Development of Novel FRET Biosensors to Detect Kinase Activity in Living Cells

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How is p38 activated?
PAR1 Induces Atypical Activation of p38

How is p38 signaling regulated?

TAB1 and TAB2 = TGFβ Activated Kinase 1, Binding Protein 1 and 2
Ge et al. (2002) Science

Grimsey, N et al. (2015) JCB
Förster Resonance Energy Transfer

B

Transmittance (%)

350 400 450 500 550 600

Wavelength (nm)

Image from researchgate.net
FRET Biosensor Design

**Constructed**

- KRAS - plasma membrane
- NLS - nuclear localization
- NES - nuclear export

**In progress**

- FYVE - early endosome
- LYN - lipid rafts [1B]
## Transfection Optimization

<table>
<thead>
<tr>
<th>GFP</th>
<th>KRAS 1:2</th>
<th>KRAS 1:3</th>
<th>KRAS 1:4</th>
<th>KRAS 1:5</th>
<th>NES 1:2</th>
<th>NES 1:3</th>
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<th>p38</th>
<th>KRAS 1:2</th>
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<th>NES 1:2</th>
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KRAS 1:4
400 ng

NES 1:5
350 ng

NLS 1:4
300 ng
FRET Assays Under Initial Parameters

Thrombin Activation of KRAS biosensor in EA Cells

Thrombin

SB inhibitor

no ligand induced change detected
FRET Assays in HeK Cells

A cohort of cells shows ligand induced change

Thrombin

PAR1 Thrombin Activation of p38 Perky in HeK Cells

Average FRET Ratio (YFP/CFP) vs. Time (Minutes)

PAR1 Thrombin Activation of p38 Perky in HeK Cells

Average FRET Ratio (YFP/CFP) vs. Time (Minutes)
FRET Assays in HeLa Cell Repeats
FRET Assays Under Optimized Parameters

FRET Response Video:
https://drive.google.com/file/d/1PcoUukQv9MTCjhAeHgNMUY7KkdHlrq/view?usp=sharing
Conclusions

FRET response matches preliminary data under optimized parameters.
Future Directions

Conduct FRET assays using constructed biosensors

Assess p38 dependent FRET activation by multiple GPCRs in multiple cell types

Use site directed mutagenesis to construct FYVE endosome biosensor

Use FRET stable cell lines to screen small molecule and peptide inhibitors
Thank you GURC and Grimsey Lab!