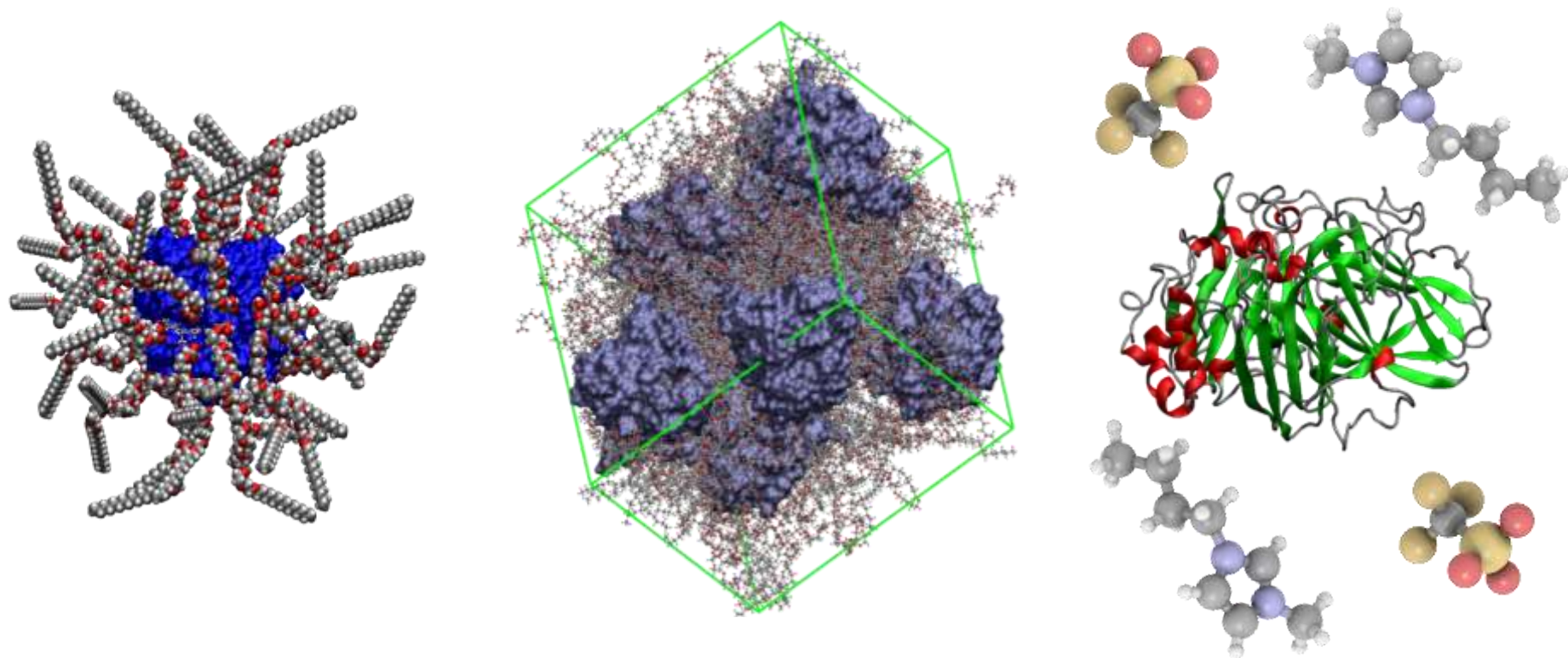


Solvent-free Functional Biofluids as a Route for Retained Structure and Improved Protein Stability in Non-aqueous Environments



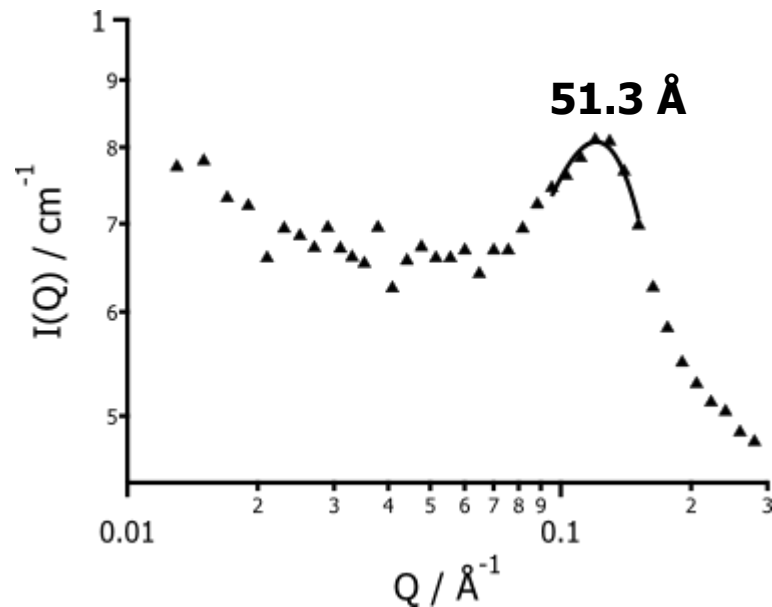
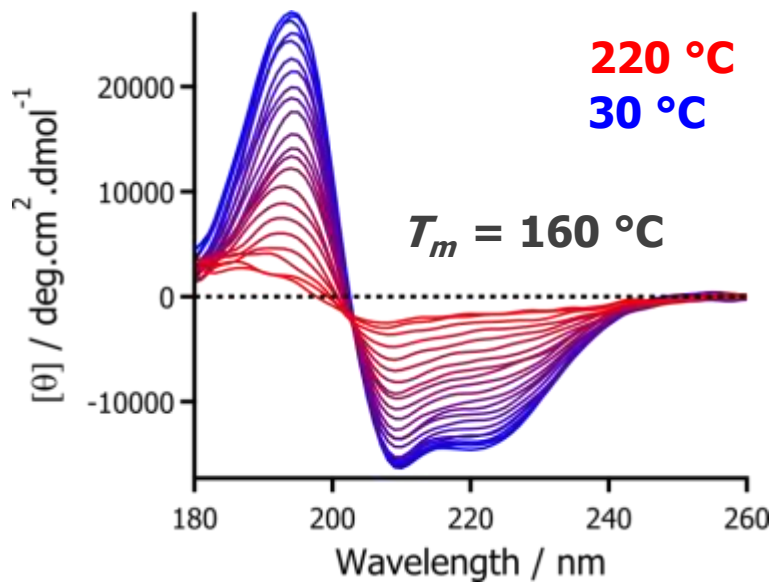
Dr. Alex P. S. Brogan

**Imperial College
London**

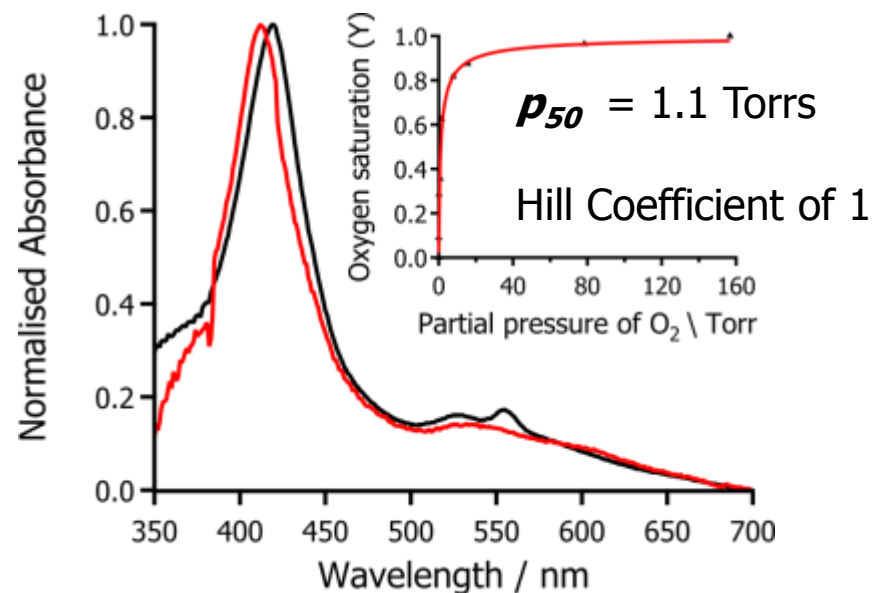
alexbrogan.co.uk/acs-2016

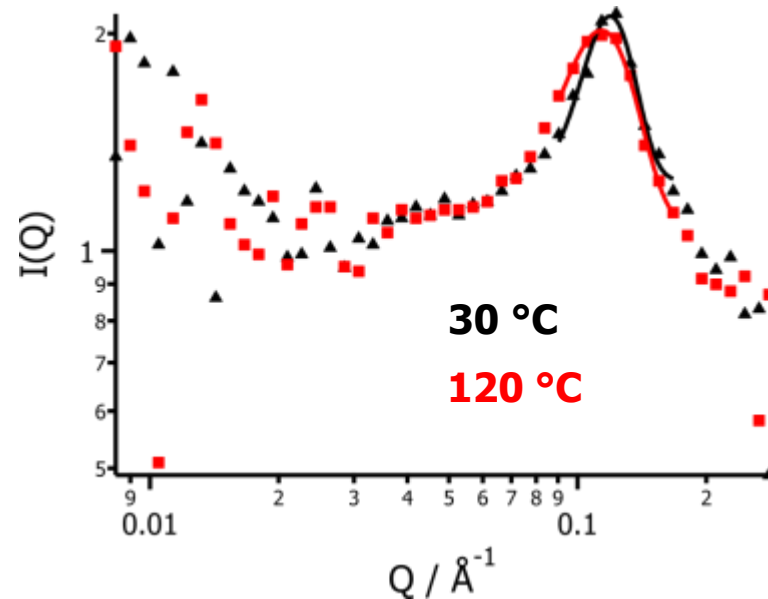
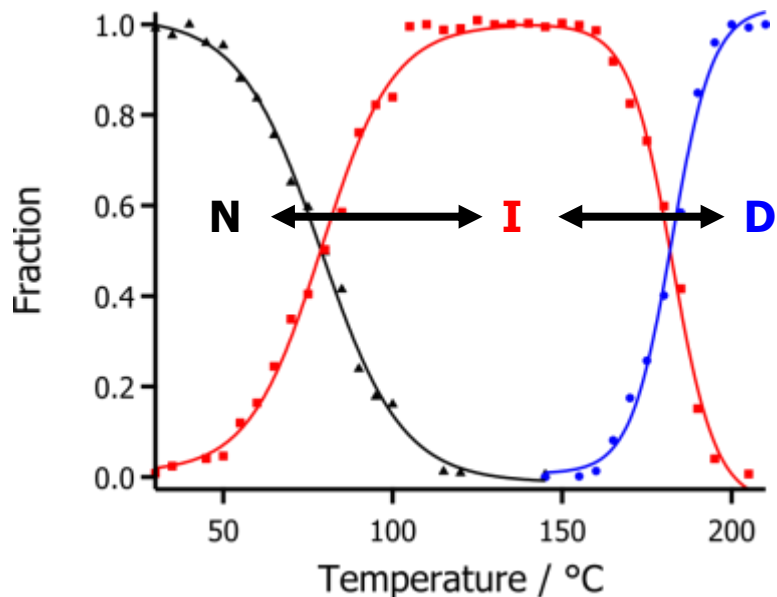
16th March 2016

ACS 251st Meeting – BIOT 458

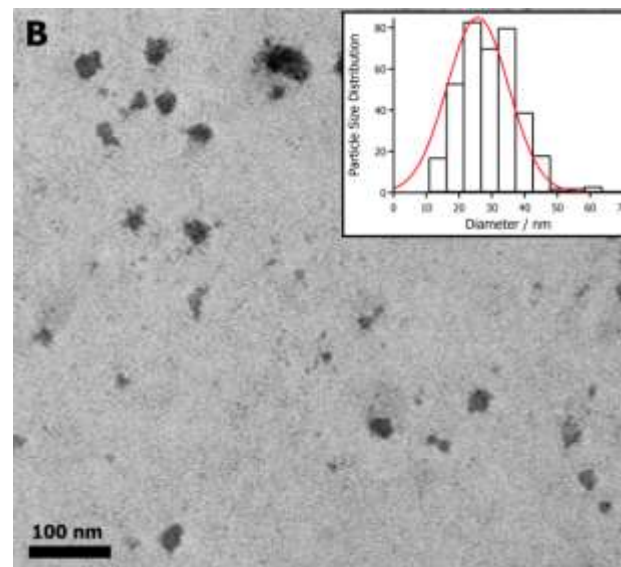


- Retained secondary structure.
- High thermal stability.
- Persistent tertiary structure.
- Biological function in absence of water.



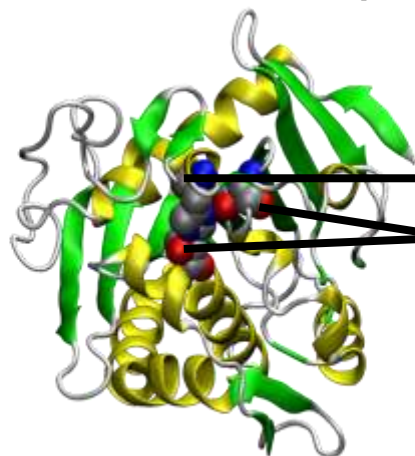


- 3-state unfolding in solvent-free liquid state.
- Intermediate stable over large temperature range.
- Intermediate only aggregates when reintroduced into water.



Rhizomucor miehei (RML)

Thermomyces lanuginosus (TLL)

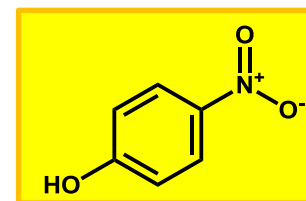
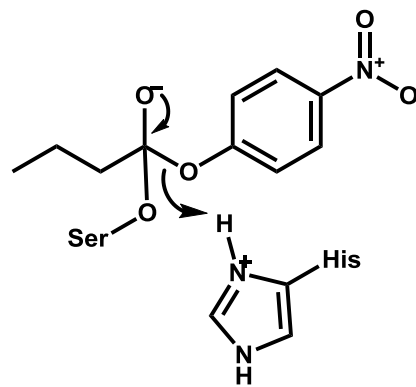
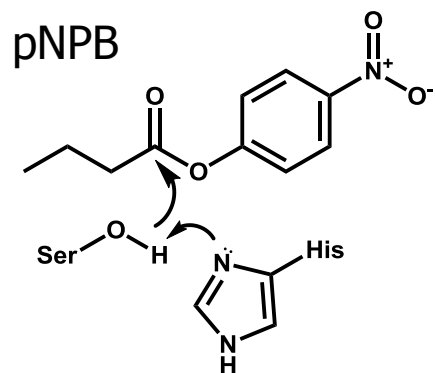
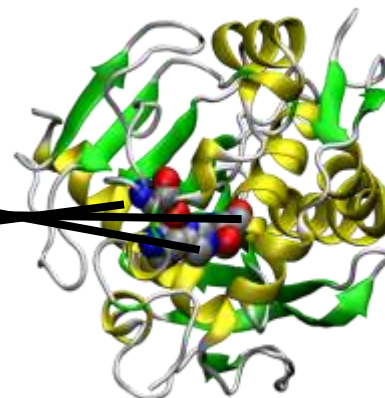


Catalytic triad

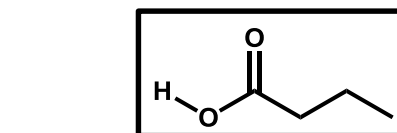
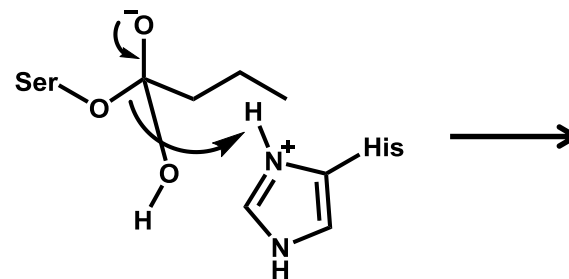
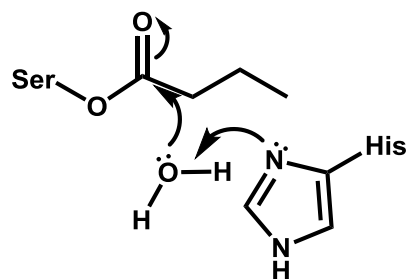
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SER

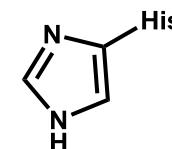
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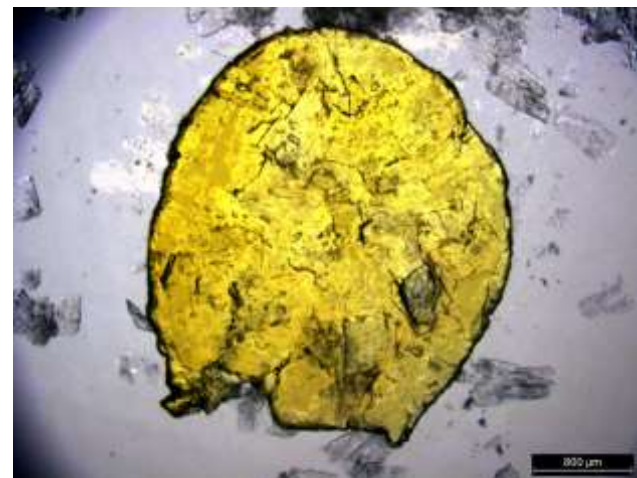
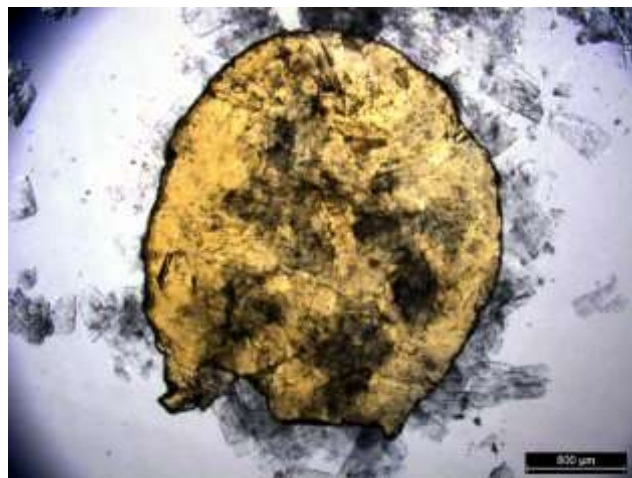
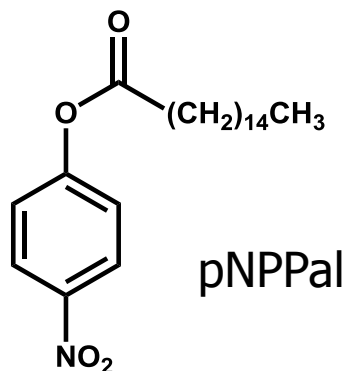


410 nm

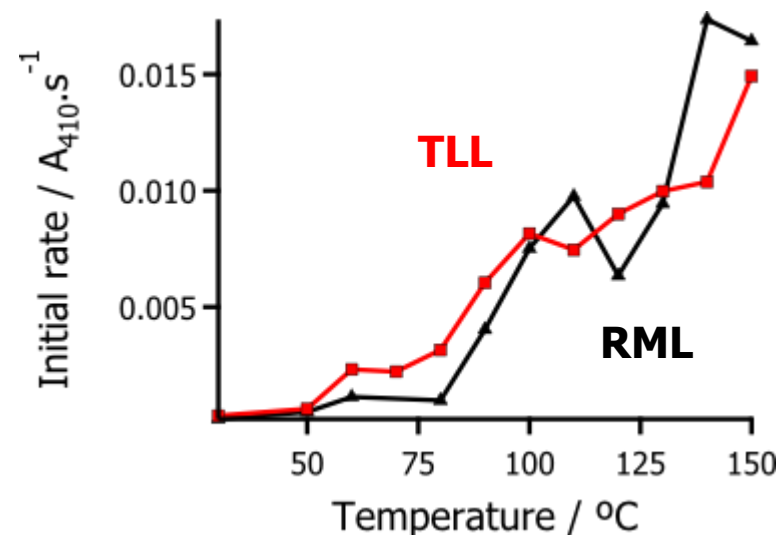


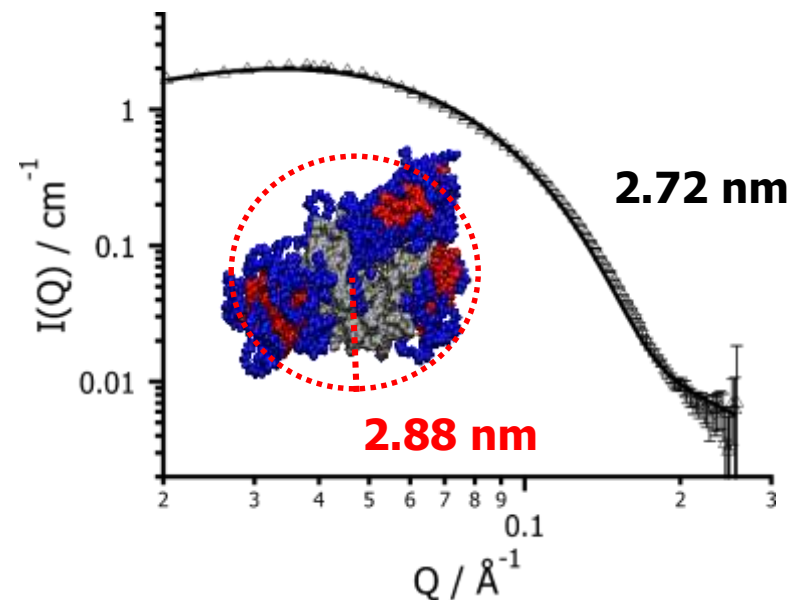
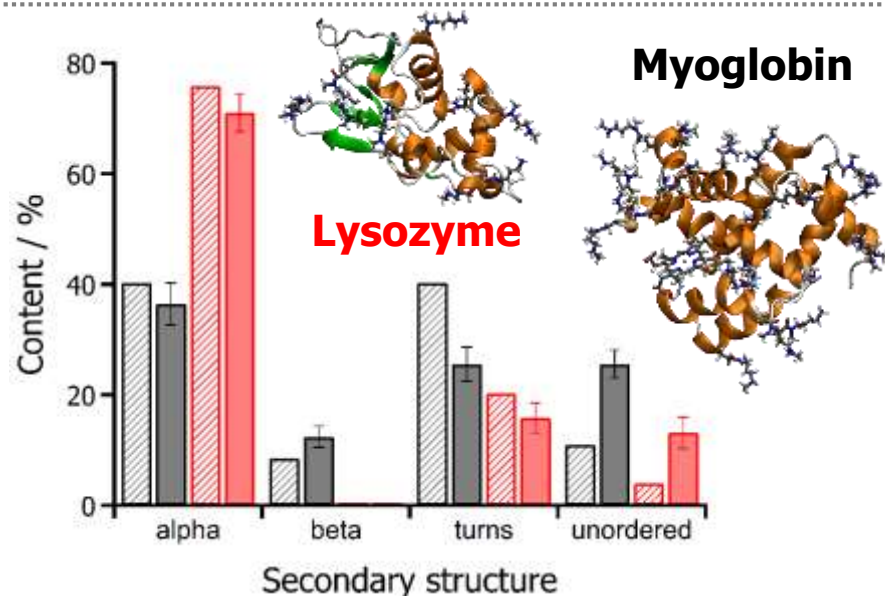
Ser-OH



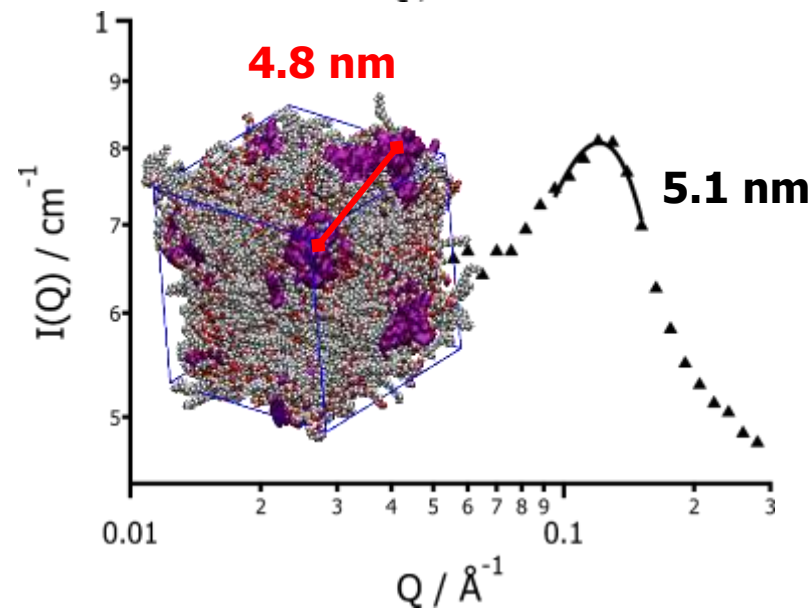


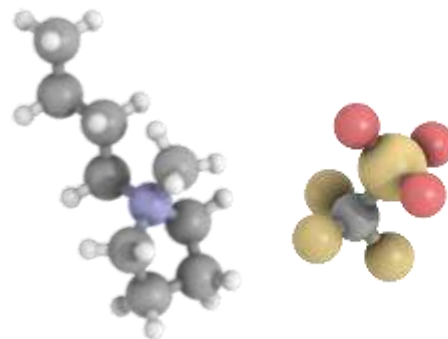
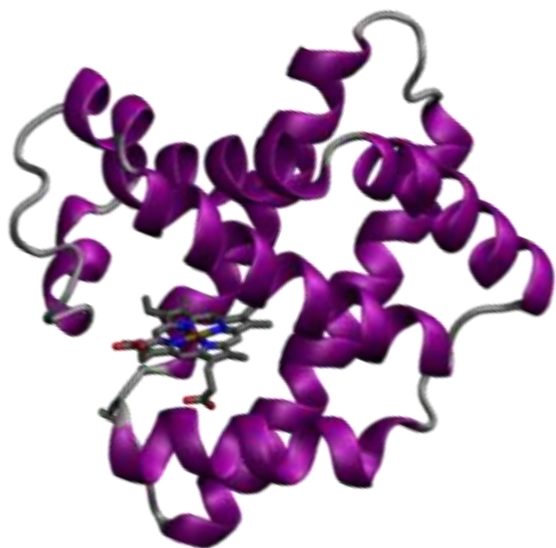
- Enzyme activity in absence of water.
- Delivery of both liquid and solid substrates to enzyme active site.
- Enhanced enzyme activity up to 150 °C





- Can use MD in absence of experimental atomistic data.
- Secondary structure of modified proteins, real and simulated, compare well.
- Computed and measured dimensions also compare well – in aqueous and solvent-free liquids.

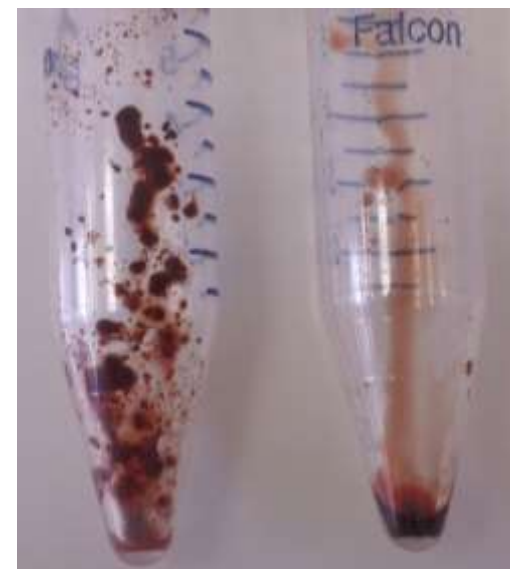




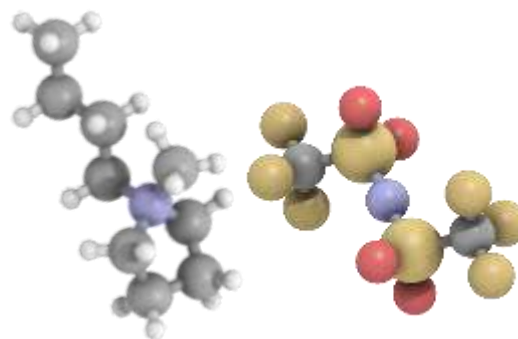
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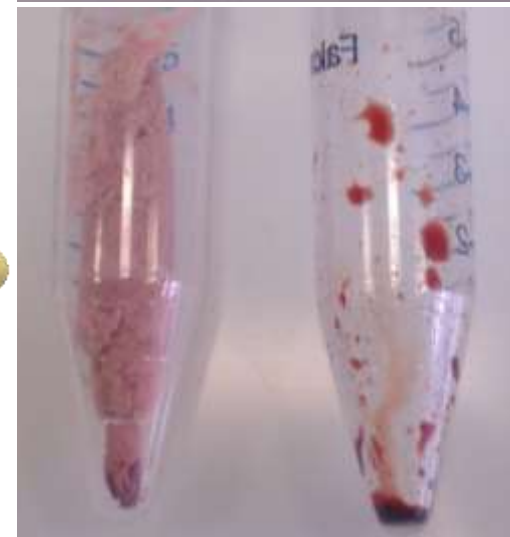
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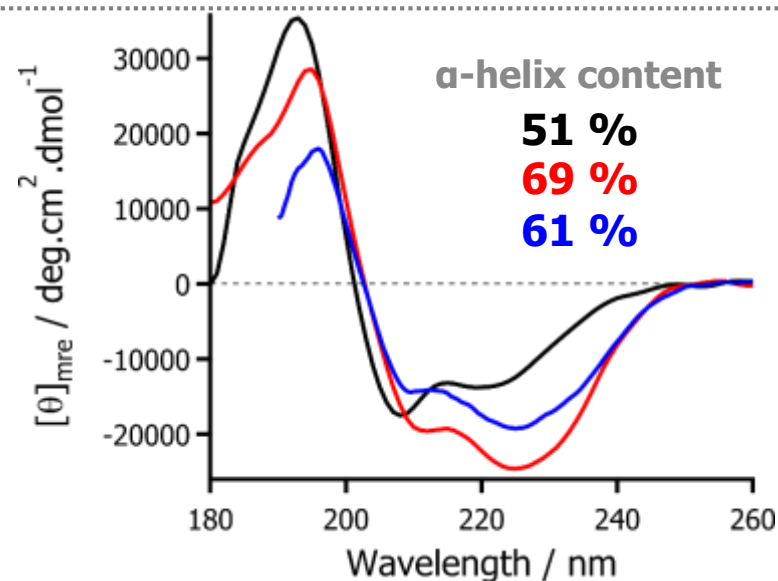
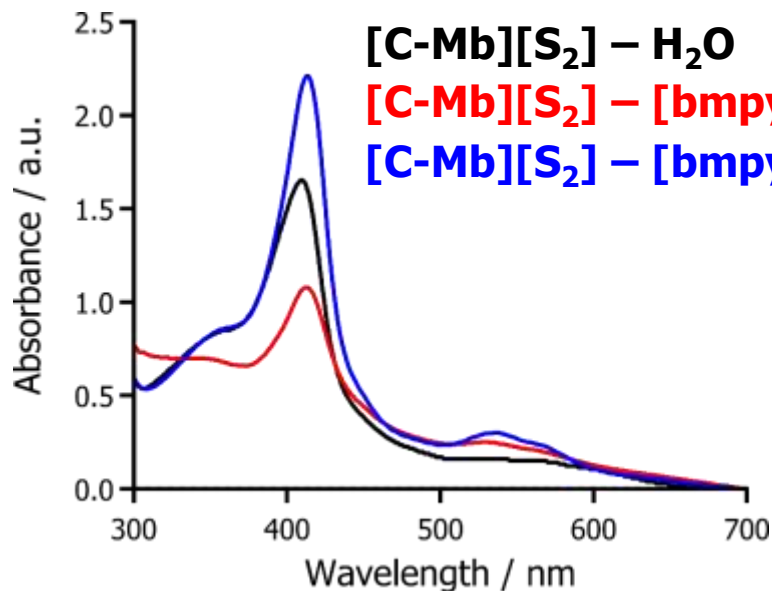


- Myoglobin as archetypal system.
- Well characterized – sensitive to environment.
- Biofluids have significant increase in IL mixing.

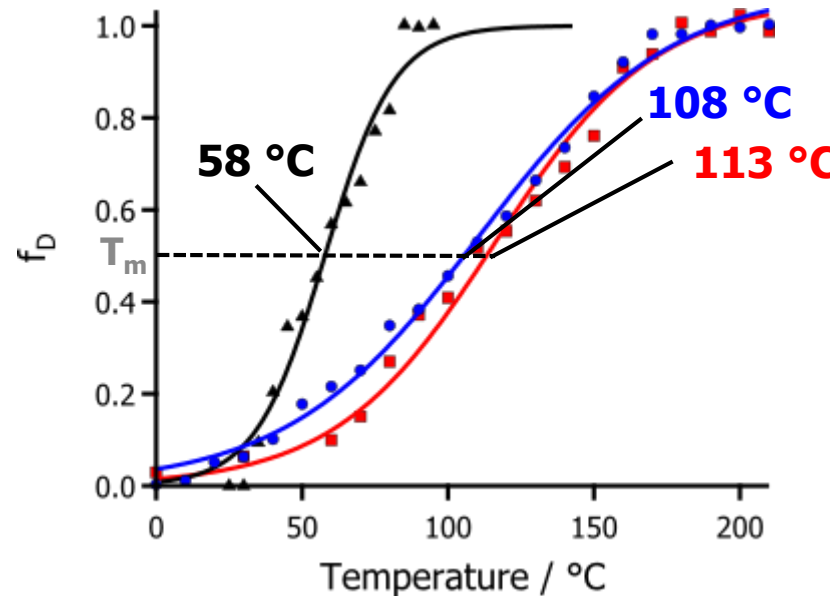


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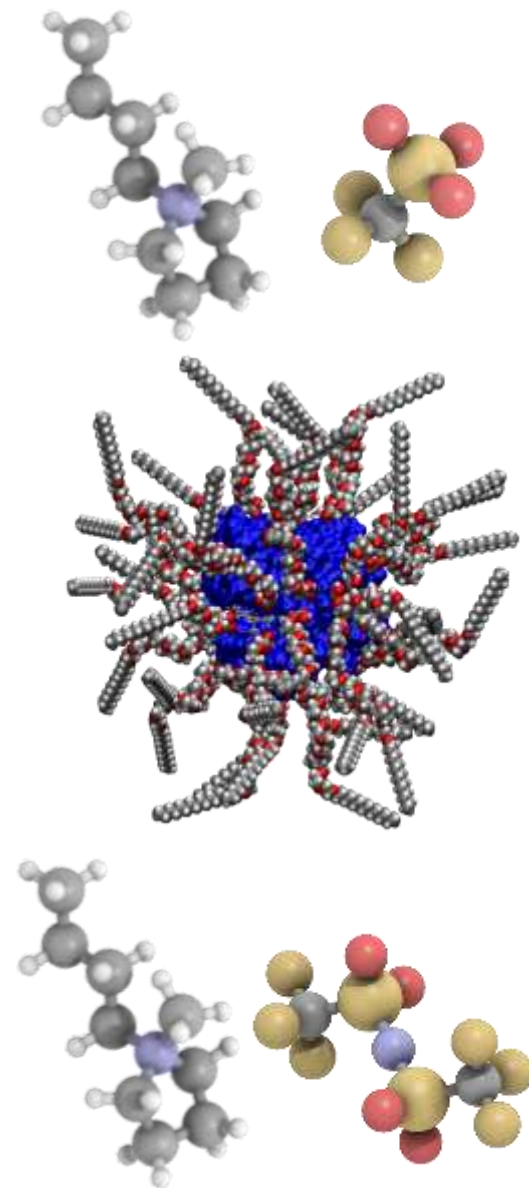




- UV/Vis shows retention of prosthetic heme in all conditions.
- SRCD indicated ionic liquids induced α -helicity
- Thermal stability of myoglobin increased significantly in ionic liquid.



- Solvent-free liquid proteins and enzymes are versatile materials with a robust synthesis.
- Good compatibility with ionic liquids.
- Protein structure highly conserved – in the absence of water.
- Thermal stability improves as compared to aqueous system.
- Promising biotechnology for potential biocatalysis in ionic liquids.



Acknowledgements

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