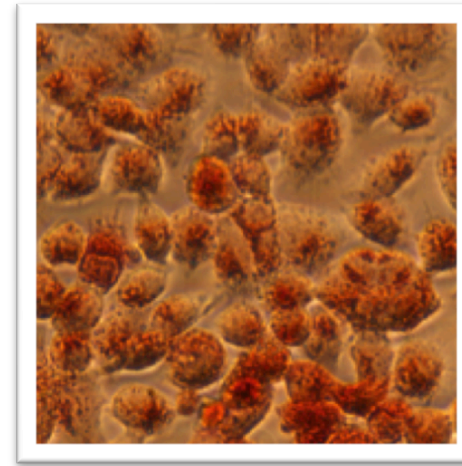
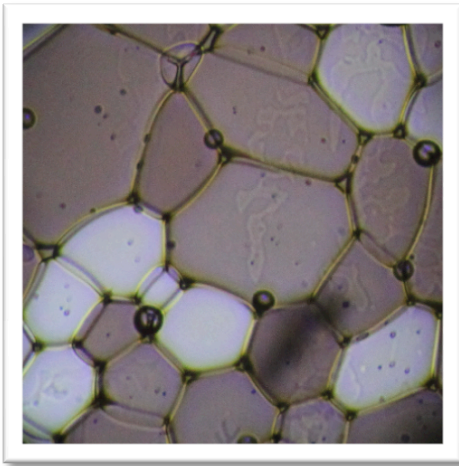
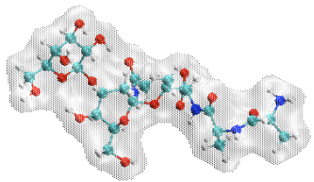


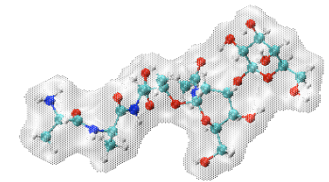
Peptidomimetic Approaches to Mimicking Antifreeze Protein Function



**Robert C. Deller, Manu Vatish, Daniel A. Mitchell
& Matthew I. Gibson**

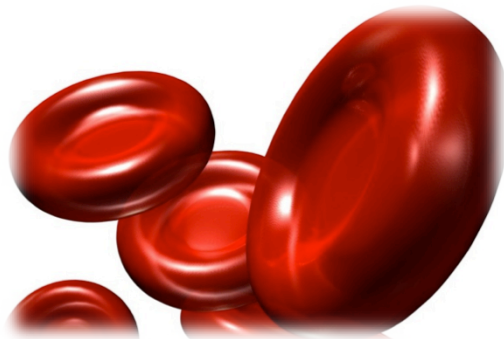


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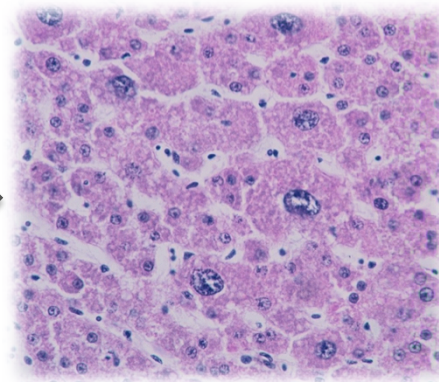
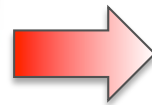
Novel cryopreservation methods

- ⊙ There is a real need for improvements in the cryopreservation of biological materials.
- ⊙ Ice recrystallisation during freeze/thawing of cells is a major contributor to cell damage during cryopreservation.



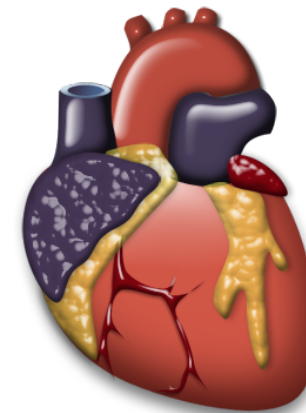
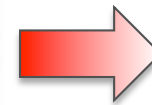
Cells

e.g. Red blood cells (42 days)



Tissues

e.g. Bone marrow tissue (72 hours)

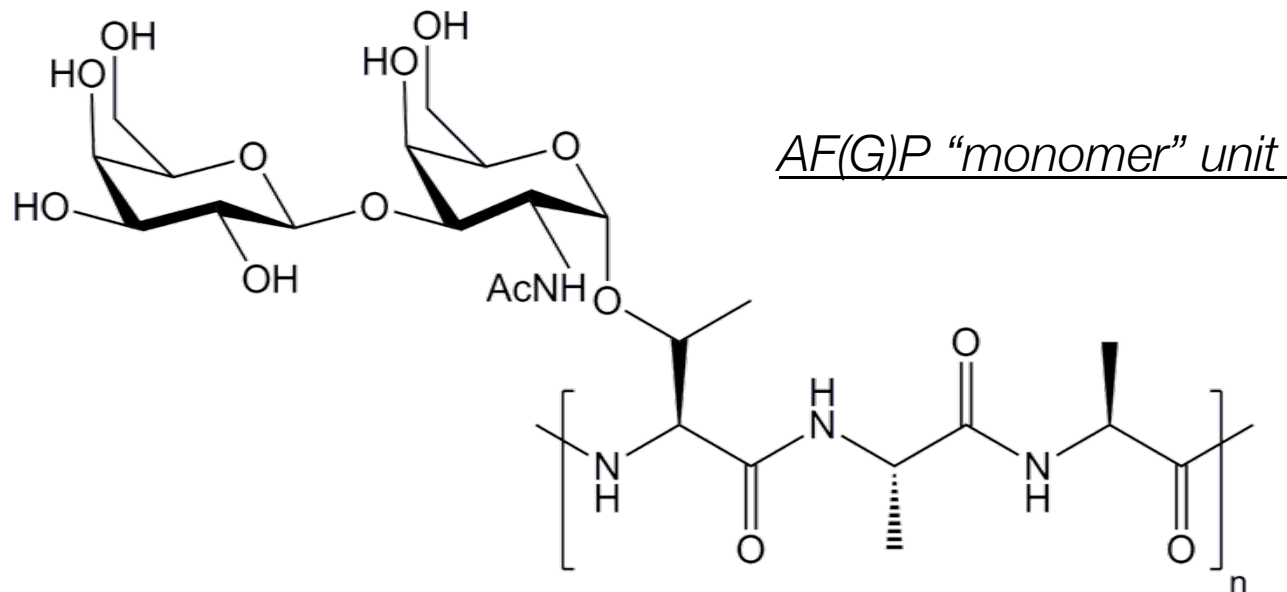


Organs

e.g. Heart (as soon as possible)

Antifreeze (glyco)proteins

⊙ Antifreeze (glyco)proteins (AF(G)Ps) are a naturally occurring class of proteins found in cold-acclimatised species that have a simple polymeric structure.

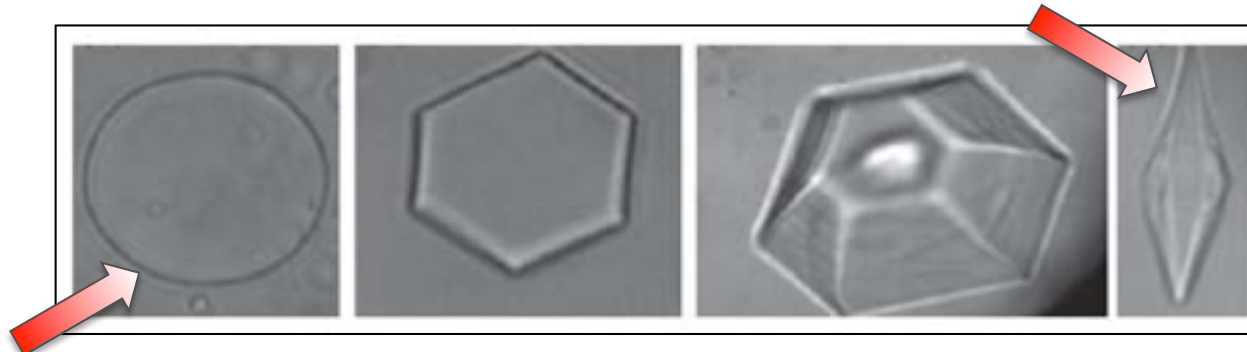


AF(G)P structure. An AAT tripeptide backbone ($n=4-55$) with an O-linked disaccharide (β -D galactosyl-(1,3)- α -N-acetyl galactosamine).

Properties of AF(G)Ps

- ⊙ AF(G)Ps display a strong recrystallisation inhibition (RI) activity. ✓
- ⊙ AF(G)Ps exhibit a thermal hysteresis (TH) activity. 🌀
- ⊙ AF(G)Ps incite dynamic ice shaping (DIS). ✗

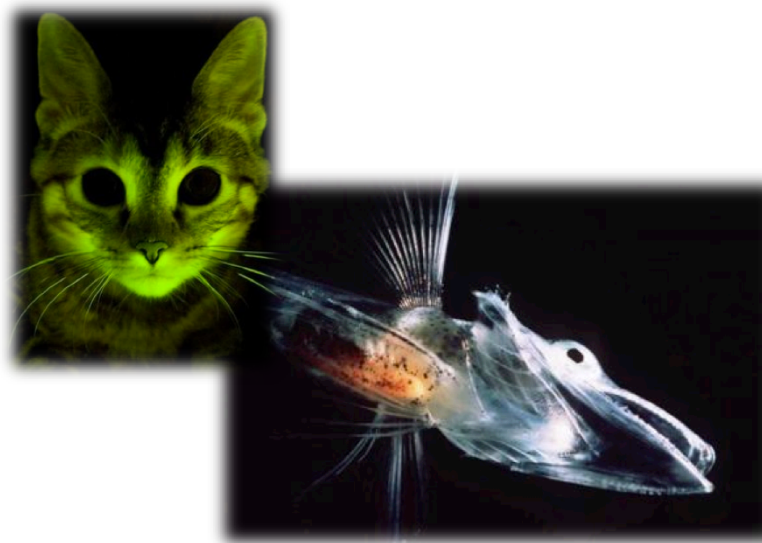
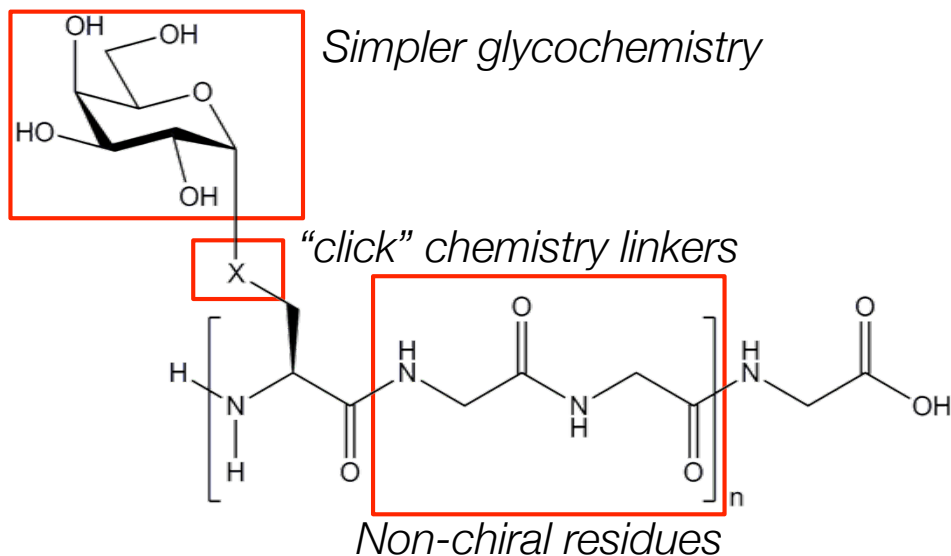
Damaging “needle-like” ice crystal



Native ice crystal morphology

Limitations of AF(G)Ps

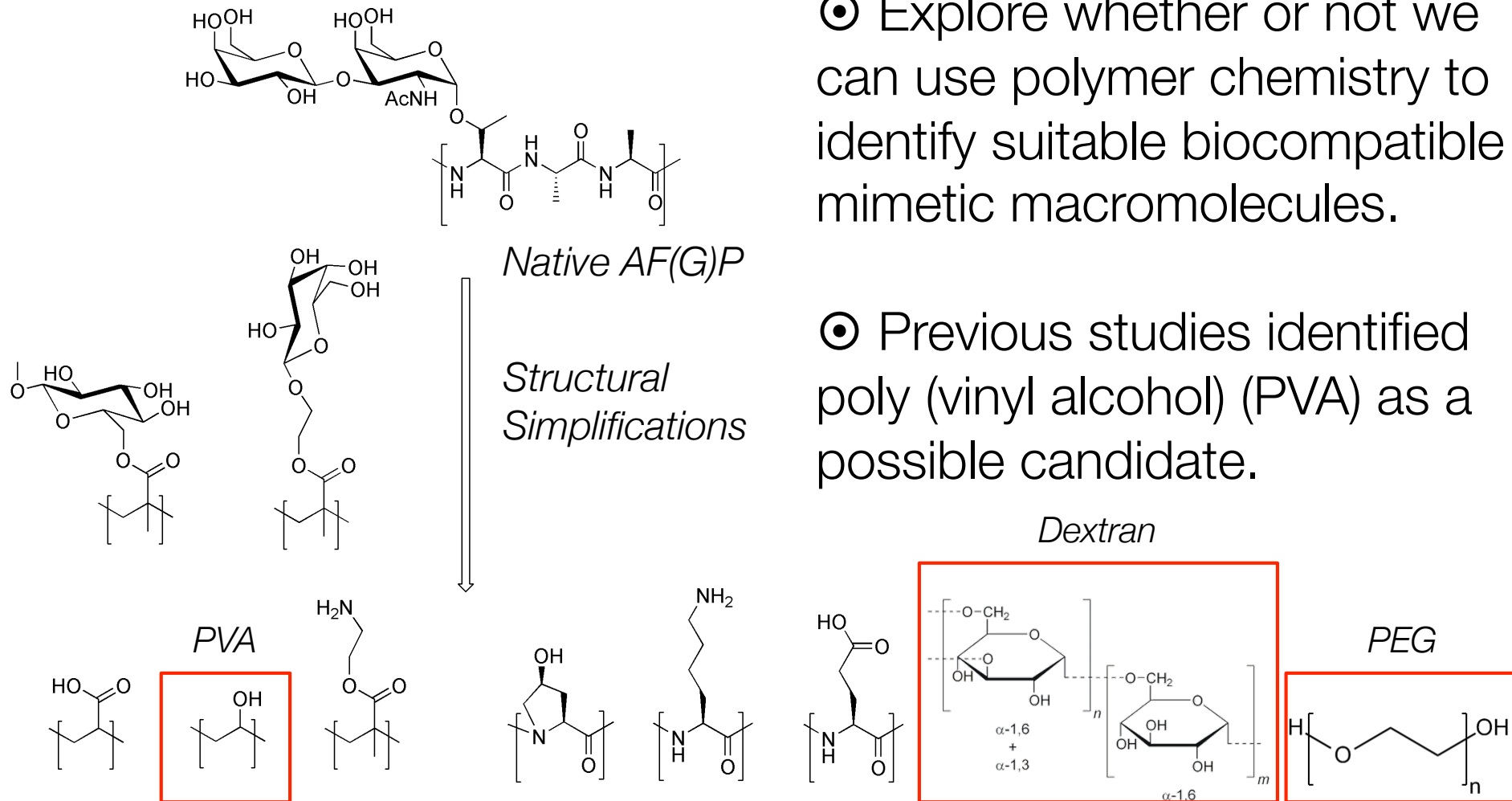
- ⊙ The isolation of AF(G)Ps in significant quantities is highly demanding and financially unviable. Transgenic and synthetic approaches have had limited success to date.
- ⊙ The application of AF(G)Ps as cryoprotectants has had varied results with numerous tissue types.



Peptidomimetic Macromolecules

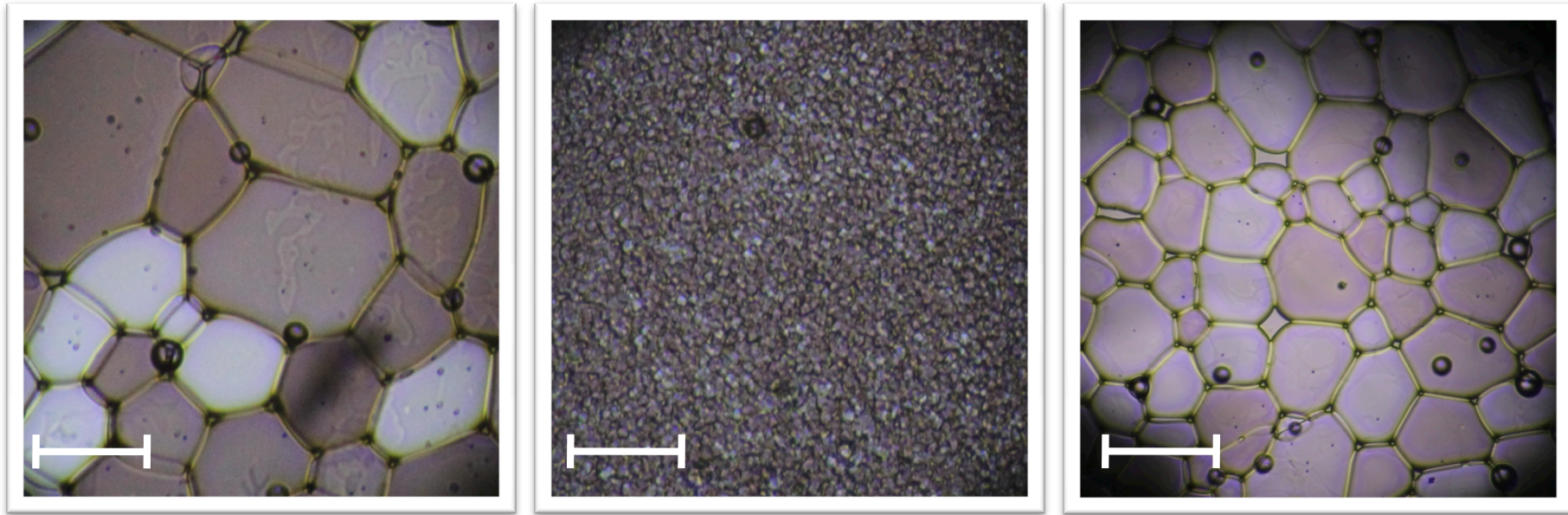
⊙ Explore whether or not we can use polymer chemistry to identify suitable biocompatible mimetic macromolecules.

⊙ Previous studies identified poly (vinyl alcohol) (PVA) as a possible candidate.



Physicochemical Properties

⊙ Perform a “splat” assay to define the mean largest grain size (MLGS) and quantify RI activity.

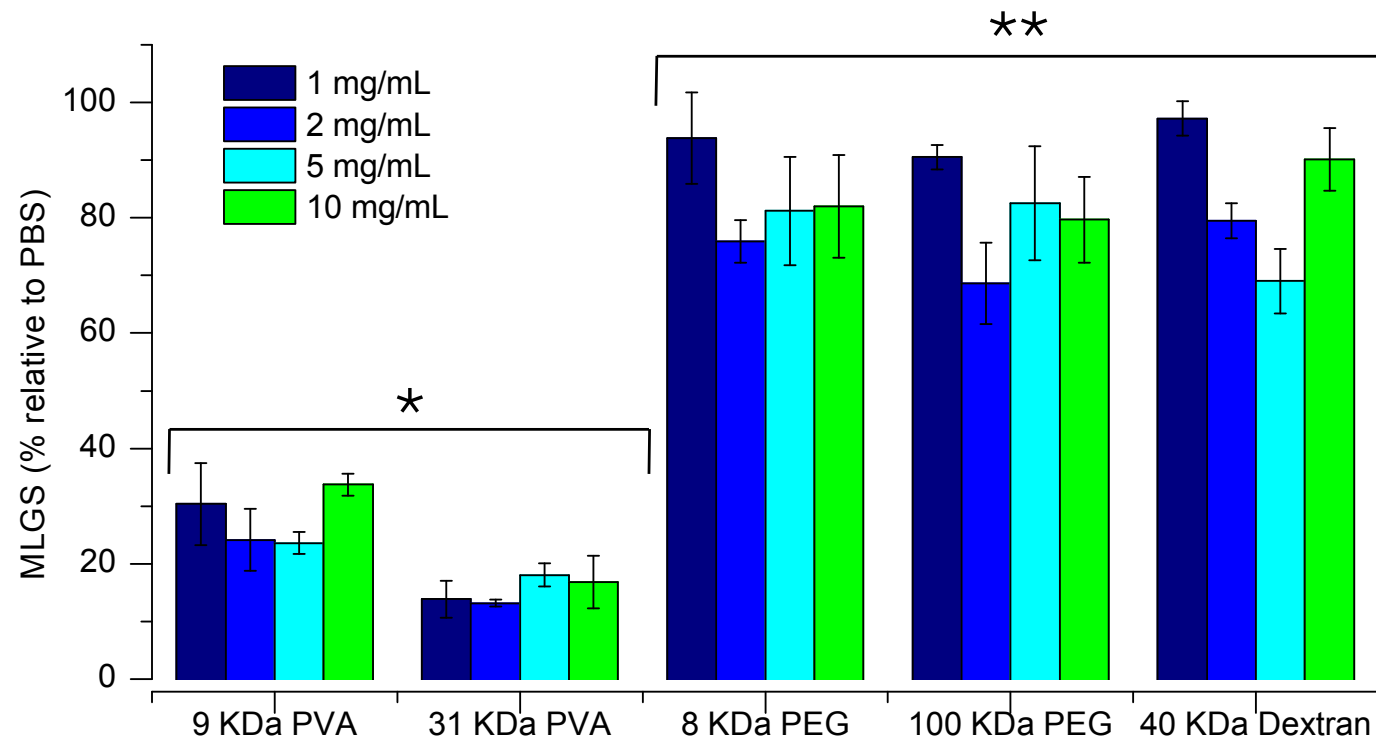


RI activity of PBS (left), 0.1 mgmL^{-1} 9 KDa PVA (centre) and 10 mgmL^{-1} 40 KDa Dextran (right.) Scale bars = $100 \mu\text{m}$.

Physicochemical Properties

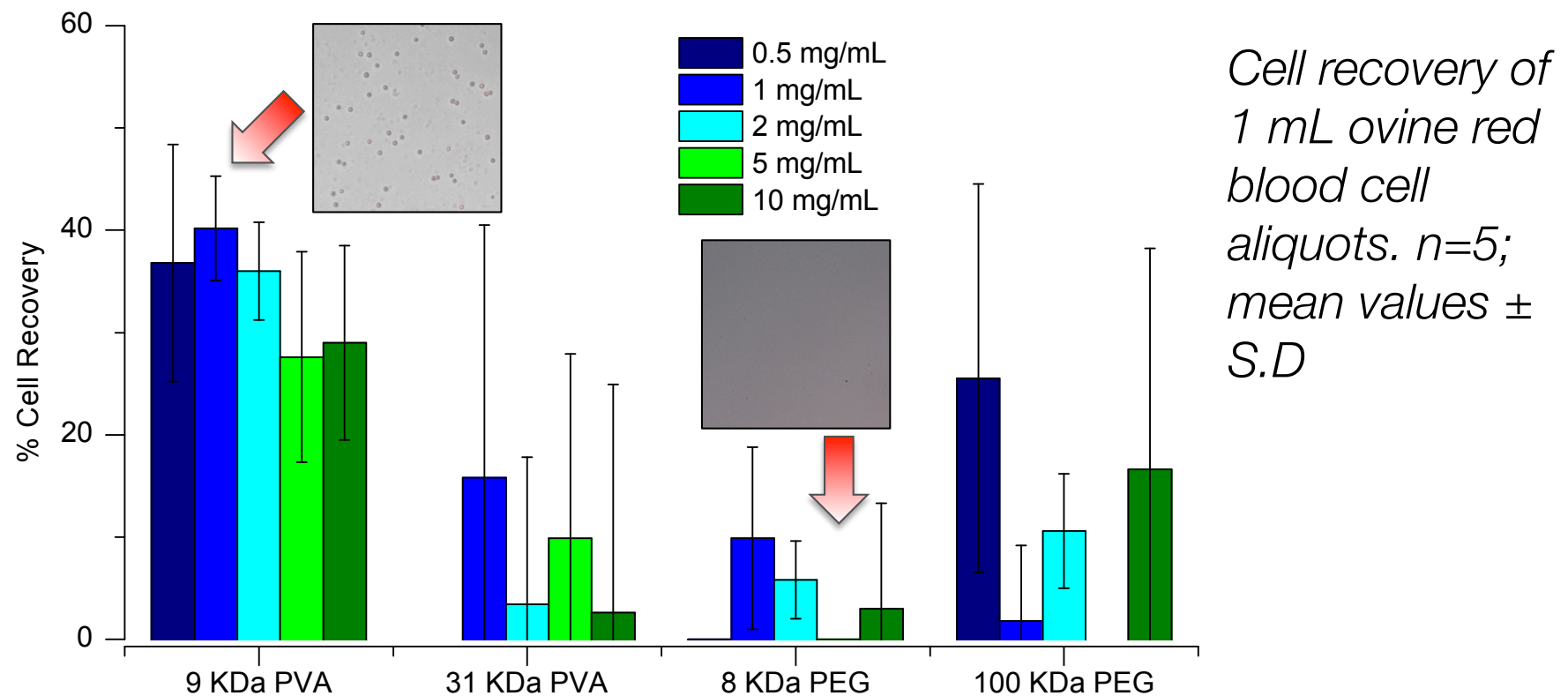
- ◎ PVA shows strong significant RI activity at both low and high molecular weights.

RI activity of several compounds relative to PBS. n=3; mean values ± S.D; time = 30 minutes.



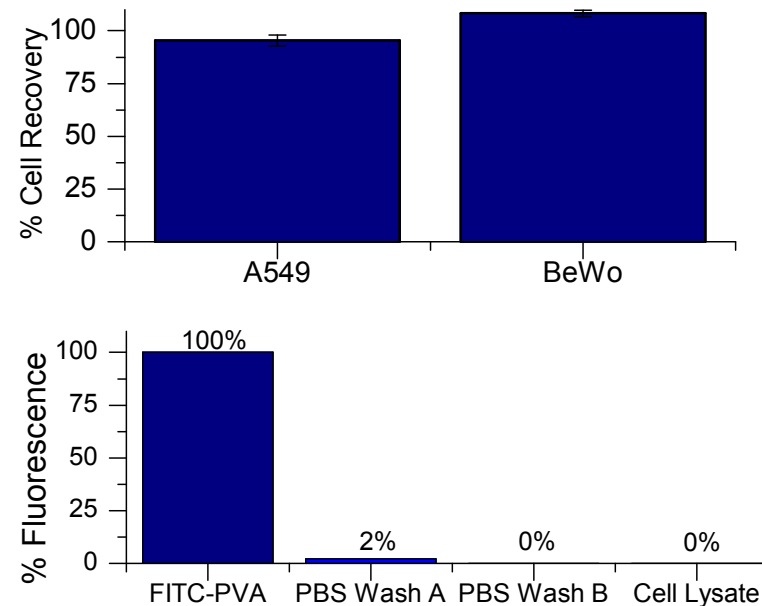
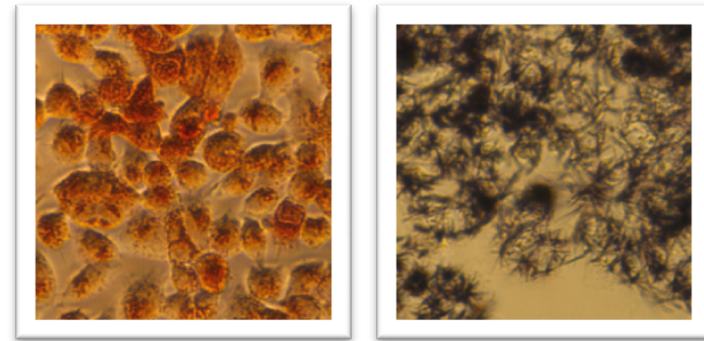
Biological Application

- ◎ PVA (0.1 wt%) has the ability to improve the cryopreservation of ovine red blood cells comparable to existing methodologies such as glycerol (40 wt%).



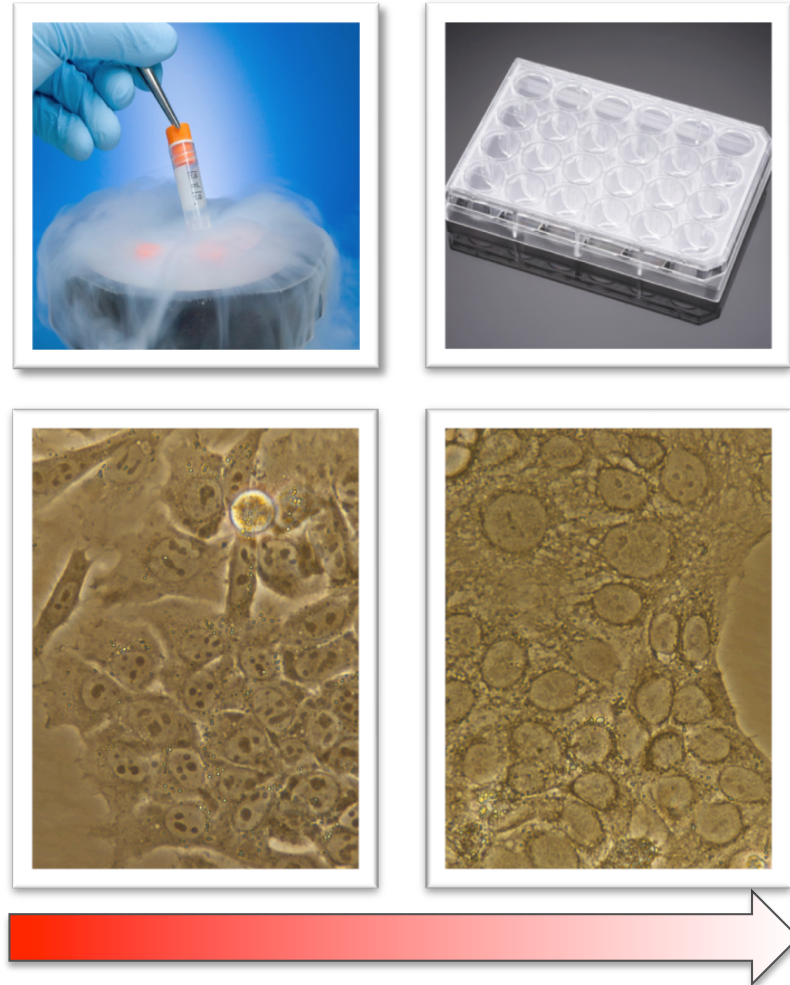
Biological Application

- ◎ 3 mammalian cell line varieties investigated for diversity.
- ◎ Minimal cytotoxic effect on cell structure and metabolism.
- ◎ FITC-labeled PVA impermeable to lung adenocarcinoma cells.



Biological Application

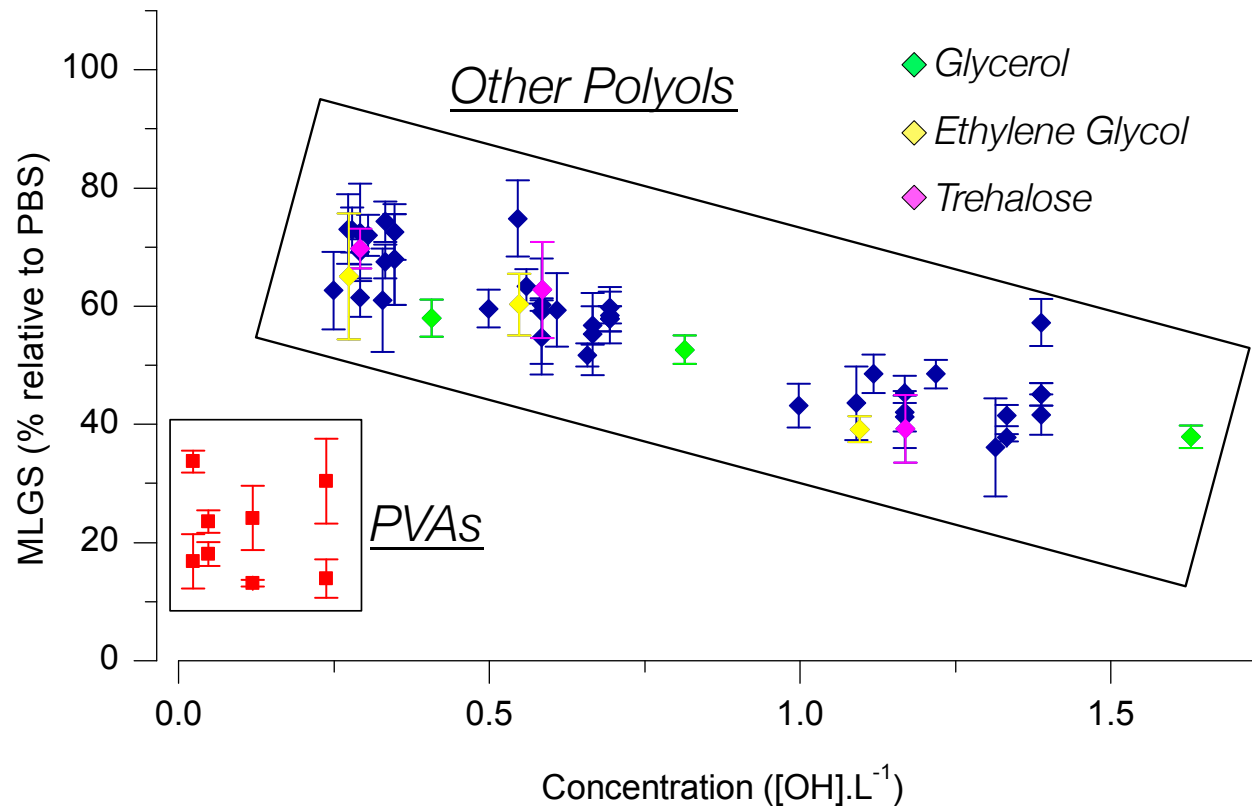
- ⊙ Freeze-thaw studies on suspended and plated cells.
- ⊙ Compare recovery rates in isolation and in conjunction with DMSO.
- ⊙ Functional assay for BeWo cells, syncytia formation and beta-hCG production.



Syncytialisation "cell fusion"

Polymeric Structure

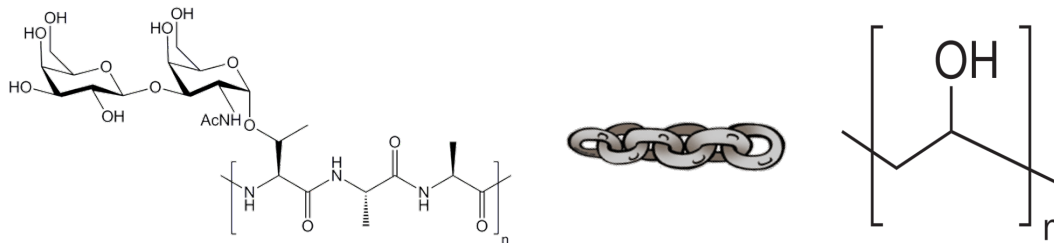
© Polymeric structure of PVA is key for RI activity rather than purely $[\text{OH}].\text{L}^{-1}$ concentration.



RI activity of numerous polyols relative to PBS. $n=3$; mean values \pm S.D; time = 30 minutes.

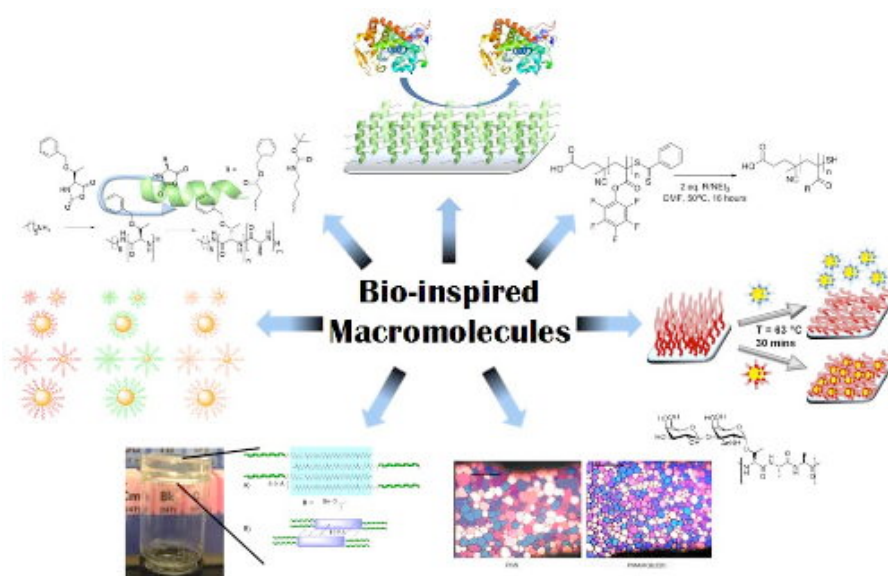
Conclusions and future studies

- ⊙ PVA displays significantly strong RI activity.
- ⊙ 9 KDa PVA has shown to improve the cryopreservation of red blood cells.
- ⊙ Future work will explore the link between RI and cryopreservation.



- ⊙ Apply findings to improving the cryopreservation of isolated primary cells.

Acknowledgements



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