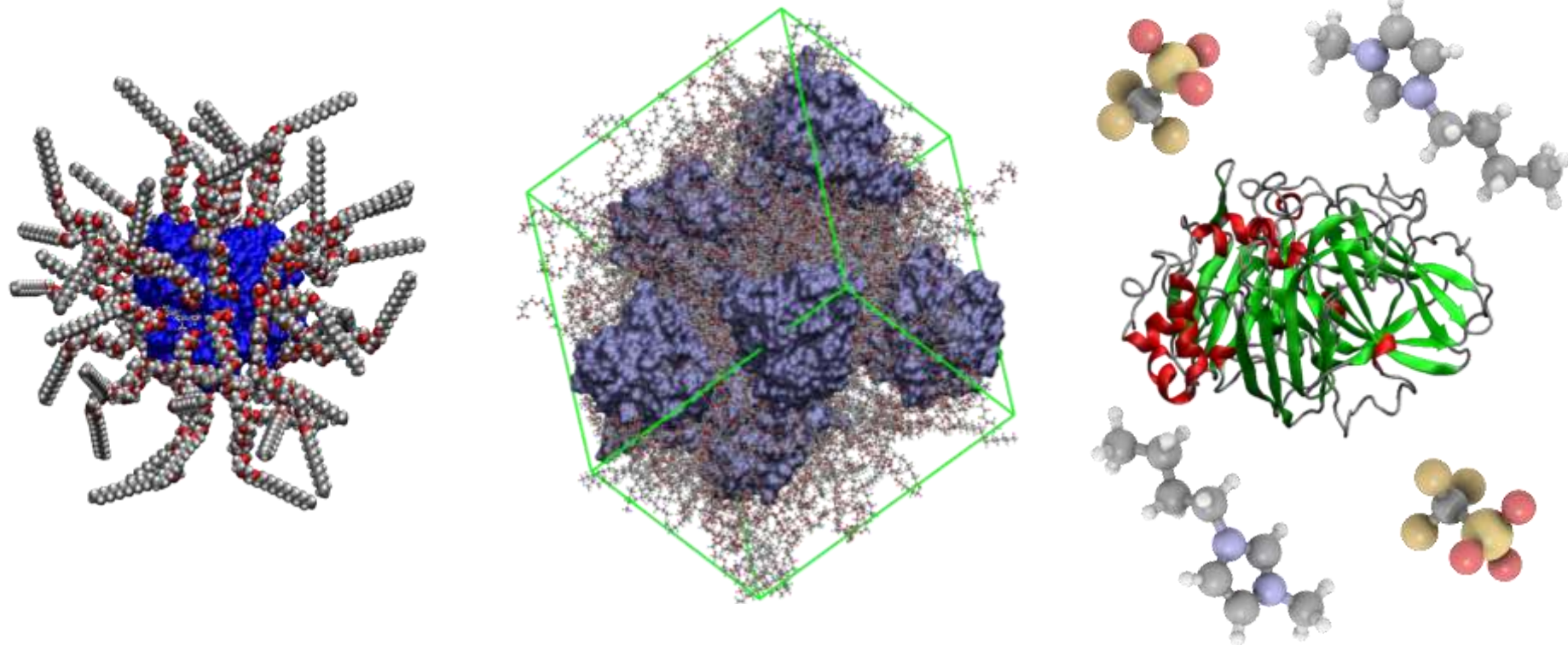


Solvent-free enzyme biofluids for anhydrous biocatalysis

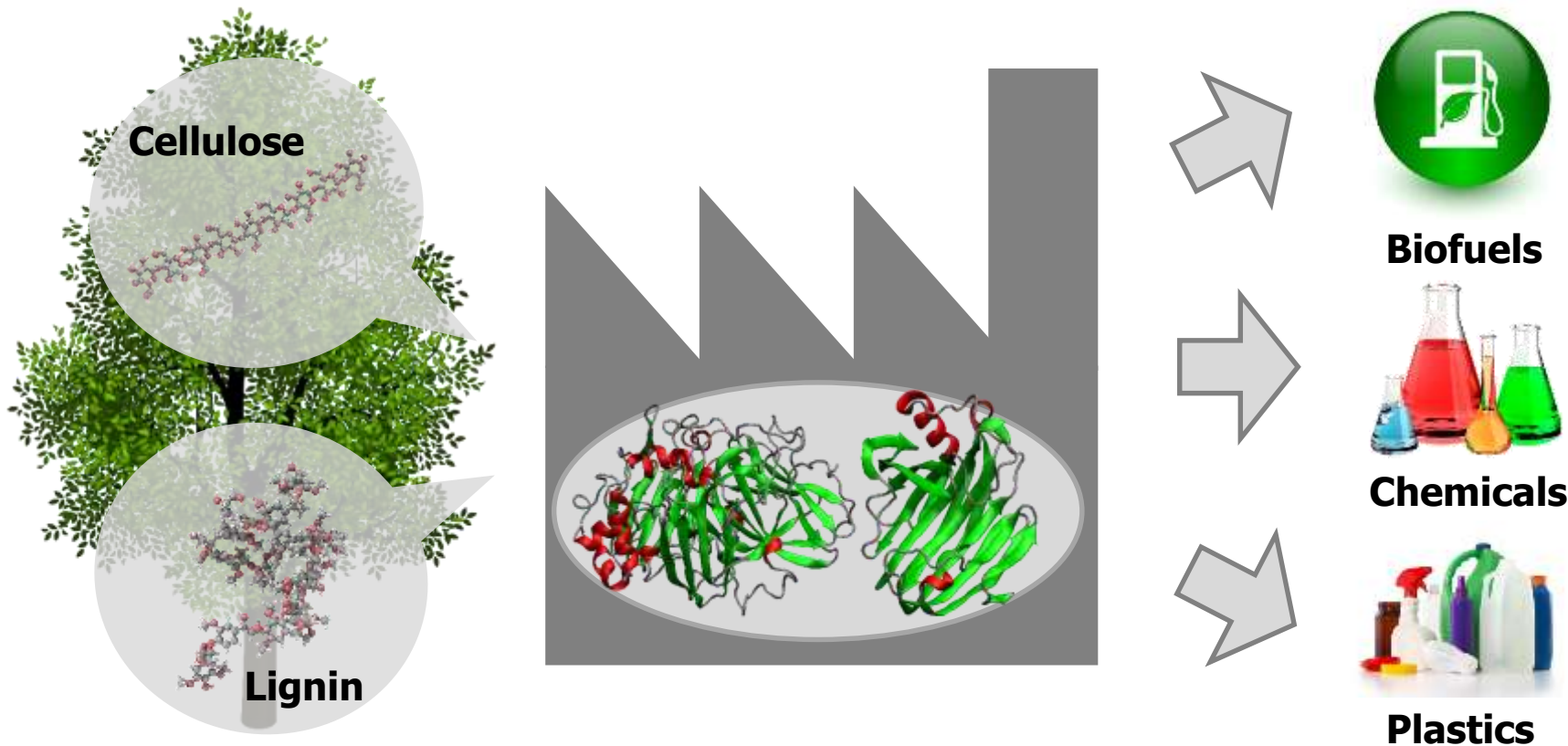


Dr. Alex P. S. Brogan

**Imperial College
London**

22nd August 2018

ACS Fall 2018 – COLL: Biomaterials & Biointerfaces
alexbrogan.co.uk/acs



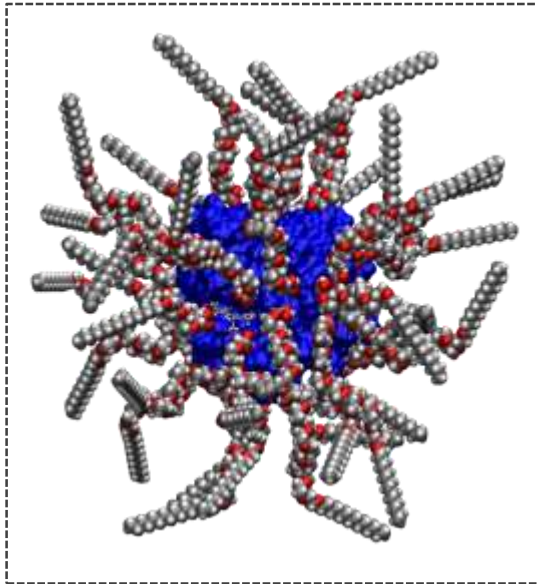
- **Ionic liquids** are a promising reaction media for industry.

→ **Biocatalysis in ionic liquids**

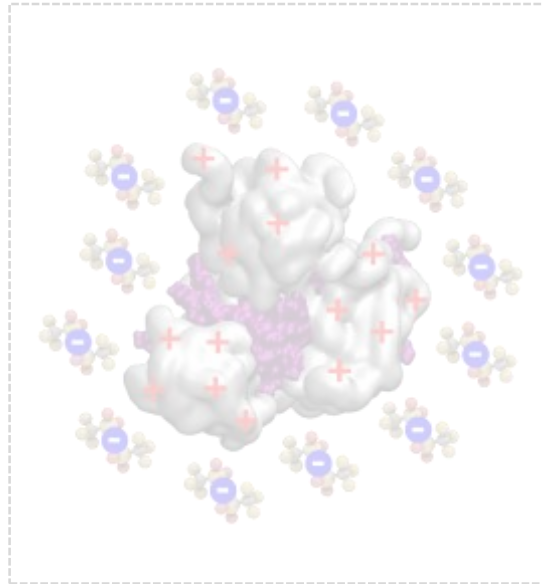
- **Enzymes** can catalyse many different industrial reactions.

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

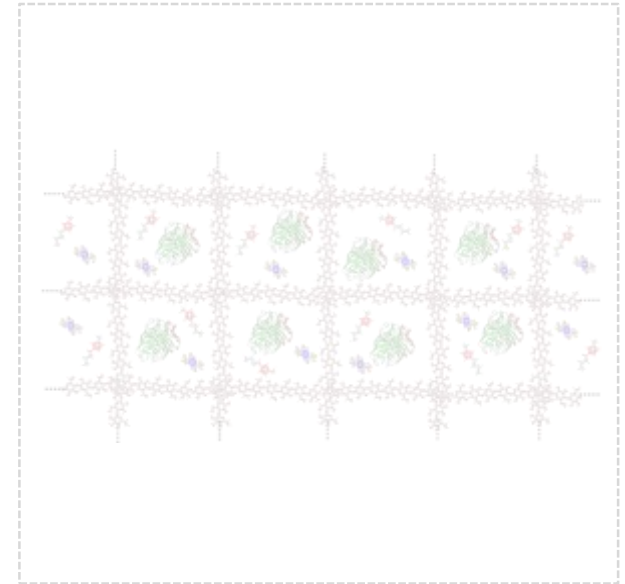
Solvent-free Enzyme Biofluids



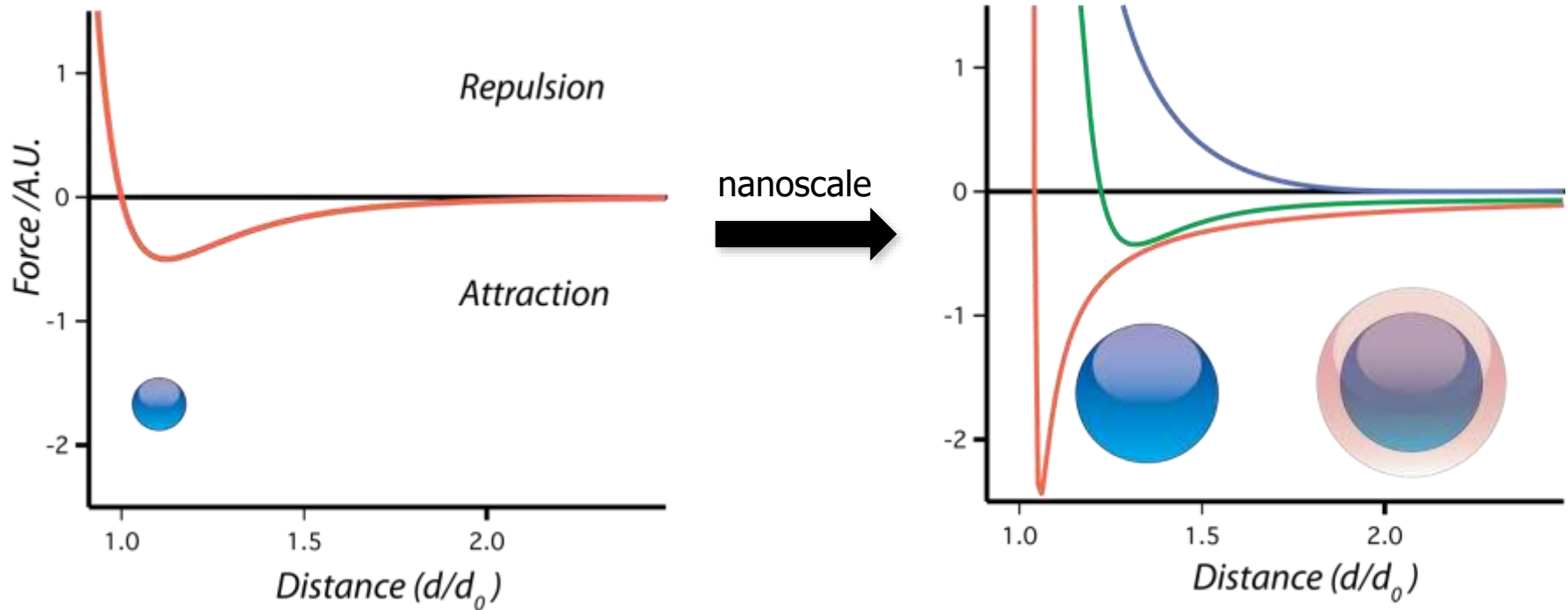
Ionic Liquid Proteins



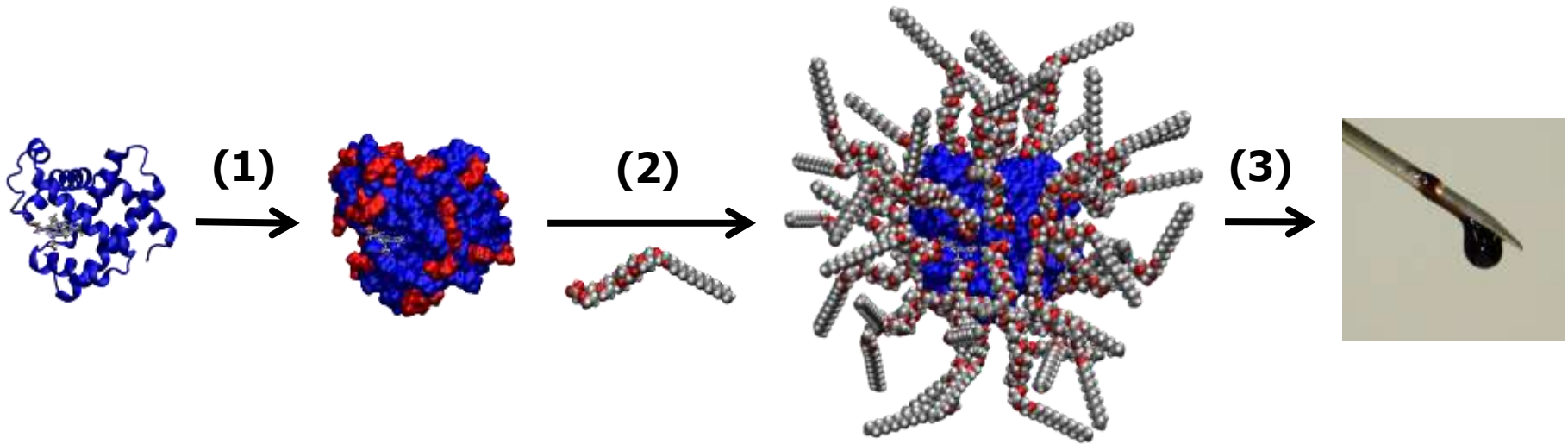
Ionogels



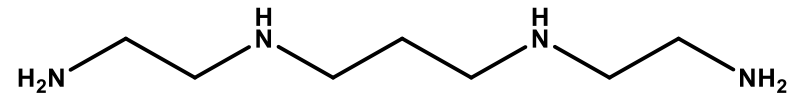
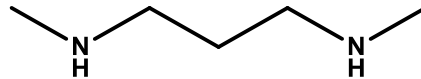
**Solubilize and stabilize enzymes in
ionic liquids**



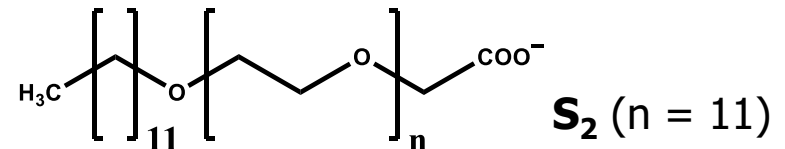
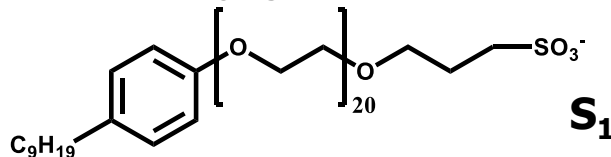
- Nanoscale objects do not have a liquid phase.
- Interparticle interactions need to be extended.



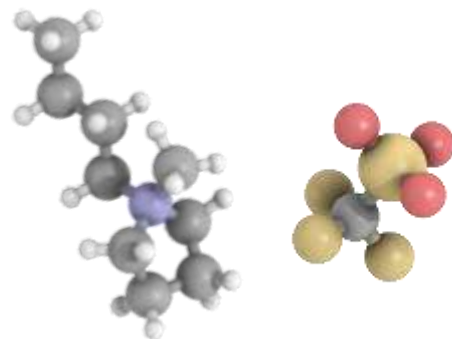
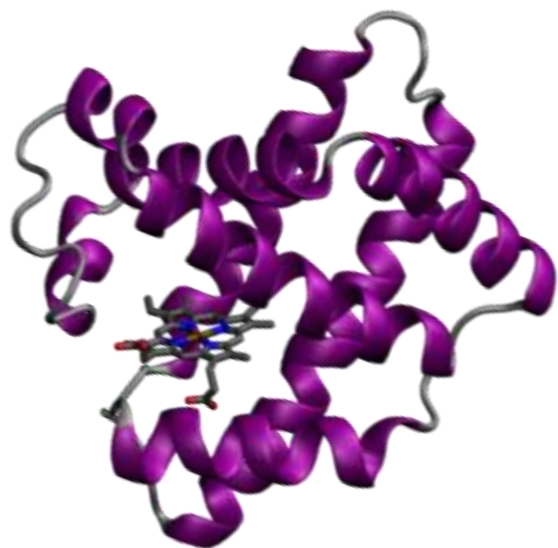
(1) Cationization of surface acidic residues with amines using the EDC reaction.



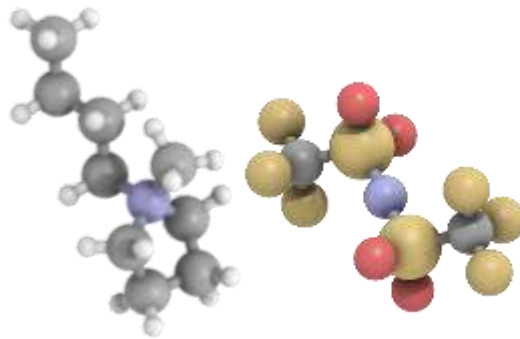
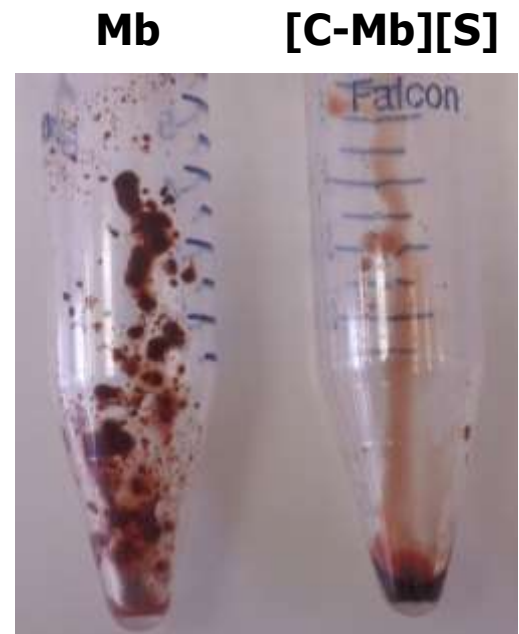
(2) Electrostatic complexation with anionic surfactant to form aqueous nanoconjugates.



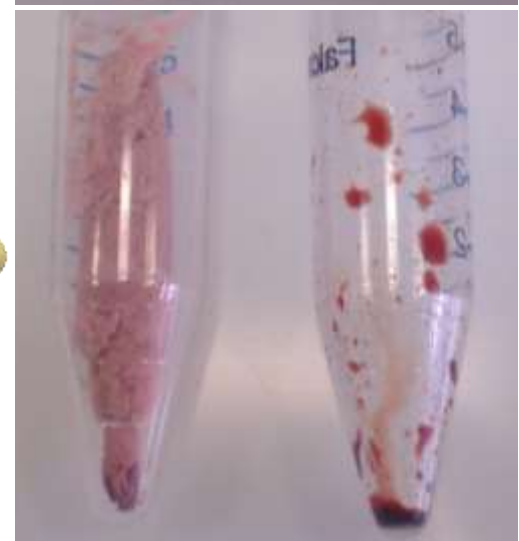
(3) Lyophilization of conjugate, followed by annealing at 60 °C to form solvent-free liquid protein.



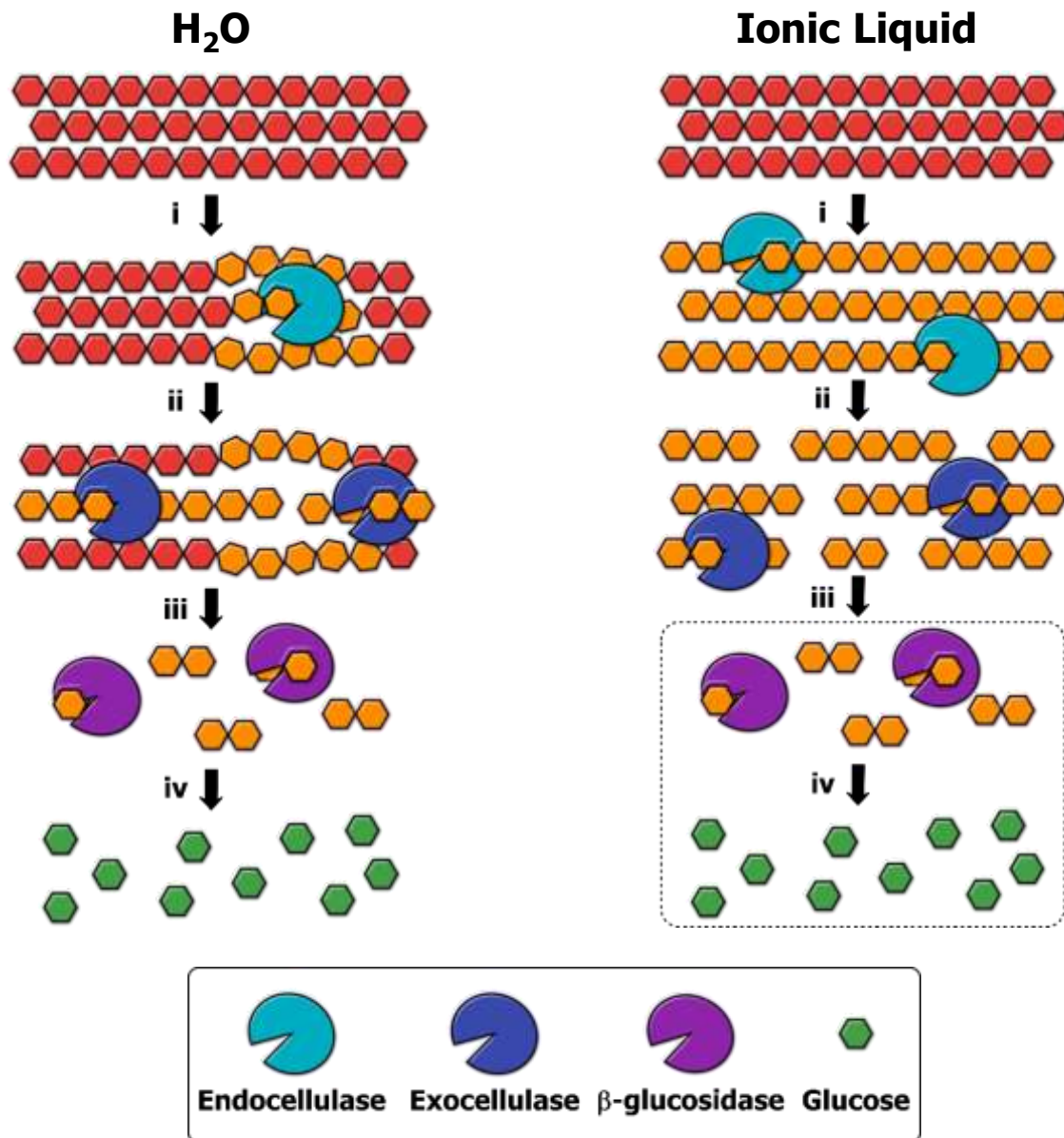
[bmpyrr][OTf]
HYDROPHILIC

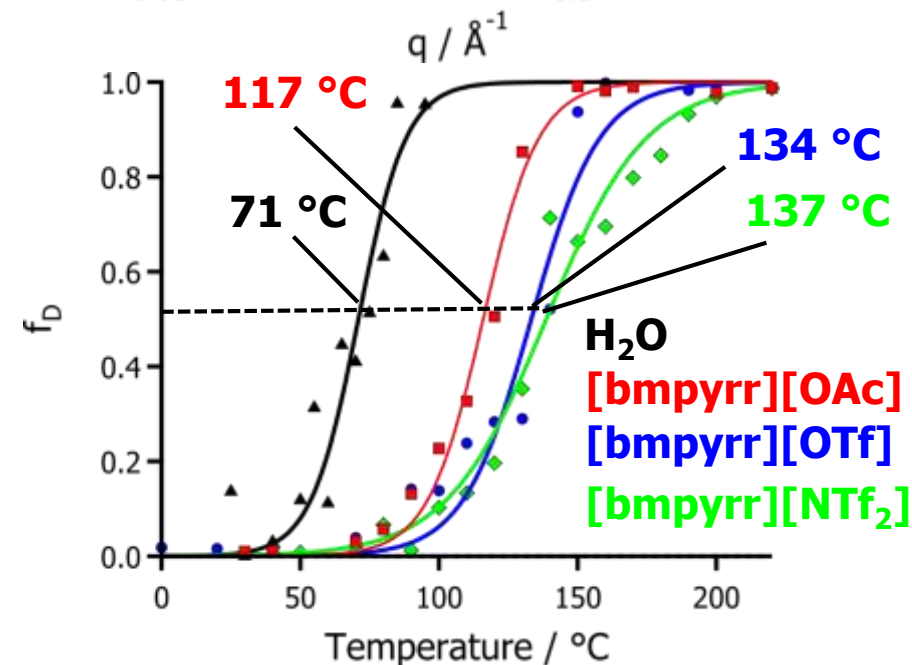
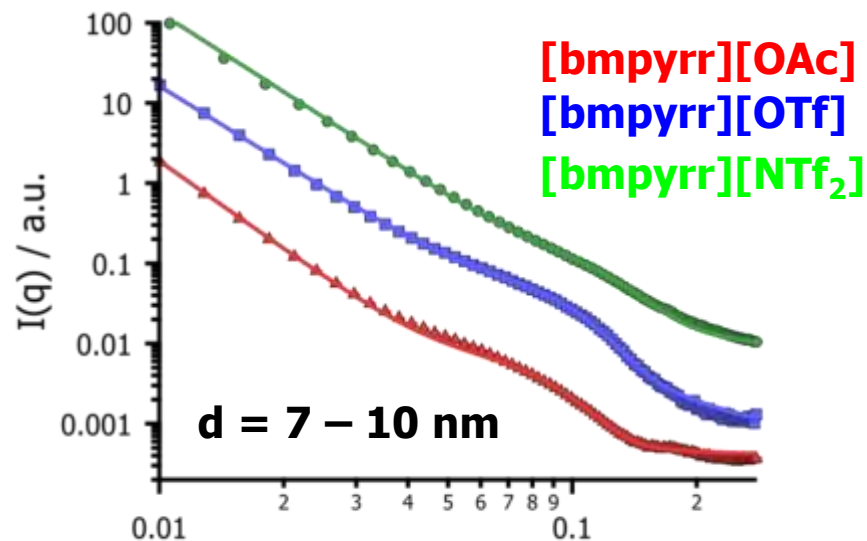
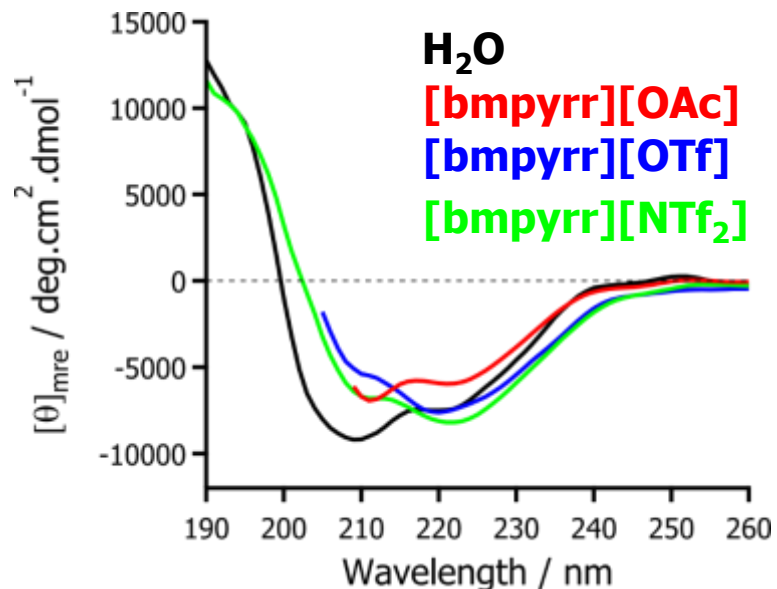


[bmpyrr][NTf₂]
HYDROPHOBIC

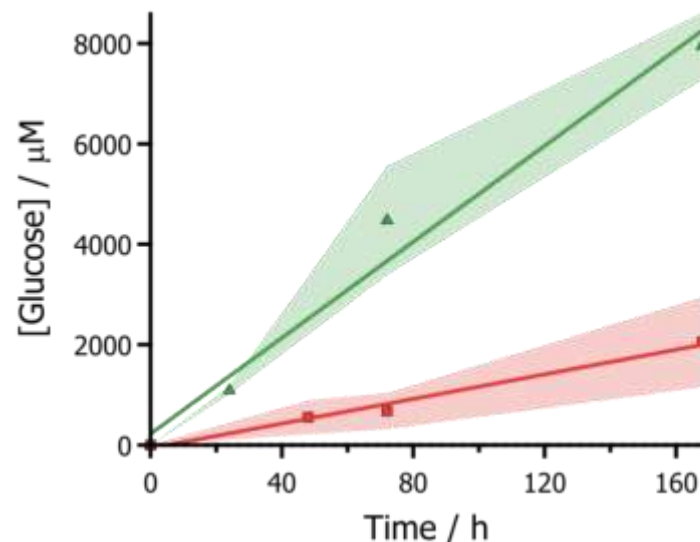
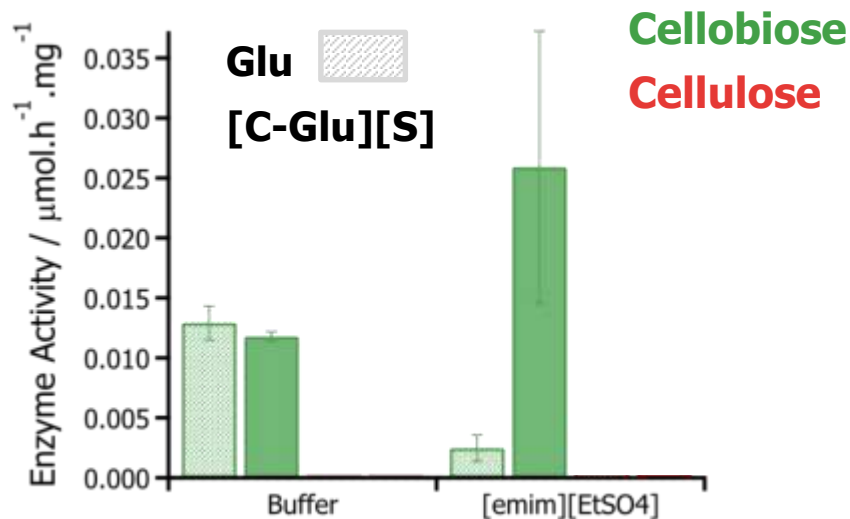


- Myoglobin as archetypal system.
- Well characterized – sensitive to environment.
- Biofluids have significant increase in IL mixing.

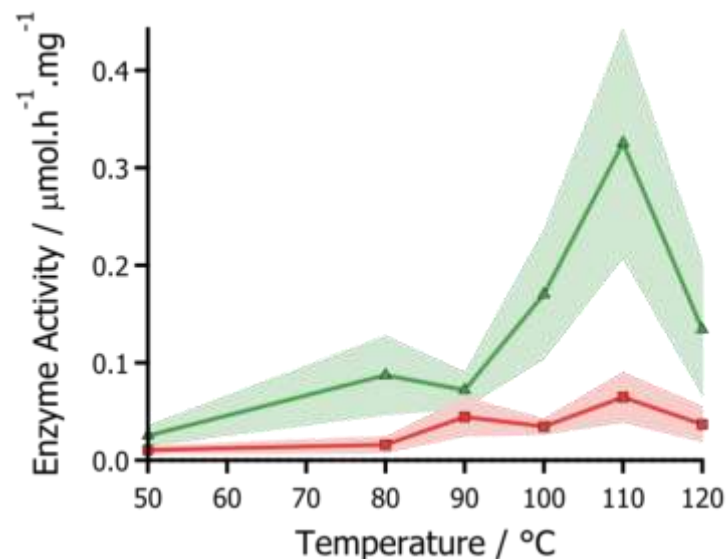


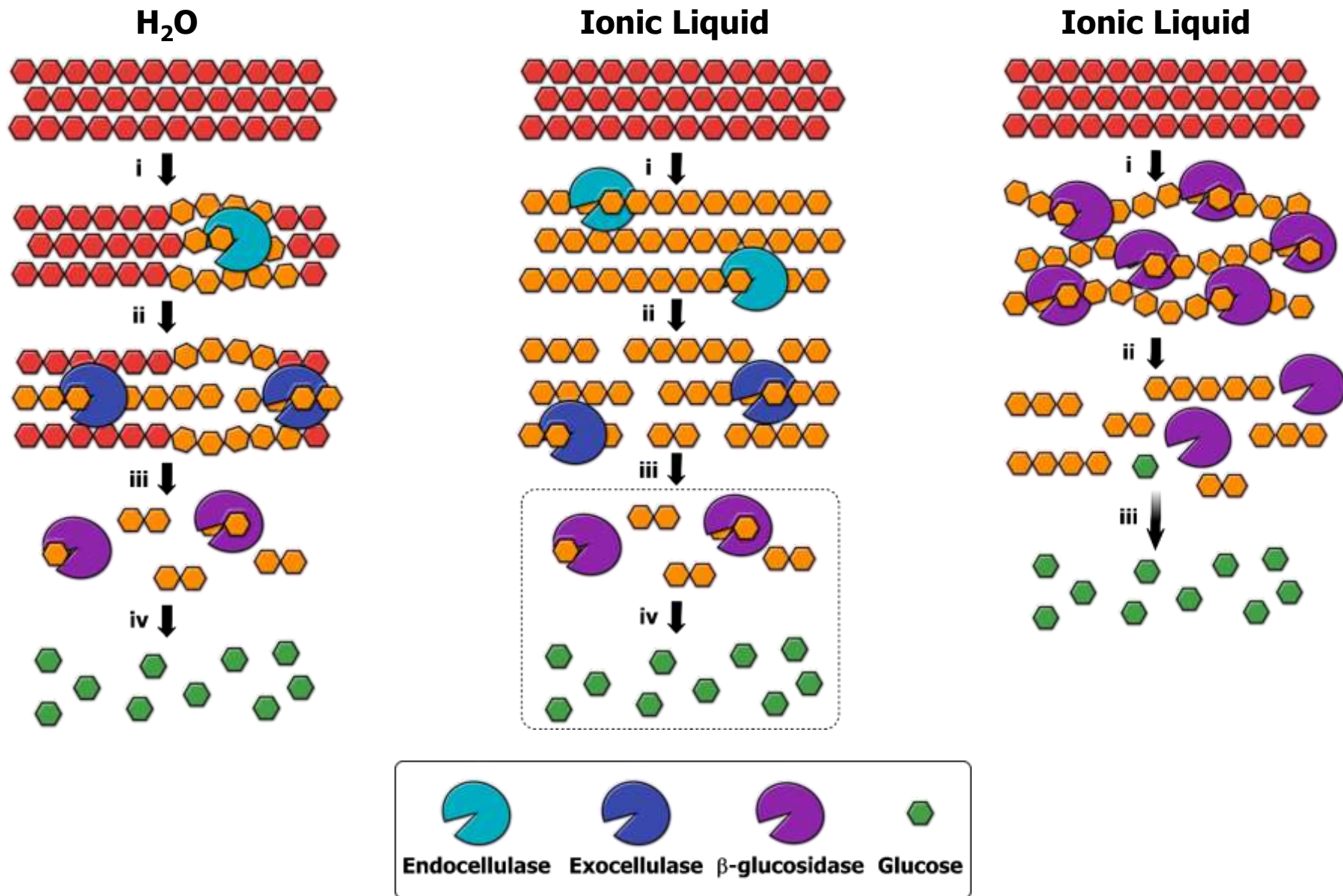


- Surface functionalization of glucosidase increases solubility in ionic liquids.
- Structure maintained (SRCD, SAXS)
- Highly stable in ionic liquids.

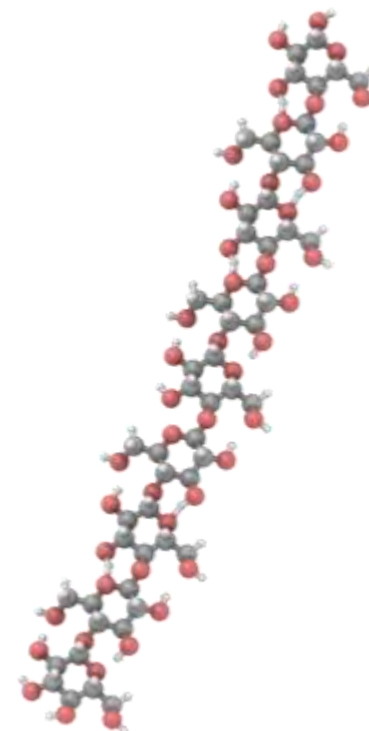
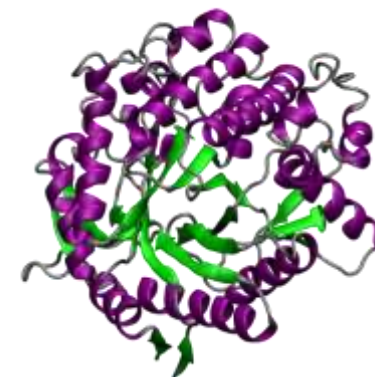


- Activity enhanced in ionic liquids – only for modified glucosidase.
- Reaction turns over at constant rate – zero order kinetics
- Enzyme activity increases by almost 30x at 110 °C





- 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

Prof. 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

