

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.

Background & Methods

Background:

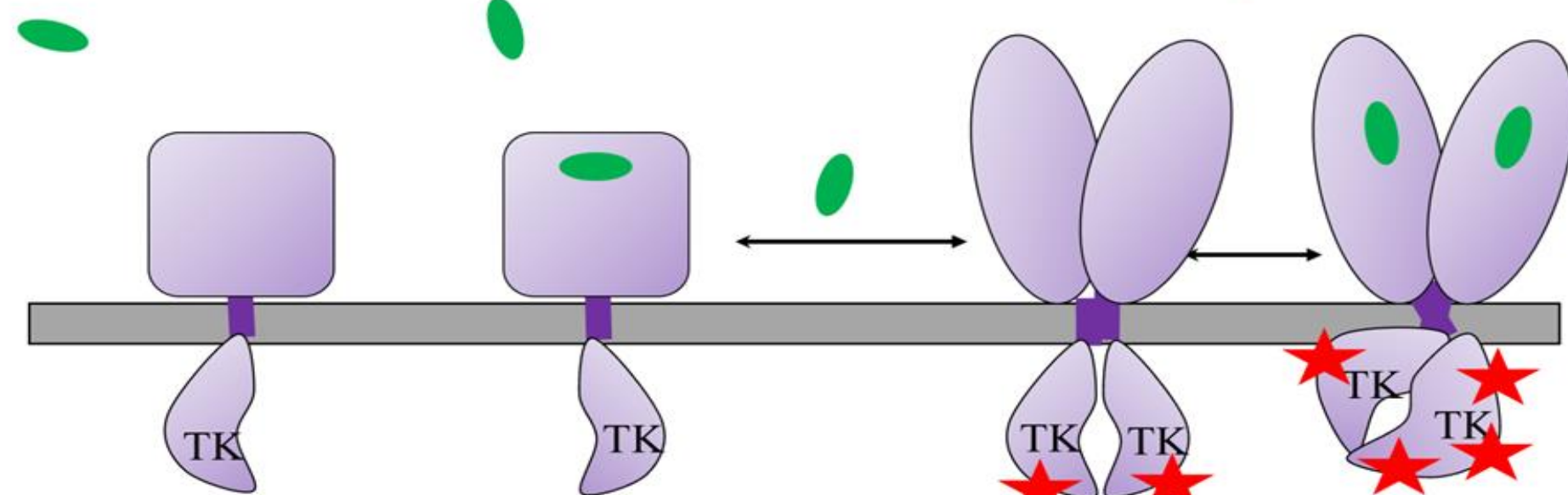


Figure 1. Mechanism of RTK Activation

FGFR2b

- Overexpressed in a variety of cancers including gastric and gastroesophageal cancers
- Contributes to tumor progression by enhancing proliferation and angiogenesis

FGFR1

- Breast, gastric, and prostate cancer
- Can promote tumor progression and metastasis

Methods:

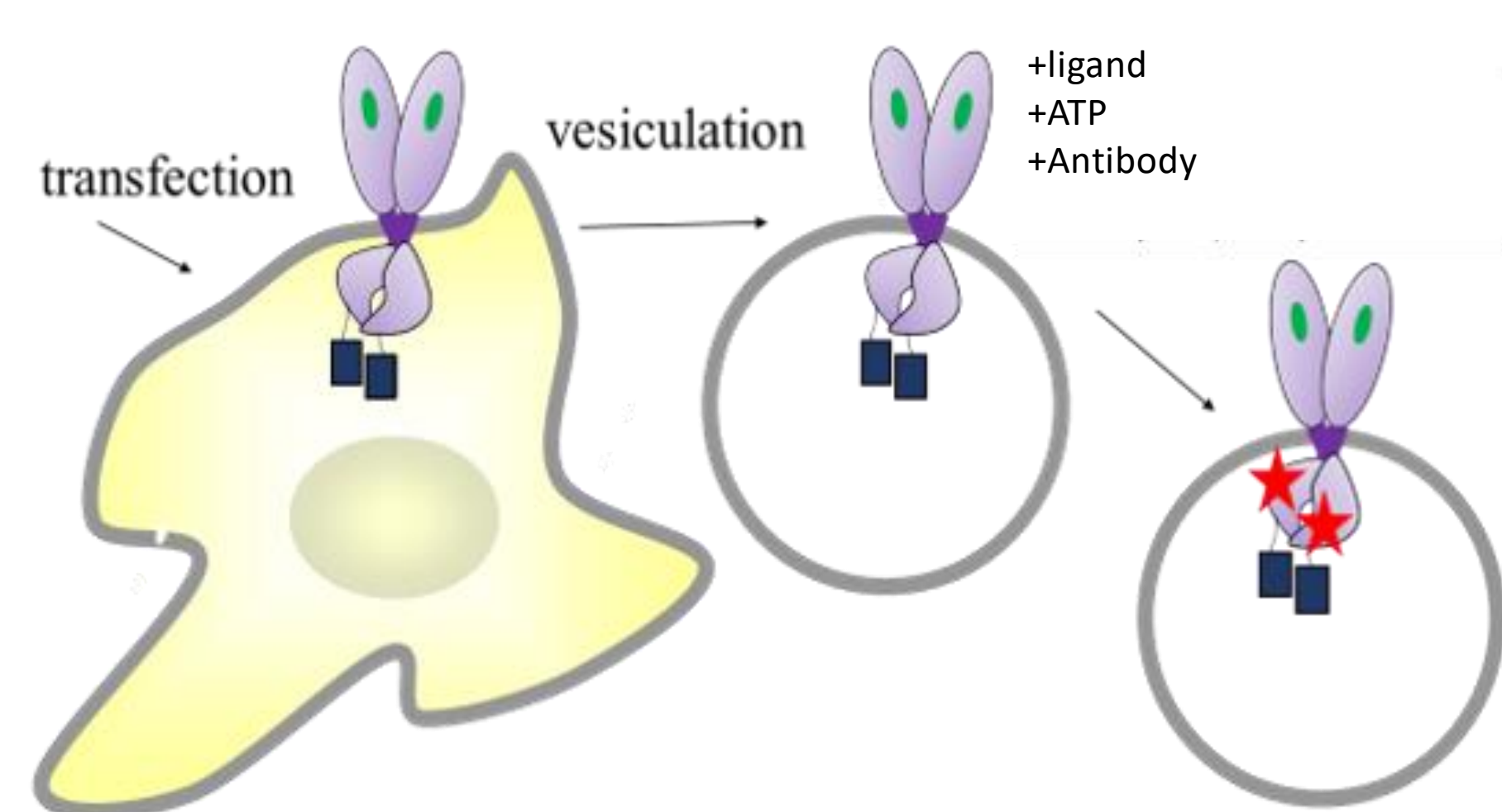


Figure 2. Vesicle Model Procedure

System Design

Model System: Osmotic Stress Derived Vesicles

Optimization Process

- DNA Transfection amount
- Antibody type
- Testing analysis program

System Overview:

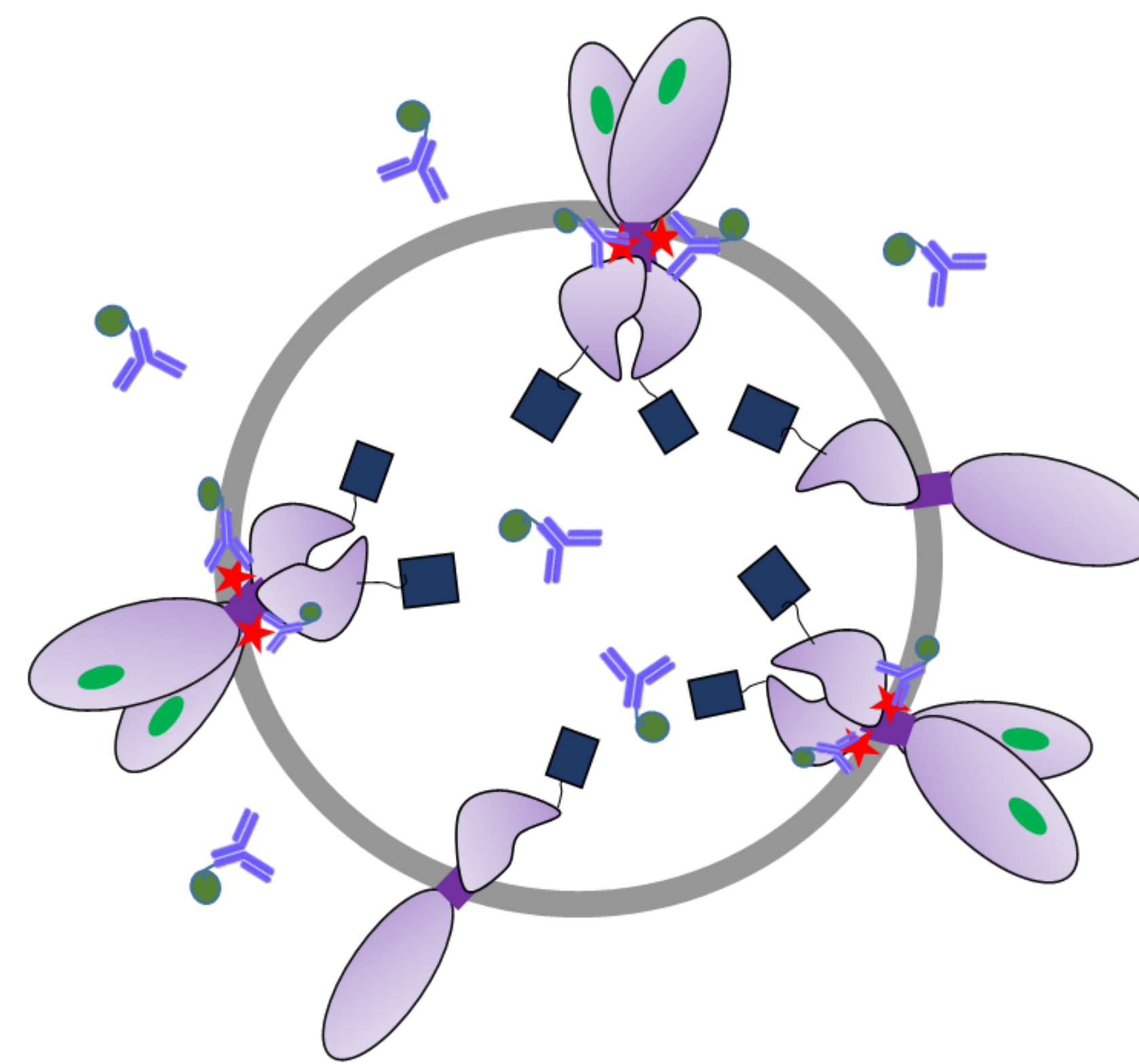
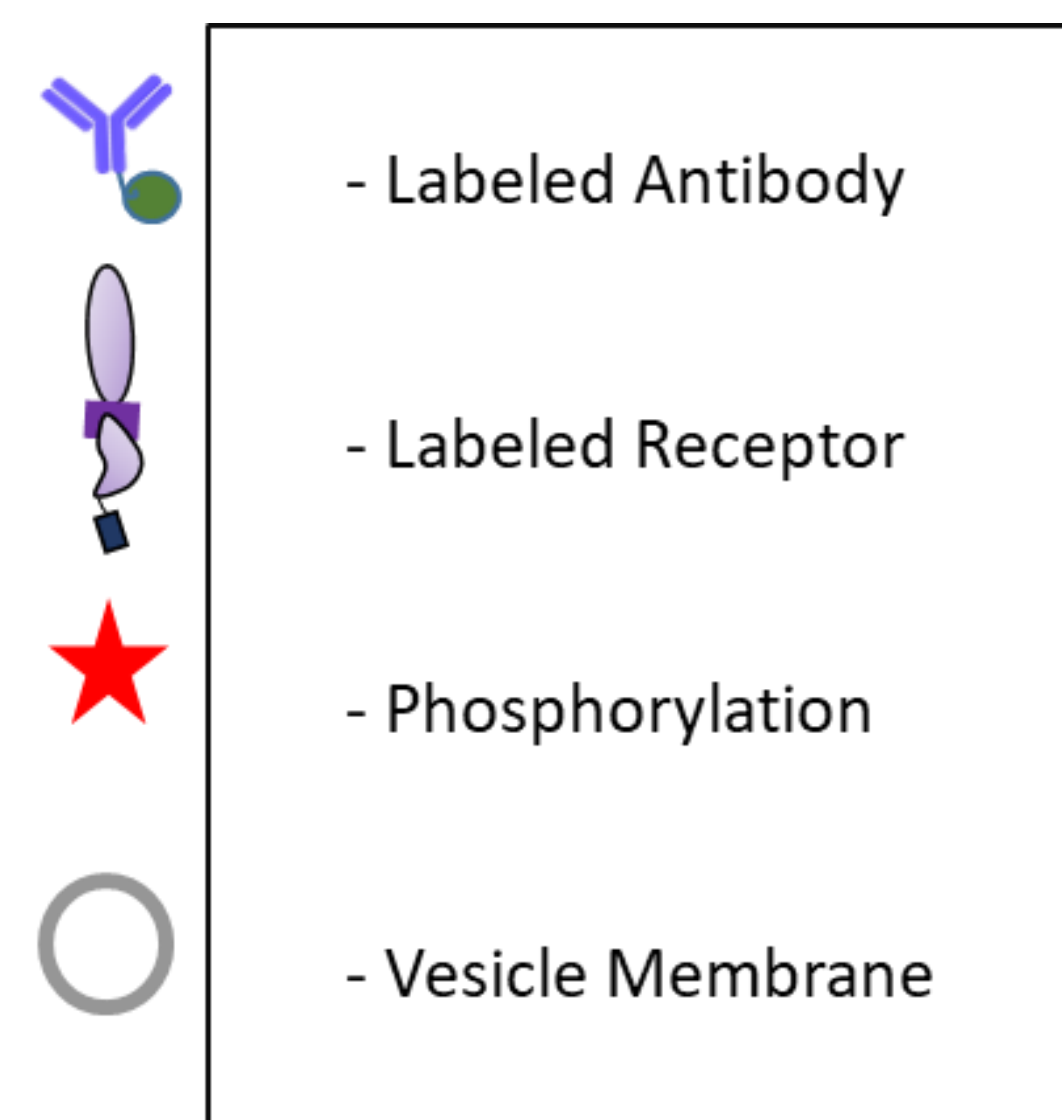


Figure 3. Model System for Ligand-Induced RTK Activation

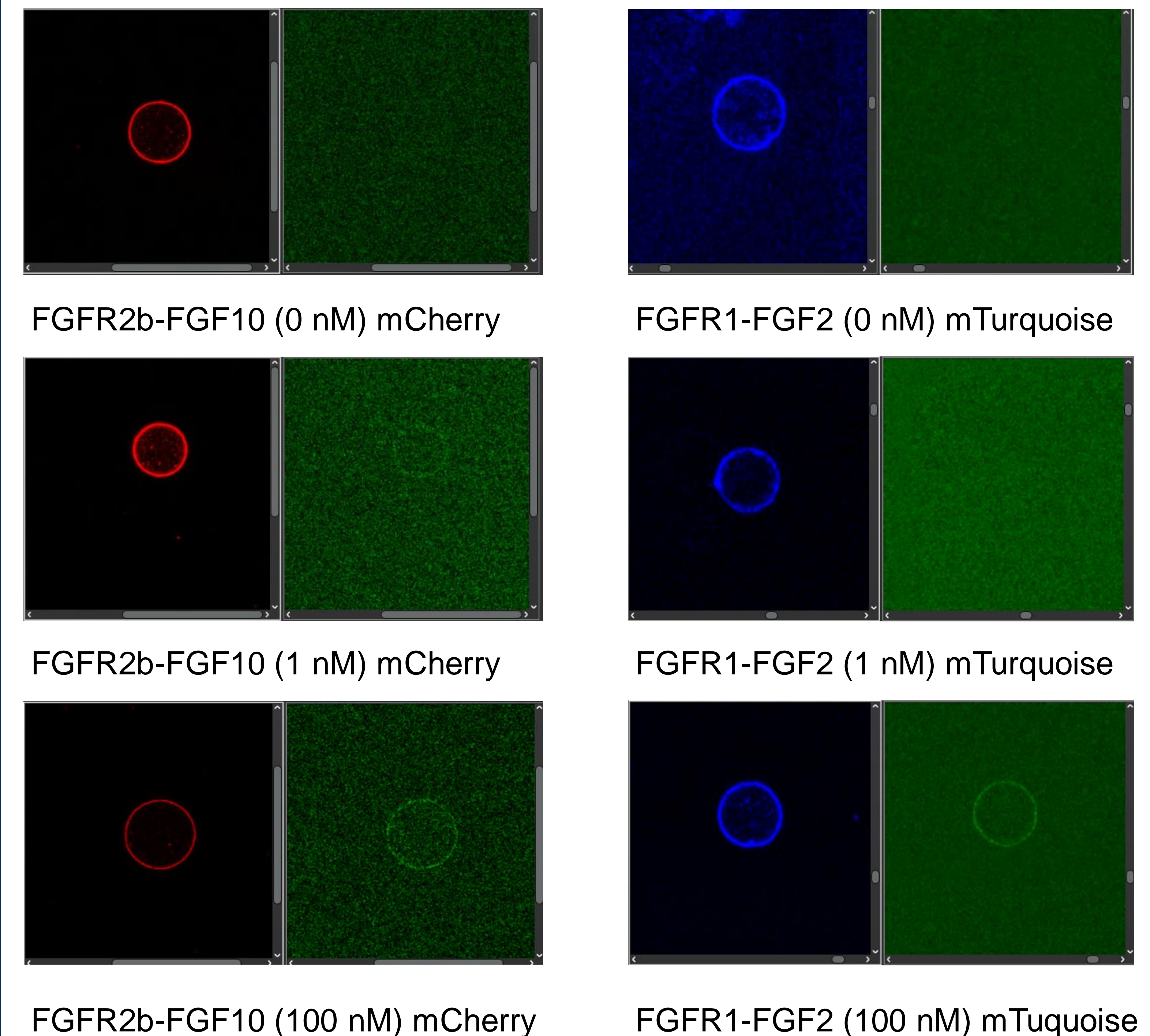
Measurements:

- Bound ligand
- Bound antibody
- Ligand concentration

System Advantages:

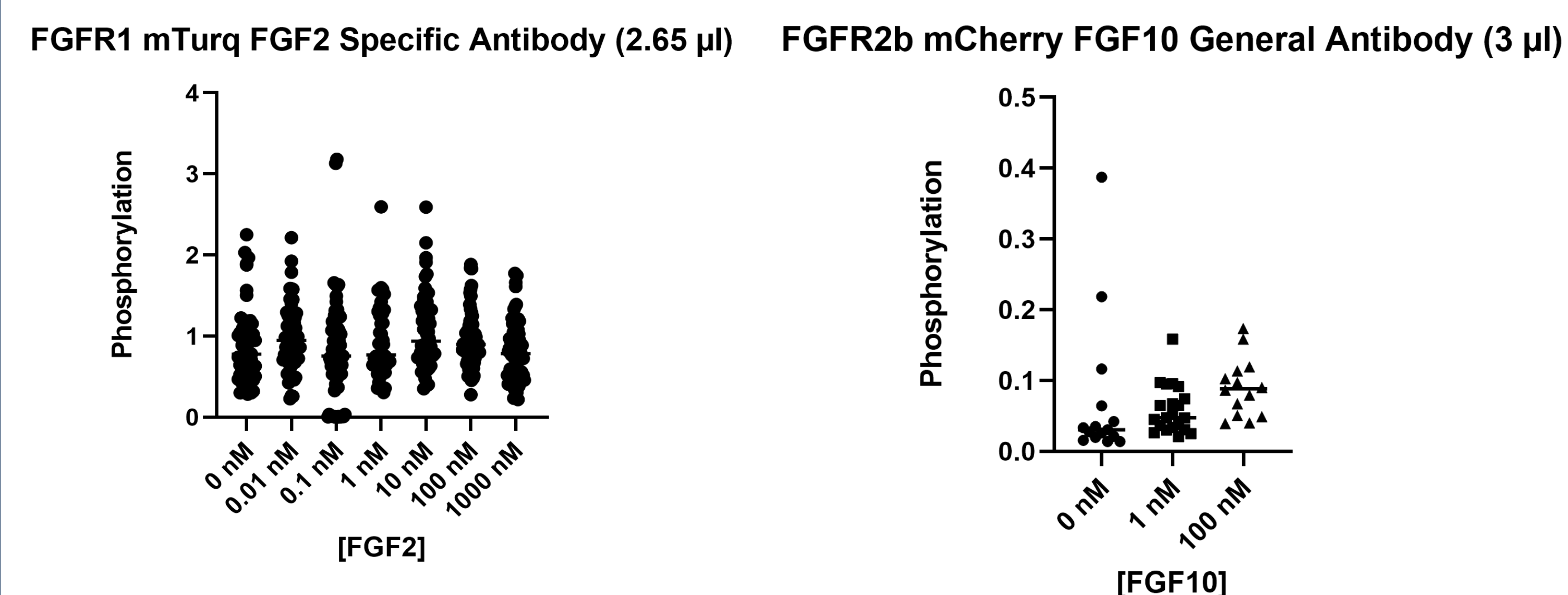
- No feedback loops
- Quantitative
- Automated

Confocal Microscopy: Effect of Ligand Concentration on Phosphorylation



Results

Phosphorylation Curves for FGFR2b and FGFR1



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:

- [1] Dai, Shuyan et al. *Cells* vol. 8,6 614. 18 Jun. 2019
- [2] Sarabipour, Sarvenaz et al. *Biochimica et biophysica acta* vol. 1848,7 (2015): 1591-8
- [3] Xie, Y., Su, N., Yang, J. et al. *Sig Transduct Target Ther* 5, 181 (2020)