

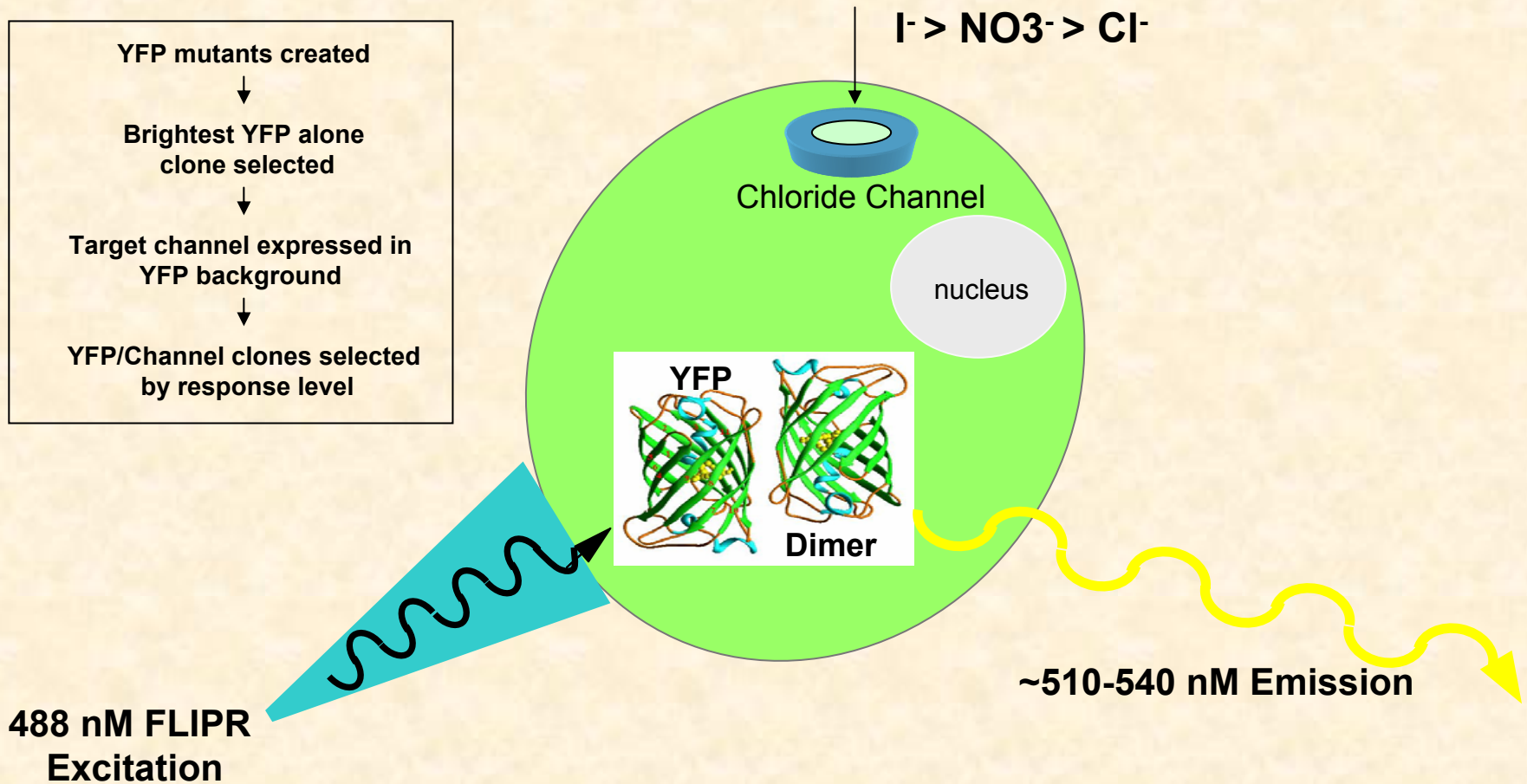
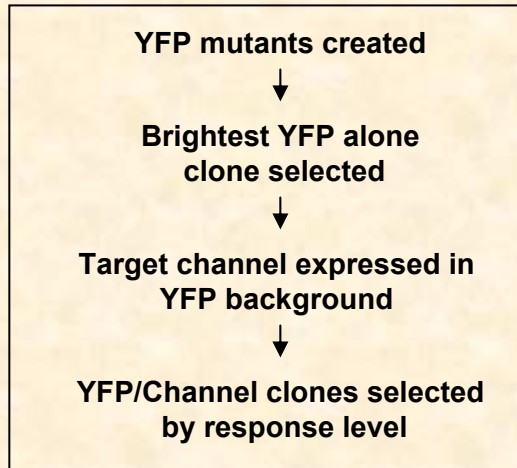
High Throughput Screening of Anion
Channels with the YFP variant
F46L/H148Q/I152L using FLIPR³⁸⁴

Mark W. Thompson

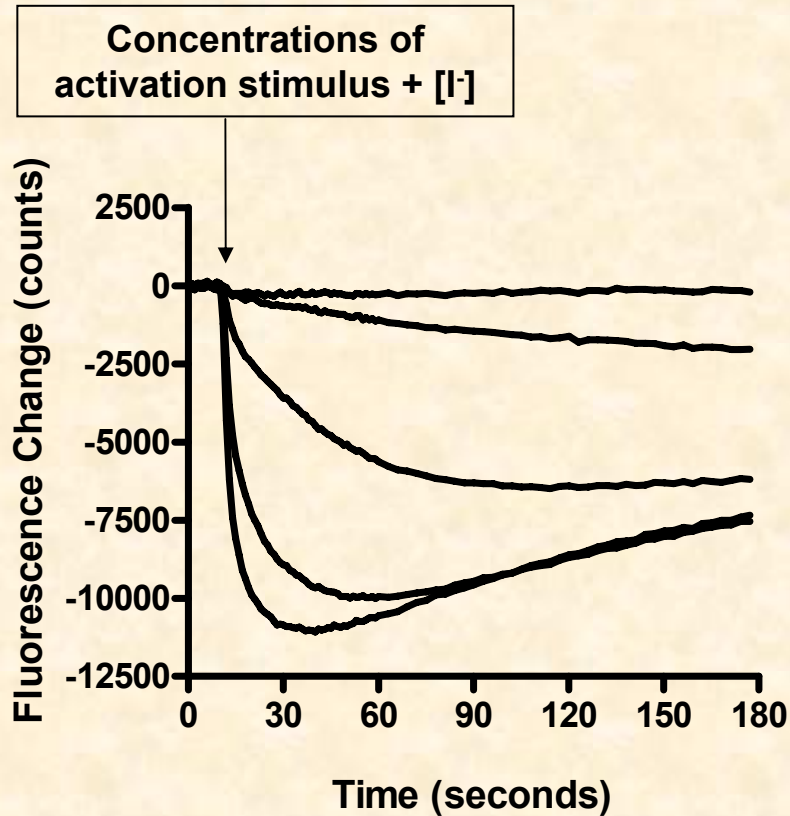
YFP Halide Indicator background

- Red-shifted form of Green Fluorescent Protein (T203Y) described by Ormo, et al. (1996) (J. Remington's Lab)
- Wachter, et al. (1998) described YFP (H148G) as a YFP with a chromophore exposed to solvent and anion sensitivity (J. Remington's Lab)
- Jayaraman, et al. (2000) report YFP (H148Q) and its enhanced halide sensitivity and utility when expressed with CFTR (A. Verkman's Lab)
- Galletta, et al. (2001) report improved halide sensing properties of YFP (H148Q/I152L) enabling practical cellular applications (A. Verkman's Lab)
- Yang, et al. (2003) report high throughput screen of 100k compound library utilizing YFP (H148Q/I152L) to identify $\Delta F508$ -CFTR potentiators (A. Verkman's Lab)

Halide Indicator Assay Overview



YFP Measurement on FLIPR



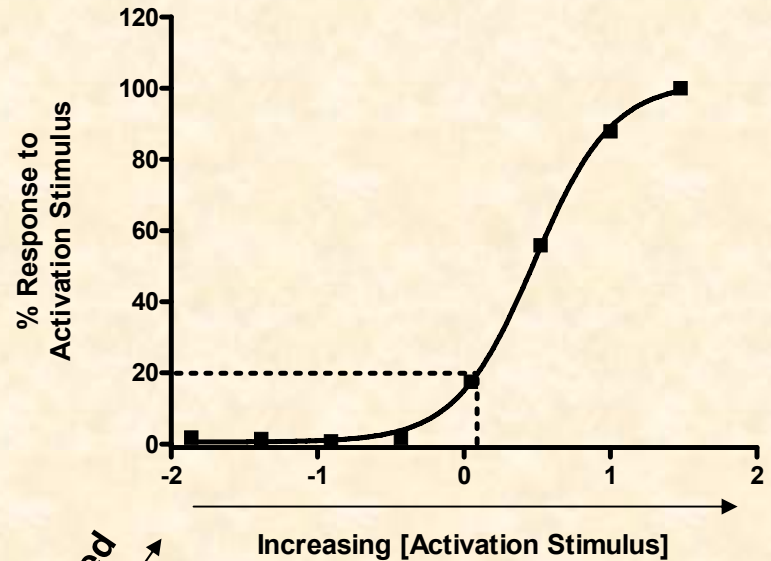
AVG

MINIMUM STATS

Data neg. control
subtracted [I-]

$$(1 - (\text{MIN}/\text{AVG}) * 100)$$

'EC20' determination
for Positive Modulation

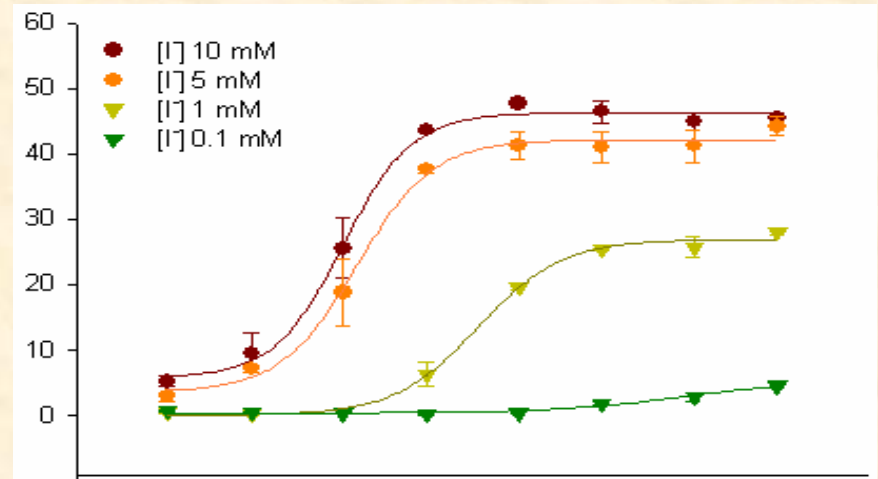


Normalized

Optimization of Assay Conditions for Positive Modulator Identification

$I/\Delta F_1 + \text{stimulus} / \Delta F_1$

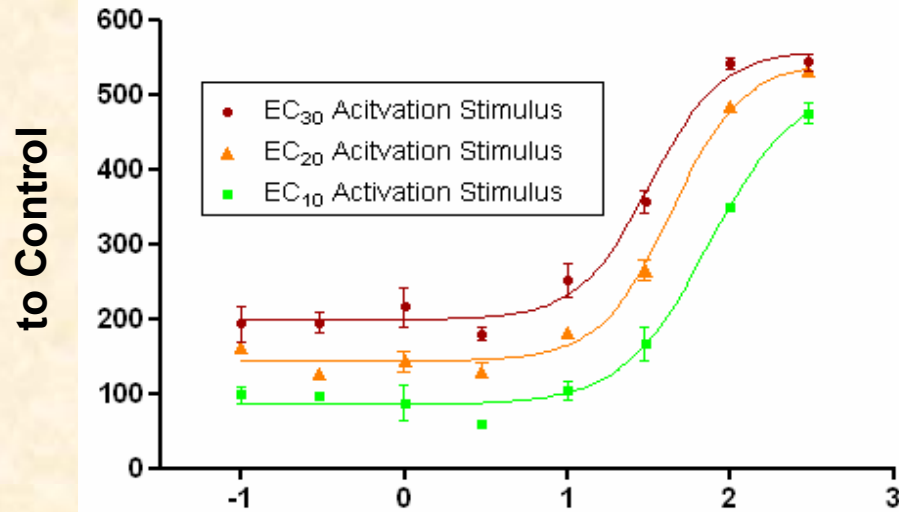
[I⁻] Optimization



Increasing [Activation Stimulus]

[Activation stimulus] Optimization

Normalized Response to Control



Log (nM) Control Compound

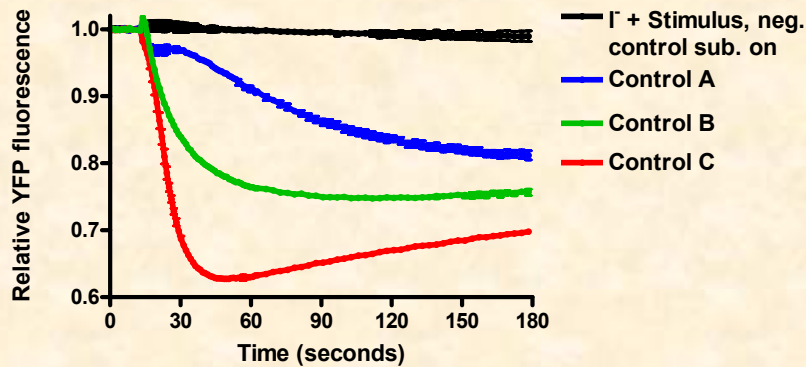
Chosen conditions for FLIPR assay:

-5 mM I⁻

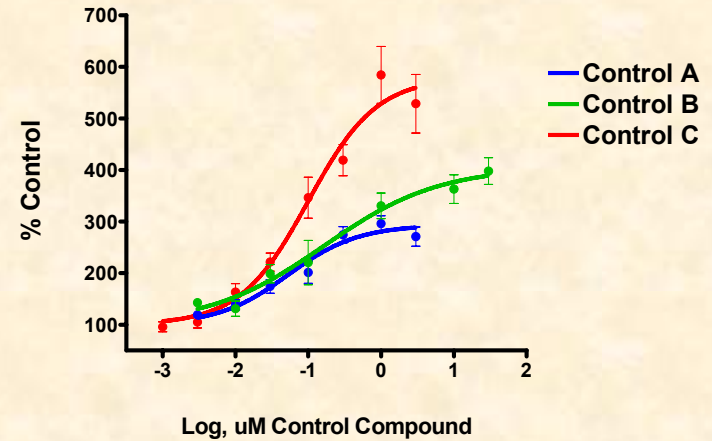
-EC20 stimulus

Control Compound Pharmacology Correlate in YFP and EP Assays

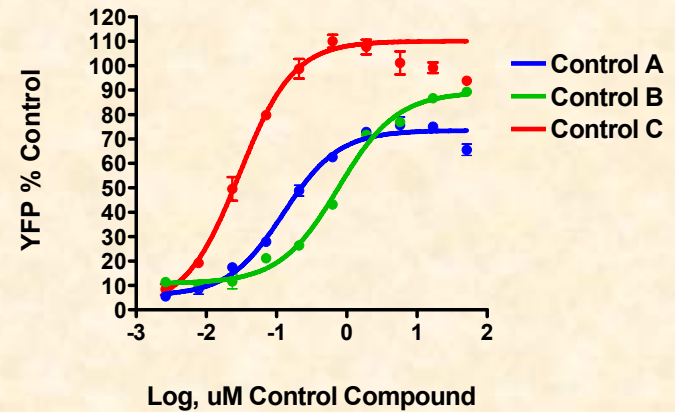
YFP FLIPR³⁸⁴ data



Electrophysiology



YFP assay



FLIPR based Halide Indicator Assay Flowchart

Plate HEK293 cells coexpressing YFP variant + Anion channel
25K cells per well in PDL-coated 384 plates

↓ Incubate @ 37°C, 5% CO₂ Overnight

Media removed and replaced with appropriate amount of Chloride Free Assay
Buffer (CFAB)

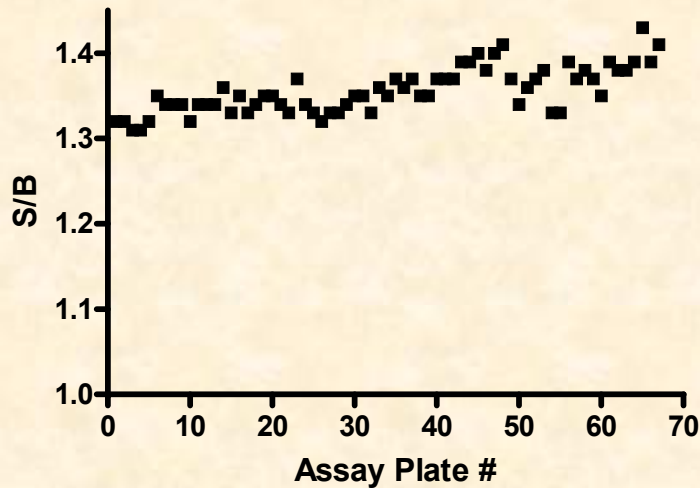
↓ Challenge cells with test compounds diluted in CFAB

↓ Incubate for 20 minutes

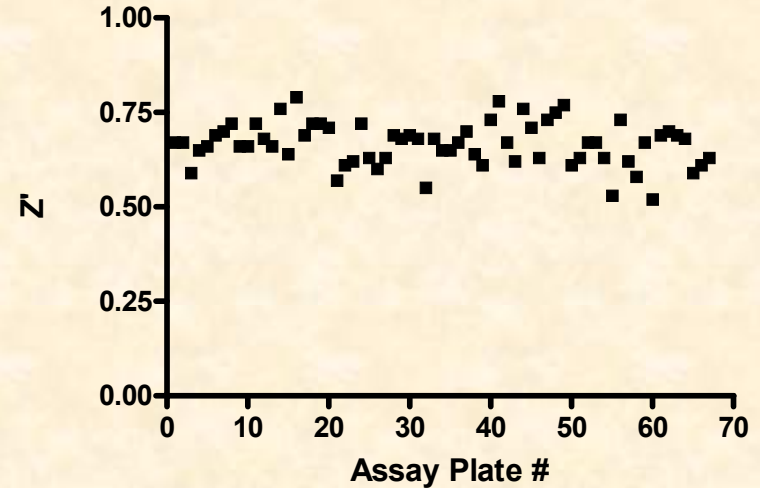
↓ Run FLIPR Assay

Screen Performance

S/B for typical screening run



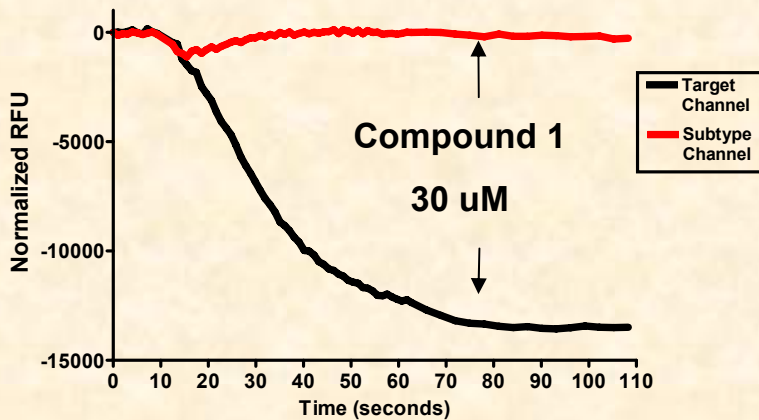
Z' values for same run
~ 0.65



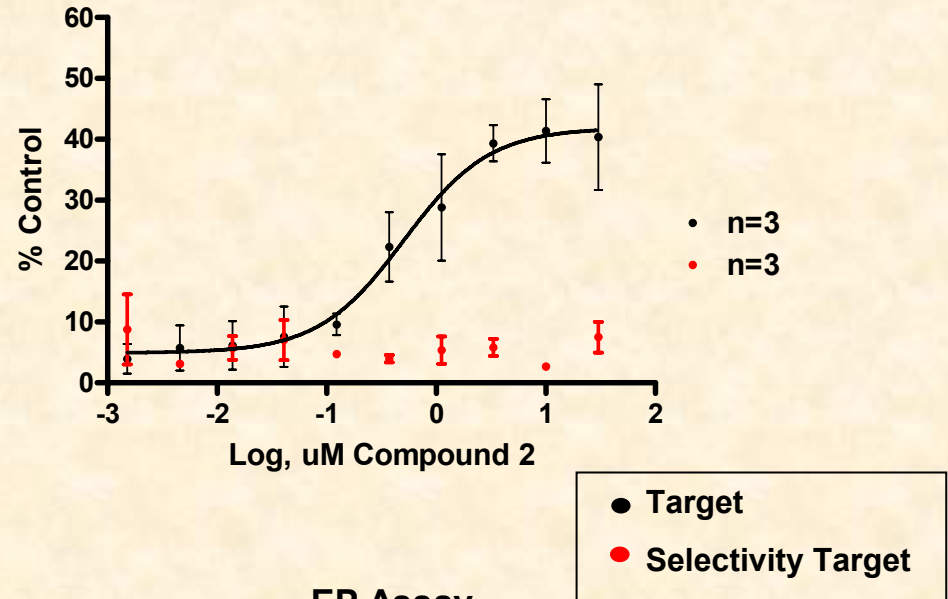
- Assay utilized for BMS primary screen
- Secondary screening:
 - Target for hit confirmation
 - Family member for selectivity
 - Pilot screening of YFP background for false positive hit rate
- Select compounds screened for selectivity in CRC mode

Assay Readily Identifies Selective Modulators

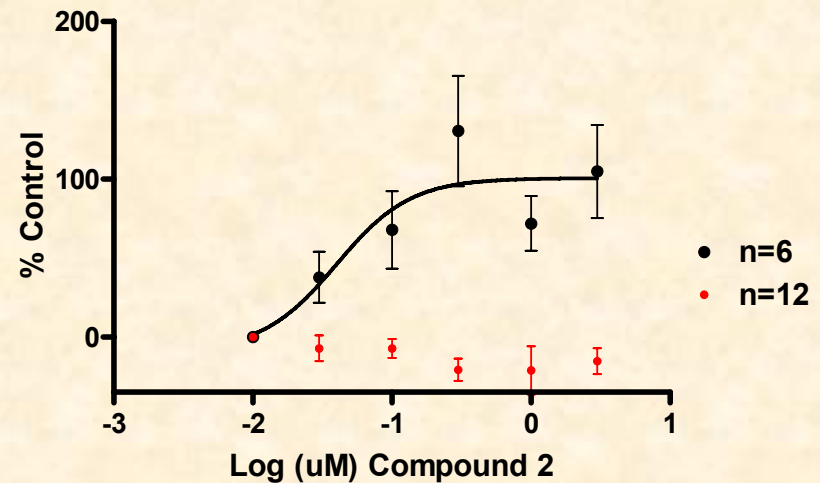
FLIPR³⁸⁴ data



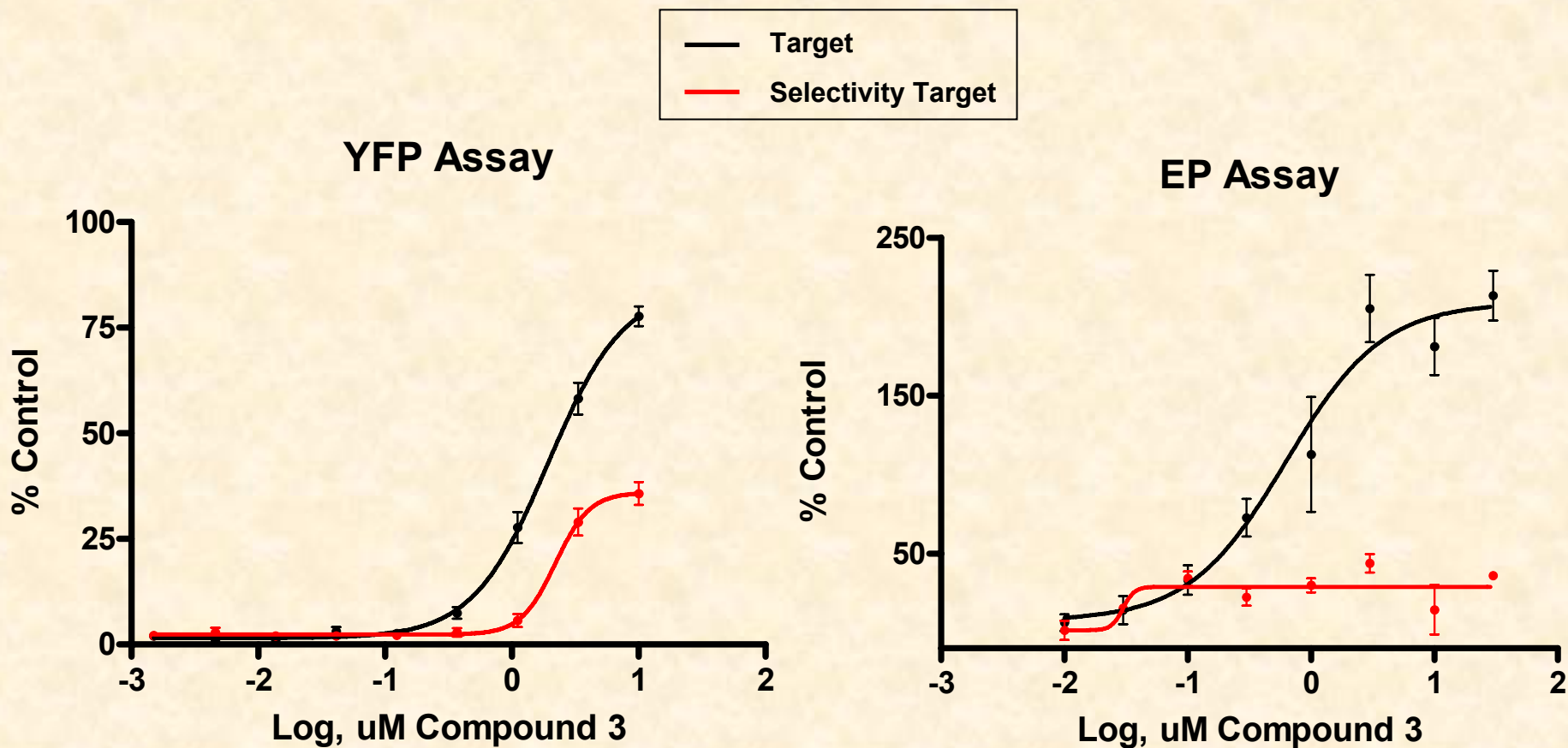
YFP Assay



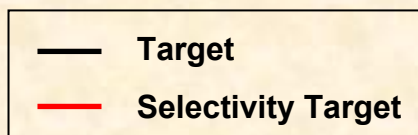
EP Assay



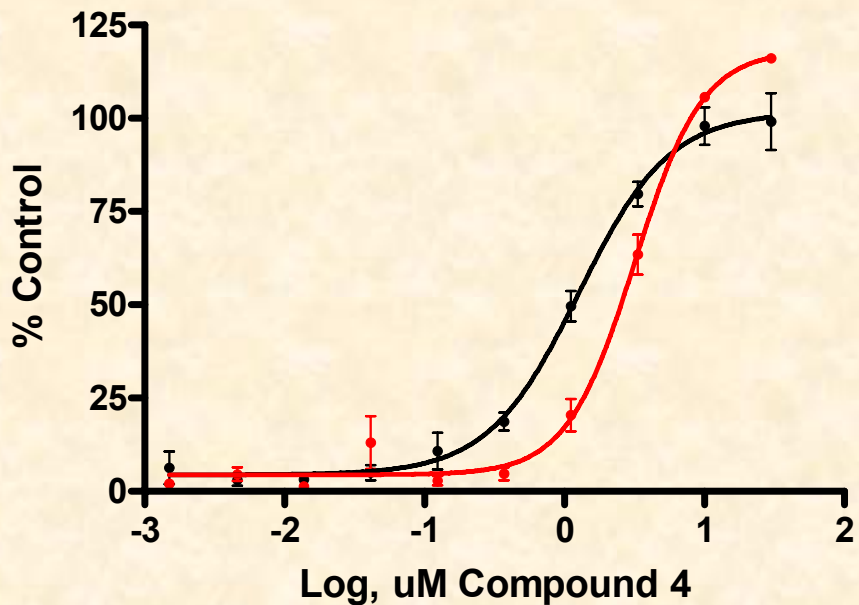
YFP Assay Identification of Partially Selective Modulators



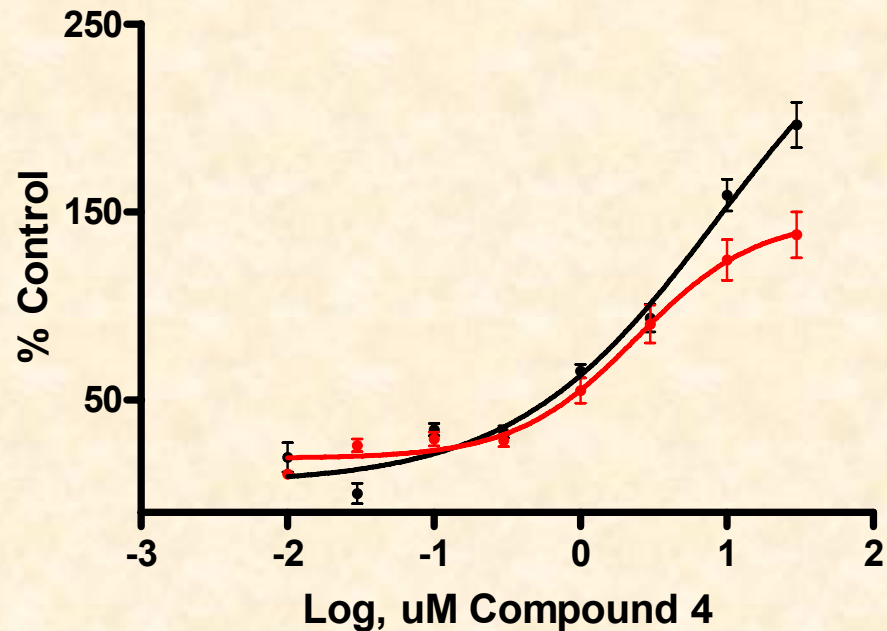
YFP Assay Identification of Non-selective Modulators



YFP Assay



EP Assay



Pros and Cons of Assay for HTS and Lead Optimization

PROS

- **High throughput method for Cl⁻ channel screening, lead evaluation and profiling**
- **Good correlation between flux assay and electrophysiology for controls and positive hits**
- **Low cost due to stably expressed indicator**
- **Functional output allows identification of all types of modulators (i.e.. agonists, allosteric modulators)**

CONS

- **False positives in HTS (eg. membrane disruptors or intracellular pH lowering)**
- **Finite dynamic range can compress SAR resolution for lead optimization**
- **Not known how well EP vs. flux data will correlate for all chemotypes**

Conclusions

- YFP halide indicator assay allows the high throughput screening of anion channel drug targets with FLIPR³⁸⁴
- The assay generates correlative pharmacological data relative to EP for the control compounds and validated hits to date
- The YFP assay is a low cost assay which can be utilized for anion channel selectivity screening, lead optimization and profiling

Acknowledgements

BMS Lead Discovery

- Meredith Arcuri
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