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Future Vaccine Manufacturing Research Hub

Technologies for Vaccine Delivery and Therostabilisation

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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

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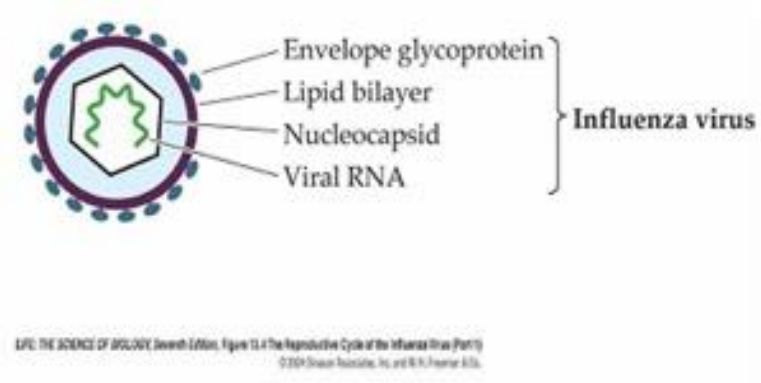
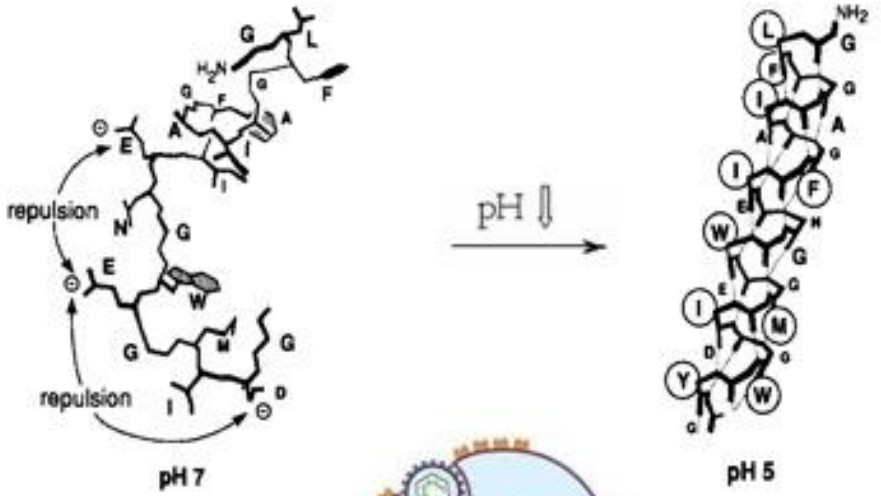
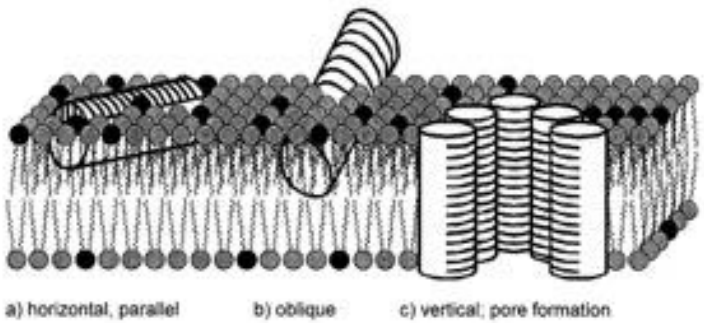
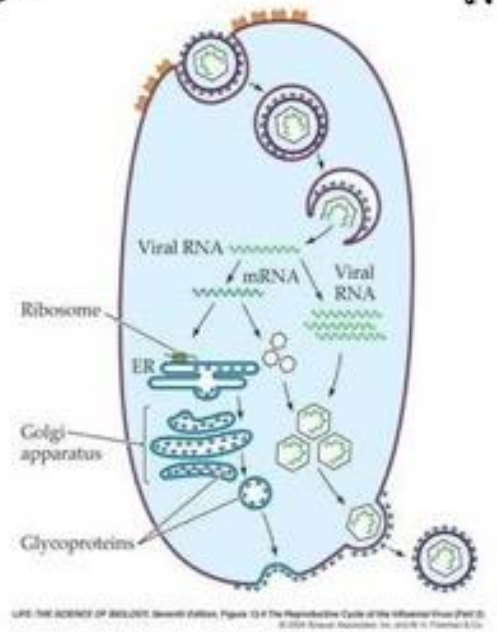
- **Potential outcomes**
  - ✓ Flexible and robust platforms for improved stability and efficacy of vaccines
  - ✓ Manufacturable formulations with optimised biostabilisation during storage

Inspiration from reproductive cycle of influenza virus

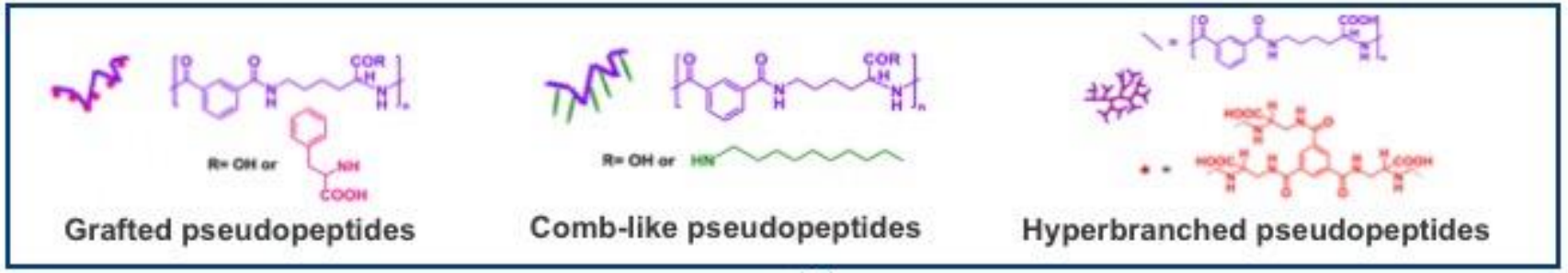


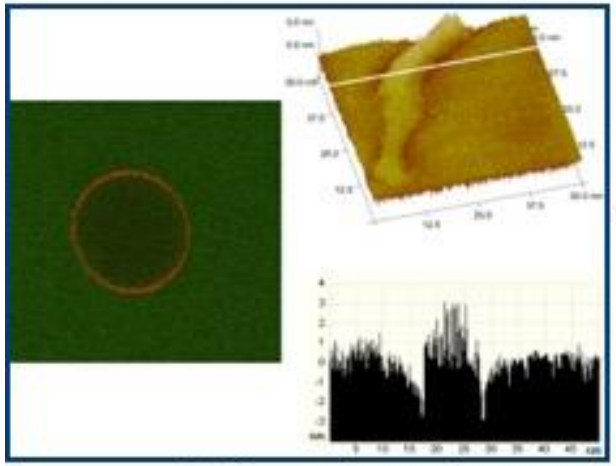




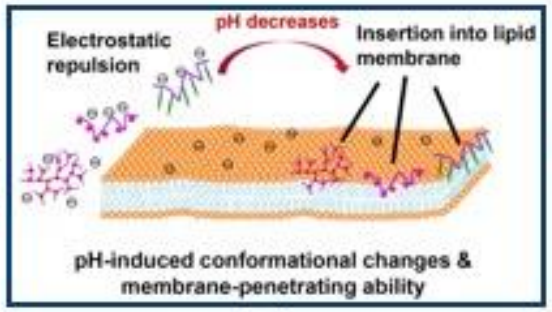


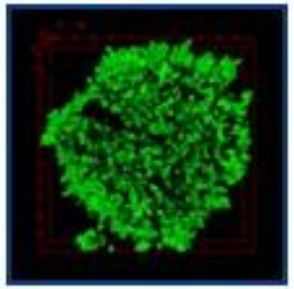
Viral peptide-mimicking, pH-responsive, pseudopeptides as delivery vehicles



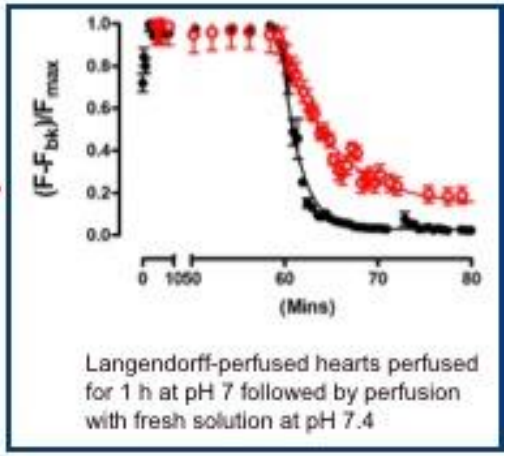


Delivery through vesicular/cellular membranes



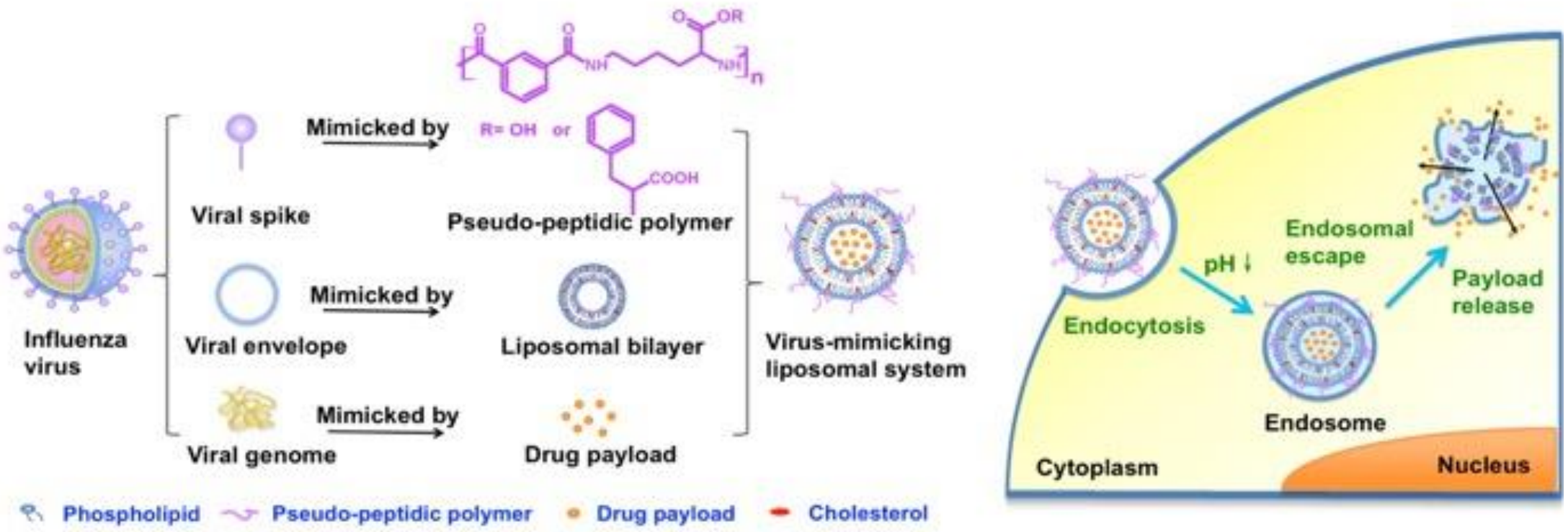


Delivery to cell spheroids



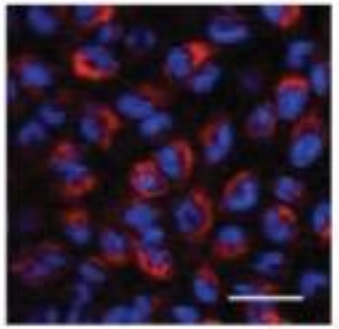
Delivery to organs

Virus-like nanoparticles as delivery vehicles

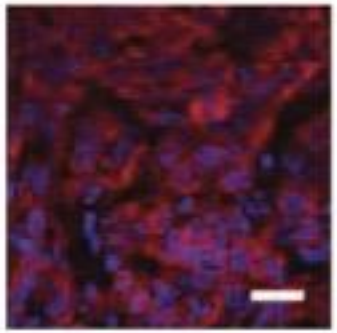


Intracellular delivery of biological molecules

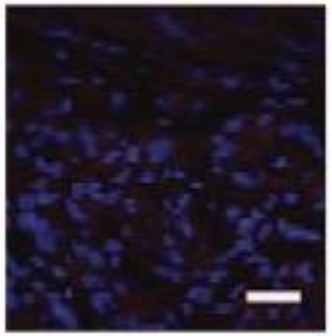
- **RNA delivery**



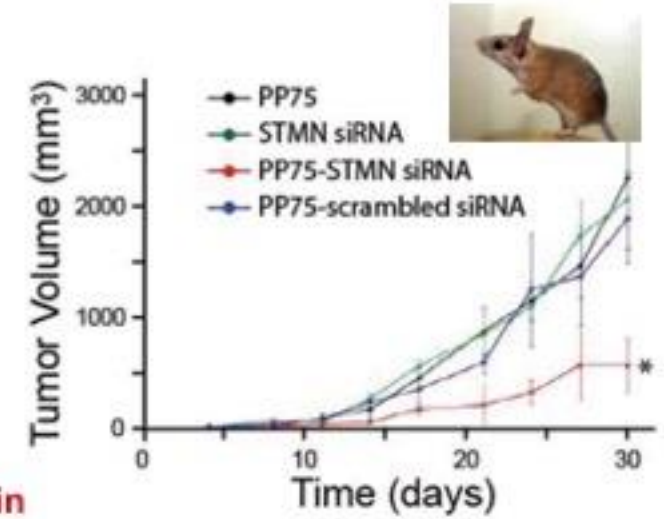
Cytoplasmic siRNA delivery



Negative Control

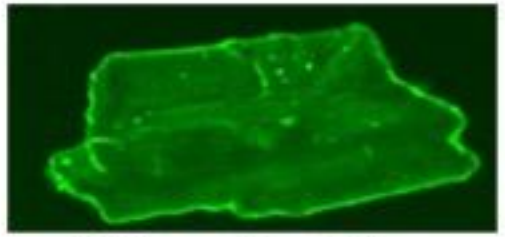


Knockdown of stathmin via siRNA delivery

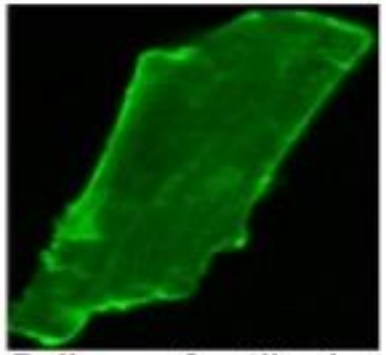


Combination therapy

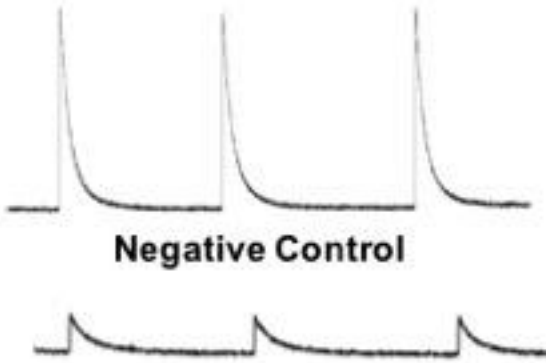
- **Protein delivery**



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



Delivery of antibody
FITC-IgG (160 kDa)



Negative Control

Delivery of functional peptide
CamBP (3.5 kDa)

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Key challenges and opportunities 2

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- **Manufacturing and storage challenge**
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- **Opportunities**
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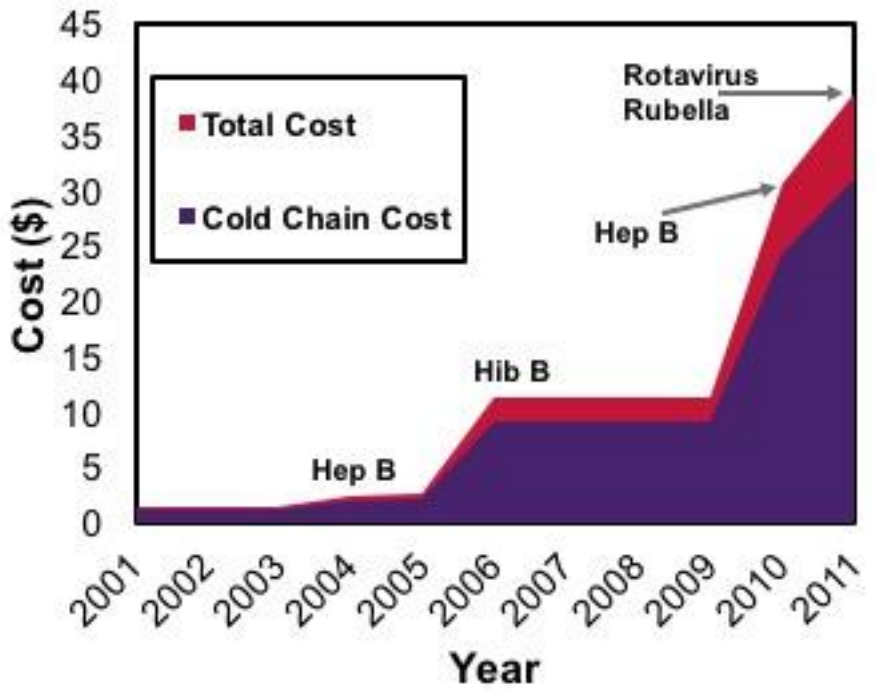
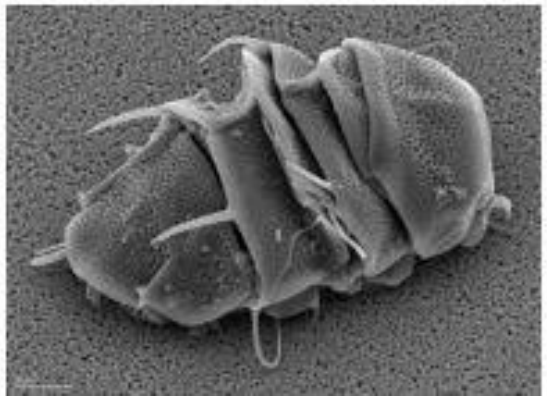
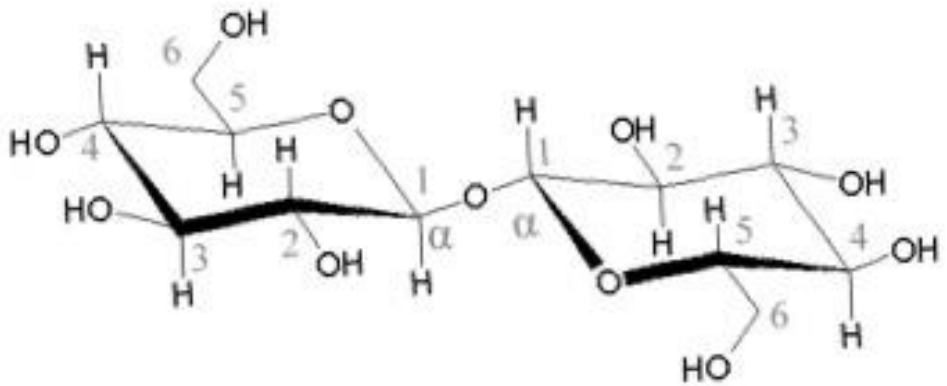
Cold Chain

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



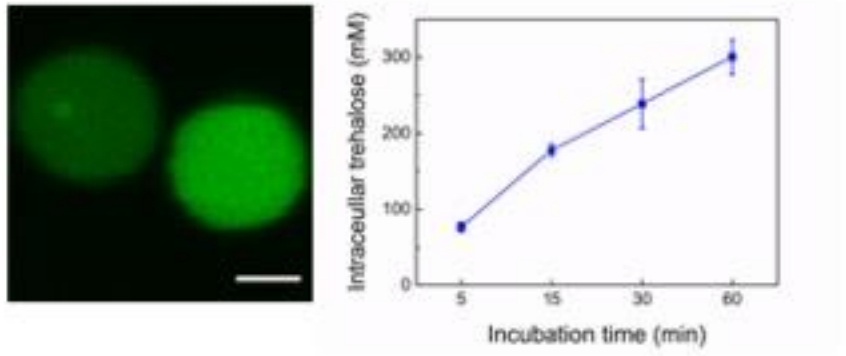
Inspiration from anhydrobiotic organisms



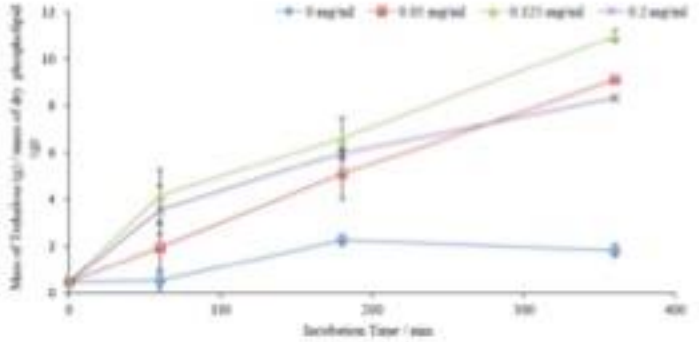


- Trehalose: non-toxic disaccharide of glucose
- Protection during freezing and drying
- Antioxidant

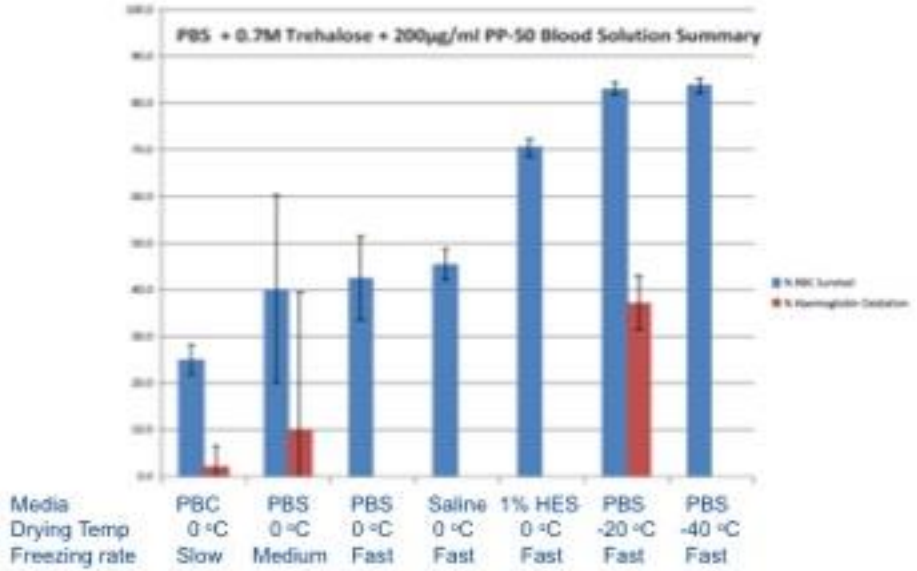
Heat-stable formulations (nanoparticle- & cell-based)



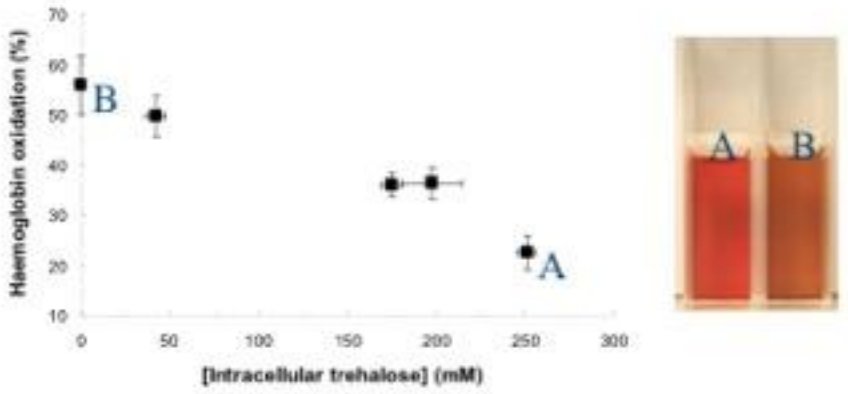
Trehalose loading into cells



Trehalose loading into virus-like liposomes



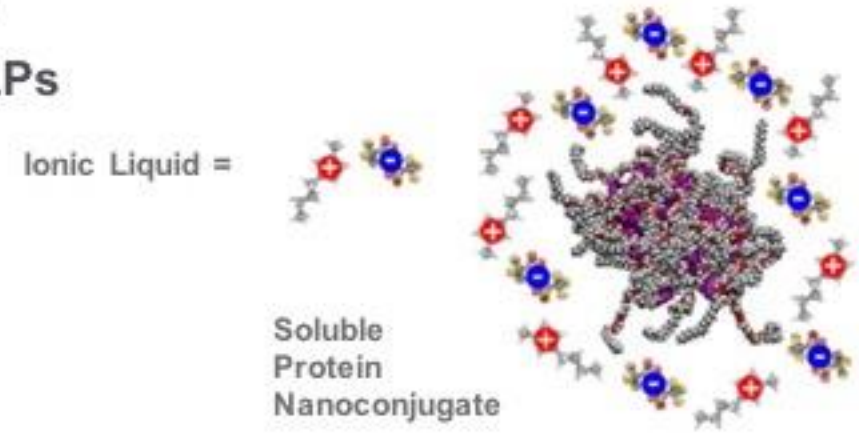
Freeze drying of red blood cells



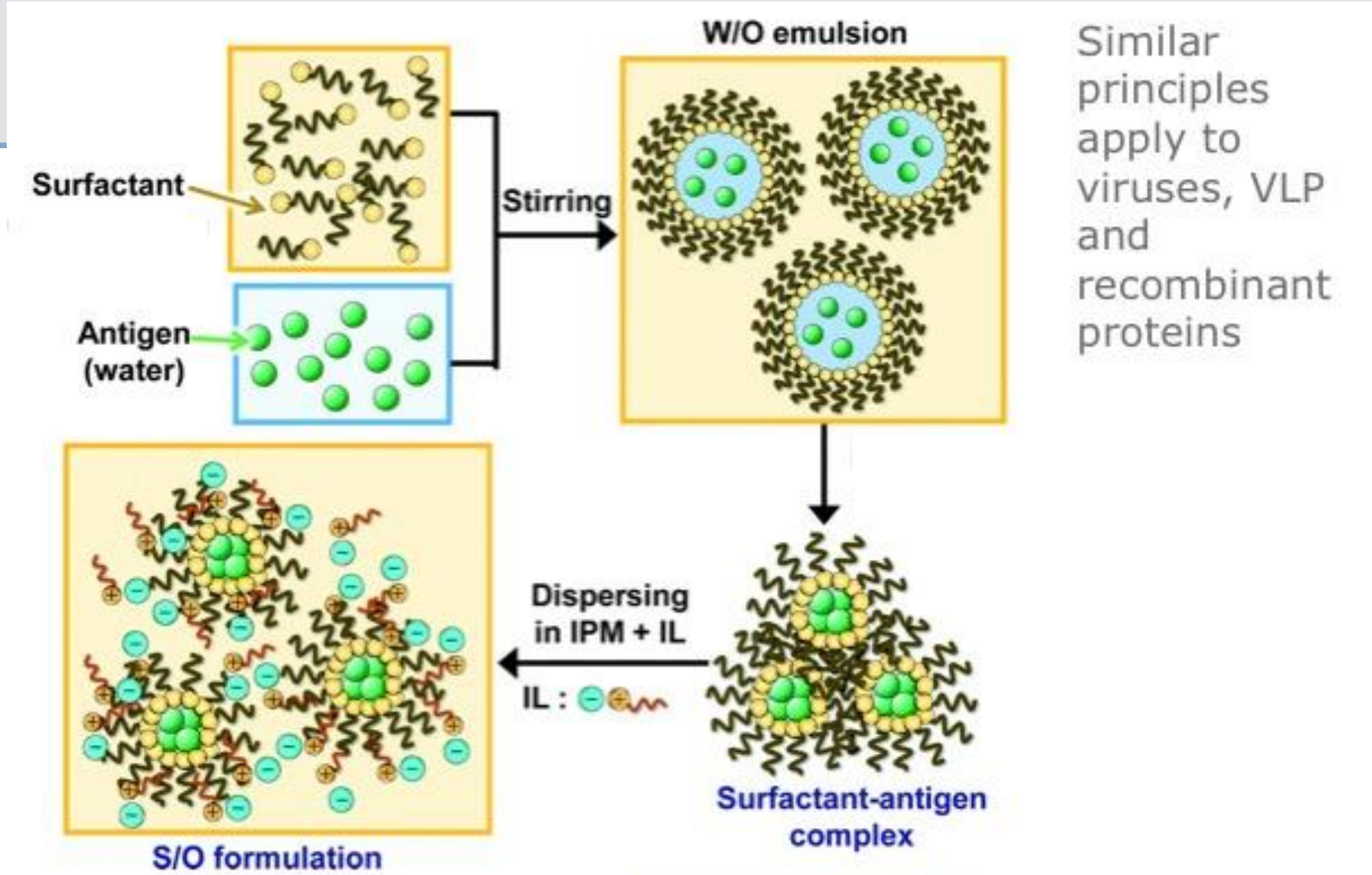
Freeze Prevention of haemoglobin oxidation in dried storage

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

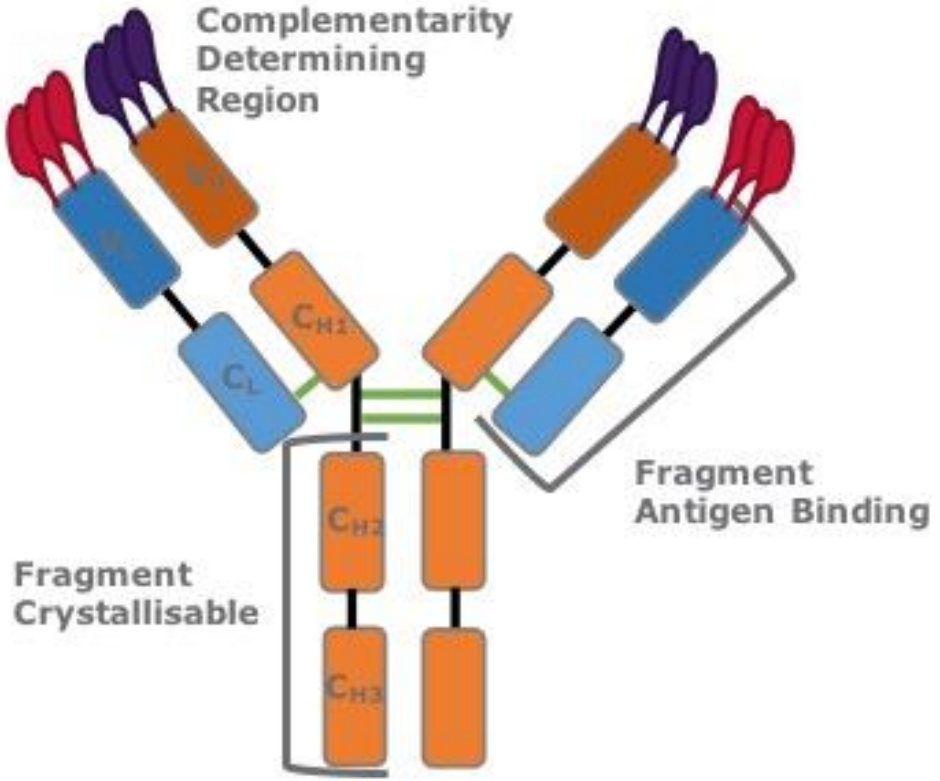
STAGE 2
Viruses

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

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

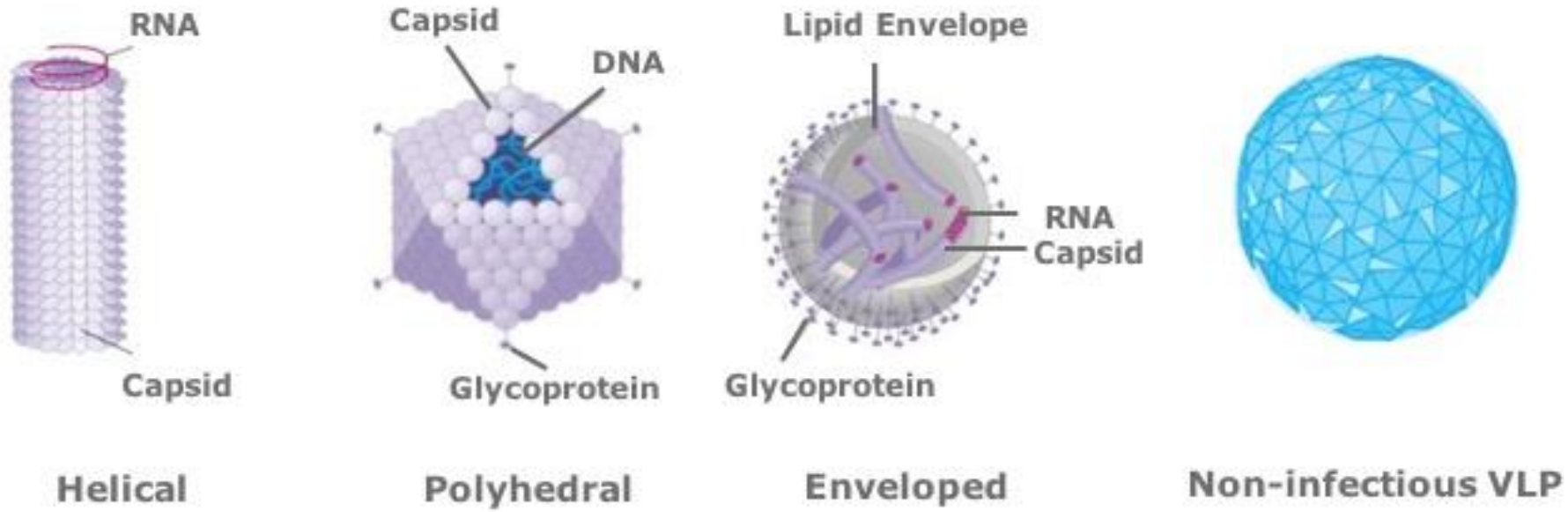


Monomer Y-shaped structure of antibodies, where V and C represents the variable and constant region. Subscripts L and H represent the light and heavy chains.[1]

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

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

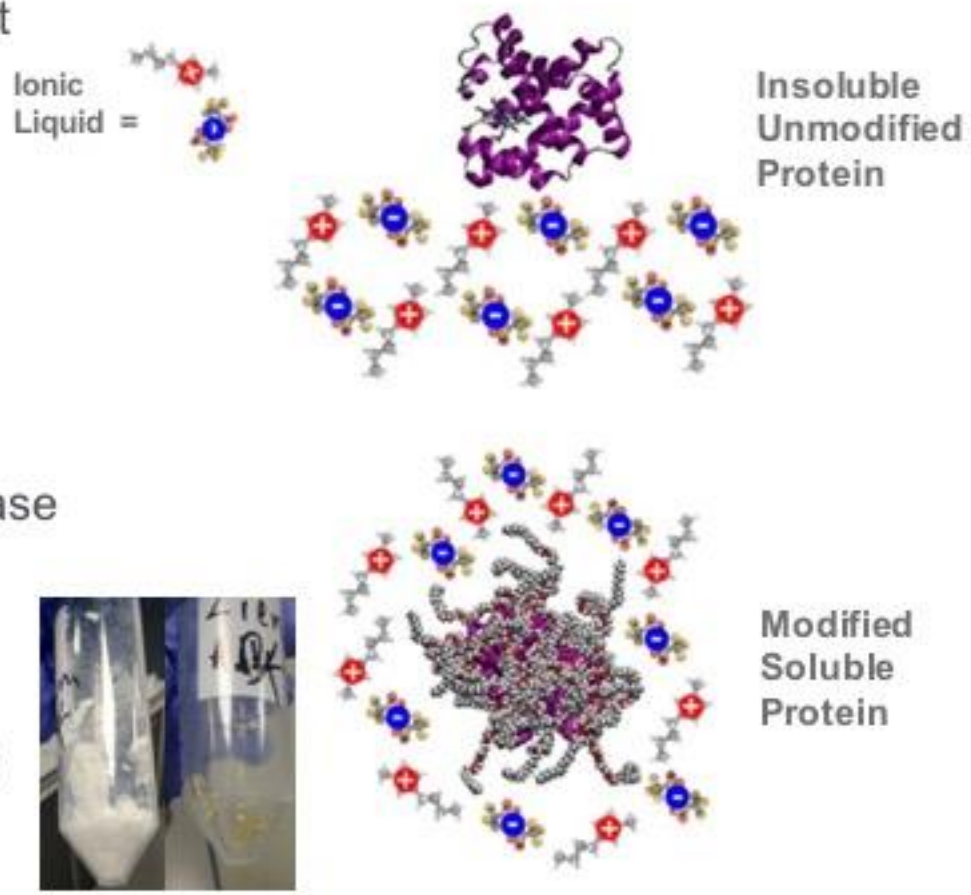


Different Viral Structures[1]

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

Proteins in Ionic Liquids

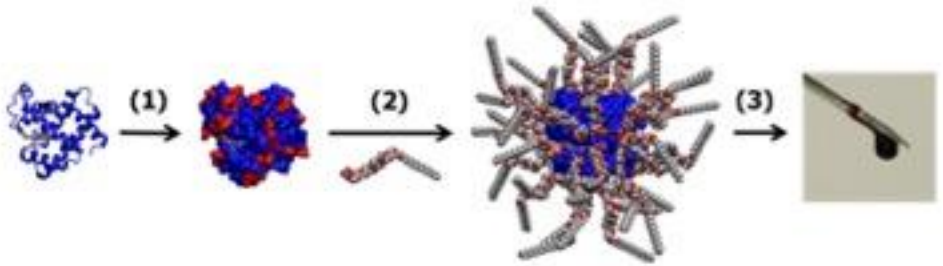
- Proteins are poorly soluble in neat ionic liquids
- Adding polymer–surfactant to the protein surface produces liquid proteins
- **Retains biological activity** of proteins, enzymes and viruses
- Modified myoglobin and glucosidase dissolved in hydrophilic and hydrophobic ionic liquids
- Increased protein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



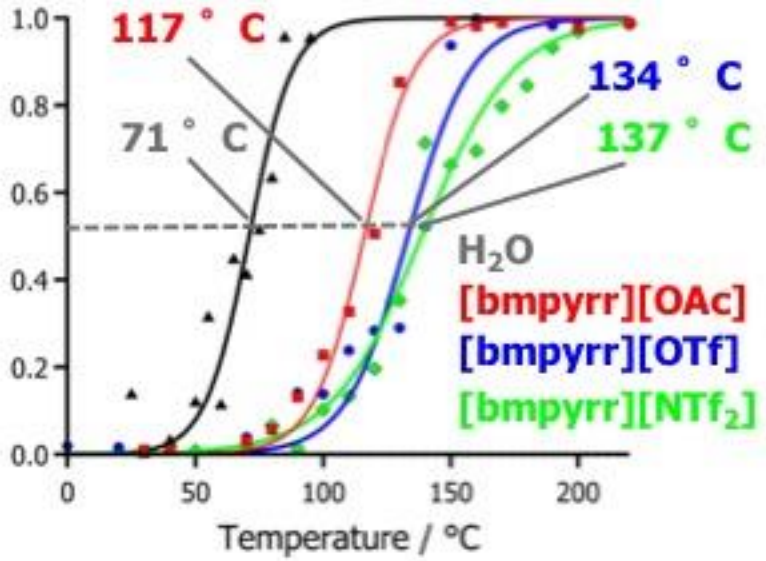
Modified proteins to allow dissolution in ionic liquids[1,2]

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

Protein stability in Ionic Liquids



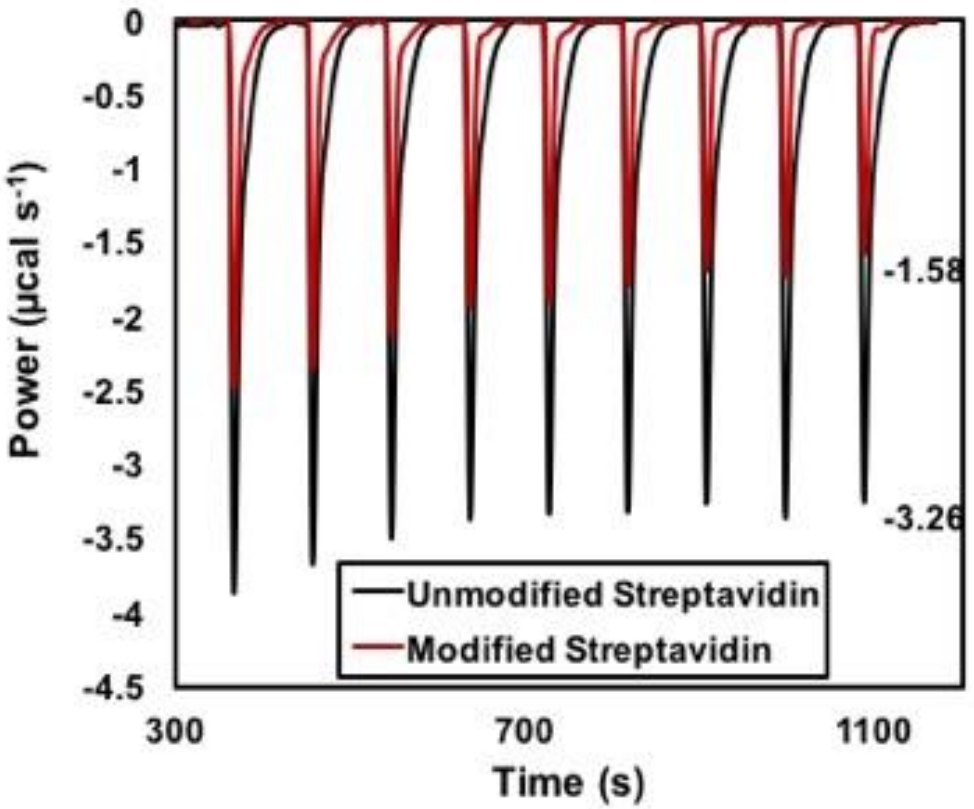
- UV/Vis shows retention of structure in all conditions
- SRCD indicated ionic liquids induced α-helicity
- Thermal stability of proteins increased significantly in ionic liquid



J. Am. Chem. Soc. 2016.

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
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- Ionic liquids for thermo-stabilisation
  - j.hallett@imperial.ac.uk

Thank you for your attention