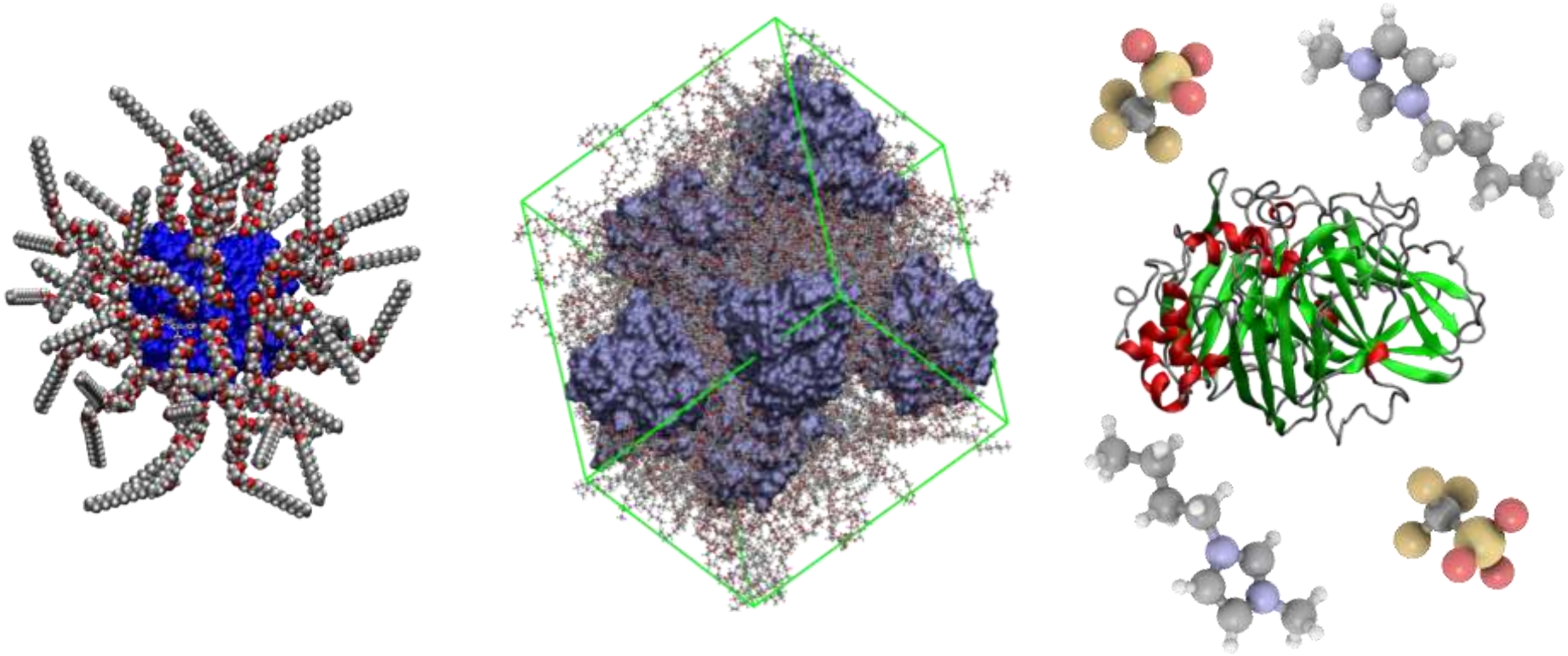
Interfacing materials with biology for non-aqueous biocatalysis



Dr. Alex Brogan

Imperial College London

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8th July 2016

MIT SynBio

Introduction

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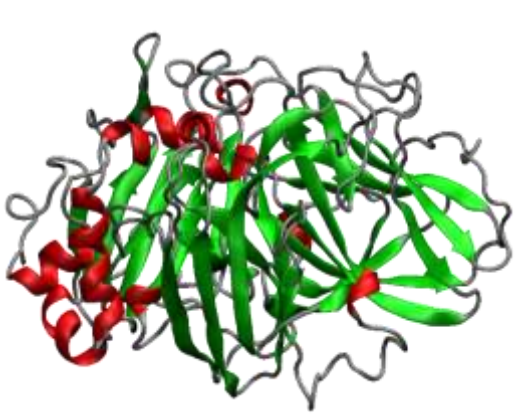
- Enzymes can catalyse many different industrial reactions.
- Ionic liquids are a promising reaction media for industry.
- Low energy, efficient routes.

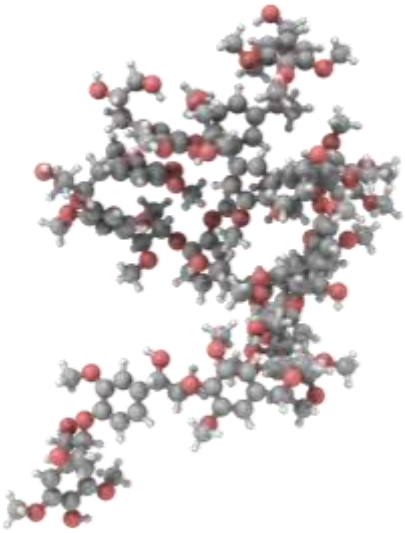
→ **Biocatalysis in ionic liquids**

- *e.g.* could take advantage of high lignocellulosic biomass solubility in ionic liquids.
- Cut out "pretreatment" and just have "treatment".
- Enzymes **insoluble** and **inactive** in common ionic liquids however…

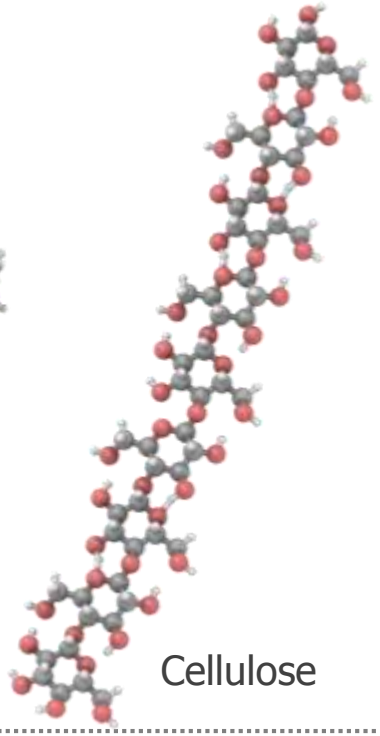


Laccase

Cellulase



Lignin



Cellulose

Solvent-free Functional Biofluids

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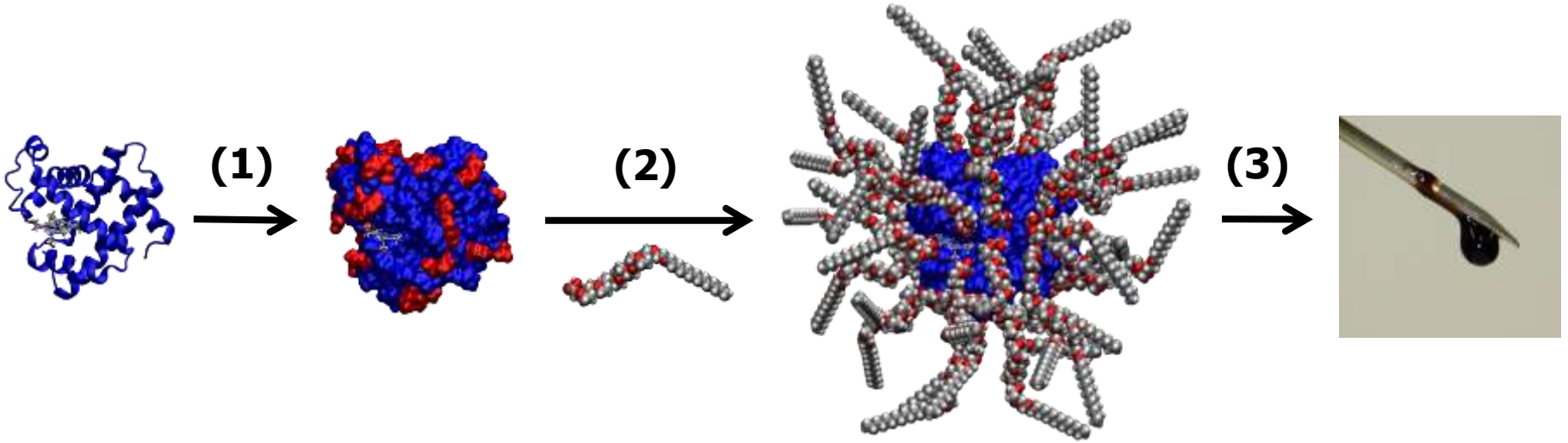
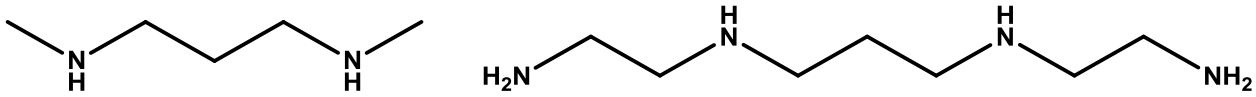
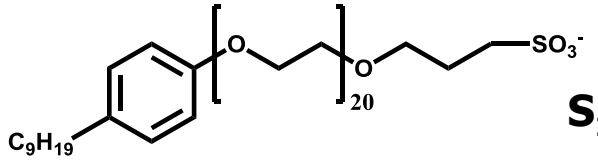


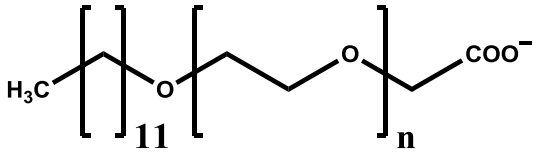
(1) Cationization of surface acidic residues with amines using the EDC reaction.



(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 form solvent-free liquid protein.

Solvent-free Liquid Myoglobin

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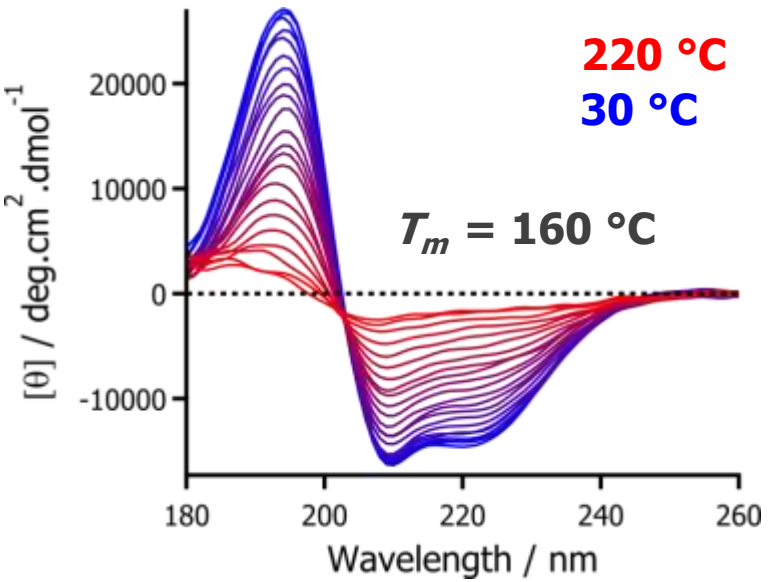
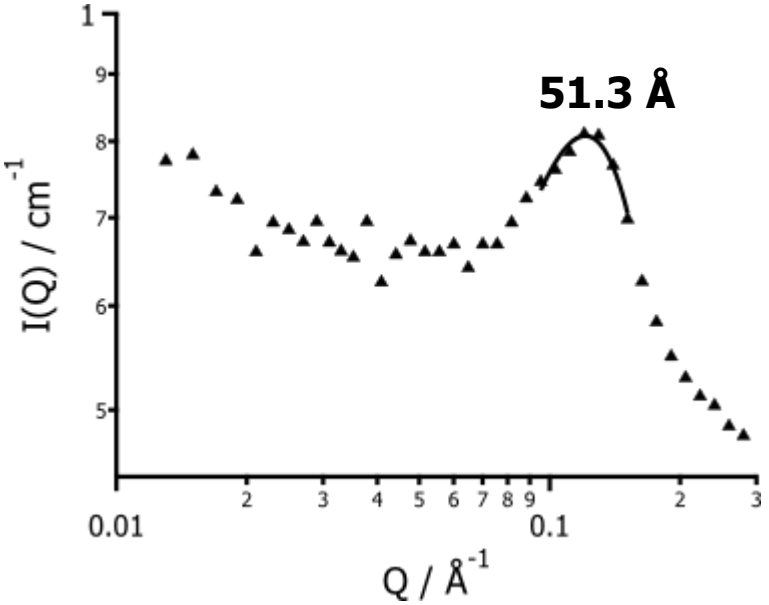
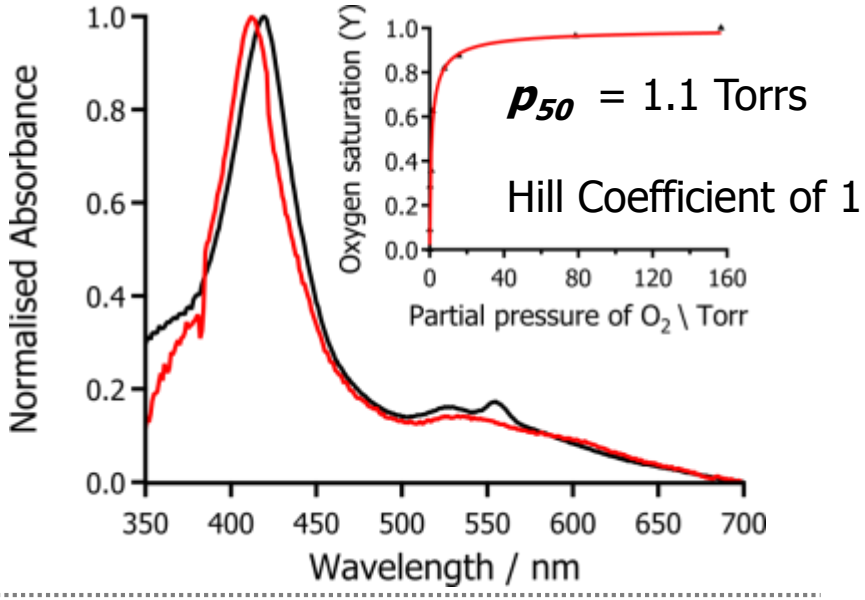




- Retained secondary structure.
- High thermal stability.
- Persistent tertiary structure.
- Biological function in absence of water.



Nat. Chem. 2010 - *Chem. Sci.* 2012

Of Dynamics and Molecular Dynamics

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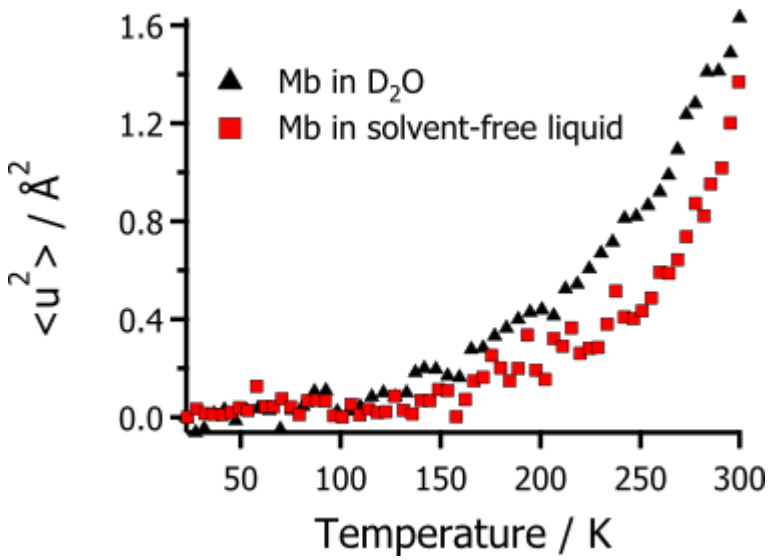
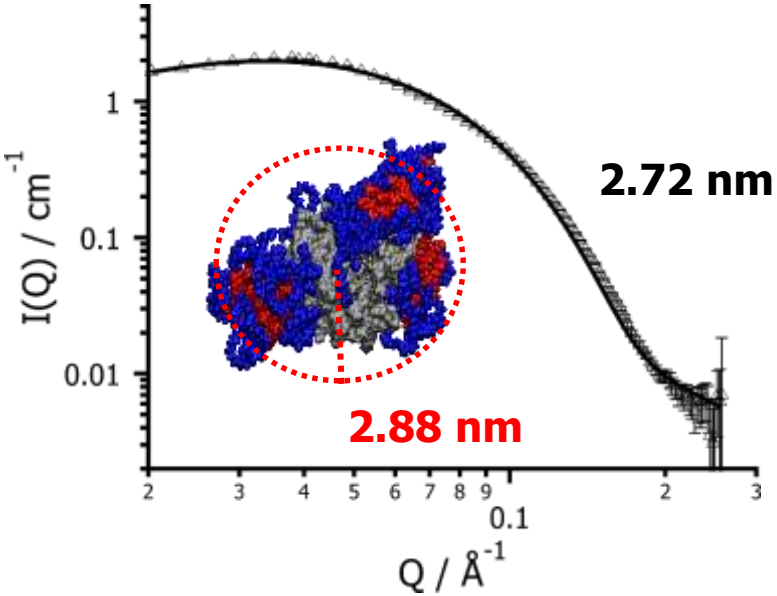
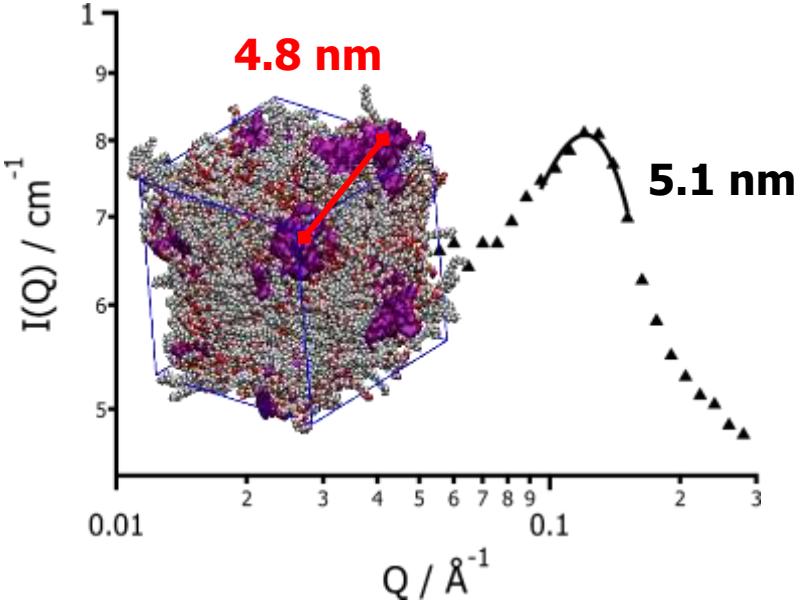




- EINS showed protein dynamics as if in water.
- Can use MD in absence of experimental atomistic data.
- Computed and measured dimensions compare well – in aqueous and solvent-free liquids.



J. Am. Chem. Soc. 2013. - *J. Am. Chem. Soc.* 2014.

Solvent-free Liquid Lysozyme

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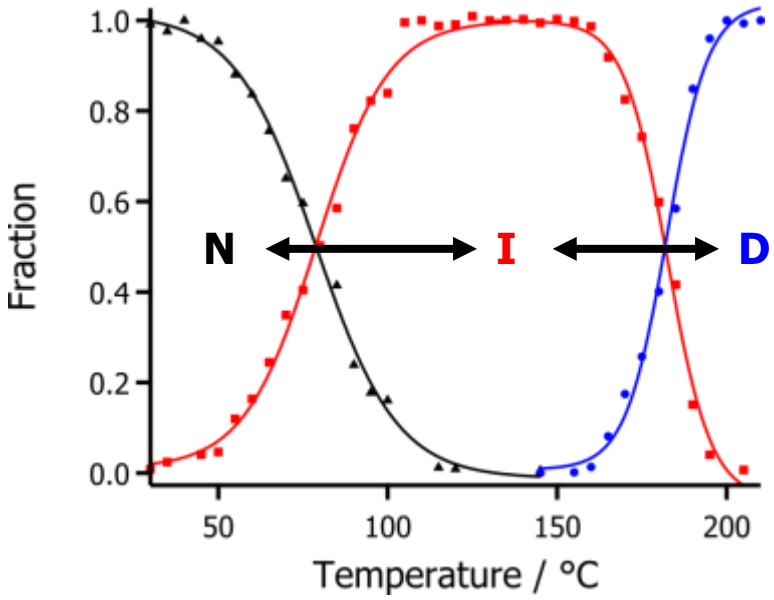
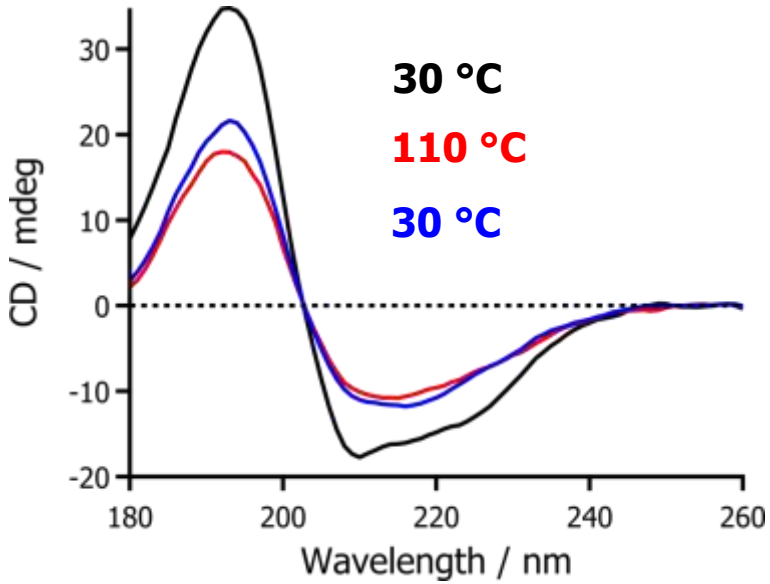
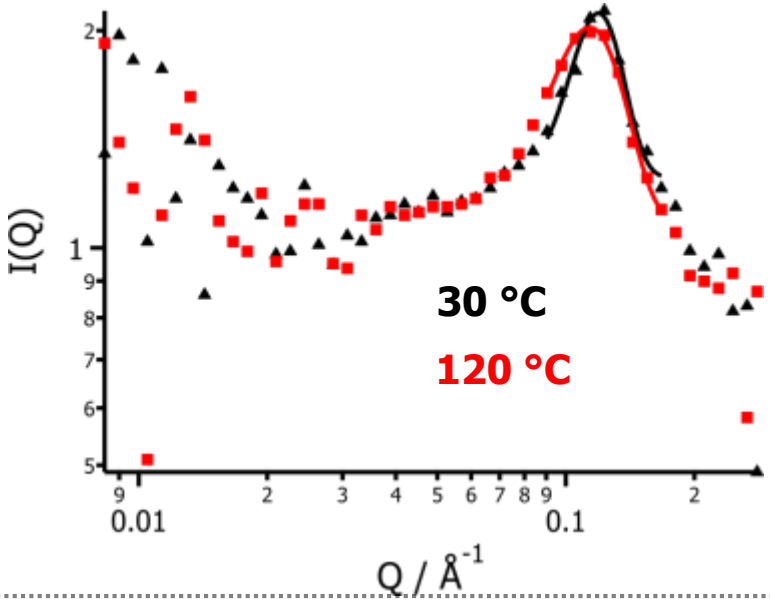




- 3-state unfolding in solvent-free liquid state.
- Intermediate shows shift to a predominately β-sheet structure.
- Intermediate stable over large temperature range.



J. Phys. Chem. B. 2013

Solvent-free Liquid Lysozyme

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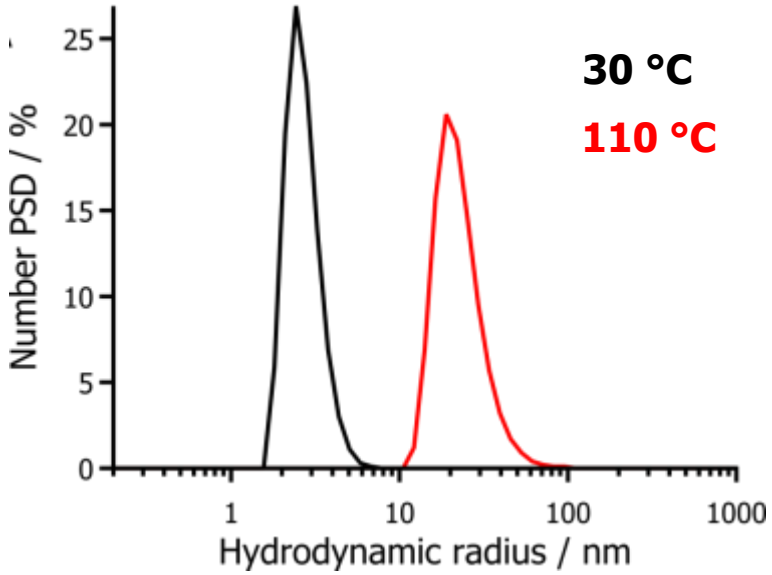
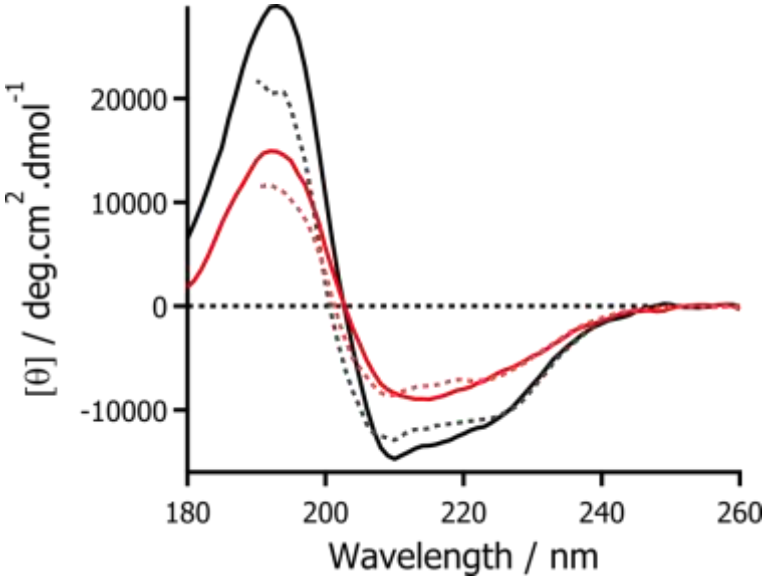
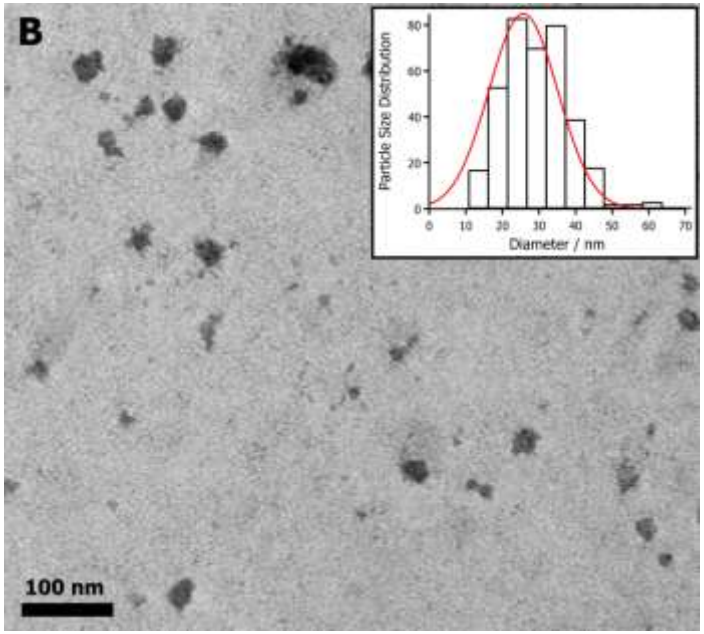




- Aggregation when intermediate introduced to water.
- β-sheet structure retained.
- Reactive intermediate of lysozyme isolated in solvent-free liquid.



J. Phys. Chem. B. 2013

Lipase

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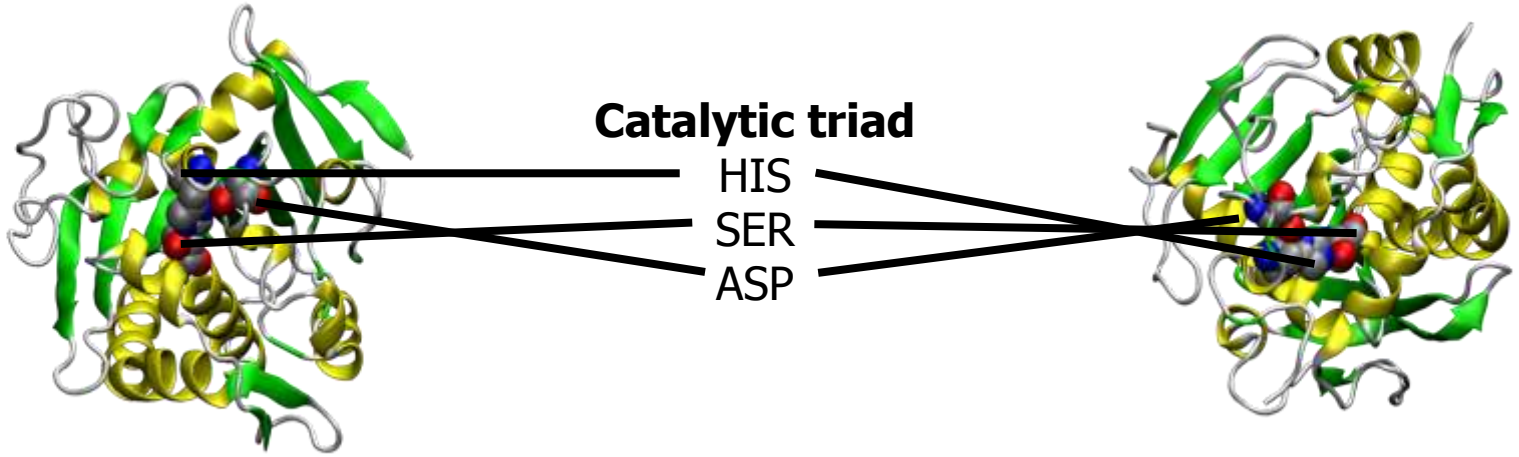
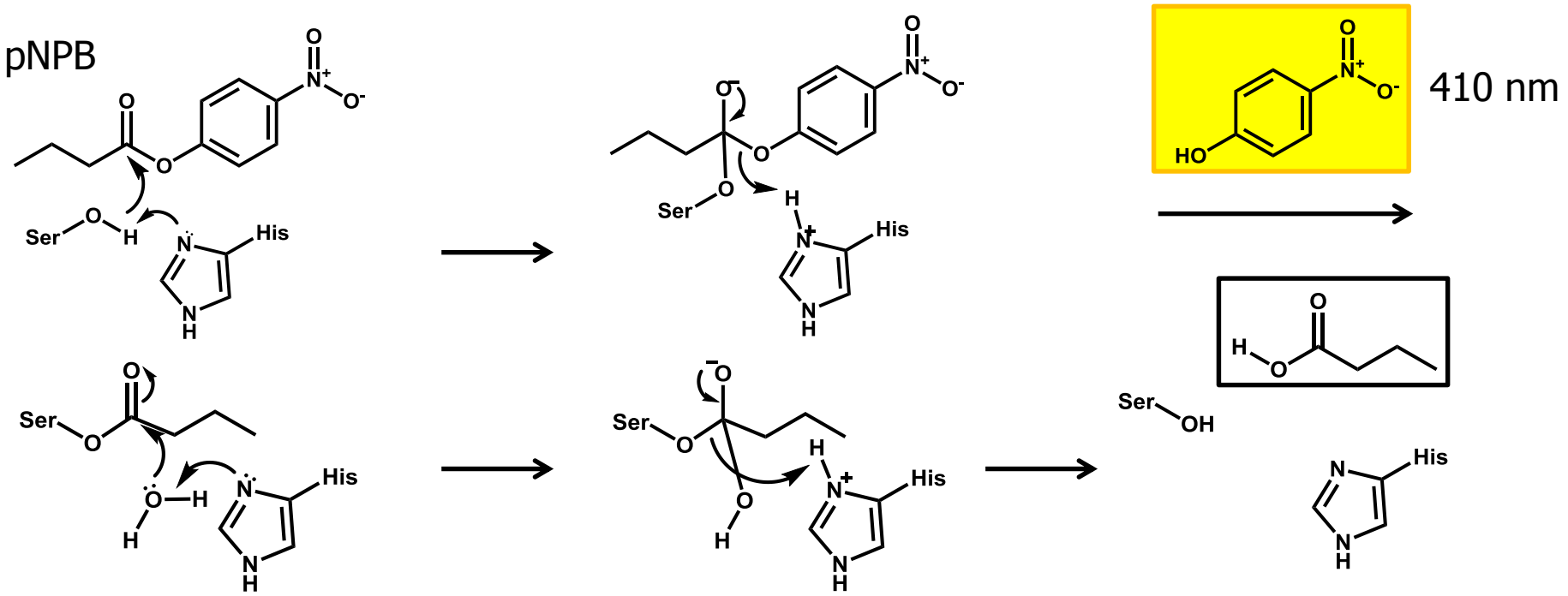
Rhizomucor miehei (RML)

Thermomyces lanuginosus (TLL)





Solvent-free Liquid Lipase

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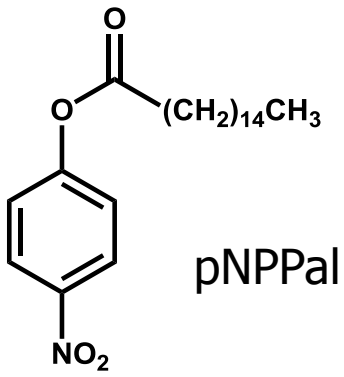
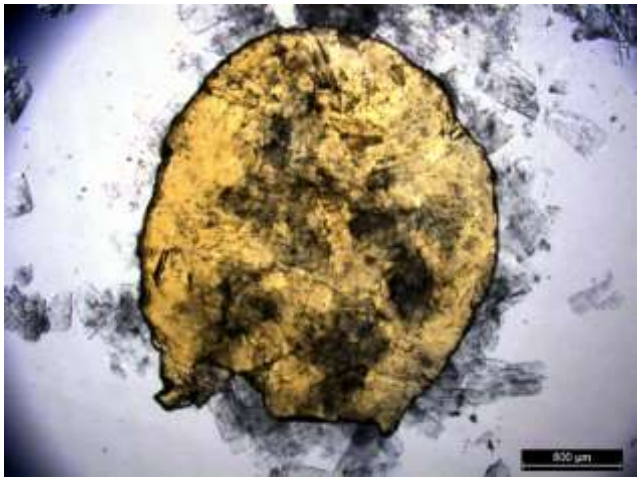
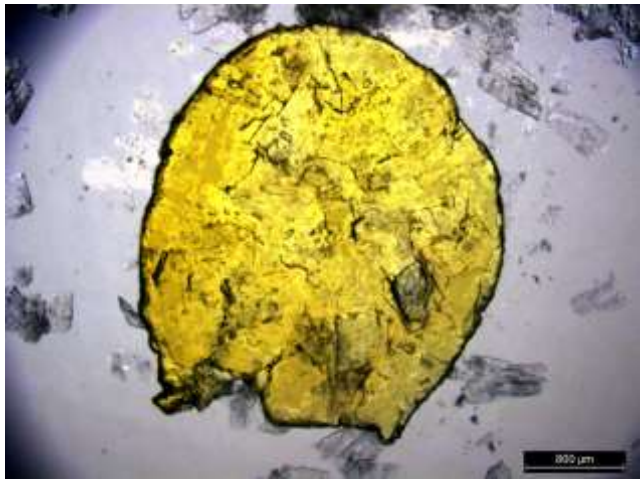
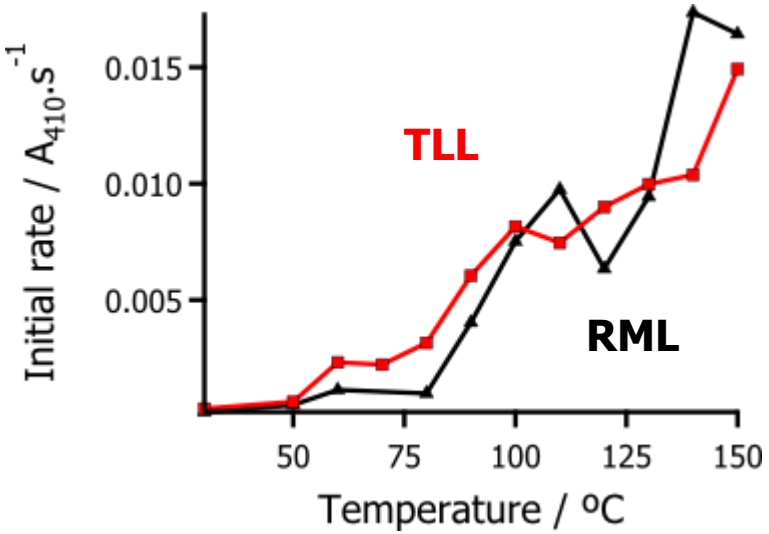


pNPPal





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



Nat. Commun. 2014

Solvent-free Biofluids and Ionic Liquids

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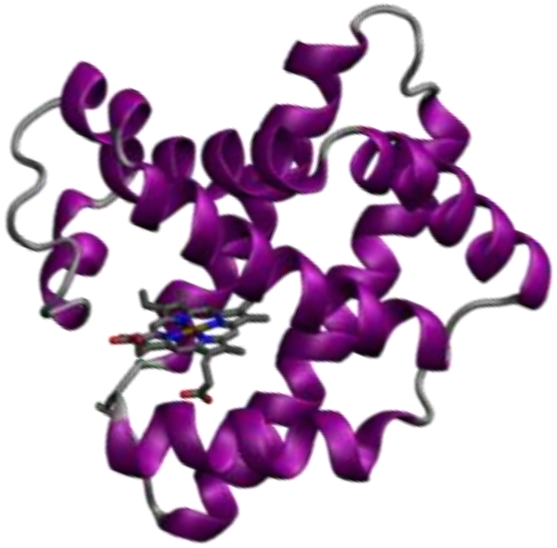
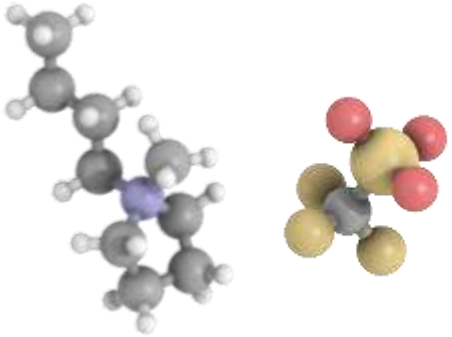
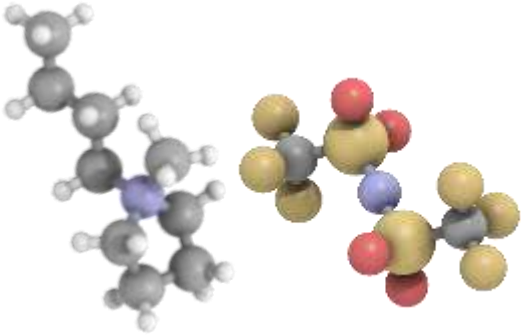


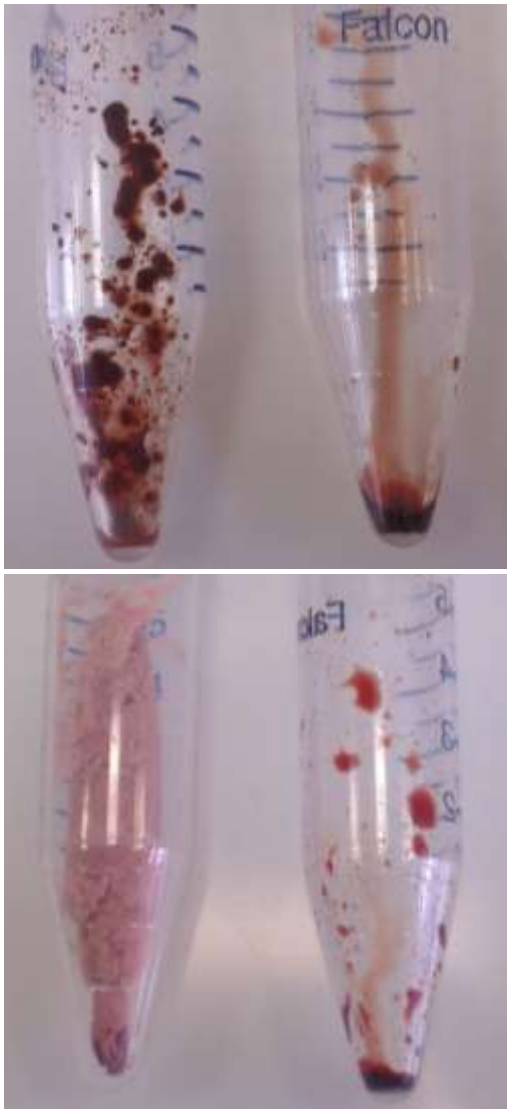


[bmpyrr][OTf]



[bmpyrr][NTf_2]

Mb **[C-Mb][S_2]**



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

Protein structure in Ionic Liquids

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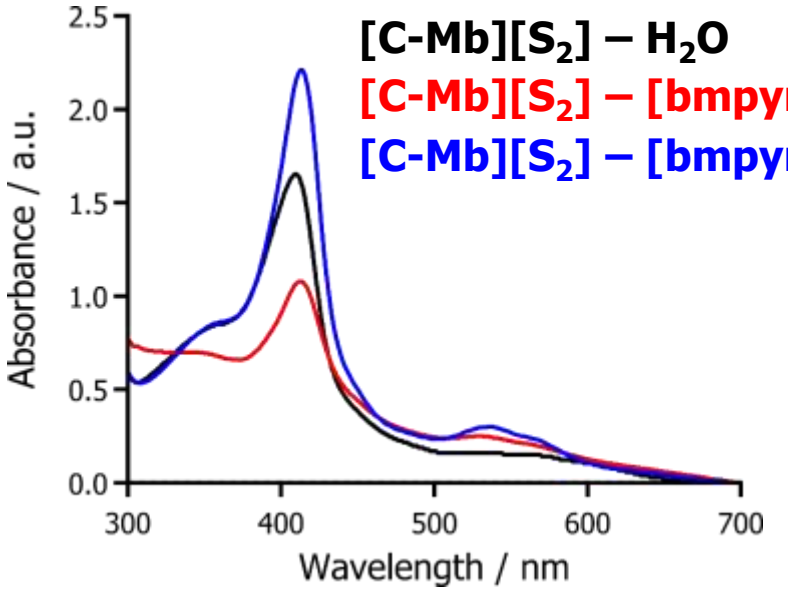
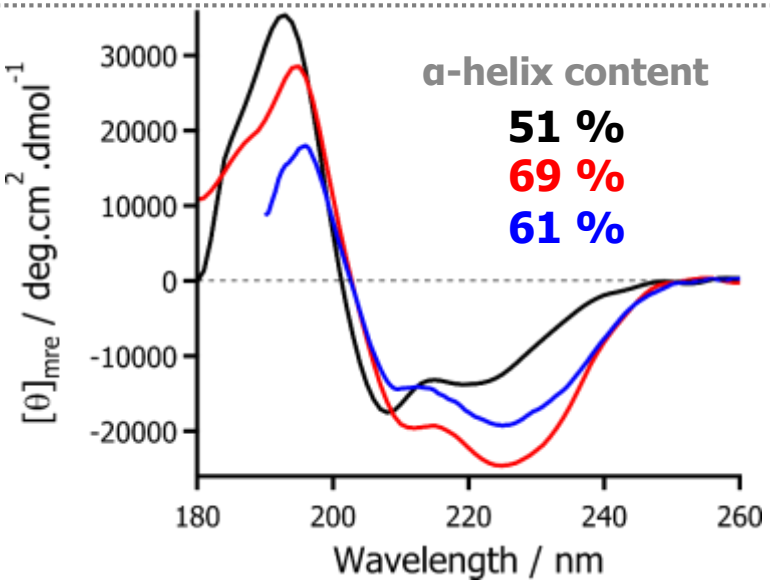
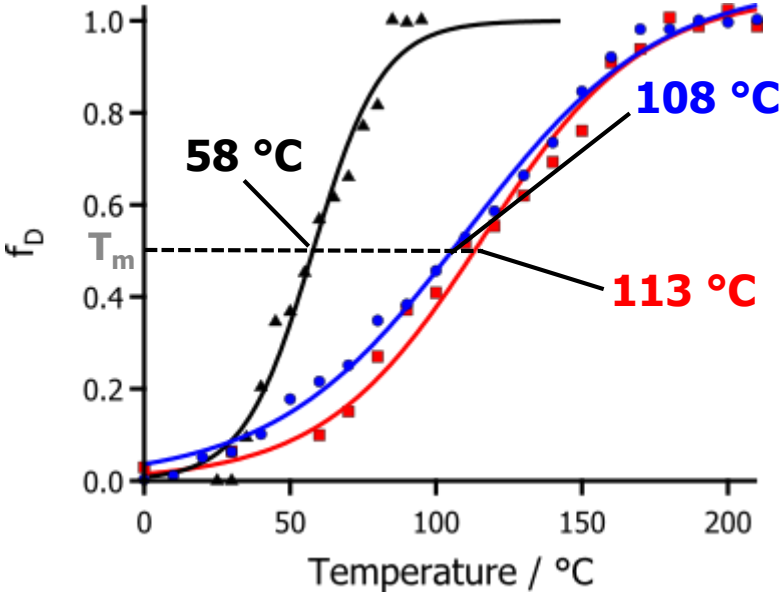




- UV/Vis shows retention of prosthetic heme in all conditions.
- SRCD indicated ionic liquids induced α-helicity
- Thermal stability of myoglobin increased significantly in ionic liquid.



J. Am. Chem. Soc. 2016.

Conclusions

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- Solvent-free liquid proteins and enzymes are versatile materials with a robust synthesis.
- Unique materials of stoichiometric protein-surfactant conjugates that have a liquid phase.
- Can design new functional biofluids with enhanced properties.
  - ❑ Hyperthermophilic-like stability
  - ❑ Stabilization of reactive intermediates
  - ❑ High temperature enzyme activity
- Good compatibility with ionic liquids.
- Increased protein structure and thermal stability compared to aqueous system.
- Promising biotechnology for potential bio-catalysis in ionic liquids.