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Future Vaccine Manufacturing Research Hub

Technologies for Vaccine Delivery and Thermostabilisation

Dr Rongjun Chen & Jason Hallett
Chemical Engineering, Imperial College
rongjun.chen@imperial.ac.uk
j.hallett@imperial.ac.uk

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Key challenges and opportunities

- **Delivery challenge**
  - ✓ Degradability of biological vaccine antigens, e.g. nucleic acids, recombinant proteins
  - ✓ To be delivered in the correct conformation
  - ✓ Lacks potential to target the immune cells
- **Manufacturing and storage challenge**
  - ✓ Reduced potency due to elevated temperature or accidental freezing
  - ✓ Vaccine stability during storage
- **Opportunities**
  - ✓ Targeted, efficient vaccine delivery formulations
  - ✓ Manufacturable, heat-stable formulations

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Proposed approaches and outcomes

- **Novel vaccine delivery formulations**
  - ✓ Bioresponsive polymers
  - ✓ Virus-like liposomes
- **Biostabilisation of vaccine delivery formulations**
  - ✓ Dry storage at room temperature
  - ✓ Sugar (trehalose, sucrose, glucose) loading by polymers/liposomes
- **Potential outcomes**
  - ✓ Flexible and robust platforms for improved stability and efficacy of vaccines
  - ✓ Manufacturable formulations with optimised biostabilisation during storage

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Proposed approaches and outcomes 1

- **Novel vaccine delivery formulations**
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  - ✓ Virus-like liposomes
- **Biostabilisation of vaccine delivery formulations**
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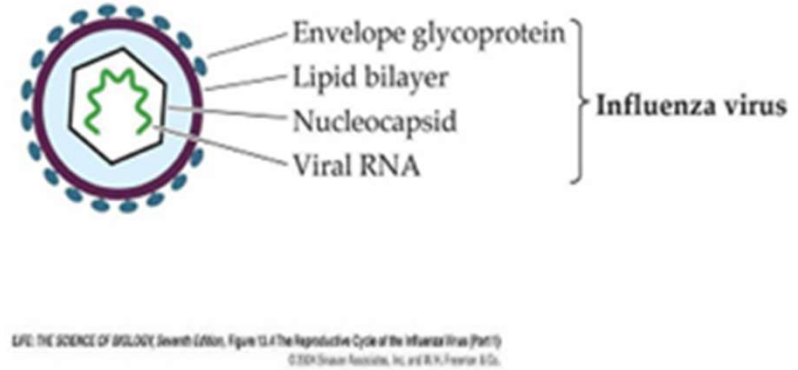
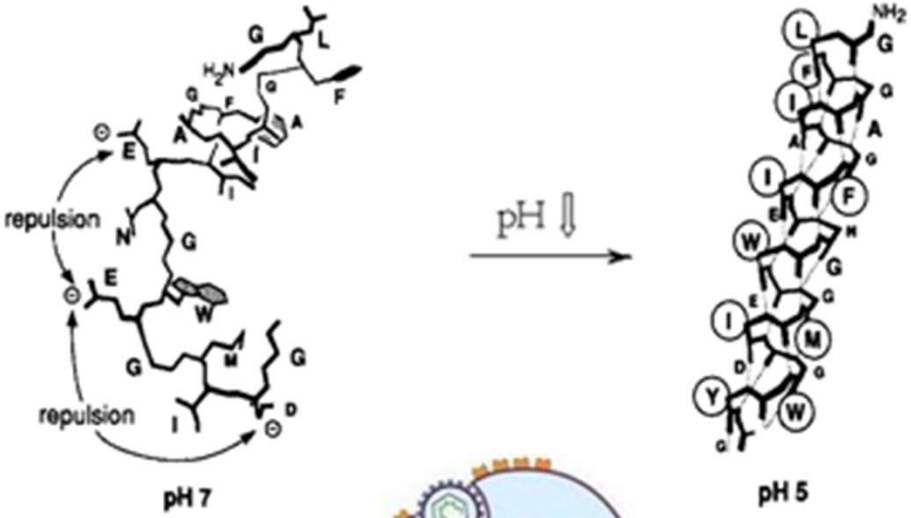
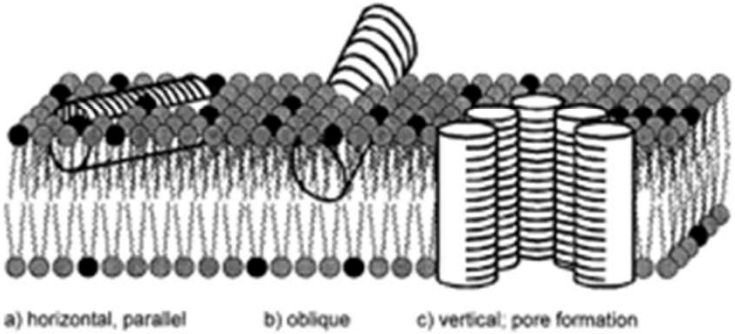
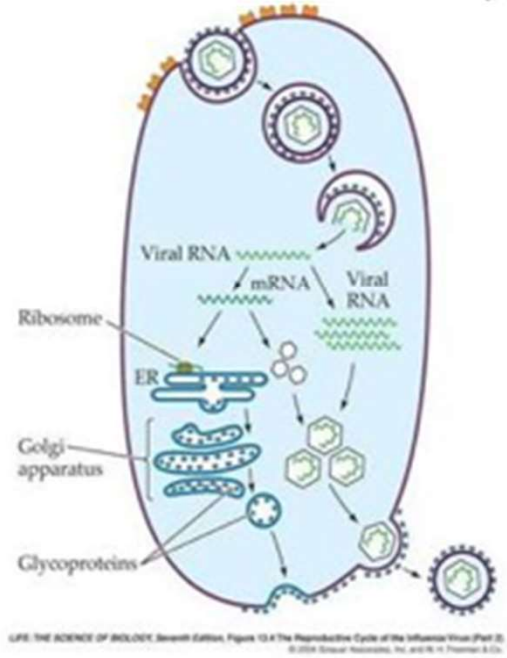
Inspiration from reproductive cycle of influenza virus









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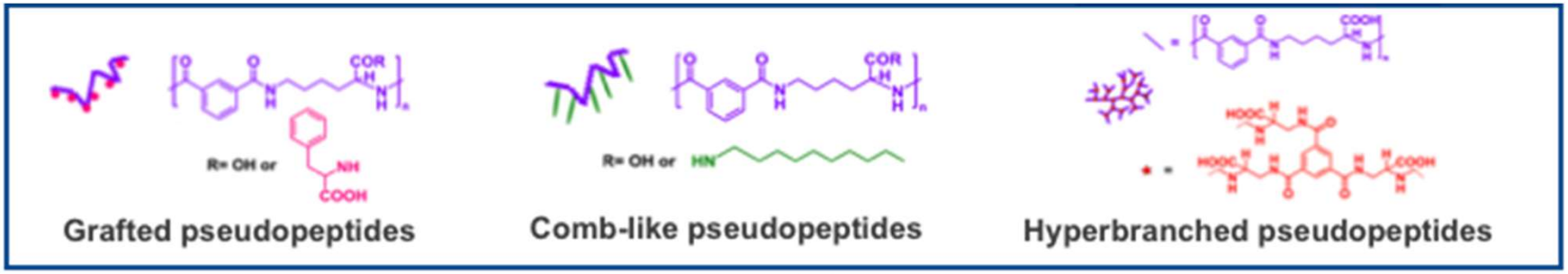
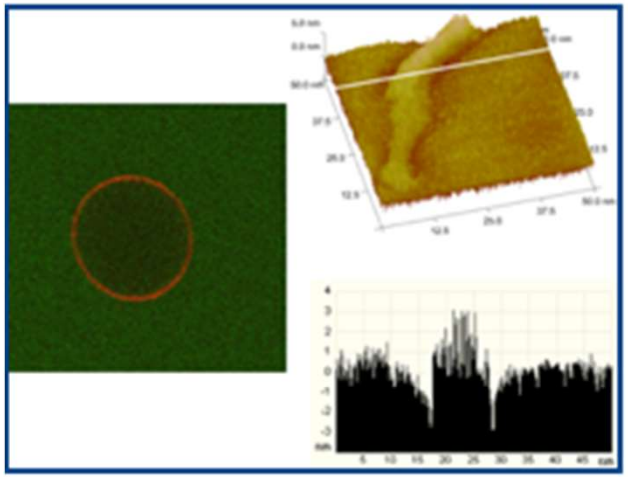
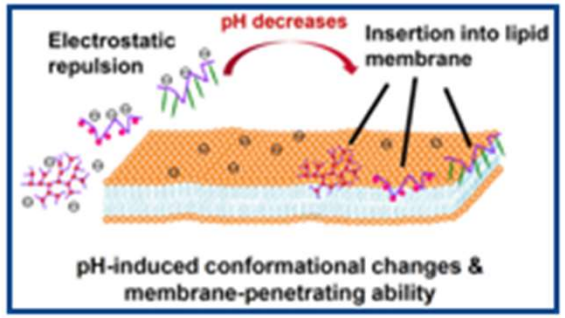
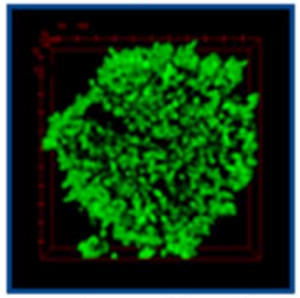
Viral peptide-mimicking, pH-responsive, pseudopeptides as delivery vehicles



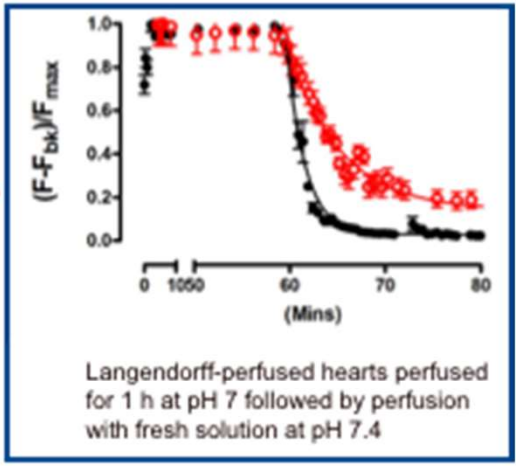


Delivery through vesicular/cellular membranes





Delivery to cell spheroids



Langendorff-perfused hearts perfused for 1 h at pH 7 followed by perfusion with fresh solution at pH 7.4

Delivery to organs

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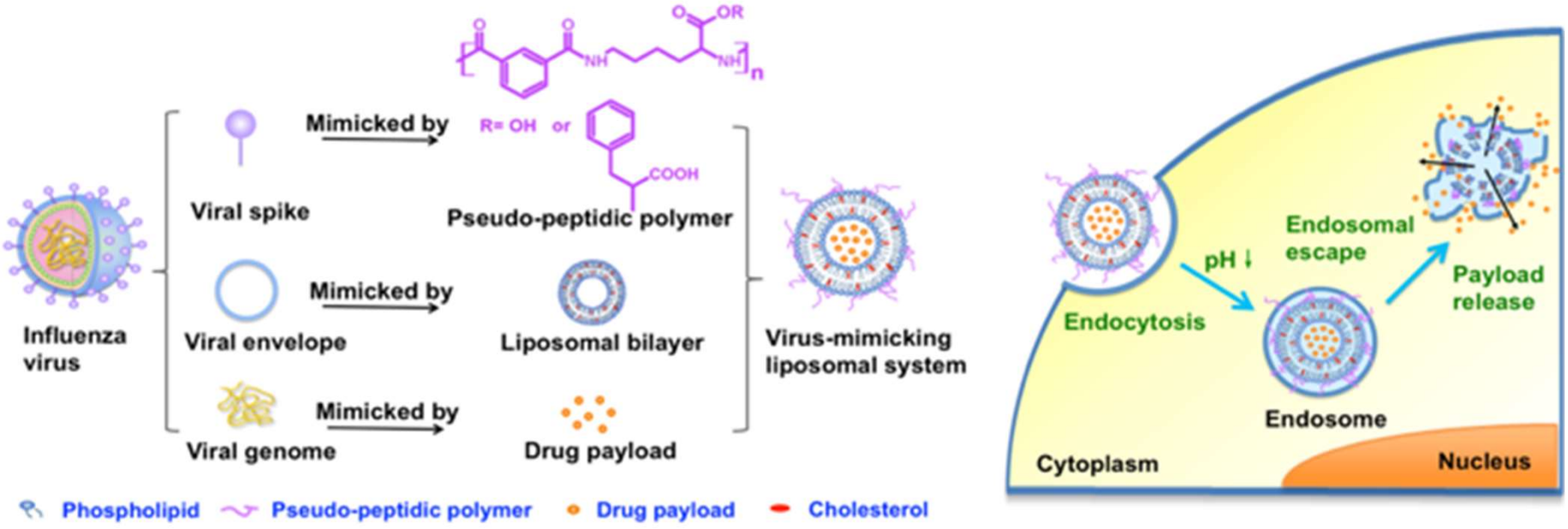
Virus-like nanoparticles as delivery vehicles



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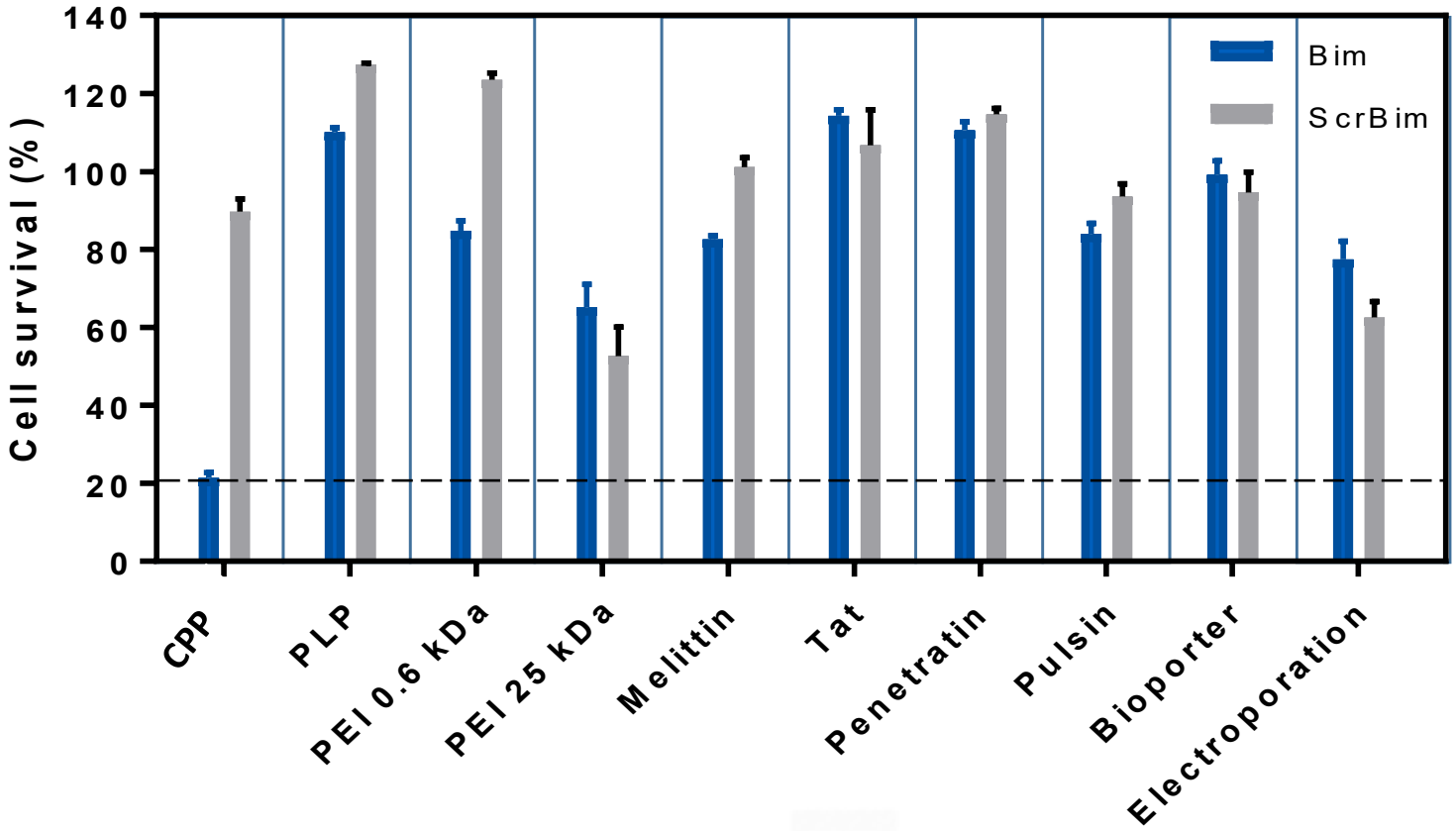
Intracellular delivery of biological molecules



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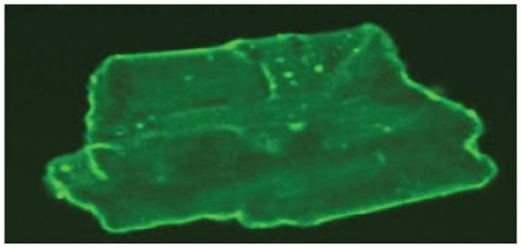
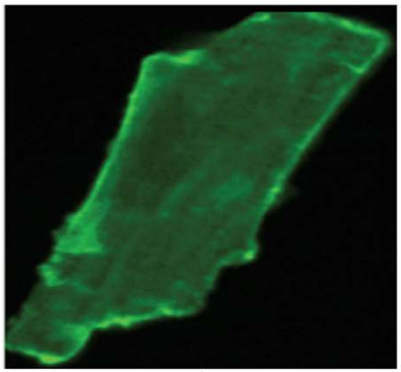
Intracellular delivery of biological molecules

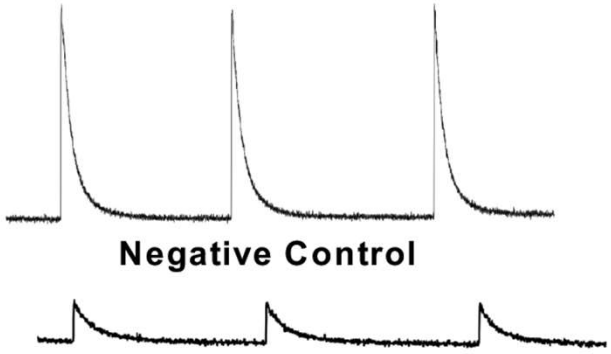
- **Protein delivery**



Delivery of peptide
PS-16-FITC (2 kDa)

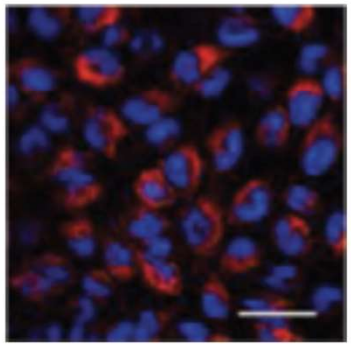


Delivery of antibody
FITC-IgG (160 kDa)

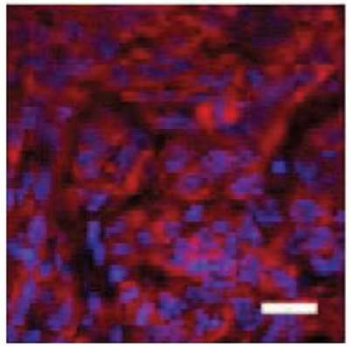


Delivery of peptide CamBP (3.5 kDa)

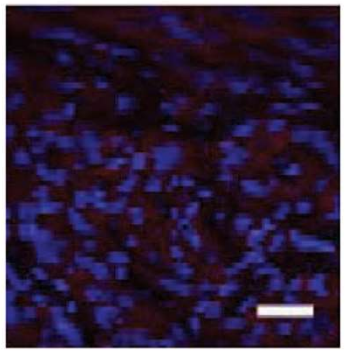
- **RNA delivery**



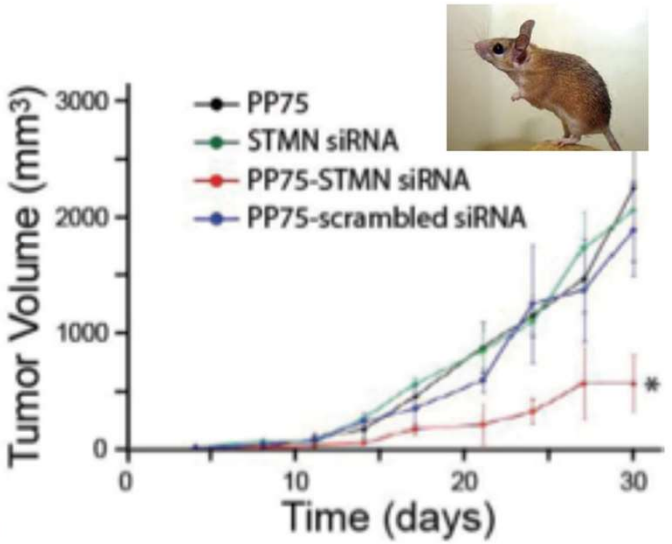
Cytoplasmic siRNA delivery



Negative Control



Knockdown of stathmin via siRNA delivery



Khormaee et al, *Advanced Functional Materials* 2013, 23, 565

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Key challenges and opportunities 2

- Delivery challenge
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  - ✓ To be delivered in the correct conformation
  - ✓ Lacks potential to target the immune cells

- **Manufacturing and storage challenge**
  - ✓ **Reduced potency due to elevated temperature or accidental freezing**
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- **Opportunities**
  - ✓ Targeted, efficient vaccine delivery formulations
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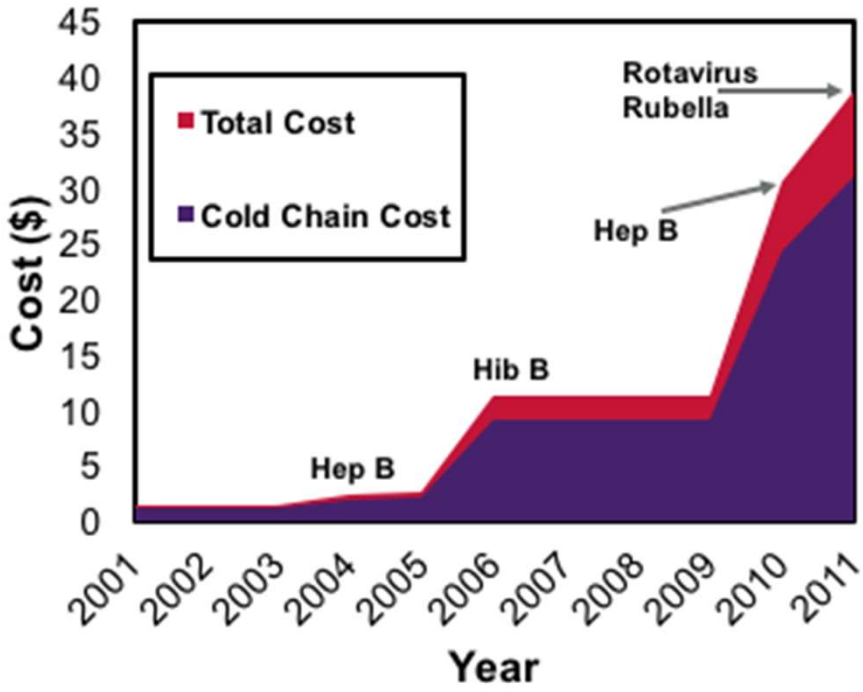
Cold Chain

- Temperature-induced risk factors for vaccines:
  - Aggregation
  - Degradation/inactivation
- Costs vaccine programmes **$200 - 300 million** per year
- **Up to 80 %** of the cost of vaccination programmes



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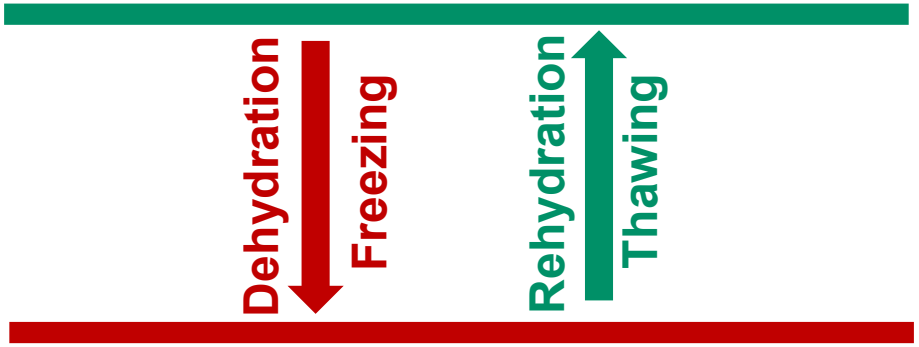
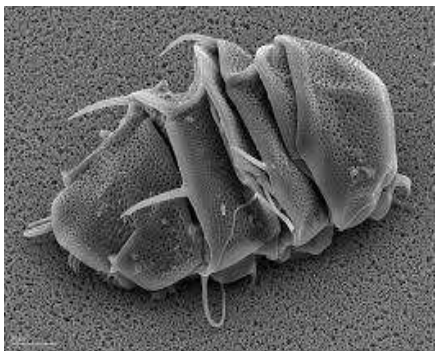
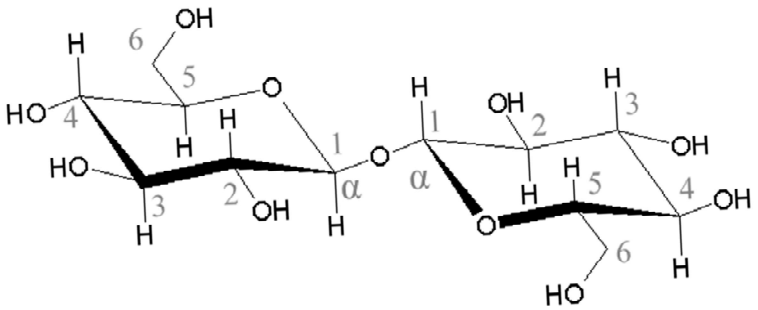
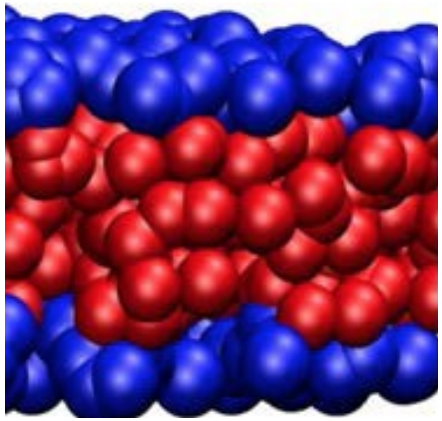
Inspiration from anhydrobiotic organisms







- Trehalose: non-toxic disaccharide
- Protection in freezing and drying
- Antioxidant



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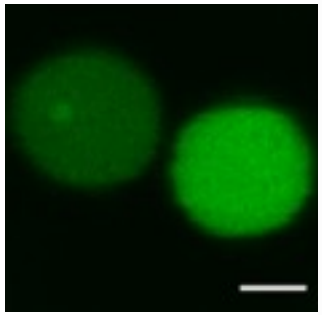
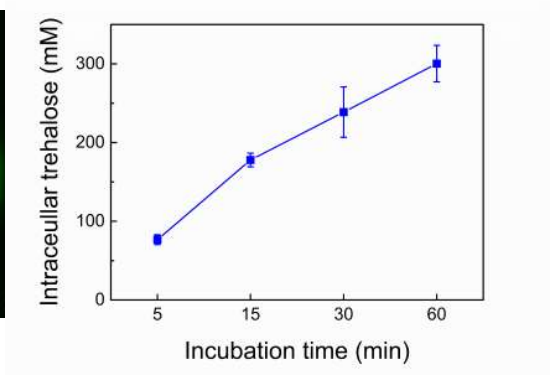
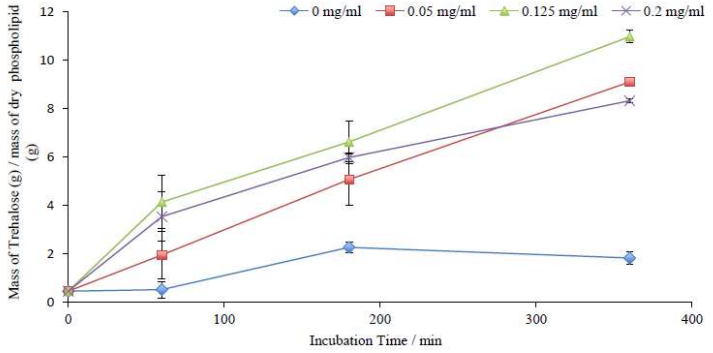
Heat-stable formulations (nanoparticle- & cell-based)

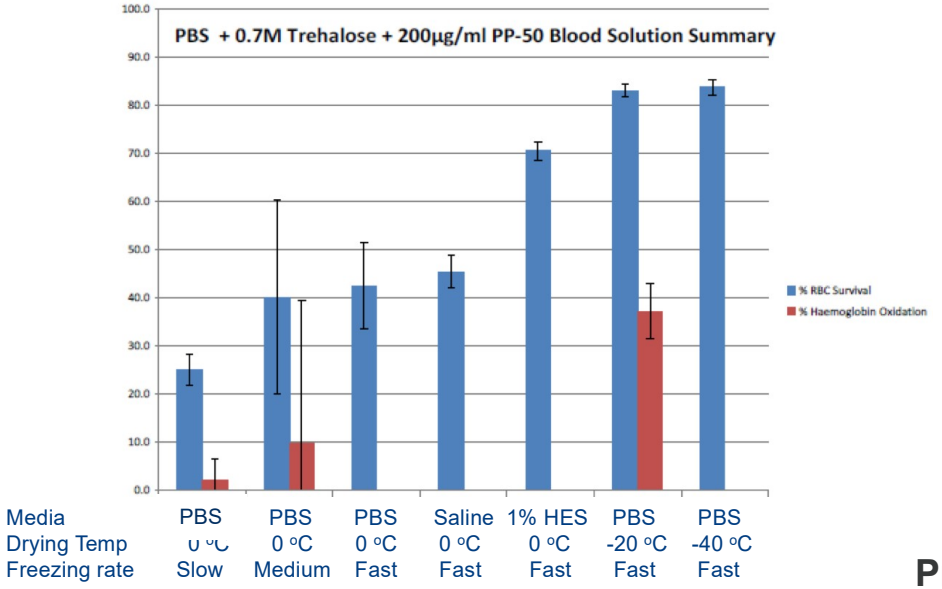




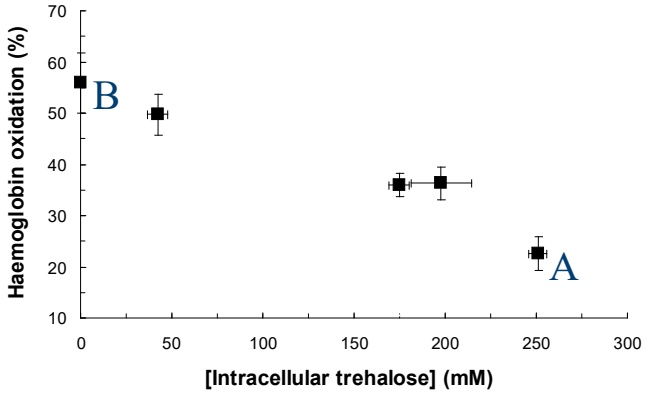
Trehalose loading into cells

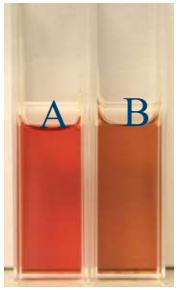


Trehalose loading into virus-mimicking liposomes



Freeze drying of red blood cells





Prevention of haemoglobin oxidation in dried storage

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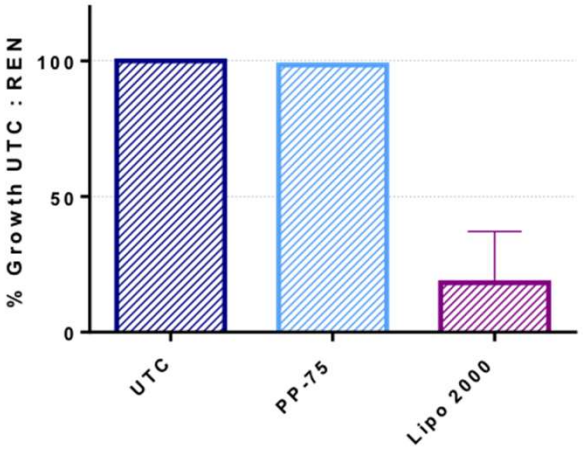
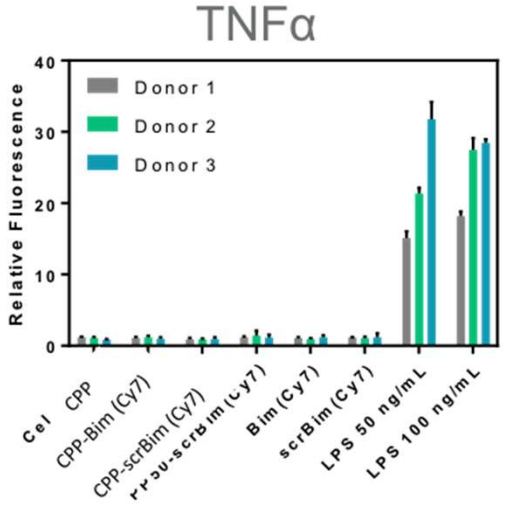
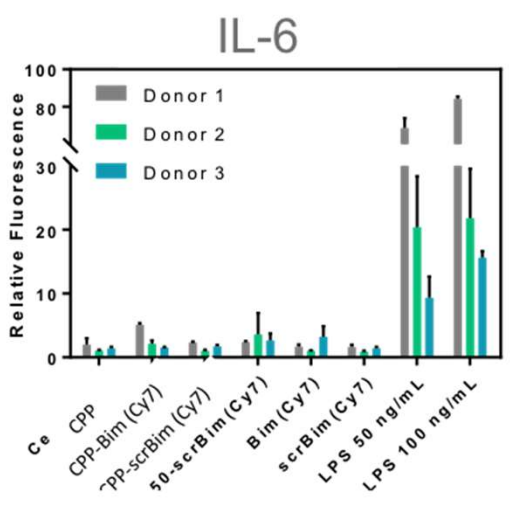
Cytotoxicity, immunogenicity and tolerability profiles

Cell cytotoxicity



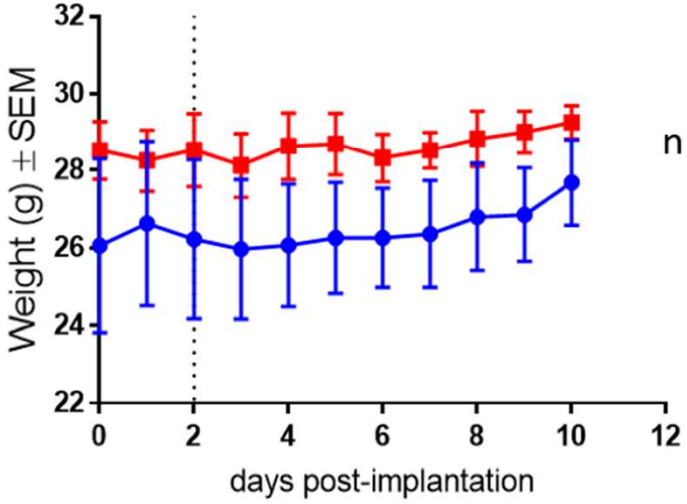
Immunogenicity





Weight change post injection (iv)

CD1-nude mice



n = 6

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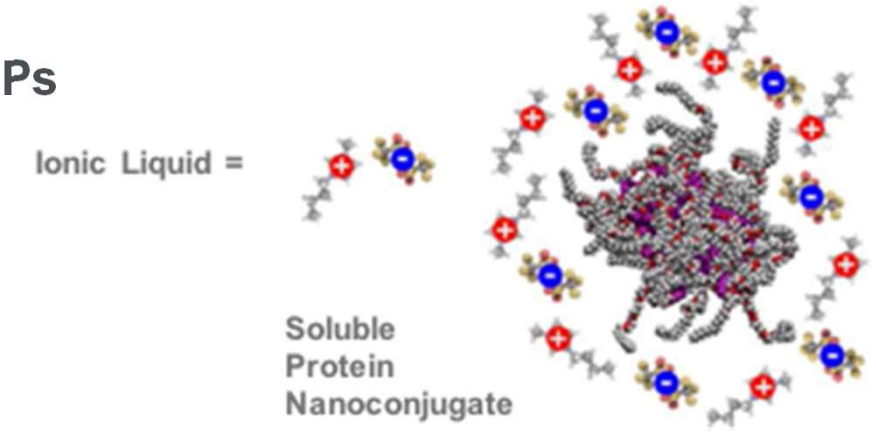
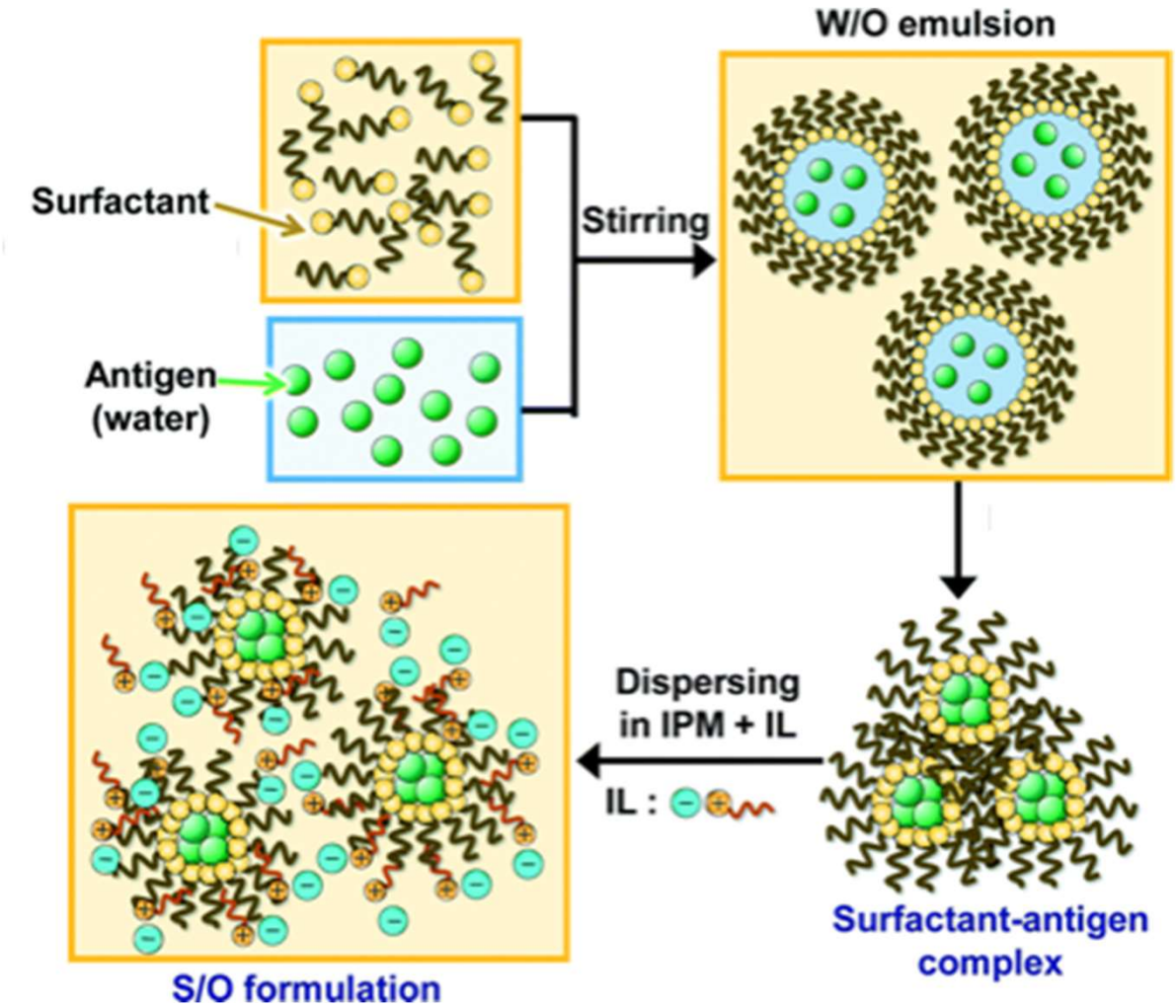
Current and Future Strategies

- **Use of biocompatible molten salts**
- **Modifying therapeutic proteins, VLPs and saRNA to be dissolved in biocompatible ionic liquids**



- Imparts higher stability to proteins (50-70 C vs native; > 100 vs aqueous)
- Demonstrated for structural proteins (stable to 180 C), enzymes (activity increased 100-1000x), antibodies (30-50x longer stability; 46% binding retained), viruses (new materials applications)
- Thermal stability increased; aggregation effectively prevented; water excluded
- Needs biocompatibility, reversibility, combination with delivery vectors
- Potential alternative to freeze drying?



Similar principles apply to viruses, VLP and recombinant proteins

Med. Chem. Commun., 2015, **6**, 2124-2128

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Research Objectives

STAGE 1
Monoclonal Antibodies

STAGE 2
Viruses

STAGE 3
Vaccines

- Improve thermal stability of antibodies to **60 ° C** for **6 months** in ionic liquids
- Retain bioavailability depending on thermal stability and if reconstitution is needed
- Achieve similar results with viruses and vaccines

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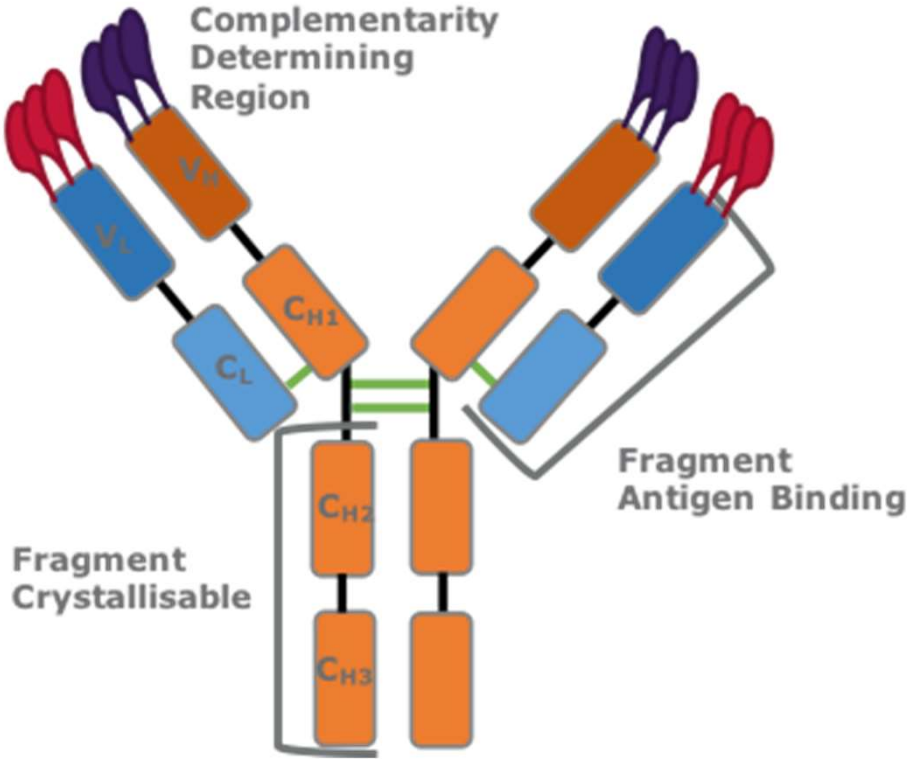
Exemplar target: Antibody Structure

- The Fc domain is constant in A.A and glycosylated for biological recognition
- Variable regions containing three antigen-binding loops each
- Variable region different in A.A sequence to maintain specificity



Monomer Y-shaped structure of antibodies, where V and C represents the variable and constant region. Subscripts L and H represent the light and heavy chains.[1]

1. Perchiacca, J.M. and P.M. Tessier, *Annual Review of Chemical and Biomolecular Engineering*, 2012

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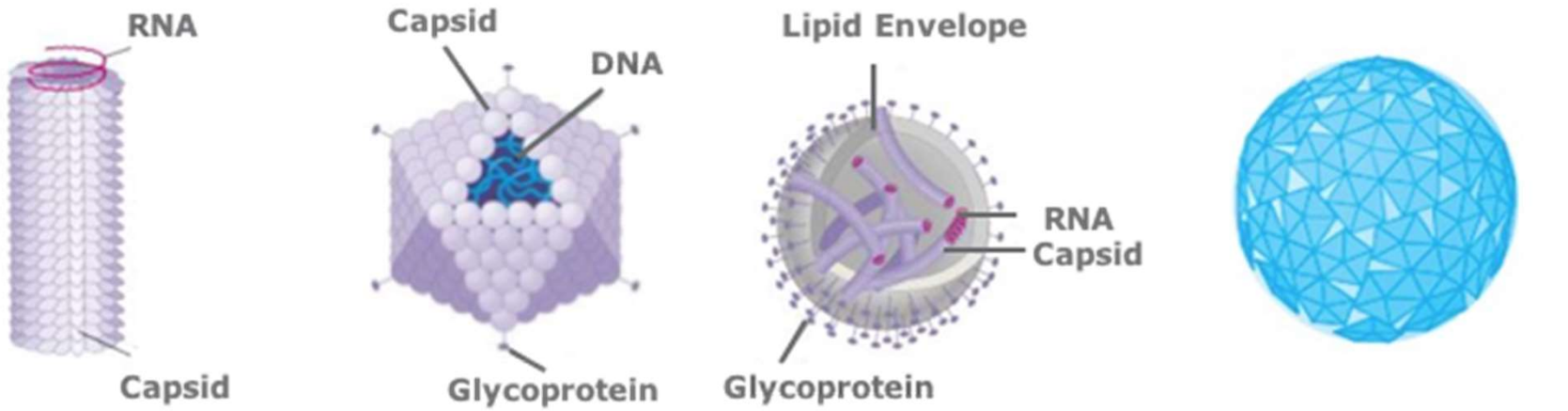
Exemplar target: Virus Structure

- Viruses are intracellular parasites containing either RNA or DNA
- Genetic material encapsulated by a protein capsid
- VLPs a potential delivery mechanism



Different Viral Structures[1]

1. Campbell, N.A., *Pearson Education Inc.*, 2008

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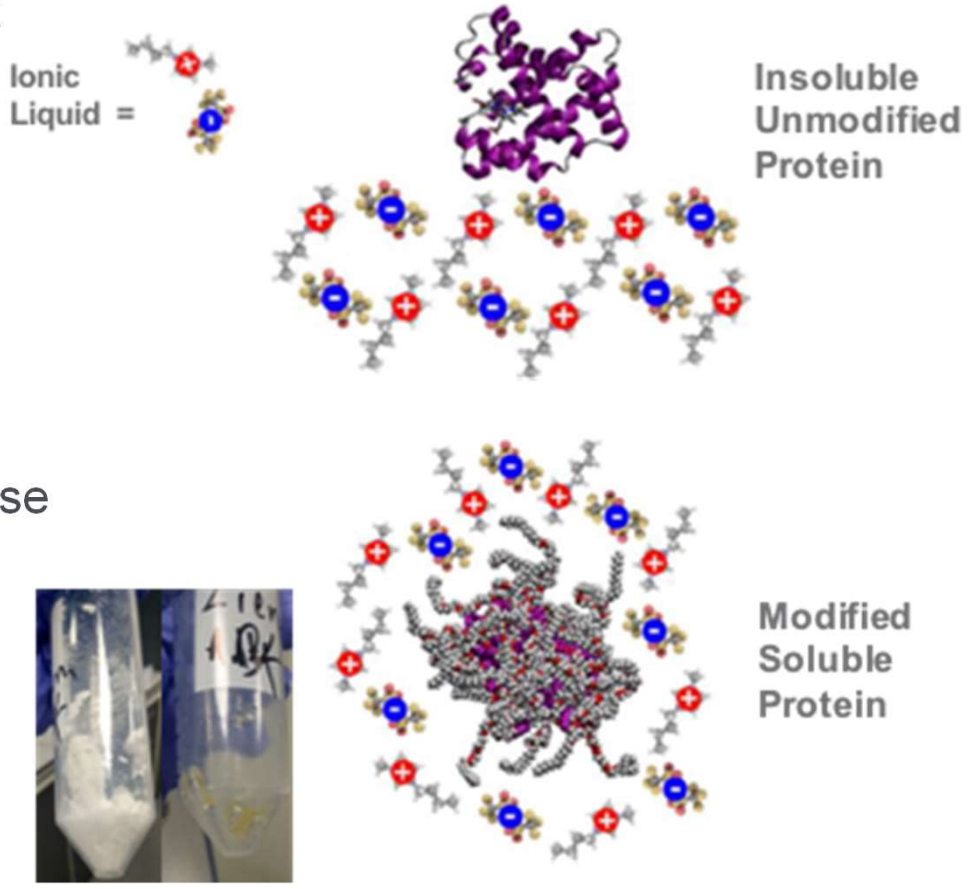
Proteins in Ionic Liquids

- Proteins are poorly soluble in neat ionic liquids
- Adding polymer−surfactant to the protein surface produces liquid proteins
- **Retains biological activity** of proteins, enzymes and viruses
- Modified myoglobin and glucosidase dissolved in hydrophilic and hydrophobic ionic liquids
- Increased protein denaturation temperature by **60° C** to **140° C** compared to aqueous solution



Modified proteins to allow dissolution in ionic liquids[1,2]

1. Brogan, A.P.S, and Hallett, J.P., *Journal of the American Chemical Society*, 2016
2. Brogan, A.P.S, Bui-Le, L., and Hallett, J.P., *Nature Chemistry*, 2018

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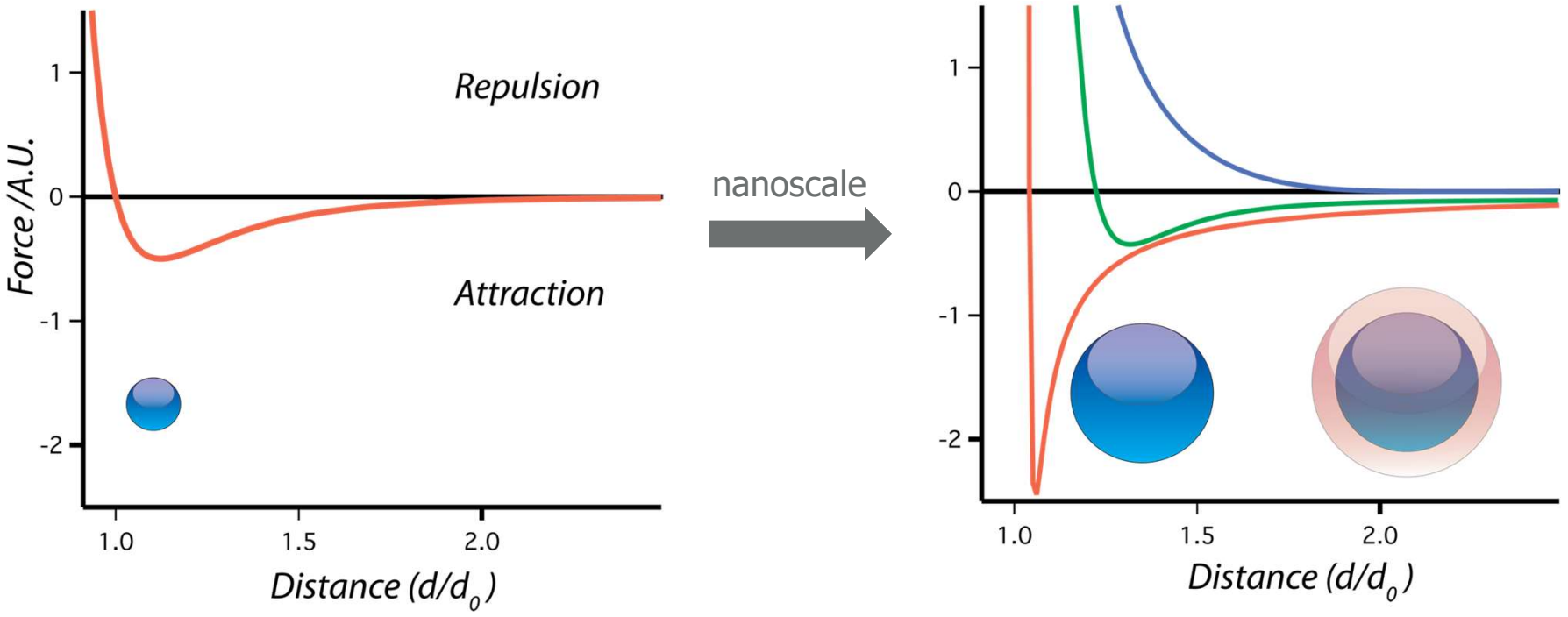
Nanoscale Liquids



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

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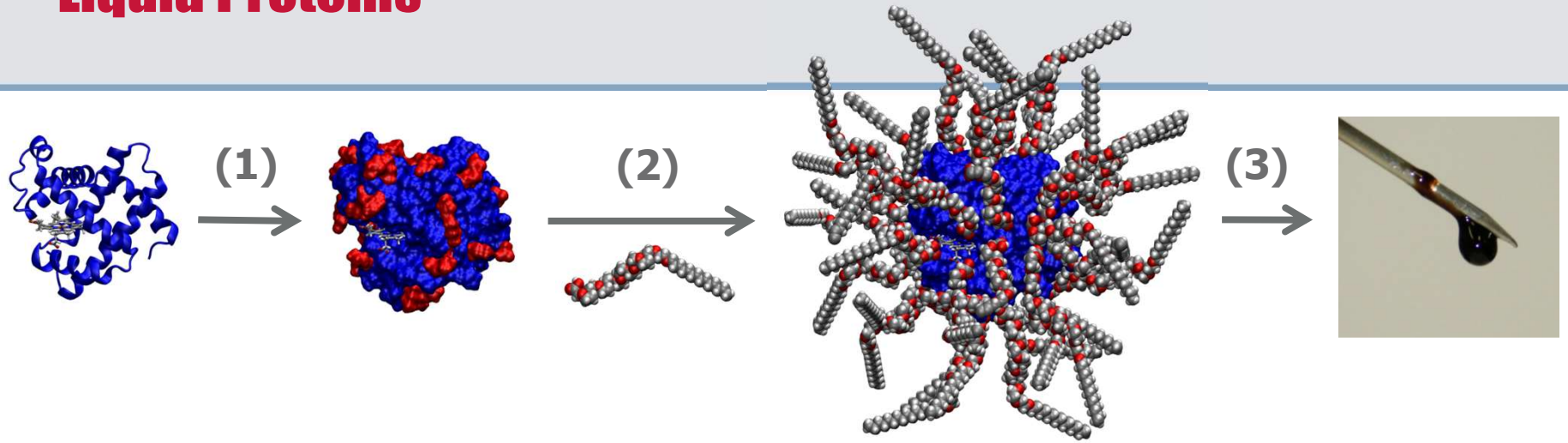
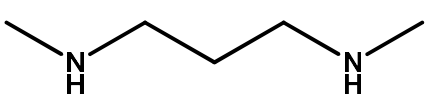
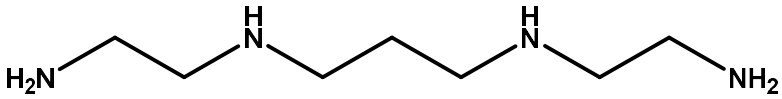
Liquid Proteins

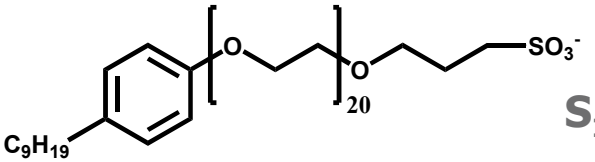


(1) Cationization of surface acidic residues with amines using the EDC reaction.

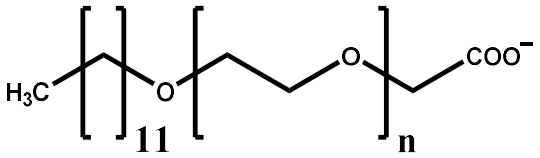




(2) Electrostatic complexation with anionic surfactant to form aqueous nanoconjugates.



S$_1$



S$_2$ (n = 11)

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

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Liquid Proteins

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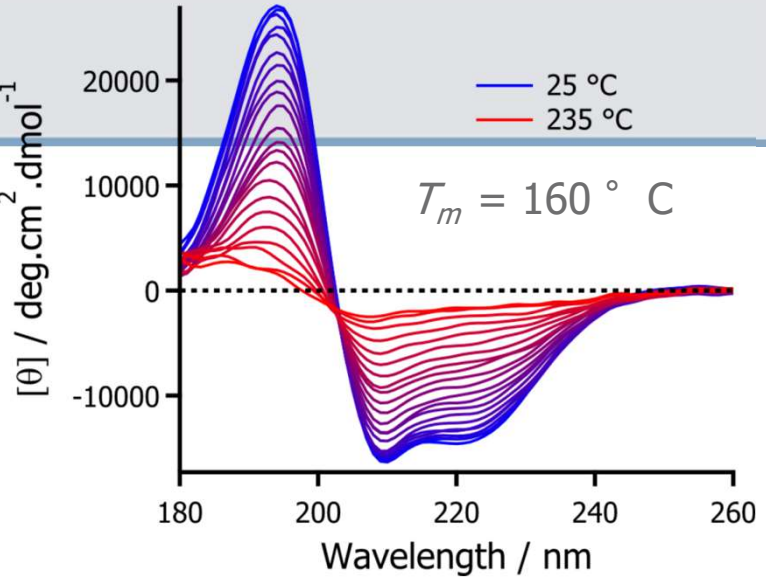
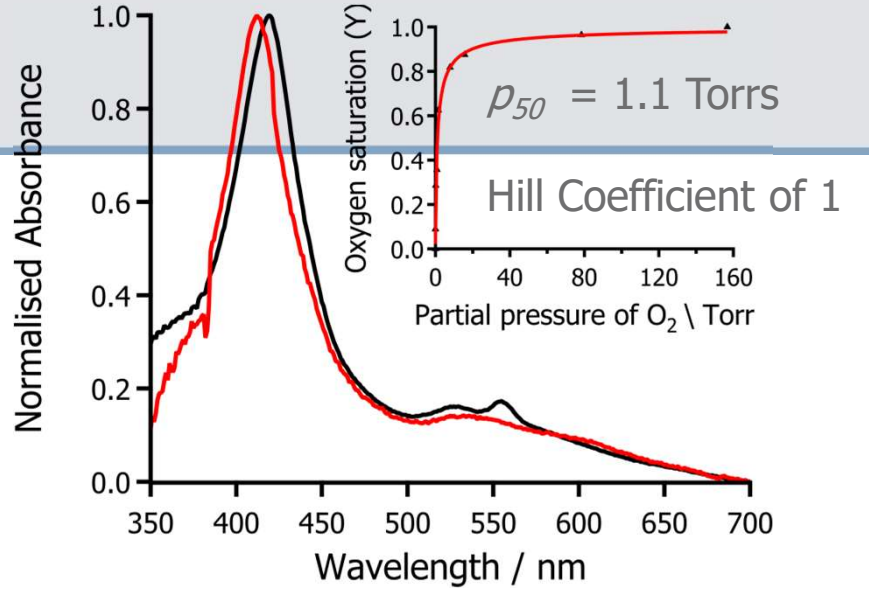
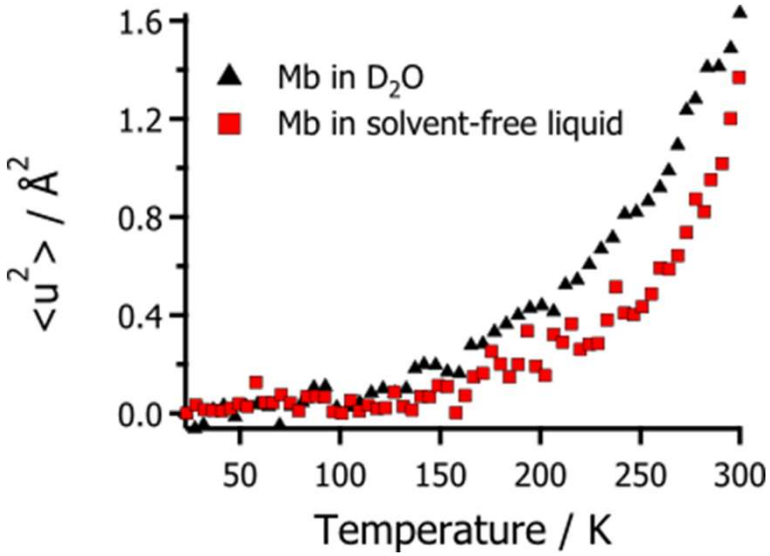




- Retained structure and hyperthermophilic-like stability.
- Biological function in the absence of water.
- Protein dynamics retained within organic corona.



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

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Liquid Structure

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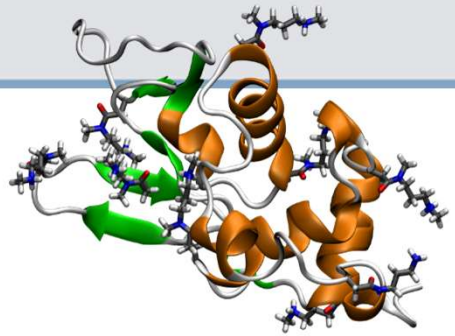
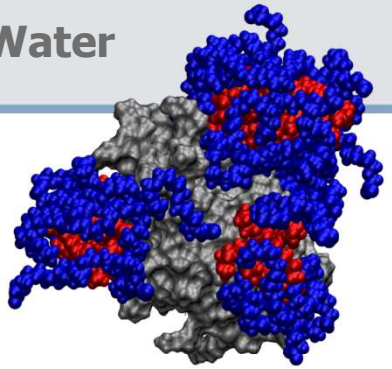
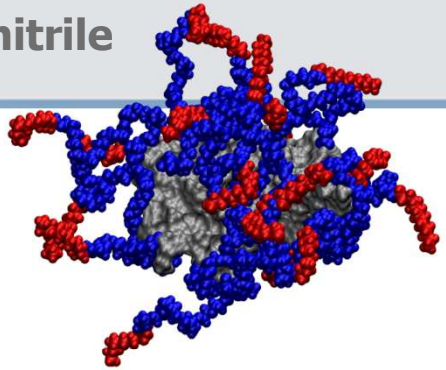


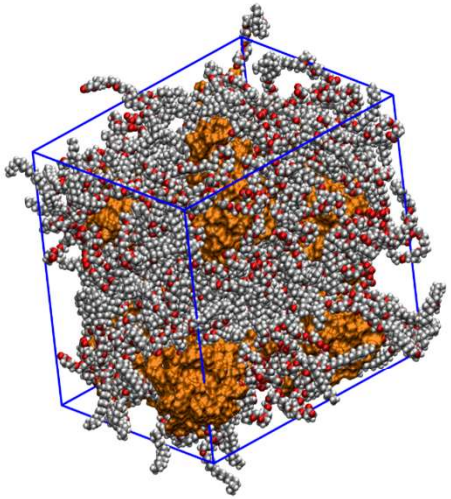
Water



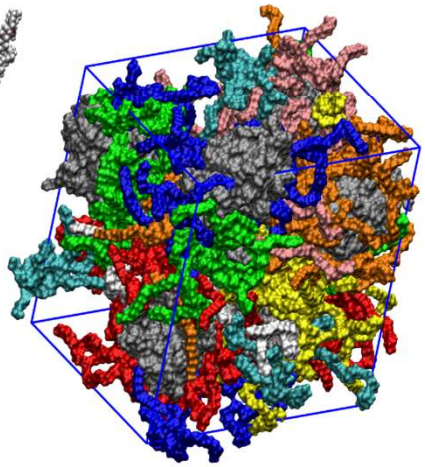
Acetonitrile



- 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

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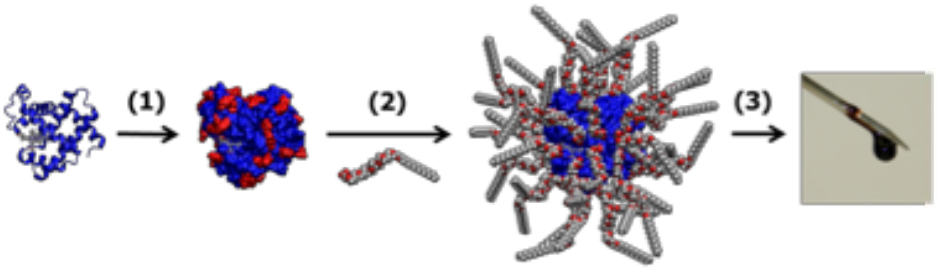
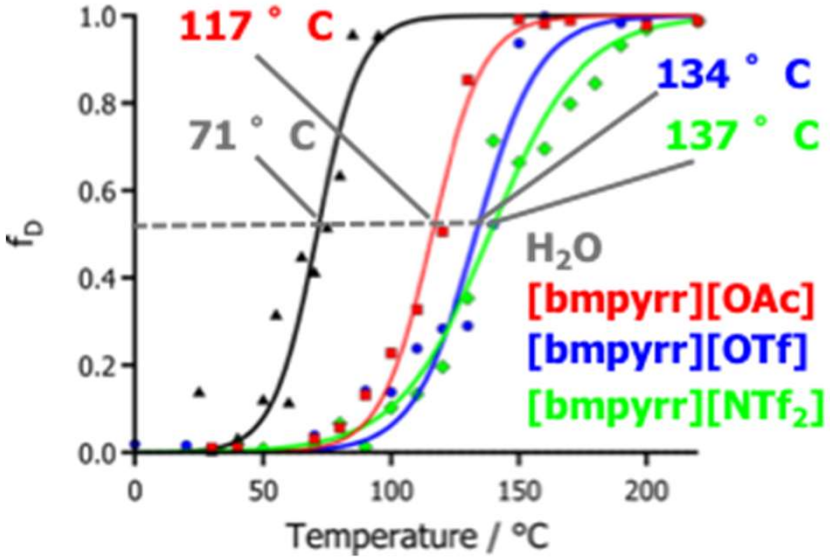
Protein stability in Ionic Liquids



- UV/Vis shows retention of structure in all conditions
- SRCD indicated ionic liquids induced α-helicity
- Thermal stability of proteins increased significantly in ionic liquid



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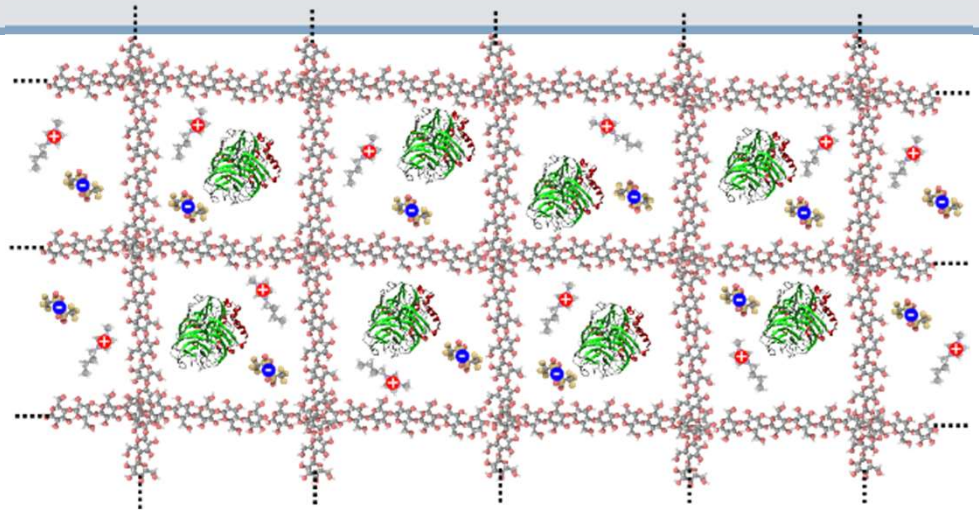
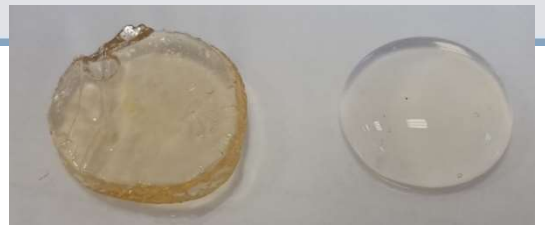
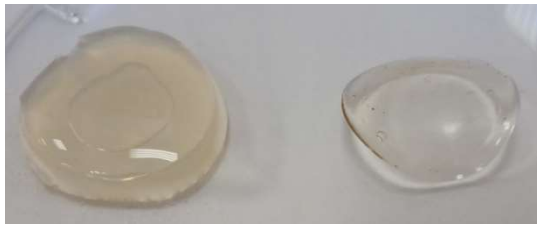
Ionogels



Ionogel Hydrogel



24 h



- 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.**

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Ionogel Structure

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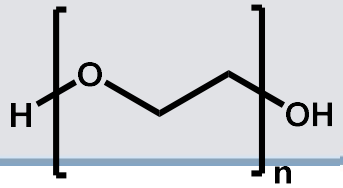
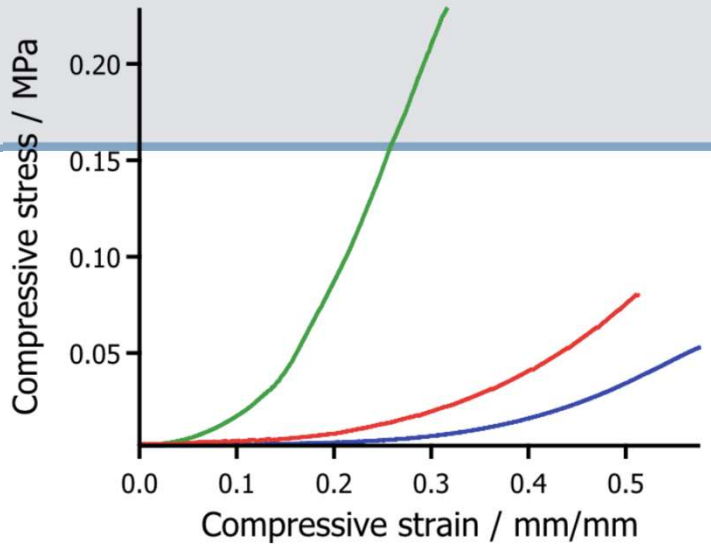
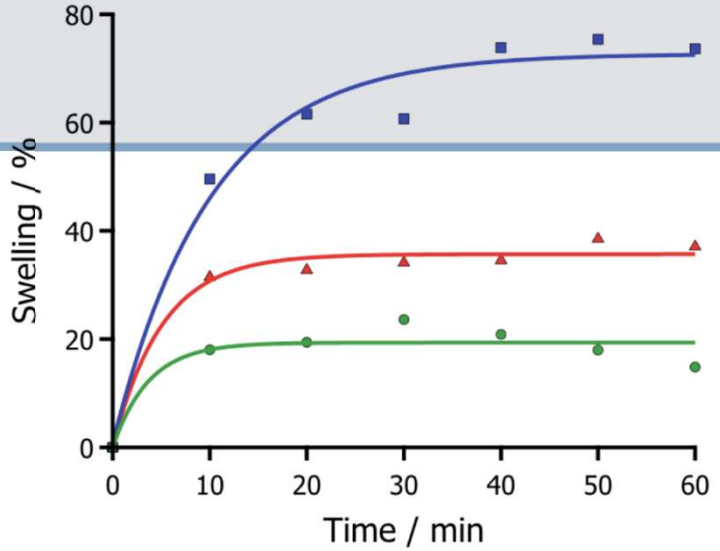


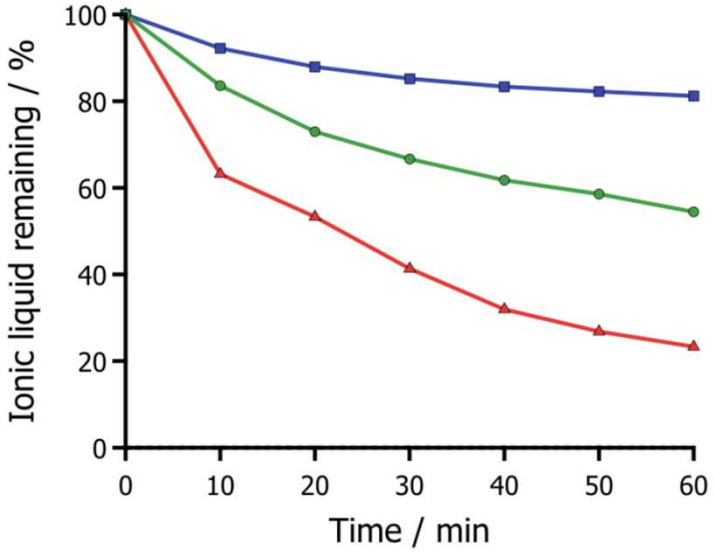
PEG

[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.



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M13 Bacteriophage

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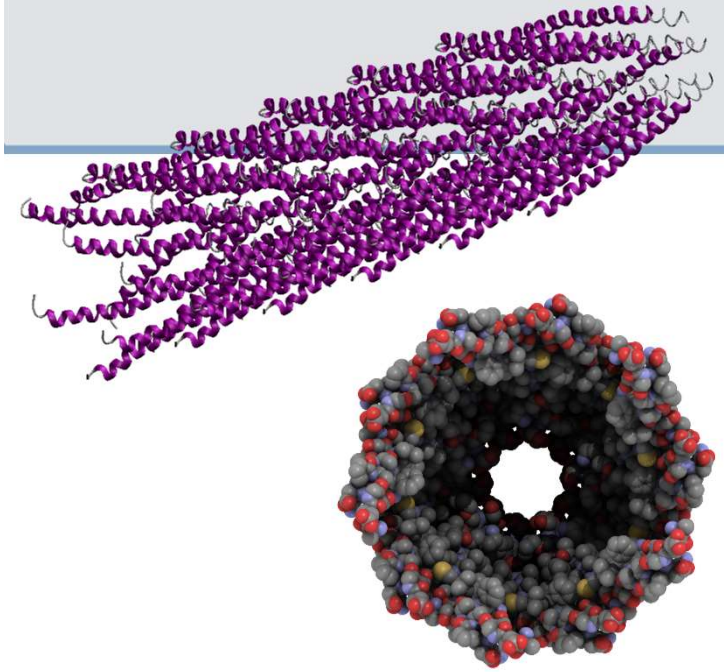

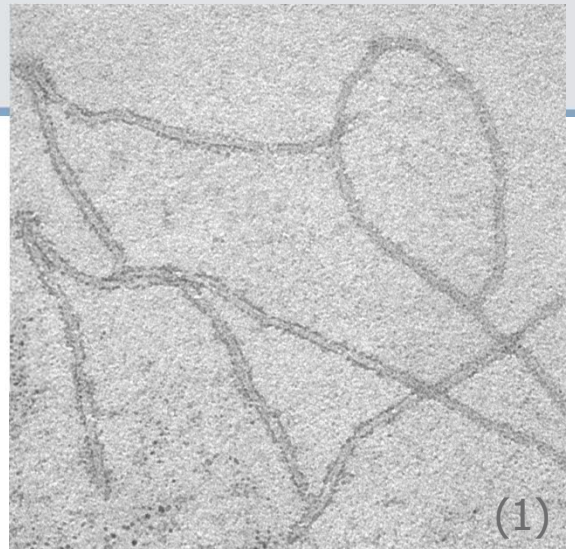

Massachusetts Institute of Technology

- 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

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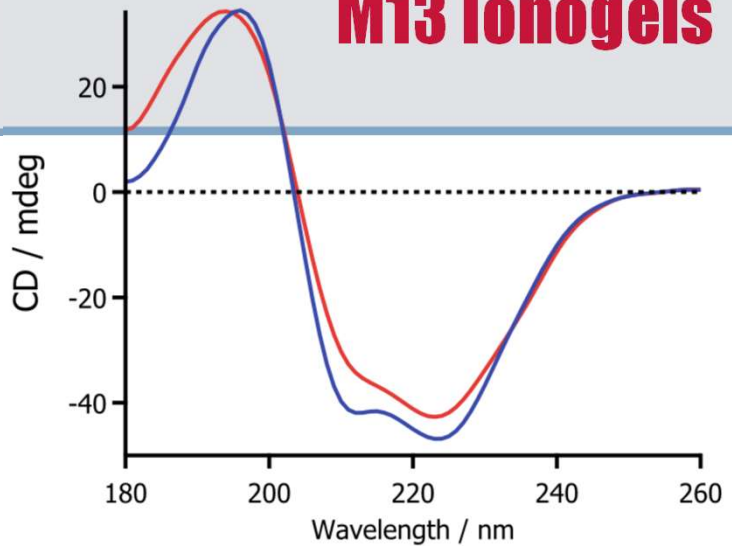
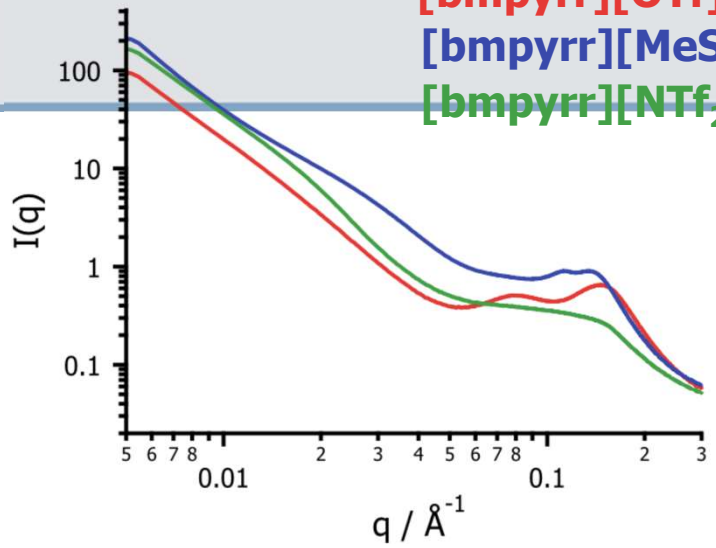
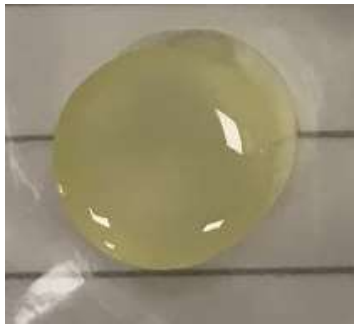
M13 Ionogels

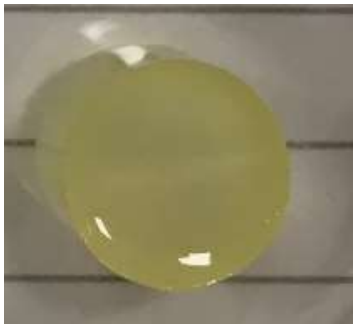


[bmpyrr][OTf]
[bmpyrr][$MeSO_4$]
[bmpyrr][NTf_2]



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





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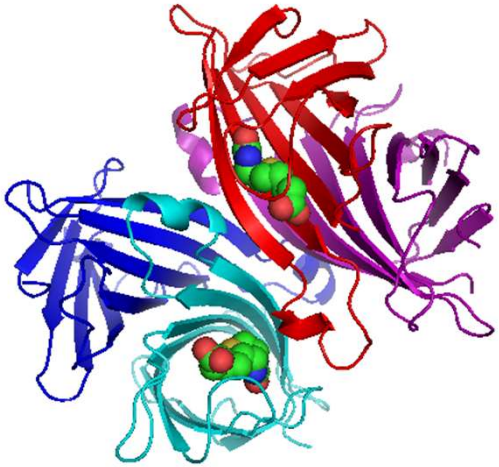
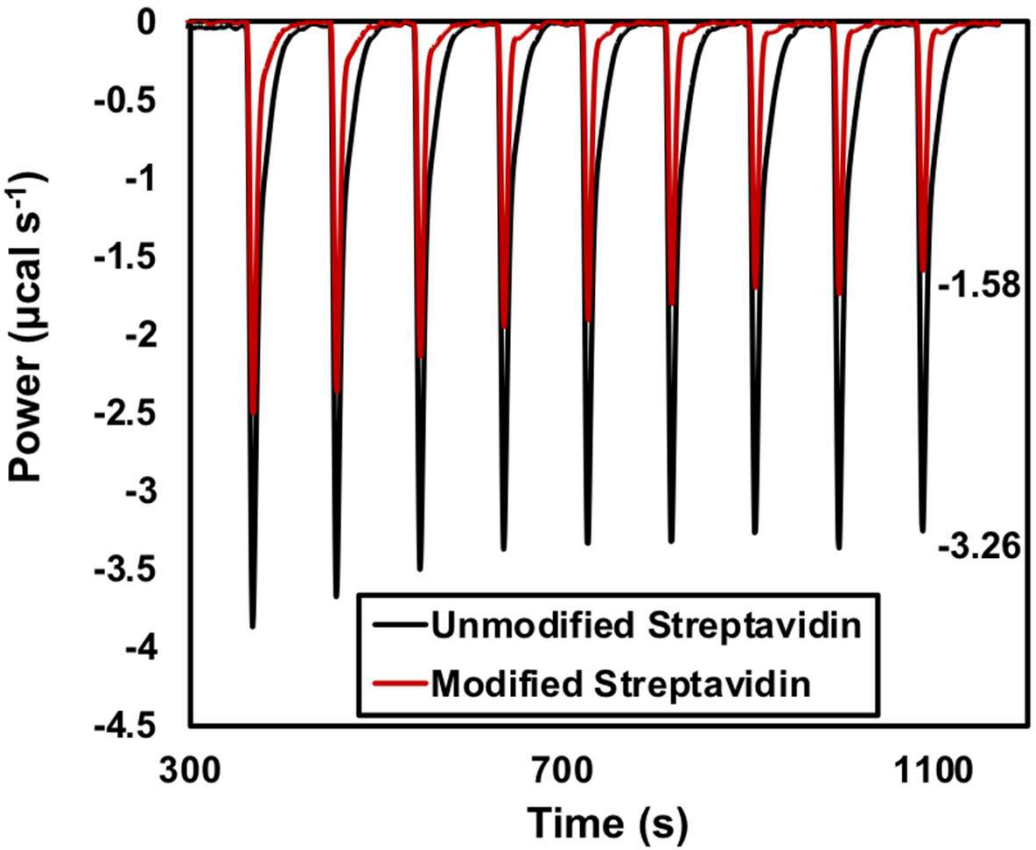
Binding studies: Antibodies

- **Strongest** non-covalent interaction
- **46 % activity** compared to native after **10 injections**



Streptavidin bound to 2 biotin molecules



Isothermal calorimetry data comparing the heat of binding for the unmodified and modified streptavidin with iminobiotin at pH 9.5

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Contacts

- Nanoparticle delivery vehicles
- Freeze dried formulations
  - rongjun.chen@imperial.ac.uk
- Ionic liquids for thermo-stabilisation
  - j.hallett@imperial.ac.uk

Thank you for your attention