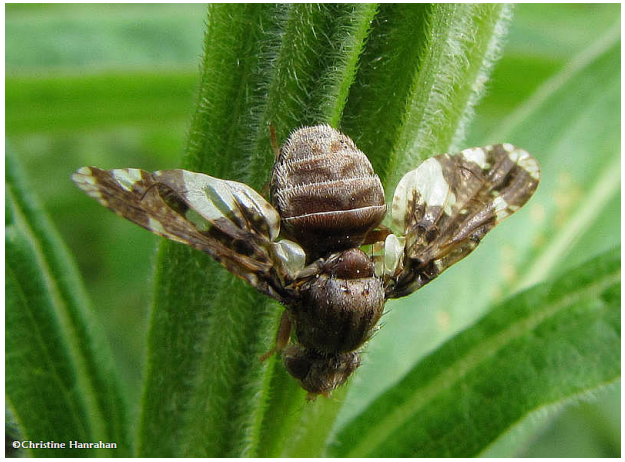
Poly-Vitrification

Vision:

Polymerization enables rapid, safe vitrification.



Steps

Starting point:

Insect fructose-glycerol-trehalose system.

1.) Enable penetrating fructose.

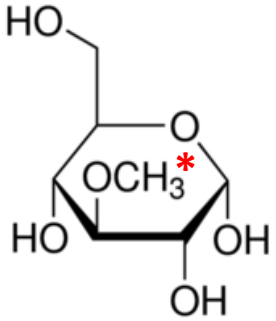
2.) Explore other sugars/derivatives for polymerization properties.

3.) Test fructose as CPA additive.

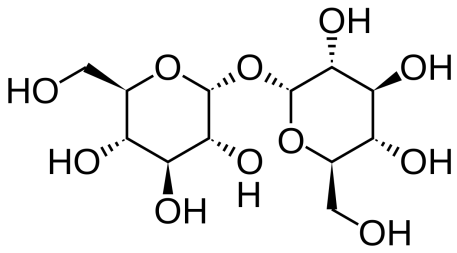
Approaches to increasing T_g

Available Sugars

- Analyze mixtures of available sugars and derivatives
  - Reduced toxicity
  - Increased Tg



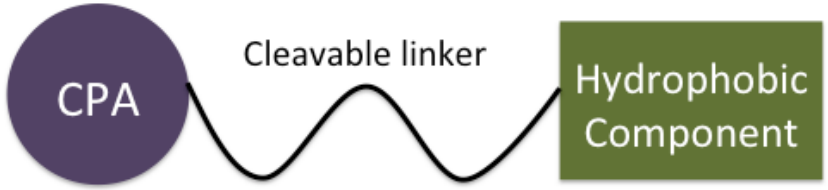
3-o-methylglucose

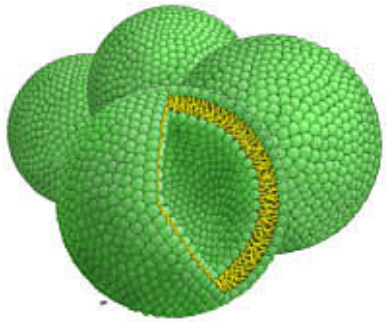


trehalose

Loading Methods

- Sugar loaded liposomes or micelles
  - Could work for low concentration sugars
- Enzymatically triggered CPA-delivery platform





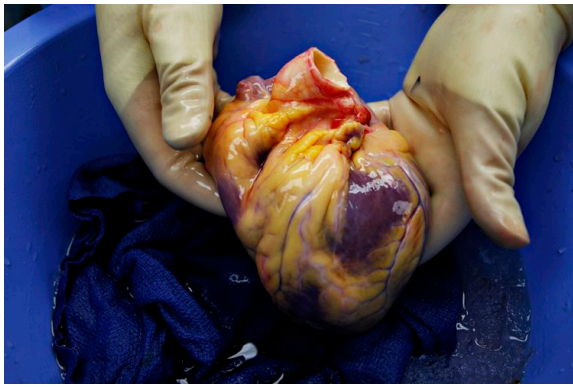
The Team: Diverse Expertise

Peter Kilbride
*Univ. College London
*Large Volume Cryopreservation

Robert McIntyre
*MIT, 21CM
*Cryobiologist

Rebecca Sandlin, PhD
*Harvard Medical School
*Chemist

Navid Manuchehrabadi, PhD
*University of Minnesota
*Biomechanical Engineer



Impact – Solves primary vitrification problems.

Merits

Feasibility – Uses established procedures.

Innovation– Fast-cooling to fast-vitrification.

The Hack – Rapidly increase viscosity at a high temperature.

References

1.) Insects at Low Temperature, Richard E. Lee Jr., David L. Denlinger, ISBN:978-1-4757-0192-0

2.) Vitrification as an approach to cryopreservation. Fahy, Cryobiology. 1984 Aug;21(4):407-26.

3.) Viscosity of cryoprotective agents near glass transition: a new device, technique, and data on DMSO, DP6, and VS55, Noday, Exp Mech. 2009 Oct;49(5):663-672.

4.) Partial glass formation: A novel mechanism of insect cryoprotection, Wasylyk, Tice & Baust, Cryobiology 10/1988; 25(5):451-458.

5.) Glass Transition Temperature of Glucose, Sucrose, and Trehalose: An Experimental and in Silico Study, Simperler et al, J. Phys. Chem. B 2006, 110, 19678-19684

Contributions

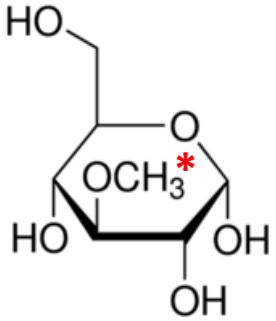
- Developed concept of **poly-vitrification**, a process by which a CPA forms polymers at low temperatures. This allows us to use very low amounts of cryoprotectants, and reduce exposure to toxic CPAs.

- Developed **plan** to create a poly-vitrification CPA by using modern chemical methods to rapidly assay potential fructose-sucrose-trehalose analogues, or to adapt fructose itself to our needs.

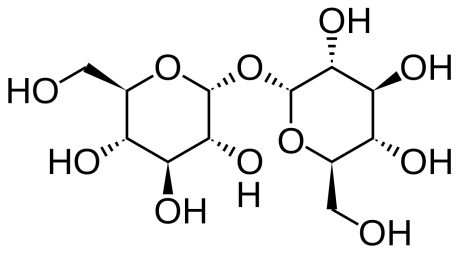
Approaches to increasing T_g

Available Sugars

- Analyze mixtures of available sugars and derivatives
  - Reduced toxicity
  - Increased Tg



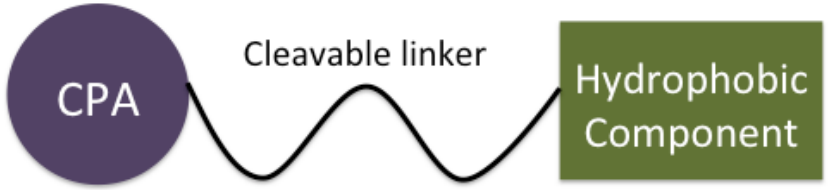
3-o-methylglucose

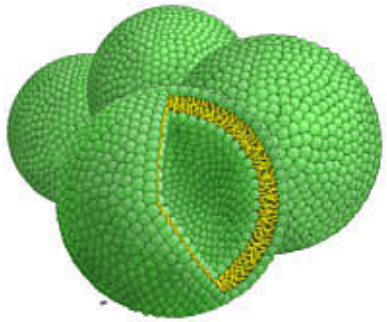


trehalose

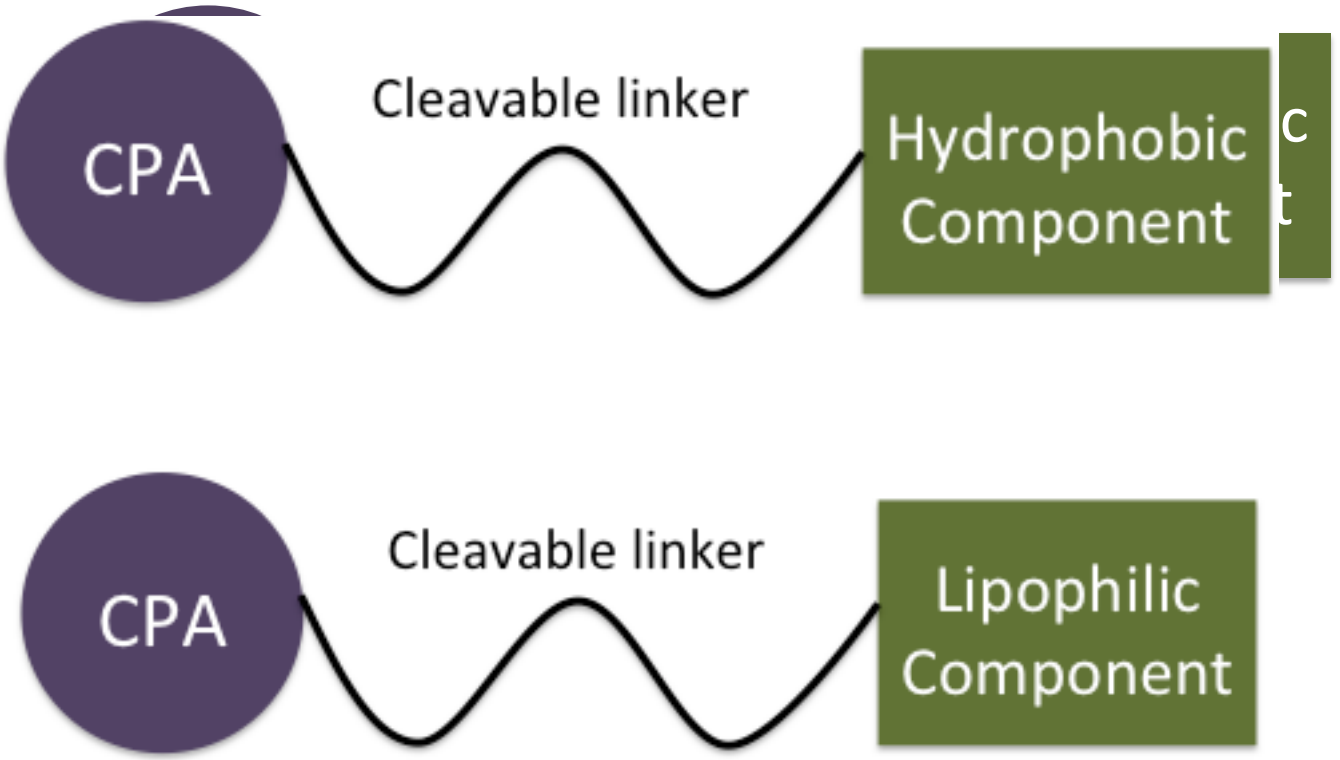
Loading Methods

- Sugar loaded liposomes or micelles
  - Could work for low concentration sugars
- Enzymatically triggered CPA-delivery platform

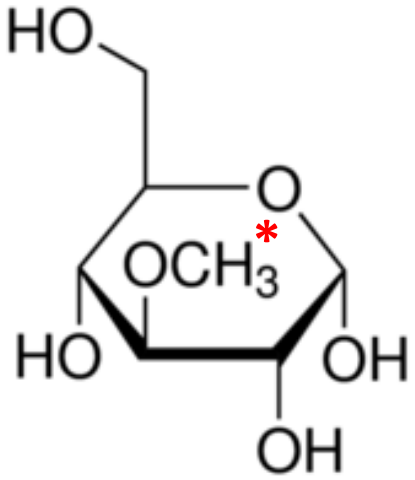




Evaluation of glass forming mixtures



Effects of structural changes on activity



3-o-methylglucose

Additive	Glass Transition (°C)
Glucose	
PVP*	-20
Sucrose	
Trehalose	
Fructose	
3-o-methylglucose	
DMSO*	-132
Propylene Glycol*	-109
M22	-110
Glycerol*	-114

-25 to -45 range at .

*at minimum concentration needed to vitrify, sucrose 86%,