

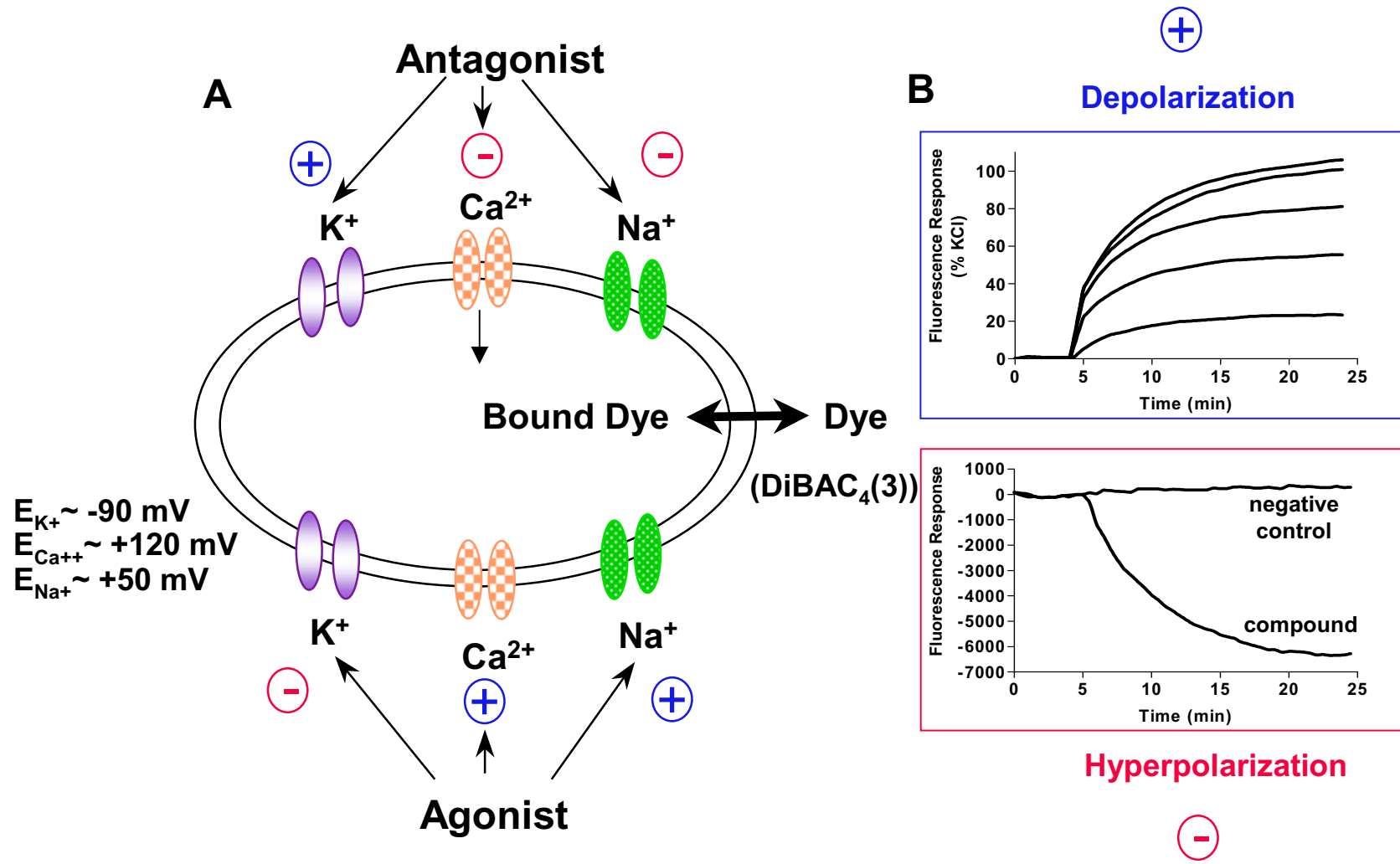


Studies on K^+ Channels in Smooth Muscle using the FLIPR Membrane Potential Dye

***Kristi Whiteaker
Abbott Laboratories***

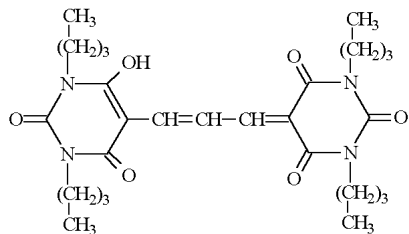


General Principles of Membrane Potential Assays





DiBAC₄(3)-based Assays in FLIPR: Advantages and Limitations



DiBAC₄(3)

- **Rapid evaluation of ion channel activity in a high-throughput manner**
- **Ligand interactions at many voltage-gated and ligand-gated channels**
- **Longer assay time (slow oxonol dye)**
- **May not be amenable to fast acting channels**
- **Quenching and fluorescence artifacts**



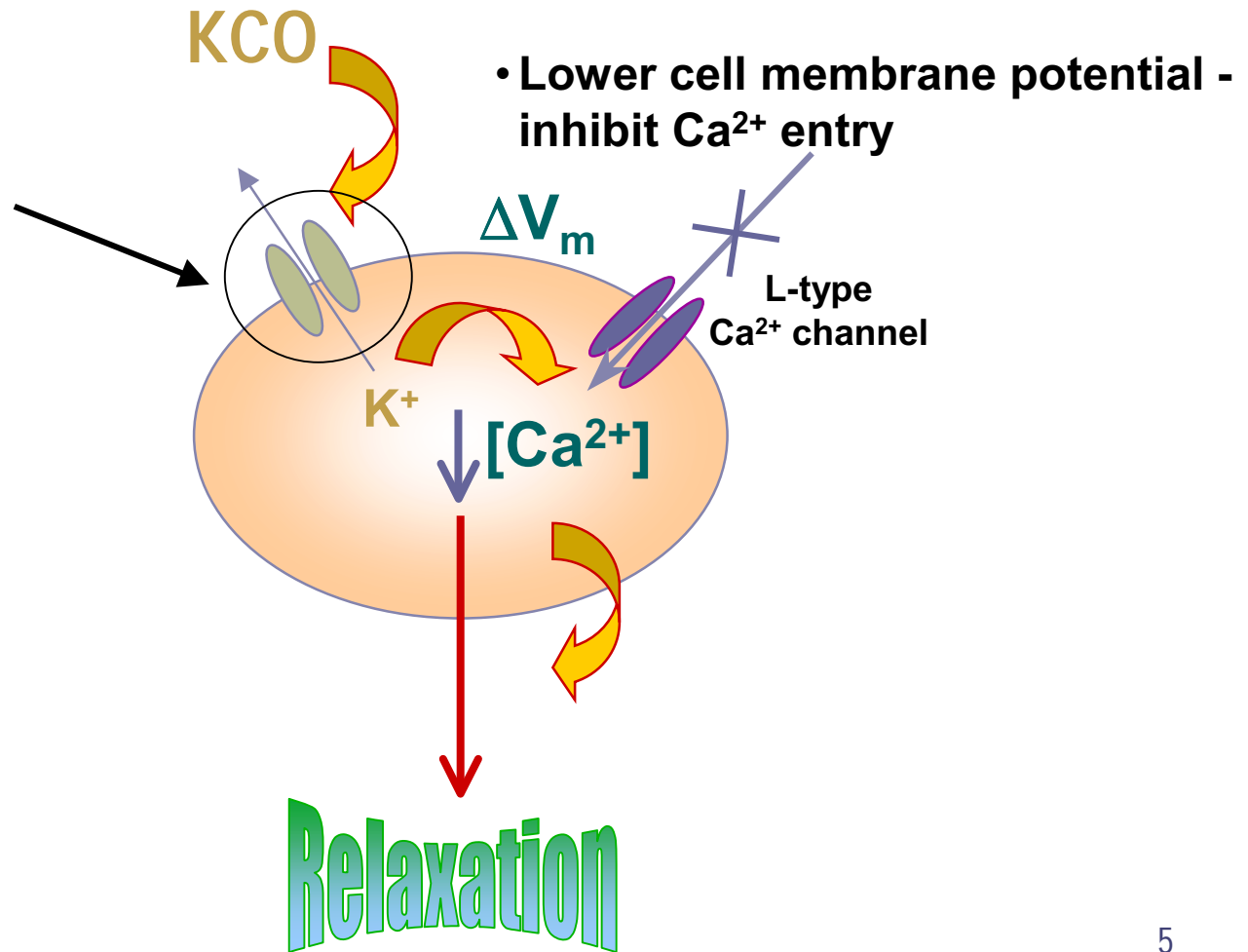
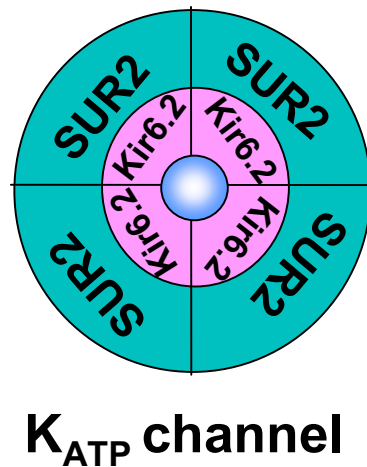
Overview

- **Model system: Smooth muscle cell line that expresses native K^+ (K_{ATP}) channels**
- **Comparison with electrophysiology**
- **Channel opener and blocker pharmacology**
- **Comparison of opener profile with DiBAC₄(3) and functional (tissue relaxation) studies**
- **HTS Considerations**



Mechanism of Action of KCOs in Smooth Muscle

- Activate K_{ATP} channels - increase K^+ permeability





FLIPR Membrane Potential (FMP) dye and DiBAC₄(3): Protocol Comparison

FMP dye

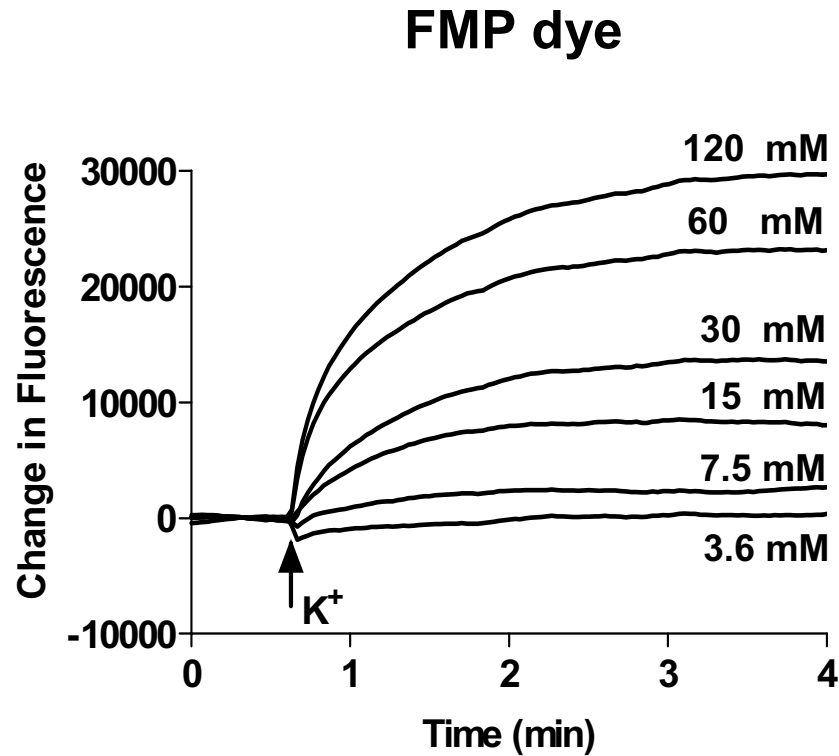
- Prepare compounds in buffer
- Add dye directly to cells
- Maximum activity in 3-4 min
- 3-4 fold increase in throughput
- Less temperature sensitive

DiBAC₄(3)

- Prepare compounds in buffer containing DiBAC₄(3)
- Wash cells twice with buffer and add dye
- Maximum activity in 15–25 min

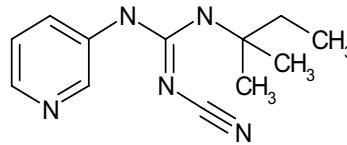


Responses to Elevated Extracellular K^+

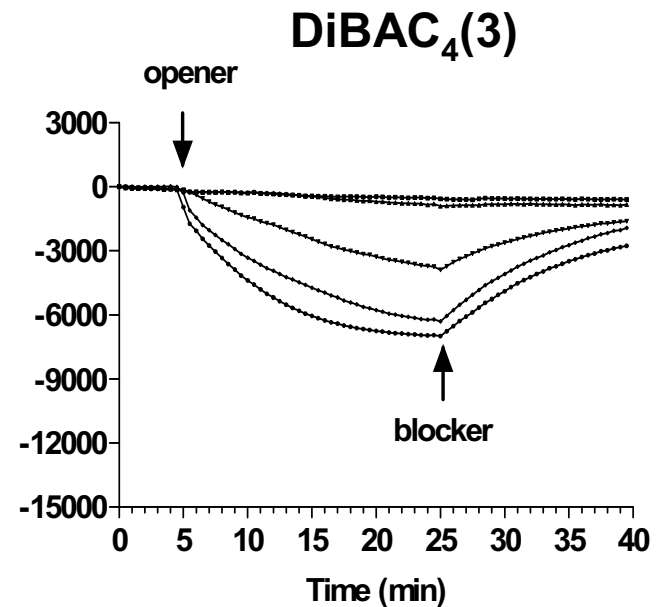
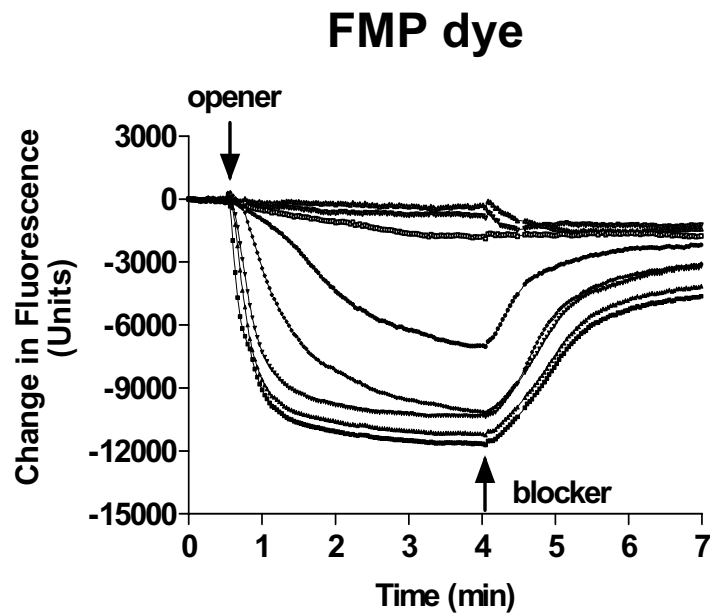


- Faster kinetics for depolarizing agents.
- Relative fluorescence changes higher with FMP.

Hyperpolarization Responses to an Opener



P1075



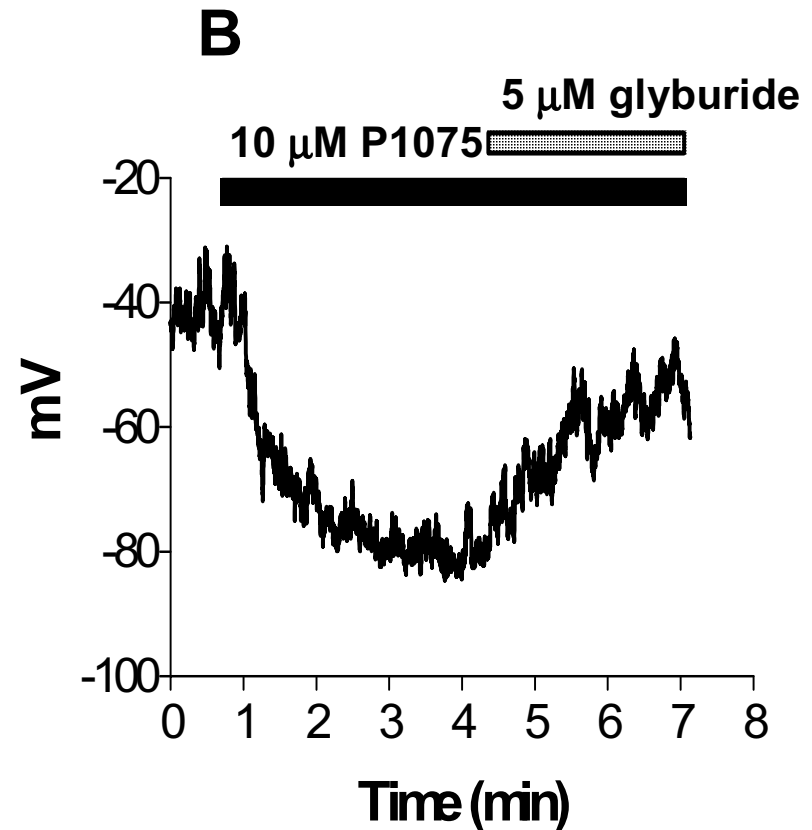
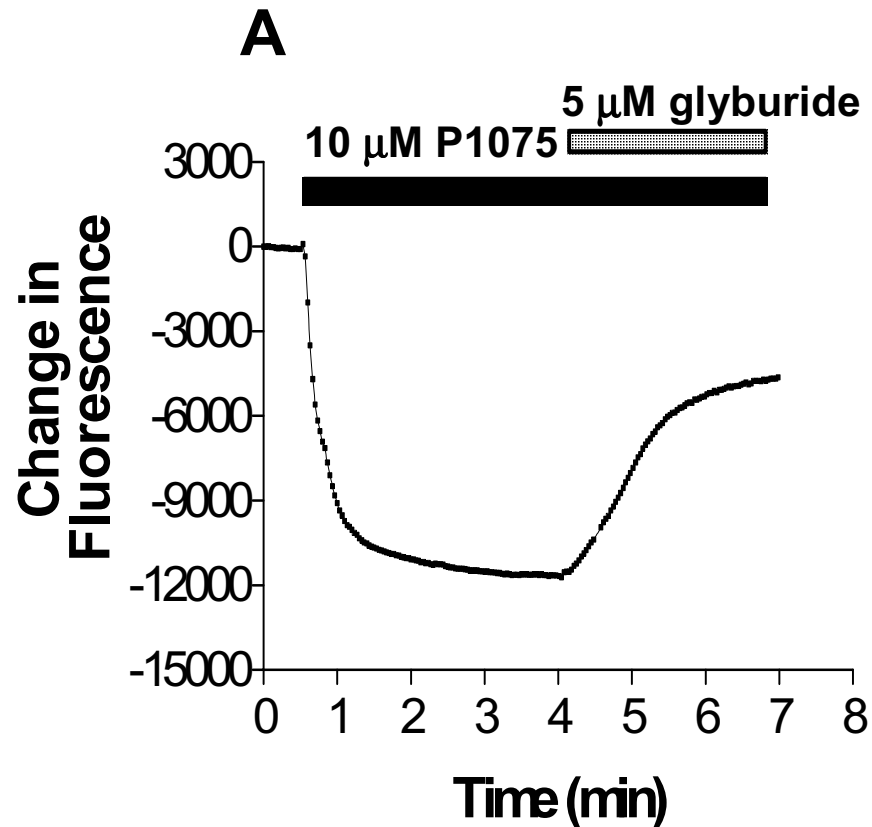
Opener: P1075 (1 nM – 10 μ M)
Blocker: glyburide (5 μ M)

- Faster kinetics for hyperpolarizing agents.
- Relative fluorescence changes higher.

K_{ATP} Channel Mediated Fluorescence Changes Compared with Membrane Potential Changes

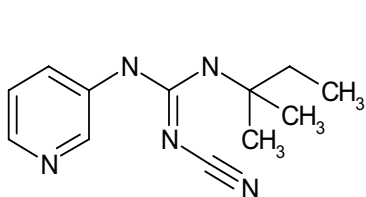
FMP dye in FLIPR

Whole-cell patch clamp

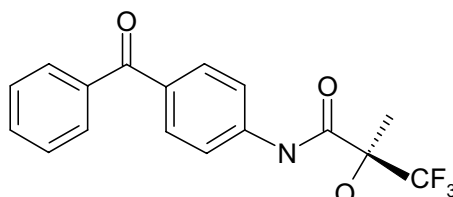


- The time course of FMP fluorescence changes are similar to real-time membrane potential changes measured electrophysiologically.

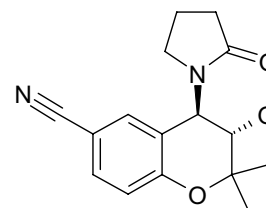
Reference Openers Evoke Concentration-Dependent Decreases in FMP Fluorescence



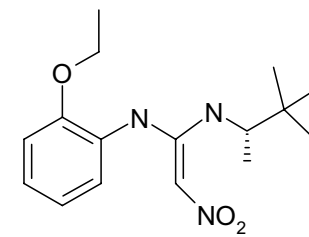
P1075



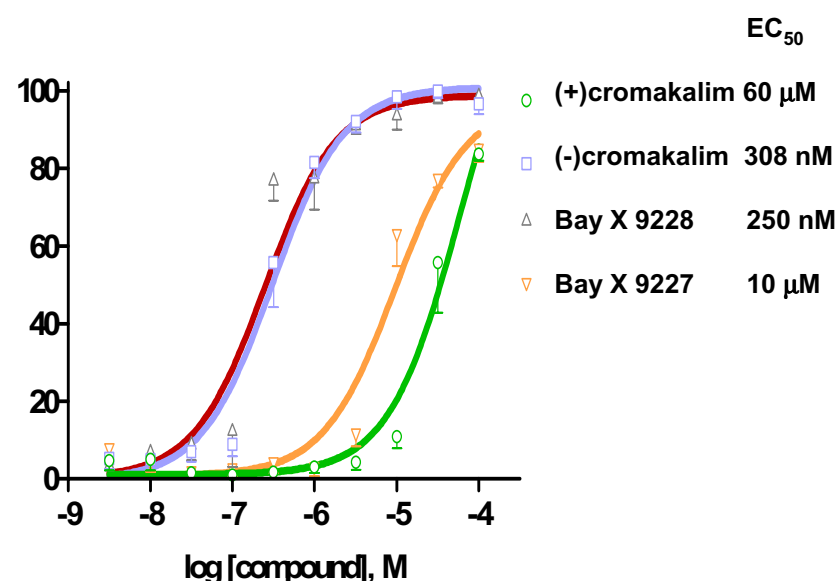
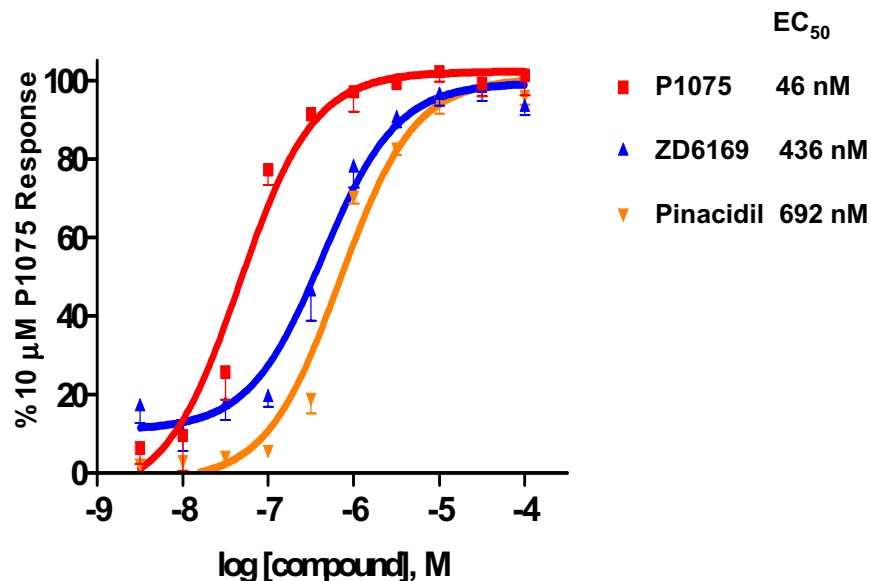
ZD-6169



cromakalim

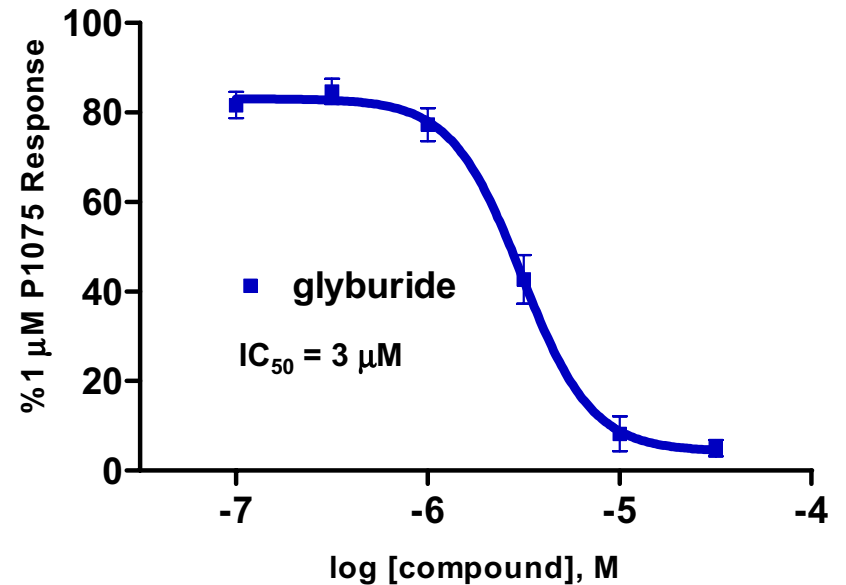
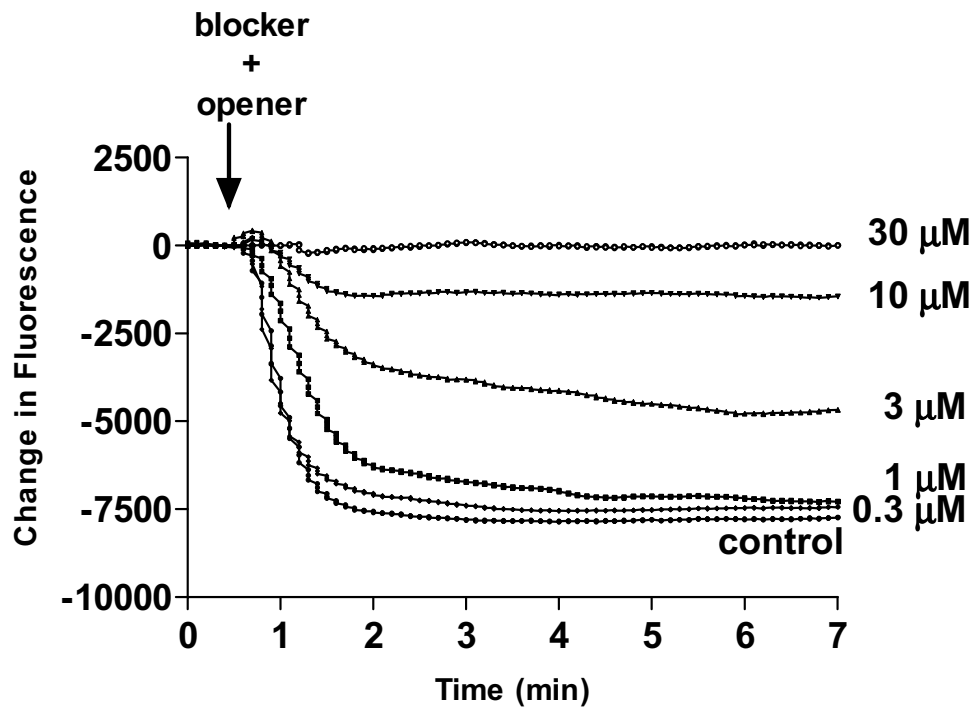


Bay X 9228



- Reference openers display concentration dependent activity in the FMP assay.

A K_{ATP} Channel Blocker Inhibits Opener Evoked Decreases in FMP Fluorescence

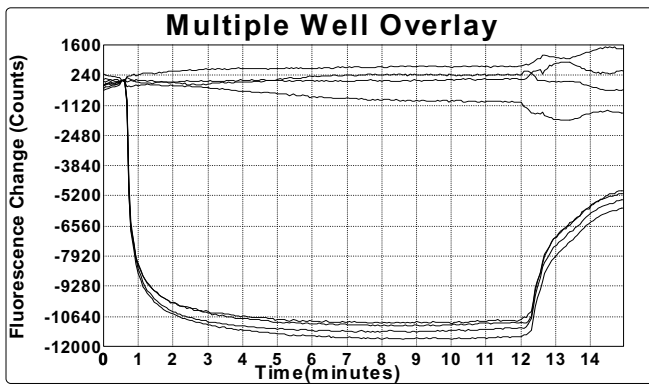


Blocker: glyburide
Opener: P1075 (1 μM)

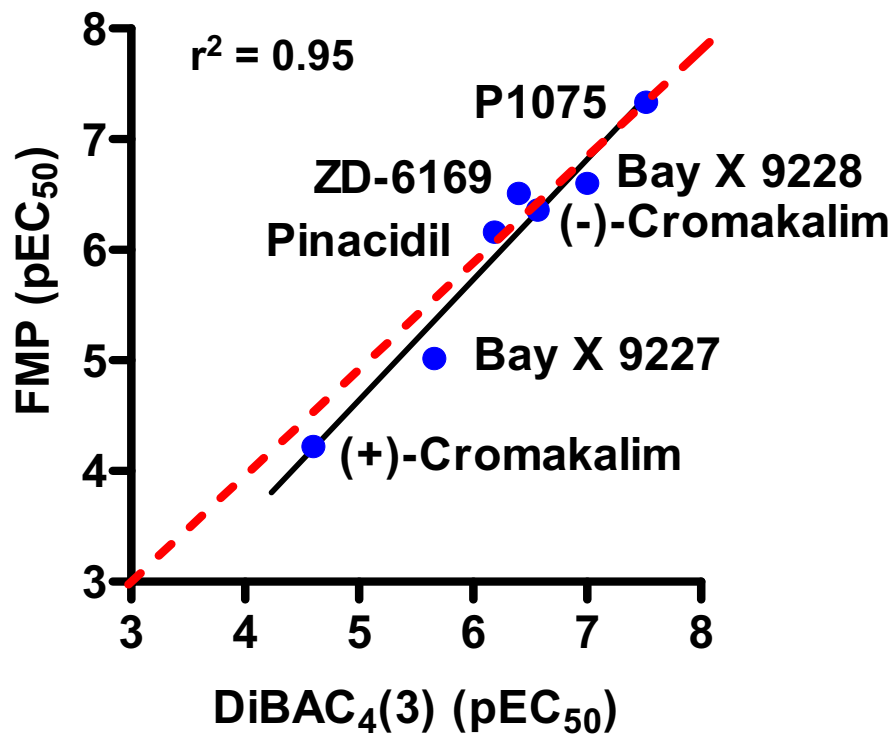
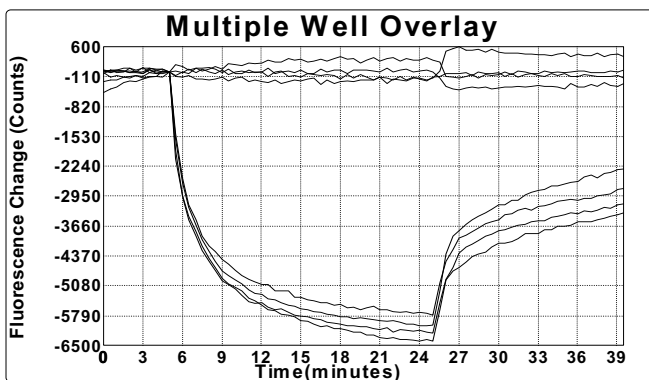
- Co-addition of a selective blocker in the presence of an opener results in concentration-dependent inhibition of opener-evoked fluorescence.

Opener Potencies Show a Good Correlation across DiBAC₄(3) and FMP Based Assays

FMP dye

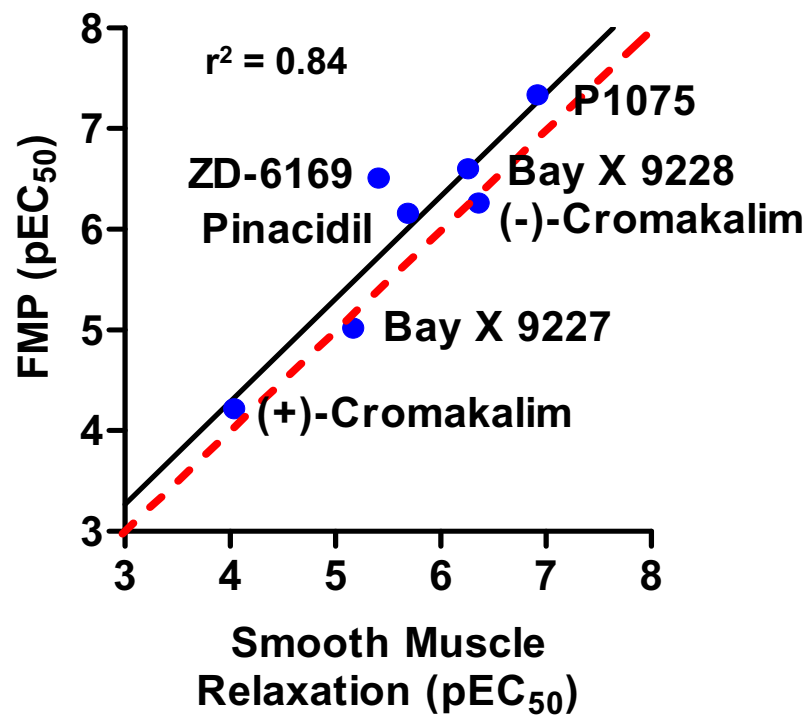
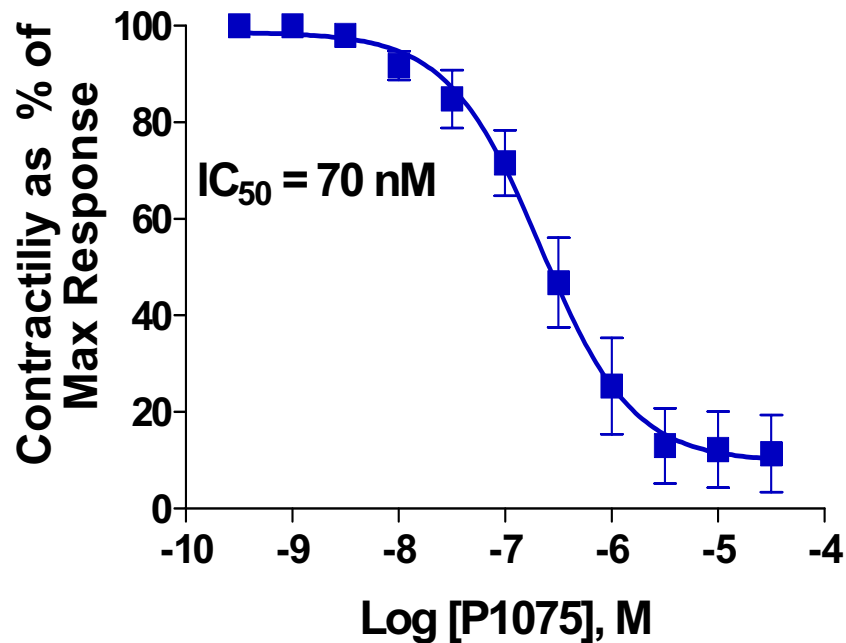


DiBAC₄(3)



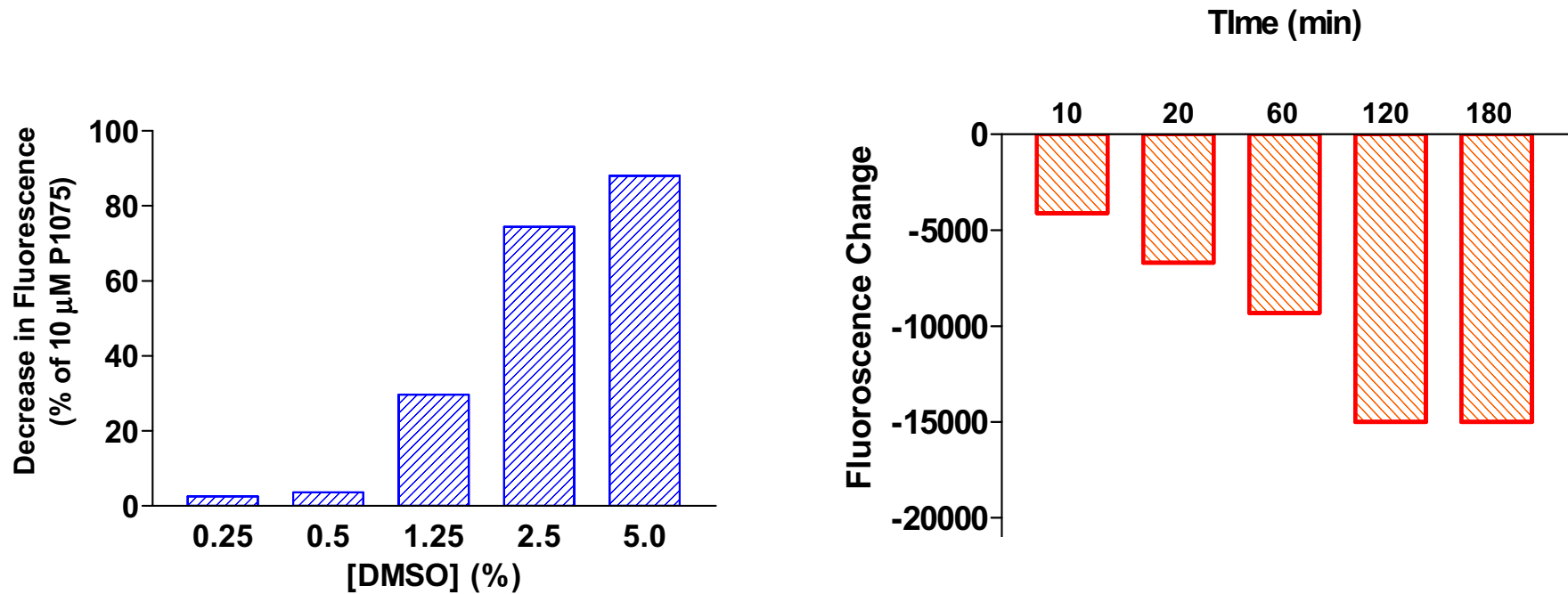
- Similar rank order of potencies was found for a variety of chemotypes.

The Potencies of Openers Show a Good Correlation with Smooth Muscle Relaxation



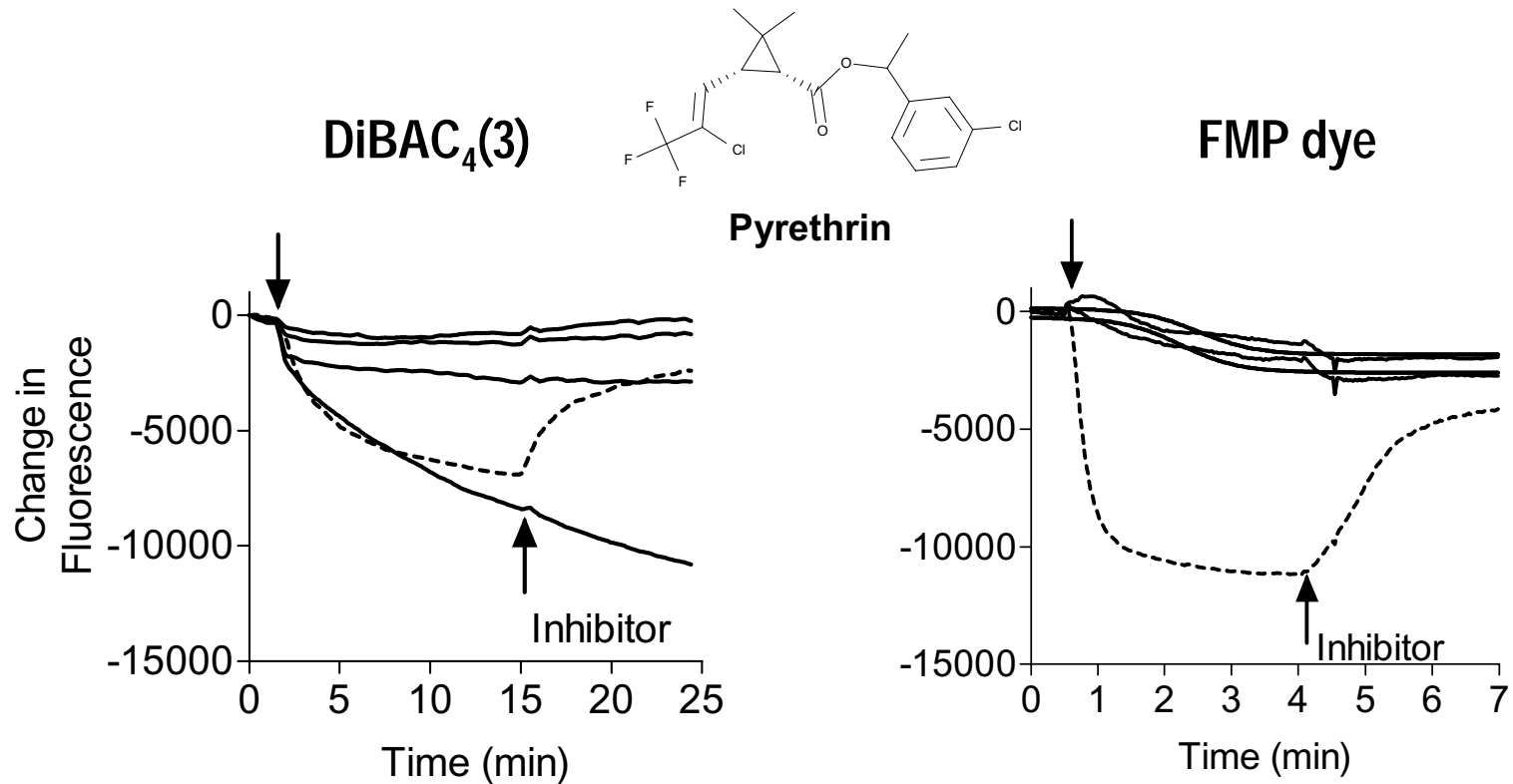
- FMP data is predictive of secondary assays.

Effect of DMSO and Dye Incubation Duration



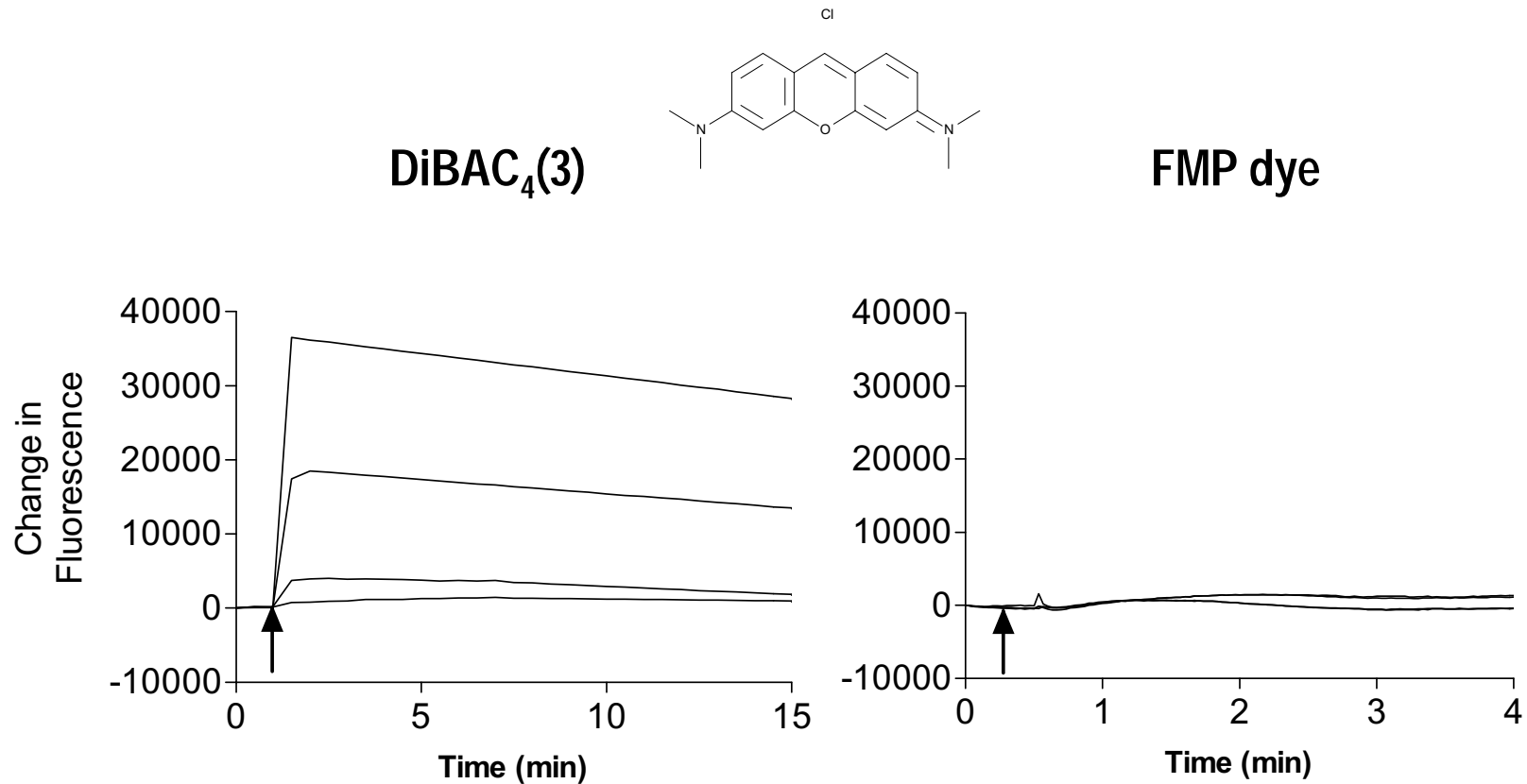
- Cells tolerate up to 0.5% DMSO with FMP.
- Adequate time for dye loading is about 40 min.
- Cells can tolerate dye loading up to 3 hr.

Evaluation of Quenching Compounds



- A known quencher of DiBAC₄(3) does not demonstrate false membrane potential activity with the FMP dye.
- Dotted line represents 10 μM P1075 effect.

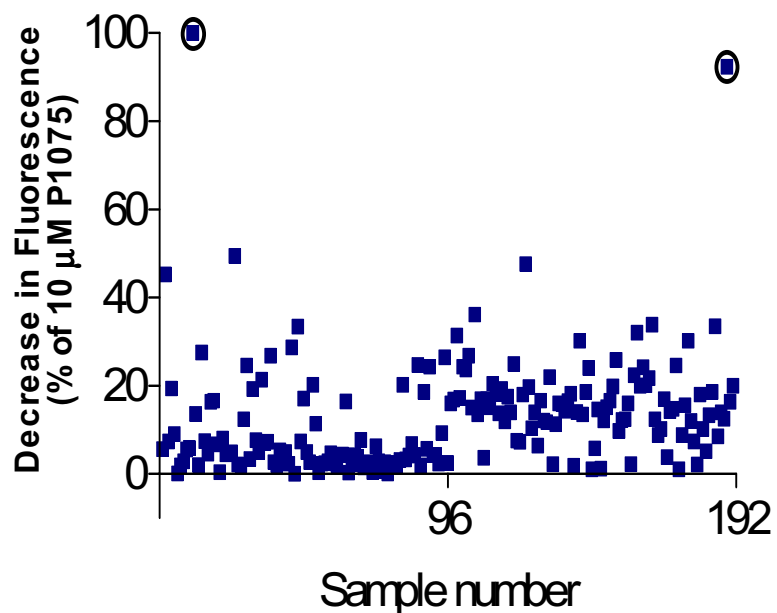
Effect of Fluorescent Compounds



- A fluorescent compound affecting DiBAC₄(3) does not demonstrate false membrane potential activity with the FMP dye.

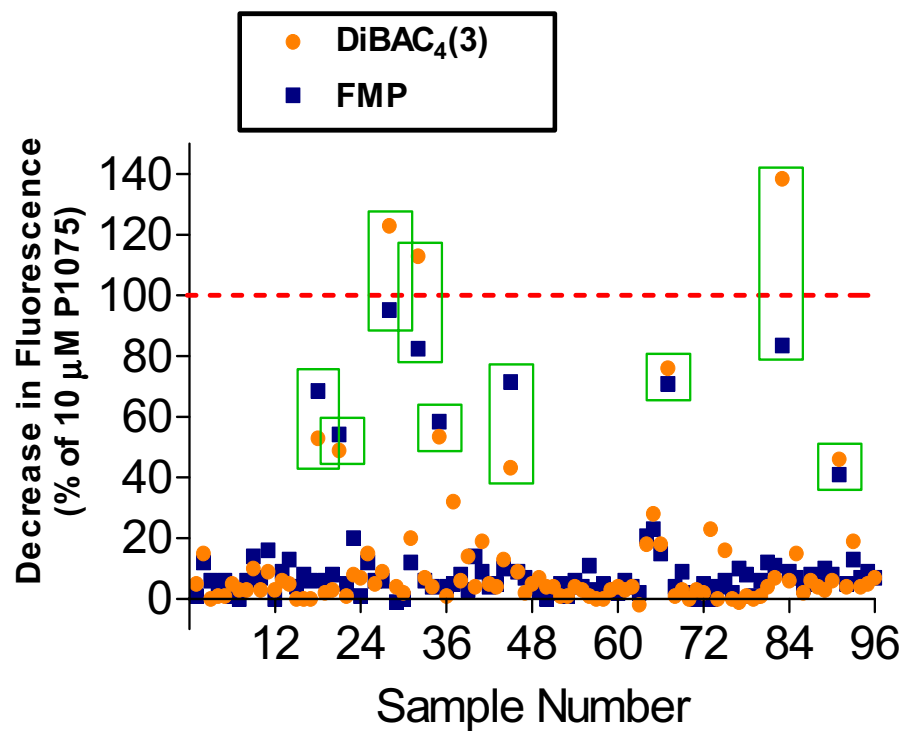


Scatter Plot of Screening Plates Containing Known Openers



- **Known activators are identified from screening plates using FMP.**

Scatter Plot of Screening Plate using FMP and DiBAC₄(3)



9 hits

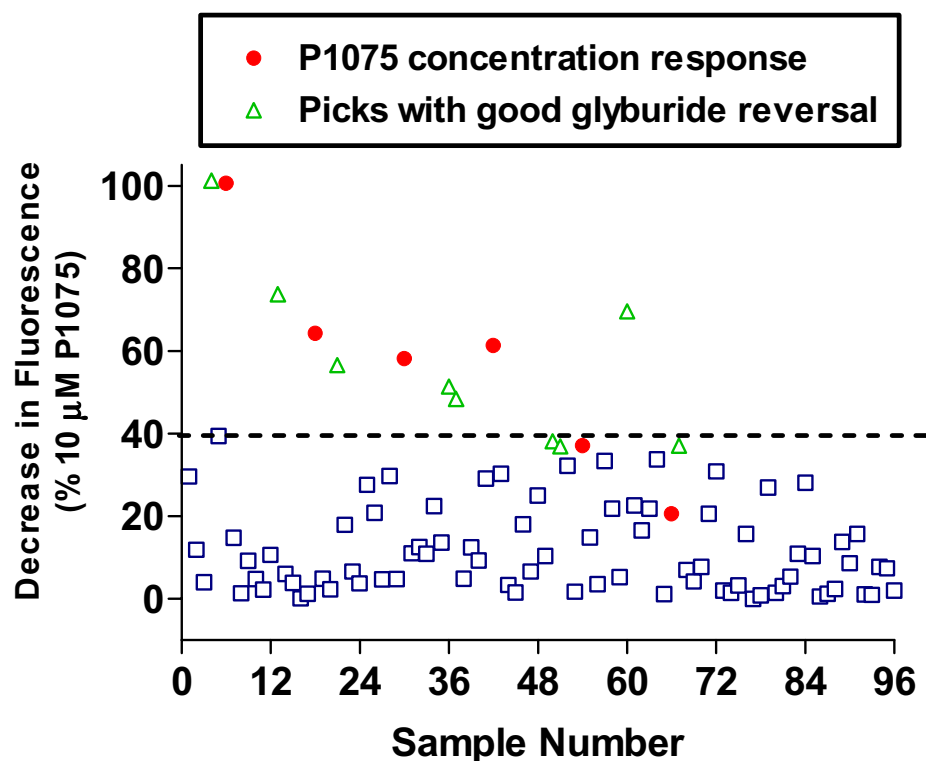
More activity in DiBAC₄(3) than FMP: **3**

More activity in FMP than DiBAC₄(3): **2**

Similar activities: **4**

- **Similar hits are identified using DiBAC₄(3) or FMP.**

Scatter Plot of Mixed Compound Screening Plate using FMP



- Hits are identified in a mixed compound plate.



Summary

- **Advantages**
 - Time comparison
 - More convenient preparation
 - Quenching and fluorescent compounds
 - Less temperature sensitive
 - Membrane potential kinetics comparable with electrophysiology data
 - Predictive of secondary assays
 - Feasible for use in HTS
- **Disadvantages**
 - Cost/Plate
 - Chemical composition undisclosed



Acknowledgements

- **Molecular Devices**
 - **Martin Kirk**
 - **Abbott Laboratories**
 - HTS Screening Group**
 - **Duke Groebe**
 - **Sujatha Gopalakrishnan**
- Project Team Members**
- **Char-Chang Shieh**
 - **Murali Gopalakrishnan**
 - **Vikki Scott**

