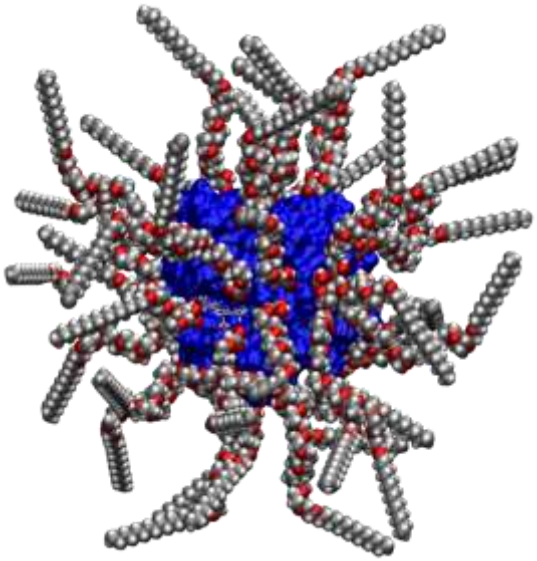
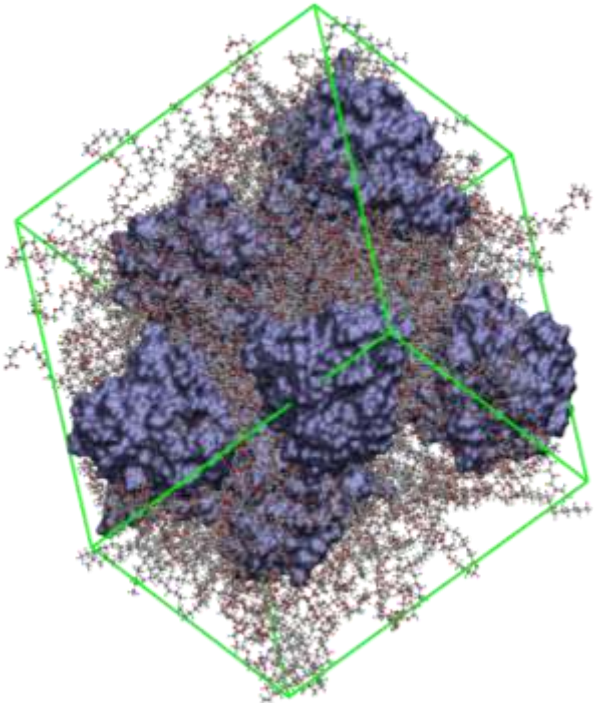
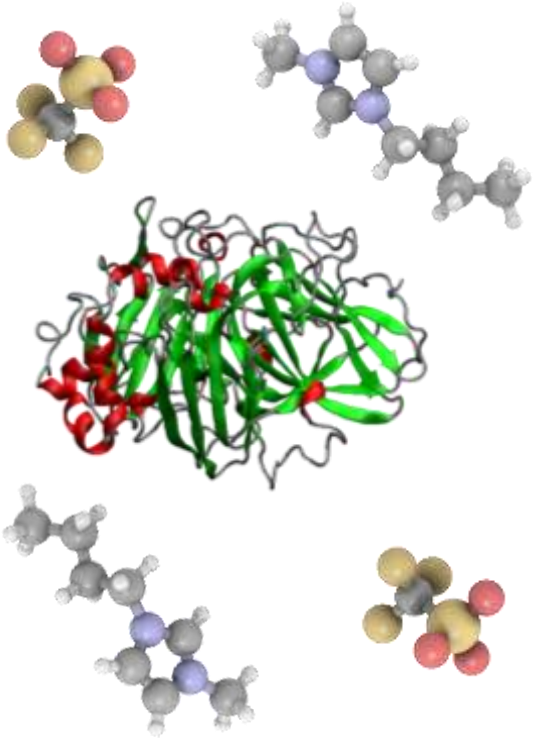
Designing Materials to Interface Biological Systems with Non-Natural Environments







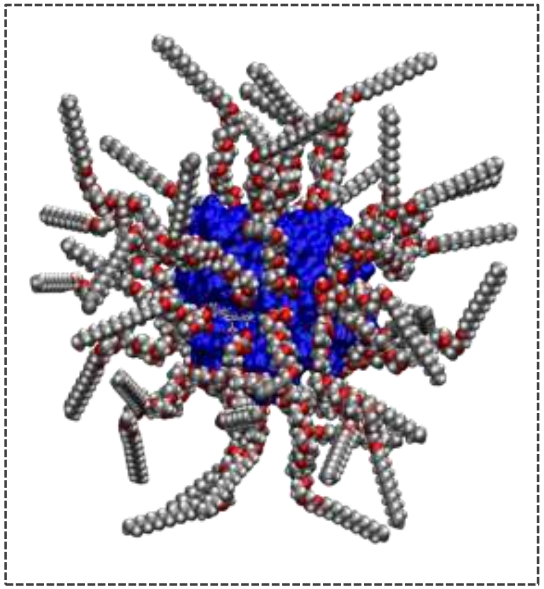
Alex P. S. Brogan
Imperial College London

6th June 2018
King's College Chemistry Seminar
alexbrogan.co.uk/kcl

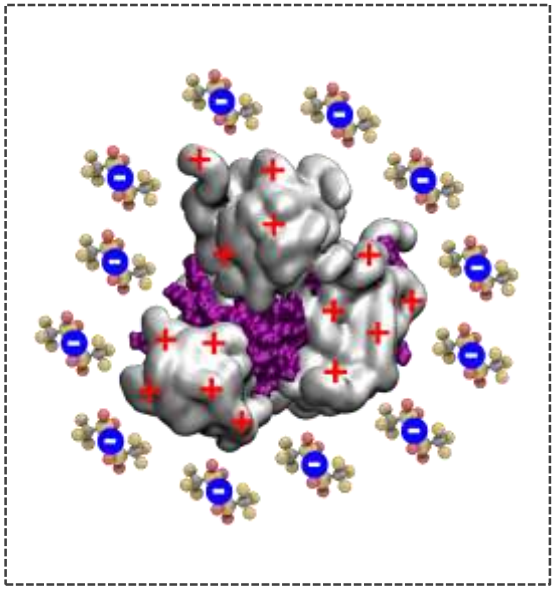
Materials

Alex Brogan

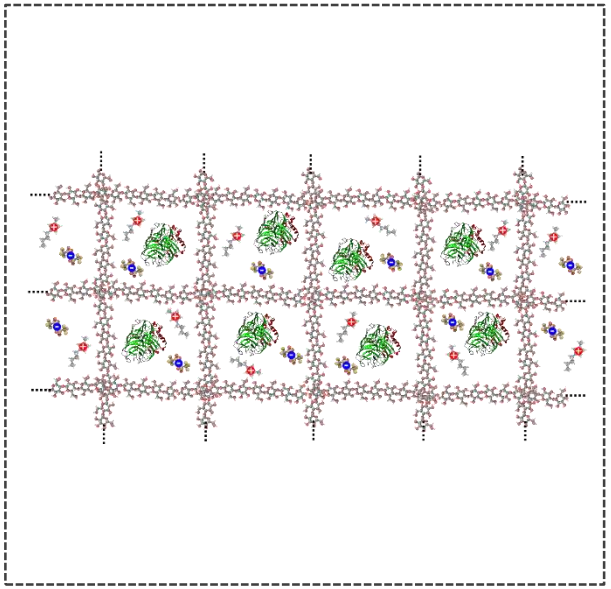
Solvent-free Enzyme Biofluids



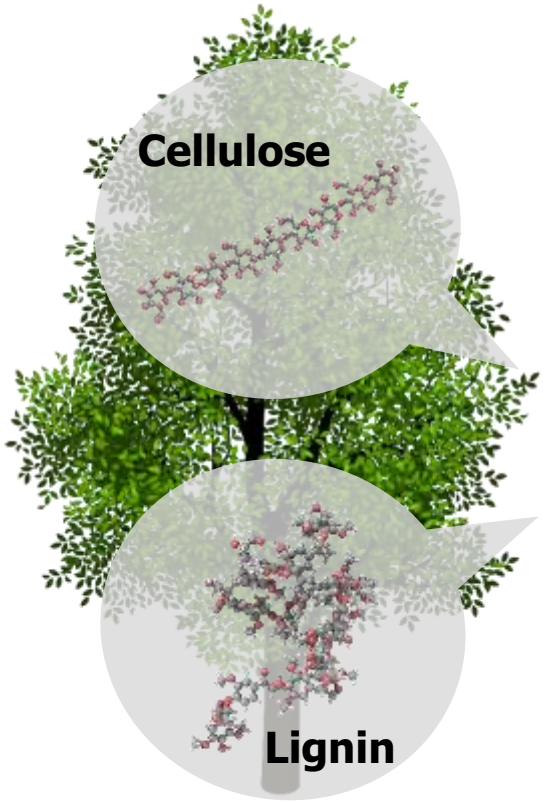
Ionic Liquid Proteins

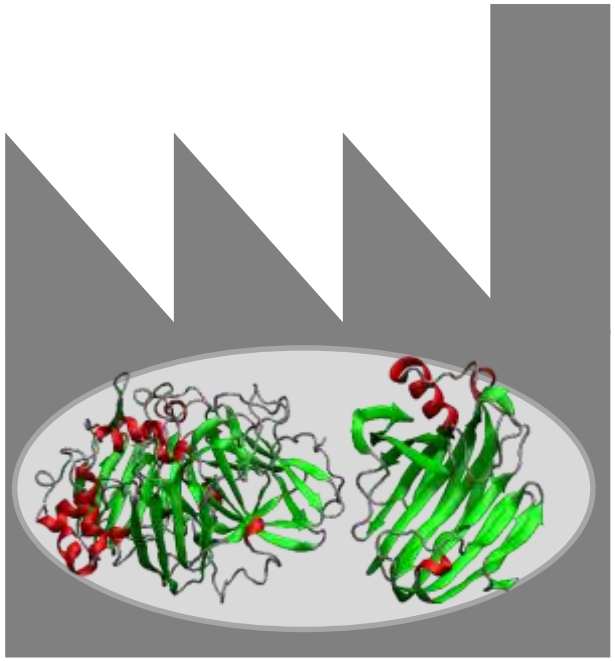


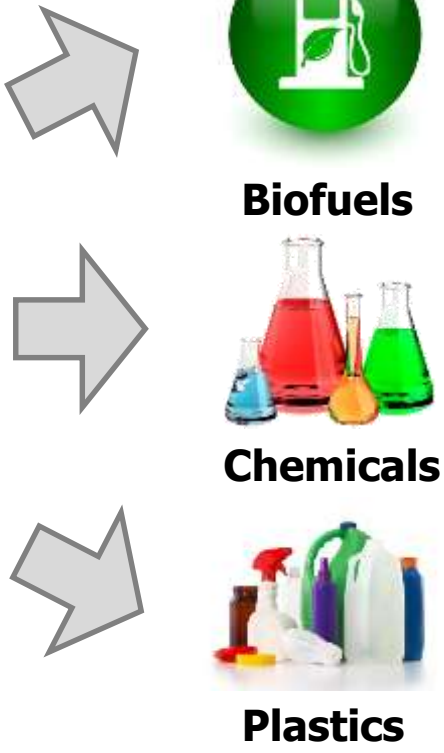
Ionogels



Primary Motivation







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

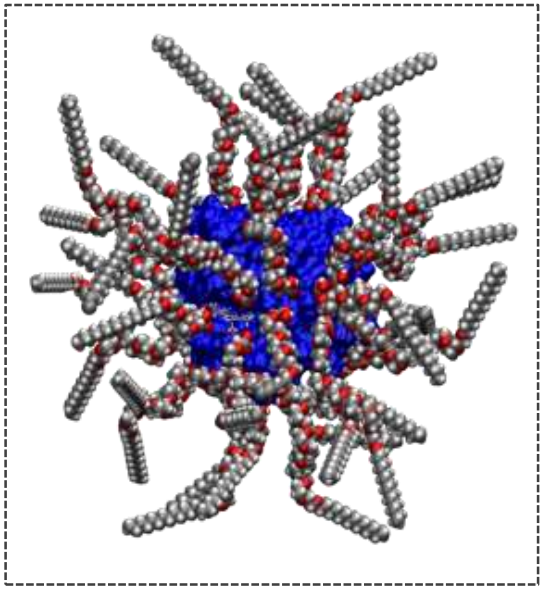
→ **Biocatalysis in ionic liquids**

- **Enzymes** can catalyse many different industrial reactions.
- Enzymes **insoluble** and **inactive** in common ionic liquids…

Pathways

Alex Brogan

Solvent-free Enzyme Biofluids

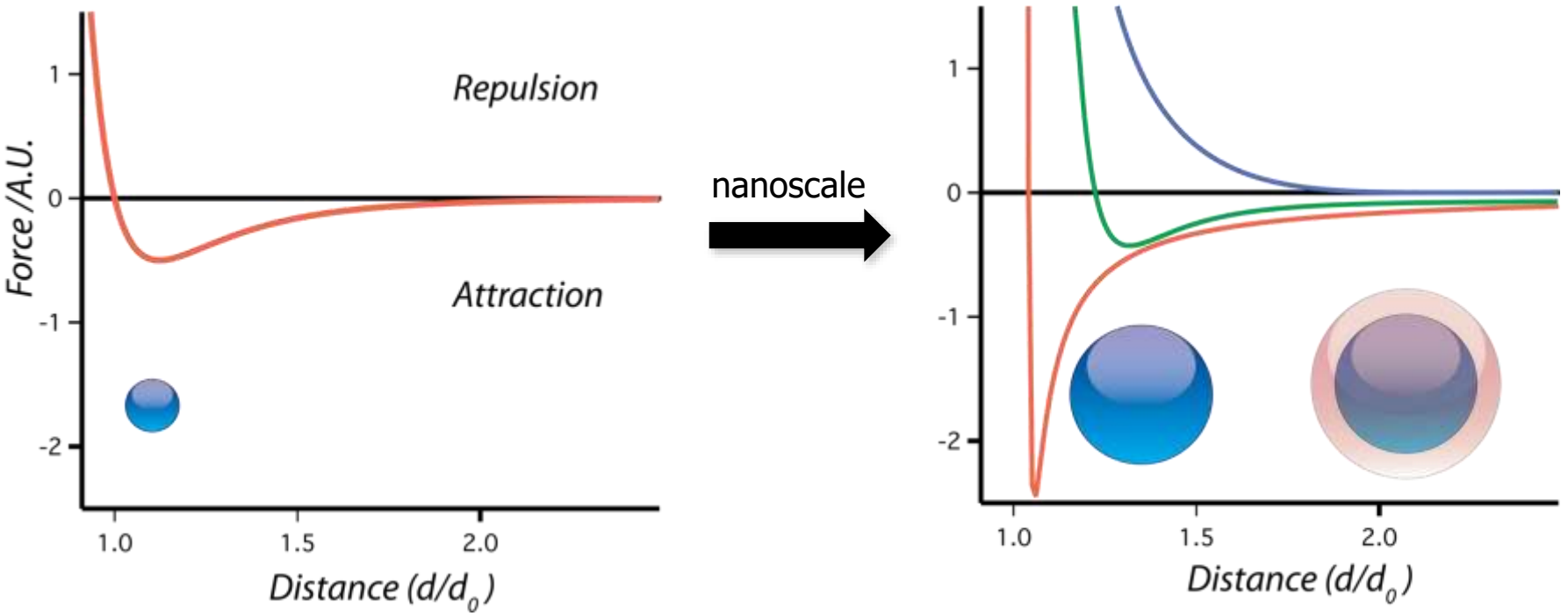


Ionic Liquid Proteins

Ionogels

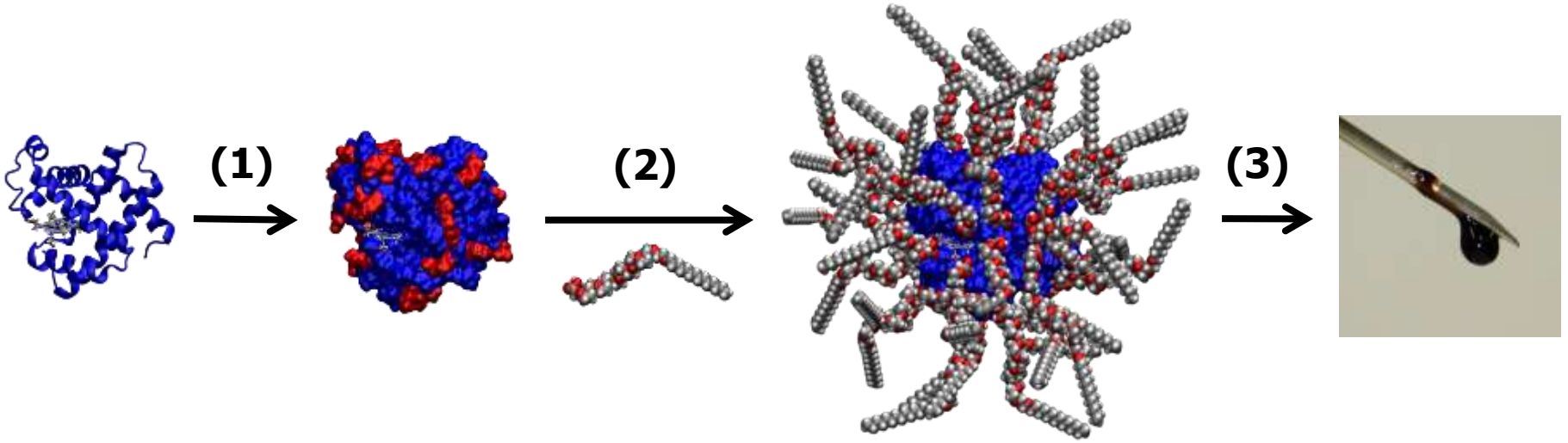
Solubilize and stabilize enzymes in ionic liquids

Nanoscale Liquids

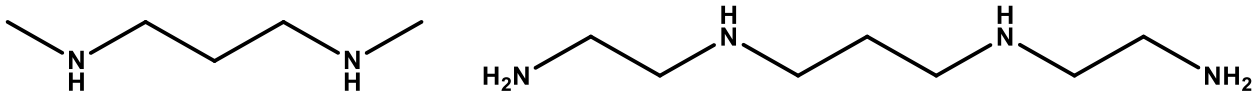


- Nanoscale objects do not have a liquid phase.
- Interparticle interactions need to be extended.

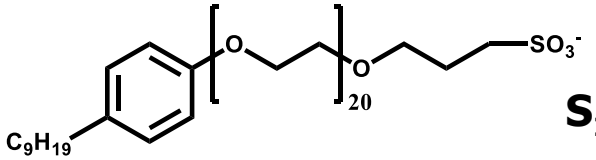
Solvent-free Enzyme Biofluids



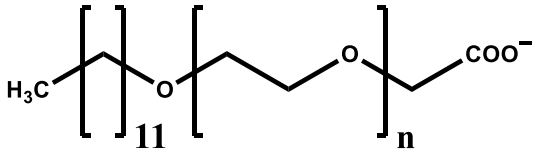
(1) Cationization of surface acidic residues with amines using the EDC reaction.



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



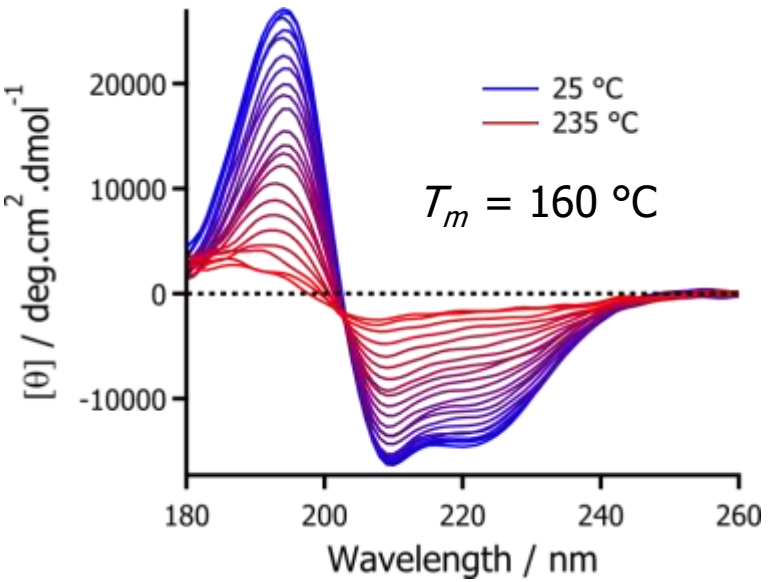
S$_1$

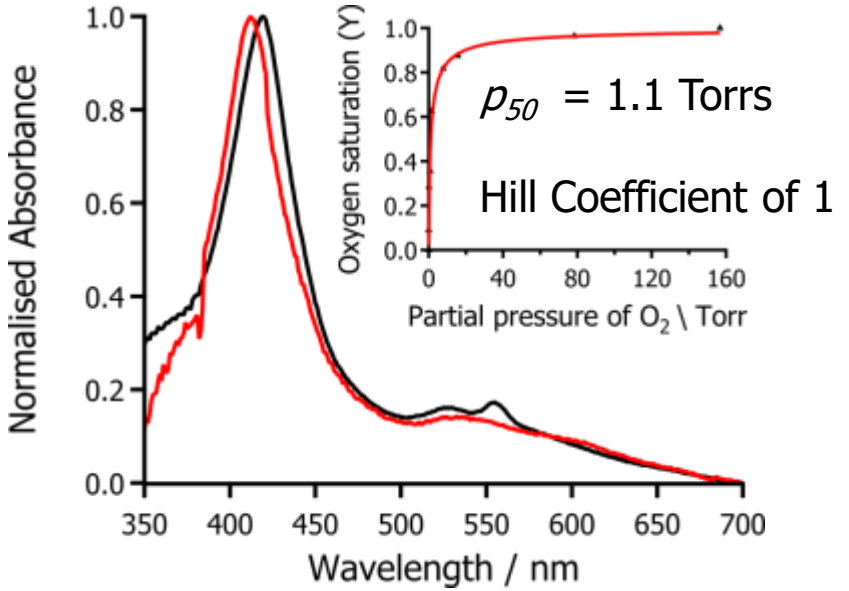


S$_2$ (n = 11)

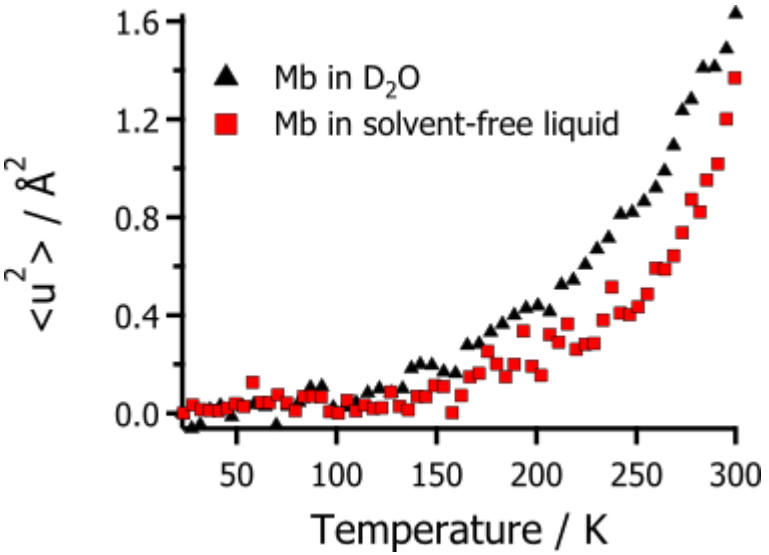
(3) Lyophilization of conjugate, followed by annealing at 60 °C to form solvent-free liquid protein.

Solvent-free Liquid Myoglobin





- Retained structure and hyperthermophilic-like stability.
- Biological function in the absence of water.
- Protein dynamics retained within organic corona.



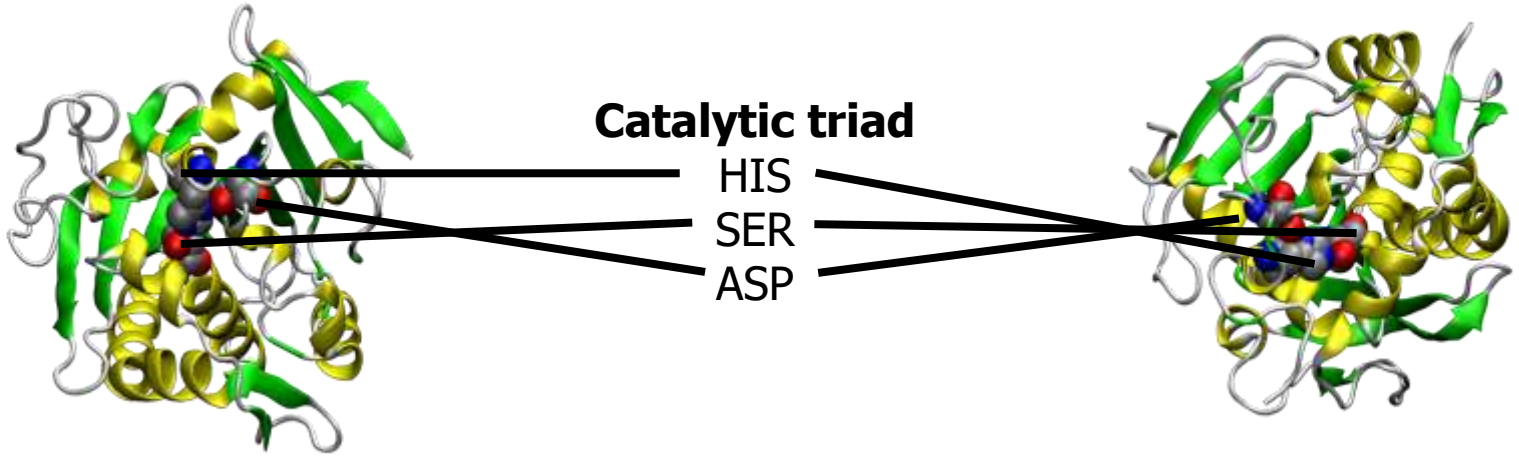
Nat. Chem. 2010 - *Chem. Sci.* 2012 – *J. Am. Chem. Soc.* 2012

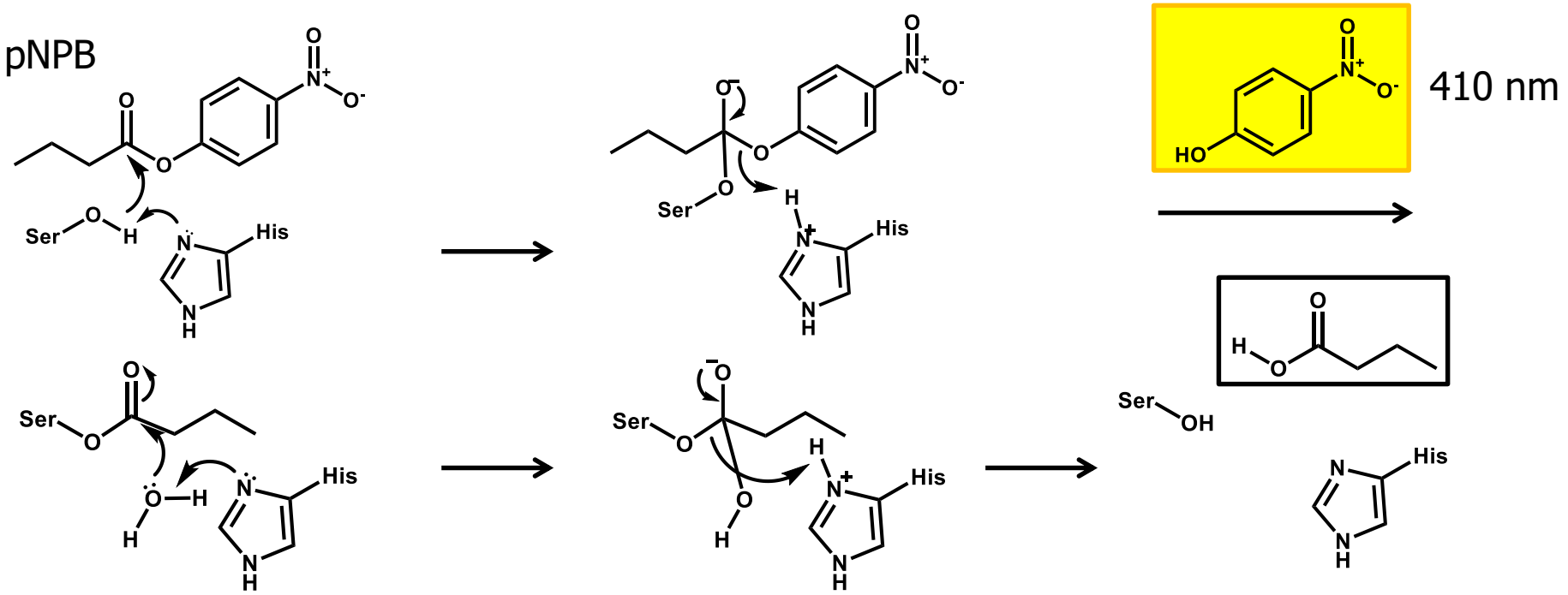
Lipase

Alex Brogan

Rhizomucor miehei (RML)

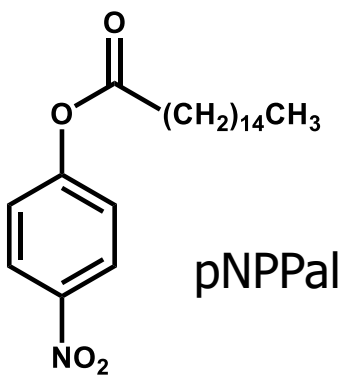
Thermomyces lanuginosus (TLL)



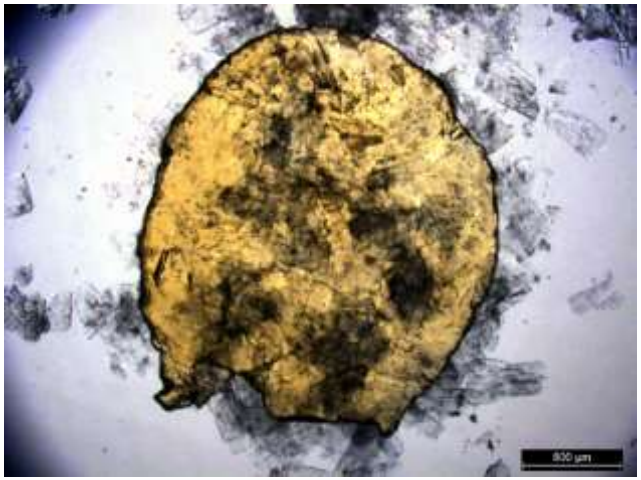


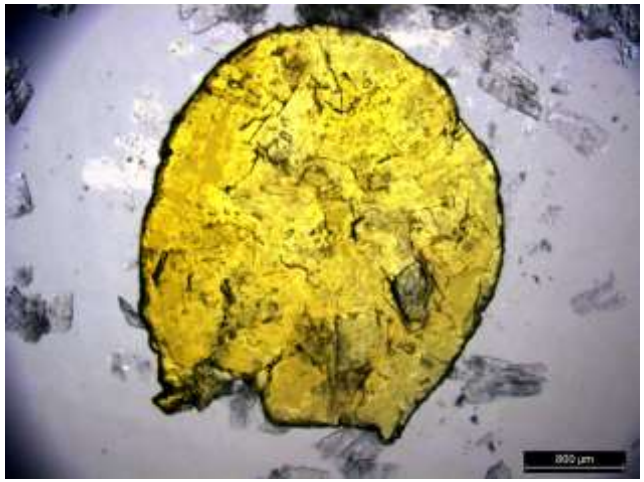
Nat. Commun. 2014

Solvent-free Liquid Lipase

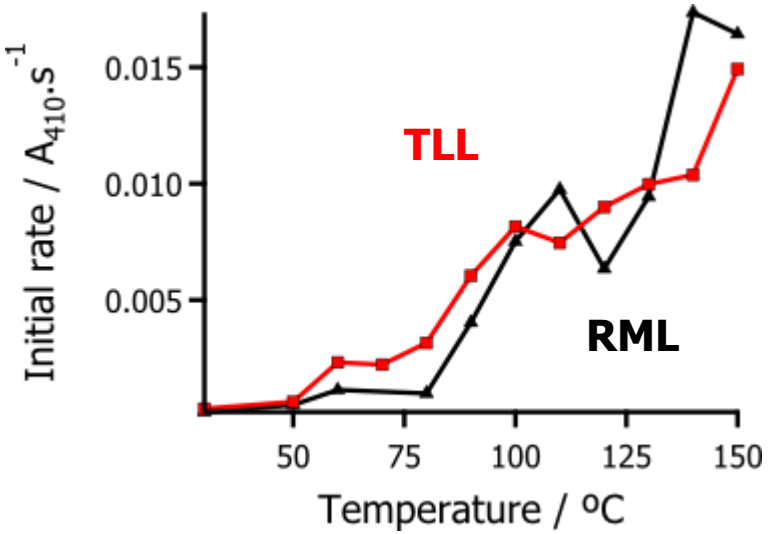


pNPPal





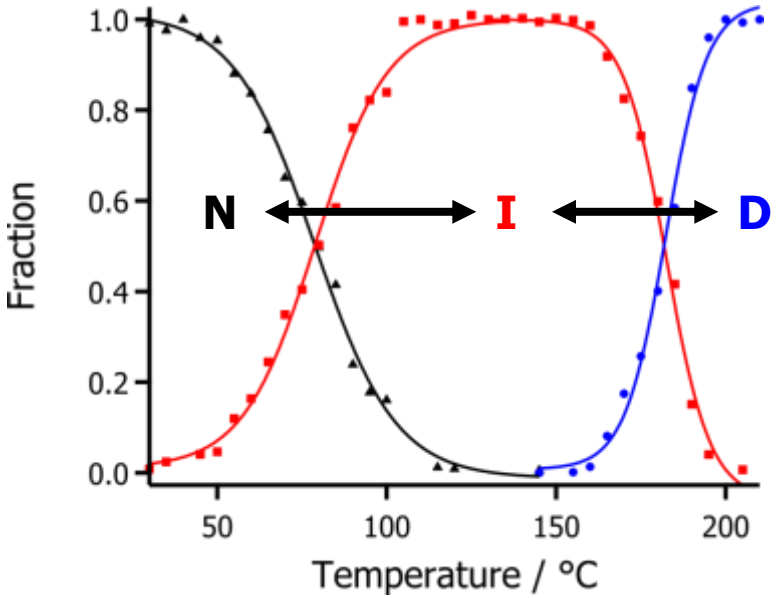
- Delivery of both liquid and solid substrates to enzyme active site.
- Enzyme activity in absence of water.
- Enhanced enzyme activity up to 150 °C

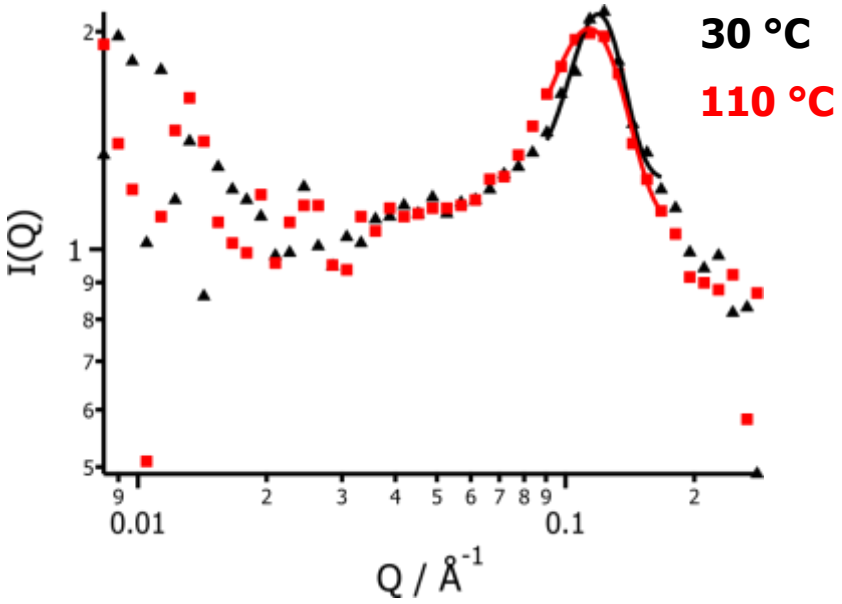


Nat. Commun. 2014

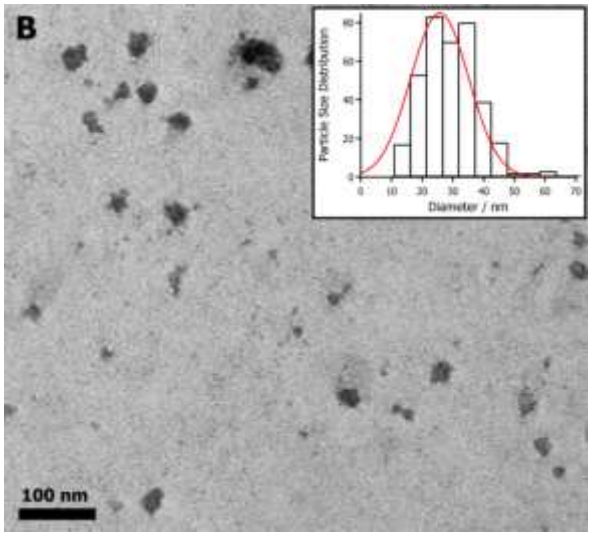
The strange case of lysozyme

Alex Brogan

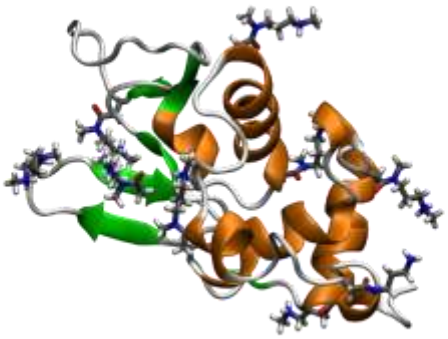




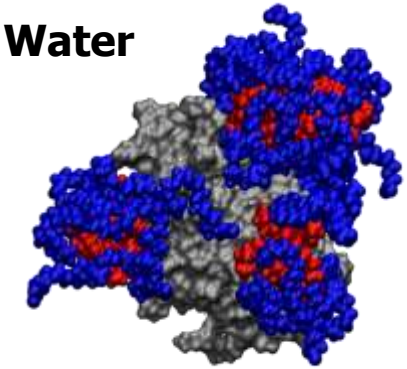
- Switch from 2-state unfolding to 3-state unfolding in solvent-free liquid state.
- Intermediate stable over large temperature range - no sign of aggregation…
- … Until introduced to water.
- Stabilization of reactive intermediate.



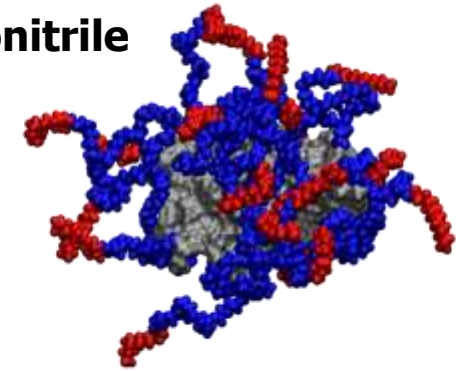
Molecular Dynamics Elucidates Structure



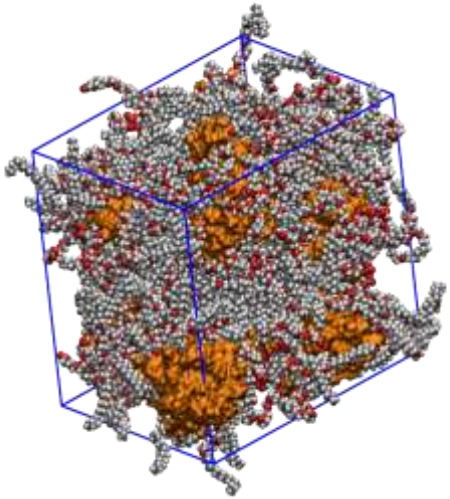
Water



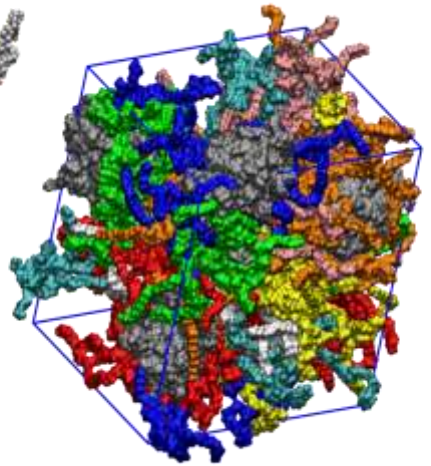
Acetonitrile



- Molecular dynamics simulations from aqueous to solvent-free state (≥100 ns).
- Observe surfactant corona behaviour in water and organic solvent.
- Discrete conjugates in solvent-free liquid with some interdigitation.
- Consistent with SANS measurements.

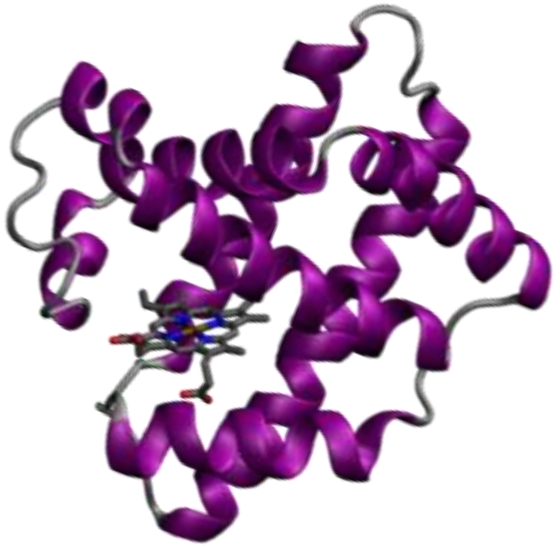


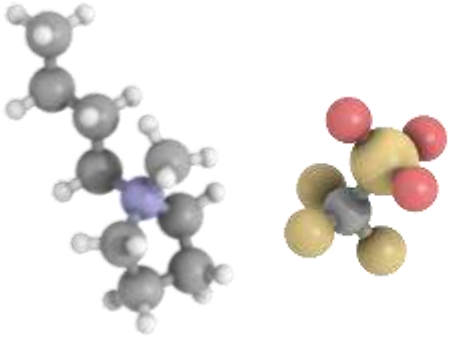
Solvent-free



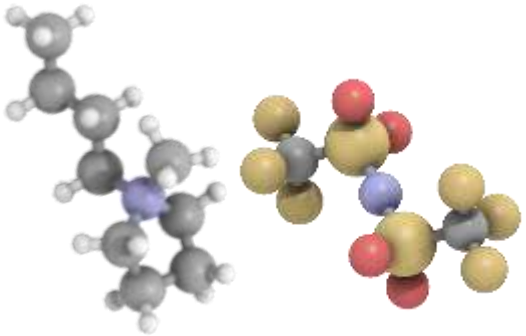
J. Am. Chem. Soc. 2014

Solvent-free Biofluids and Ionic Liquids





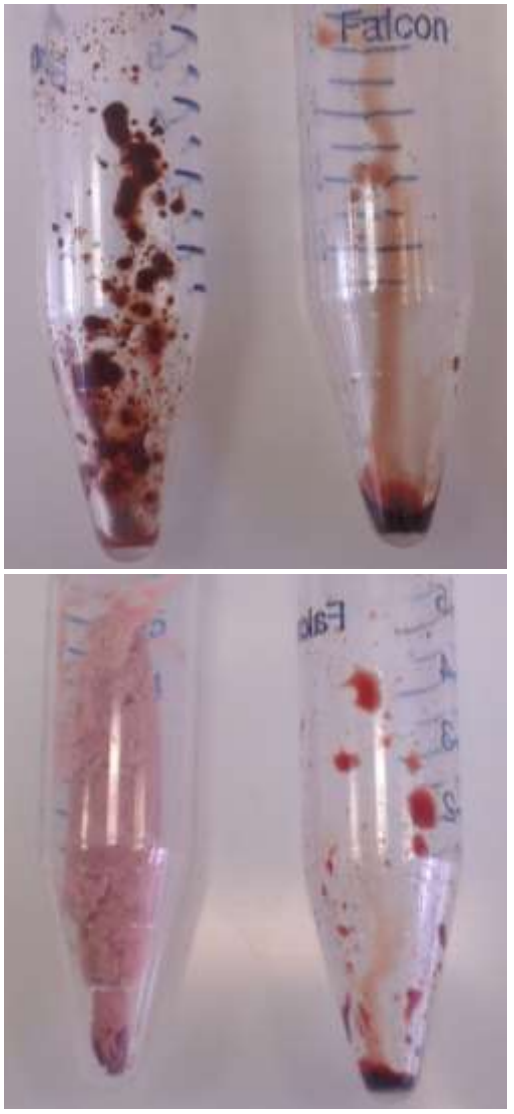
[bmpyrr][OTf]
HYDROPHILIC



[bmpyrr][NTf_2]
HYDROPHOBIC

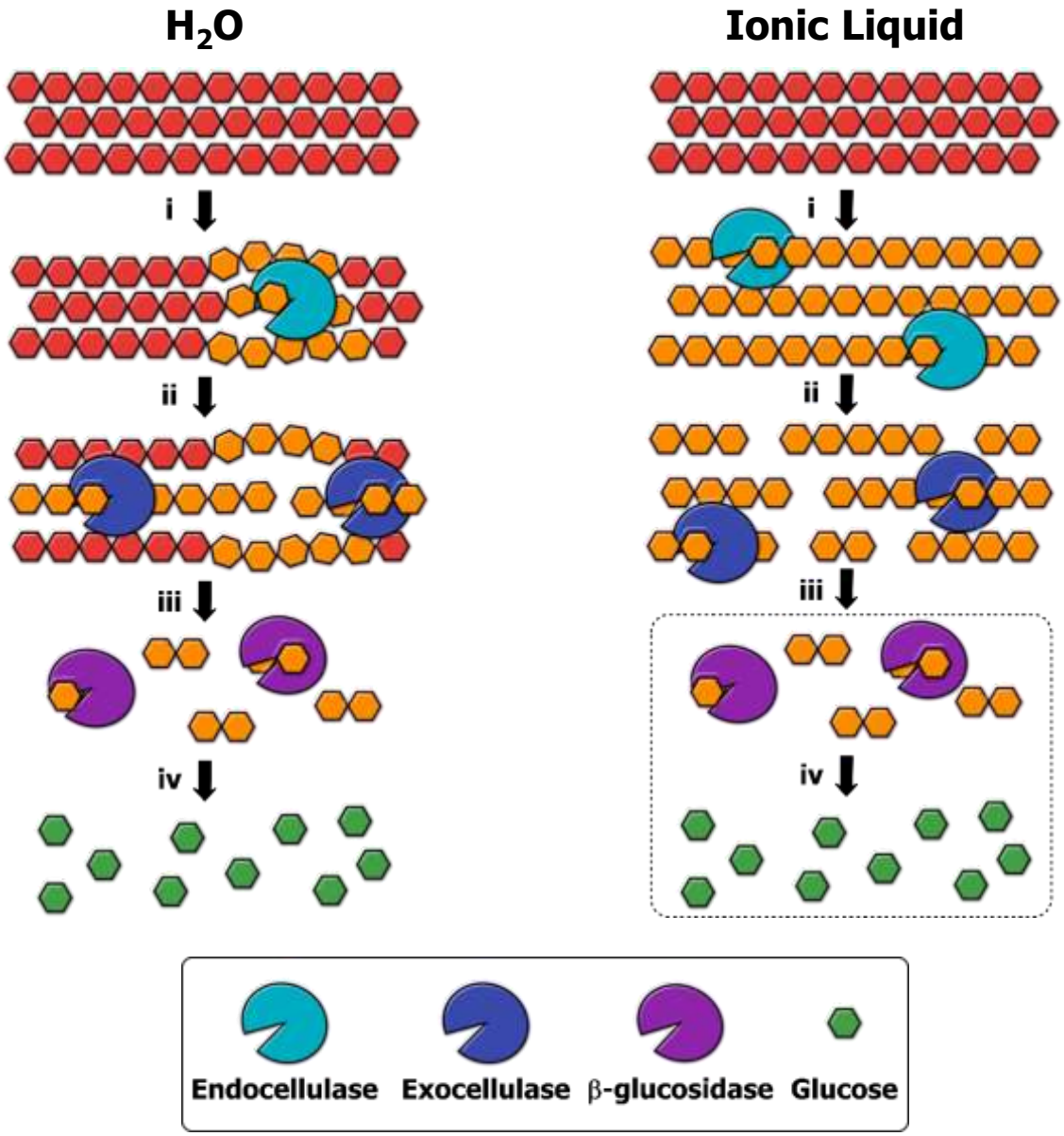
- Myoglobin as archetypal system.
- Well characterized – sensitive to environment.
- Biofluids have significant increase in IL mixing.

Mb **[C-Mb][S]**



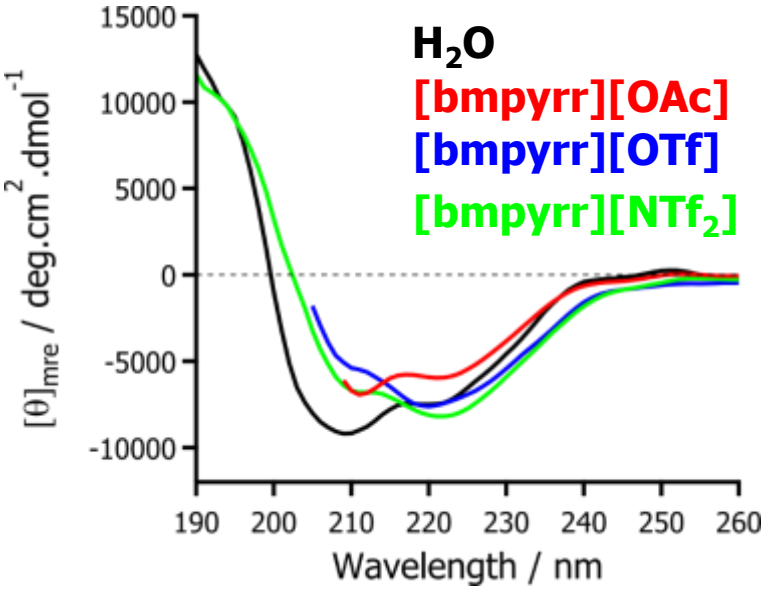
J. Am. Chem. Soc. 2016.

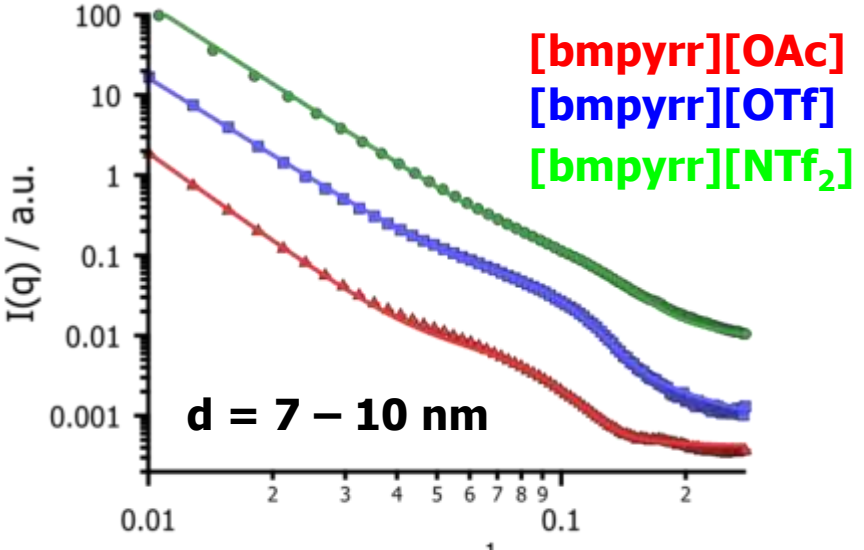
Glucosidase in Cellulase Cocktails



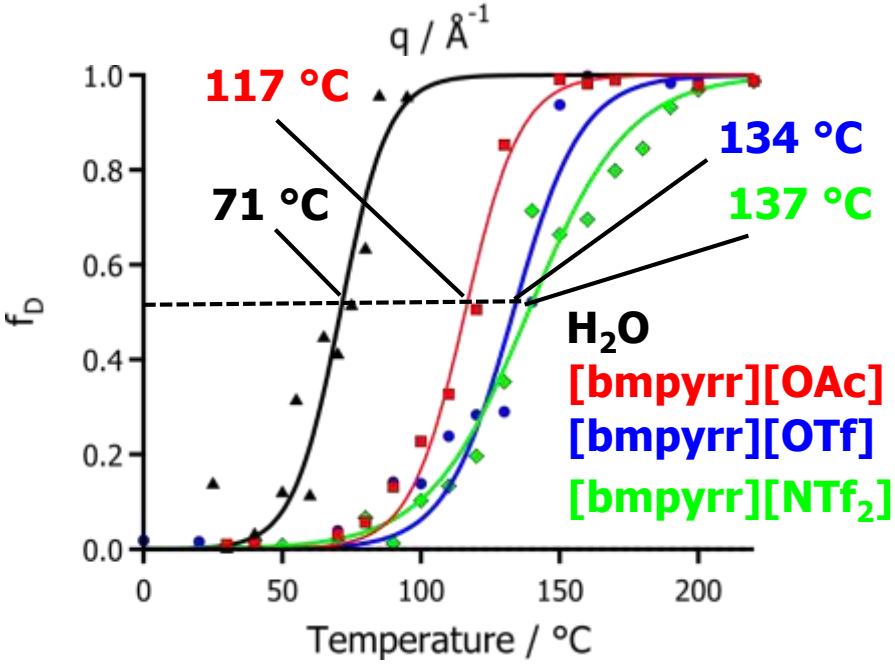
Nat. Chem. 2018

Glucosidase Biofluids and Ionic Liquids



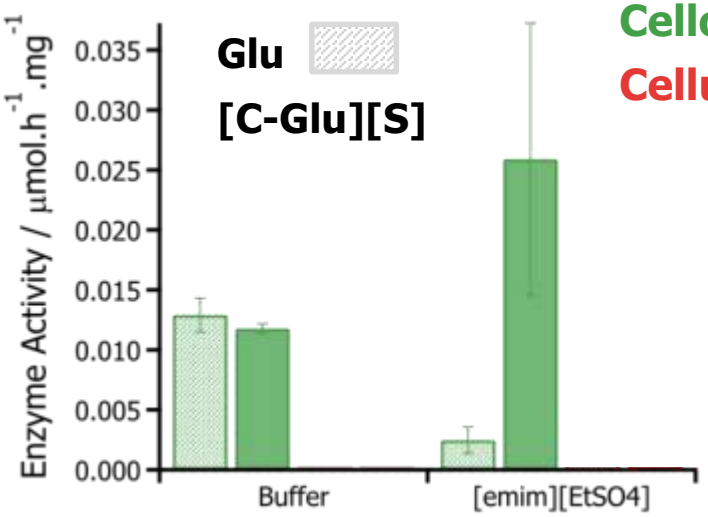


- Surface functionalization of glucosidsase increases solubility in ionic liquids.
- Structure maintained (SRCD, SAXS)
- Highly stable in ionic liquids.



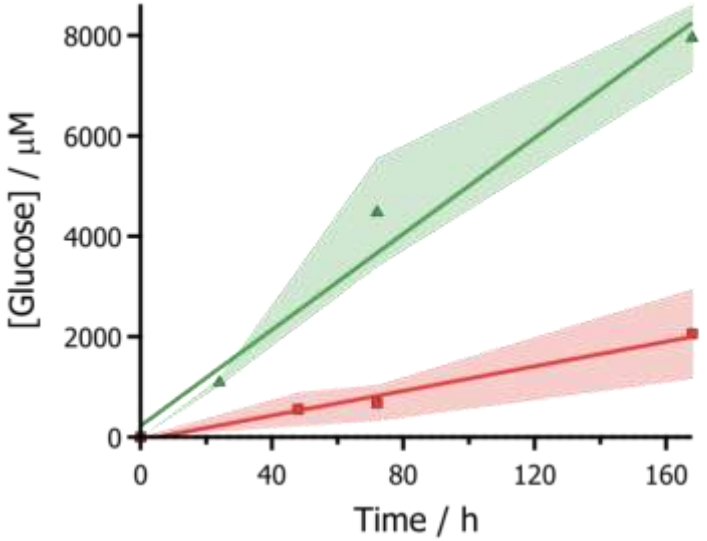
Nat. Chem. 2018

Glucosidase Activity in Ionic Liquids

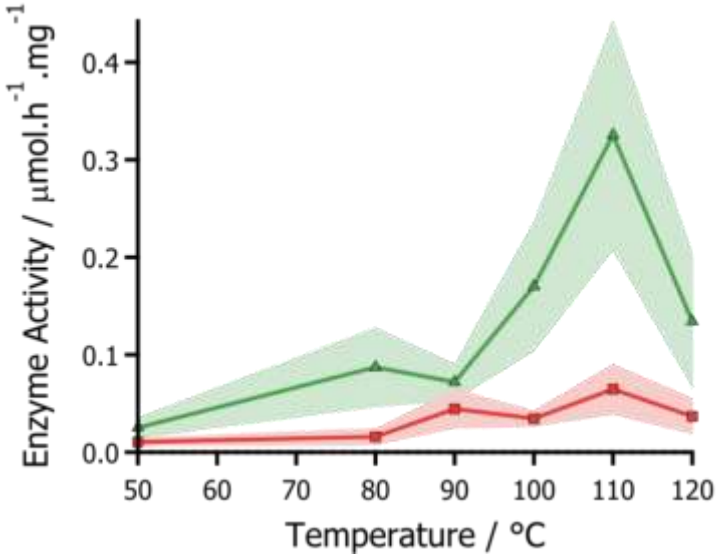


Cellobiose

Cellulose



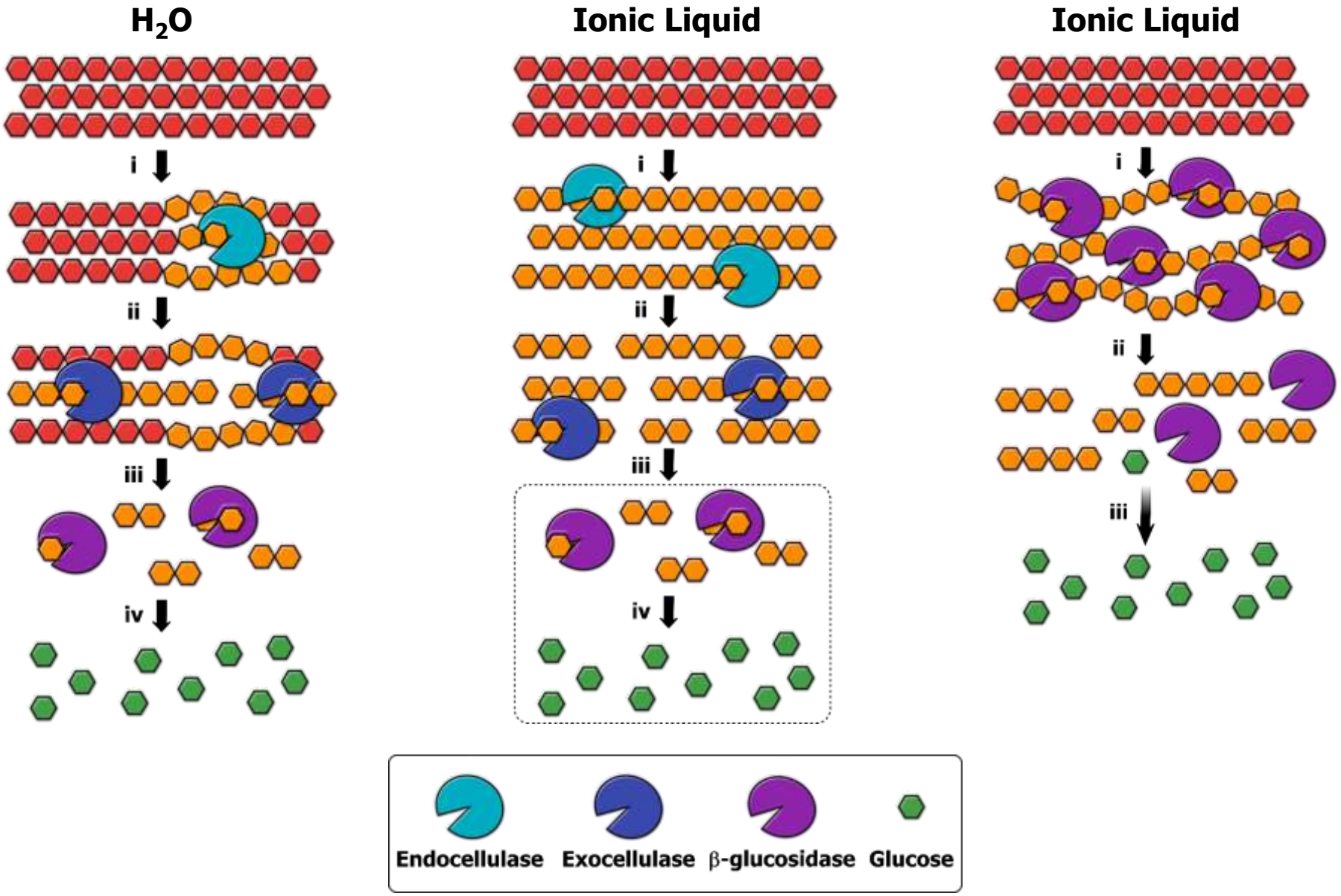
- Activity enhanced in ionic liquids – only for modified glucosidase.
- Reaction turns over at constant rate – zero order kinetics
- Enzyme activity increases by almost 30x at 110 °C



Nat. Chem. 2018

Glucosidase Activity in Ionic Liquids

Alex Brogan



Nat. Chem. 2018

Non-aqueous Biocatalysis – Where Next?

Alex Brogan

Biopolymer Degradation: Biomass Utilization



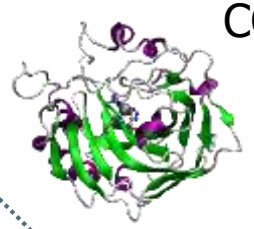
Cellulose processing from biomass.

Lignin processing using **laccases** and **peroxidases**.

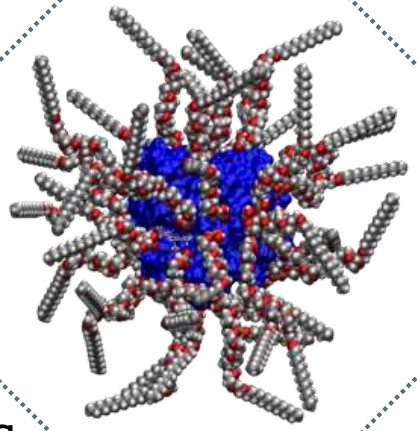
Waste processing with cocktail of enzymes.

Polymers from Renewable Sources

FDCA from glucose as feedstock for polyesters – **glucose oxidase**.



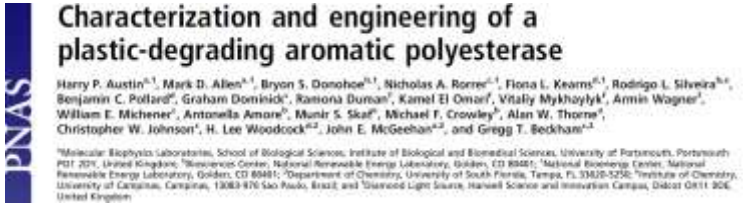
CO_2 as an alternative to phosgene for production of polycarbonates – **carbonic anhydrase**



Polymer Degradation: Plastic Recycling

Poly Lactic Acid degradation using **lipases** and **proteinases**.

Improving new enzyme utility.



PNAS

Characterization and engineering of a plastic-degrading aromatic polyesterase

Harry P. Austin[a,1], Mark D. Allen[a,1], Bryon S. Donohoe[b,1], Nicholas A. Rorrer[c,1], Fiona L. Kearns[d,1], Rodrigo L. Silveira[b,e], Benjamin C. Pollard[d], Graham Dominick[c], Ramona Duman[f], Kamel El Omari[g], Vitaliy Mykhaylyk[g], Armin Wagner[g], William E. Michener[c], Antonella Amore[h], Munir S. Skaf[e], Michael F. Crowley[b], Alan W. Thorne[a], Christopher W. Johnson[a], H. Lee Woodcock[d,2], John E. McGeehan[a,2], and Gregg T. Beckham[c,2]

[a]Molecular Biophysics Laboratories, School of Biological Sciences, Institute of Biological and Biomedical Sciences, University of Portsmouth, Portsmouth PO1 2DY, United Kingdom; [b]Biosciences Center, National Renewable Energy Laboratory, Golden, CO 80401; [c]National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80401; [d]Department of Chemistry, University of South Florida, Tampa, FL 33620-5250; [e]Institute of Chemistry, University of Campinas, Campinas, 13083-970 Sao Paulo, Brazil; and [f]Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, United Kingdom

Chemo-enzymatic

Enzyme reactions performed in organic solvents.

Couple with catalysts not stable in water.

→ Build unnatural biosynthetic cascades.

Solvent-free Biofluids – Prospective

Alex Brogan

Non-Aqueous Biocatalysis

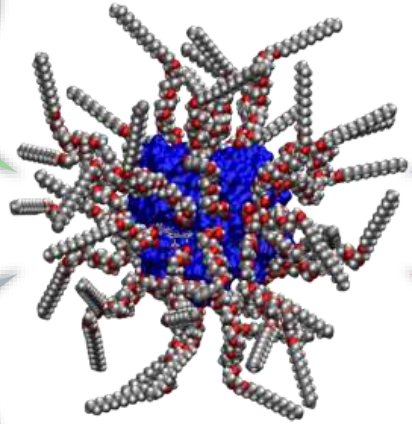
- Biomass utilization
- Plastic recycling
- Chemoenzymatic

Stabilization of Therapeutic Biomolecules

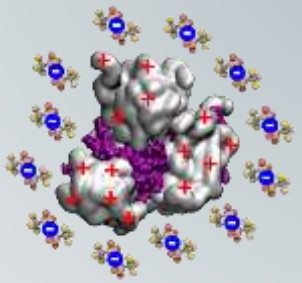
- Antibodies, viruses, RNA
- Cold-chain free storage

PhD Student
Liem Bui-Le

Imperial College London



Ionic Liquid Proteins



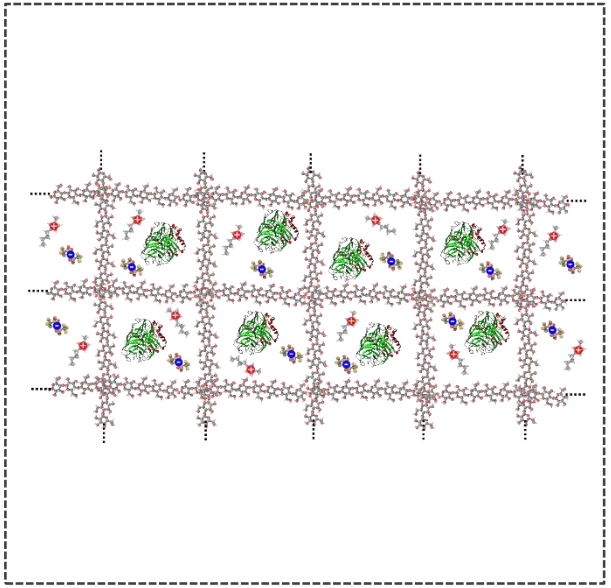
Cloaking Device for Drugs

- Greater lifetime of bioconjugate in body
- Increase accumulation of drugs at disease sites?

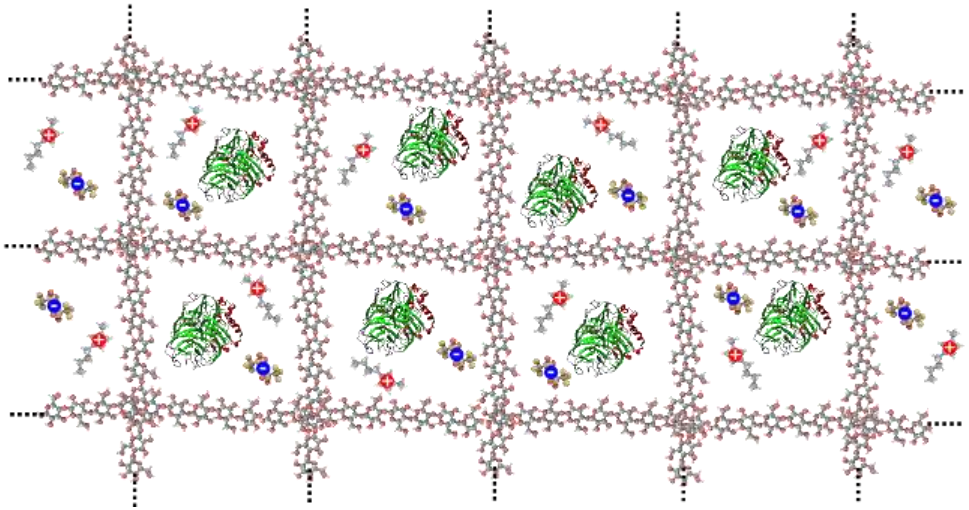
Solvent-free Enzyme Biofluids

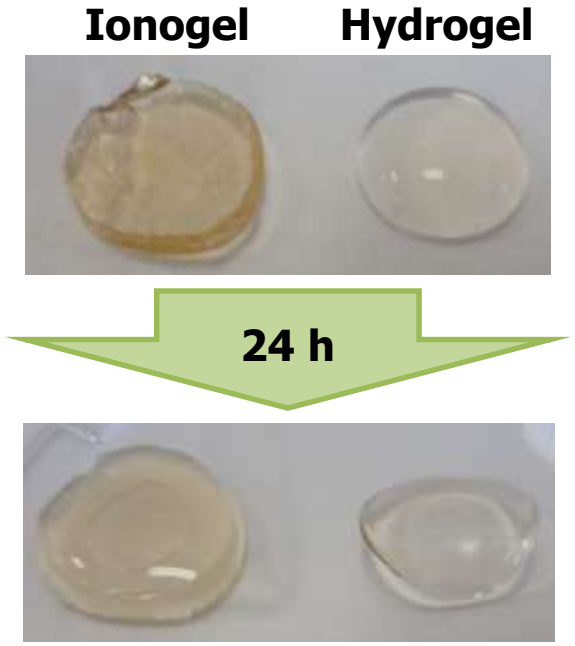
Ionic Liquid Proteins

Ionogels



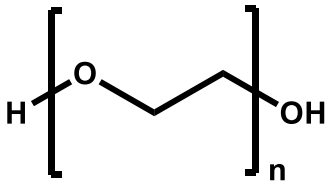
Ionogels – Ionic Liquid Based Gel Materials





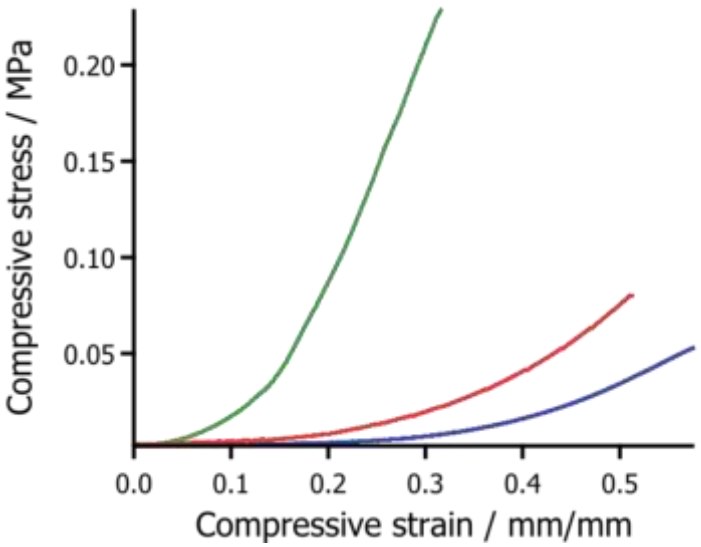
- Relatively new material – come into prominence over past decade.
- Gel system of ionic liquid encapsulated by polymer matrix.
- Current research concentrating on soft electronics – **little to no research on biocatalysis or biointerfacing.**

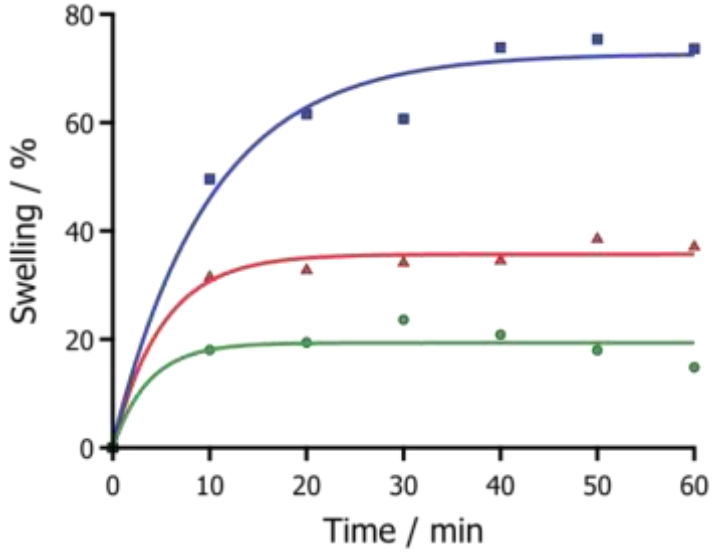
Ionogels – Physical Characterisation



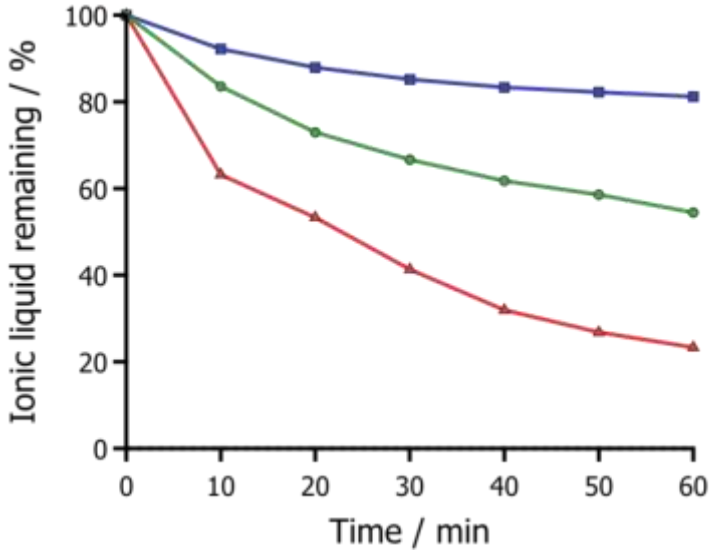
PEG

[emim][EtSO$_4$]
[emim][OAc]
[emim][OTf]



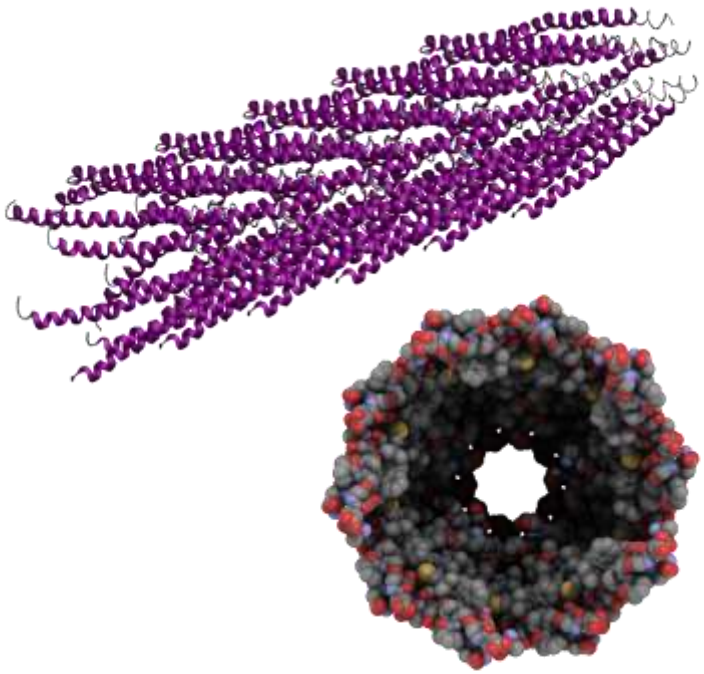


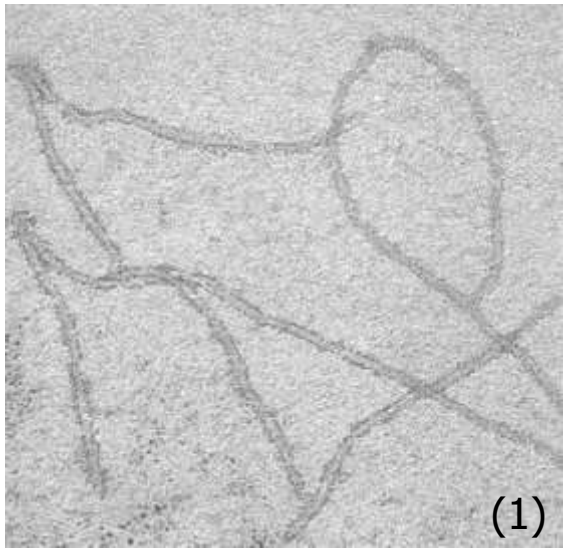
- Changing ionic liquids affects properties – modular.
- Mechanical properties dictated by ionic liquid (and polymer content)
- Very stable to temperature – and some stability towards solvents.



Artemis Charalambidou, Sophie Hyer

Virus Ionogels – M13 Bacteriophage



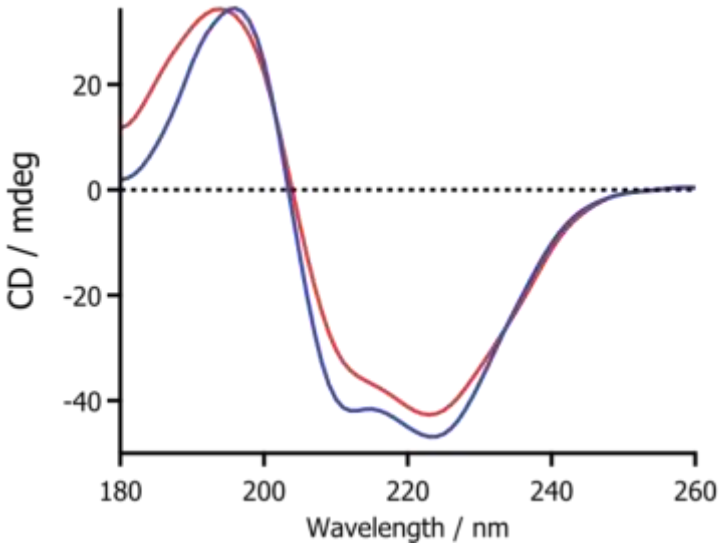


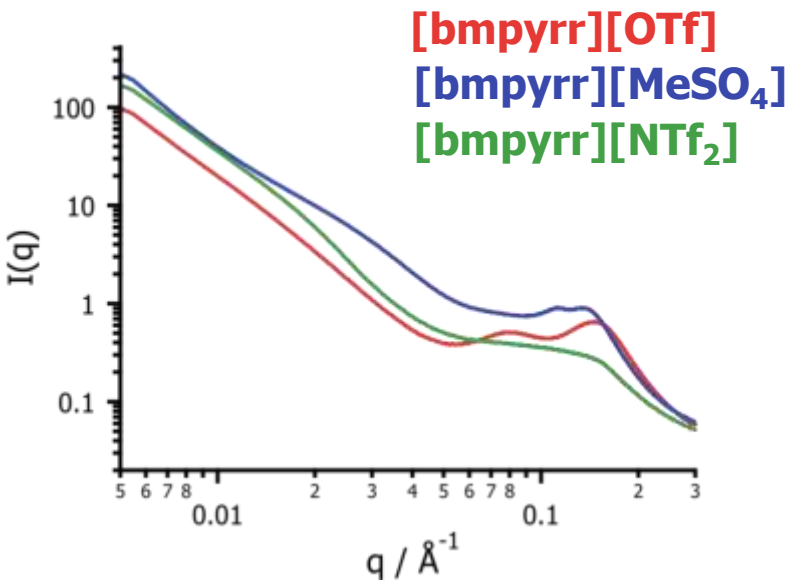
Massachusetts Institute of Technology

- M13 is a filamentous bacteriophage with extremely high aspect ratio – 10 nm wide by 900 nm long.
- Used by Belcher Lab (MIT) as highly versatile scaffold for templating various materials.
- Prospective uses as soft batteries and catalytic materials.

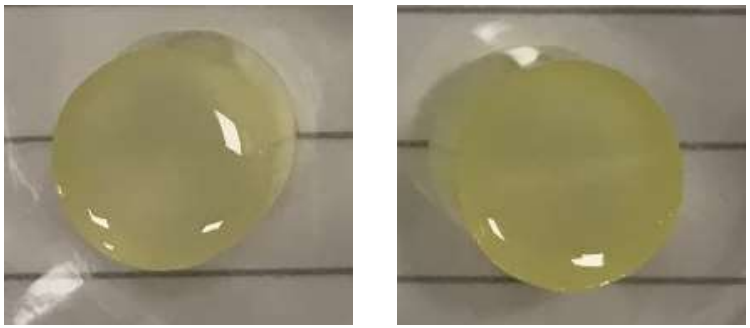
(1) UMC Utrecht Phage Library

M13 in Ionic Liquids and Ionogels





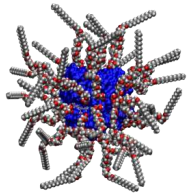
- Possible to make mixtures of M13 biofluid and ionic liquids.
- Structure maintained in the ionic liquids.
- Ionogels with M13 inside can be created.



Ionogels - Prospective

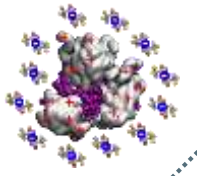
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Fundamentals



Physical properties – effect of components.

Solvent stability / release profiles.



Integration of functional components.

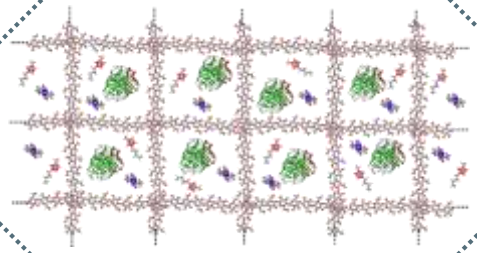
→ Solvent-free biofluids

Soft Bioelectronics

Limited research into ionogels for soft bioelectronics.

→ Potential for both **fundamental** and **applied** research

Ionic conductivity – ideal for biointerfacing / biosensing



Biocatalysis

Modular biocatalytic devices.

→ Cascade reactions

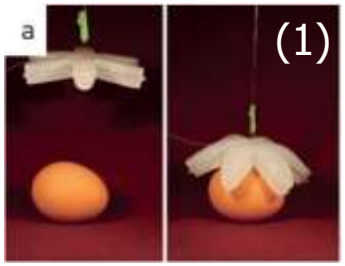
Enzymatic electrodes – cofactor free bioelectrocatalysis.

Couple with photon scavangers – photobiocatalysis.

→ Rechargable materials

Soft Robotics

Limited research into ionogels as materials for soft robotics.



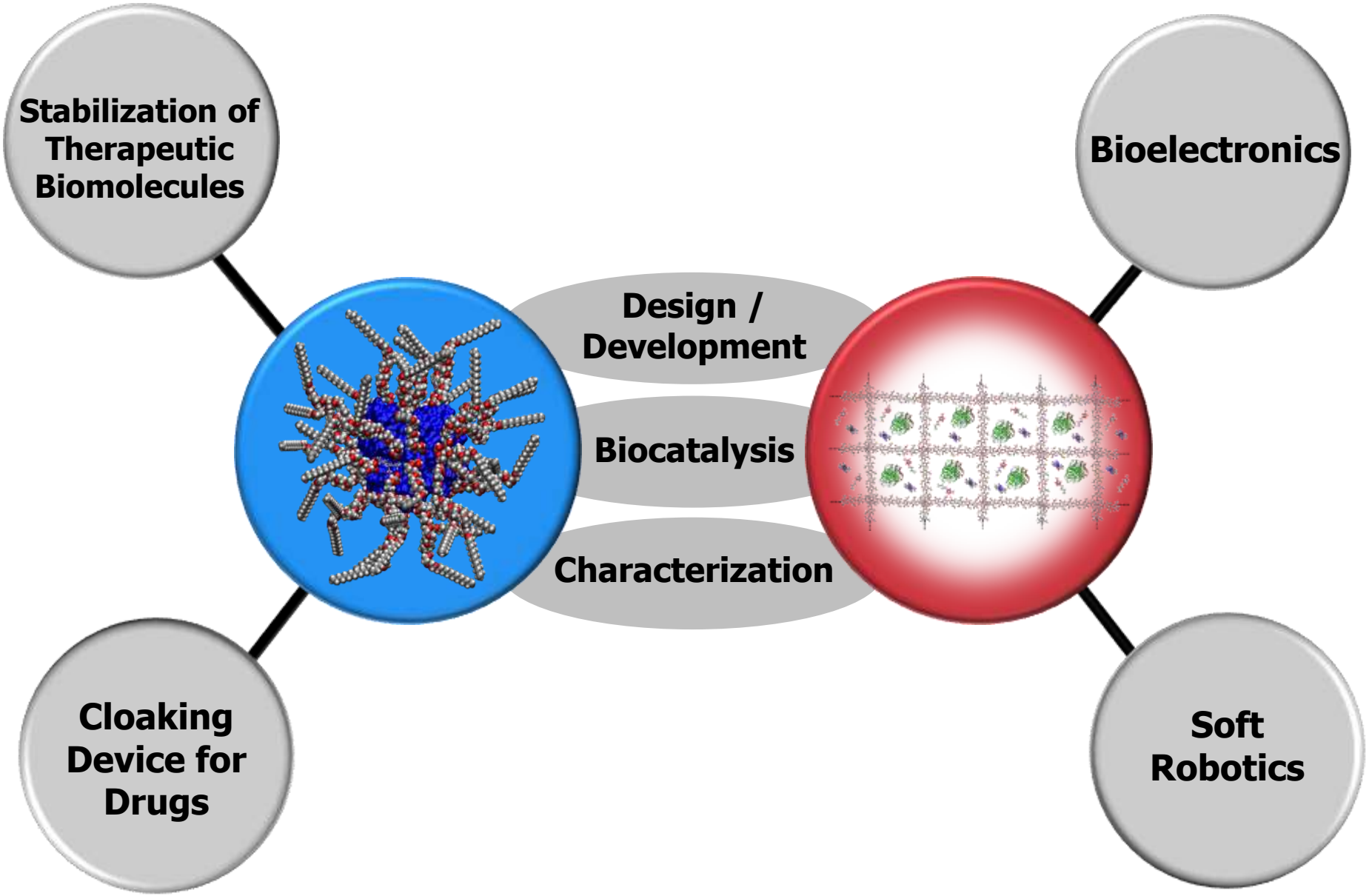
Increase complexity – functionality - with (bio)nanotechnology.

→ Local order to intrinsically disordered material

(1) G. M. Whitesides, *Angew. Chem. Int. Ed.* 2018.

Summary

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Acknowledgements

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Science & Technology Facilities Council
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Dr. François-Xavier Gallat, Dr. Martin Weik

ILL Neutrons for Science

Prof. Stephen Mann, Dr. Adam Perriman

University of BRISTOL

EPSRC
Engineering and Physical Sciences Research Council

MISTI
MIT International Science & Technology Initiatives

biome bioplastics

BBSRC
bioscience for the future

ROYAL SOCIETY OF CHEMISTRY