

The Cryopreservation of Biological Materials

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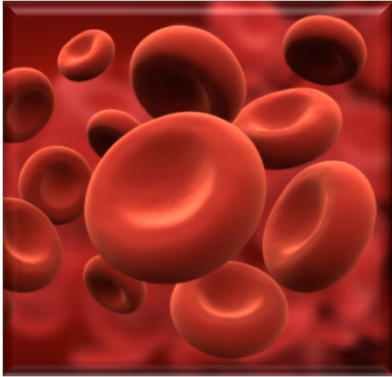
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 @LabGibson



Biological Materials

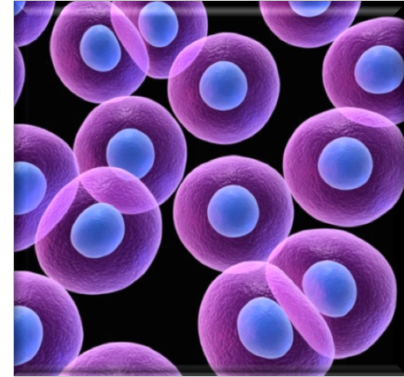
Blood



Glycerol

- Slow preparation
- Time consuming rigorous removal process

Cells

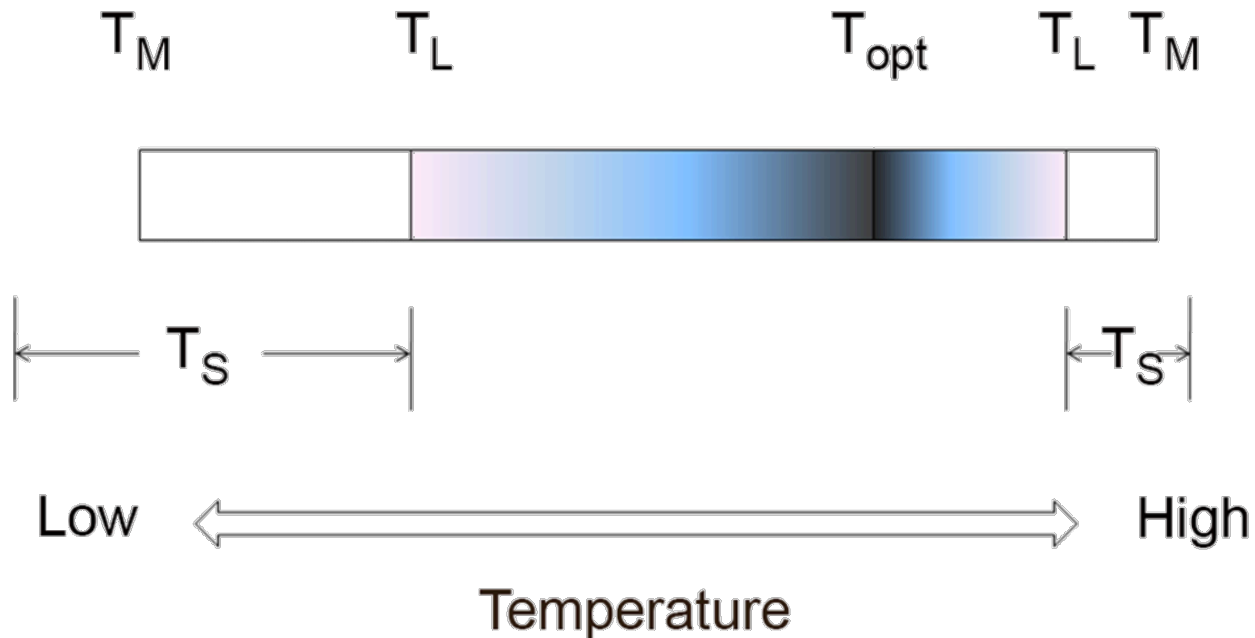


DMSO

- Toxic to cells at room temperature [1]
- Frozen in solution

Limitations

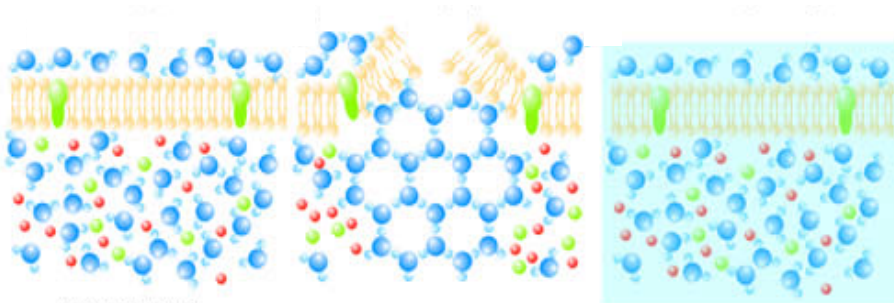
- Between T_M and T_S in a state of suspended animation
- Resume metabolism once the temperature re-crosses the T_M threshold [2]



Problems

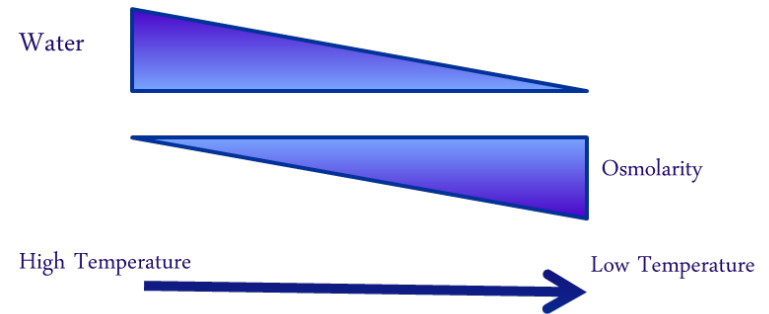
Ice

- Lipid bilayer disruption
- Ice nucleation proliferates
- Internal ice is almost always lethal

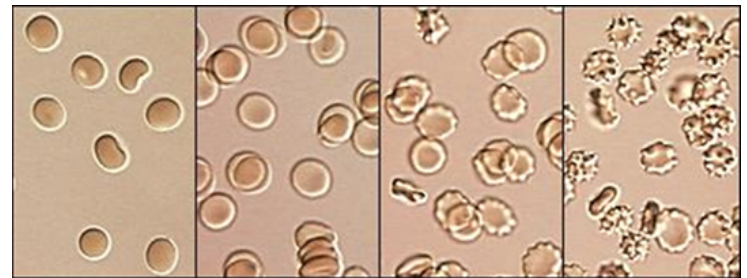


Osmolarity

- As ice forms, solute concentration changes

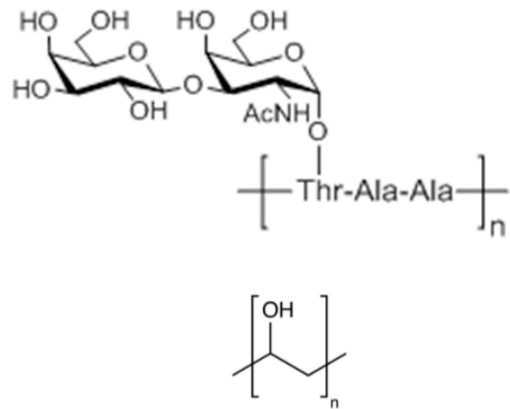
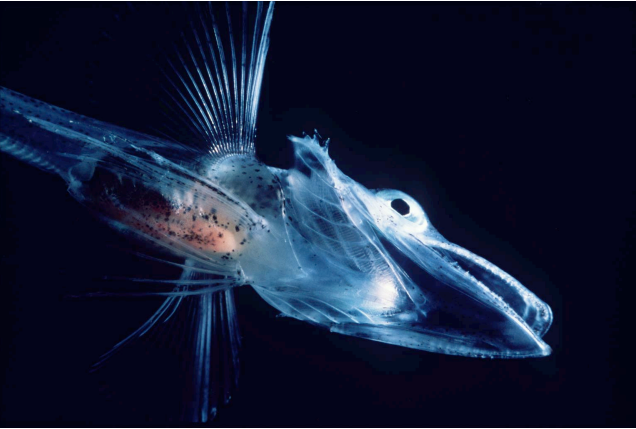


- Water rushes out of the cell

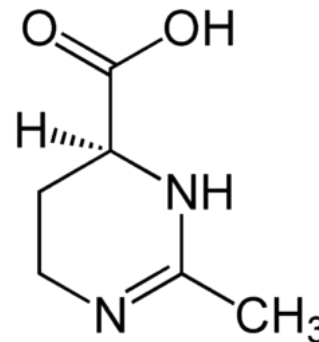


Strategies

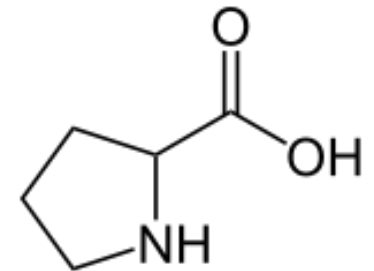
Antifreeze Proteins



Protective Osmolytes



Amino Acids



Solution Testing w/ Blood

	% Lysis	SEM	% Recovery
50 mM Proline + 50 mM Ectoine + 1% DMSO + PVA	36.2%	10.6%	63.8%
50 mM Ectoine + 1% DMSO	37.2%	8.4%	62.8%
50 mM Proline + 50 mM Ectoine + 1% DMSO	40.1%	5.2%	59.9%
50 mM Proline + 50 mM Ectoine + 10% DMSO	43.4%	5.8%	56.6%
50 mM Proline + 50 mM Ectoine	44.1%	4.4%	55.9%
5 mM Proline + 50 mM Ectoine + 1% DMSO	47.6%	7.8%	52.4%
50 mM Proline + 50 mM Ectoine + 1% DMSO	48.1%	6.3%	51.9%
5 mM Proline + 50 mM Ectoine + 1% DMSO	48.4%	9.2%	51.6%
5 mM Proline + 50 mM Ectoine	49.4%	6.1%	50.6%
50 mM Ectoine + 1% DMSO	49.7%	2.6%	50.3%
5 mM Proline + 50 mM Ectoine + 1% Glycerol	50.1%	6.3%	49.9%
50 mM Proline + 50 mM Ectoine + 10% Glycerol	50.1%	4.7%	49.9%
50 mM Proline + 1% DMSO	50.3%	4.7%	49.7%
50 mM Proline + 50 mM Ectoine + 1% Glycerol	50.4%	8.8%	49.6%
1% DMSO	50.6%	8.2%	49.4%
200 mM Proline	50.6%	3.3%	49.4%
50 mM Ectoine	51.7%	9.2%	48.3%
50 mM Proline + 50 mM Ectoine + 10% DMSO	52.0%	10.3%	48.0%
50 mM Proline + 50 mM Ectoine + 1% DMSO + PVA	52.1%	8.2%	47.9%
100 mM Proline	52.7%	1.4%	47.3%
50 mM Proline + 50 mM Ectoine	52.9%	5.8%	47.1%
PVA	54.3%	7.5%	45.7%
50 mM Proline + 50 mM Ectoine + 1% Glycerol + PVA	55.4%	6.2%	44.6%
50 mM Proline + 10% DMSO + PVA	55.6%	4.9%	44.4%
5 mM Proline + 10% DMSO + PVA	56.1%	5.6%	43.9%
50 mM Ectoine + 1% DMSO + PVA	56.3%	9.9%	43.7%
5 mM Proline + 10% DMSO	56.8%	4.3%	43.2%
5 mM Proline + 1% DMSO + PVA	57.2%	6.2%	42.8%
5 mM Proline + 1% DMSO	58.3%	5.2%	41.7%
50 mM Ectoine + 1% Glycerol	59.3%	5.7%	40.7%
50 mM Ectoine + 10% DMSO + PVA	59.8%	9.3%	40.2%
50 mM Proline + 50 mM Ectoine + PVA	60.2%	2.2%	39.8%
50 mM Proline + 10% DMSO	60.3%	4.0%	39.7%
10% DMSO	62.3%	9.7%	37.7%
50 mM Proline + 1% DMSO + PVA	62.6%	7.5%	37.4%
50 mM Ectoine + 10% DMSO	66.1%	4.4%	33.9%
5 mM Proline + 50 mM Ectoine + 10% DMSO	66.3%	1.6%	33.7%
5 mM Proline + 50 mM Ectoine + 10% DMSO + PVA	67.1%	3.2%	32.9%
5 mM Proline + 50 mM Ectoine + 1% DMSO + PVA	71.5%	3.8%	28.5%

Solution Testing w/ Blood

	% Lysis	SEM	% Recovery
50 mM Proline + 50 mM Ectoine + 1% DMSO + PVA	36.2%	10.6%	63.8%
50 mM Ectoine + 1% DMSO	37.2%	8.4%	62.8%
50 mM Proline + 50 mM Ectoine + 1% DMSO	40.1%	5.2%	59.9%
50 mM Proline + 50 mM Ectoine + 10% DMSO	43.4%	5.8%	56.6%
50 mM Proline + 50 mM Ectoine	44.1%	4.4%	55.9%
5 mM Proline + 50 mM Ectoine + 1% DMSO	47.6%	7.8%	52.4%
50 mM Proline + 50 mM Ectoine + 1% DMSO	48.1%	6.3%	51.9%
5 mM Proline + 50 mM Ectoine + 1% DMSO	48.4%	9.2%	51.6%
5 mM Proline + 50 mM Ectoine	49.4%	6.1%	50.6%
50 mM Ectoine + 1% DMSO	49.7%	2.6%	50.3%
5 mM Proline + 50 mM Ectoine + 1% Glycerol	50.1%	6.3%	49.9%
50 mM Proline + 50 mM Ectoine + 10% Glycerol	50.1%	4.7%	49.9%
50 mM Proline + 1% DMSO	50.3%	4.7%	49.7%

Permeability

Proline Kd: 0.718 min^{-1}

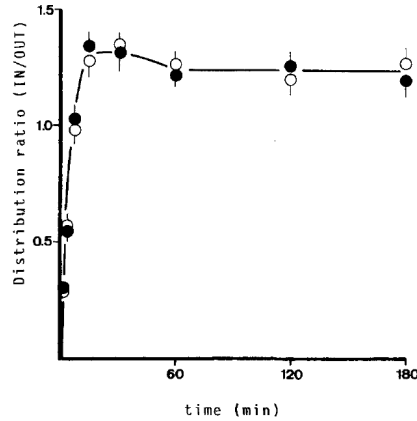


Fig. 1. Time course of L-proline uptake by rat erythrocytes. L-proline was used at 0.1 mM . For experimental details see Experimental section. ● Na^+ medium; ○ K^+ medium. Data points are the mean \pm SE of 3 experiments each done in triplicate.

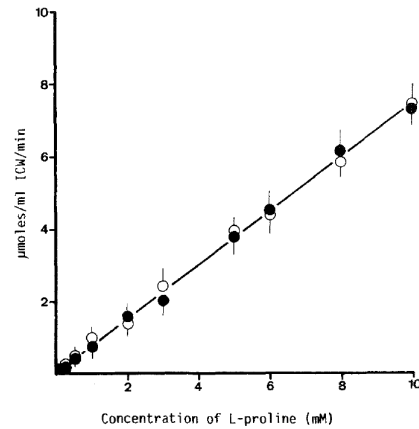


Fig. 2. Concentration dependence of L-proline uptake into rat red blood cells. The initial rate of L-proline transport was measured at 1 min. ● Na^+ medium; ○ K^+ medium. Values are the mean \pm SE of 3 experiments each done in triplicate.

Lysine Kd: 0.191 min^{-1}

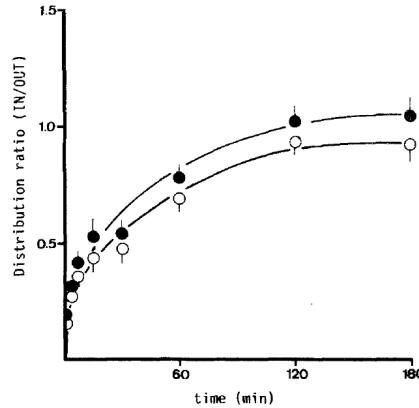


Fig. 3. Time course of L-lysine uptake by rat erythrocytes. L-lysine was used at 0.1 mM . For experimental details see Experimental section. ● Na^+ medium; ○ K^+ medium. Data points are the mean \pm SE of 3 experiments each done in triplicate.

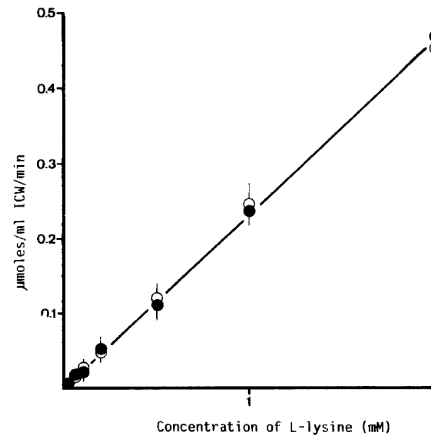


Fig. 4. Concentration dependence of L-lysine uptake into rat red blood cells. The initial rate of L-lysine transport was measured at 1 min. ● Na^+ medium; ○ K^+ medium. Values are the mean \pm SE of 3 experiments each done in triplicate.

Glutamate Kd: 0.067 min^{-1}

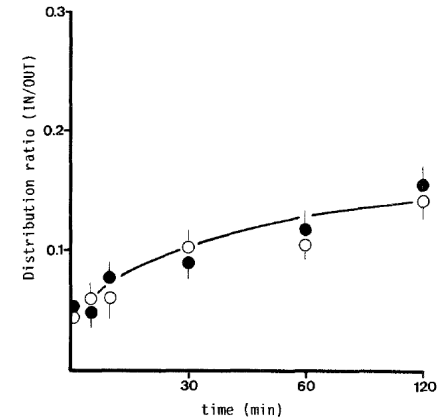


Fig. 5. Time course of L-glutamate uptake by rat erythrocytes. L-glutamate was used at 0.2 mM . For experimental details see Experimental section. ● Na^+ medium; ○ K^+ medium. Data points are the mean \pm SE of 3 experiments each done in triplicate.

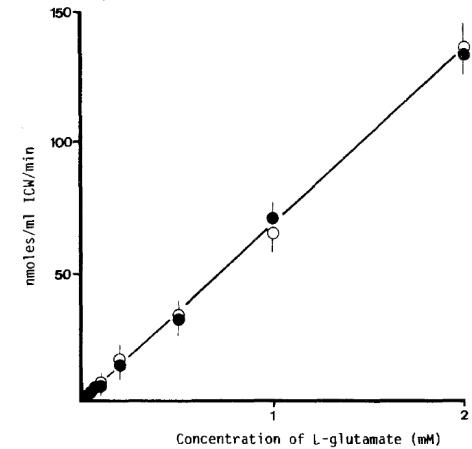
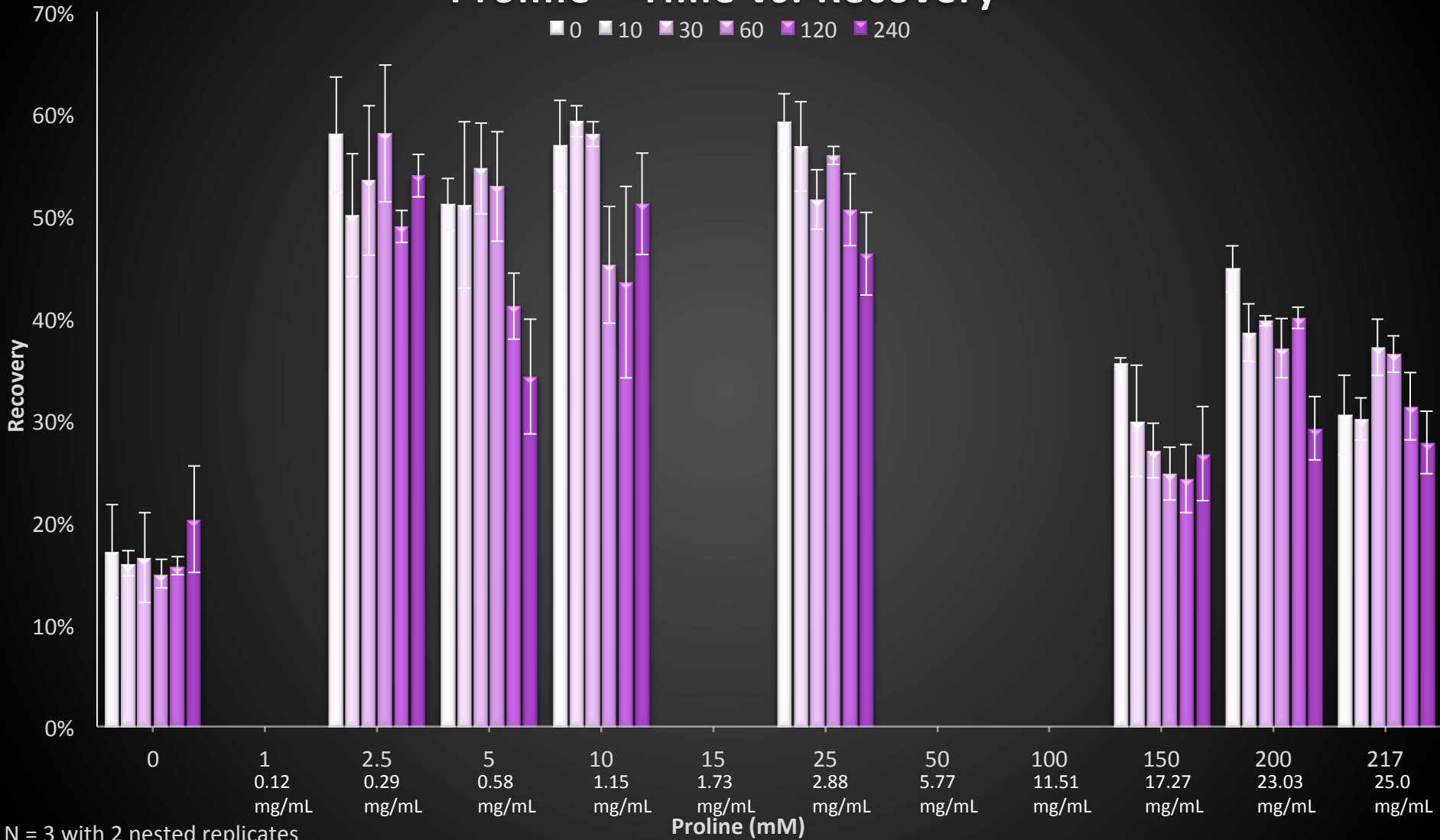


Fig. 6. Concentration dependence of L-glutamate uptake into rat red blood cells. The initial rate of L-glutamate transport was measured at 1 min. ● Na^+ medium; ○ K^+ medium. Values are the mean \pm SE of 3 experiments each done in triplicate.

Solution Testing w/ Blood

Proline – Time vs. Recovery

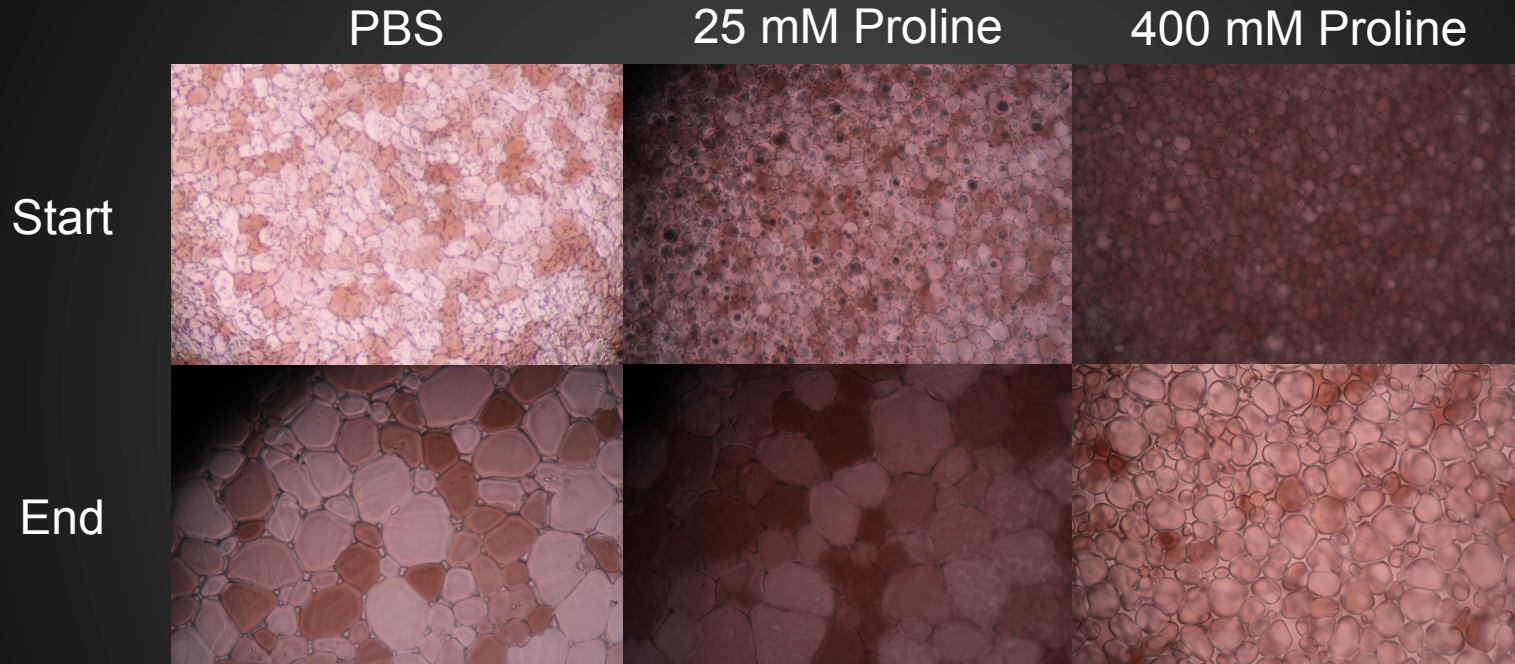
■ 0 ■ 10 ■ 30 ■ 60 ■ 120 ■ 240



N = 3 with 2 nested replicates

Proline (mM)

Splat Assay



Future Blood Work

- Complete proline concentrations
- Evaluate best proline concentration with PVA additions to find best combination
- Splat assay best combination
- Different freezing and thawing conditions



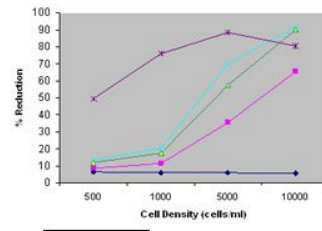
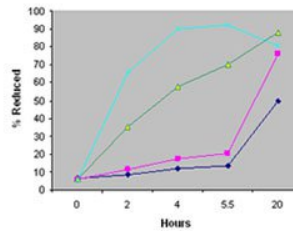
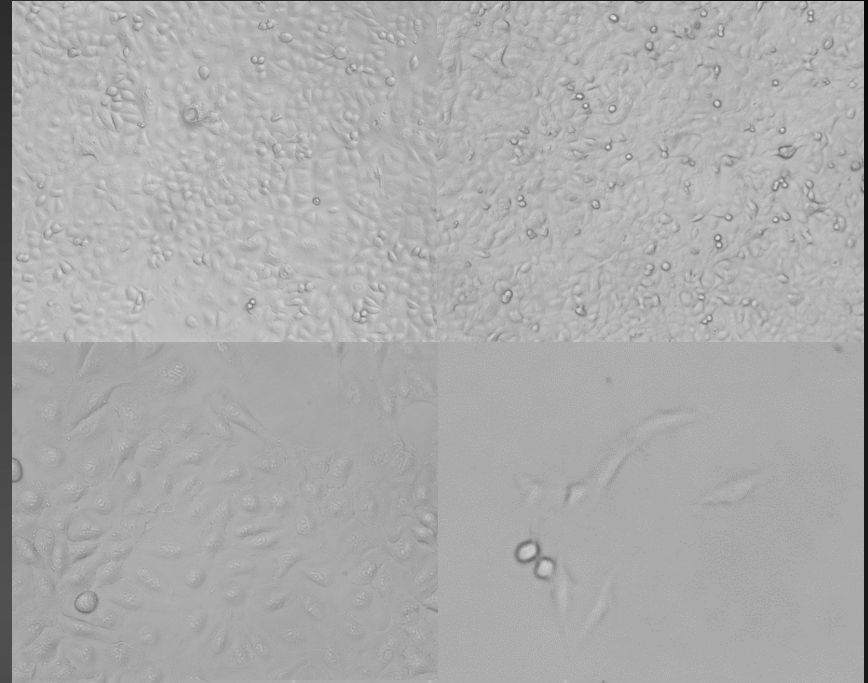
CELLS



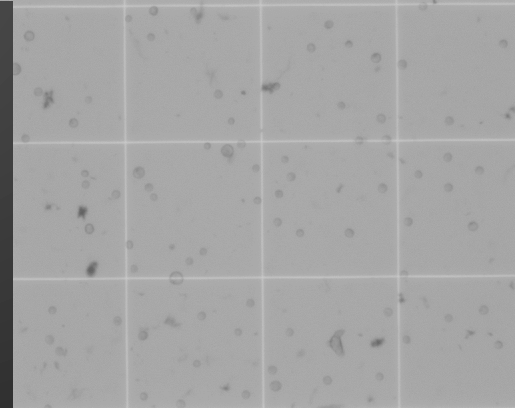
CELLS

A549 Cells

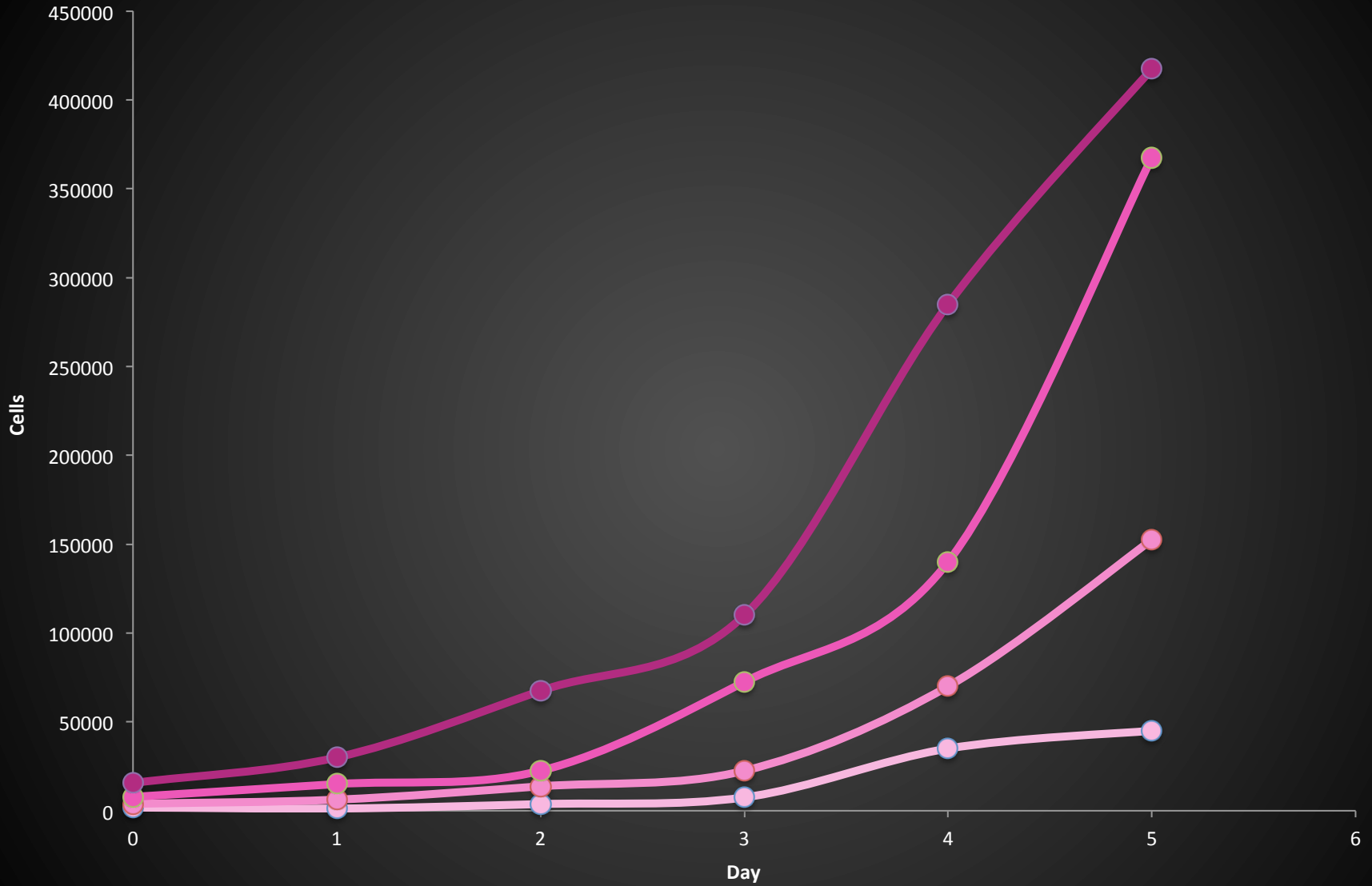
- Human carcinoma epithelial cells
- Adherent
- Contact-dependent



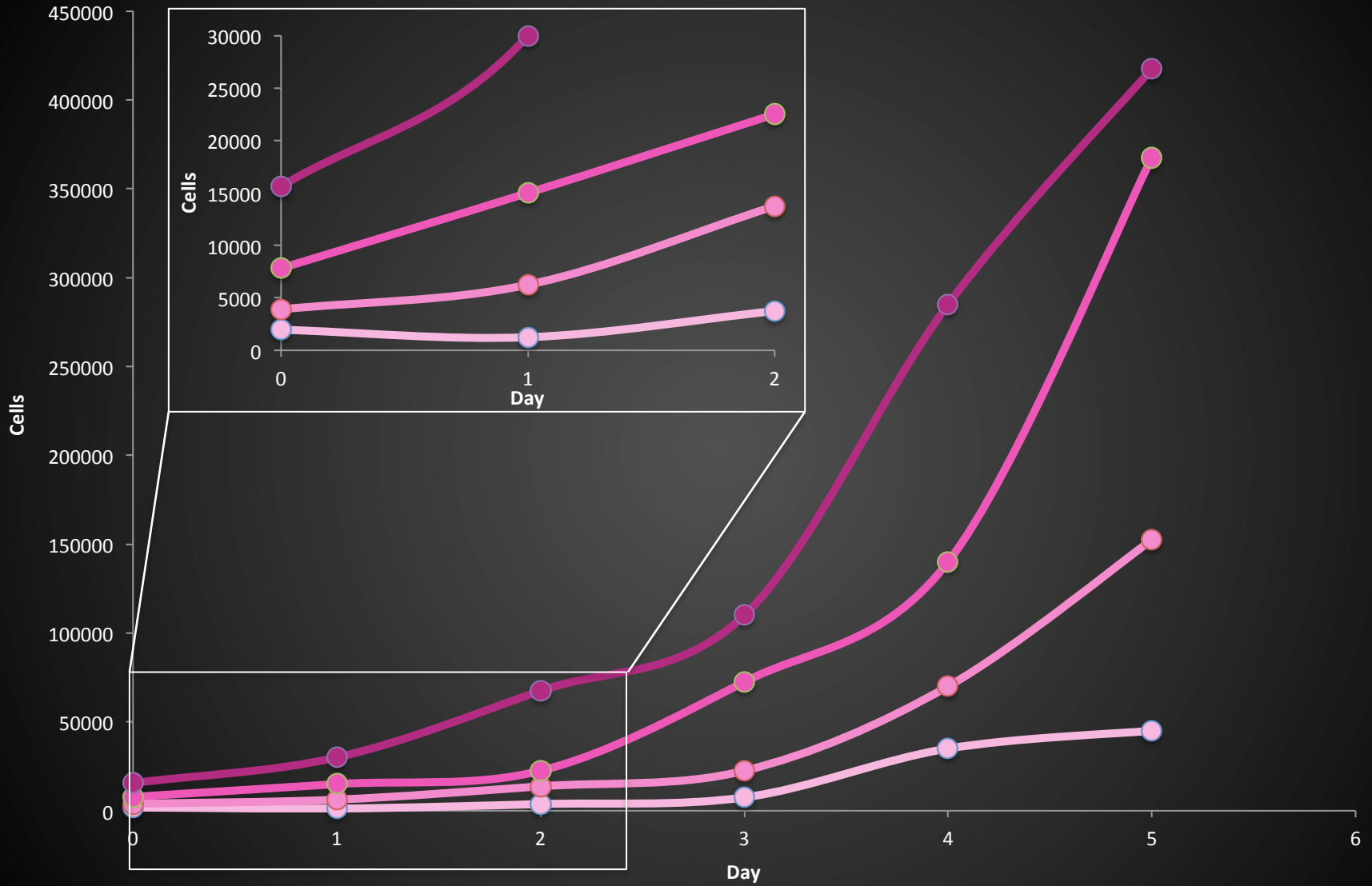
Graphs showing percentage of AlamarBlue® reduction for A549 cells with different incubation periods (Figure 1a) and at different initial cell densities (Figure 1b).



Cell Growth

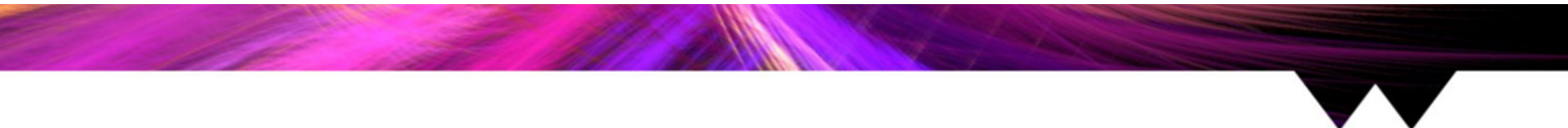


Cell Growth



Future Cell Work

- Evaluate freezing attachment
- Freeze with proline, ectoine, PVA combinations
- Splat assay solutions
- Solution effects on growth and protein expression



Acknowledgements



Principal Investigator: Matt Gibson



European Research Council

Established by the European Commission

Extra bits in case you need them.