CORNING

Epic® system

Epic® Cell Assays for Probing Endogenous Ion Channels

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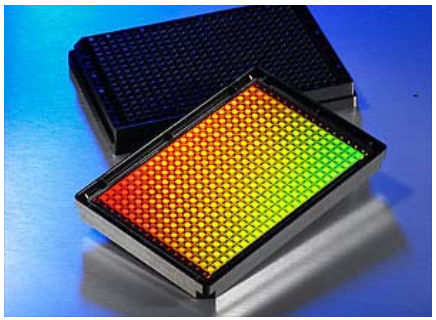
Abstract

Ion channels remain a singularly under-exploited class of targets. Realization of the full potential of ion channels as drug targets is partly hammered by the limited availability of physiologically relevant high throughput screening technologies. We describe a novel label-free cellular assay technology, centered on non-invasive and manipulation-free optical biosensor, for screening compounds against endogenous ion channels. We will discuss the rationale of our approach, and provide data for cellular mechanisms of optical responses mediated through the opening of an endogenous potassium ion channel.

Corning® Epic® System

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



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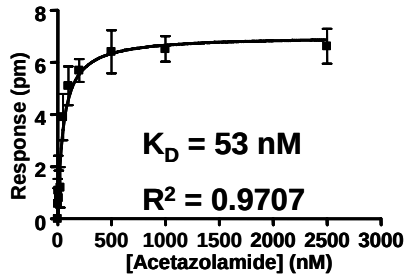


Microplate
- 384-well format
- Optical biosensor in each well
- Surface chemistry

Plate Reader
- Compatible w/ HTS automation
- ≥ 40,000 wells/8hrs
- Sensitivity of 5pg/mm^2

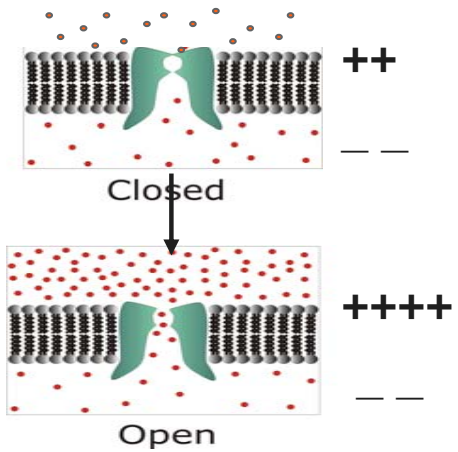
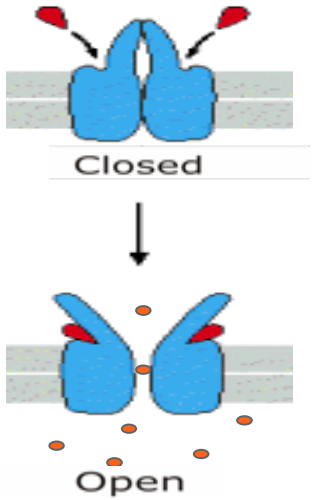
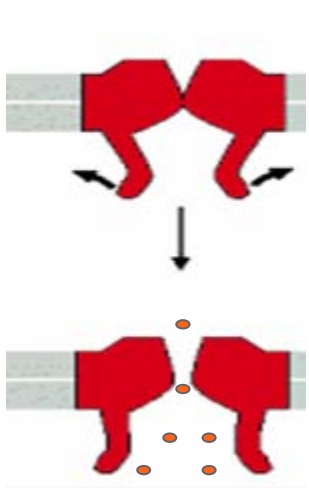
(300Da drug to 75kDa target)

Binding Data
- Manipulated and analyzed by customer

Importance of Ion Channels

- Ion channels
  - Are membrane proteins that control the flow of inorganic ions into and out of cells
  - Are an important class of drug targets
    - Ion channels account for ~8% of data points screened
  - Are important for drug safety
    - hERG potassium channel, a voltage-gated ion channel, is the most studied ion channel today
      - FDA requires cardiac safety screening for all new drugs
      - Pharma has lost billions of dollars because of cardiac-related recalls
      - ~ 200 drugs on the market today have cardiac risks

There are Three Classes of Ion Channels Based on Mode of Action

Voltage-Gated	Ligand-Gated	Mechanically-Gated
		

Four Main Types of Ions

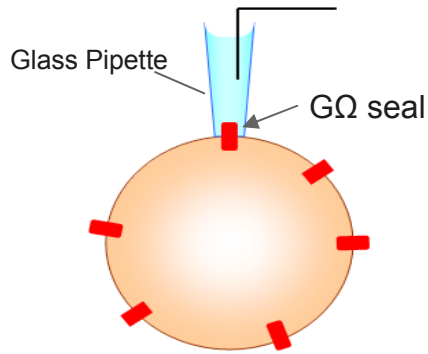
K^+ Na^+ Ca^{2+} Cl^-

Electrophysiology is the Gold Standard for Ion Channels

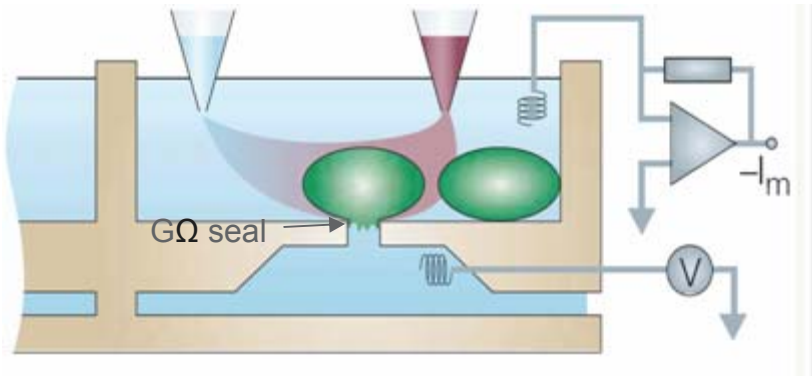
- Patch clamping measures tiny currents across the cell membrane at the single cell level

- Automated patch clamping
  - Moderate throughput: 200 – 2000 data points / 8 hrs
  - High consumable cost: $1.50 - $3.00/well
  - Key limiting factor is unpredictable GΩ seal rate (50-70%)

Patch clamping



Automated patch clamping



Lacking Physiologically Relevant HTS Tools Limits the Upside Potential of Ion Channels as Drug Targets

- Ion channels remain a singularly under-exploited class of targets

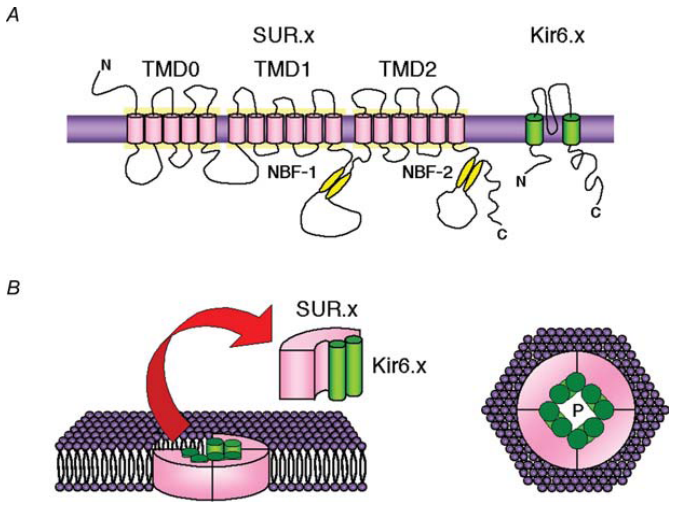
- The lack of physiologically relevant screening tools is the limiting factor
  - Patch clamping
    - Low-medium throughput
    - Single cell assay – cellular response is highly heterogeneous
    - Invasive – sealing may perturb the cellular structure of ion channel complexes
    - Artificial system – requires the use of engineered cells, and/or artificial assay environment
  - Ion flux/fluorescence
    - High throughput
    - Poor hit quality – high false positives and negatives
    - Invasive – pre-loading dyes or ions may alter cellular backgrounds
    - Artificial system – the use of engineered cells, and/or dye labeled molecules

Label-free Epic® Cell Assays for Ion Channel Assays

- Epic® cell assays utilize label-free and non-invasive optical biosensor technology to probe the dynamic redistribution of cellular material within the sensing zone mediated through ion channels
- The resultant signal is an integrated cellular response, and follows the entire evolution of ion channel activity
- The real time kinetics enables classification of mode of action of modulators acting on ion channels and their regulatory proteins
- *As demonstrated in Epic® whole cell GPCR assays, Epic® ion channel assays may offer novel insights for ion channel modulators that link ion channel activity to cell physiology*

A Model System Utilizing an Endogenous ATP-Sensitive K Ion Channel

- ATP-sensitive potassium channel K_{ATP}
  - Responds to fluctuations in intracellular levels of ATP and ADP
  - Acts as a sensor of cell metabolism: Couples changes in cellular metabolism to membrane excitability
  - Is a drug target for angina pectoris and hypertensive crises
  - Consists of four pore-forming subunits Kir6.x and four regulatory subunits SUR
    - Kir 6.x is an inward rectifier K+ channel
    - SURs is a member of the ATP Binding Cassette family of membrane proteins (ABC transporters)
  - Multiple locations in cells: sarcolemmal ($sarcK_{ATP}$), mitochondrial ($mitoK_{ATP}$), or nuclear ($nucK_{ATP}$)

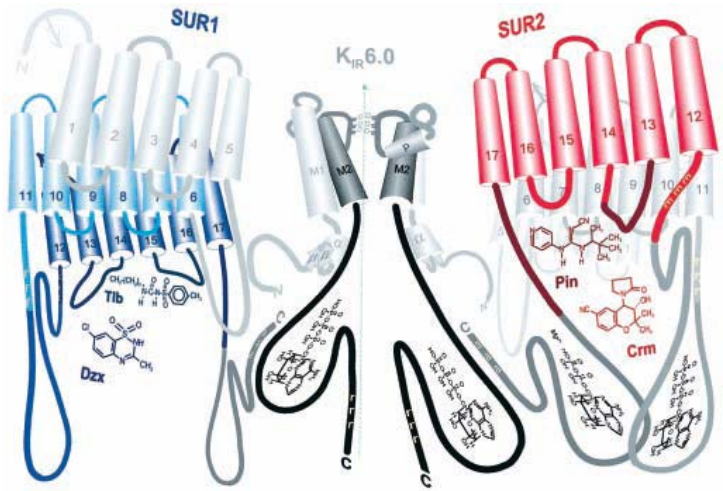


Cells	Type of K_{ATP}
• Pancreatic β cells	• Kir6.2/SUR1
• Cardiomyocyte	• Kir6.2/SUR2A
• Smooth muscle	• Kir6.2/SUR2B
• Vascular smooth muscle	• Kir6.1.SUR2B

Aguilar-Bryan, L. et al. *Endocrine Rev.* 1999, 20, 101-135.
Teramoto, N. *J. Physiol.* 2006, 571, 617-624.

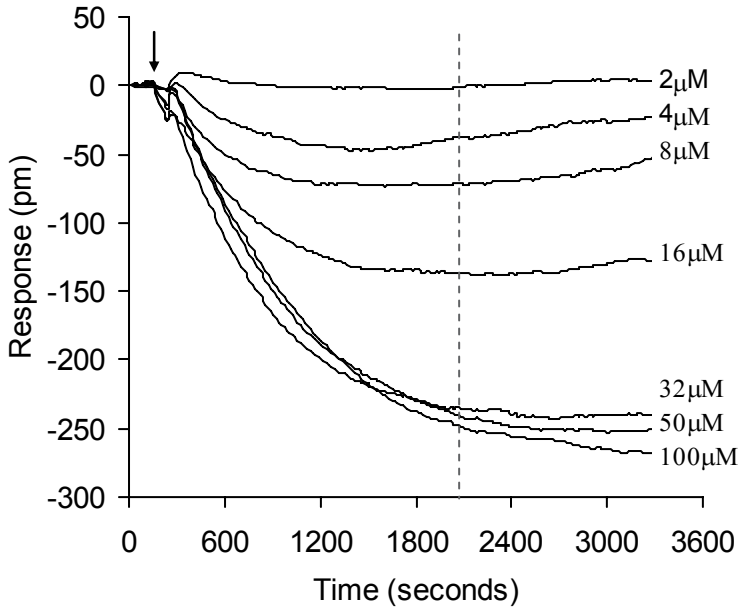
Pharmacological Characteristics of K_{ATP}

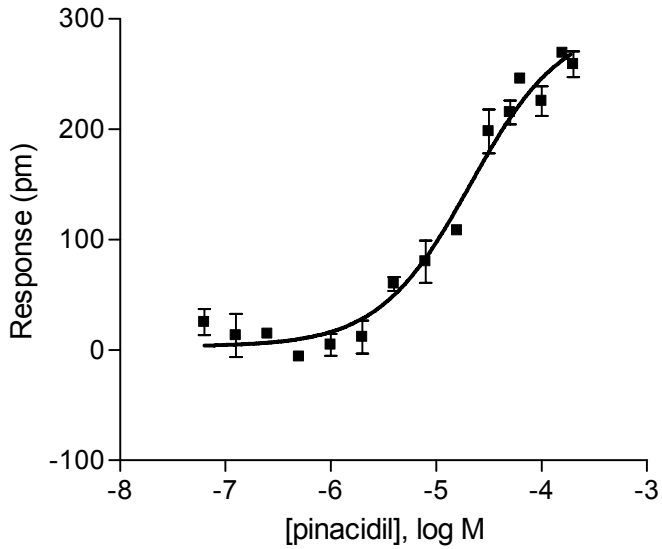
- K_{ATP} ion channel
  - Kir6.x mediates channel inhibition by ATP
  - SUR subunit mediates the stimulatory effects of Mg^{2+}ADP as well as channel response to sulfonylureas and potassium channel openers (KCOs)
  - Each SUR molecule contains two of the nucleotide binding domains (NBD) that are highly conserved in all ABC transporters
- K^+ channel openers
  - Comprise a structurally diverse group of drugs with a broad spectrum of potential therapeutic applications
  - Pinacidil, cromakalim, and nicorandil preferentially target the SUR2A and SUR2B isoforms, but has little or no effects on SUR1-based channels
  - Diazoxide preferably activates SUR1, and only activates SUR2A and SUR2B under certain conditions



Babenko, A. P. *et al. J. Biol. Chem*. 2000, 275, 717-720.

Pinacidil Induces a Dose-Dependent and Saturable Epic® Signal

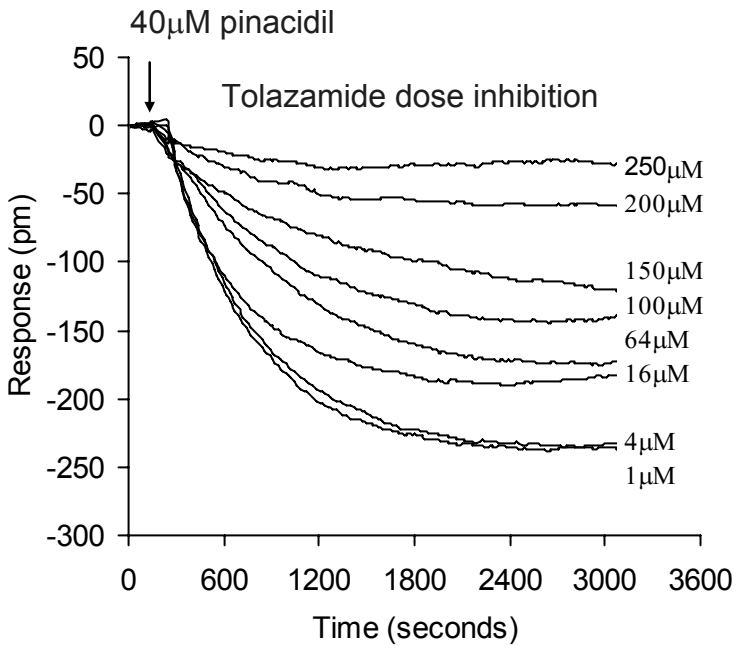


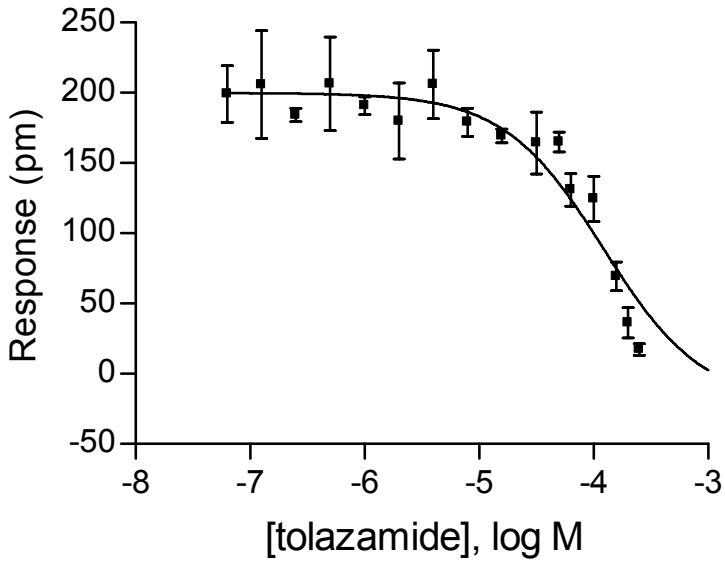


EC_{50} = 21.1±3.5μM

- Agreement with electrophysiology data

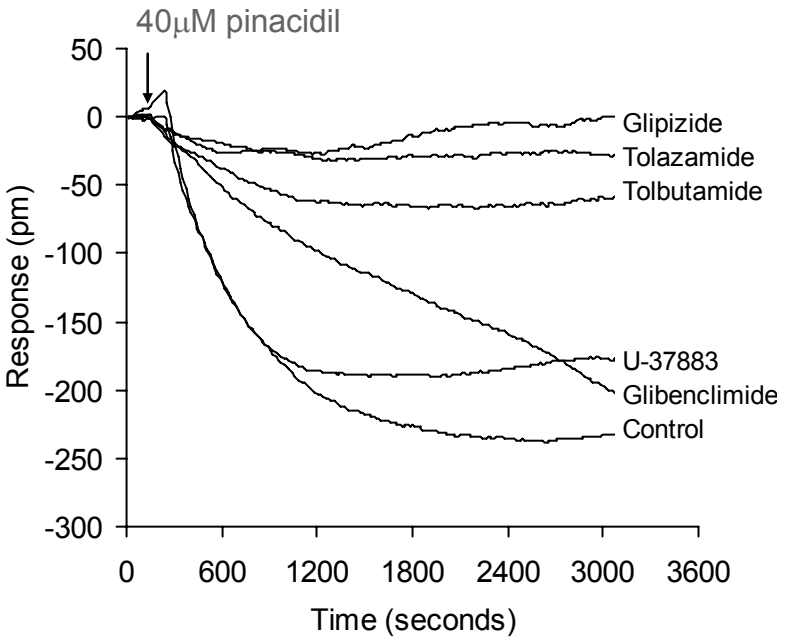
K_{ATP} Blockers Dose-Dependently Inhibit the Pinacidil Response

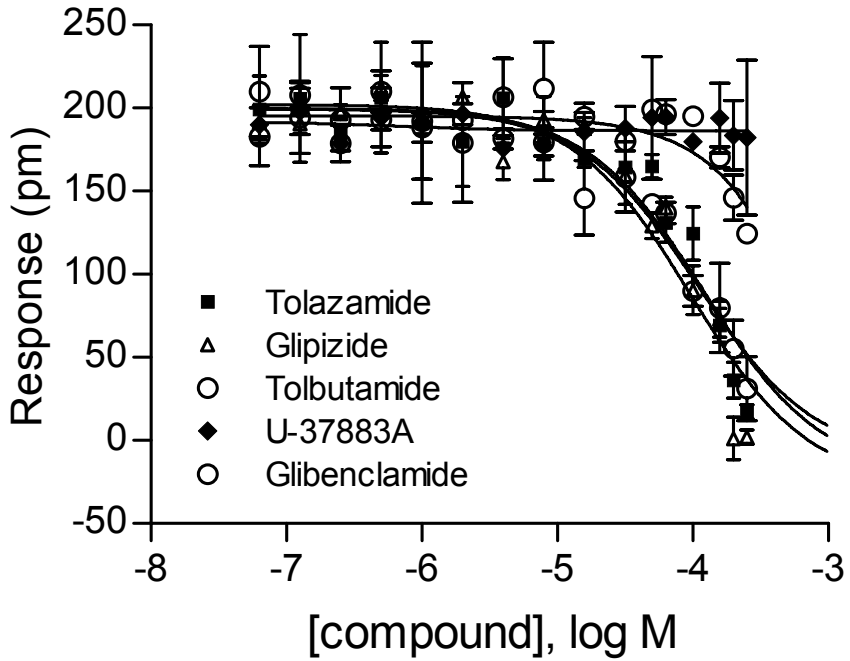




- Selectivity profiles indicate Kir6.2/SUR2 K_{ATP}

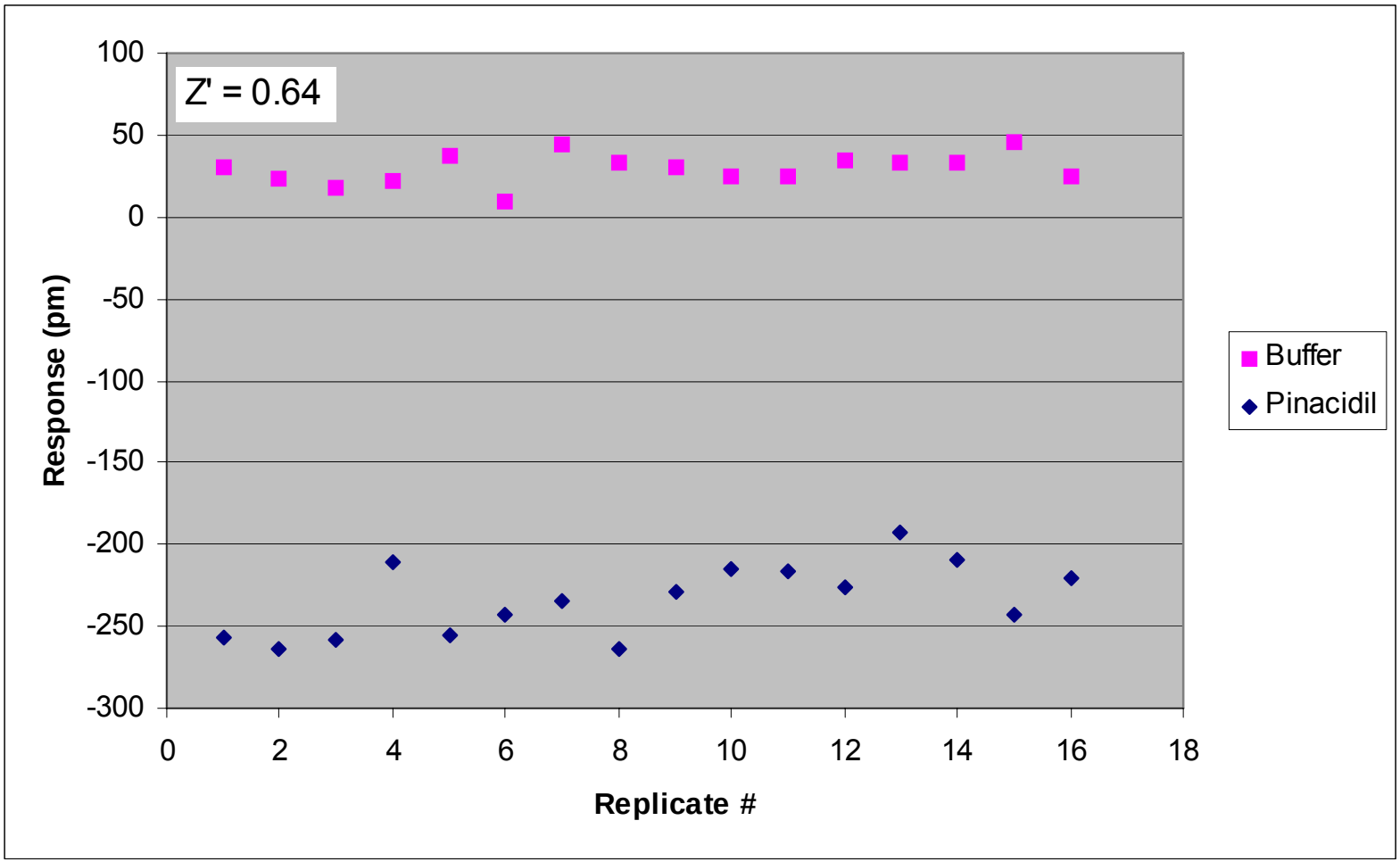
K_{ATP} Blockers Dose-Dependently Inhibit the Pinacidil Response



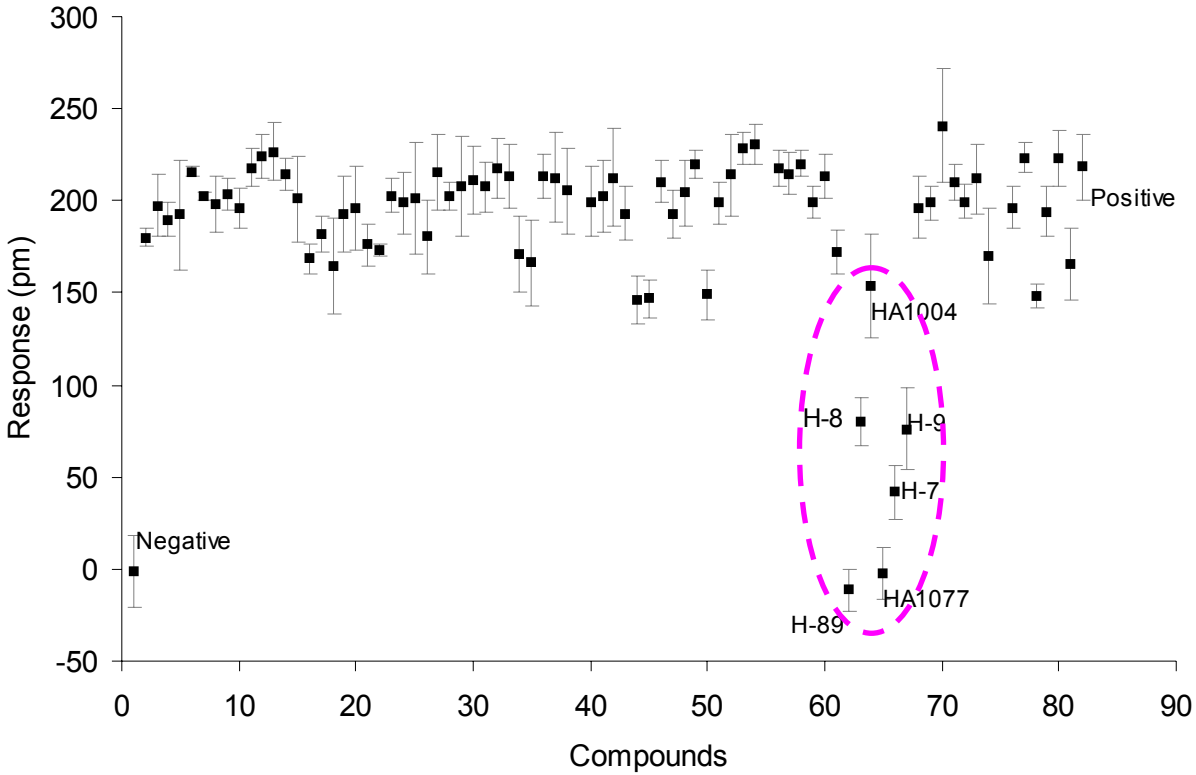


- Selectivity profiles indicate Kir6.2/SUR2 K_{ATP}

The K_{ATP} Assay is Robust



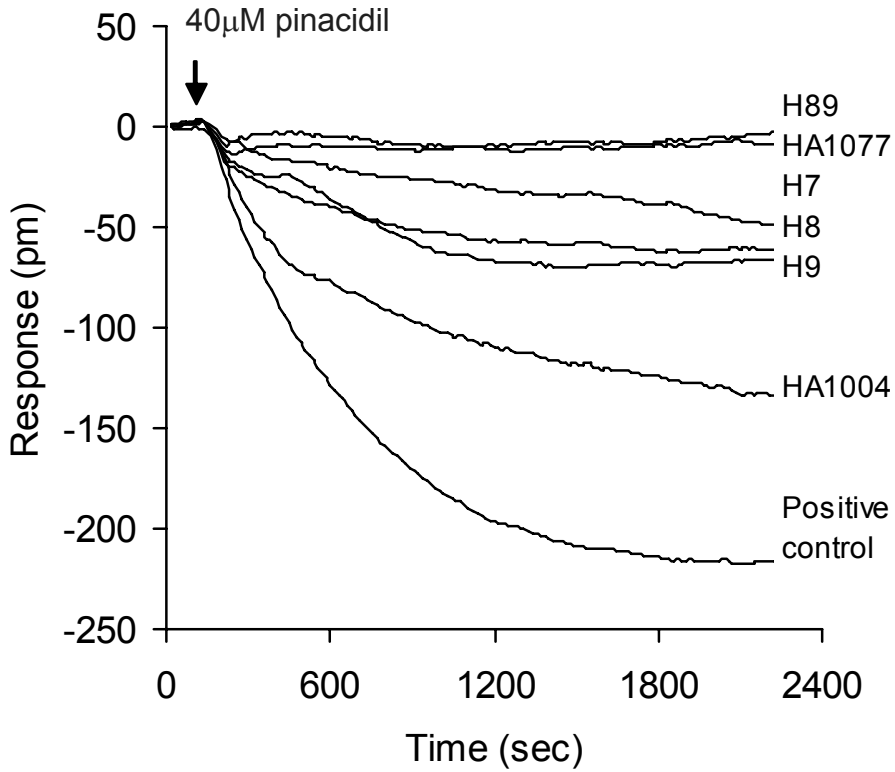
Kinase Modulation Profiles Identified Several Modulators of the Pinacidil Response



- All PKA inhibitors examined inhibited the pinacidil response.
  - PKA is upstream of K_{ATP}

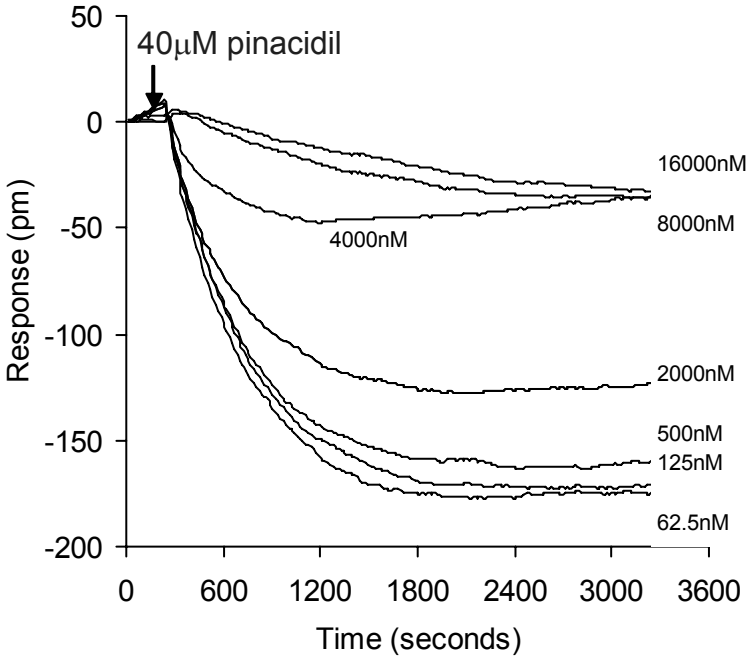
The Kinase Inhibitors Display Three Types of Modulation

- Impact of distinct kinase inhibitors is different
  - Little or no effect
  - Attenuation
  - Alteration in kinetics



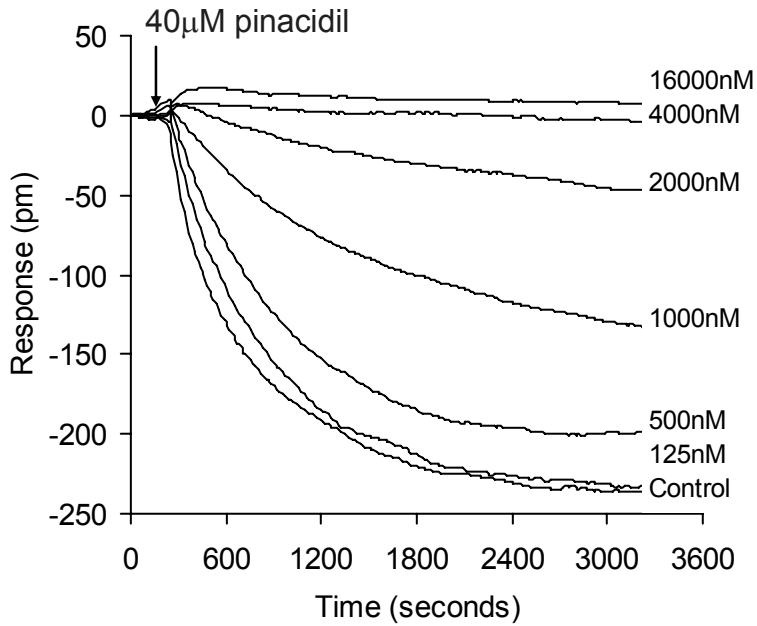
The Pinacidil Response is Sensitive to Actin Filament Disruption

Cytochalasin B



IC_{50} = 5.7±1.5μM

Latrunculin A



IC_{50} = 9.9±2.7μM

- Both actin filament disruption agents inhibited the pinacidil response.
  - Cytochalasin B caps microfilaments
  - Latrunculin A sequesters actin monomers

Summary

- Epic® cell assays enable the detection of endogenous and non-functional ligand gated ion channel K_{ATP}
- The K_{ATP} assay is robust with a Z' of > 0.6
- The kinase modulation profiles identified several PKA inhibitors that attenuate the pinacidil response
- The pinacidil response is sensitive to the disruption of actin filaments
- Electrophysiology, RT-PCR and RNAi knockout studies are underway