

The Cryopreservation of Biological Materials

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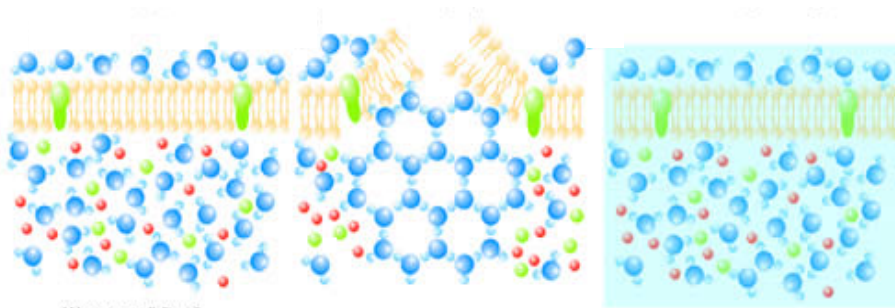
Problems

- Donor cells and tissues essential for modern medicine
 - Donor bone marrow (leukemia)
 - Regenerative medicine requires stem cells
- Finite lifetime
- Storage and transport

Limitations

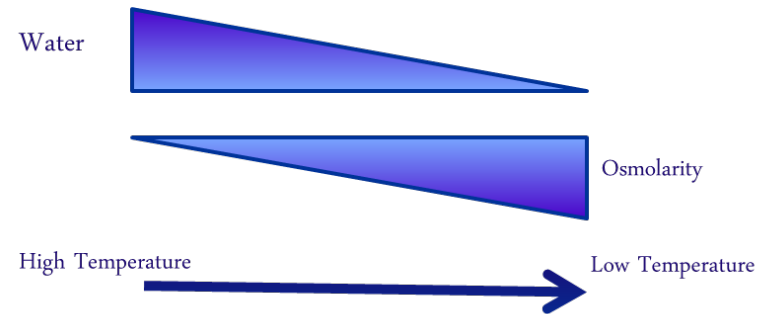
Ice

- Lipid bilayer disruption
- Internal ice is almost always lethal
- Ice recrystallization upon thawing

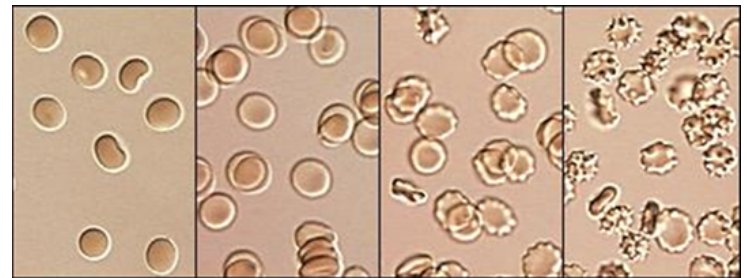


Osmolarity

- As ice forms, solute concentration changes

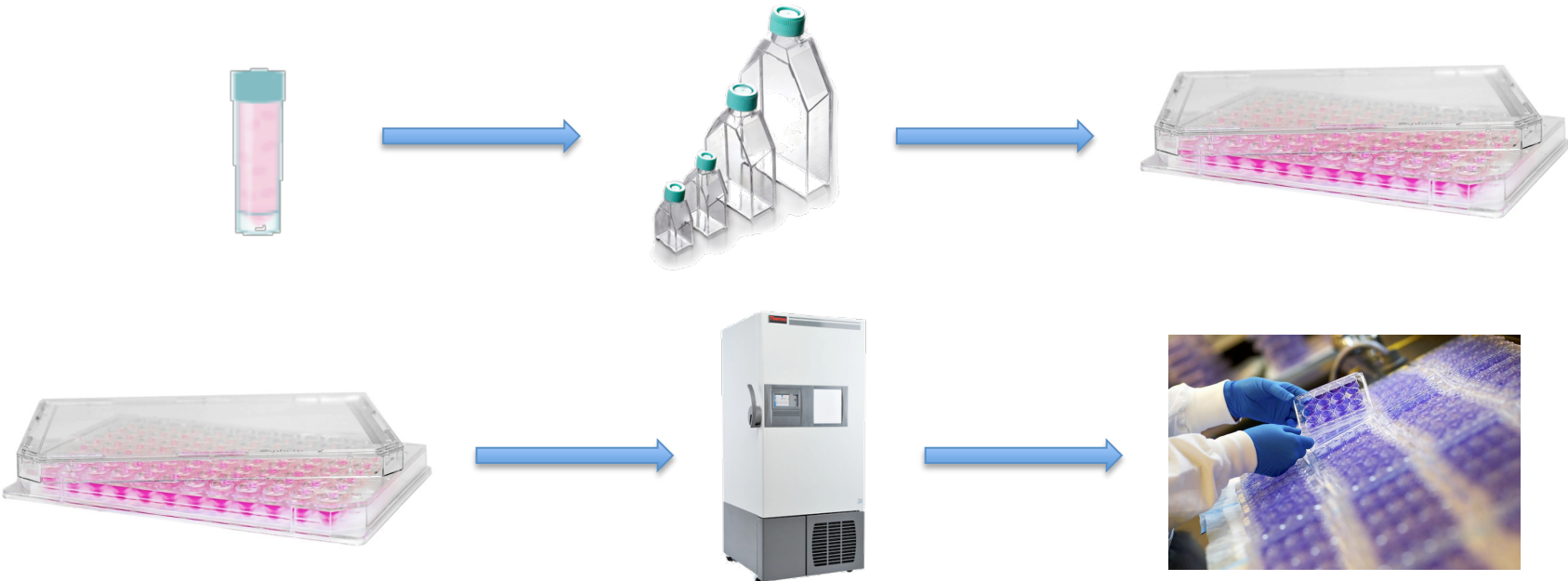
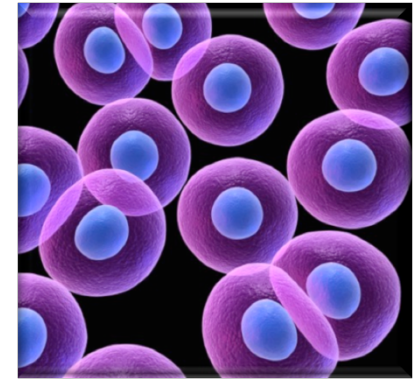


- Water rushes out of the cell



Current Cell Cryopreservation

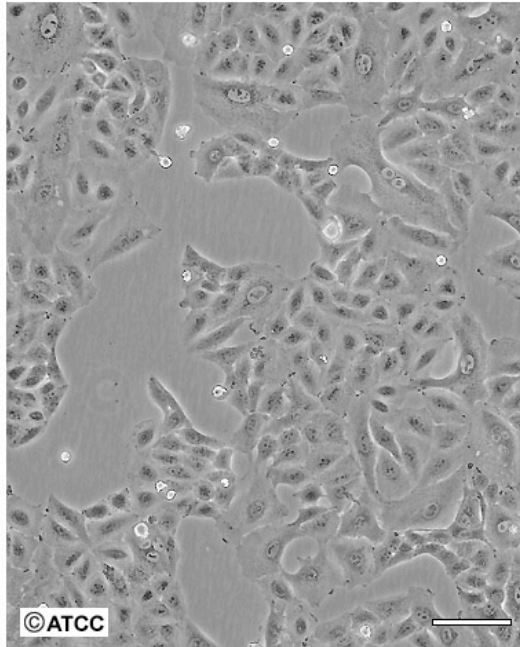
- Dimethyl Sulfoxide (DMSO)
 - Toxic at room temperature^[13]
- Frozen in solution
 - Phenotypic changes



Cell Lines

A549

- Human lung cells
- Epithelial carcinoma



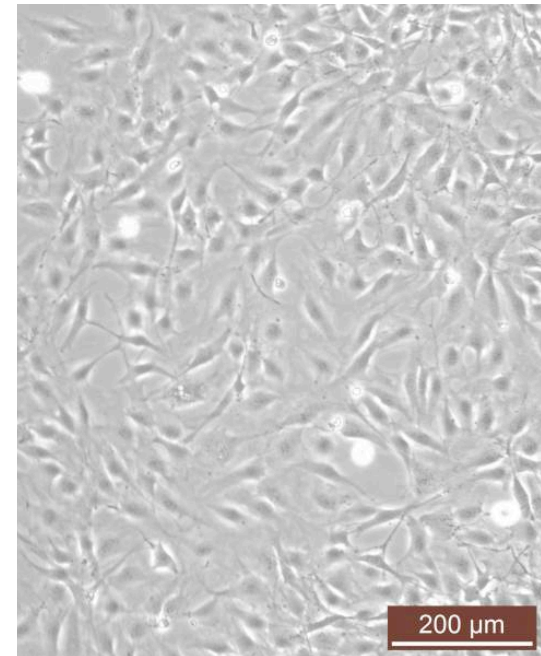
© ATCC

High Density

Scale Bar = 100µm

MC-3T3

- Mouse bone cells
- Fibroblast preosteoblast

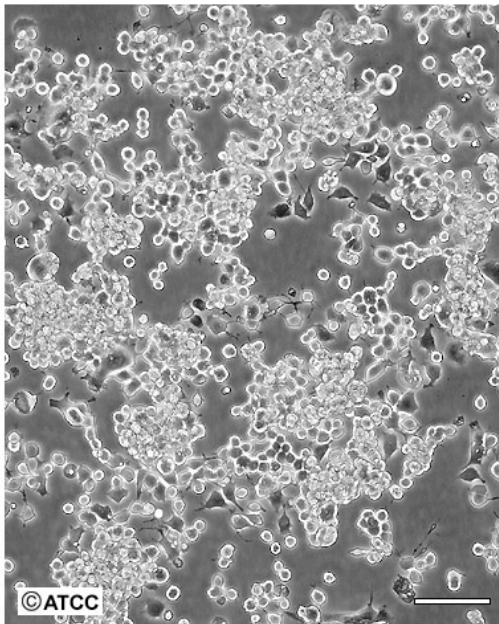


200 µm

Cell Lines

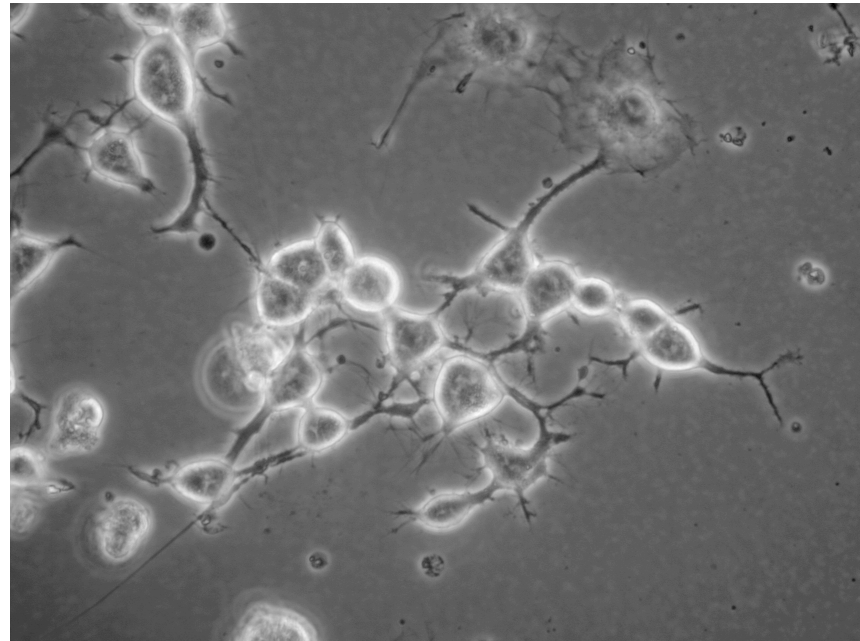
Neuro-2a

- Mouse brain cells
- Neuroblastoma

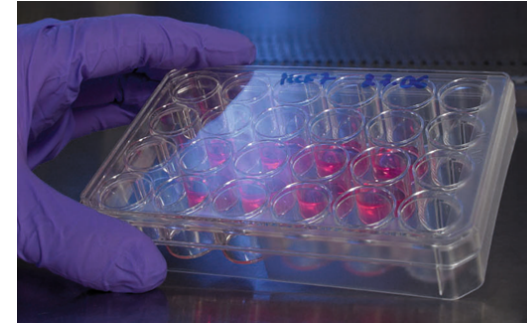


High Density

Scale Bar = 100µm



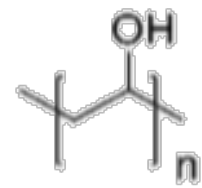
Freezing Viability



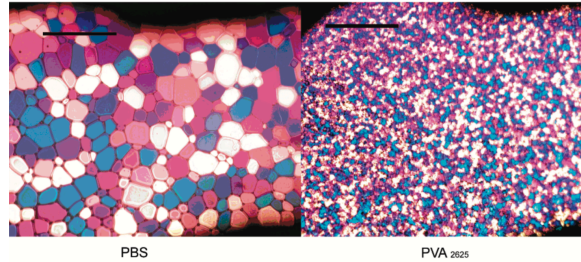
- 24 well plates
- Cells are plated for 2 hours
- Incubated with solutes for 24 hours
- CPA applied for 10 min then removed
- Placed into passive freezing device (-1 °C/min) for 24 hours
- Quickly thawed with 37 °C medium
- Incubated for 24 hours
- Counted for viability



Poly(vinyl alcohol) (PVA)

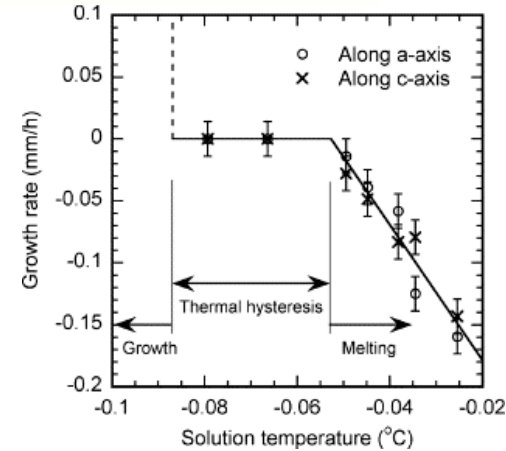


- RI Activity^[6]
 - Dilute range ($<1 \text{ mg mL}^{-1}$)

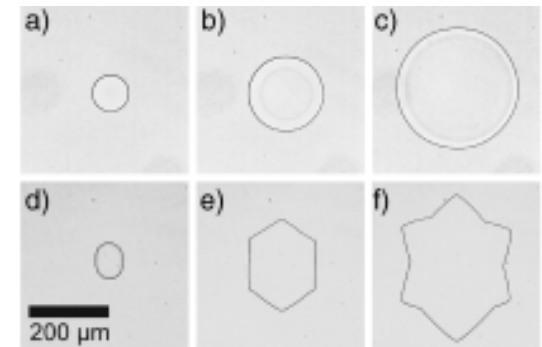


- Weight Dependent RI^[7]
 - Higher M_w gives more RI

- Small TH Activity^[8]



- Dynamic Ice Shaping Behavior^[9]

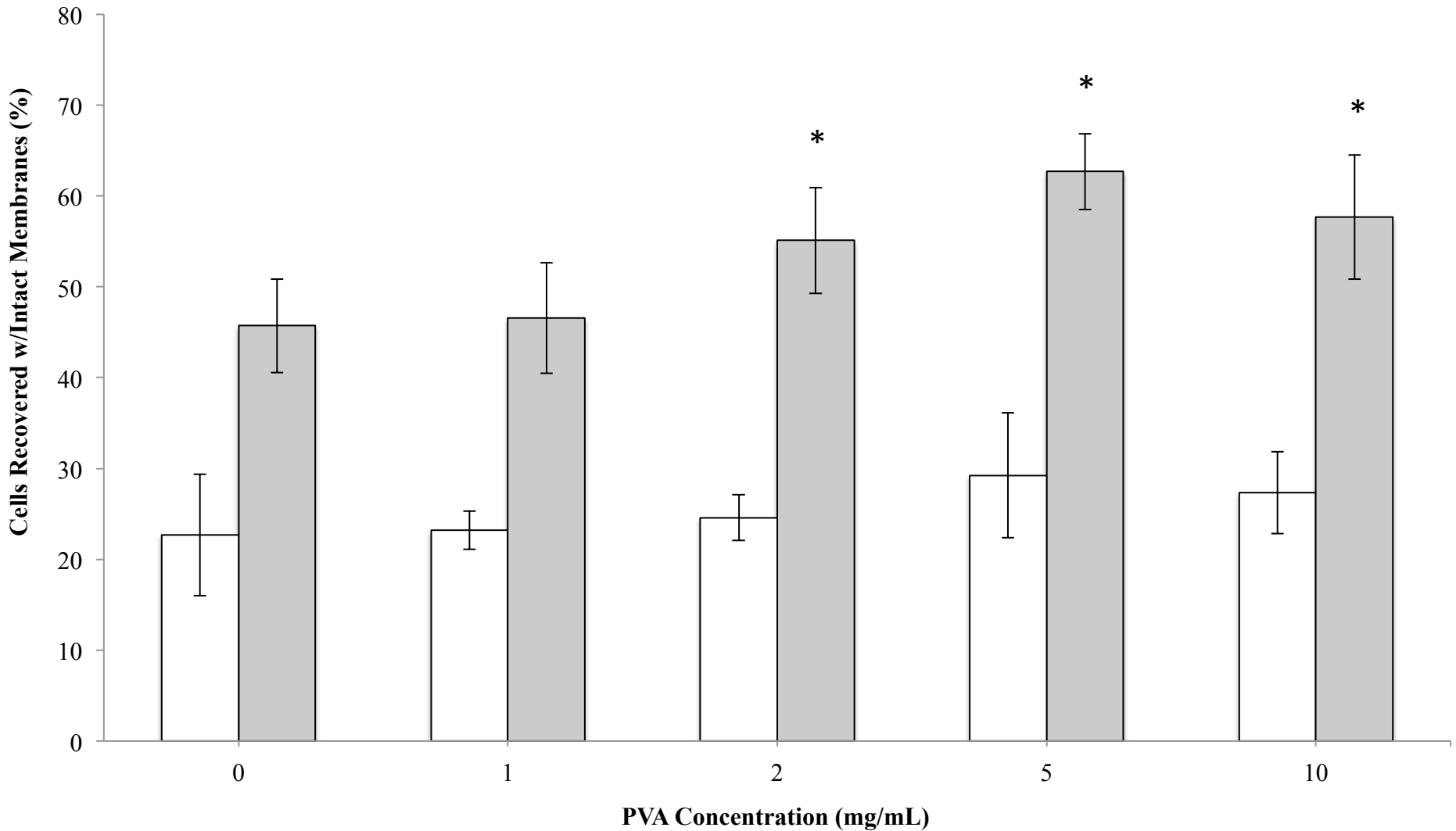


- Hydroxyl Group Spacing^[9]

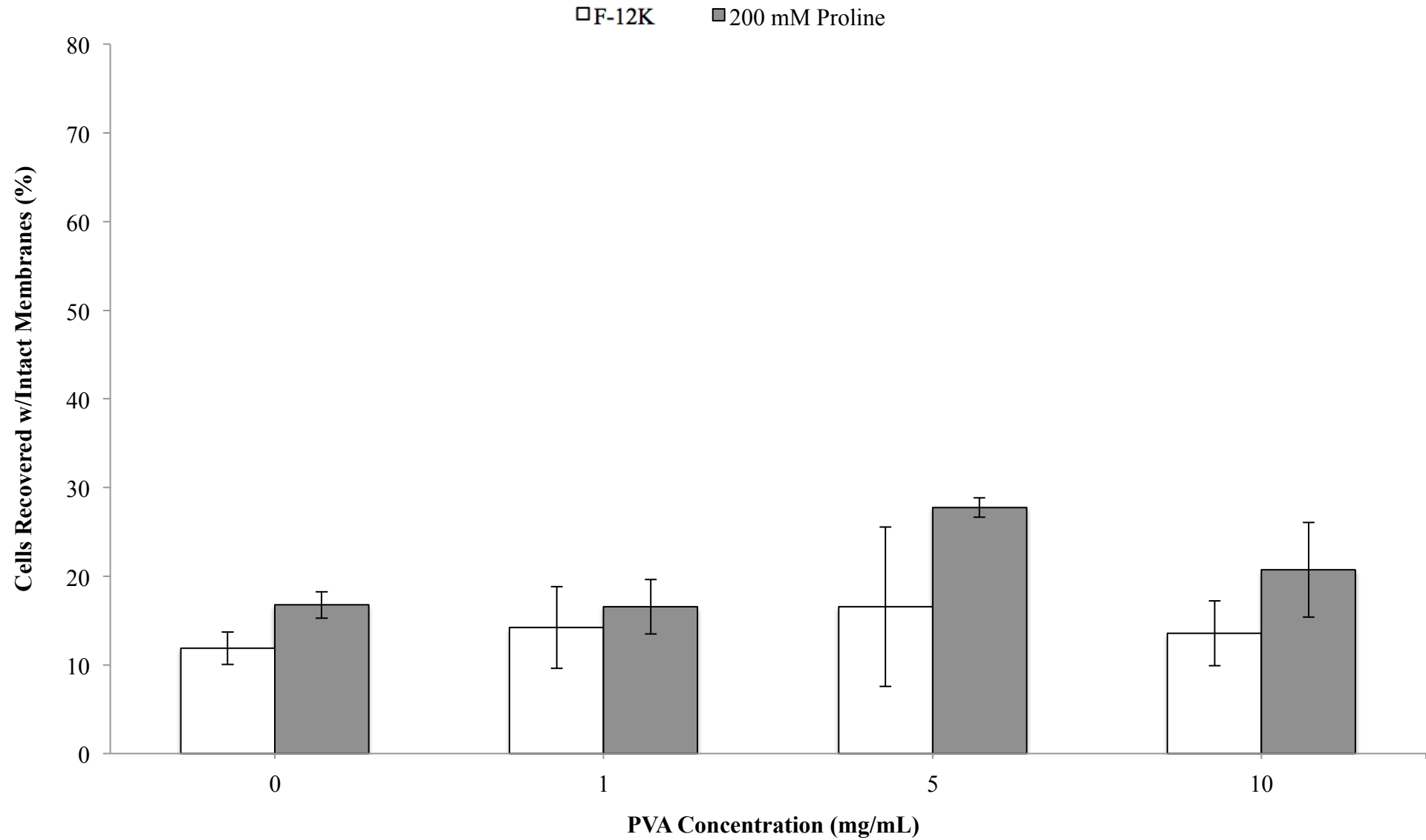
- Closely matched to prism planes of hexagonal ice

A549 Cryopreservation with Proline & PVA

□ F-12K □ 23.1 mg/mL Proline

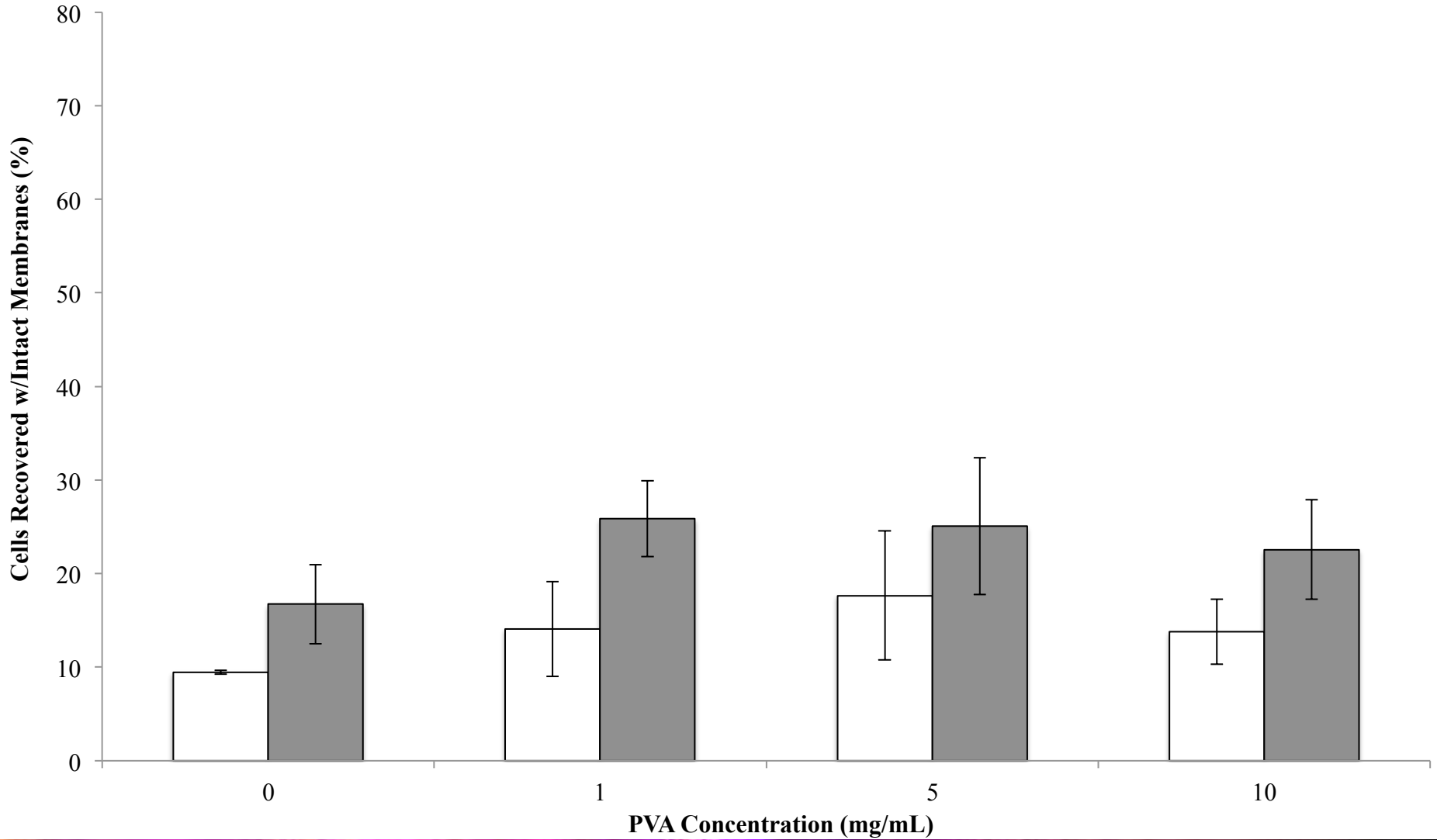


MC-3T3 Cell Cryopreservation with PVA

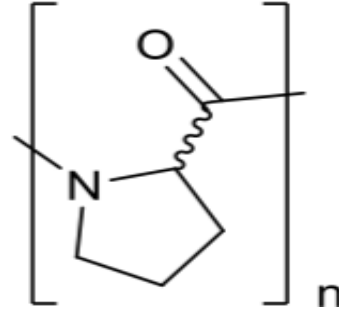


Collagen MC-3T3 Cell Cryopreservation with PVA

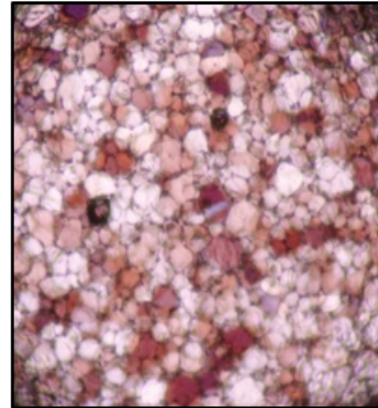
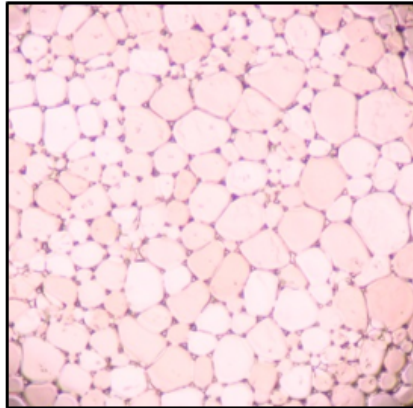
□ F-12K ■ 200 mM Proline



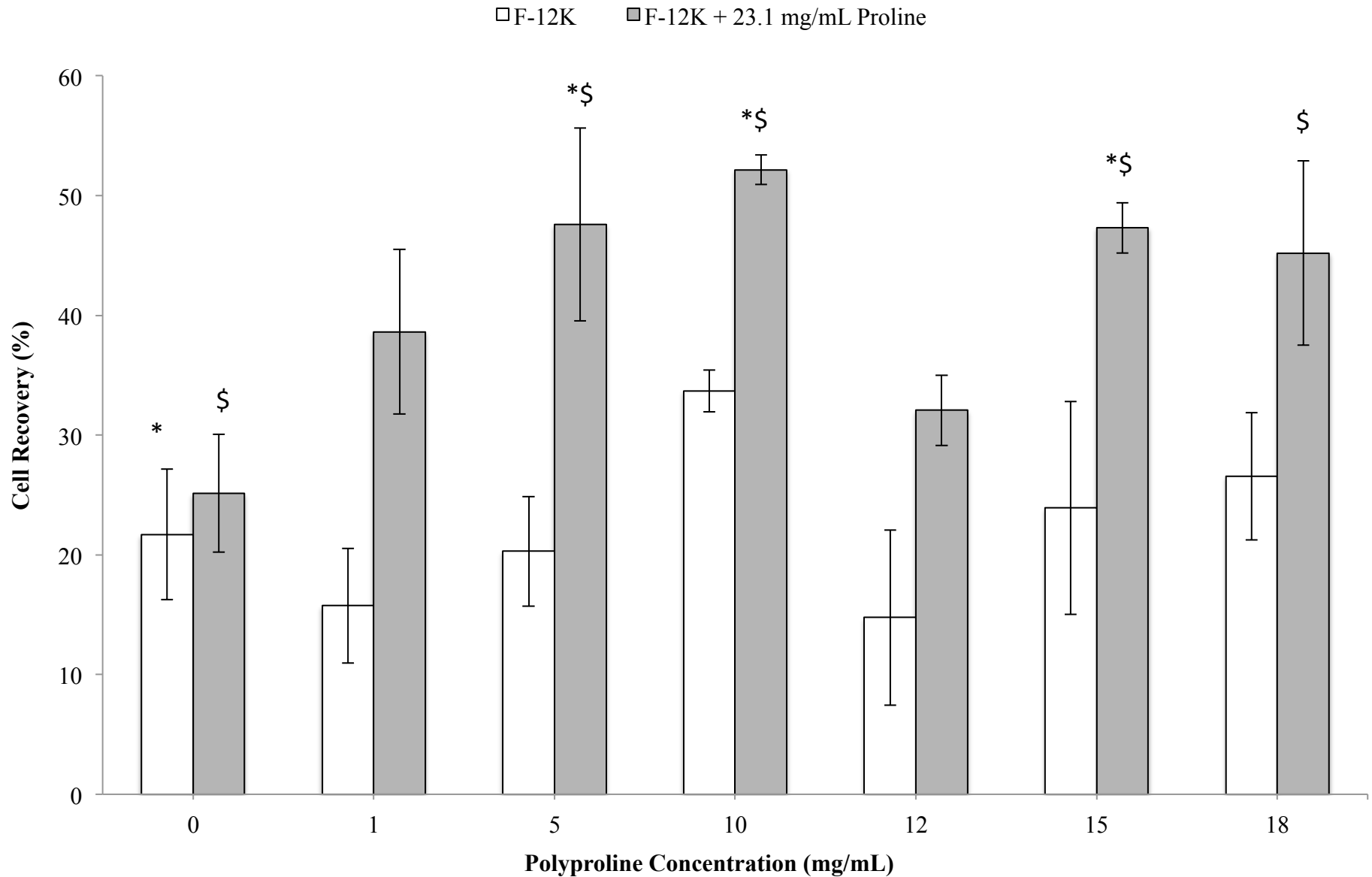
Polyproline



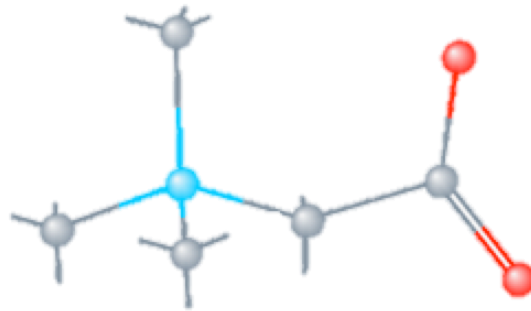
- IRI Activity



A549 Cell Cryopreservation with Polyproline



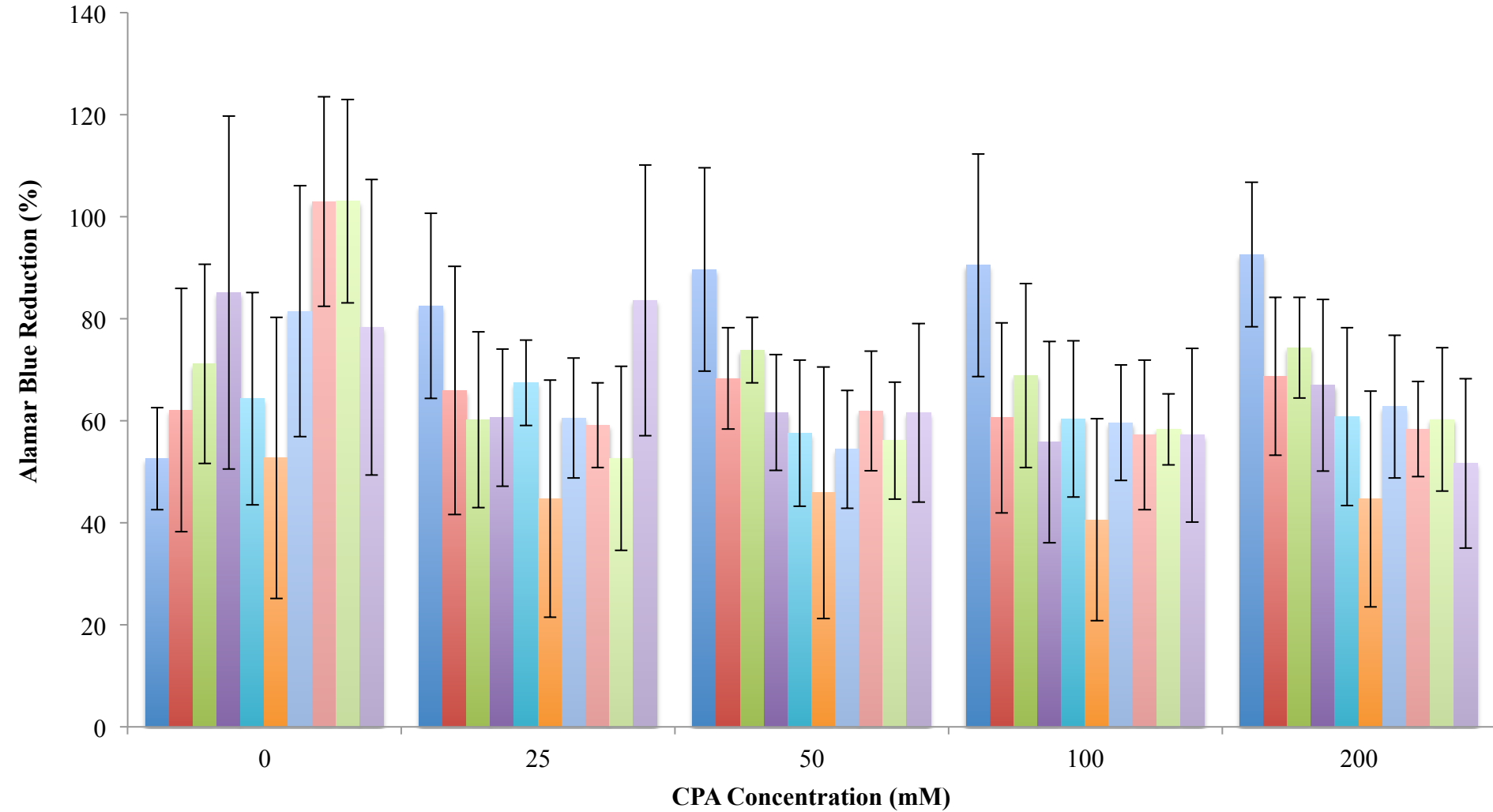
Betaine



- Osmoregulating compound
- Recently shown to protect cells during ultra-rapid freezing

A549 Betaine Screen

0 25 50 100 200 0+P 25+P 50+P 100+P 200+P

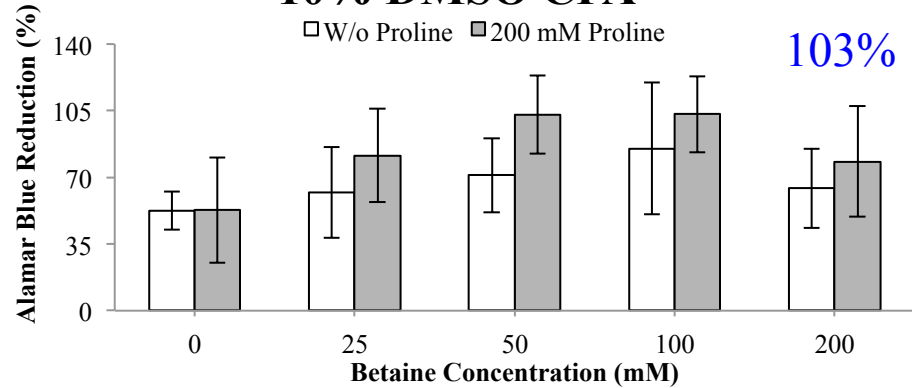


A549 Betaine Screen

10% DMSO CPA

□ W/o Proline ■ 200 mM Proline

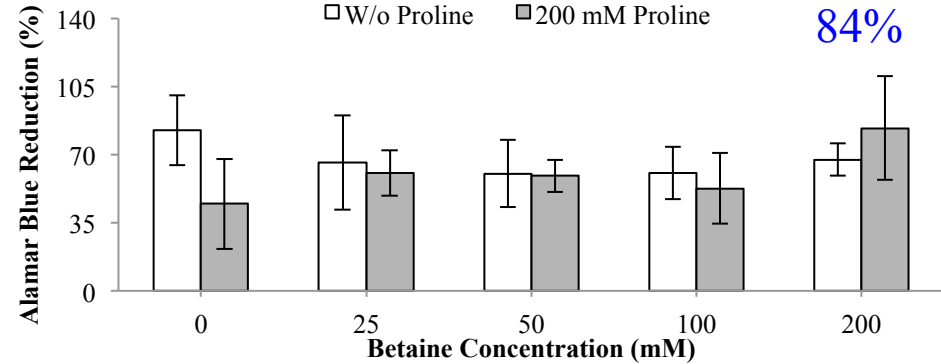
103%



25 mM Betaine + 10% DMSO CPA

□ W/o Proline ■ 200 mM Proline

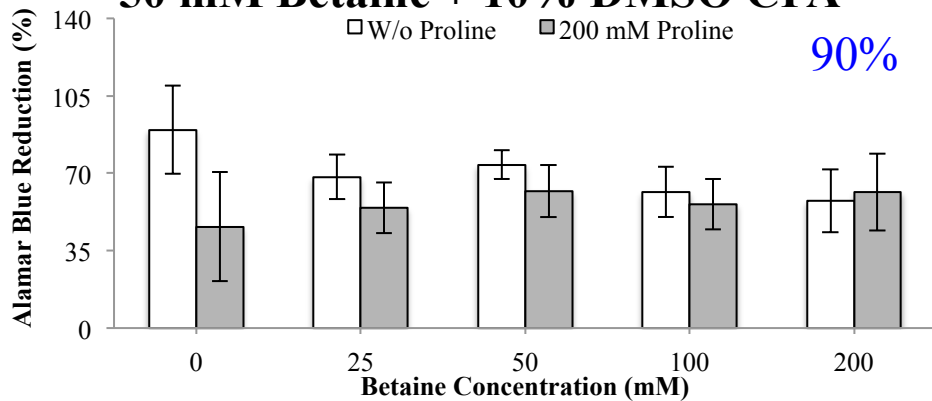
84%



50 mM Betaine + 10% DMSO CPA

□ W/o Proline ■ 200 mM Proline

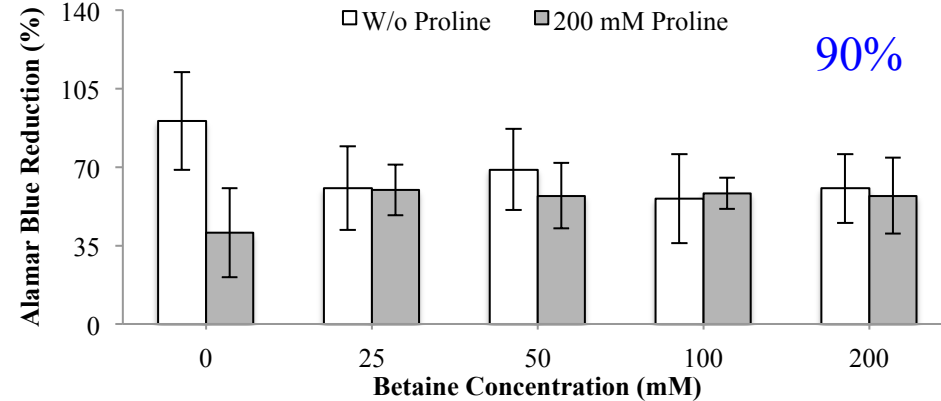
90%



100 mM Betaine + 10% DMSO CPA

□ W/o Proline ■ 200 mM Proline

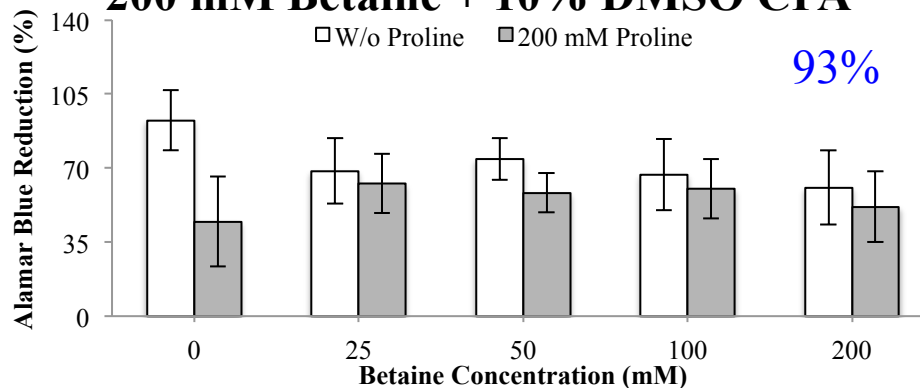
90%



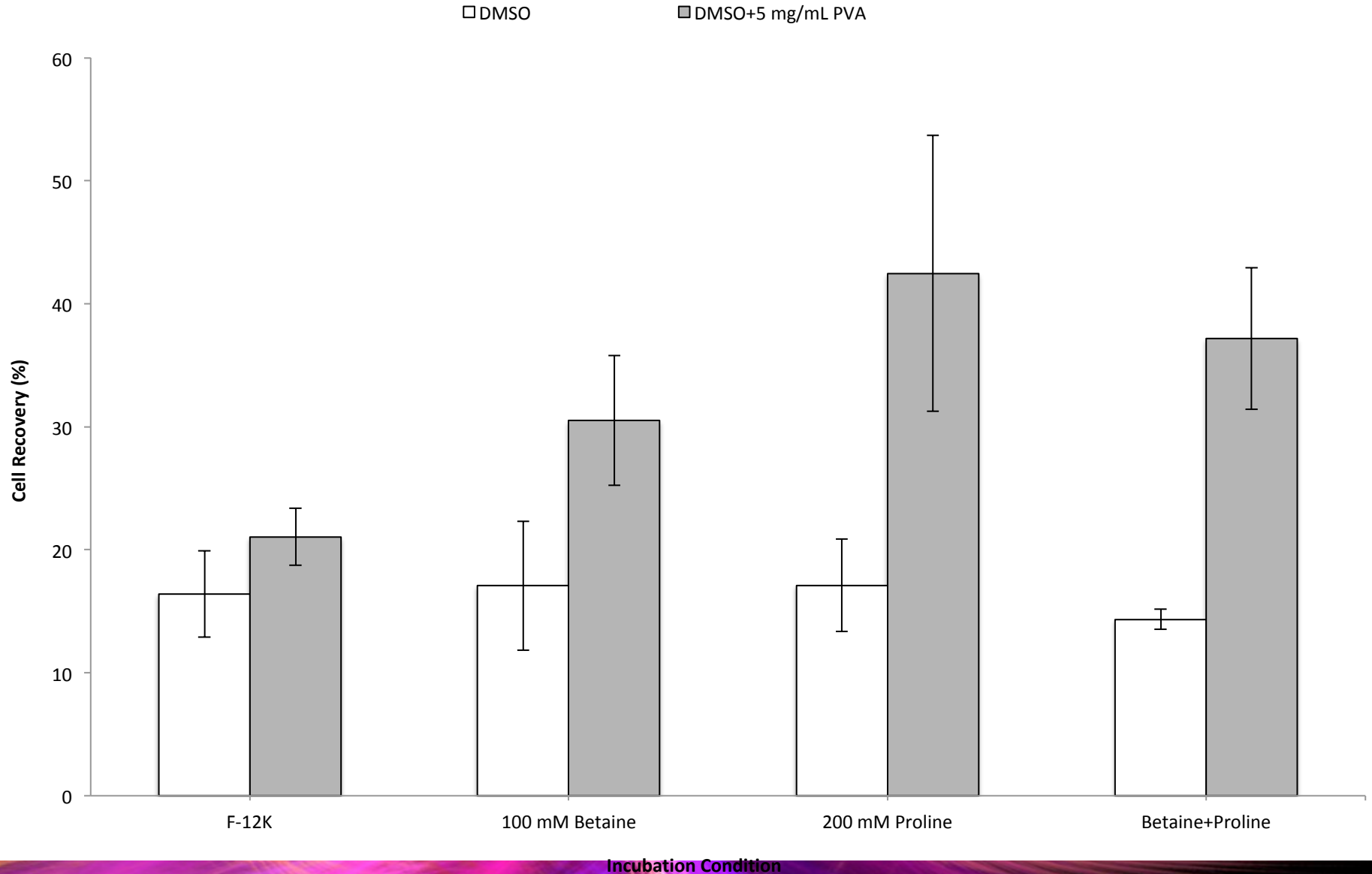
200 mM Betaine + 10% DMSO CPA

□ W/o Proline ■ 200 mM Proline

93%

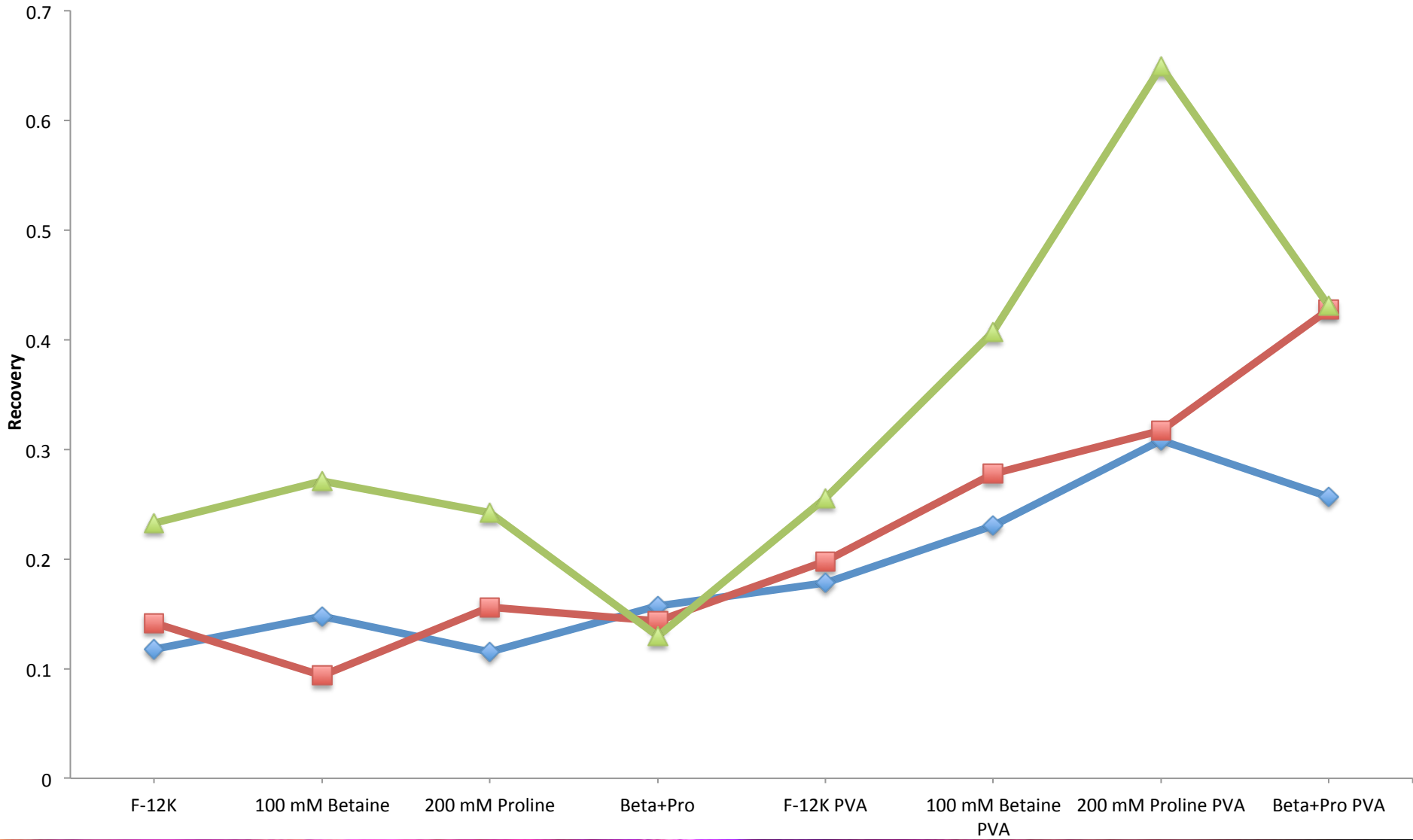


A549 Betaine Plate Freezing



A549 Betaine Plate Freezing

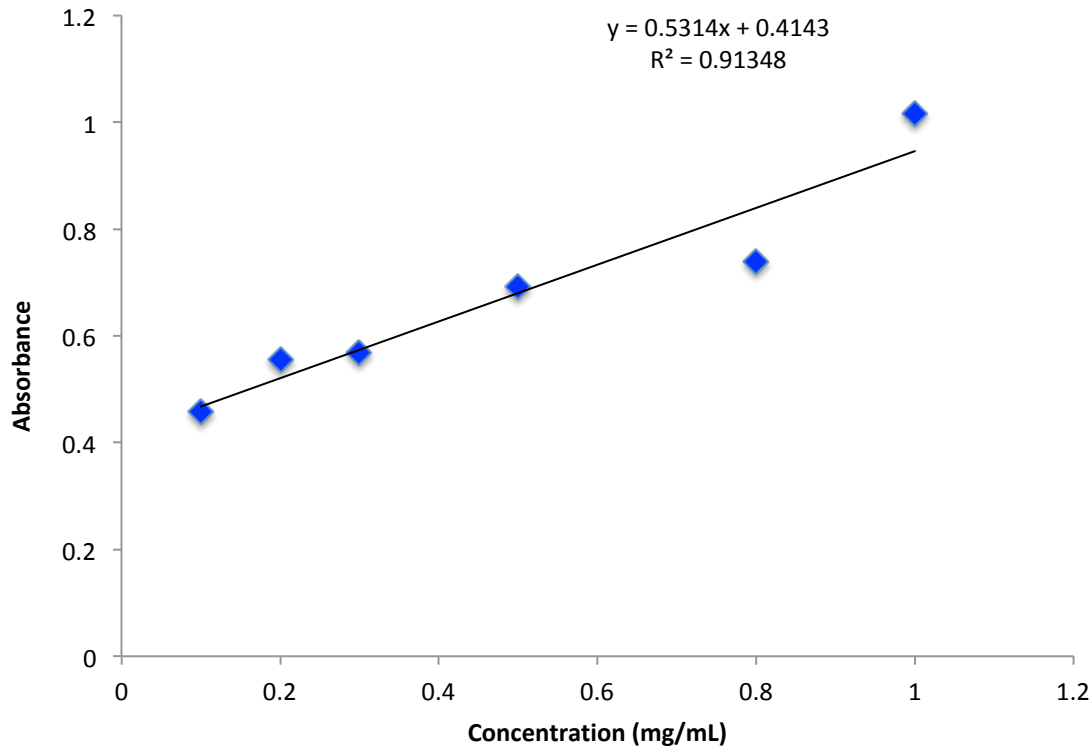
1 2 3



Proteomics

- Whole Cell Proteomics

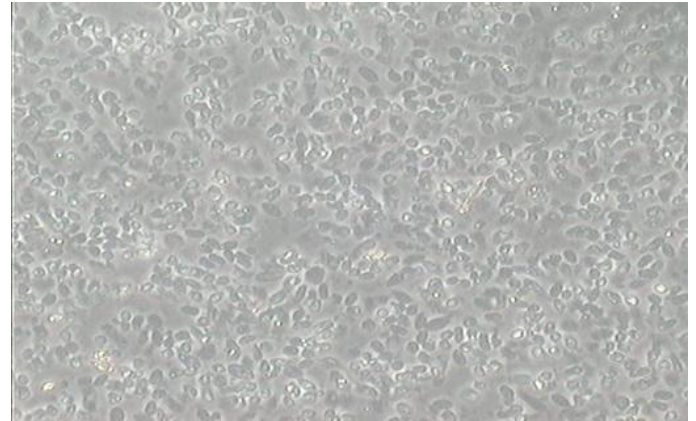
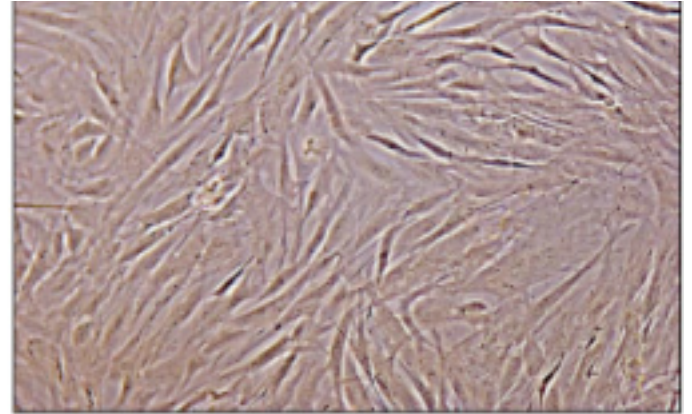
- Probing for protein differences between incubation conditions



Control	254 ug
Betaine	139 ug
Proline	390 ug

University Hospital

- Primary endometrial cells
 - Stromal & epithelial
- Vial freezing
 - 90% serum

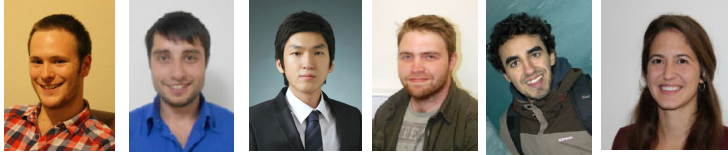


Next

- Proteomics for control, proline, and betaine incubation
- Finish betaine plate freezing– precursor for Chris' polymers
- Collagen coating 24 well plates for Neuro-2a freezing
- UH freezing
- Ben's sugar assay and imaging

Acknowledgements

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Citations

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- [13] G. M. Fahy, "Cryoprotectant toxicity neutralization," *Cryobiology*, vol. 60, no. 3 SUPPL., pp. S45–S53, Jul. 2010.
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Extra bits in case you need them.