
Dr. Alex Brogan
Imperial College
London

alexbrogan.co.uk/grs
10th July 2016
Biocatalysis (GRS) 2016

- Enzymes can catalyse many different industrial reactions.
- Ionic liquids are a promising reaction media for industry.
- Low energy, efficient routes.

→ Biocatalysis in ionic liquids

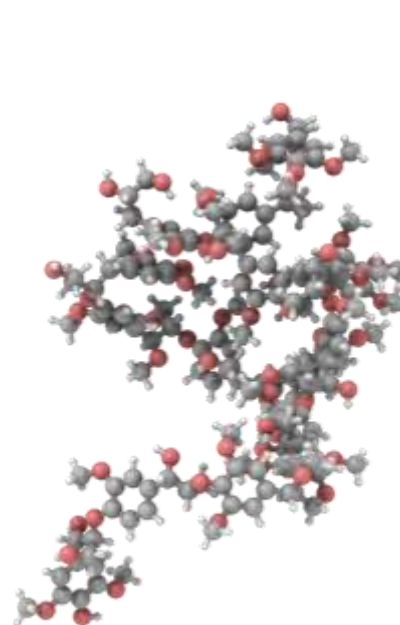
- *e.g.* could take advantage of high lignocellulosic biomass solubility in ionic liquids.
- Cut out “pretreatment” and just have “treatment”.
- Enzymes **insoluble** and **inactive** in common ionic liquids however...



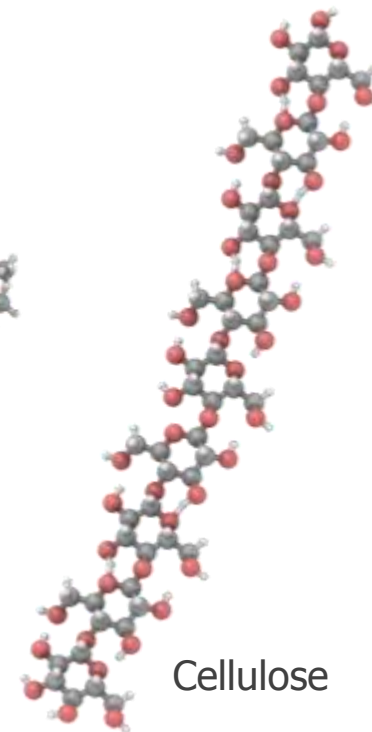
Laccase



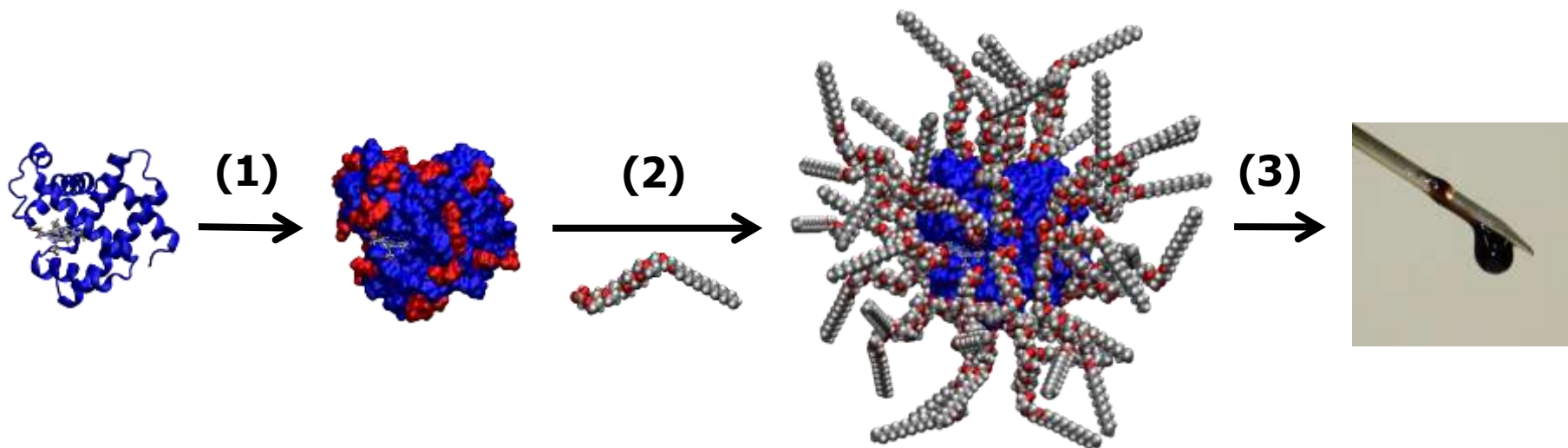
Cellulase



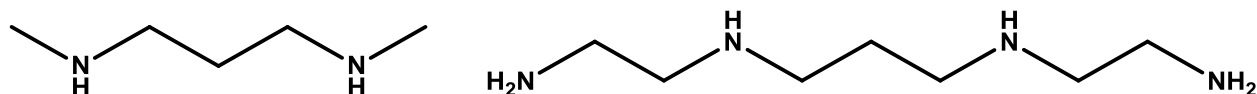
Lignin



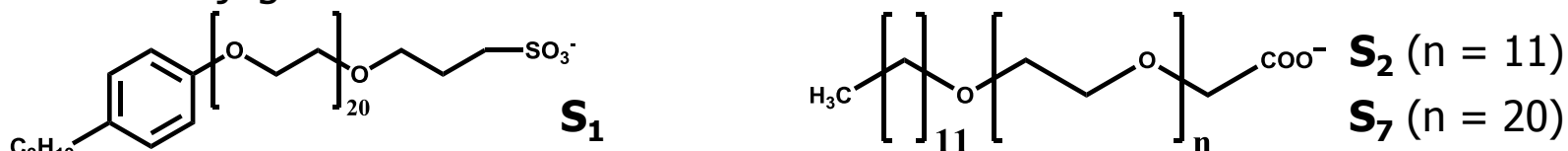
Cellulose



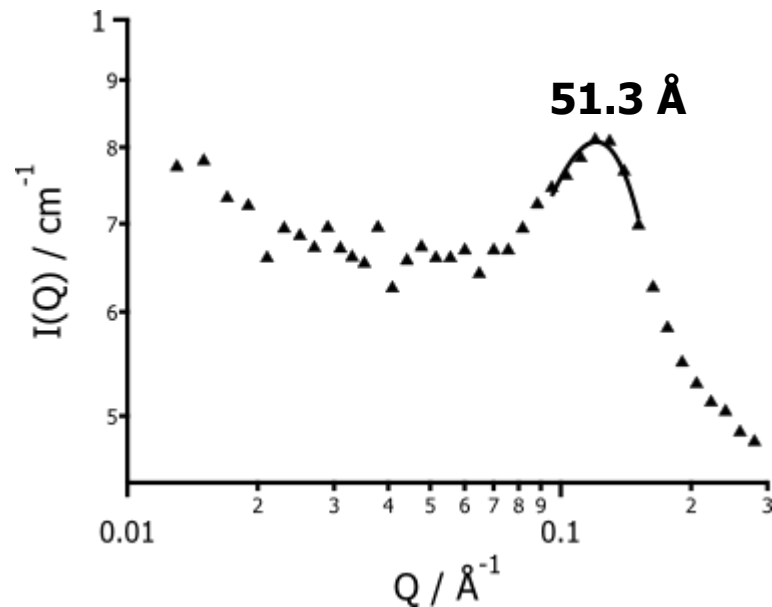
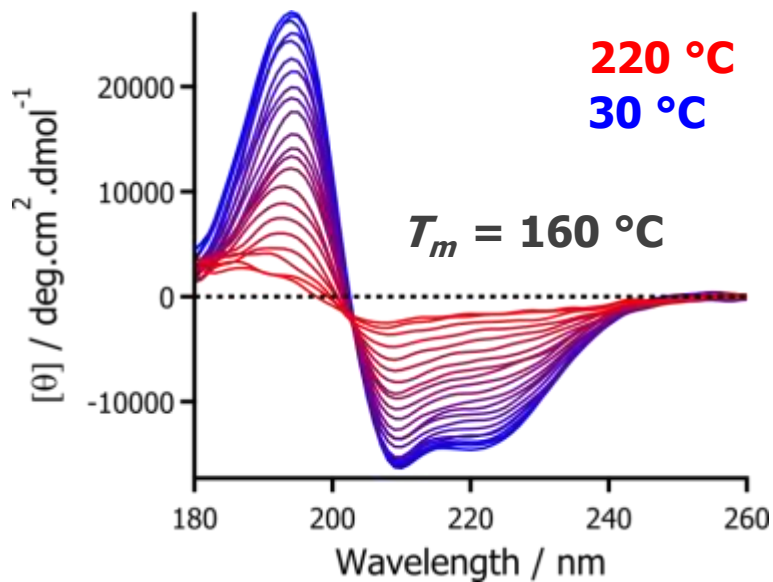
(1) Cationization of surface acidic residues with amines using the EDC reaction.



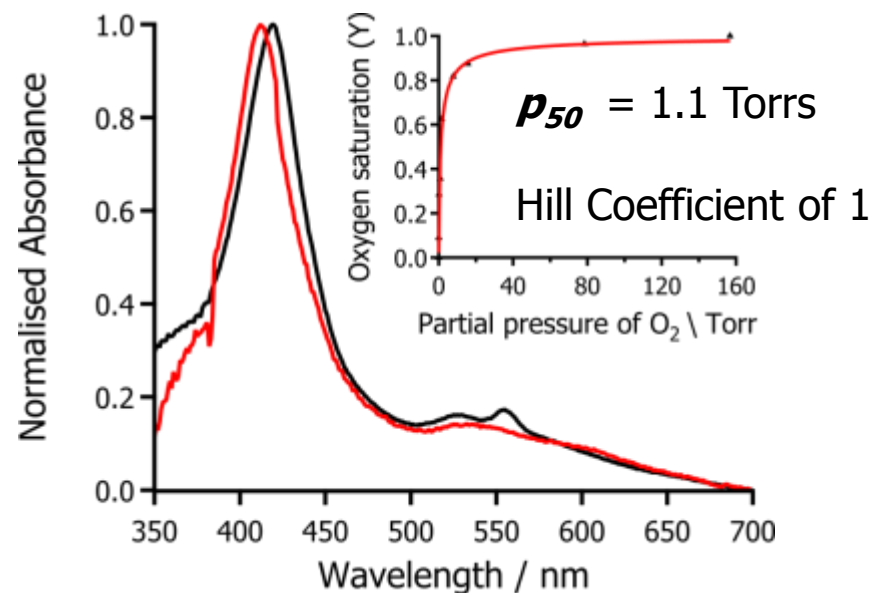
(2) Electrostatic complexation with anionic surfactant to form aqueous nanoconjugates.



(3) Lyophilization of conjugate, followed by annealing at 60 °C to form solvent-free liquid protein.

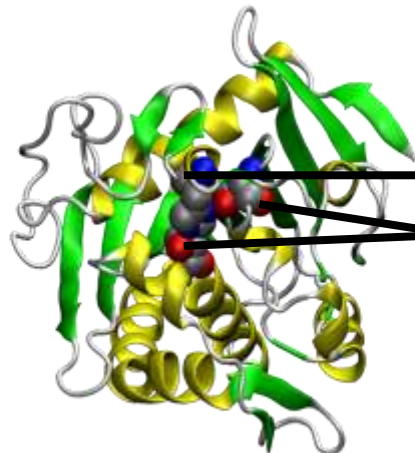


- Retained secondary structure.
- High thermal stability.
- Persistent tertiary structure.
- Biological function in absence of water.



Rhizomucor miehei (RML)

Thermomyces lanuginosus (TLL)

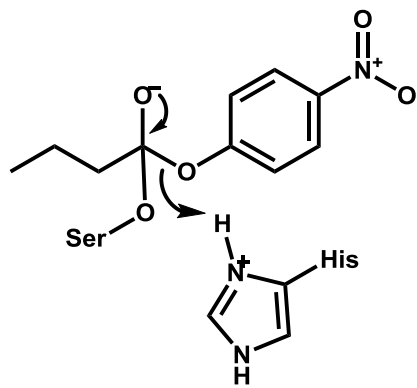
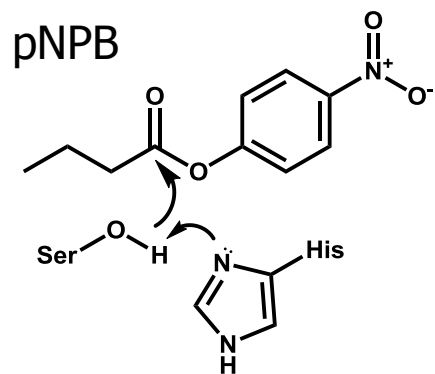
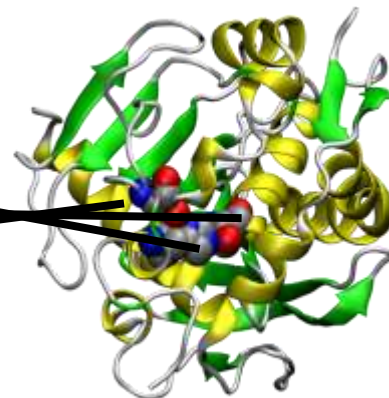


Catalytic triad

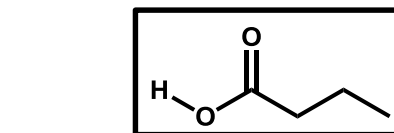
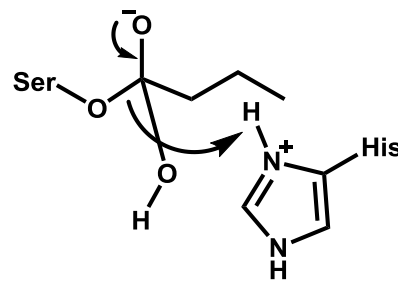
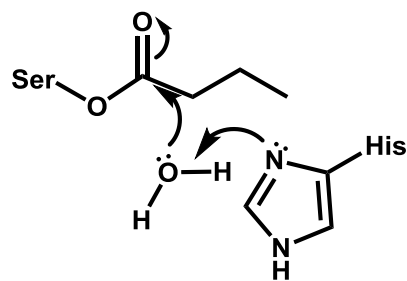
HIS

SER

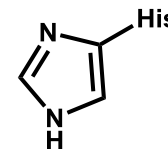
ASP

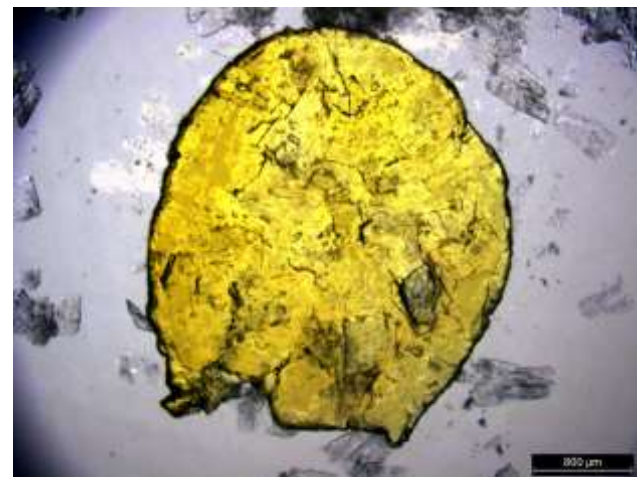
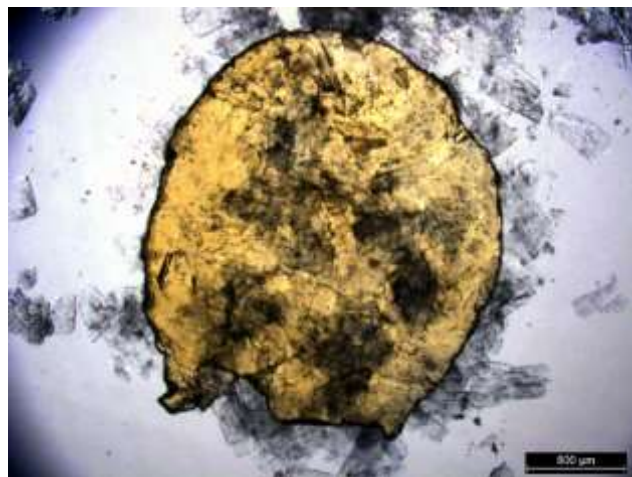
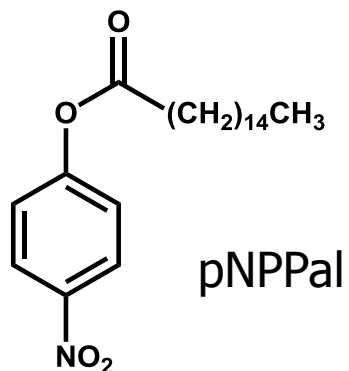


410 nm

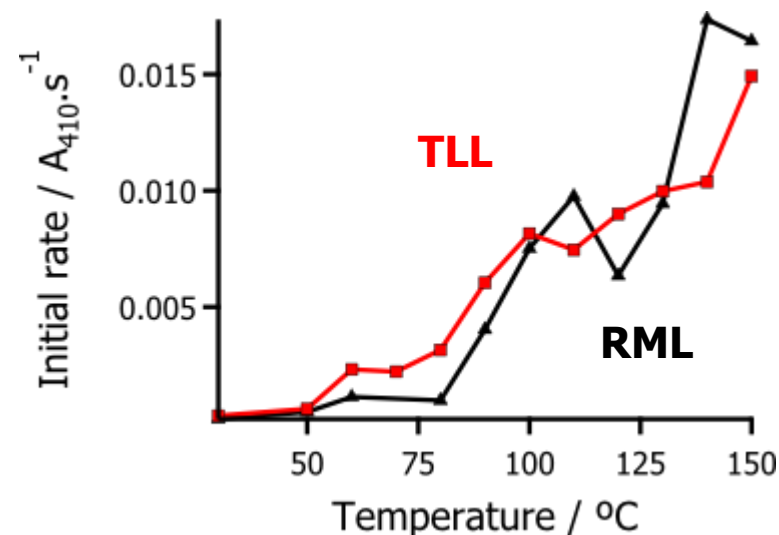


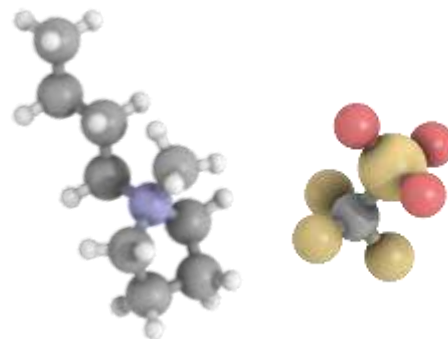
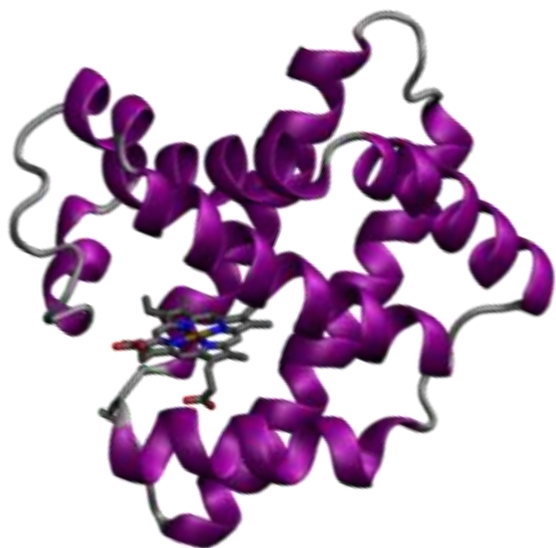
Ser-OH





- Enzyme activity in absence of water.
- Delivery of both liquid and solid substrates to enzyme active site.
- Enhanced enzyme activity up to 150 $^{\circ}\text{C}$

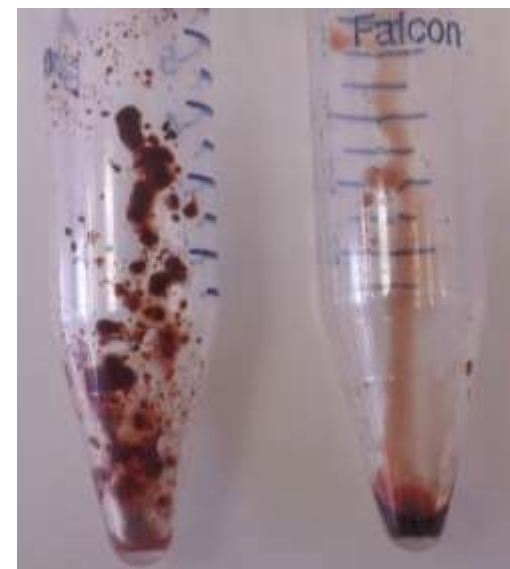




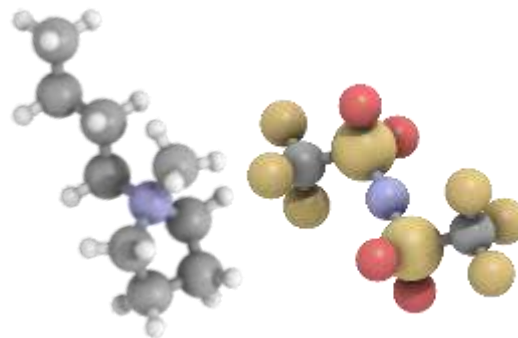
[bmpyrr][OTf]

Mb

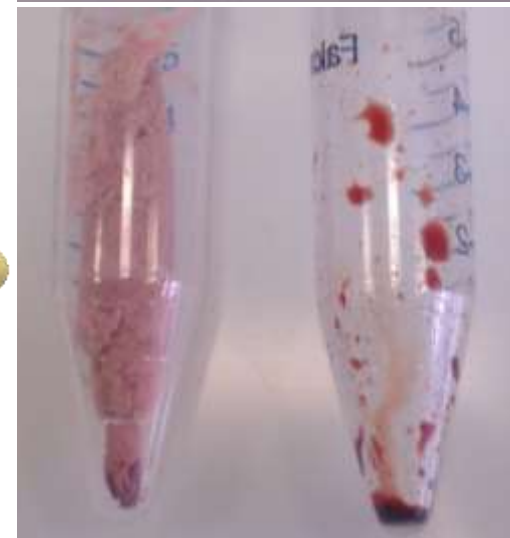
[C-Mb][S₂]

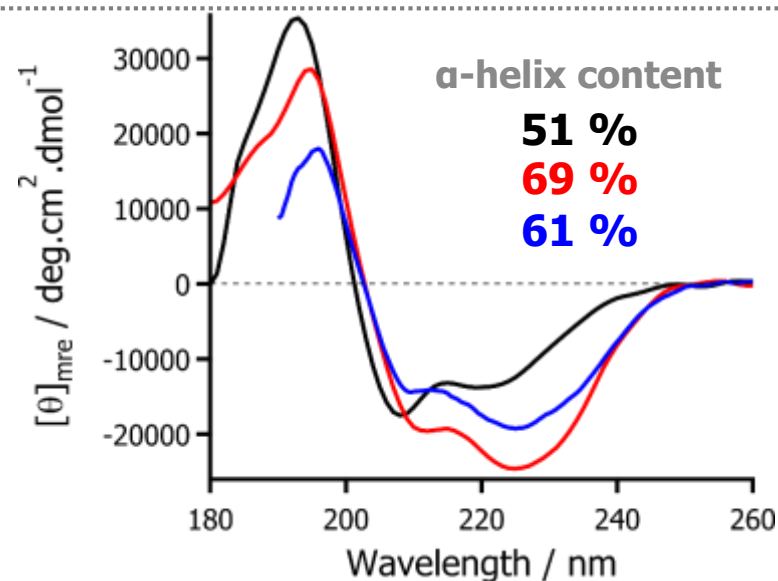
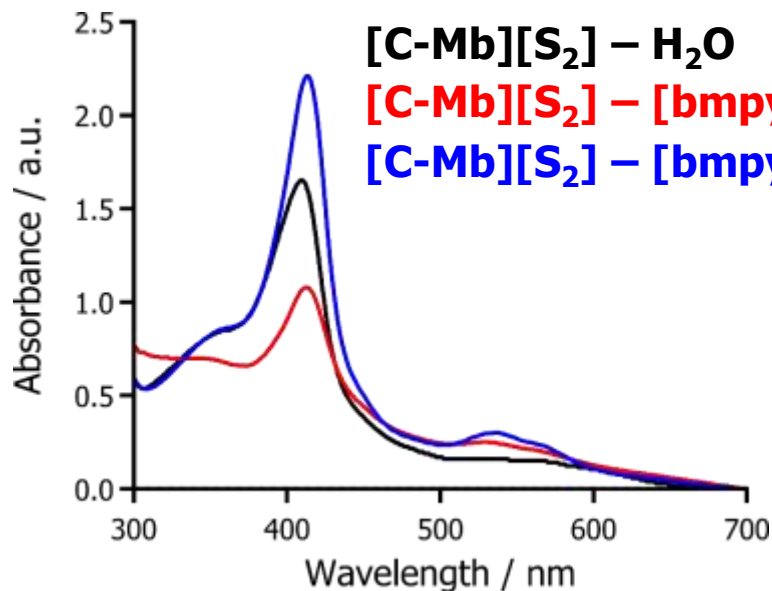


- Myoglobin as archetypal system.
- Well characterized – sensitive to environment.
- Biofluids have significant increase in IL mixing.

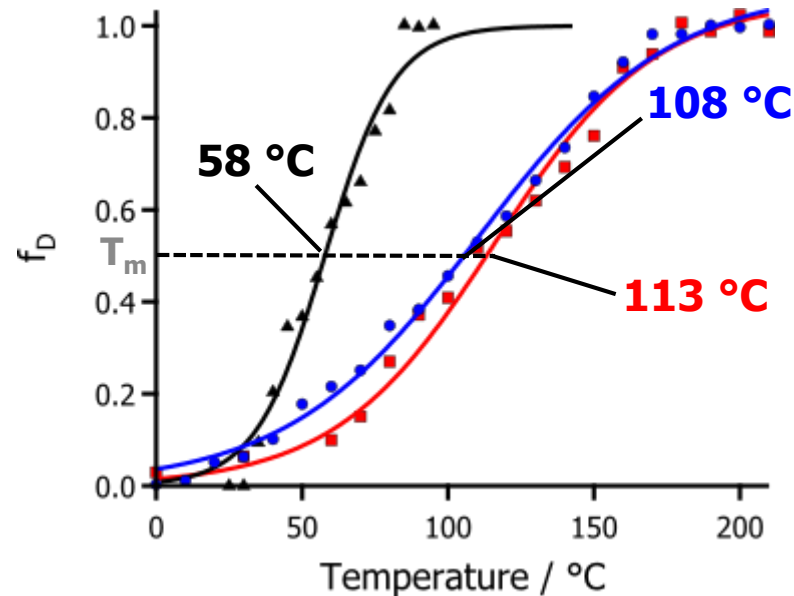


[bmpyrr][NTf₂]

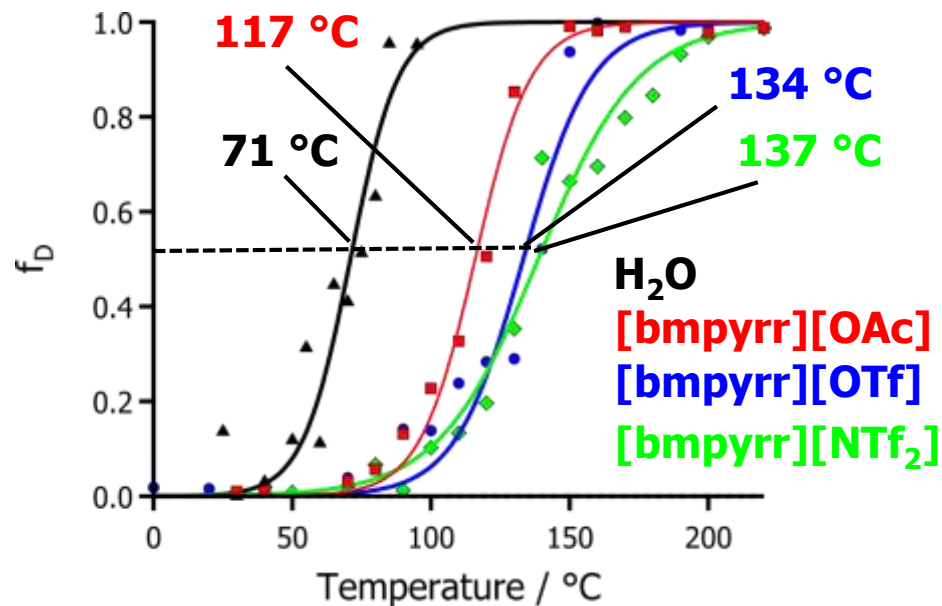
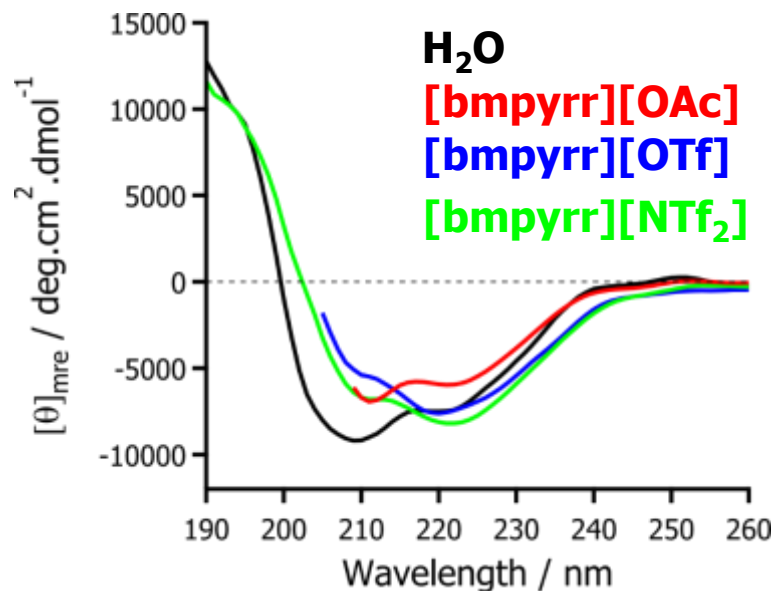




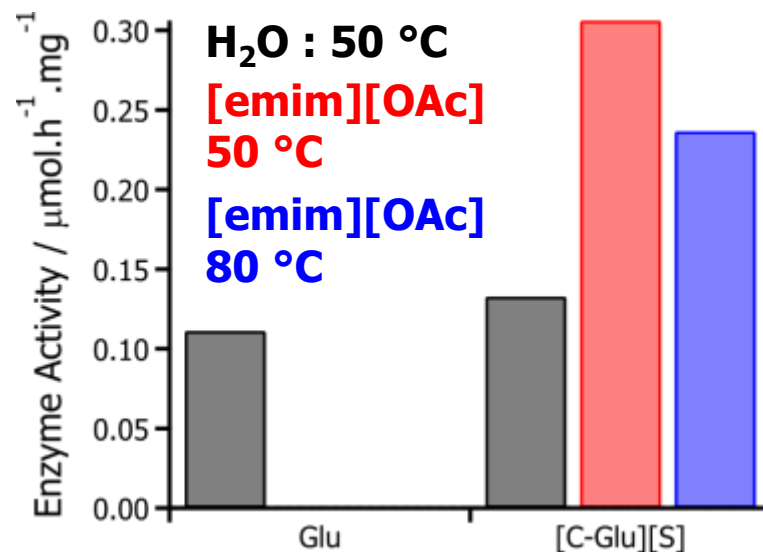
- UV/Vis shows retention of prosthetic heme in all conditions.
- SRCD indicated ionic liquids induced α -helicity
- Thermal stability of myoglobin increased significantly in ionic liquid.



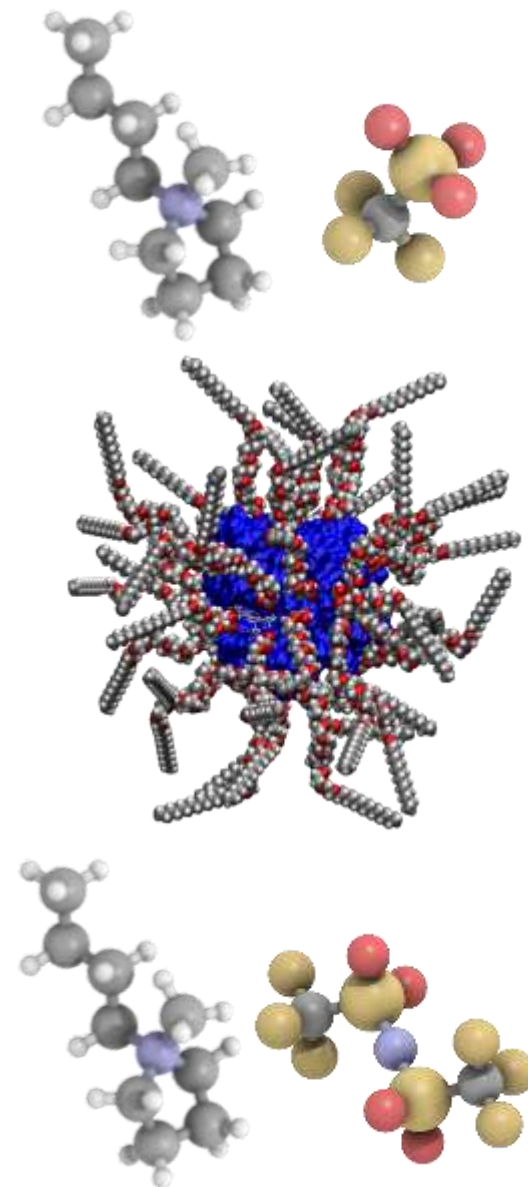
β -Glucosidase



- Surface functionalization of β -Glucosidase increases solubility in ionic liquids.
- Highly stable in ionic liquids – both secondary (SRCD) and tertiary (SAXS).
- Activity towards cellobiose retained in ionic liquids.



- Solvent-free liquid proteins and enzymes are versatile materials with a robust synthesis.
- Unique materials of stoichiometric protein-surfactant conjugates that have a liquid phase.
- Good compatibility with ionic liquids.
- Increased protein structure and thermal stability compared to aqueous system.
- Promising biotechnology for potential bio-catalysis in ionic liquids.



Acknowledgements

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The Hallett Group

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