Protein-polymer surfactant conjugates for anhydrous stability and function

Alex P. S. Brogan*, Jason P. Hallett*, Adam W. Perriman†, Stephen Mann‡

*Department of Chemical Engineering, Imperial College, London, UK, SW7 2AZ.

†School of Cellular and Molecular Medicine, University of Bristol, Bristol, UK, BS8 1TD. ‡School of Chemistry, University of Bristol, Bristol, UK, BS8 1TS.

alexbrogan.co.uk/posters

Imperial College London

EPSRC Engineering and Physical Sciences Research Council

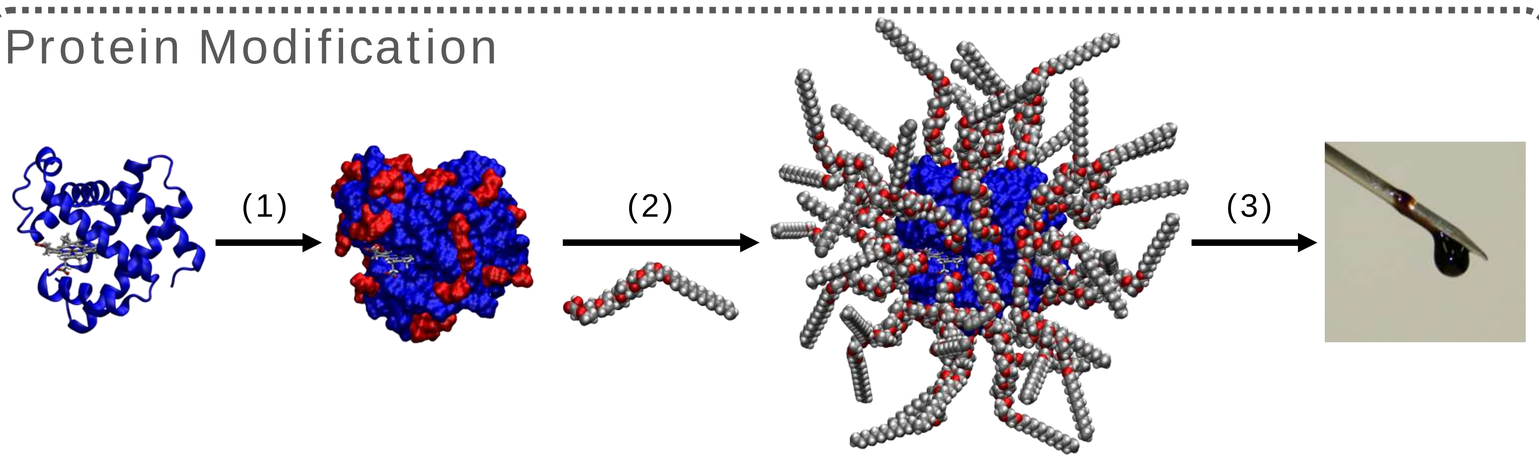
Introduction

Through the chemical modification of protein/enzyme surfaces, via attachment of polymer surfactants, the previously inaccessible liquid phase of solvent-free enzymes can been accessed.

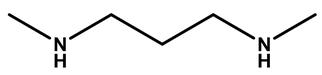
Despite extremely low water contents (0.1—0.3 wt%), protein architecture is highly conserved and protein dynamics remain as if hydrated. Furthermore, solvent-free liquid proteins exhibit high thermal stability, with enzyme activity up to 150 °C.

Here, we show that these modified proteins have high solubility in anhydrous liquids, with retained structure, improved thermal stability, and increased enzyme activity. Such technology could be used to develop ionic liquid stable biocatalysts.

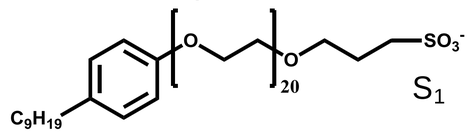
Protein Modification



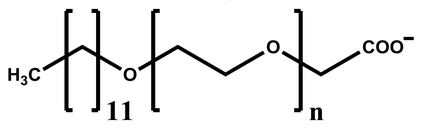
(1) Cationization of surface acidic residues with DMPA using EDC reaction.



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



S_1

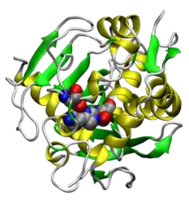


S_2 (n = 11)

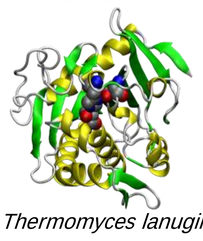
S_7 (n = 20)

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

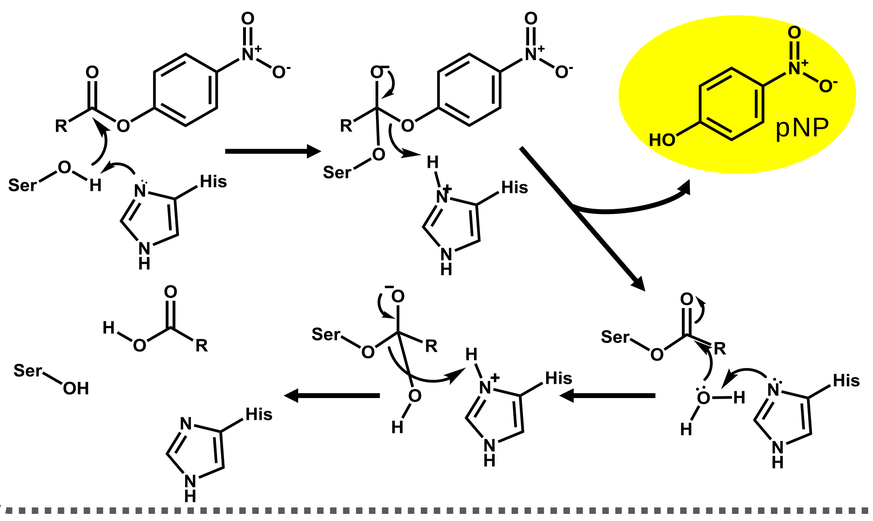
Lipase fluids with enzyme activity up to 150 °C

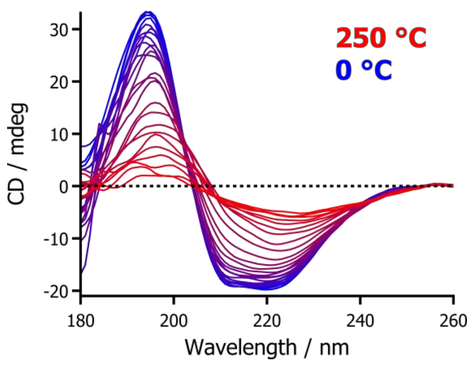


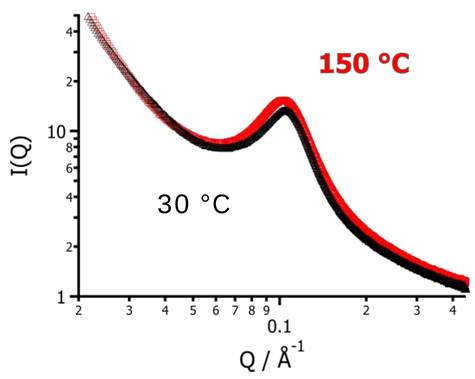
Lipase from *Rhizomucor miehei* (RML)

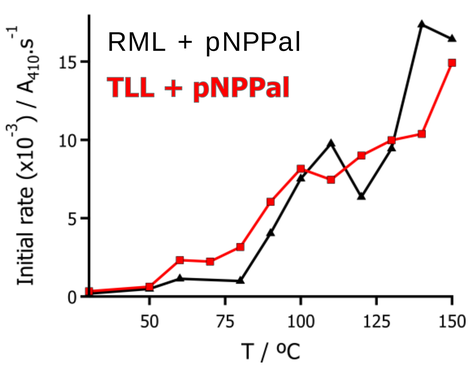


Lipase from *Thermomyces lanuginosus* (TLL)

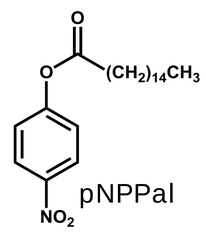


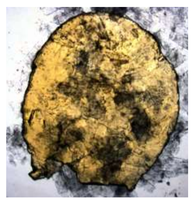




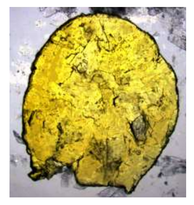


- Solvent-free liquids of lipase from two organisms successfully synthesized.
- High conservation of secondary structure (SRCD) and globular architecture (SAXS).
- Liquid lipase can mix with liquid substrates, and solubilize solid substrates.
- Enzyme activity increases with temperatures up to 150 °C.



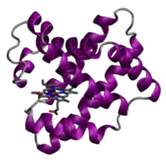


RML + pNPPal



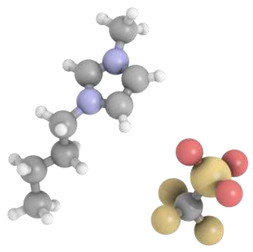
t = 24 h

Solubility and stability in anhydrous ionic liquids



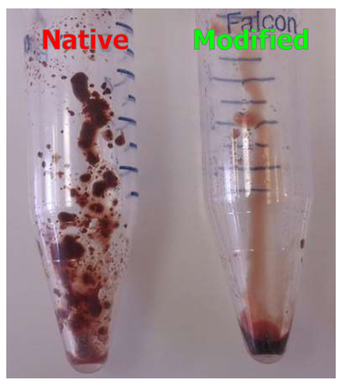
Myoglobin

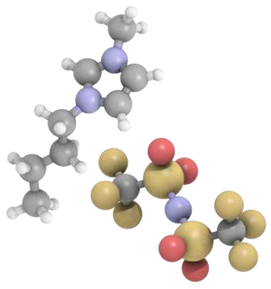
Key: Aqueous
[bmpyrr][OTf]
[bmpyrr][NTf$_2$]



[bmpyrr][OTf]

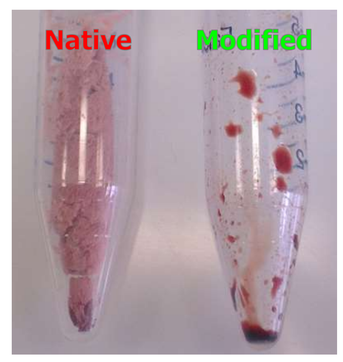
HYDROPHILIC



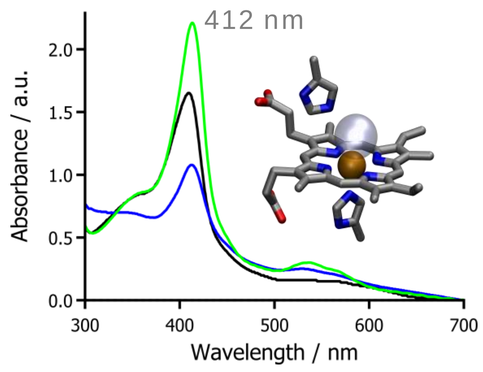


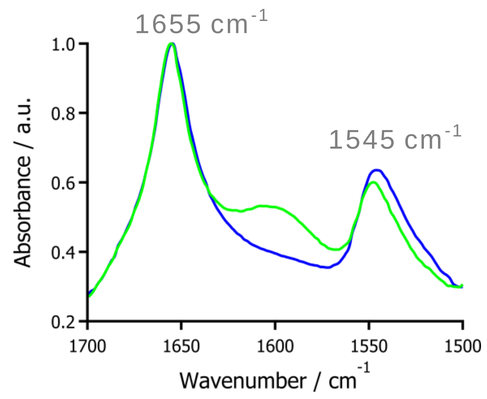
[bmpyrr][NTf$_2$]

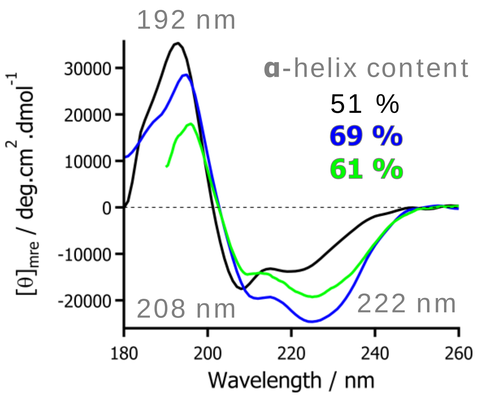
HYDROPHOBIC

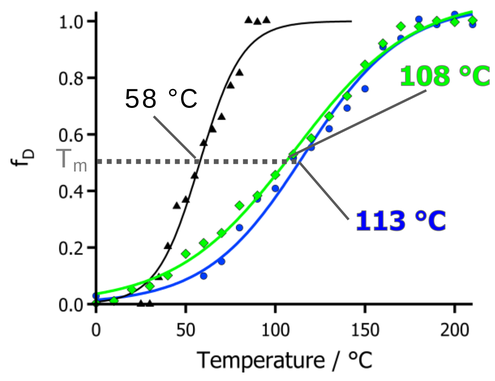


Surface modified myoglobin highly soluble in both hydrophobic and hydrophilic ionic liquids.

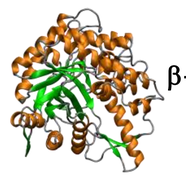






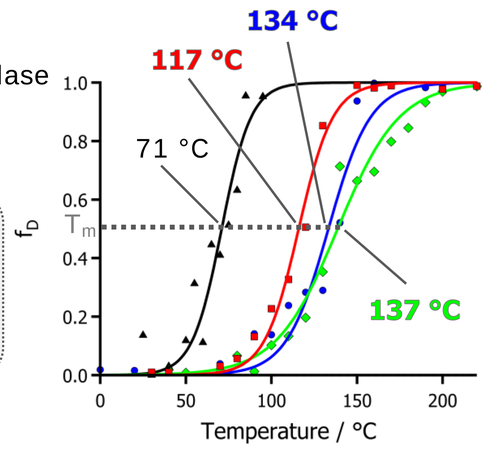


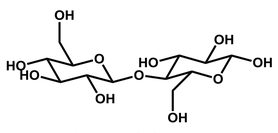
- Characteristic Soret band indicating retained tertiary structure (UV/Vis).
- Secondary structure retained as predominately α-helix (FTIR, CD).
- 55 °C increase in thermal stability in ionic liquids.



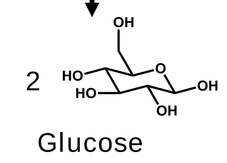
β-glucosidase

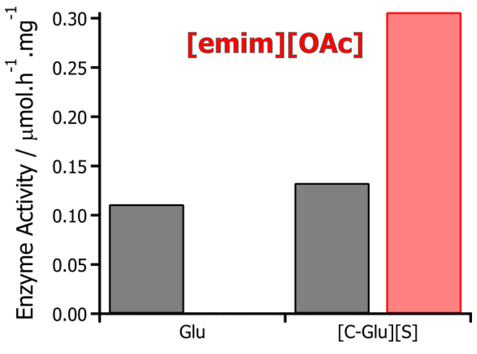
Key: Aqueous
[bmpyrr][OTf]
[bmpyrr][NTf$_2$]
[bmpyrr][OAc]





50 °C





- β-Glucosidase soluble in range of ionic liquids.
- Thermal stability increase greater ionic liquids with non-interacting anions.
- Modified enzyme more active in ionic liquids than aqueous conditions.

Conclusions

Non-aqueous enzymatic biofluids of lipase have been shown to retain structure and enzymatic function in the absence of water, exhibiting hyperthermophilic-like behaviour.

Using solvent-free liquid myoglobin as an archetypal system, these modified proteins show great miscibility with anhydrous ionic liquids. In addition, the protein has increased secondary structure, and improved thermal stability as compared to aqueous solution. Additionally, β-glucosidase shows increased enzyme activity in ionic liquids. As a result, this technology has potential in the design of ionic liquid stable biocatalysts for use in absence of water.

References

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