

Comparison of Three Different Dye Systems at a Voltage- gated Potassium Channel and a Ligand-gated Ion Channel Using FLIPR^{Tetra}

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Limitations of Techniques for Studying Ion Channels

- Ion channels traditionally studied using manual *in vitro* electrophysiology techniques, such as mammalian patch-clamping or oocyte recording
 - Throughput low, although data quality is high
 - Medium-throughput electrophysiology systems now available, such as IonWorks and PatchExpress
- Calcium-sensitive fluorophores allow study of channels that are permeable to calcium ions in a high-throughput plate-based format
 - Most but not all voltage-gated channels are impermeable to calcium
 - Most but not all ligand-gated channels are permeable to calcium

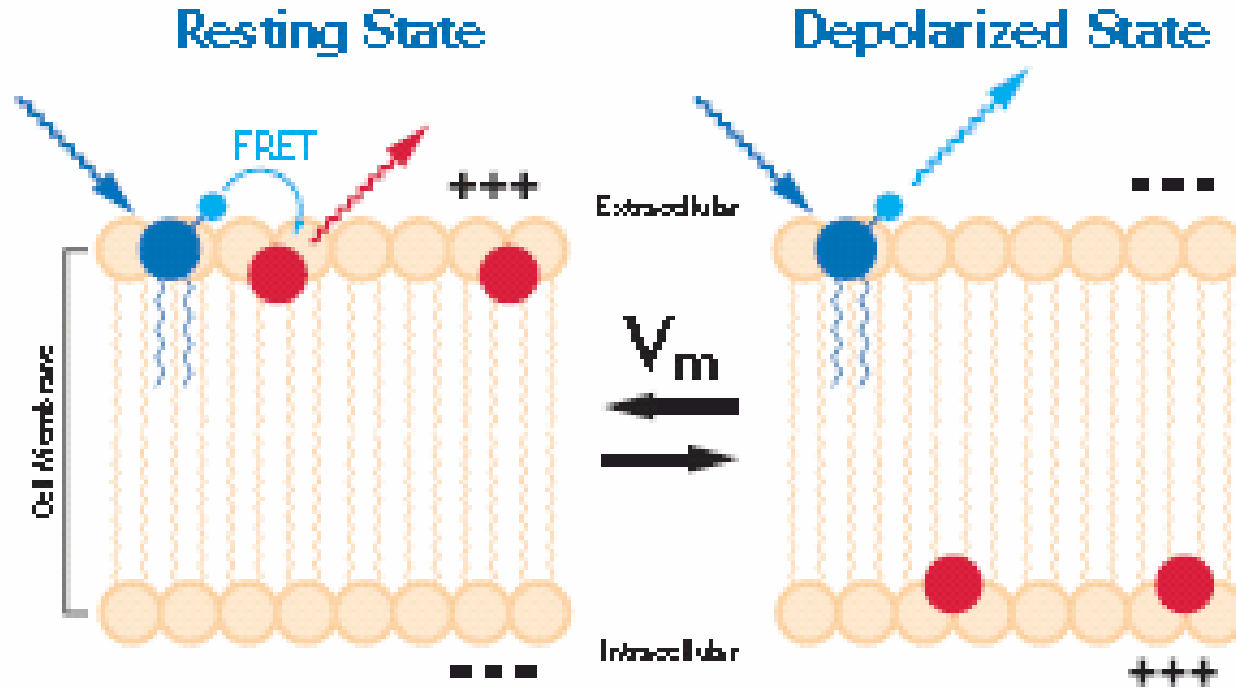
Objectives

- To gain experience with FLIPR^{Tetra} to maximize ability to screen ion channel targets
- To compare three different dye systems on a voltage-gated potassium channel
- To develop voltage-sensitive assay for a ligand-gated ion channel
- To compare assay parameters for the three dye systems, including sensitivity, signal:noise ratio, and quality of information gleaned from each technology

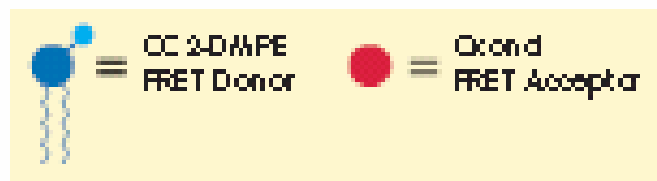
Voltage Sensor Probe Dye System (VSP)

- In vitro
- Measures voltage change across the cell membrane
- Sub-second timescale detection
- Sensitive (detects >5 mV changes)
- Ratiometric data format reduces well-to-well variability
- Can be used on multiple instruments, including FLIPR^{Tetra}
- Excitation wavelength = 400 nm
- Emission wavelengths = 460 nm and 580 nm

Voltage Sensor Probe Dye System



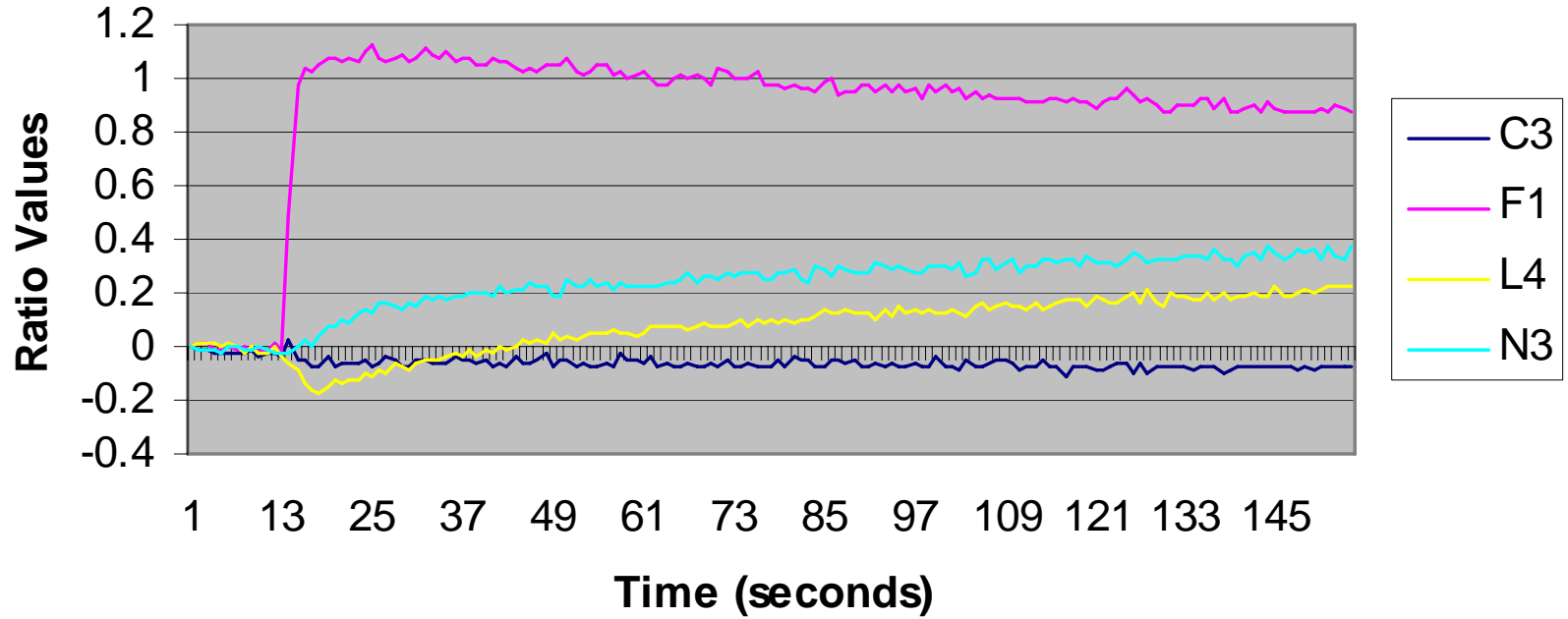
$$\text{Emission Ratio} = \frac{\text{Donor (CC 2-DMPE) Emission (460 nm)}}{\text{Acceptor (Oxonol) Emission (580 nm)}}$$



Voltage Sensor Probe Conditions for Potassium Channel Experiments

- VSP-1 buffer with KCl [4.5 mM]
- Cell-free wells used as negative correction
- 5-minute recordings following compound addition
- Max-Min measurement used to calculate responses

Treatment Effects on Voltage Sensor Probe Signal

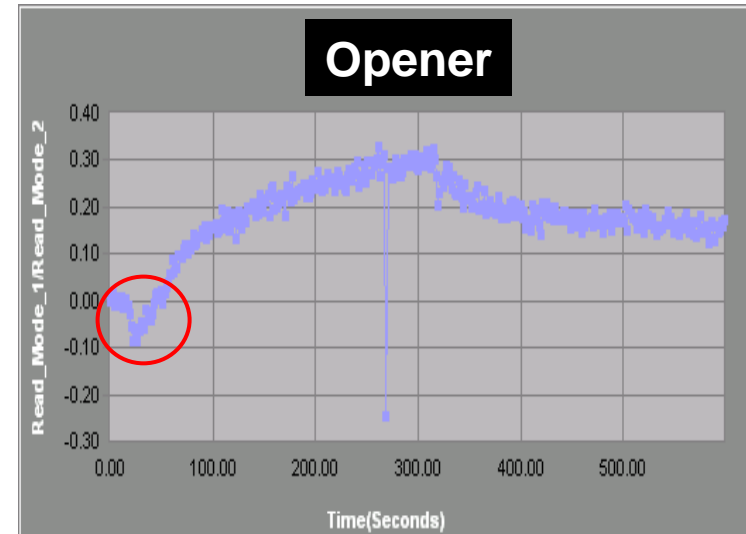


Buffer

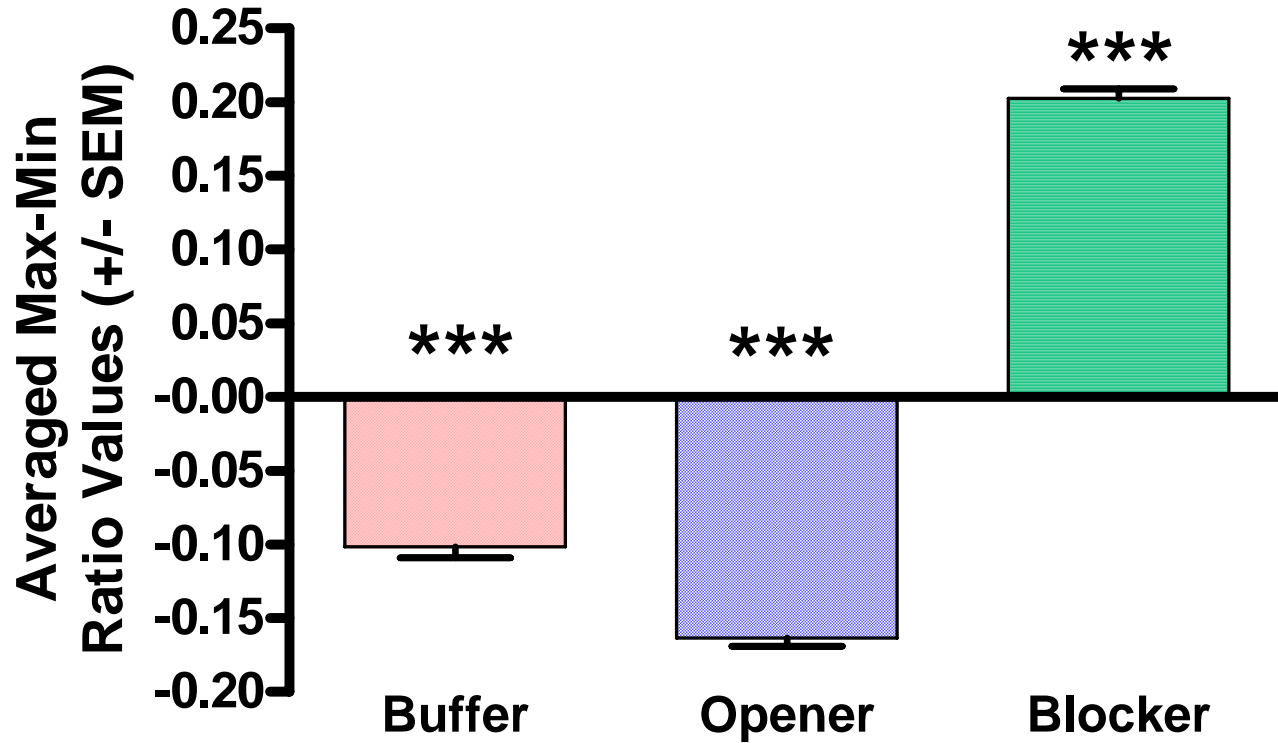
Blocker

KCI

Opener



Treatment Effects on Voltage Sensor Probe Signal

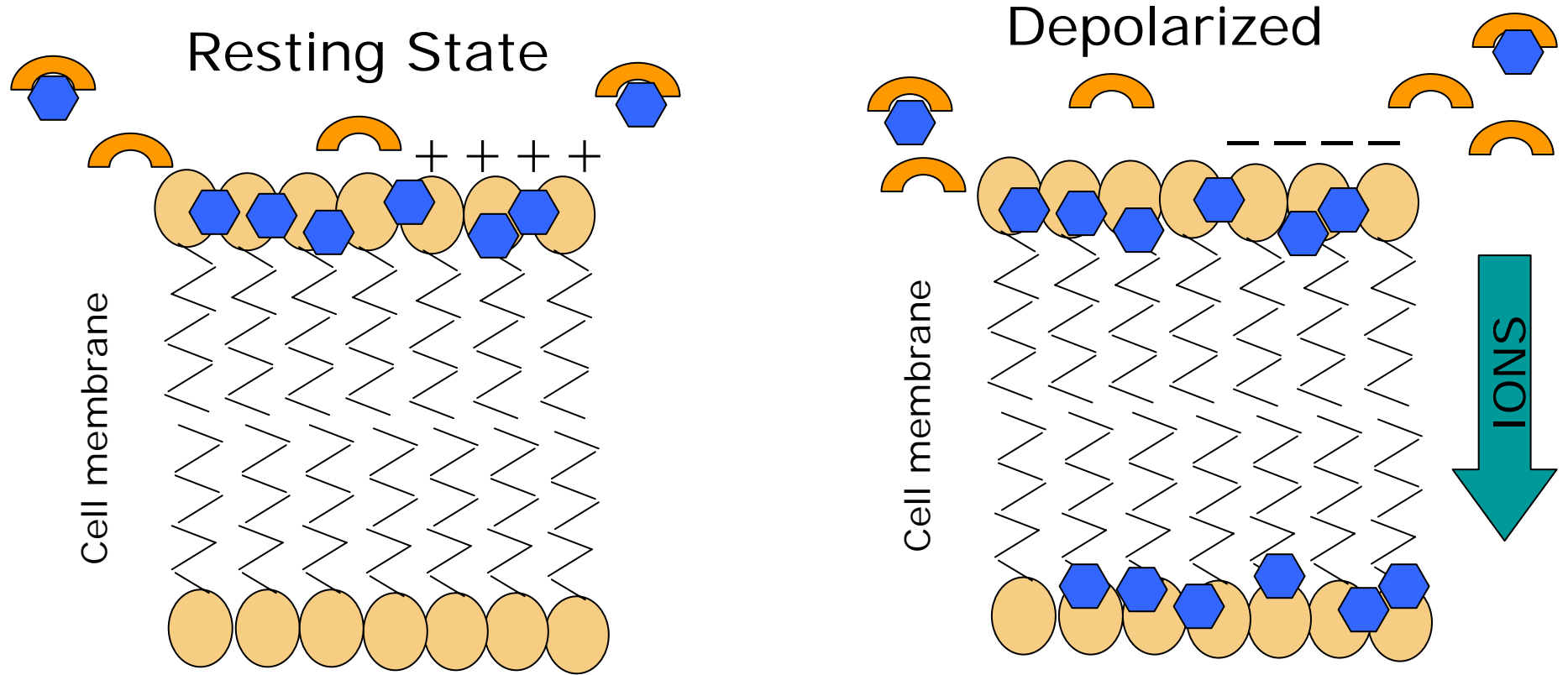


*** = $p < 0.01$, $n = 24$

Membrane Potential Dye System

- Molecular Devices
- Measures voltage change across the cell membrane
- Fast response times enable more accurate measurements
- No wash protocol provides higher throughput than some other methods
- Compatible with FLIPR and FLEXstation instruments from Molecular Devices
- Uses Filter #2 on FLIPR³⁸⁴--Excitation = 488 nm (argon ion laser)
- For FLIPR^{Tetra}:
 - Excitation wavelength = 530 nm
 - Emission wavelength = 565 nm

Membrane Potential Blue Dye System

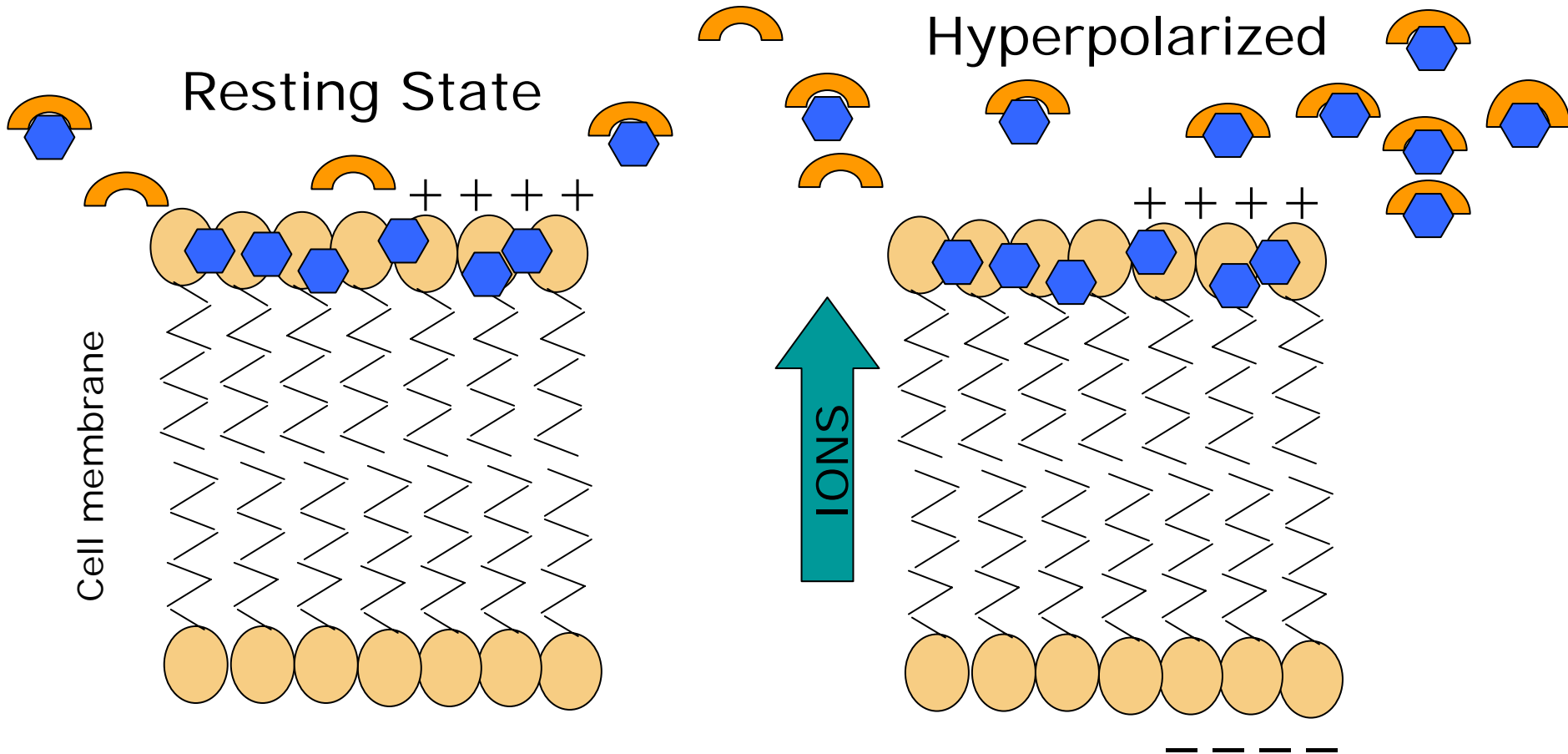


----- Legend: + + + +

Membrane Potential Dye Signal Increases

Quencher

Membrane Potential Blue Dye System



Legend:

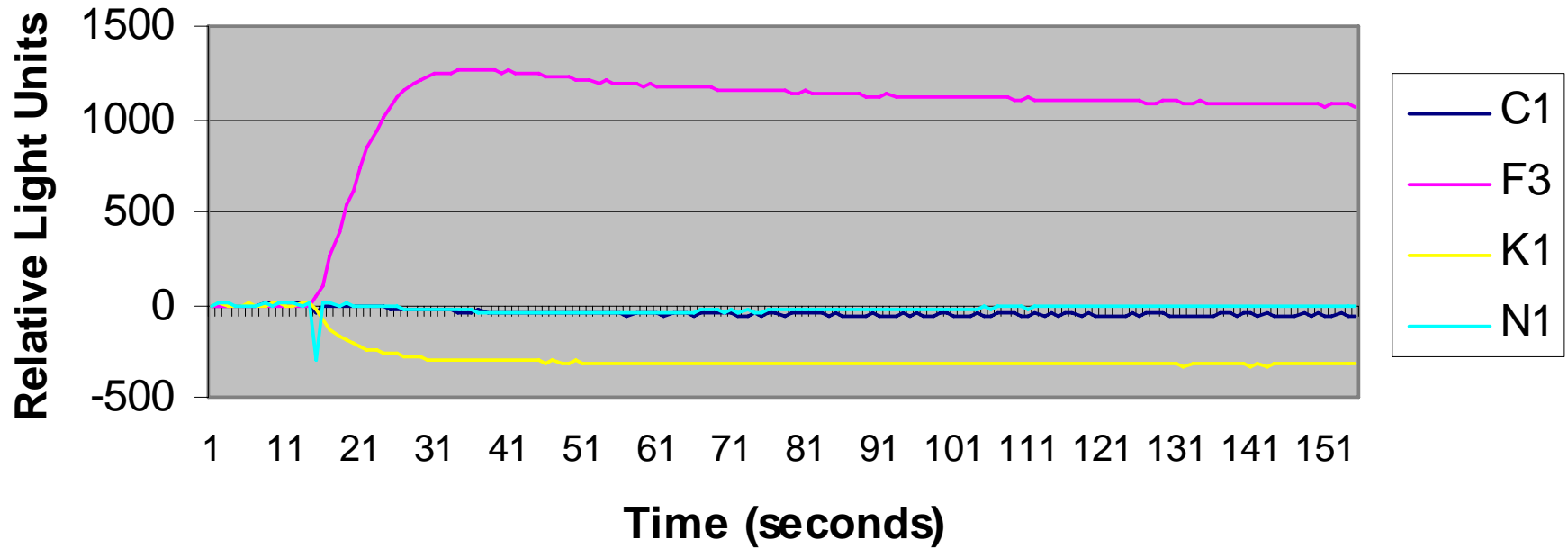
Membrane
Potential Dye

Quencher

Signal Decreases



Treatment Effects on Membrane Potential Blue Signal



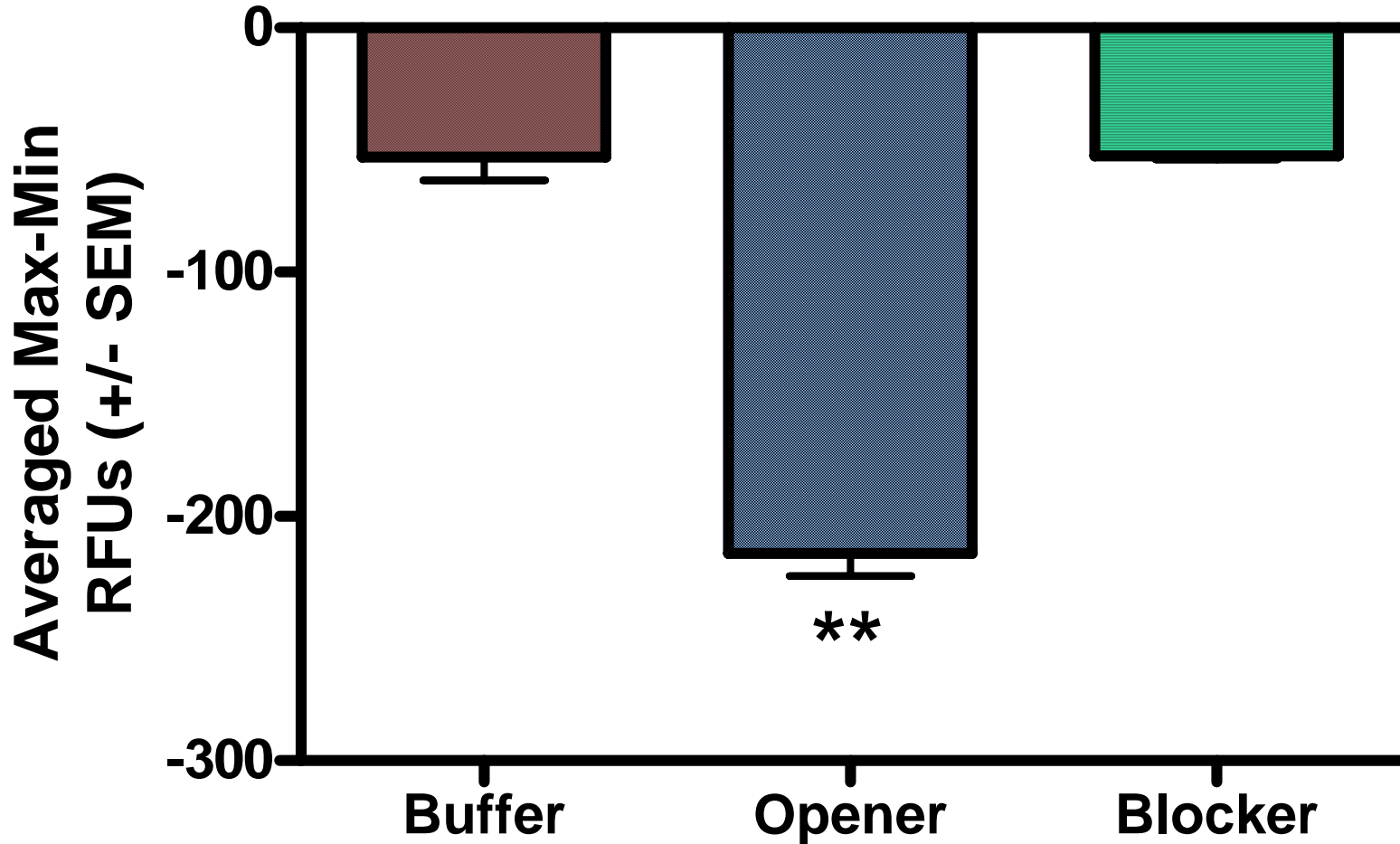
— Buffer

— Blocker

— KCl

— Opener

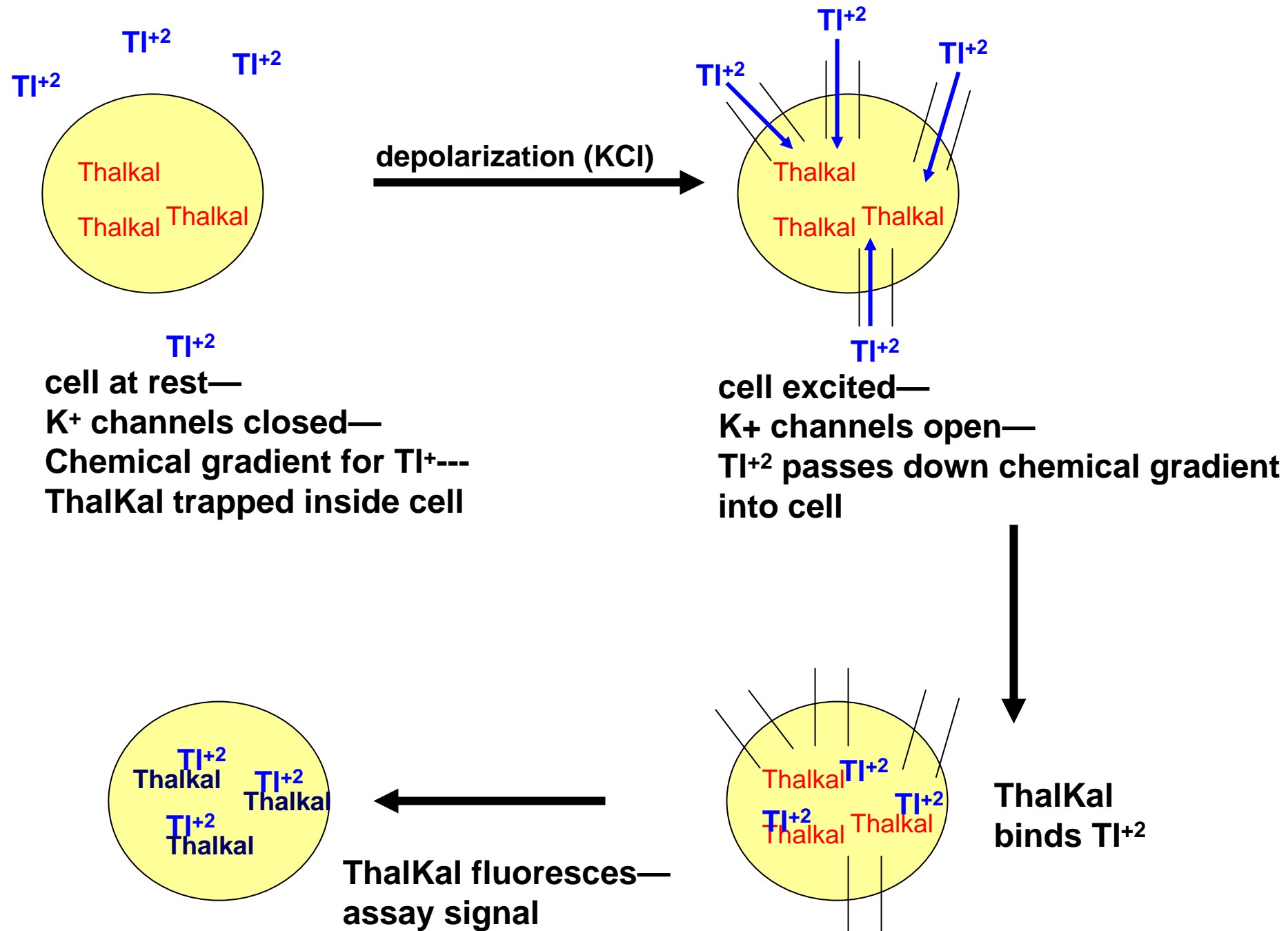
Treatment Effects on Membrane Potential Blue Signal



** = $p < 0.001$, $n = 24$

ThalKal: Potassium Channel Dye System

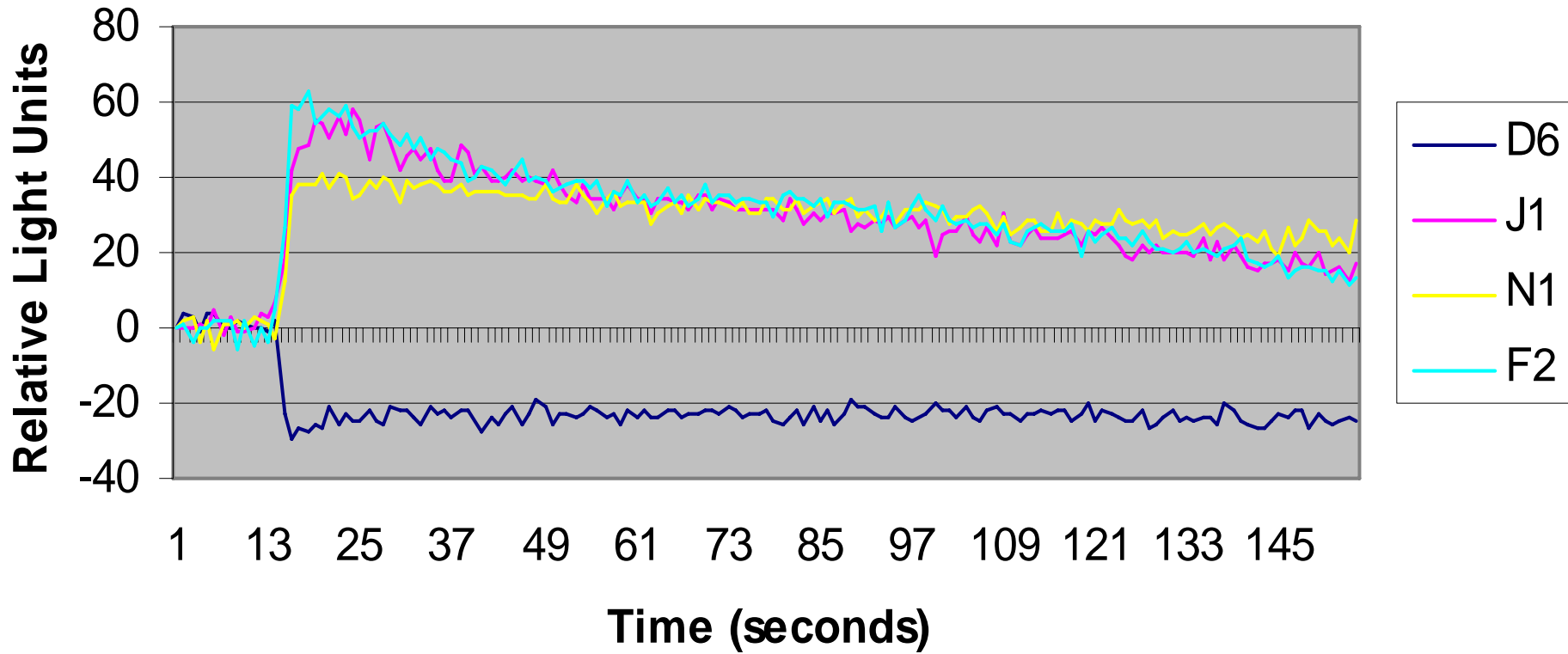
- Molecular Devices
- Measures activity of potassium channels via the permeability of thallium ions by using a thallium-sensitive fluorophore
- No wash protocol provides higher throughput than some other methods
- Compatible with FLIPR and FLEXstation instruments from Molecular Devices
- Uses Filter #2 on FLIPR³⁸⁴--Excitation = 488 nm (argon ion laser)
- For FLIPR^{Tetra}:
 - Excitation wavelength = 470-495 nm
 - Emission wavelength = 515-575 nm



ThalK_{al} Conditions for Potassium Channel Experiments

- Chloride-free assay buffer
 - K⁺ gluconate [2.5 mM]
- Tl₂SO₄ [3 mM] in buffer and drug applications
- Tl₂SO₄ buffer control used as negative correction
- Five-minute recordings with compound addition
- Max-Min measurement used to calculate responses

Treatment Effects on ThalKal Signal



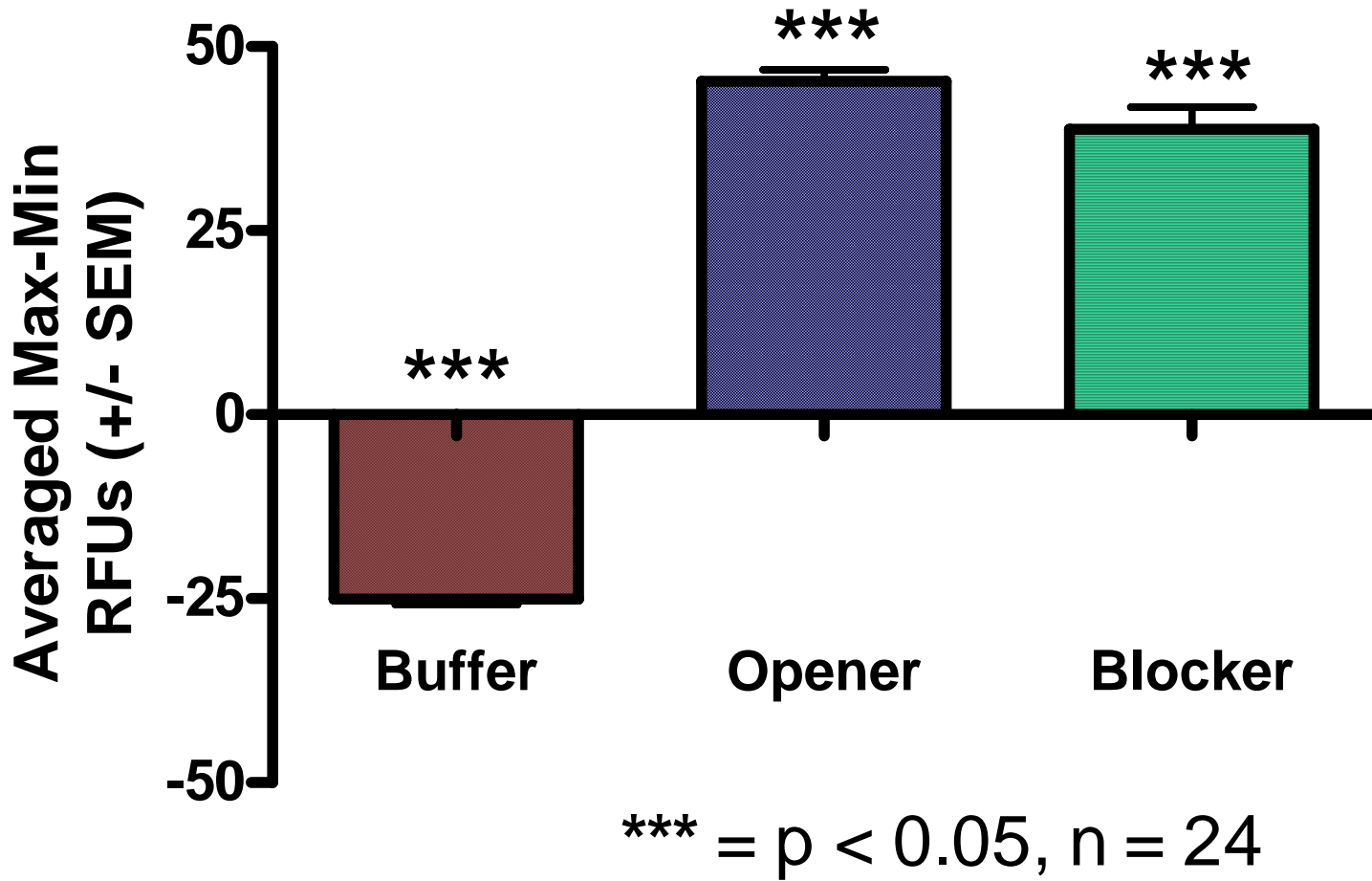
Buffer

K₂SO₄

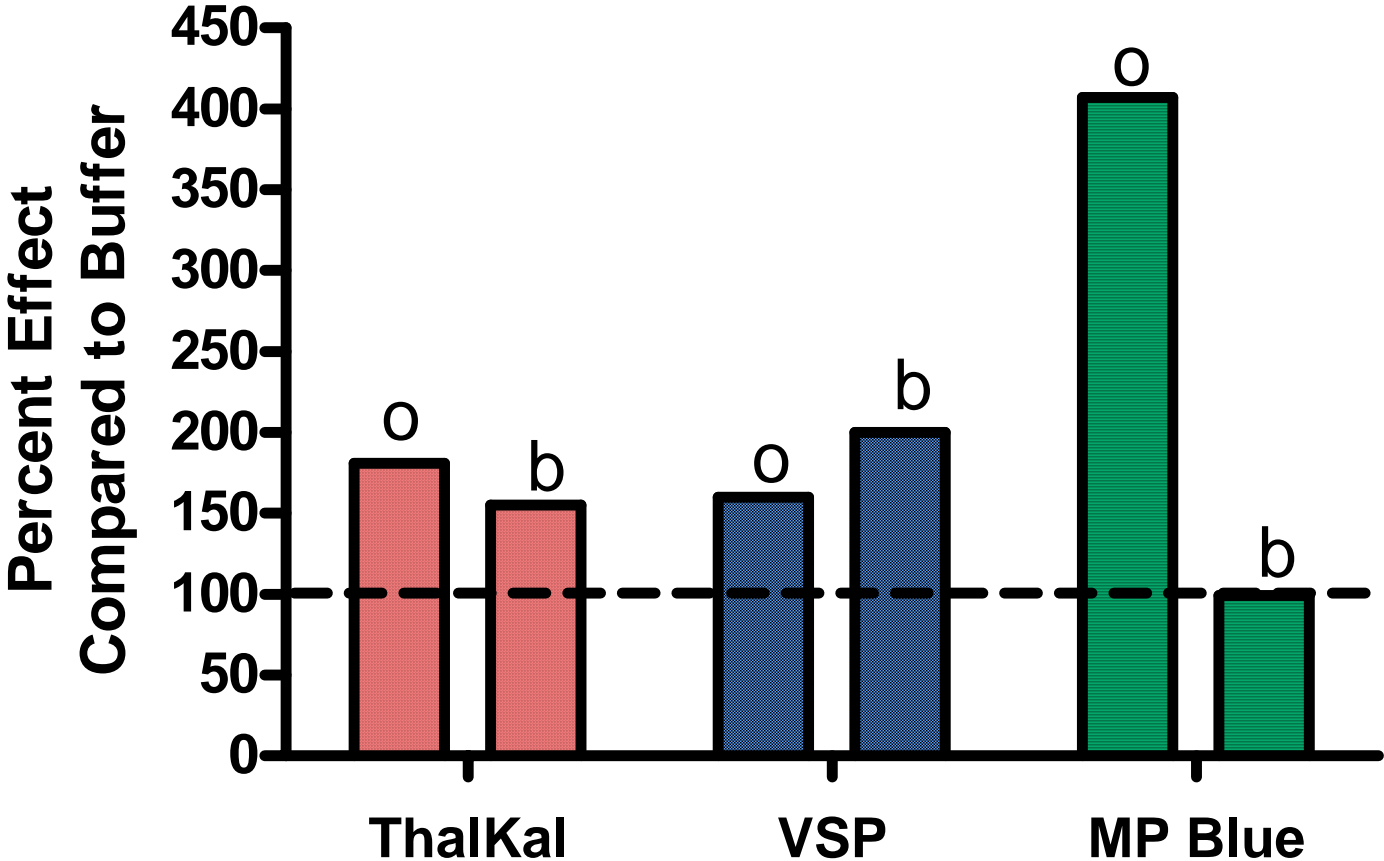
Opener

Blocker

Treatment Effects on ThalKal Signal



Comparison of Effect Sizes



o = opener
b = blocker

Comparison of 3 Dye Systems for K⁺ Channel Assay

- ThalKal
 - **Signal:noise = 1.8**
 - Direct measure of K⁺ channel activity
 - Sustained responses reach steady-state
- VSP
 - **Signal:noise = 1.6**
 - Opener effect is transient
 - Not best assay for hyperpolarization
 - Dynamic range small, so that blocker effect appeared large
- Membrane Potential Blue
 - **Signal:noise = 4.1**
 - Sustained responses reach steady-state
 - Sensitive to both hyperpolarization and depolarization

*****Membrane Potential Blue and ThalKal
produce useful information for this assay**

Voltage Sensor Probe System with a Ligand-gated Ion Channel

Analysis Transfer Fluid Read With TF Transfer Fluid Read With TF

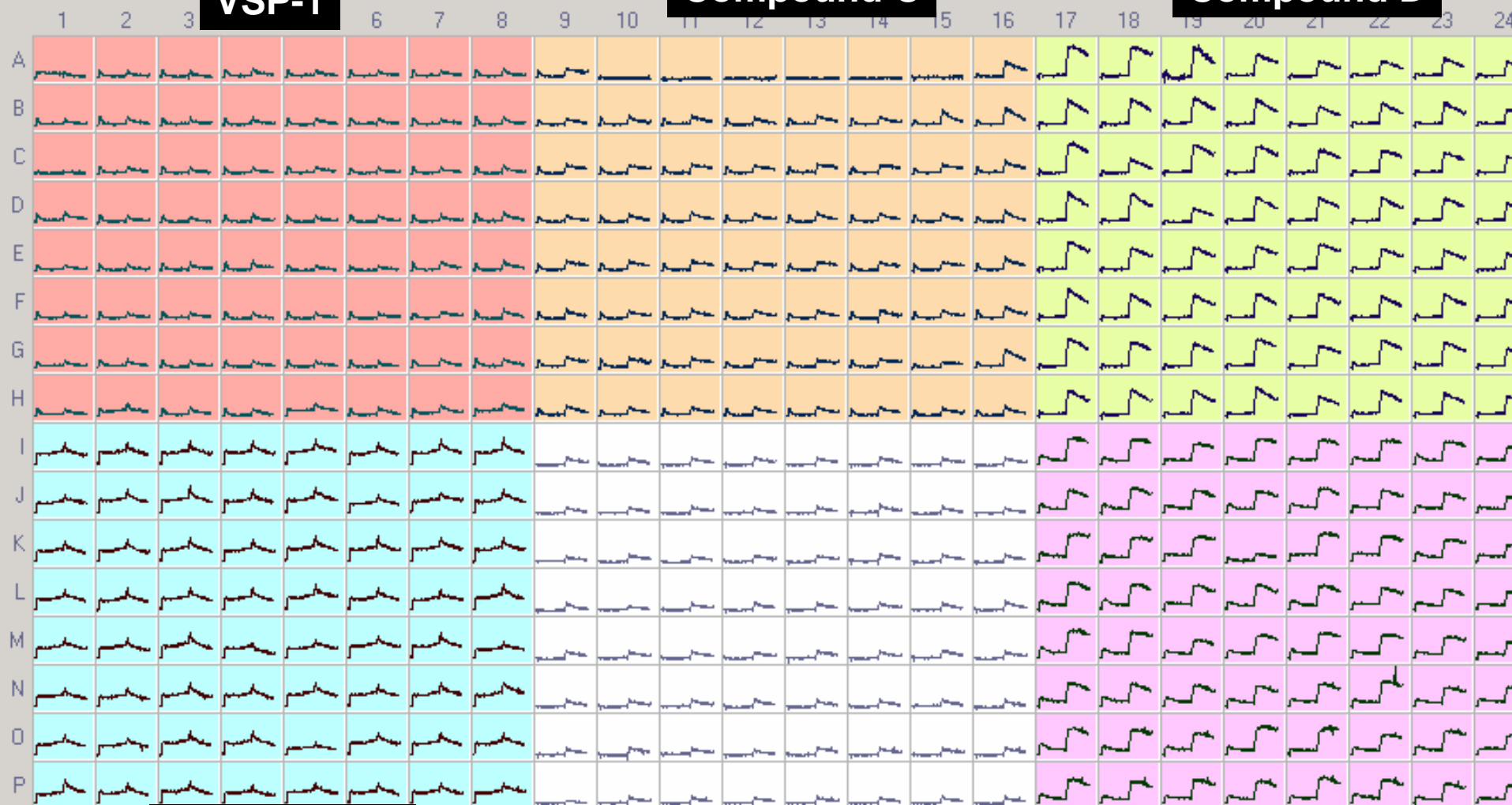
ing Reduction Export Image Notes Display: Ratio

Min Value: 0.46
Max Value: 4.07

VSP-1

Compound C

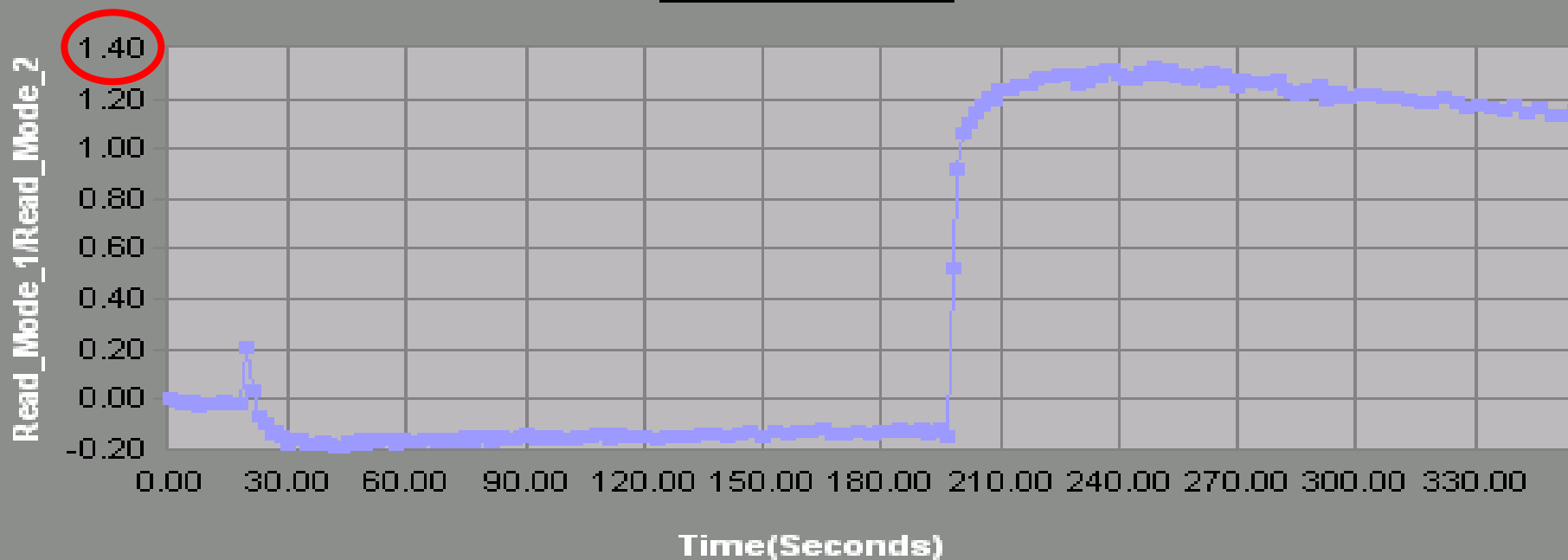
Compound D



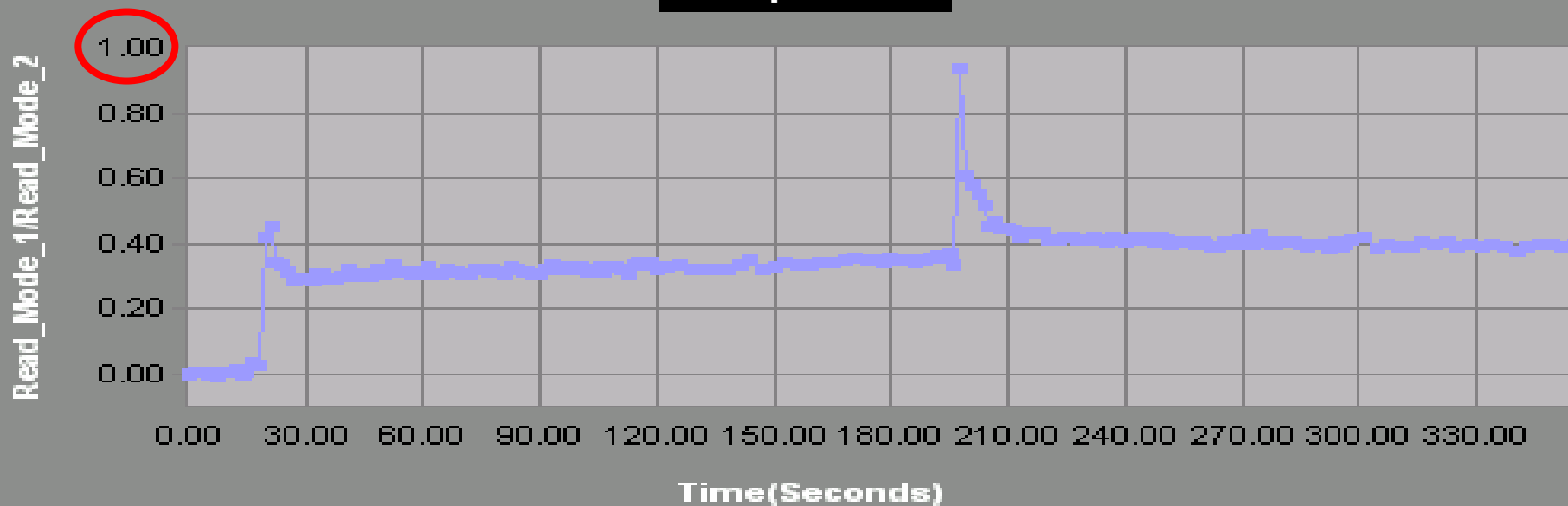
Compound E

Compound F

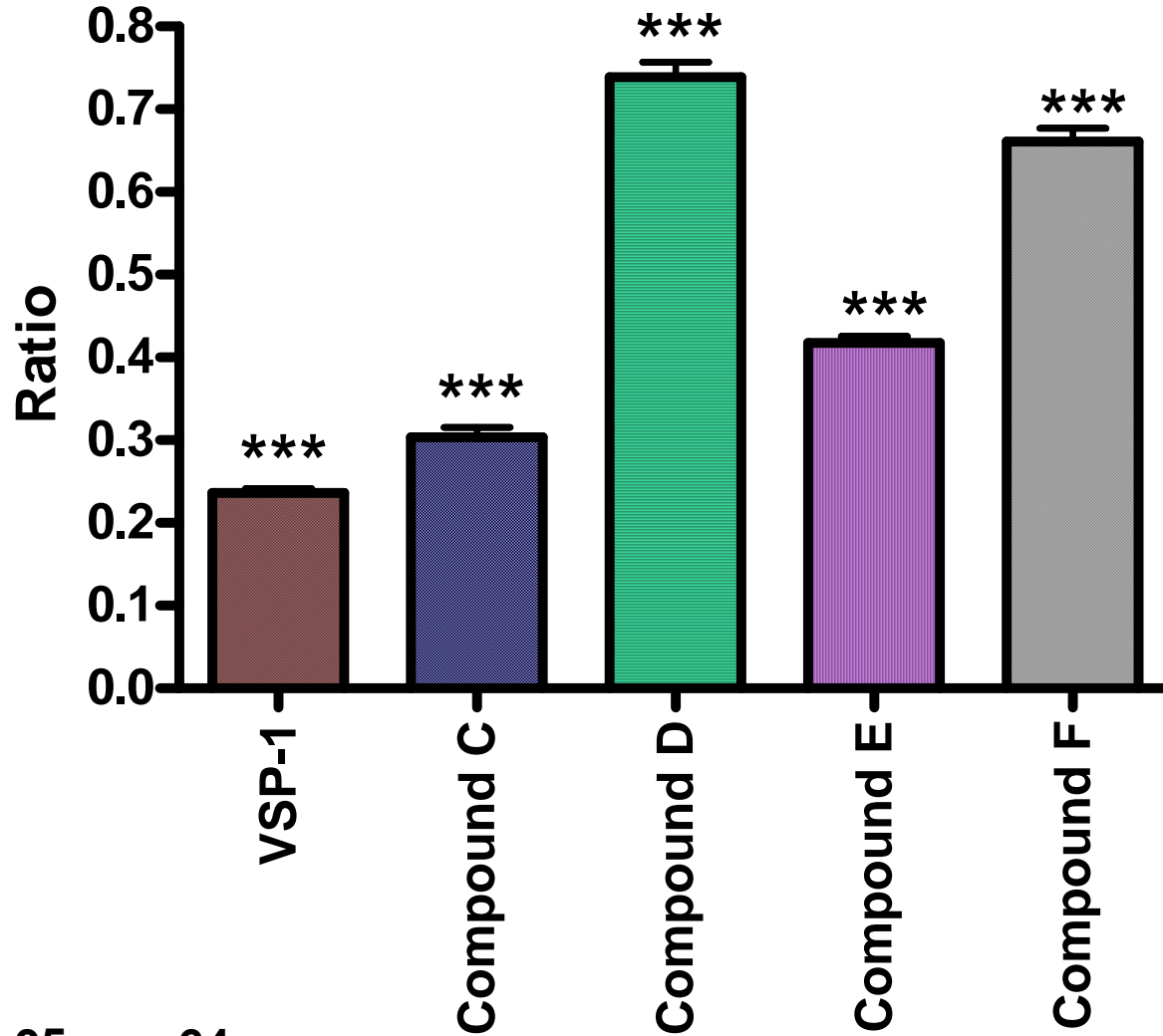
Compound D



Compound E



Treatment Effects on Ligand-gated Channel



*** = $p < 0.05$, $n = 64$

Analysis of Voltage Sensor Probe System for Ligand-gated Ion Channel

- **Signal:noise = 3.1**
- Compound effects reach steady-state
- Only looking for depolarization signals (no need for detection of hyperpolarization)
- Very sensitive assay
 - Able to detect compounds of varying efficacies
 - Able to detect compounds with different kinetics

*****Voltage Sensor Probe dye system is very well suited for this assay**

Acknowledgements

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