BIOM2013 Practical 1 Macropinocytosis

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Introduction

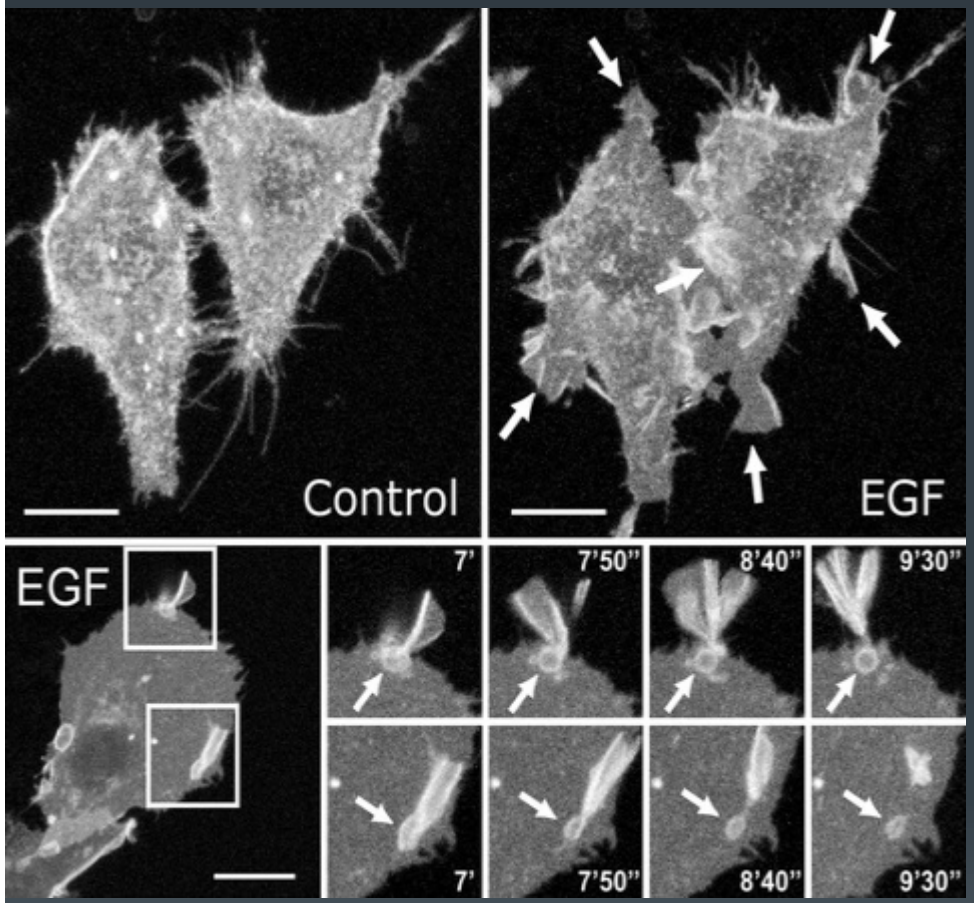


Image 1: EGF induces membrane ruffling and macropinocytosis.
Two top images = 5min after EGF, arrows indicate actin ruffling.
Large lower = formation of macropinocytosis arrows
Time lapsed = macropinocytosis formation

- Endocytosis – uptake of fluid into vacuoles (macropinosomes)
- Actin mediated process
- HPTS assay (solvent green) – measure fluorescence and absorbance
- Fluorescence- records actin filaments initiated
- Absorbance – measures number of cells
- Cells studied – Hek293 (human embryonic kidney 293 cells) and HeLa (cervical cancer cells – immortal cell line)
- Hek293 = ↑ fluorescence = would allow inhibition
- HeLa = ↓ absorbance = would allow for enhancement of dye
- EGF – enhances macropinocytosis by increasing formation of membrane ruffling
- Wortmannin – inhibits macropinocytosis – reducing uptake of dye by inhibiting P13Kinase – Inhibits Polymerisation

Image: http://www.springerimages.com/Images/MedicineAndPublicHealth/1-10.1007_s00418-008-0401-3-3
Baker, S.A., et al. (1995) Wortmannin blocks lipid and protein kinase activities associated with PI 3-kinase and inhibits a subset of responses induced by Fc epsilon R1 cross-linking. *Molecular Biology of the Cell, 6*(9), 1145-58
Clague, M.J., et al. (1995) Phosphatidylinositol 3-kinase regulation of fluid phase endocytosis. *Federation of European Biomedical Societies, 367*(3), 272-4.
Lopez, A.F., et al. (1986) Recombinant Human Granulocyte-Macrophage Colony-stimulating Factor Stimulates In Vitro Mature Human Neutrophil and Eosinophil Function, Surface Receptor Expression, and Survival. *The Journal of Clinical Investigation, 78*(5), 1220 – 1228.
Roepstorff, K., et al. (2008) Endocytotic downregulation of ErbB receptors: mechanisms and relevance in cancer. *Histohemistry and Cell Biology, 129*, 563–578.

Aims and Hypotheses

-Overall aim:

- to observing and analyse macropinocytosis :

-Enhancement (EGF)

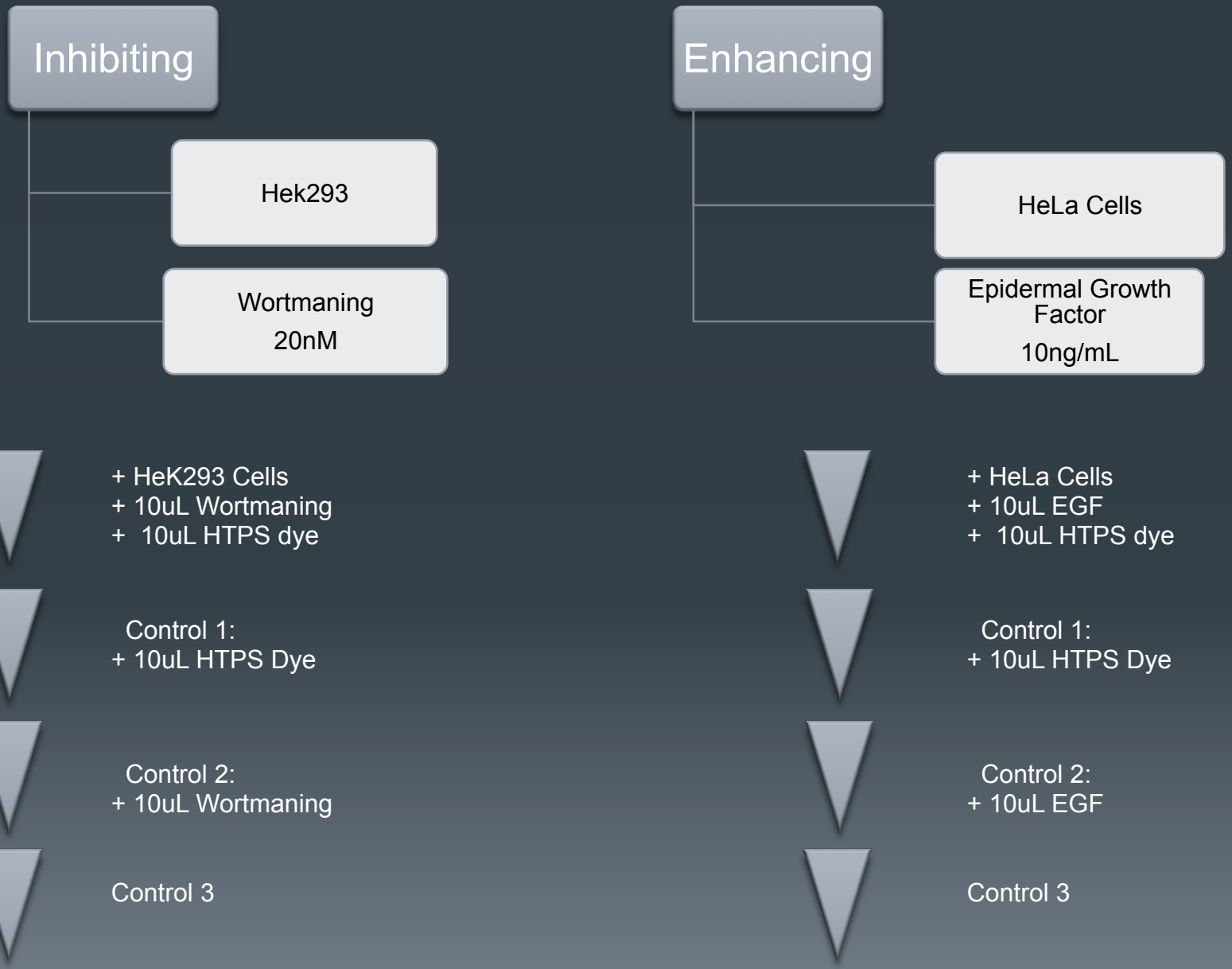
-Inhibition (Wortmannin)

-Hypotheses:

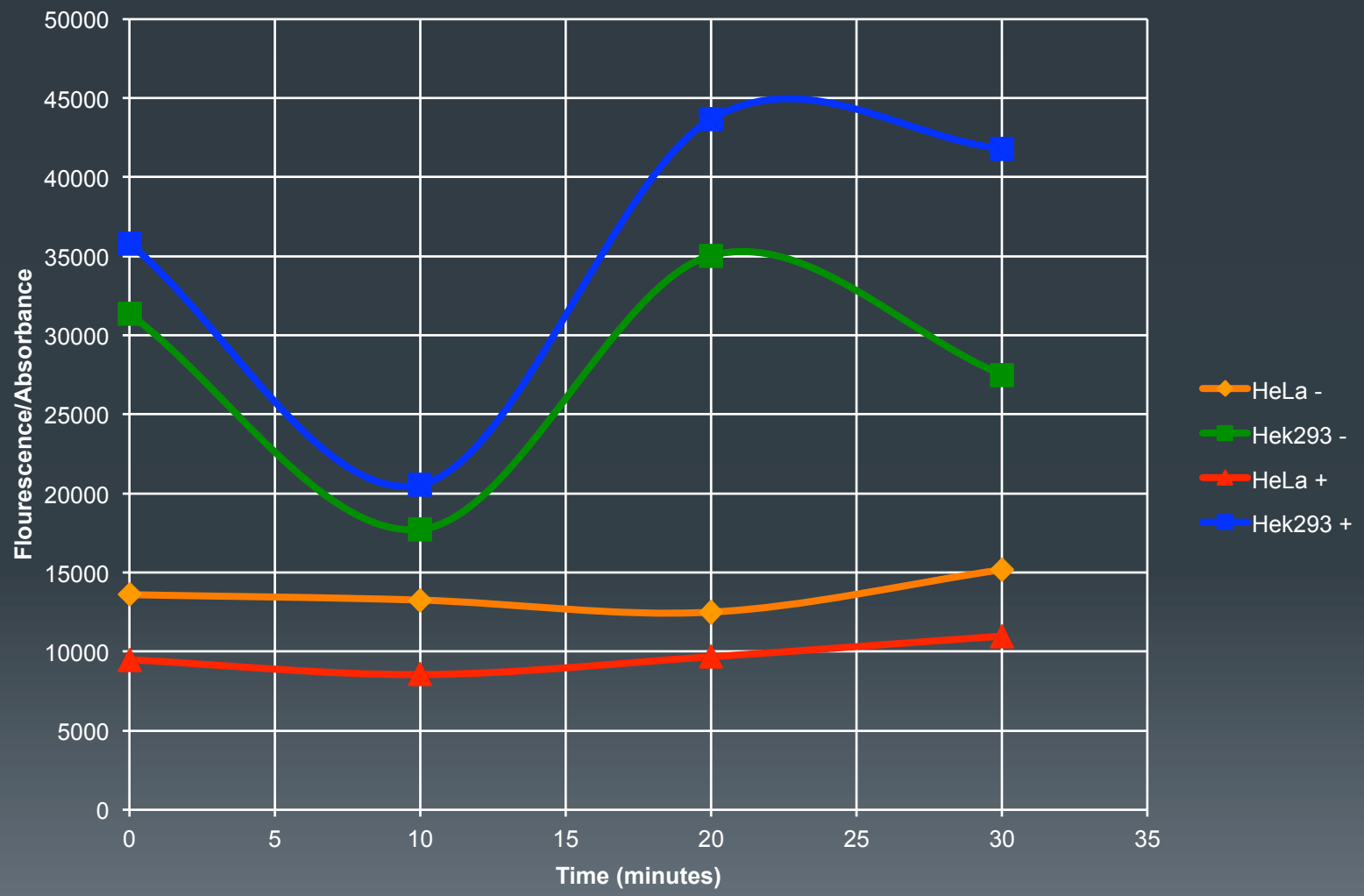
-Uptake of dye in cells.

- ↑ fluorescence in EGF treated cells
- ↓ fluorescence in Wortmannin treated cells

Method

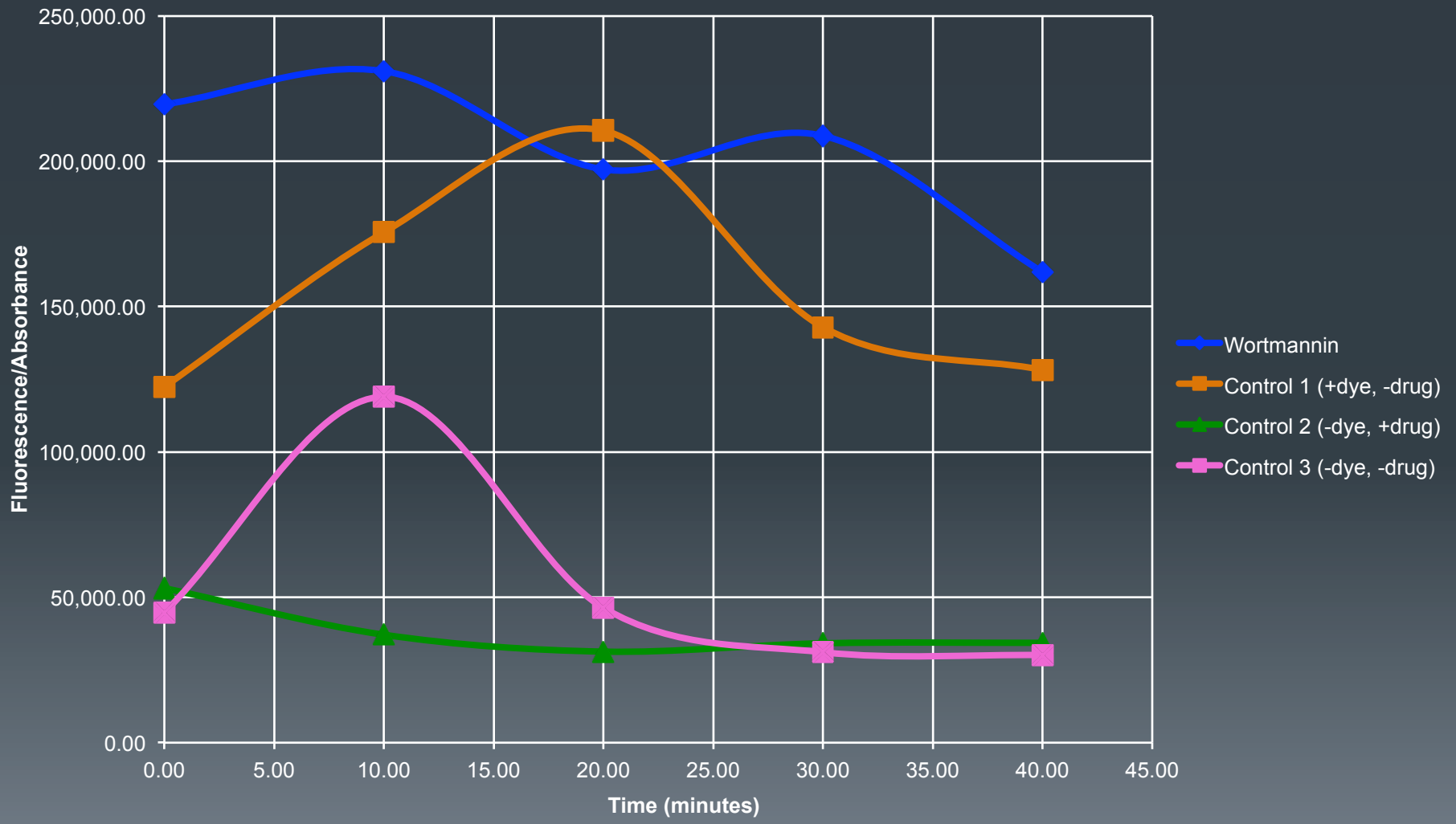

Inhibiting
Hek293
Wortmaning
20nM
+ HeK293 Cells
+ 10uL Wortmaning
+ 10uL HTPS dye
Control 1:
+ 10uL HTPS Dye
Control 2:
+ 10uL Wortmaning
Control 3
Enhancing
HeLa Cells
Epidermal Growth Factor
10ng/mL
+ HeLa Cells
+ 10uL EGF
+ 10uL HTPS dye
Control 1:
+ 10uL HTPS Dye
Control 2:
+ 10uL EGF
Control 3

Preliminary results



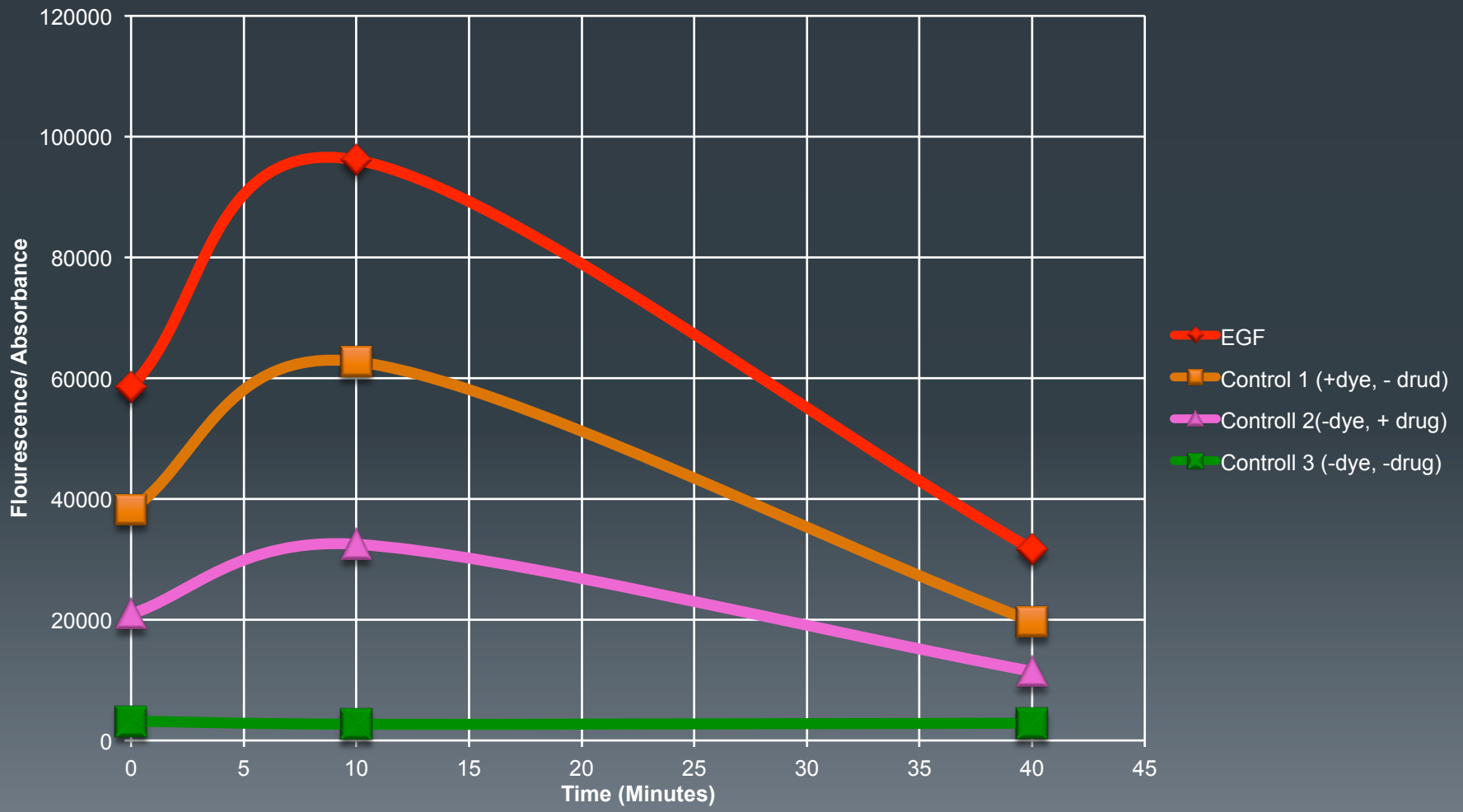
Results

Inhibition of Hek293 Cells : Data results.



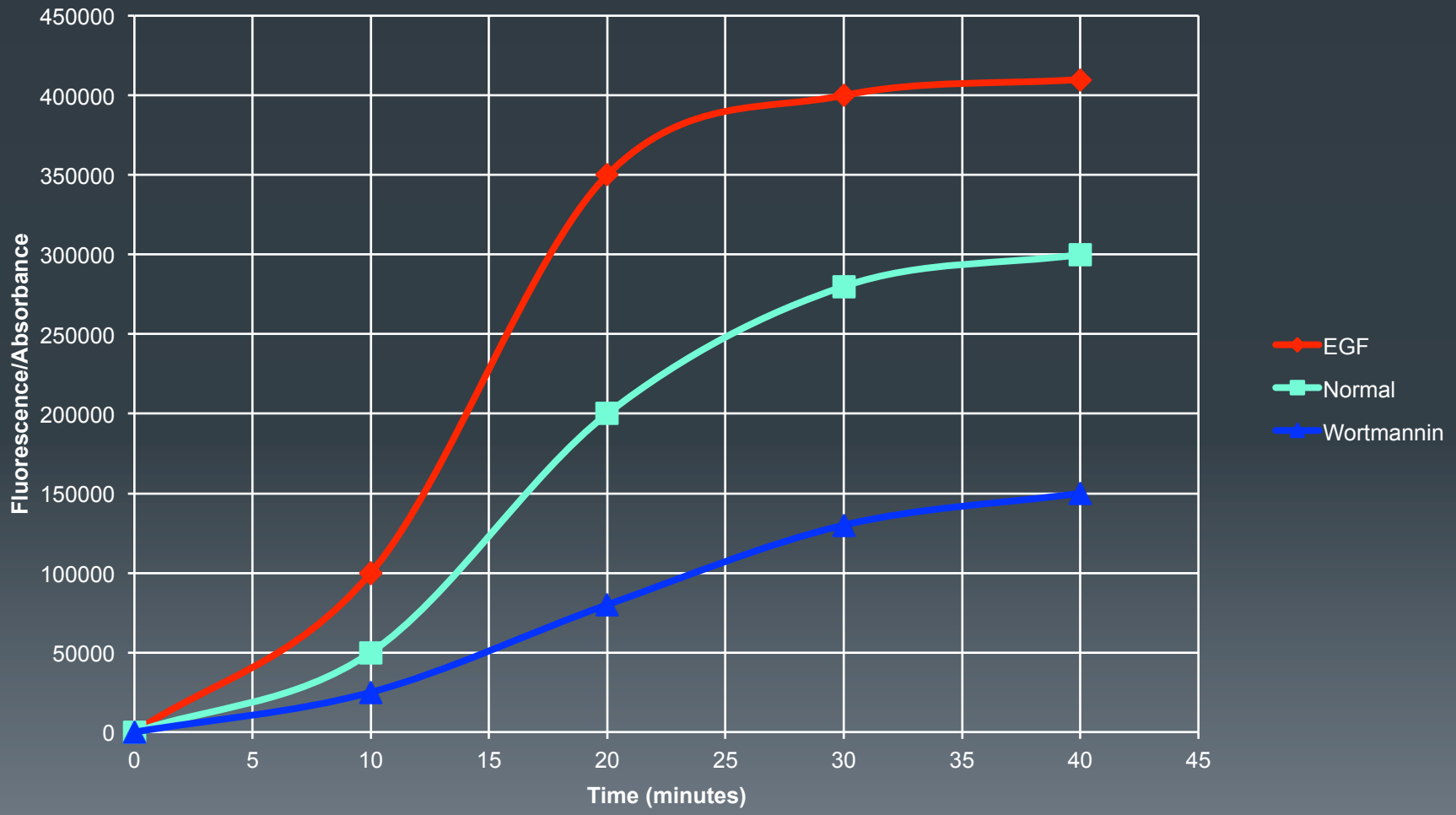
Results

Enhancement of HeLa Cells: Data results.



Results

Expected results.



Sources of Error

What may have gone wrong?

-HPTS dye used affected by pH:
- -pH change late in macropinocytosis
- -Other alternatives include *Lucifer yellow*

-Wortmannin concentration levels:
- -100nM to 200nM ideal
- -20nM was used

-Experimental and human error:
- -Labelling of tubes

Araki, N. et al. (1996) A role for phosphoinositrde 3-Kinase in the completion of macropinocytosis, *Journal of cell biology, 135.5,* 1249 – 1260.
Blommaart, E.F.C. et al. (1997) Phosphatidylinositol 3-Kinase inhibitors wortmannin and LY294002 inhibits autophagy in isolated rat hepatocytes, *European journal of Biochemistry*.
Kerr, M.C., & Teasdale, R.D. (2009) Defining Macropinocytosis, *Traffic, 10,* 364 – 371
Han, J. & Burgess, K. (2009) Fluorescent indicators for intracellular pH, *Chemistry Review*

Conclusions

- HeK293 hypothesised to be inhibited by wortmannin
  - However results did not prove this.
- HeLa hypothesised to be enhanced by EGF
  - Data varied from hypothesis.
- Overall aim was achieved
  - Administration of drugs did show changes in macropinocytosis.

Future Directions

- PI-3 Kinase and cell proliferation
- Wortmannin concentrations
- Other dyes

Mizuno, M. et al. (2003) Phosphatidylinositol 3-kinase: a molecule mediating BDNF-dependent spatial memory formation, *Molecular Psychiatry,* 8, 217-224
Araki, N. et al. (2006) Effect of 3-methyladenine on the fusion process of macropinosomes in EGF-stimulated A431 Cells. *Cell Structure and Function, 31,* 145 – 157.
Zhang, Y. et al. (2010) Construction of a high-EGFR expression cell line and its biological properties comparing with A431 cell. *African Journal of Biotechnology, 9*(30), 4674-4680.
Donepudi, M., & Resh, M.D. (2008) c-Src trafficking and co-localizationwith the EGF receptor promotes EGF ligand-independent EGF receptor activation and signaling. *Cellular Signalling, 20,* 1359 - 1367