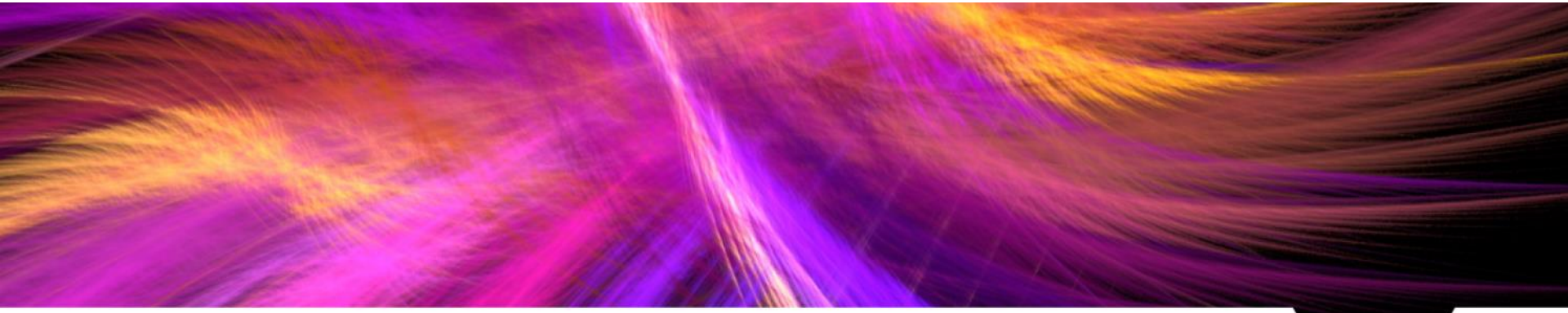
The Cryopreservation of Biological Materials

Trisha L. Bailey
Department of Chemistry,
University of Warwick, UK

t.bailey.1@warwick.ac.uk www.warwick.ac.uk/go/gibsongroup

@LabGibson



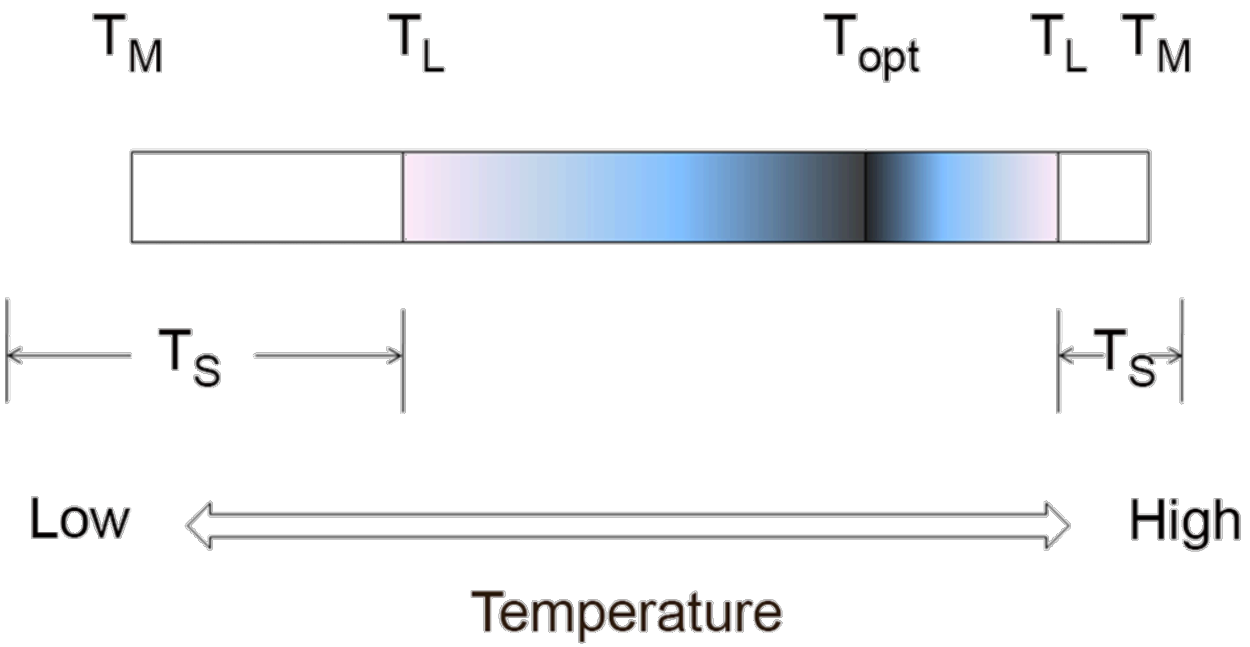
WARWICK

Problems

- Donor cells and tissues essential for modern medicine
  - 30 mil units of blood transfused annually in US
  - Donor bone marrow (leukemia)
  - Regenerative medicine requires stem cells
- Finite lifetime
- Storage and transport

Limitations

- Between T_M and T_S is a state of suspended animation
- Resume metabolism once the temperature re-crosses the T_M threshold[1]

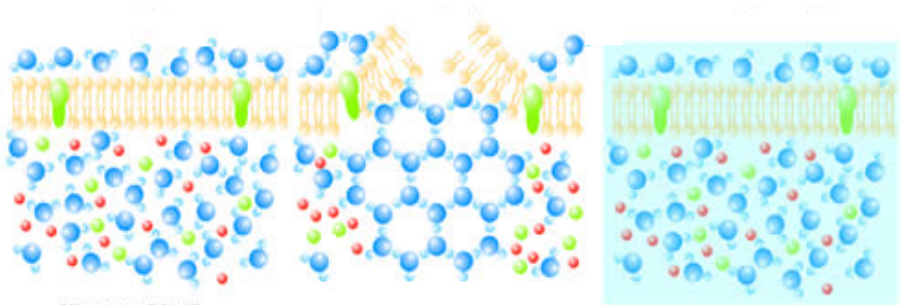


[1] A. Clarke, et al., *PLoS One*, 2013.

Limitations

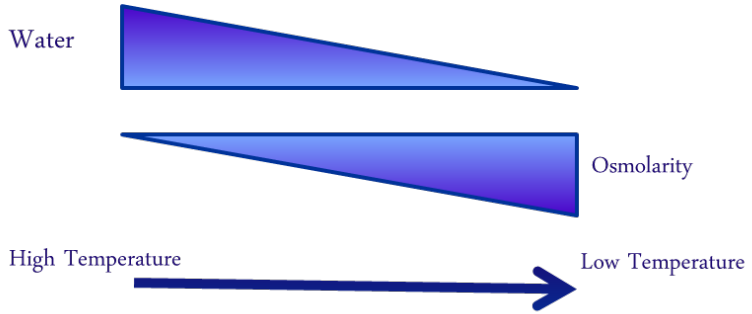
Ice

- Lipid bilayer disruption
- Internal ice is almost always lethal

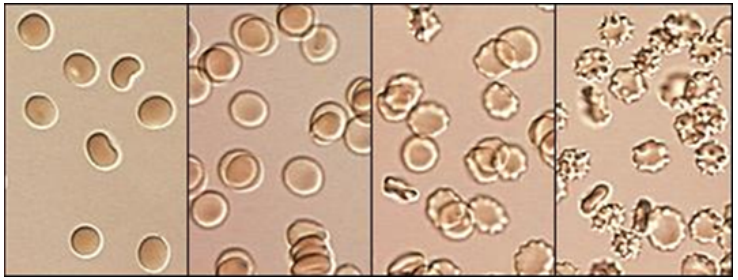


Osmolarity

- As ice forms, solute concentration changes

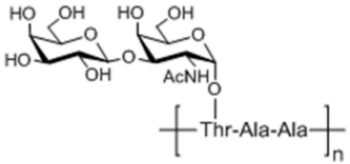


- Water rushes out of the cell



Nature's Protection

[2]

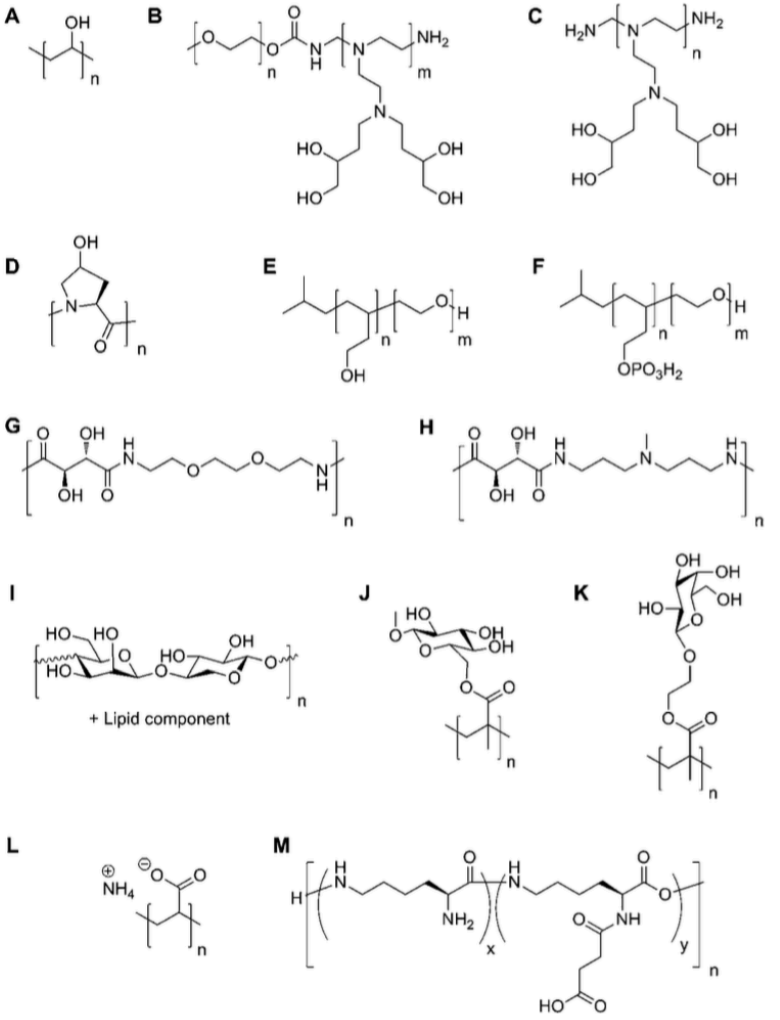
Characteristic	AFGP	Type I AFP	Type II AFP	Type III AFP	Type IV AFP
Mass (kDa)	2.6 - 33	3.3 – 4.5	11 – 24	6.5	12
Key Properties	AAT repeat; disaccharide	Alanine-rich α-helix	Disulfide bonded	β-sandwich	Alanine rich; helical bundle
Representative Structure					
Natural Source	Antarctic Notothenioids; northern cods	Right-eyed flounders; sculpins	Sea raven; smelt; herring	Ocean pout; wolfish; eel pout	Longhorn sculpin

[2] C. J. Capicciotti, et al, *Recent Dev. Study Recryst.*, 2013.

Antifreeze Glycoproteins (AFGPs)

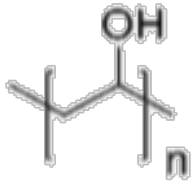
- Problems
  - Obtaining large quantities
  - Prevents bacterial expression
  - Immunogenicity/Toxicity
  - Bipyramidal Shaped Ice
- Synthetic Polymers
  - Highly tunable
    - Composition
    - Architecture
    - Molecular weight

[5]

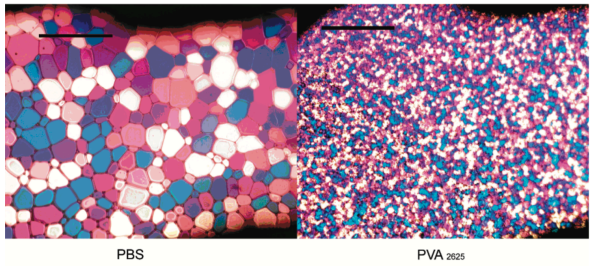


[5] M. I. Gibson, *Polym. Chem.*, 2010.

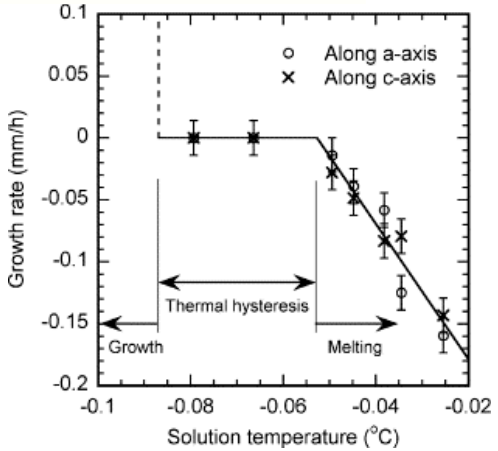
Poly(vinyl alcohol) (PVA)



- RI Activity[6]
  - Dilute range (<1 mg mL^{-1})

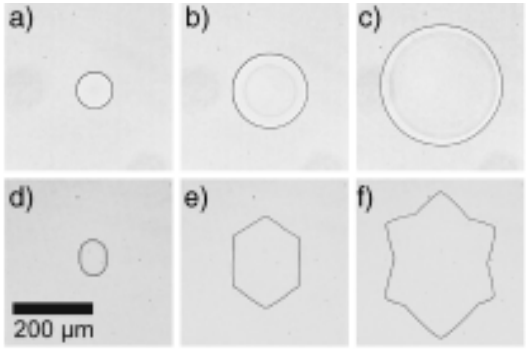


- Weight Dependent RI[7]
  - Higher M_w gives more RI



- Small TH Activity[8]

- Dynamic Ice Shaping Behavior[9]

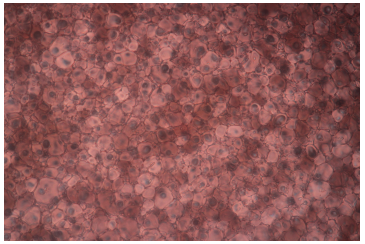


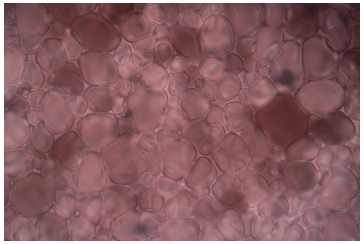
- Hydroxyl Group Spacing[9]
  - Closely matched to prism planes of hexagonal ice

[6] M. I. Gibson et al., *Biomacromolecules*, 2009. [7] Inada and S.-S. Lu, *Cryst. Growth Des.*, 2003.
[8] T. Inada and S. S. Lu, *Chem. Phys. Lett.*, 2004. [9] C. Budke and T. Koop, *ChemPhysChem*, 2006.

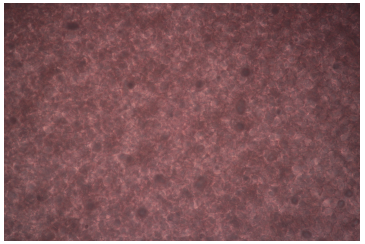
SPLAT Assay

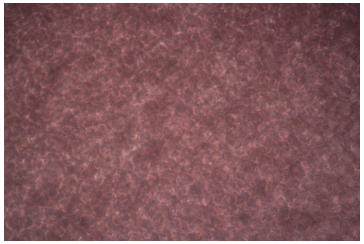
PBS



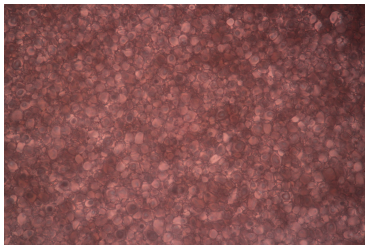


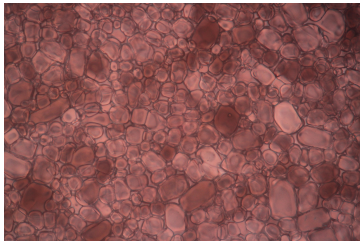
10 mg/mL PVA in F-12K



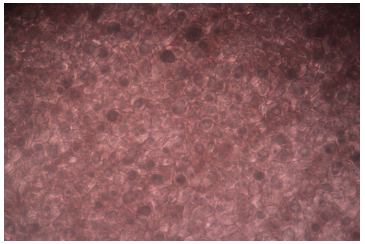


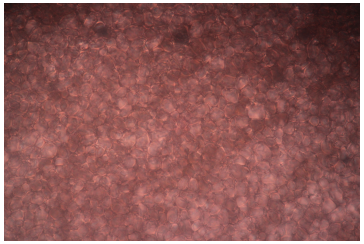
200 mM Proline in F-12K



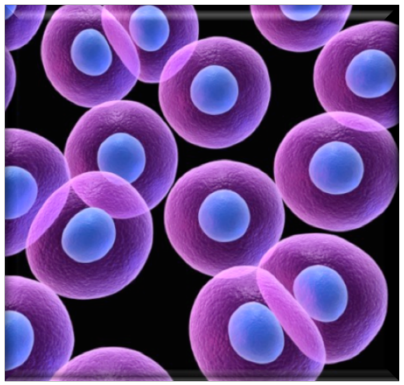


200 mM Proline + 10 mg/mL PVA in F-12K

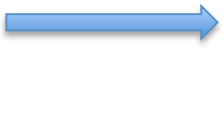


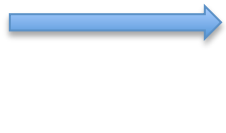


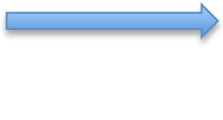
Current Cell Cryopreservation

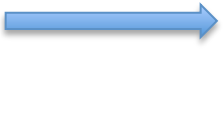


- Dimethyl Sulfoxide (DMSO)
  - Toxic at room temperature[13]
- Frozen in solution
  - Phenotypic changes







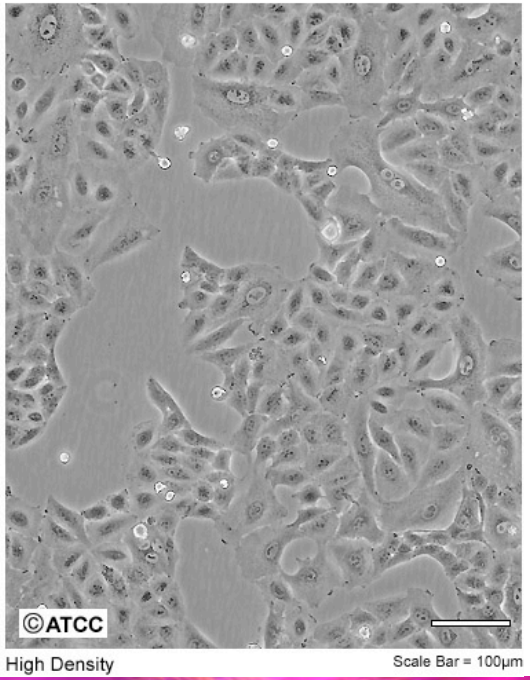


[13] G. M. Fahy, *Cryobiology*, 2010.

Cell Lines

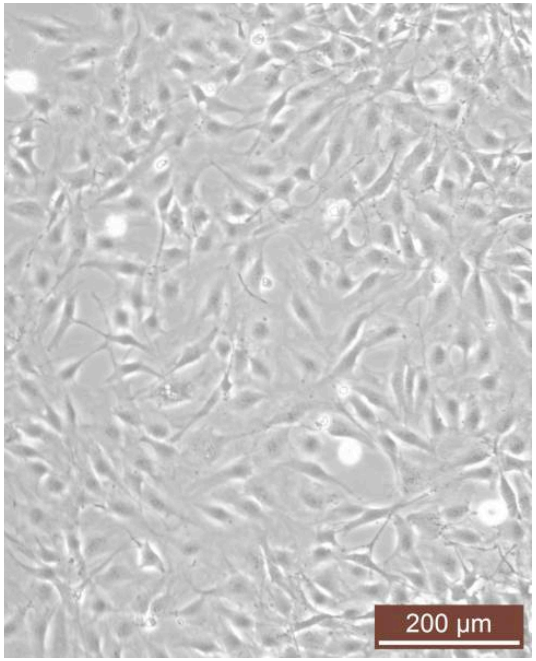
A549

- Human lung cells
- Epithelial carcinoma

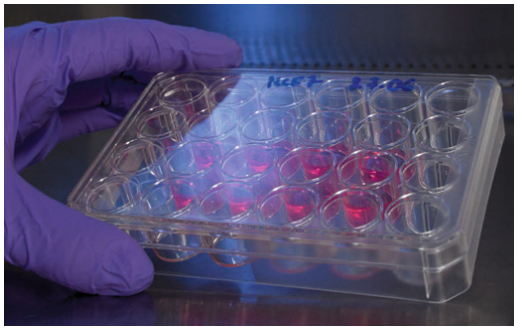


MC-3T3

- Mouse bone cells
- Fibroblast preosteoblast



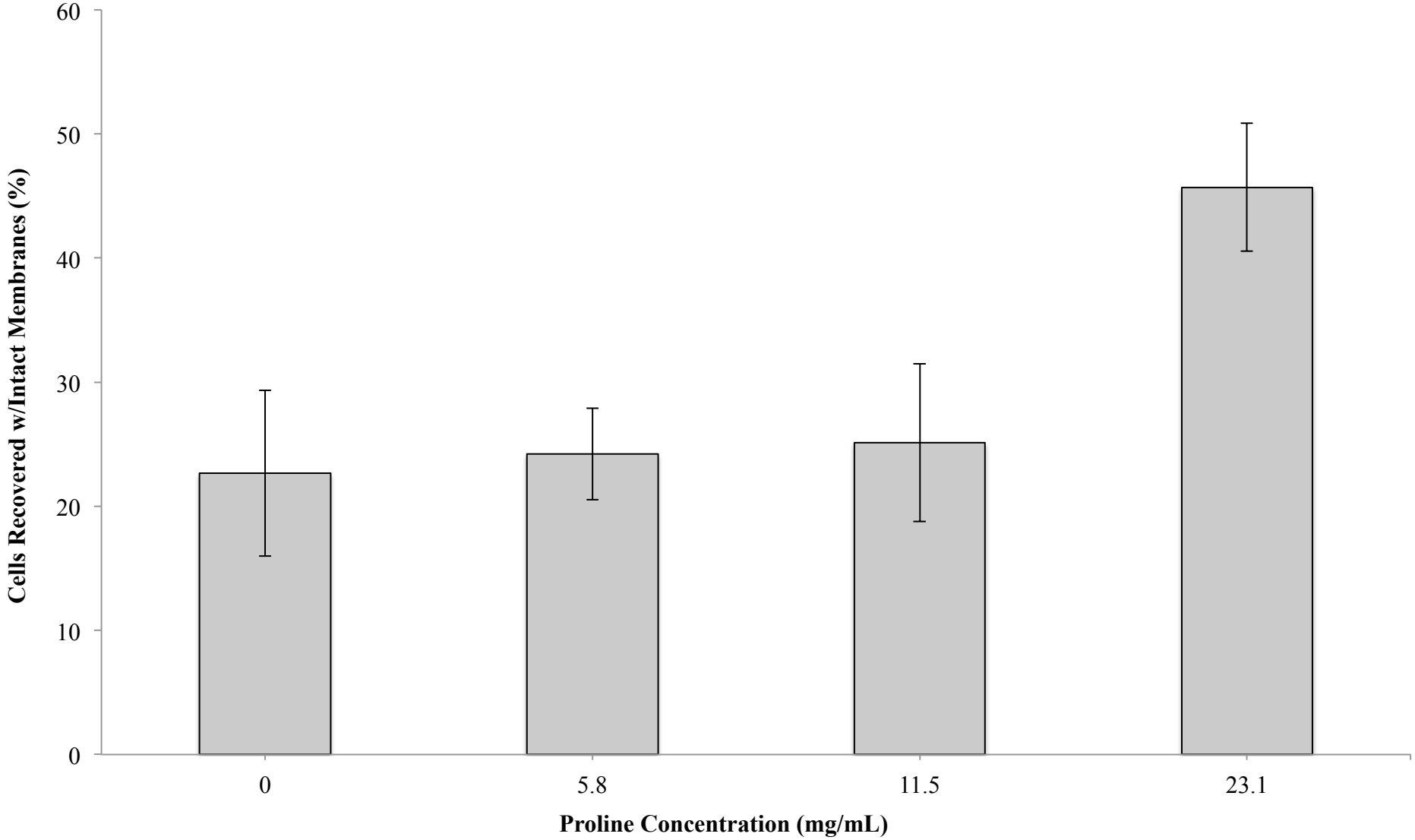
Freezing Viability



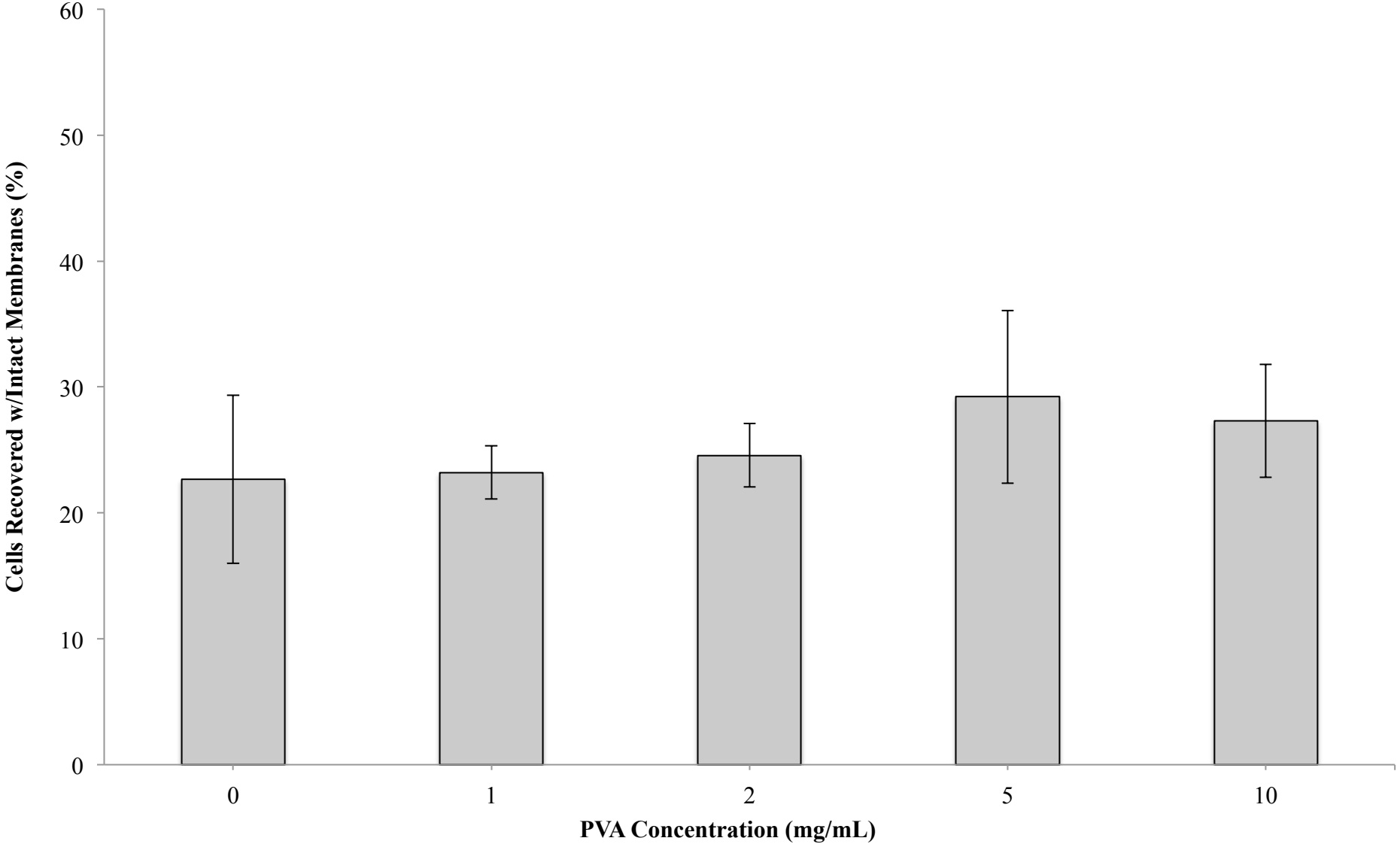
- 24 well plates
- Cells are plated for 2 hours
- Incubated with solutes for 24 hours
- CPA applied for 10 min then removed

- Placed into passive freezing device (-1 ºC/min) for 24 hours
- Quickly thawed with 37 ºC medium
- Incubated for 24 hours
- Counted for viability

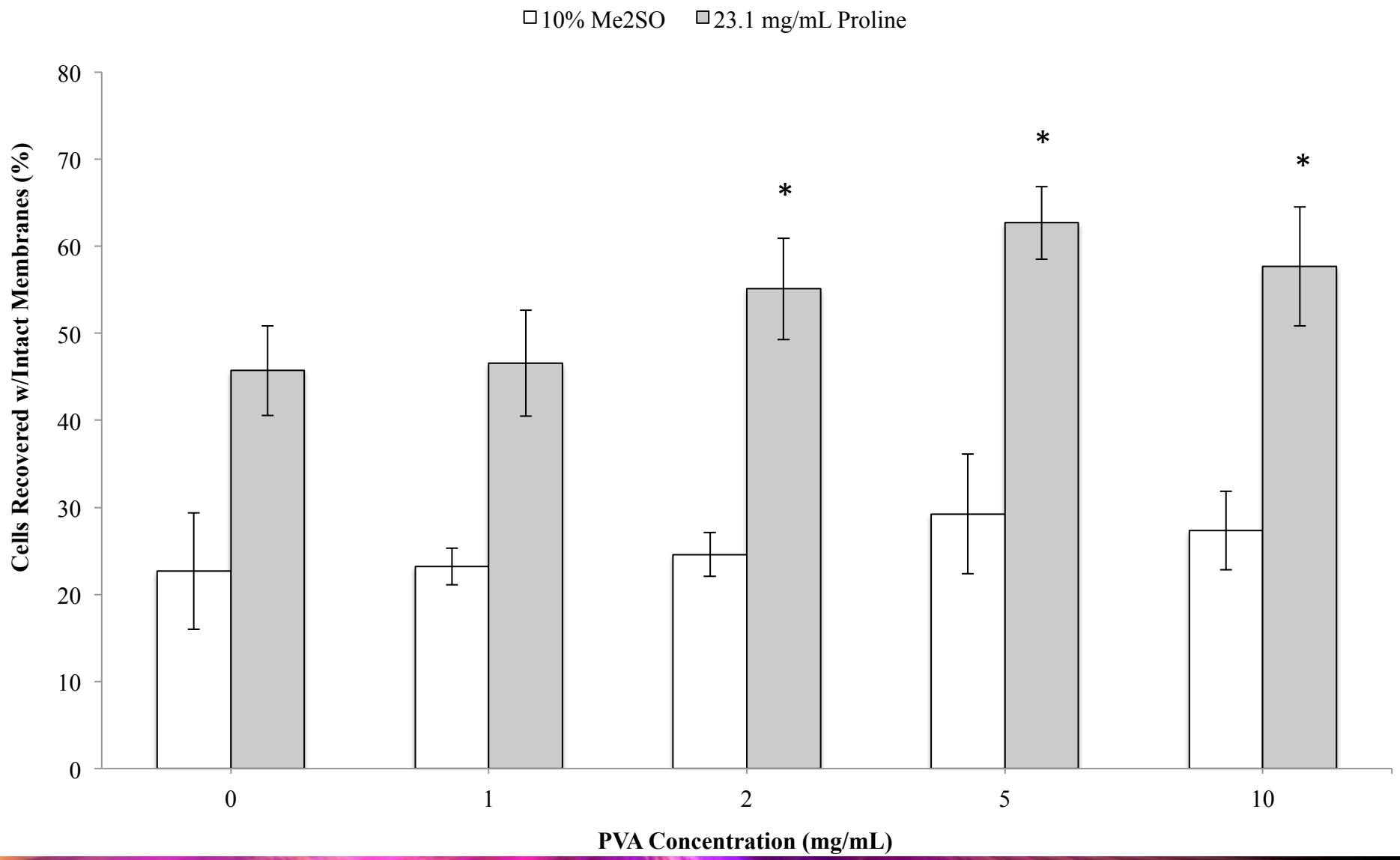
A549 Cell Cryopreservation with Proline



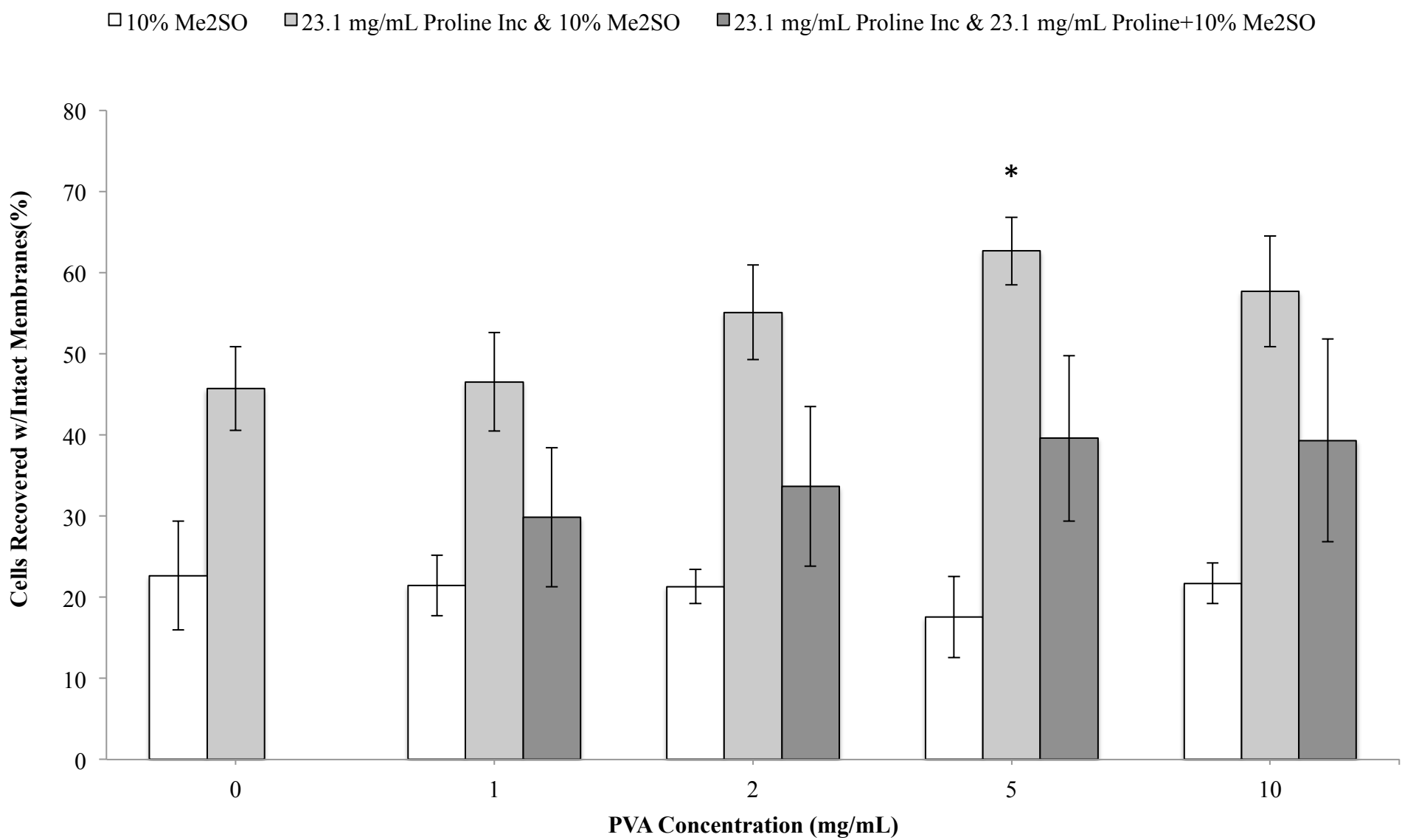
A549 Cell Cryopreservation with PVA



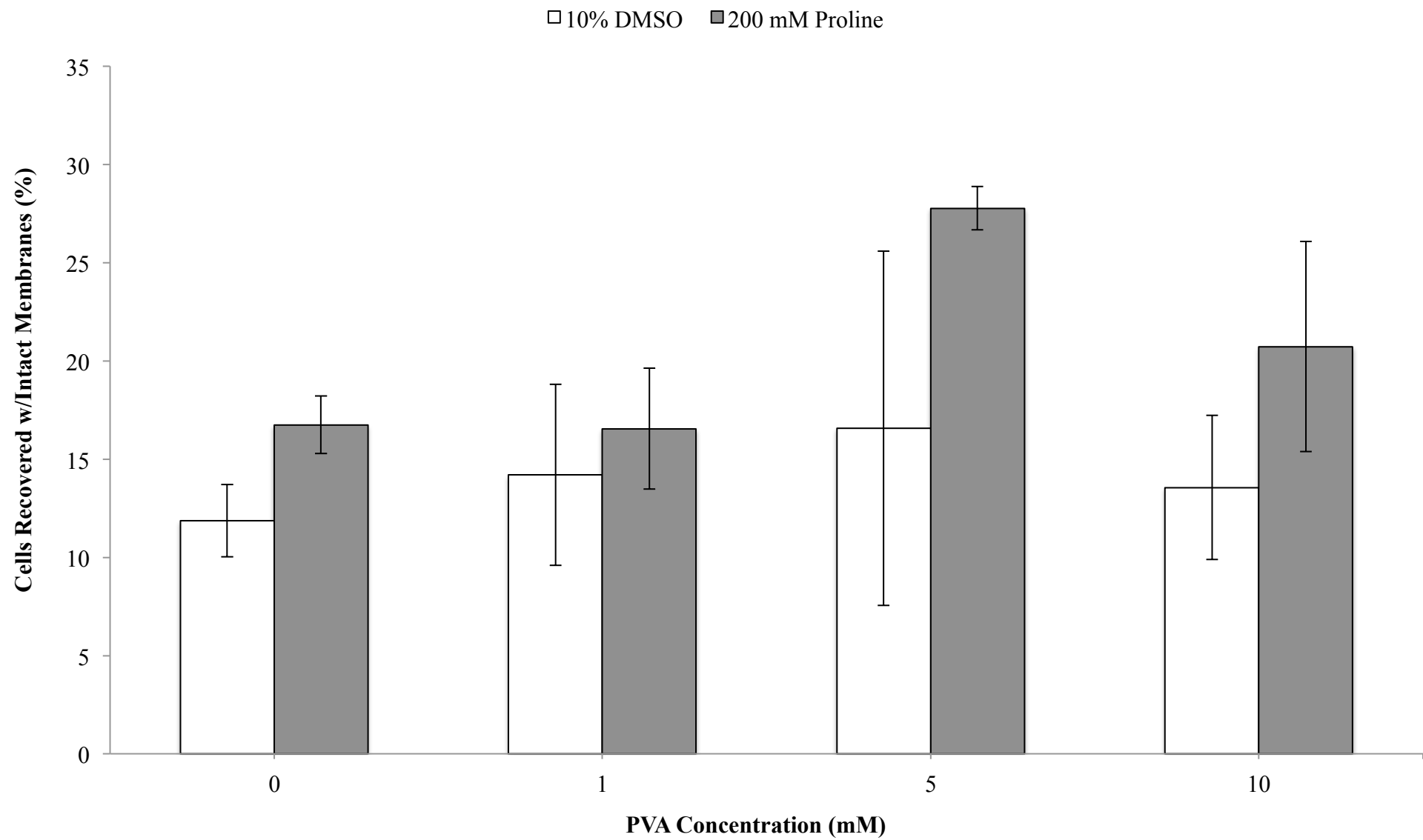
A549 Cryopreservation with Proline & PVA



A549 Cryopreservation Proline & Proline+PVA



MC-3T3 Cell Cryopreservation with PVA



Next

- Collagen coating 24 well plates for MC-3T3 freezing
- Differentiate MC-3T3 cells post-freezing to additionally asses viability
- DSC solutions to see if/when T_g is formed

Acknowledgements

Principal Investigator:
Matt Gibson

THE ROYAL SOCIETY

The Leverhulme Trust

erc

European Research Council

Established by the European Commission

Citations

[1] A. Clarke, G. J. Morris, F. Fonseca, B. J. Murray, E. Acton, and H. C. Price, “A Low Temperature Limit for Life on Earth,” *PLoS One*, vol. 8, no. 6, p. e66207, Jan. 2013.

[2] C. J. Capicciotti, D. Malay, and R. N. Ben, “Ice Recrystallization Inhibitors: From Biological Antifreeze to Small Molecules,” *Recent Dev. Study Recryst.*, pp. 177–224, 2013.

[3] Y. Wu, J. Banoub, S. V. Goddard, M. H. Kao, and G. L. Fletcher, “Antifreeze glycoproteins: Relationship between molecular weight, thermal hysteresis and the inhibition of leakage from liposomes during thermotropic phase transition,” *Comp. Biochem. Physiol. - B Biochem. Mol. Biol.*, vol. 128, no. 2, pp. 265–273, 2001.

[4] M. Griffith and M. W. F. Yaish, “Antifreeze proteins in overwintering plants: A tale of two activities,” *Trends Plant Sci.*, vol. 9, no. 8, pp. 399–405, 2004.

[5] M. I. Gibson, “Slowing the growth of ice with synthetic macromolecules: beyond antifreeze(glyco) proteins,” *Polym. Chem.*, vol. 1, no. 8, p. 1141, 2010.

[6] M. I. Gibson, C. A. Barker, S. G. Spain, L. Albertin, and N. R. Cameron, “Inhibition of ice crystal growth by synthetic glycopolymers: Implications for the rational design of antifreeze glycoprotein mimics,” *Biomacromolecules*, vol. 10, no. 2, pp. 328–333, 2009.

[7] T. Inada and S.-S. Lu, “Inhibition of Recrystallization of Ice Grains by Adsorption of Poly(Vinyl Alcohol) onto Ice Surfaces,” *Cryst. Growth Des.*, vol. 3, no. 5, pp. 747–752, Sep. 2003.

[8] T. Inada and S. S. Lu, “Thermal hysteresis caused by non-equilibrium antifreeze activity of poly(vinyl alcohol),” *Chem. Phys. Lett.*, vol. 394, no. 4–6, pp. 361–365, 2004.

[9] C. Budke and T. Koop, “Ice recrystallization inhibition and molecular recognition of ice faces by poly(vinyl alcohol),” *ChemPhysChem*, vol. 7, no. 12, pp. 2601–2606, 2006.

[10] J. P. Nolan and M. G. Mythen, “Hydroxyethyl starch: Here today, gone tomorrow,” *Br. J. Anaesth.*, vol. 111, no. 3, pp. 321–324, 2013.

[11] R. C. Deller, M. Vatish, D. a Mitchell, and M. I. Gibson, “Synthetic polymers enable non-vitreous cellular cryopreservation by reducing ice crystal growth during thawing.,” *Nat. Commun.*, vol. 5, p. 3244, 2014.

[12] D. E. Mitchell, J. R. Lovett, S. P. Armes, and M. I. Gibson, “Combining Biomimetic Block Copolymer Worms with an Ice-Inhibiting Polymer for the Solvent-Free Cryopreservation of Red Blood Cells,” *Angew. Chemie - Int. Ed.*, vol. 55, no. 8, pp. 2801–2804, 2016.

[13] G. M. Fahy, “Cryoprotectant toxicity neutralization,” *Cryobiology*, vol. 60, no. 3 SUPPL., pp. S45–S53, Jul. 2010.

[14] R. C. Deller, J. E. Pessin, M. Vatish, D. A. Mitchell, and M. I. Gibson, “Enhanced non-vitreous cryopreservation of immortalized and primary cells by ice-growth inhibiting polymers,” *Biomater. Sci.*, vol. 4, no. 7, 2016.

WARWICK
THE UNIVERSITY OF WARWICK

Extra bits in case you need them.