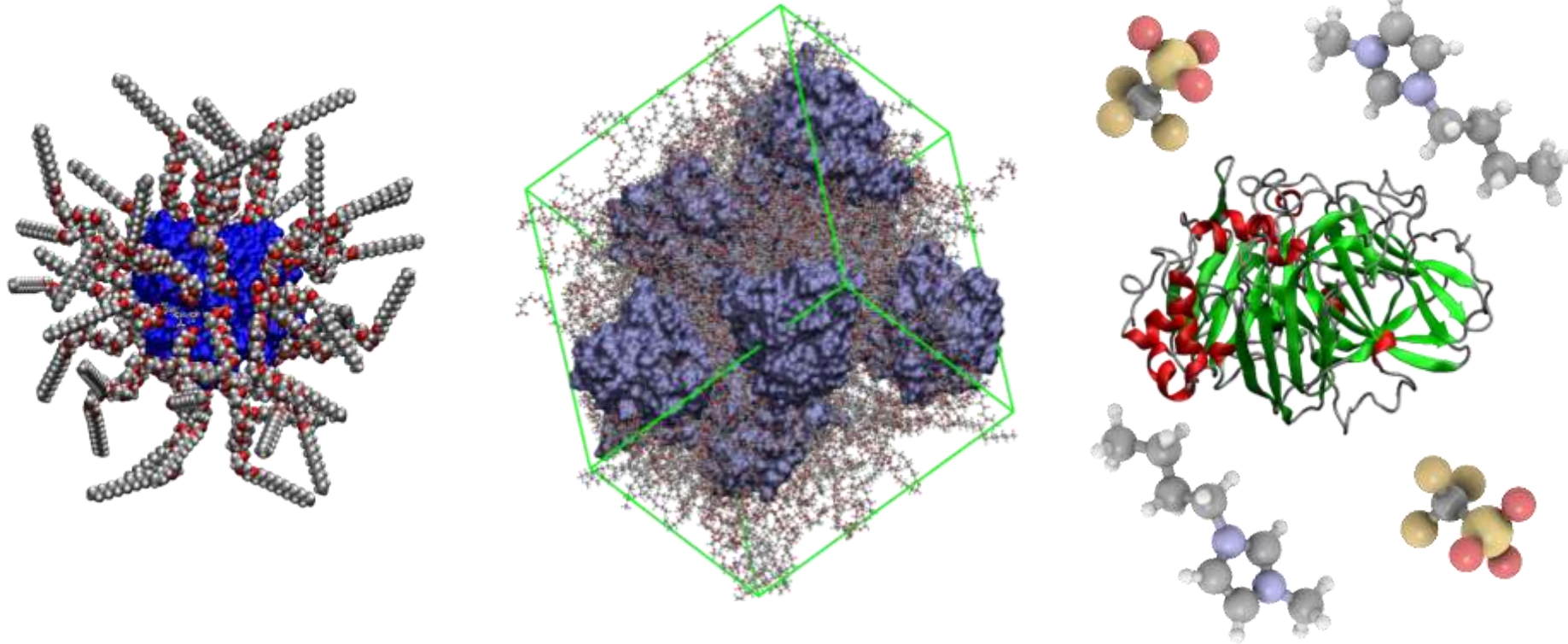


Solvent-free enzyme biofluids for anhydrous biocatalysis



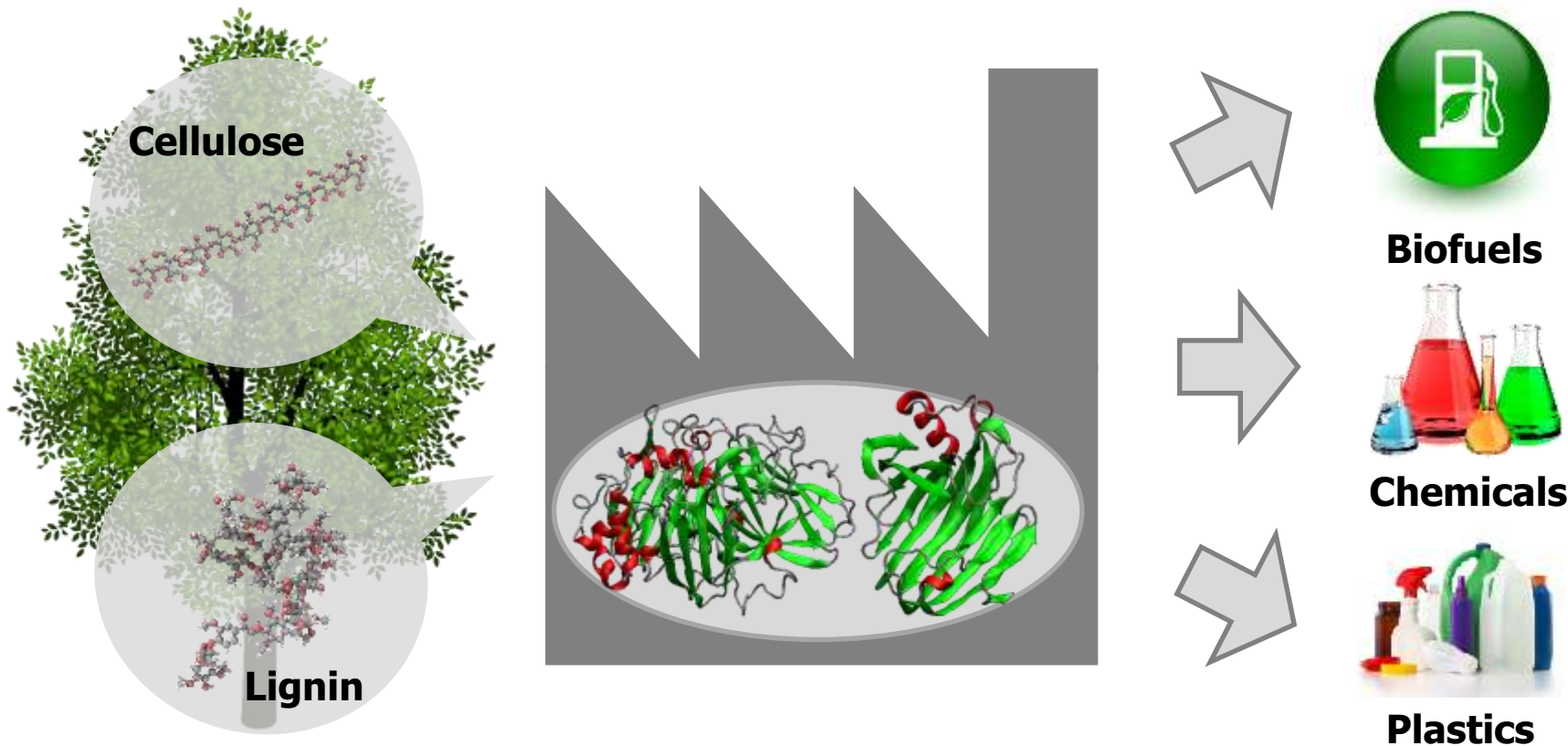
Dr. Alex P. S. Brogan

**Imperial College
London**

5th April 2018

MRS Spring 2018

alexbrogan.co.uk/mrs



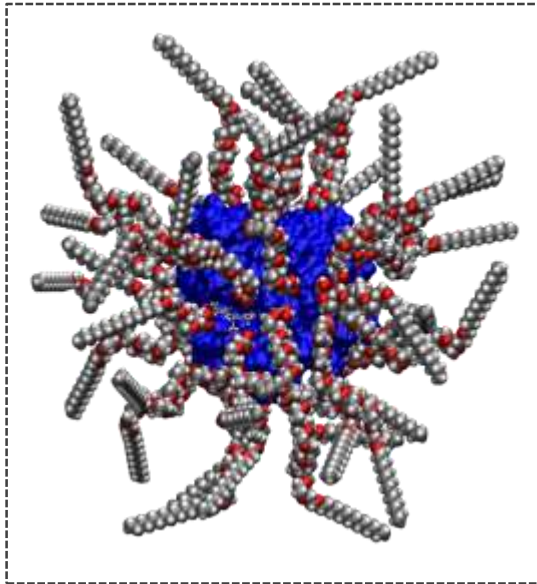
- **Ionic liquids** are a promising reaction media for industry.

→ **Biocatalysis in ionic liquids**

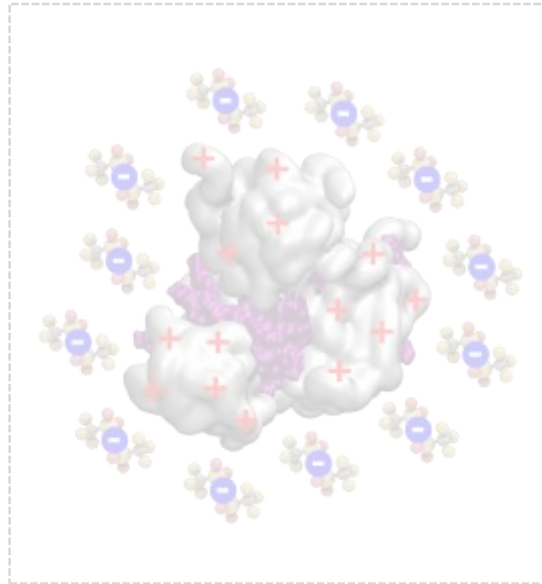
- **Enzymes** can catalyse many different industrial reactions.

- Enzymes **insoluble** and **inactive** in common ionic liquids...

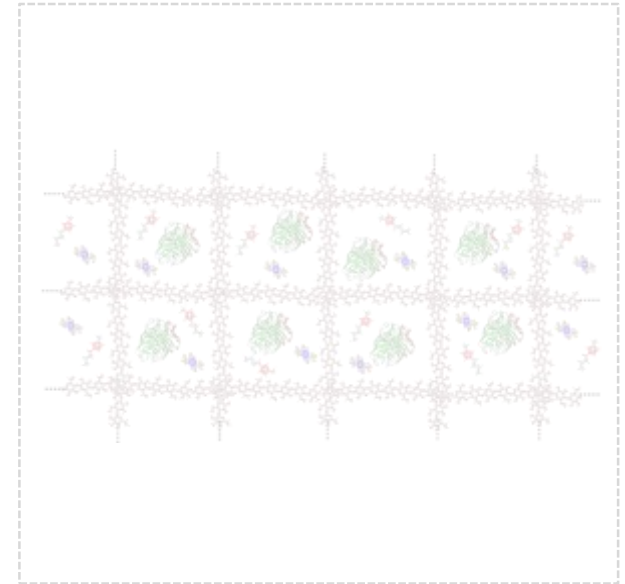
Solvent-free Enzyme Biofluids



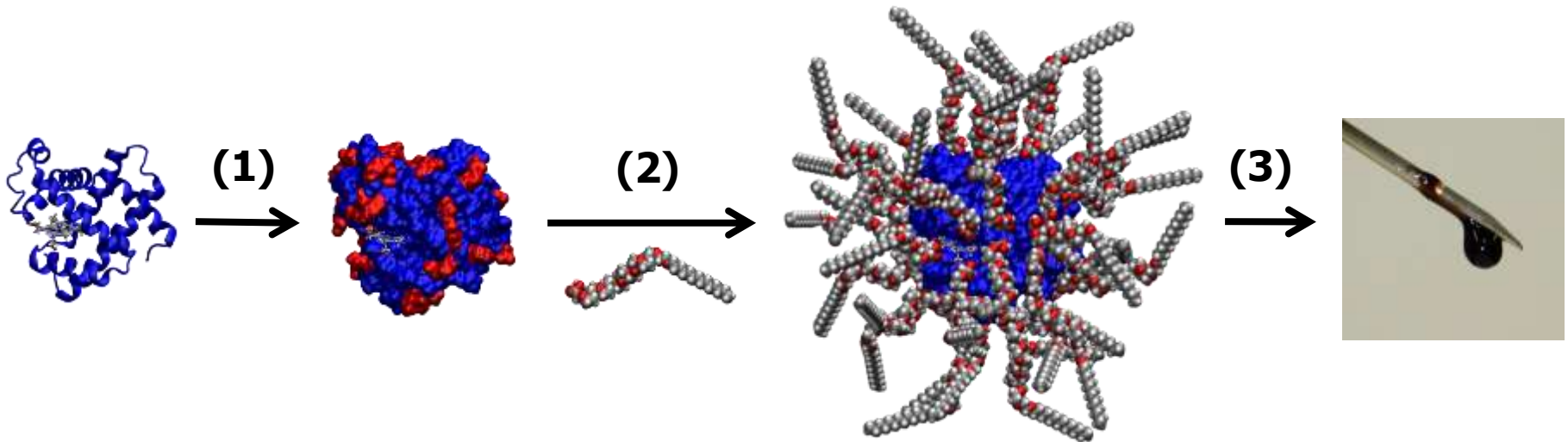
Ionic Liquid Proteins



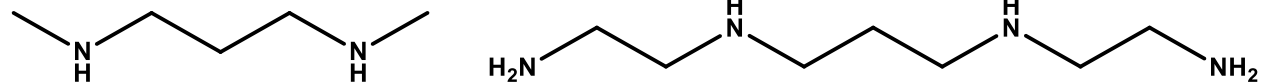
Ionogels



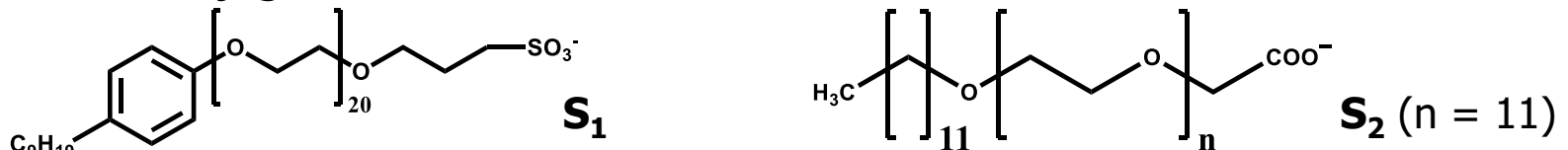
**Solubilize and stabilize enzymes in
ionic liquids**



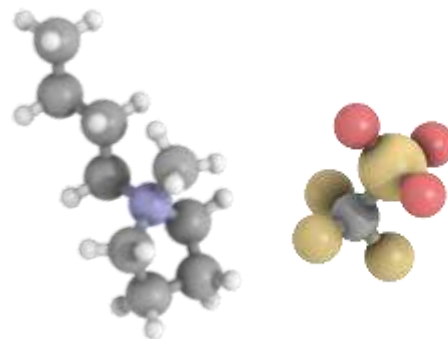
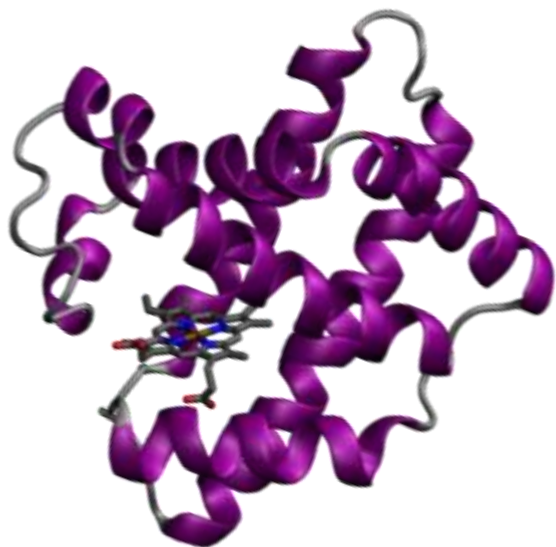
(1) Cationization of surface acidic residues with amines using the EDC reaction.



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



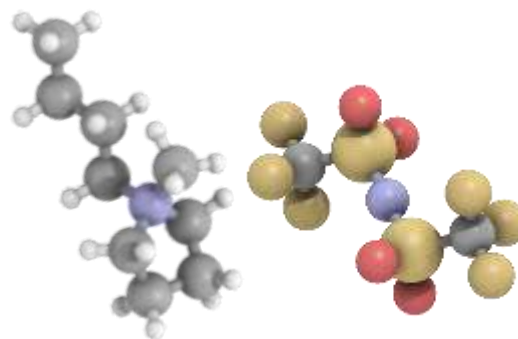
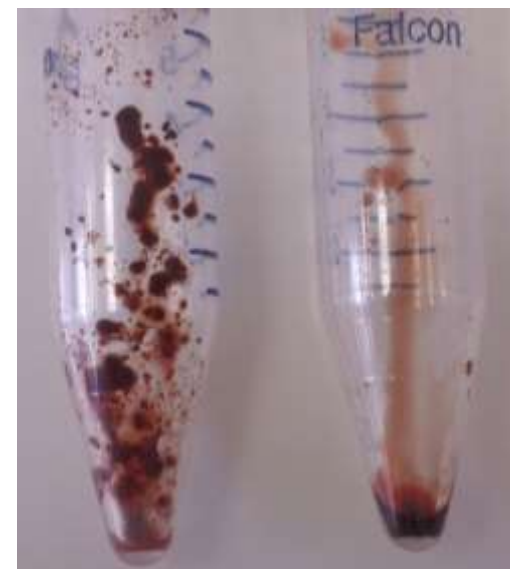
(3) Lyophilization of conjugate, followed by annealing at 60 °C to form solvent-free liquid protein.



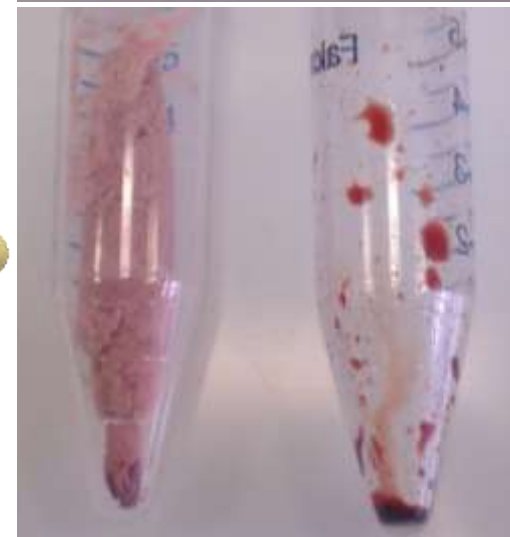
[bmpyrr][OTf]
HYDROPHILIC

Mb

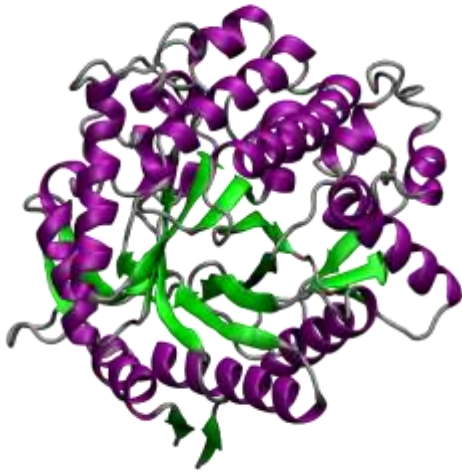
[C-Mb][S]



[bmpyrr][NTf₂]
HYDROPHOBIC



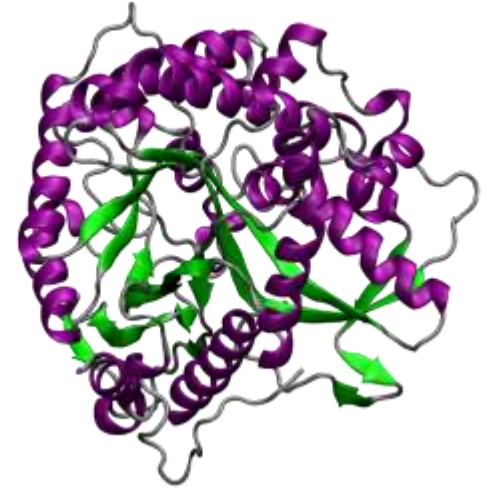
- Myoglobin as archetypal system.
- Well characterized – sensitive to environment.
- Biofluids have significant increase in IL mixing.



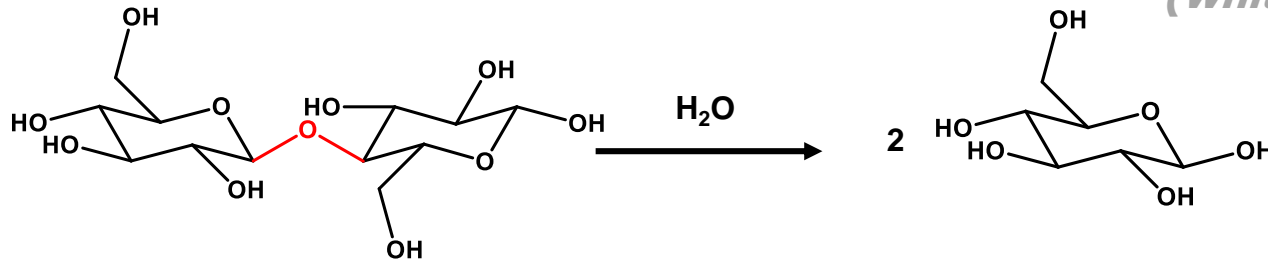
Streptomyces sp.



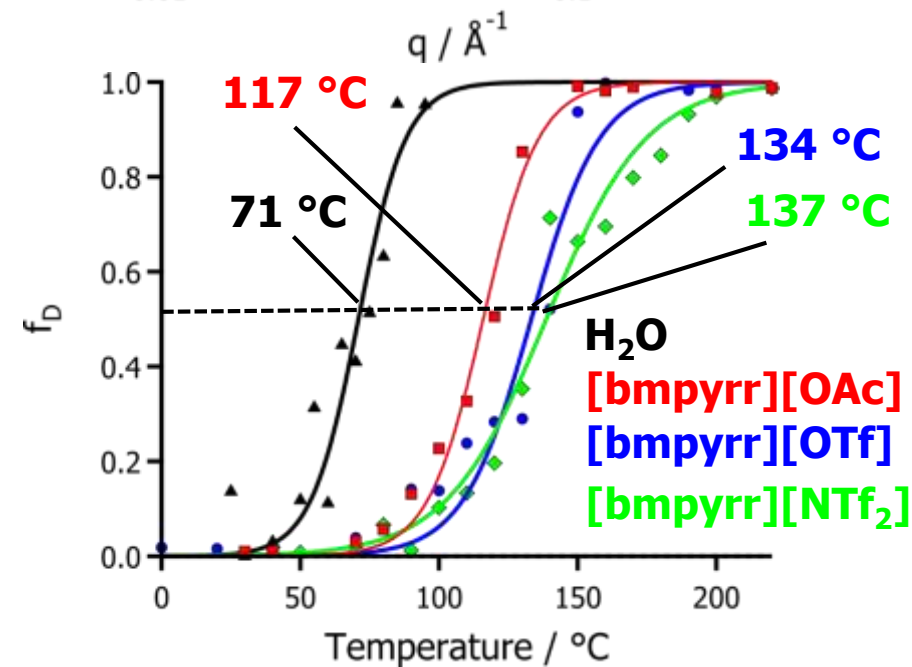
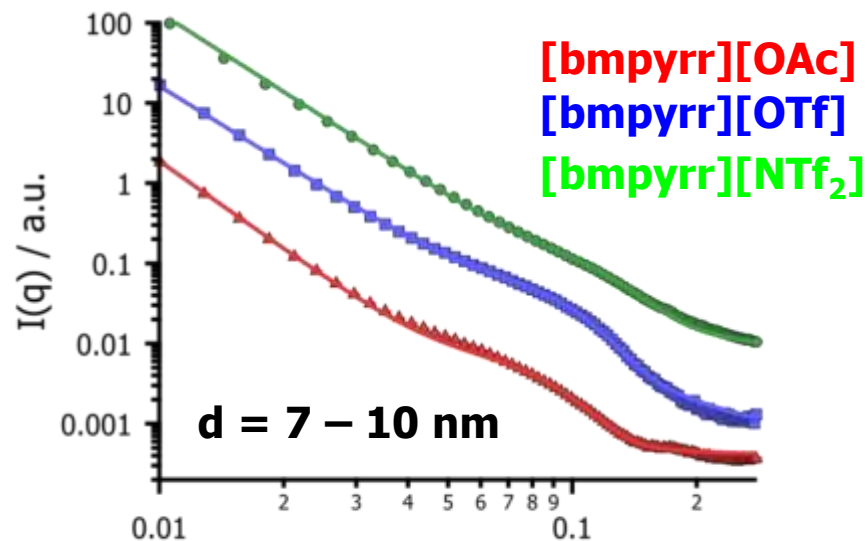
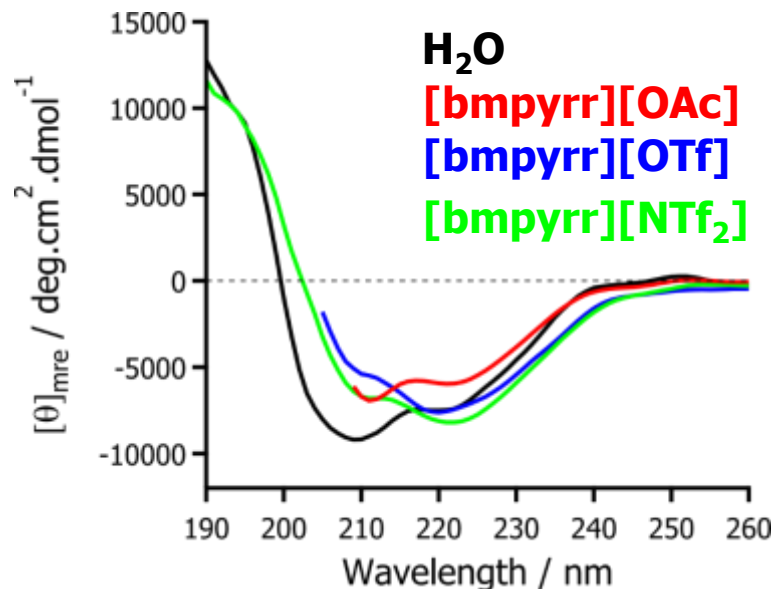
Thermotoga maritima



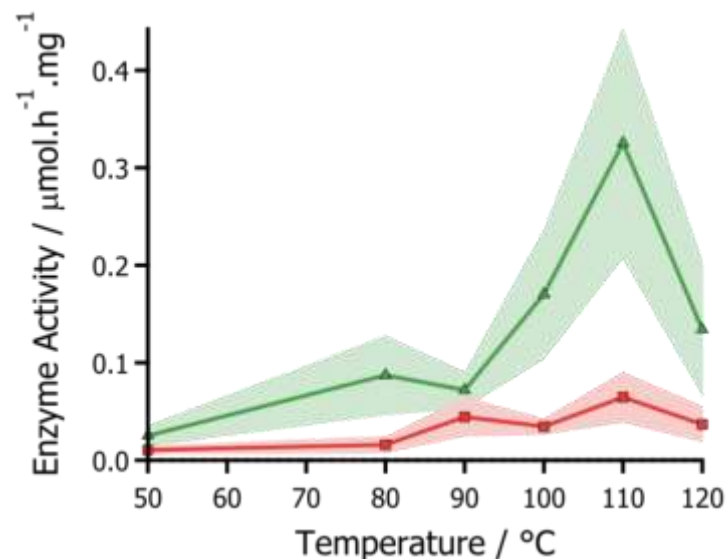
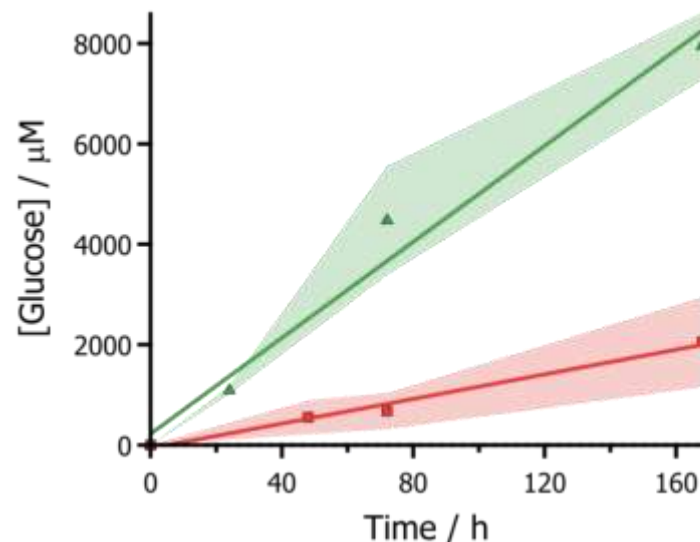
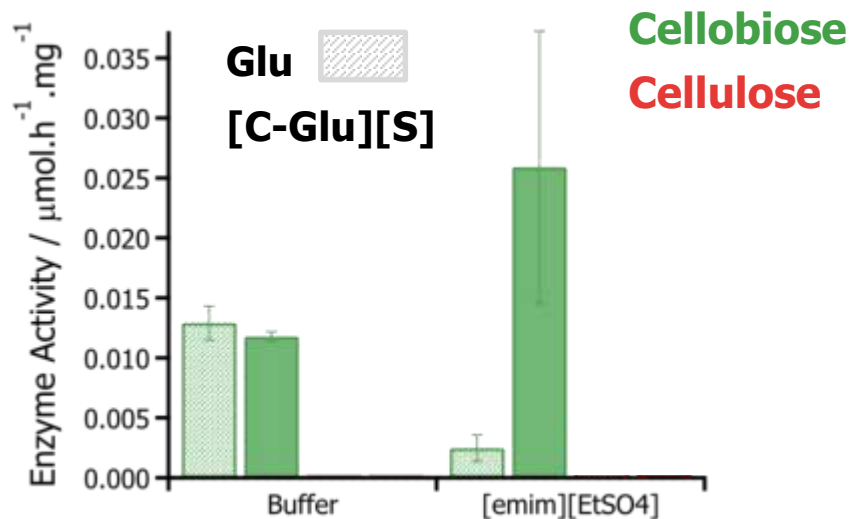
Trifolium repens
(white clover)



- Catalyses hydrolytic cleavage of $\alpha/\beta(1-4)$ linkages between sugar units.
- β -glucosidases most abundant – one of the cellulase trio of enzymes.
- Conserved structural motifs and catalytic centres (2 glutamate and 1 asparagine)

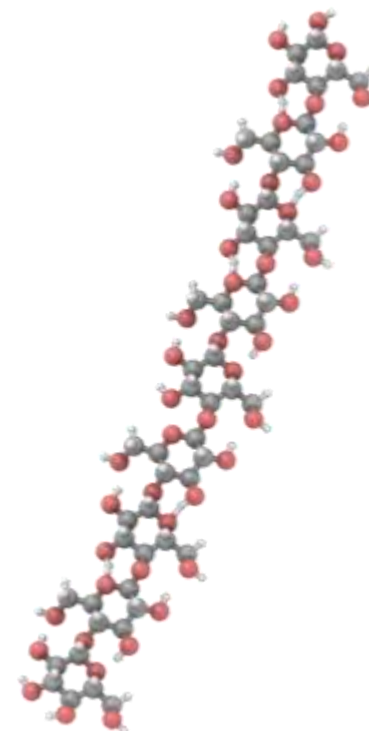
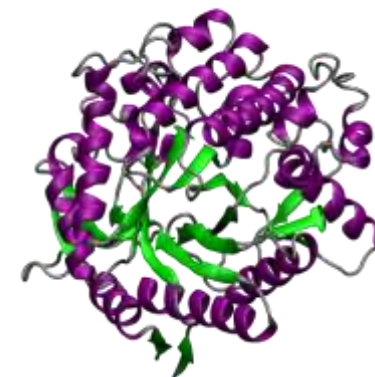


- Surface functionalization of glucosidase increases solubility in ionic liquids.
- Structure maintained (SRCD, SAXS)
- Highly stable in ionic liquids.



- Activity enhanced in ionic liquids – only for modified glucosidase.
- Reaction turns over at constant rate – zero order kinetics
- Enzyme activity increases by almost 30x at 110 $^{\circ}\text{C}$

- Robust synthesis for chemically modified proteins and enzymes.
- Good compatibility with ionic liquids.
- Protein structure highly conserved – in the absence of water.
- Thermal stability improves as compared to aqueous system.
- Enzyme activity of glucosidase enhanced in ionic liquids.
- Solvent-induced promiscuity of glucosidase towards cellulose.



Acknowledgements

Dr. Jason Hallett

Liem Bui-Le, Artemis Charalambidou,
Sophie Hyer

The Hallett Group

Dr. Nimrod Heldman & Prof. Angela Belcher
(MIT)

Diamond Light Source (B23 & I22)



Warwick Bromley/
Nature Chemistry

EPSRC

Engineering and Physical Sciences
Research Council

