A Model System for Quantifying Receptor Tyrosine Kinase Activation

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Objective & Motivation

**Objective:** To optimize and test a model system and analysis method for quantifying receptor tyrosine kinase (RTK) activation in response to ligand binding.

**Motivation:** RTKs are important drug targets because their dysregulation has been implicated in numerous cancers and developmental disorders. Quantification of their activation is necessary to design precision drugs that can achieve the desired response level.

**System Design**

Model System: Osmotic Stress Derived Vesicles

**Optimization Process**
- DNA Transfection amount
- Antibody type
- Testing analysis program

**System Overview:**
- Labeled Antibody
- Labeled Receptor
- Phosphorylation
- Vesicle Membrane

**Measurements:**
- Bound ligand
- Bound antibody
- Ligand concentration

**System Advantages:**
- No feedback loops
- Quantitative
- Automated

**Results**

**Phosphorylation Curves for FGFR2b and FGFR1**

<table>
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<tr>
<th>FGFR1 mTurquoise Specific Antibody (2.65 μM)</th>
<th>FGFR2b mCherry FGF10 General Antibody (3 μM)</th>
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Results of the neural network analysis program developed by Daniel Wirth in the Hristova lab to analyze the intensity data from the model system.

**Conclusions and Future Work**

**Conclusion:** The model system achieves higher phosphorylation at higher ligand concentrations as seen by eye. However, the analysis program needs to be further optimized as this increase is not accurately measured in the phosphorylation curves.

**Future Work:** Fix the analysis program and re-analyze the current data in addition to applying it to new receptors.

**References:**