Enzyme-surfactant nanoconjugates for non-aqueous production of biofuels from triglycerides

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Introduction

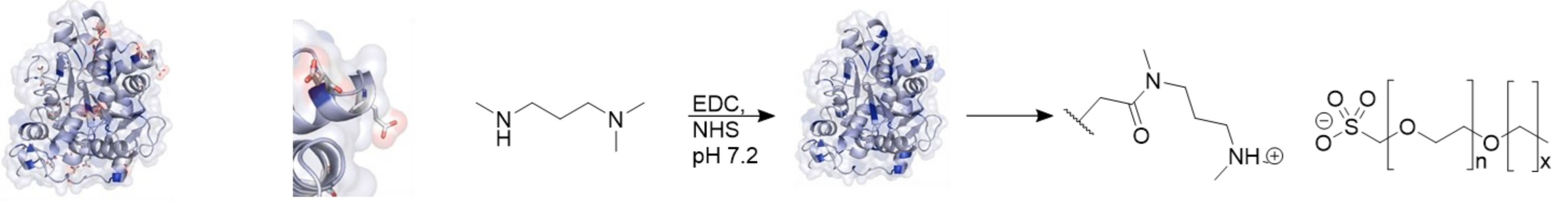
By chemically modifying the surface of a protein via the conjugation of polymer surfactants, Brogan et al. have previously demonstrated the formation of solvent-free biofluids[1].

These biofluids have been shown to be soluble in anhydrous ionic liquids while conserving protein structure and dynamics[2].

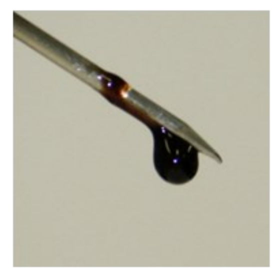
Previous work has focused on stabilising hydrolytic enzymes in ionic liquids[3]. This work aims to apply this stabilisation technique to P450 fatty acid decarboxylase, allowing for the enzyme to be used in ionic liquids to form terminal alkenes from fatty acids[4]. These terminal alkenes can be used as drop-in biofuels in conventional petrodiesel engines[5].

This enzymatic process has previously been hindered by the low solubility and critical micelle concentration of the fatty acid substrates in water and it is hoped that it can be improved by performing the reaction in non-aqueous media.

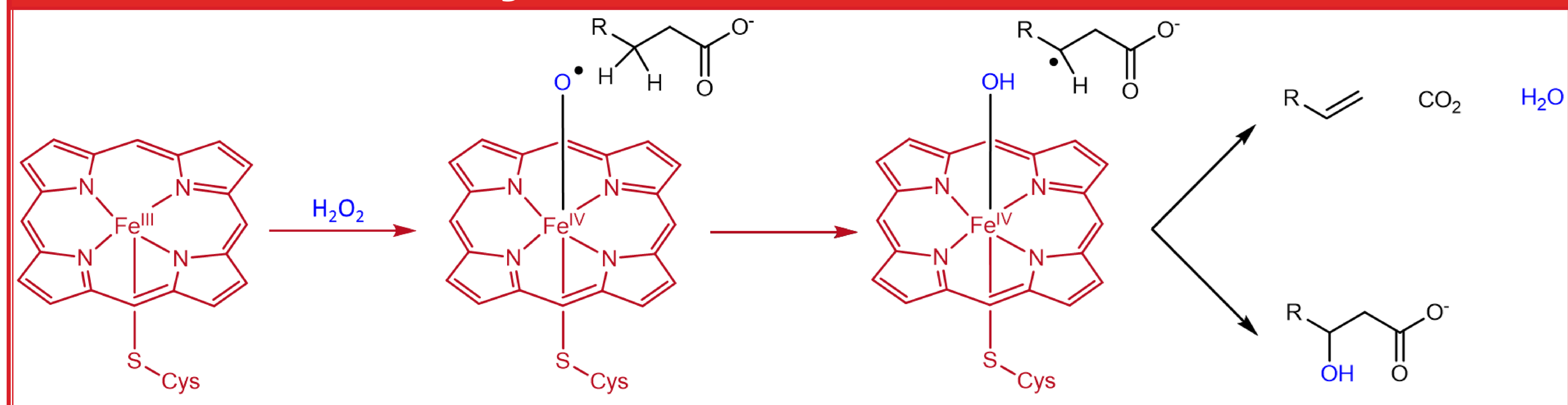
Protein modification



- The surface of the protein is cationised via chemical coupling of polyamines to aspartate and glutamate residues
- Anionic surfactants are then conjugated to the protein surface forming a stabilising corona
- The resulting protein-surfactant nanoconjugate is then lyophilised and thermally annealed to form a solvent-free biofluid[1]

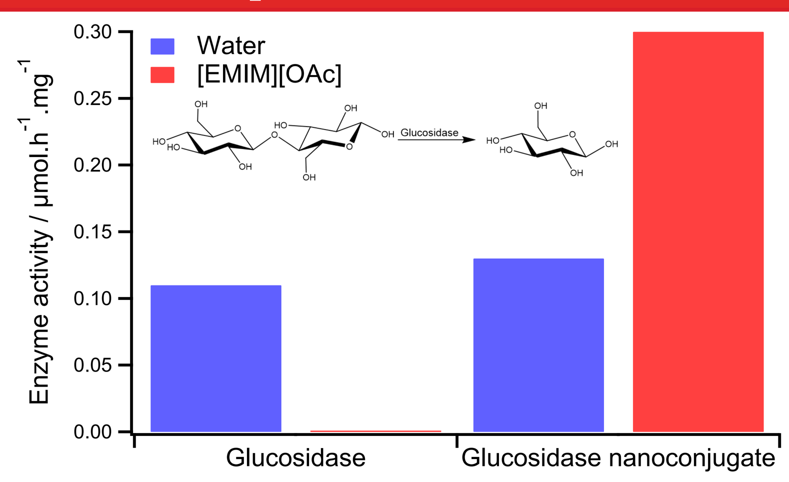


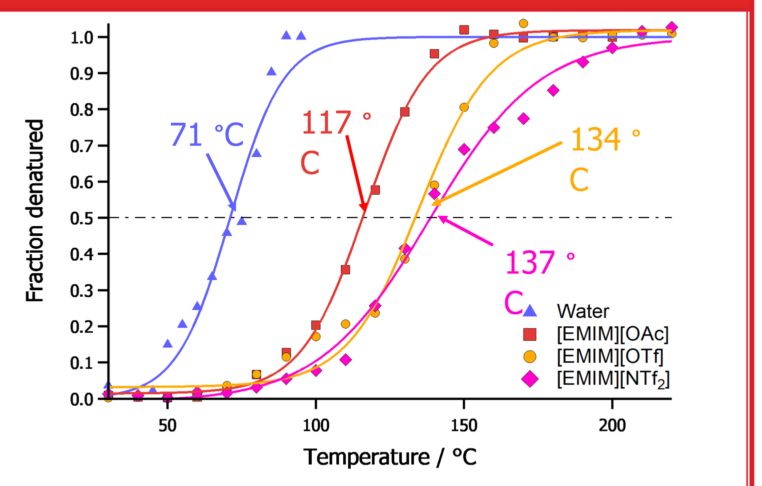
P450 decarboxylase



- A P450 decarboxylase is an enzyme which can be used to decarboxylate fatty acids to form terminal alkenes.
- The mechanism involves oxidation of the heme group of the protein by H_2O_2 to form an Fe(IV) radical species which abstracts a hydrogen atom from the substrate
- The radical substrate species then either decarboxylates forming an alkene or is hydroxylated by the enzyme[4]

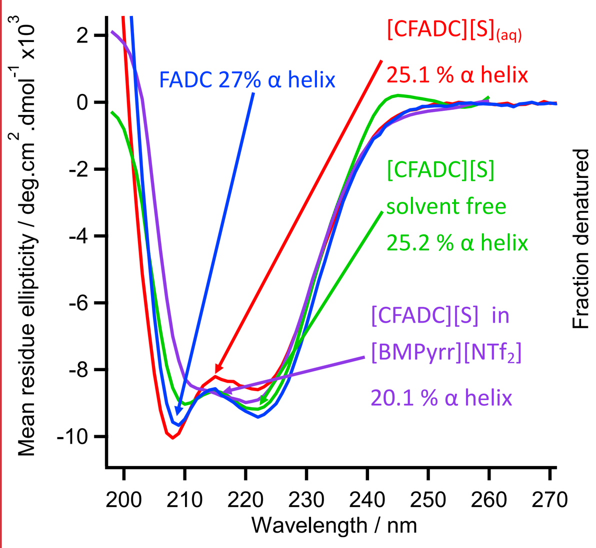
Ionic liquids

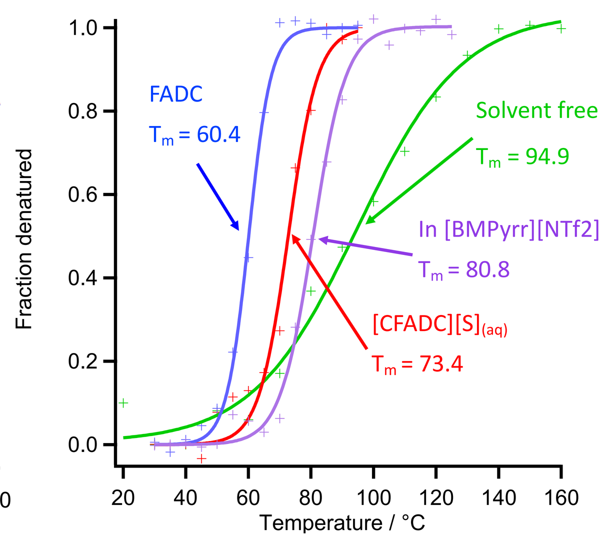




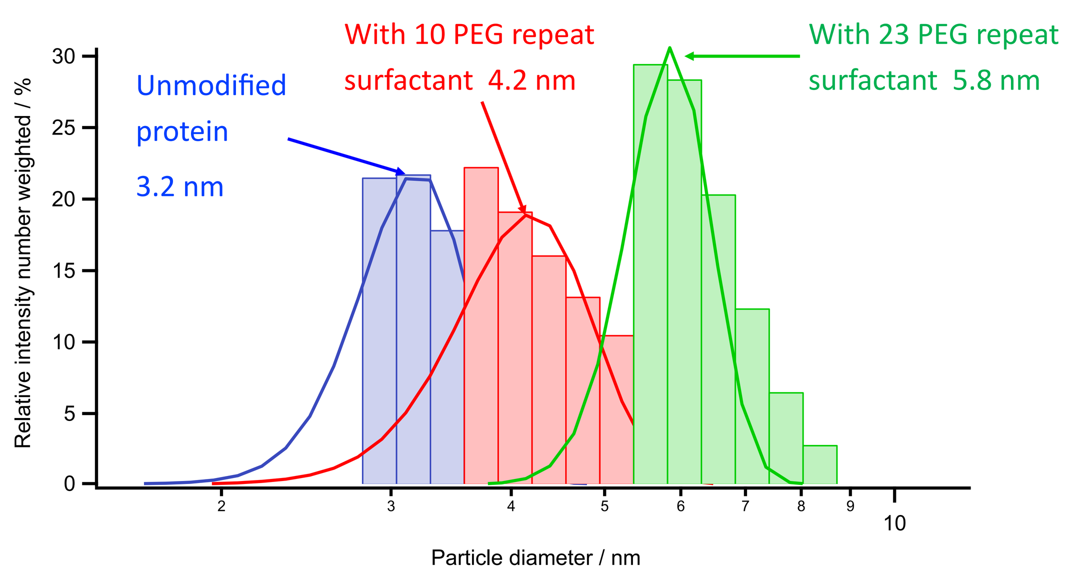
- Ionic liquids have a range of desirable solvent properties such as tuneable polarity and hydrophobicity, high stability, non-flammability, and negligible vapour pressure
- Protein nanoconjugates have demonstrated increased activity and thermal stability in ionic liquids relative to unmodified enzymes in aqueous media
- The solvent properties of ionic liquids has also led to the demonstration of solvent induced substrate promiscuity[3]

Structure



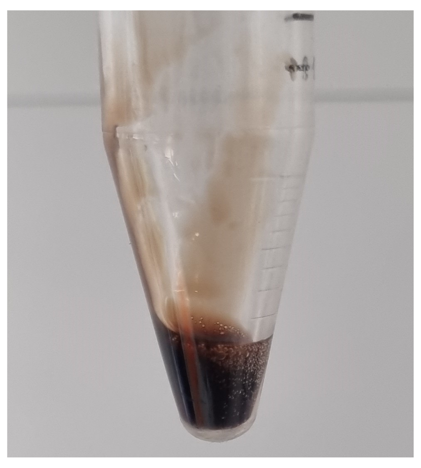


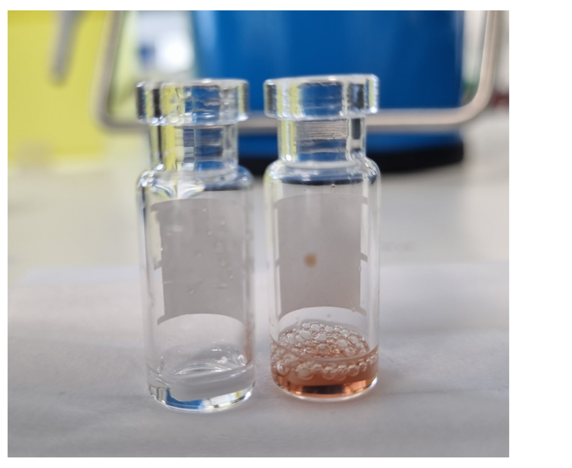
- Chemical modification and conjugation of surfactant had minimal effect on the secondary structure of the enzyme
- Modification of the protein improved its thermal stability as shown by an increase in T_m
- The thermal stability was higher in non-aqueous environments



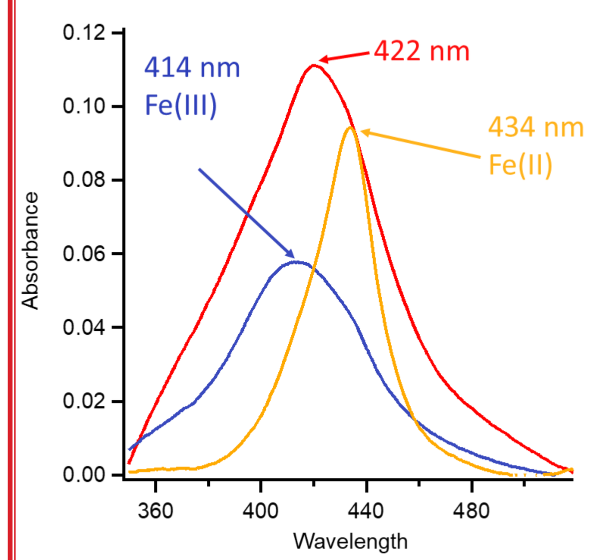
- Conjugation of surfactant increased the hydrodynamic diameter of the protein with larger surfactants causing a greater increase in diameter

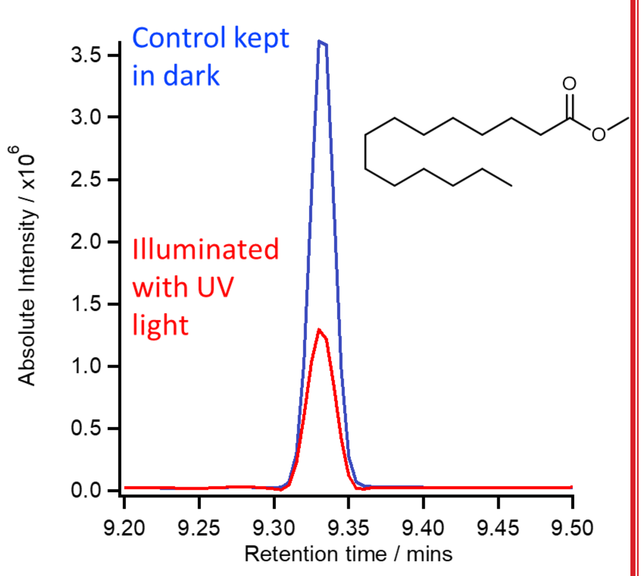
Catalysis

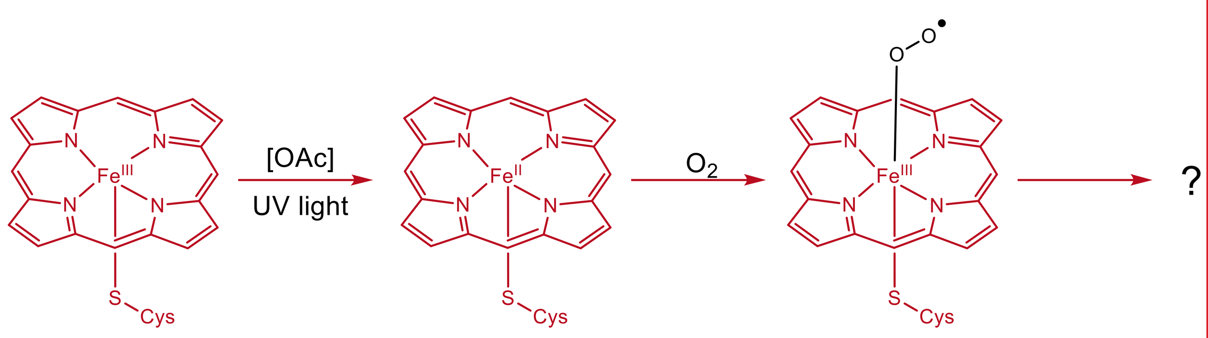




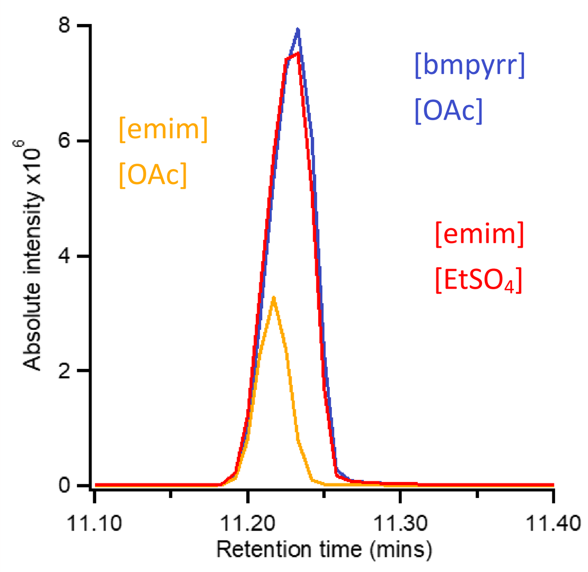
- The protein nanoconjugate formed a solvent-free biofluid which was soluble in ionic liquids

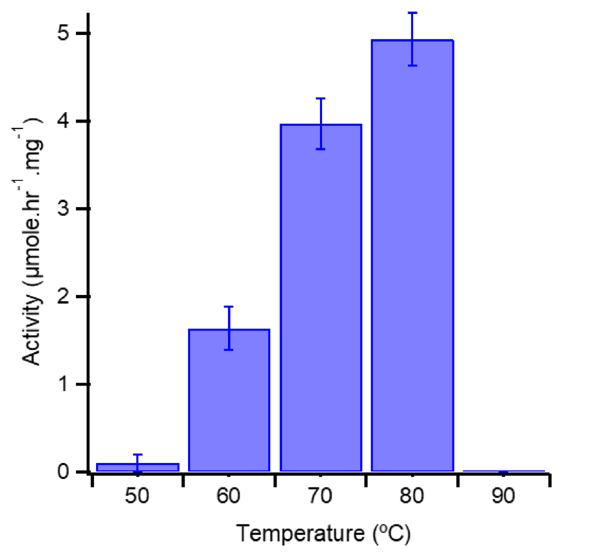


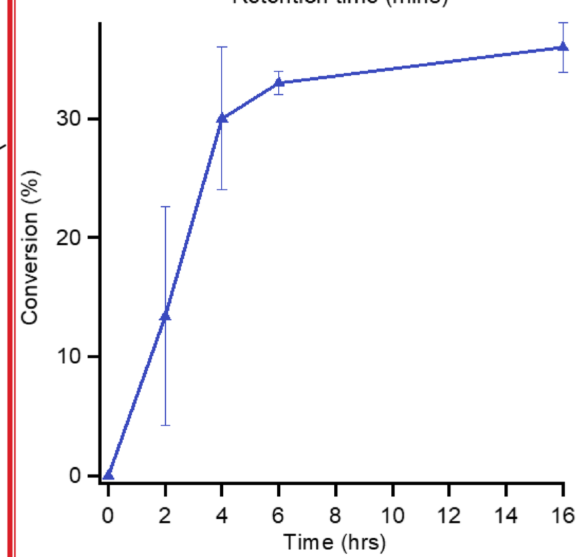


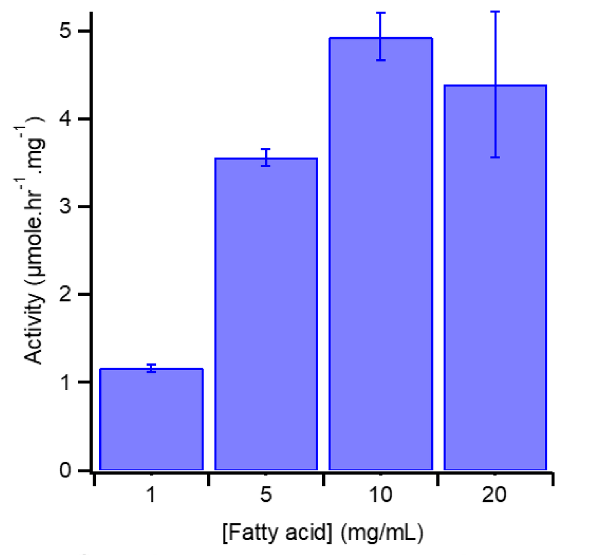


- The soret band of the nanoconjugate was found to respond to UV light in [OAc] containing ionic liquids
- The peak shifts suggest a photoreduction occurred followed by a reaction with O_2
- UV driven reaction was able to consume substrate









- Photocatalytic consumption of substrate was only observed in [emim][OAc], it was hypothesised to be linked to spontaneous carbene formation
- Optimisation of the reaction showed maximum activity at 80 °C and at 10 mg/mL of myristic acid
- The reaction appears to essentially stop after 4 hours which coincided with photobleaching of the heme cofactor
- Future work will focus on the use of different light sources to prolong the lifetime of the reaction.

Conclusion

- A polymer surfactant enzyme nanoconjugate has been formed using a P450 decarboxylase
- The nanoconjugate is soluble in a range of ionic liquids and the fatty acid substrate
- The thermal stability of the proteins secondary structure has been significantly improved while retaining enzyme activity
- The H_2O_2 driven reaction requires optimisation in ionic liquids
- A new mode of photochemical reactivity has been observed and will be investigated further

References

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