



A label-free Perspective on GPCR Drug Discovery

**Novel Applications and Cutting Edge Technologies for
GPCR Screening and Profiling**

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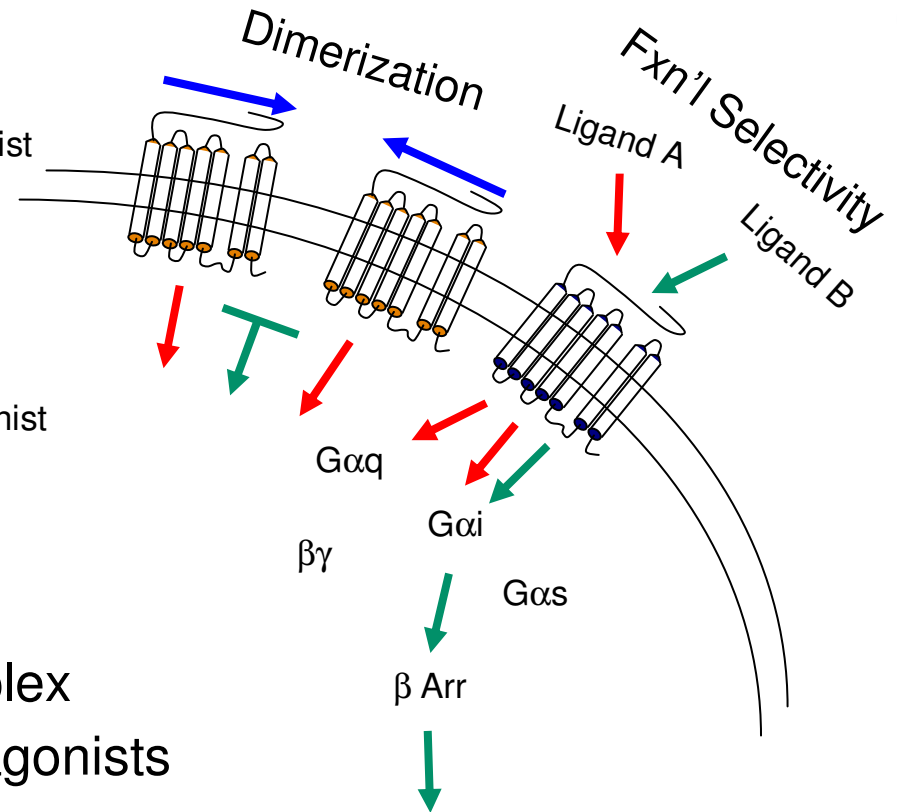
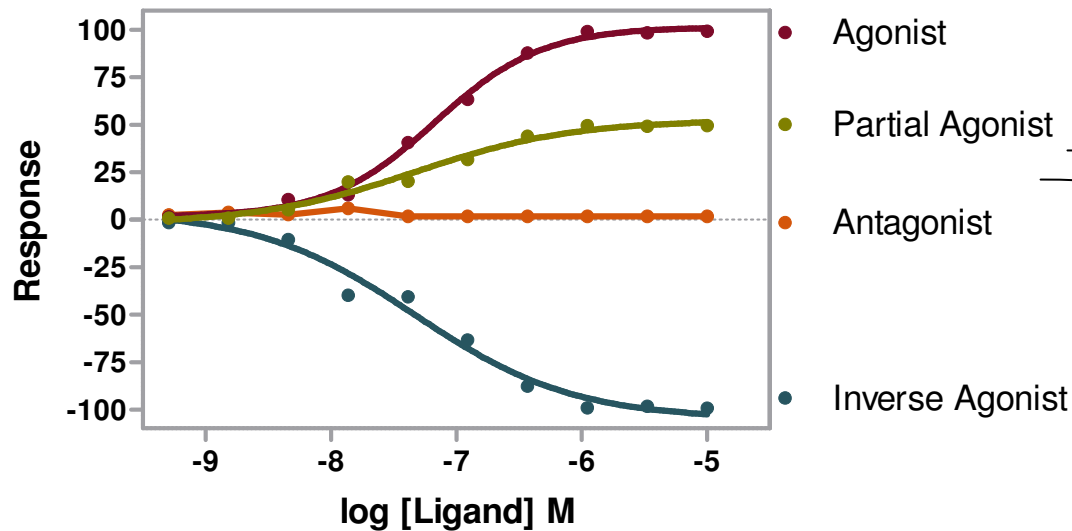
View of Early Drug Discovery

- Biology provides the Targets
- Chemistry provides the Tools
- Pharmacology provides the Rules
- Assays provide the Lens

Continued evolution

- Targets
 - Our understanding of GPCR biology is changing dramatically
- Tools
 - Compound libraries continue to diversify and improve
- Rules
 - Evolve along with empirical observations
- Lenses
 - Label-free cell based assays represent an important leap

Targets: The complexity keeps on coming



- GPCR's and their ligands are complex
 - Agonists, antagonists, inverse agonists
 - Allosteric modulators
 - Ligand-biased signaling
 - Multimers
 - Signaling diversity

Lenses: Coming full-circle

- Tissue slices for drug profiling and pharmacology
 - Low throughput
 - Expensive
 - Experts only
- Reductionism
 - Clear linkage between chemistry and targets
 - Loss of physiological context
 - Major advances in throughput - industrialization
- Cell-based assays: single-point
 - More biology – But not quite enough
 - Maintain throughput and reductionist principles
- Cell-based assays – Label-free
 - Integrated-responses
 - Closer to tissue-based experimentation
 - Medium throughput in kinetic mode
 - Non-expert

Cell based assays: Coming full-circle

“It is important to consider the properties of the pharmacological target, namely 7TM receptors, to optimize the design of a 7TM receptor cellular assay.”

Terry P. Kenakin

Nature Reviews Drug Discovery. August, 2009

- When taking into account the complexity of natural GPCR behavior ...

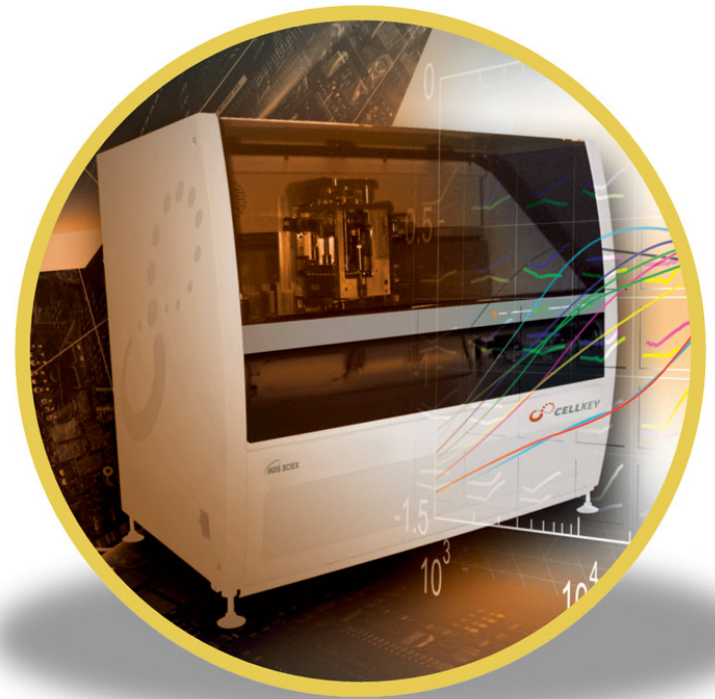
What is any single mediator's relationship to the overall cellular response?

Pushing cell based assays further Label-free

- With their integrated readouts & unique sensitivity to endogenous targets
 - Label-free cell based assays will be among the most effective tools for investigating nuanced GPCR characteristics.
- GPCR biology can be tested
 - At natural expression levels,
 - Along side potential multimer partners (also expressed at natural levels),
 - Attached to native pathways
 - In cell types closely aligned to the disease process under investigation.

CellKey™ System: a cell-based assay platform

- **Label free technology**
 - Measures impedance using Cellular Dielectric Spectroscopy
- **Real time, kinetic measurements**
- **Automated fluid handling**
 - Simultaneous compound addition and read
- **Thermal control**
 - RT to 37°C
- **Software package for data analysis**
- **Lab automation system integration**
- **In 96W and 384W formats**
 - Standard and small volume 96 W



Target and pathway independent

- Sensitivity enables routine evaluation of endogenous targets
- Universal platform compatible with a wide range of targets

	GPCR			TKR	Chemokine	Cytokine	Other
Endogenous	Prostanoid Calcitonin Adenosine A _{2B} β-adrenergic Dopamine VPAC ₁ Apelin Cholecystokin Cannabinoid CGRP1, CGRP2 GABA mGLUR	Serotonin (5HT _{1B}) CXCR ₄ α2-adrenergic CXCR2 Dopamine MCHR1 Urotensin Histamine H ₁ EDGE PAR	P2Y Bradykinin Endothelin Muscarinic m ₃ PAF α1-adrenoceptor Bombesin Tachykinin Orexin Oxytocin	EGF IGF-1 FGF HGF PDGF NGF	CXCR3 CXCR4 CCR1 CCR4 CCR5 CCR2 CCR3 D6 US28 DARC ECRF3 KSHV	IL-1 IL-2 IL-3 IL-6 TNFRSF1 TNFRSF2	FcεRI Integrins Ion channels
Transfected	β3-adrenoceptor Dopamine D1,D5	Dopamine D2L,D4 Urotensin	Muscarinic m ₁ Muscarinic m ₃				

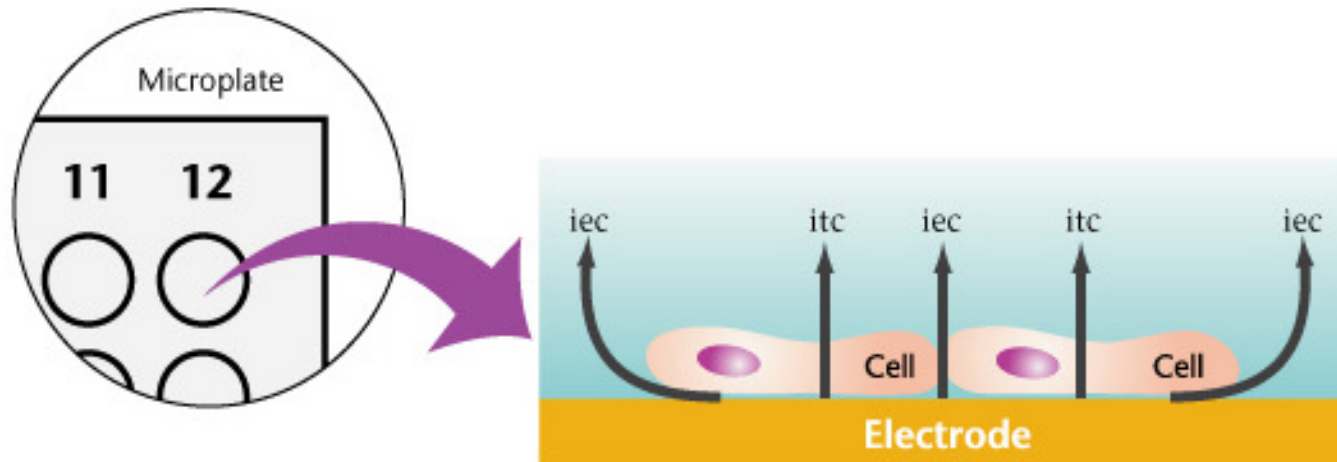
Cell type independent

- Amenable to the use of cell lines and primary cells, both **adherent** and **non-adherent** cells
 - epithelial; endothelial; fibroblast; muscle; neuronal; lymphoblast; monocytes etc

A-10	HL-60	RMS13
A-431	HUVEC	SH-SY5Y
A549	Jurkat	TE-671
CHO	MCF7	THP-1
COS-7	MDS-MB-231	U-937
HEK 293	Neuro-2A	U-2 OS
HeLa	PC-3	V79-4
MDCK	RBL-2H3	WI-38
KELLY	NIH/3T3	K-562

Mast cells
Neutrophils
Osteoblasts
Prostate stromal cells
Prostate smooth muscle cells
Prostate epithelial cells
BSMC
PBMC
CD4 ⁺ T cells

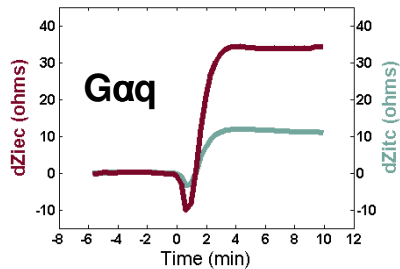
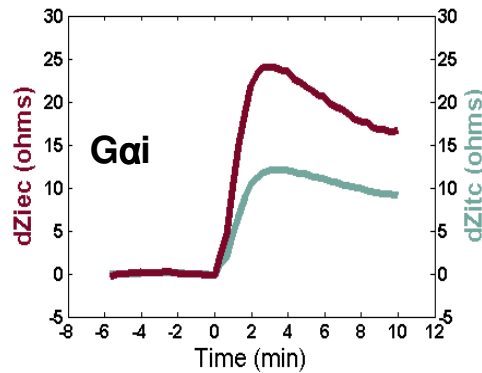
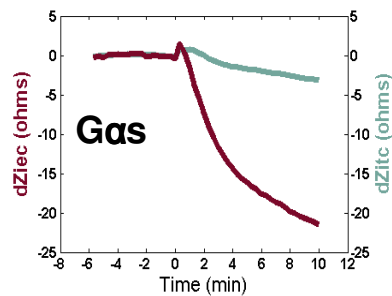
Information rich data from every assay



$$Z \approx V/I$$

- Transcellular (Z_{itc})
- Extracellular (Z_{iec})

CellKey System Data Analysis



- **Quantitative pharmacological analysis**

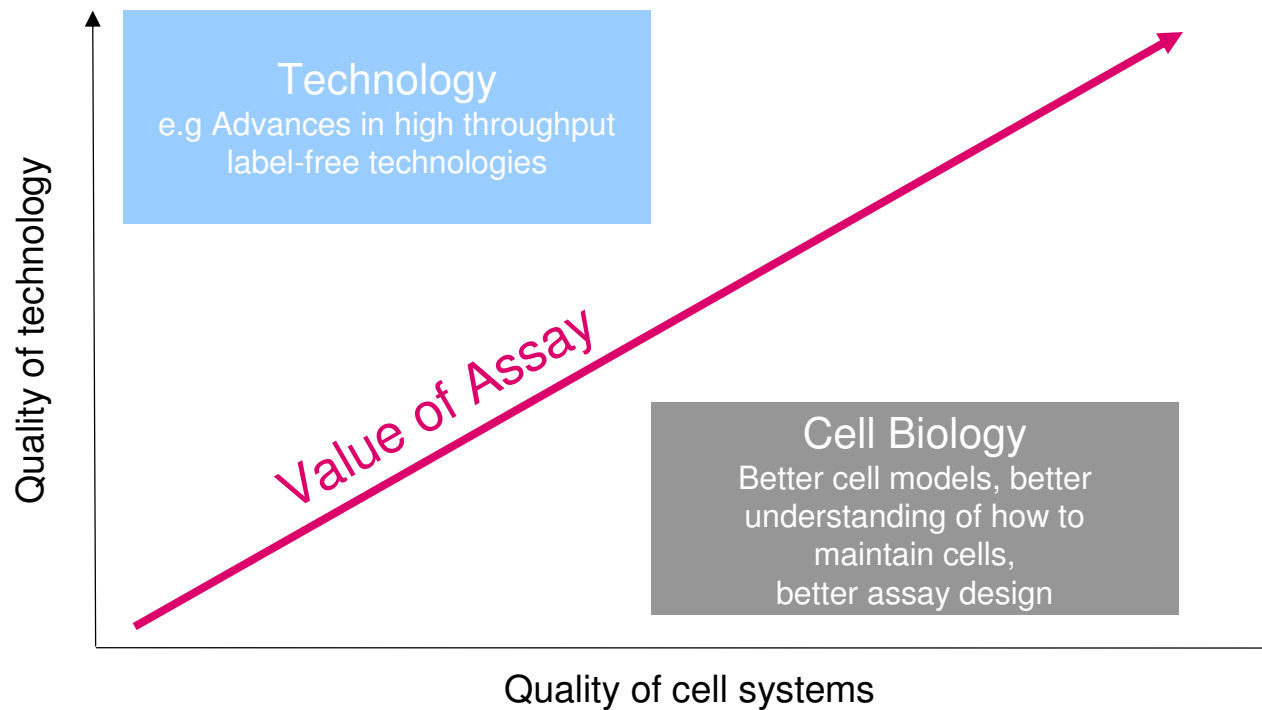
- partial and inverse agonism
- antagonists and modulators
- complex Schild analysis

- **Qualitative analysis**

- CellKey™ response profile
- insight into MOA

Lonza Collaboration

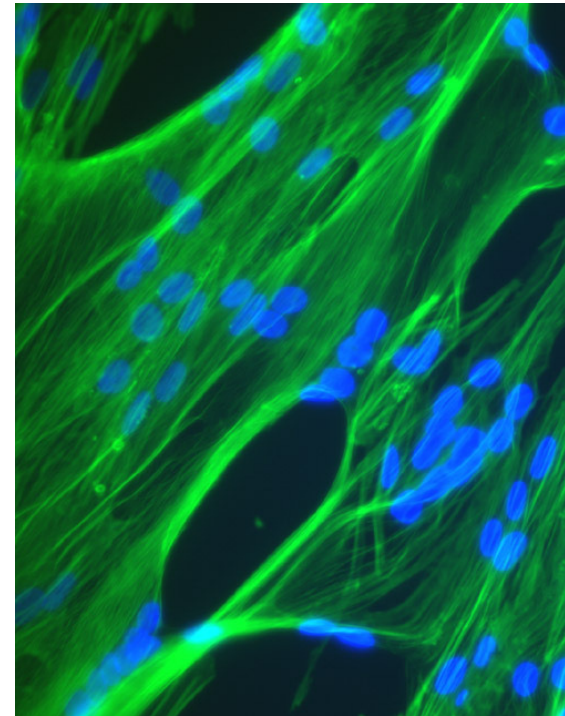
Label-free analysis of primary cells



- Biorelevant target identification and validation:
 - Hu Skeletal Muscle Myoblasts (HSMM)
 - Hu Umbilical Vein Endothelial Cells (HUVEC)

Benefits of utilizing Human Skeletal Muscle Myoblasts

- Easy to use and reproducible system for basic research into muscle physiology
 - Muscle mass and function
 - Myotube formation and tissue repair
- Important in models of muscular dystrophy and other muscular and neuromuscular degenerative disorders
- Critical to understanding and treating the metabolic consequences of Type II diabetes
 - Insulin uptake or resistance

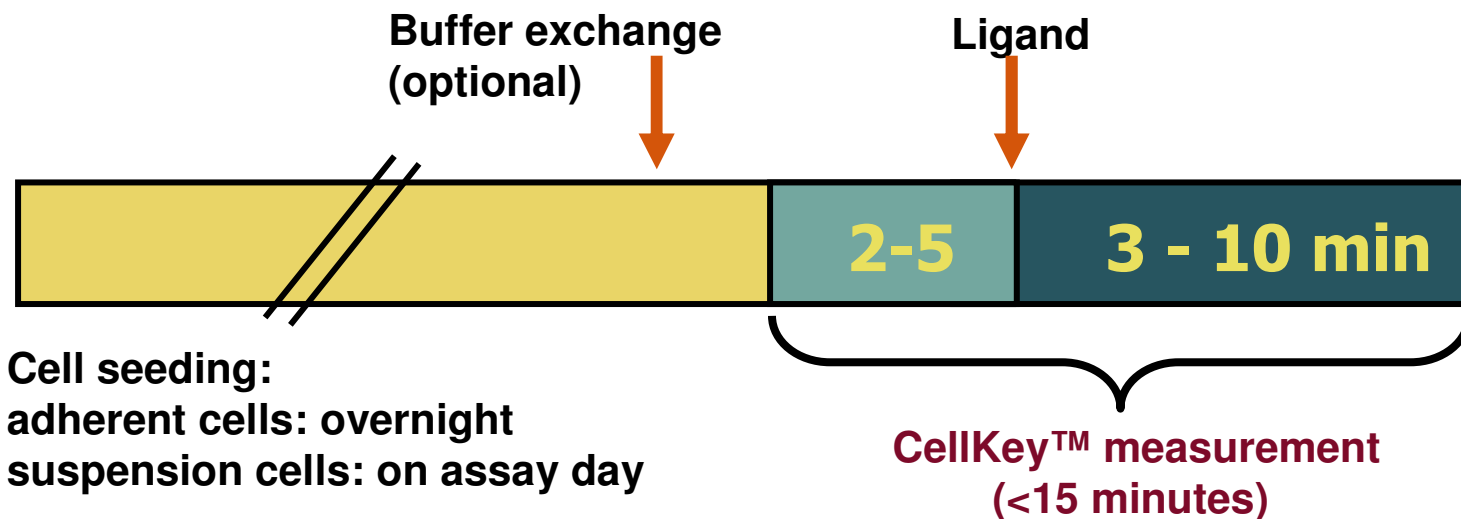


The CellKey™ System assay development & method

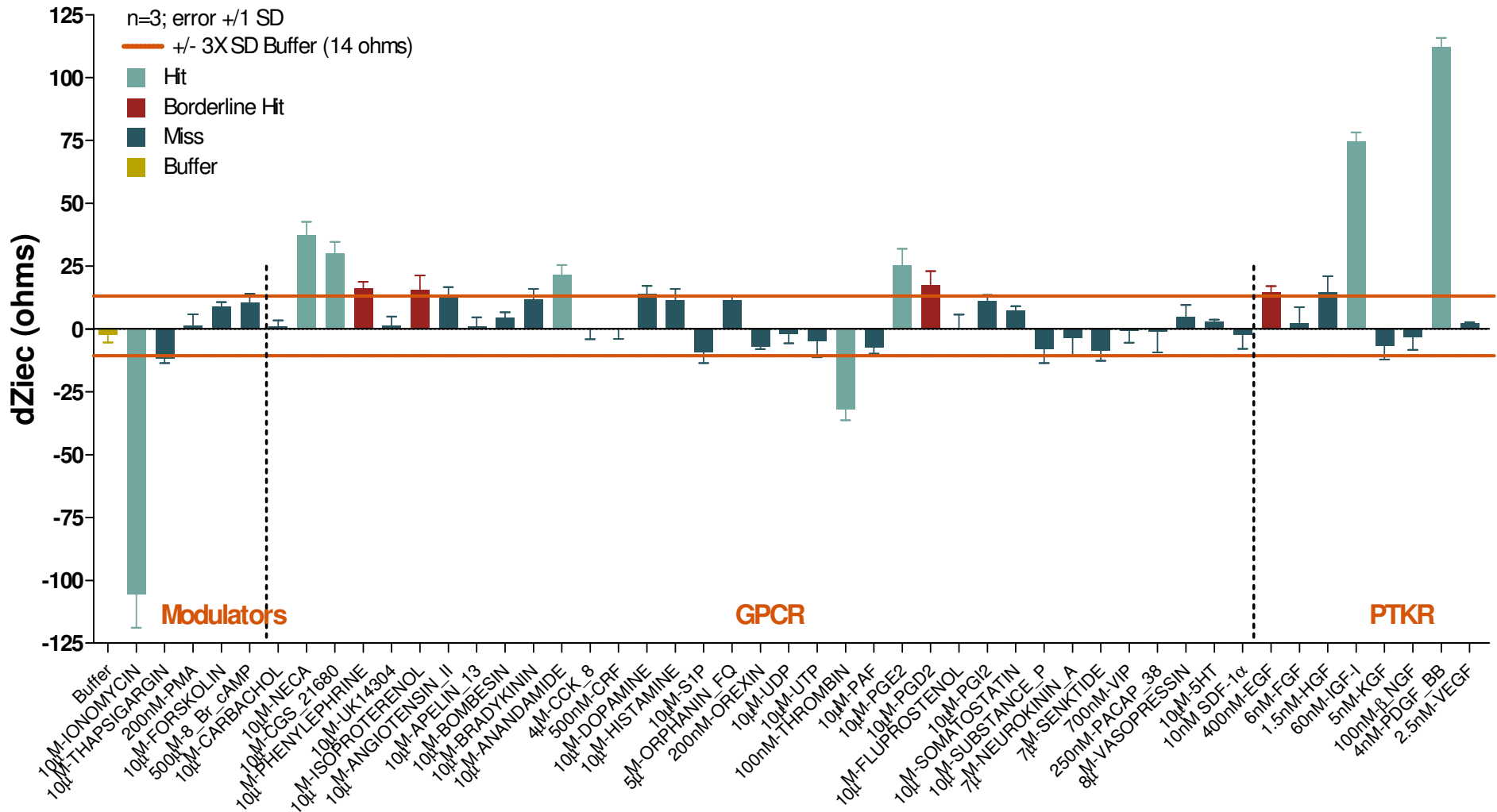
- **Assay development with the CellKey™ System**

- Cell titration
- Cell plating conditions/fluidics
- Assay temperature
- Ligand concentration
- Measurement duration

- **Typical assay protocol for the CellKey™ System**



Receptor Panning on HuSMM



Experimental Conditions: Human Skeletal Muscle Myoblasts

- Lonza's optimized culture conditions translated well to the CellKey System, making assay development easy
 - Cells cultured in SkGM-complete media
 - Seeded 50,000 cells/well in CellKey Standard 96W plates evenings prior to experimentation
 - No plate coating required
 - All experiments performed at 37°C on the CellKey System reader
 - Prior to each experiment, cells were washed into 135 µL assay buffer using onboard fluidics

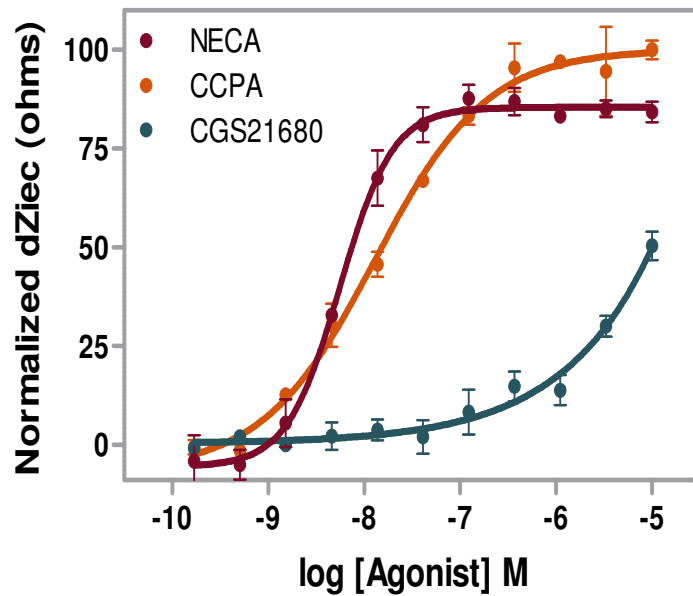
Receptor Panning Benefits

- Identify active endogenous GPCR's, TKR's etc on ANY cell type.
- Analysis of multiple receptor types across many functional families is simply and simultaneously accomplished by adding receptor ligands to cells using the automated CellKey™ System platform; no specialized reagents or genetic manipulation is required.
- A panel of cell lines can easily be screened for functional expression of a specific receptor of interest.
- Receptor panning can be performed in primary cells, which permits rapid development of secondary screening assays that complement and confirm primary screens.
- This assay development application provides the information necessary for choosing the most appropriate biorelevant cell context for secondary screening.

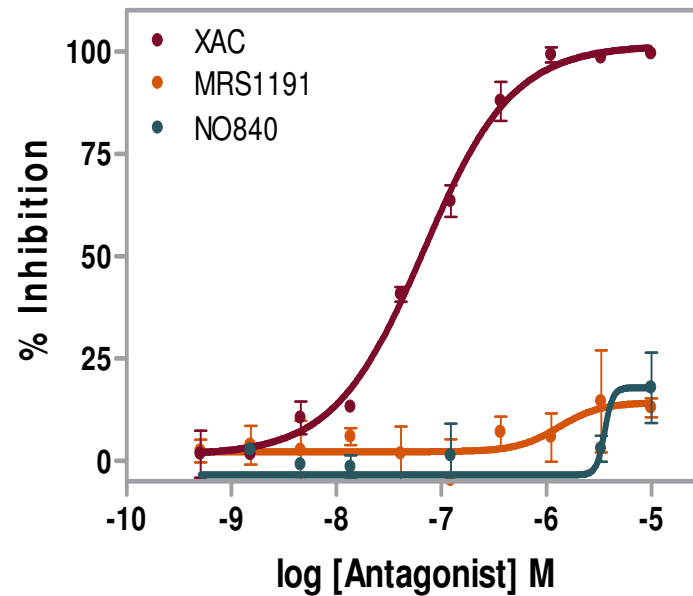
Robust Pharmacology

Endogenous adenosine receptor

Agonist CRC

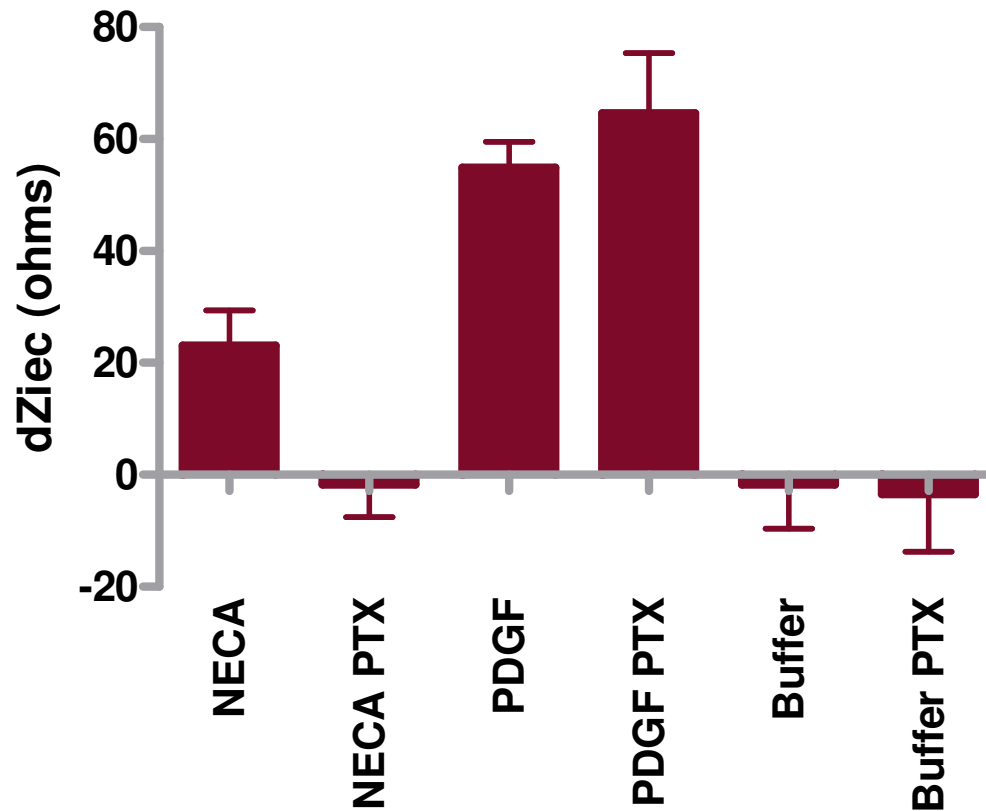


Antagonist CRC



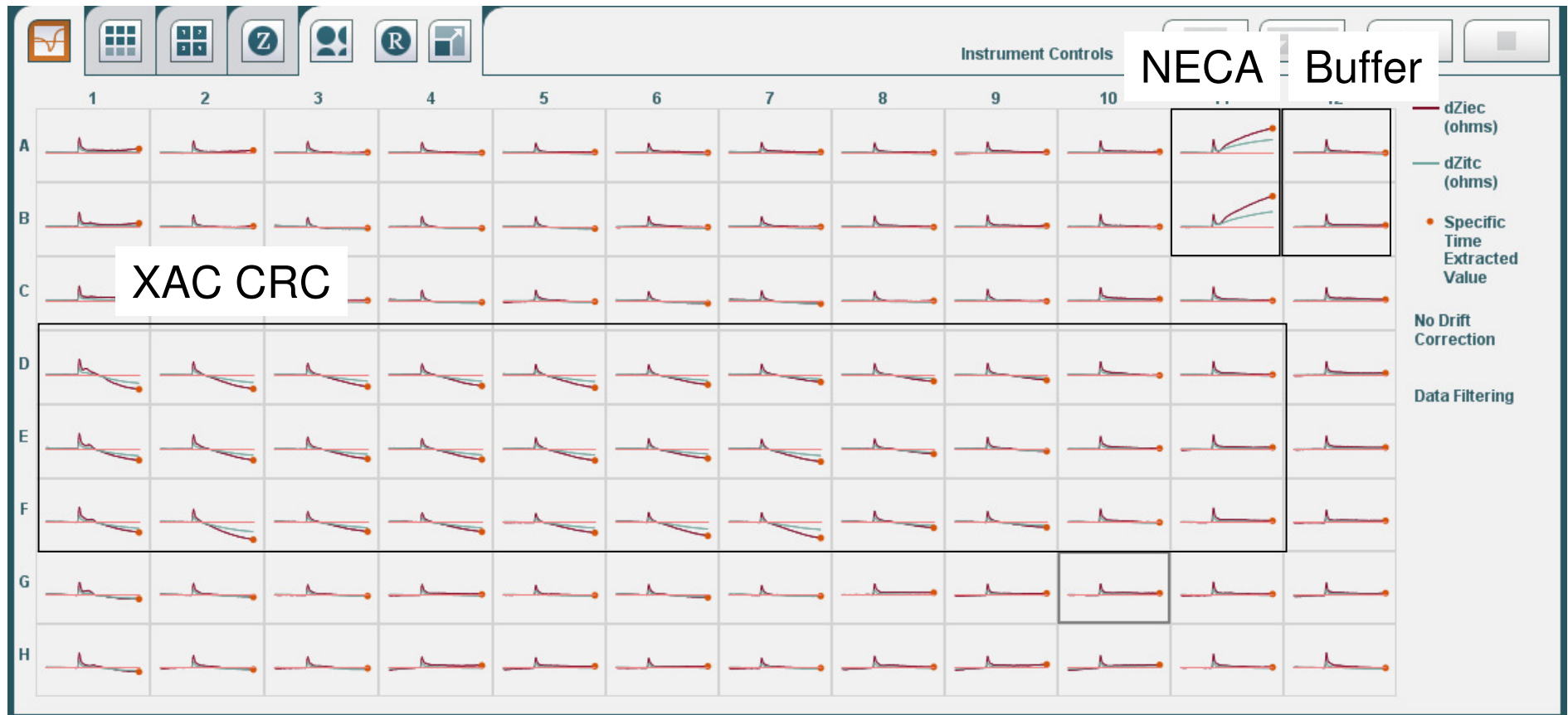
HSMM: Biorelevant pathway analysis

Endogenous adenosine receptor: Simple validation of $G_{\alpha i}$ -coupled pathway



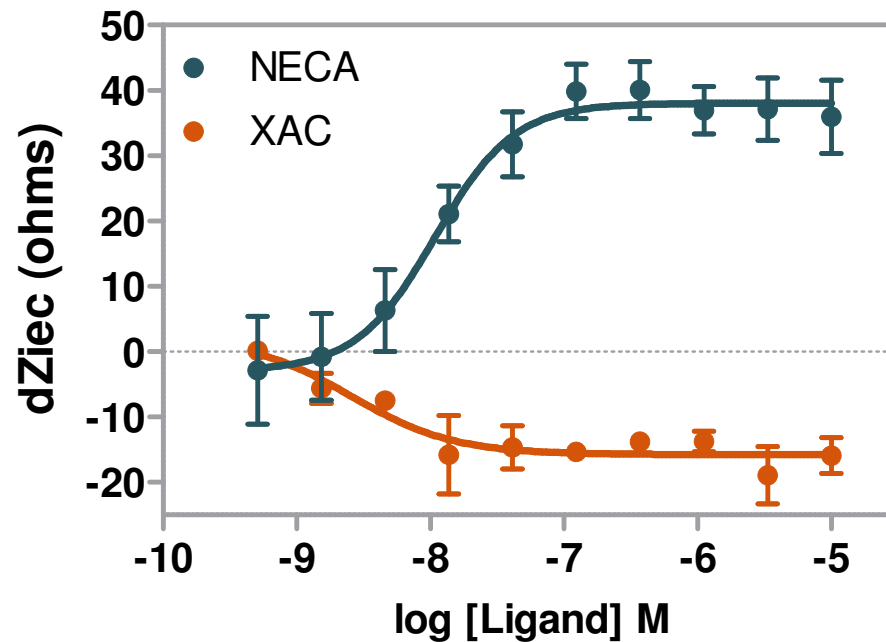
Identification of interesting compound effects

- XAC - Possible inverse agonist in primary cell type?
 - Universality and sensitivity enables easy identification



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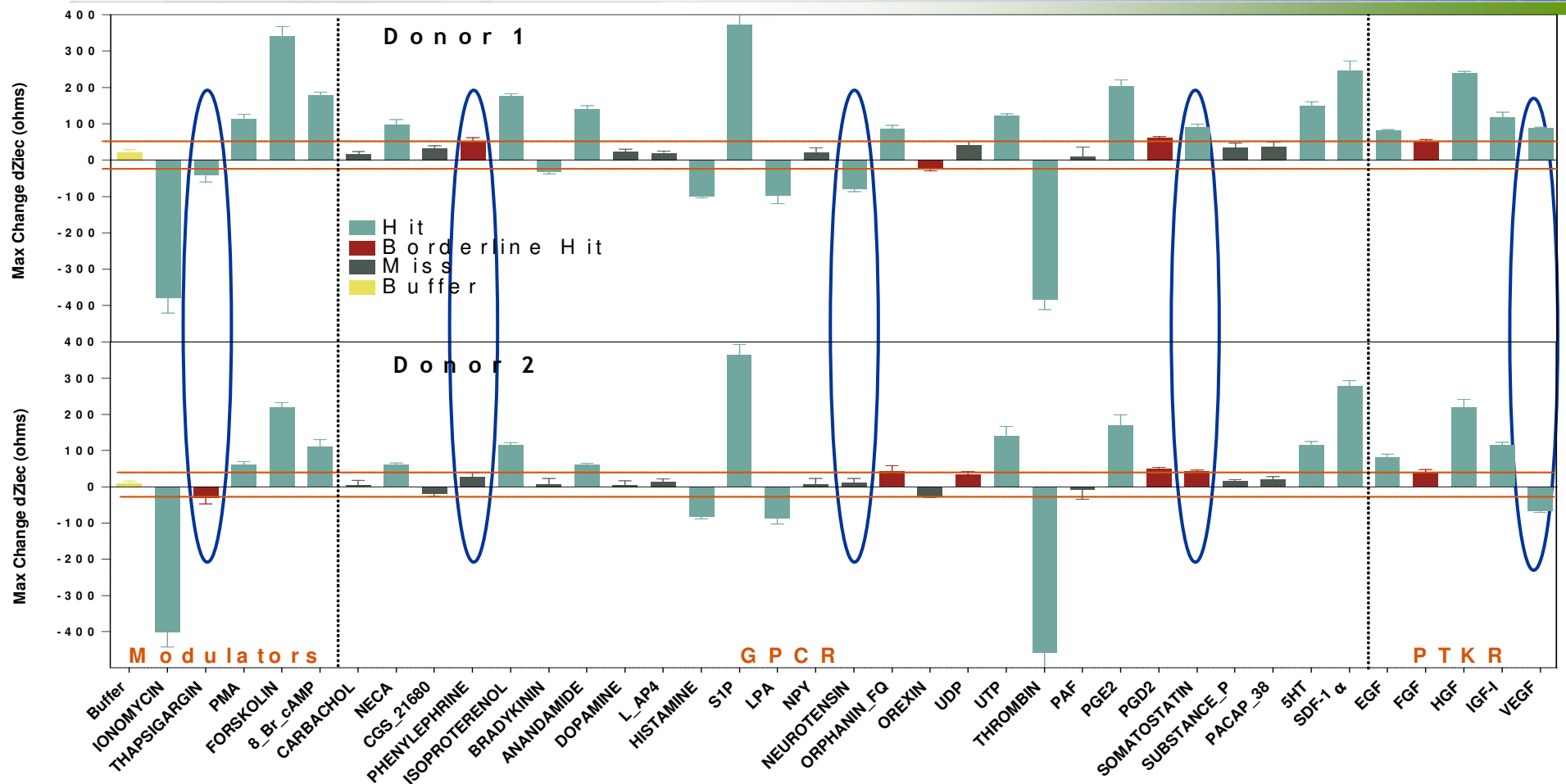
Human Skeletal Muscle Myoblasts: Summary

- Identified a number of interesting active endogenous receptors through receptor panning
- Endogenous Adenosine receptor pharmacology
 - CellKey response profile indicates G α i-coupled response
 - Pertussis toxin sensitivity validates pathway
 - Pharmacological subtyping possible
 - Possible inverse agonist to an endogenously-expressed G α i-coupled GPCR ID'd

Benefits of utilizing HUVEC

- Normal endothelial tissue is essential to numerous physiological processes
- Dysfunction of endothelial cells has serious consequences with regard to the onset of many diseases, including cancer and atherosclerosis, two major causes of death worldwide.
- HUVEC are commonly used for experimental applications in cardiovascular pharmaceutical development and vascular pathology,
 - COPD, such as emphysema
 - Angiogenesis, anti-angiogenesis (anti-VEGF tx's for example)
 - Basic physiology research
- Lonza's HUVEC are available
 - from multiple donors, pooled or single donor-derived
 - at early passages
 - 10 million/cryovial size will be available as standard product soon

Receptor Panning on HUVEC from 2 different donors

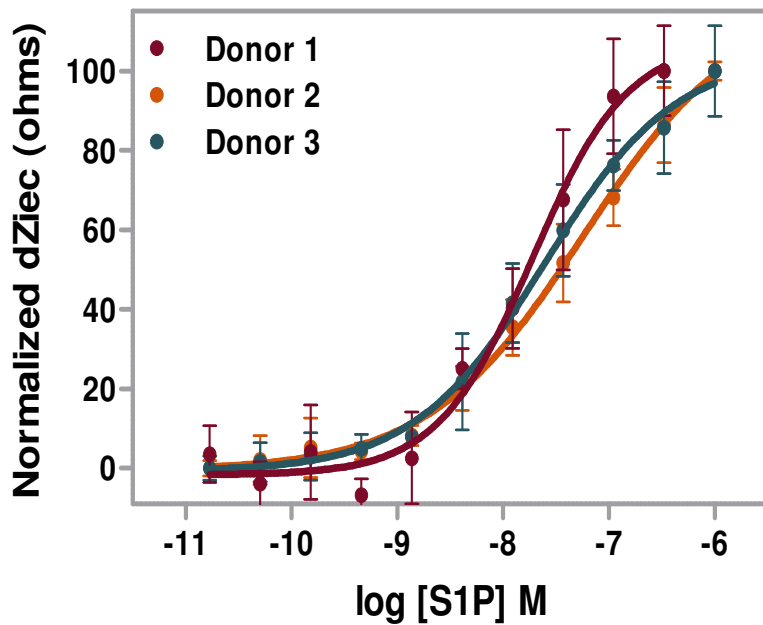


- Data suggests CellKey may be able to detect differences in receptor expression profiles based on donor populations. New way to use receptor panning.
- Functional assay method, as opposed to PCR. No requirement for lysis of cells, etc. Faster results turnaround, easier workflow.

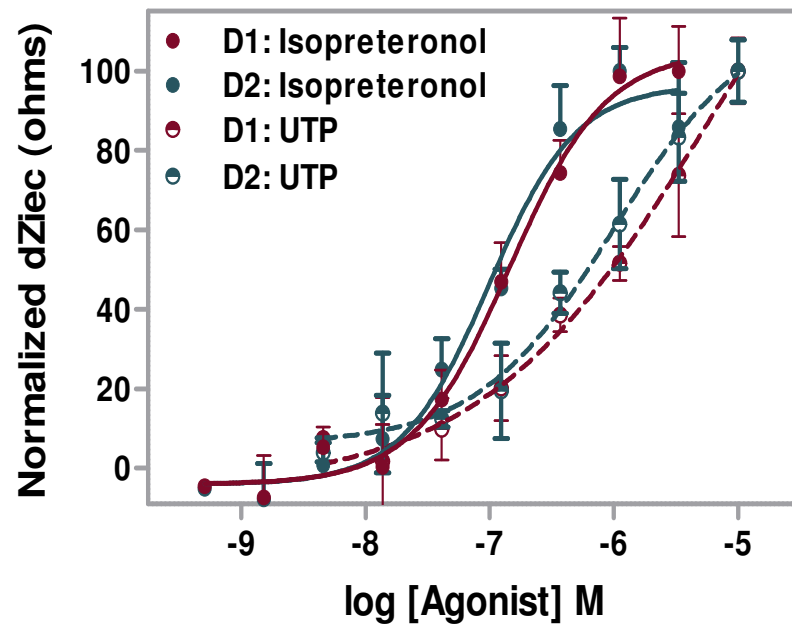
HUVEC Target validation: Agonist pharmacology

GPCR targets across donors

EDG receptor



Adrenergic and Purinergic receptors



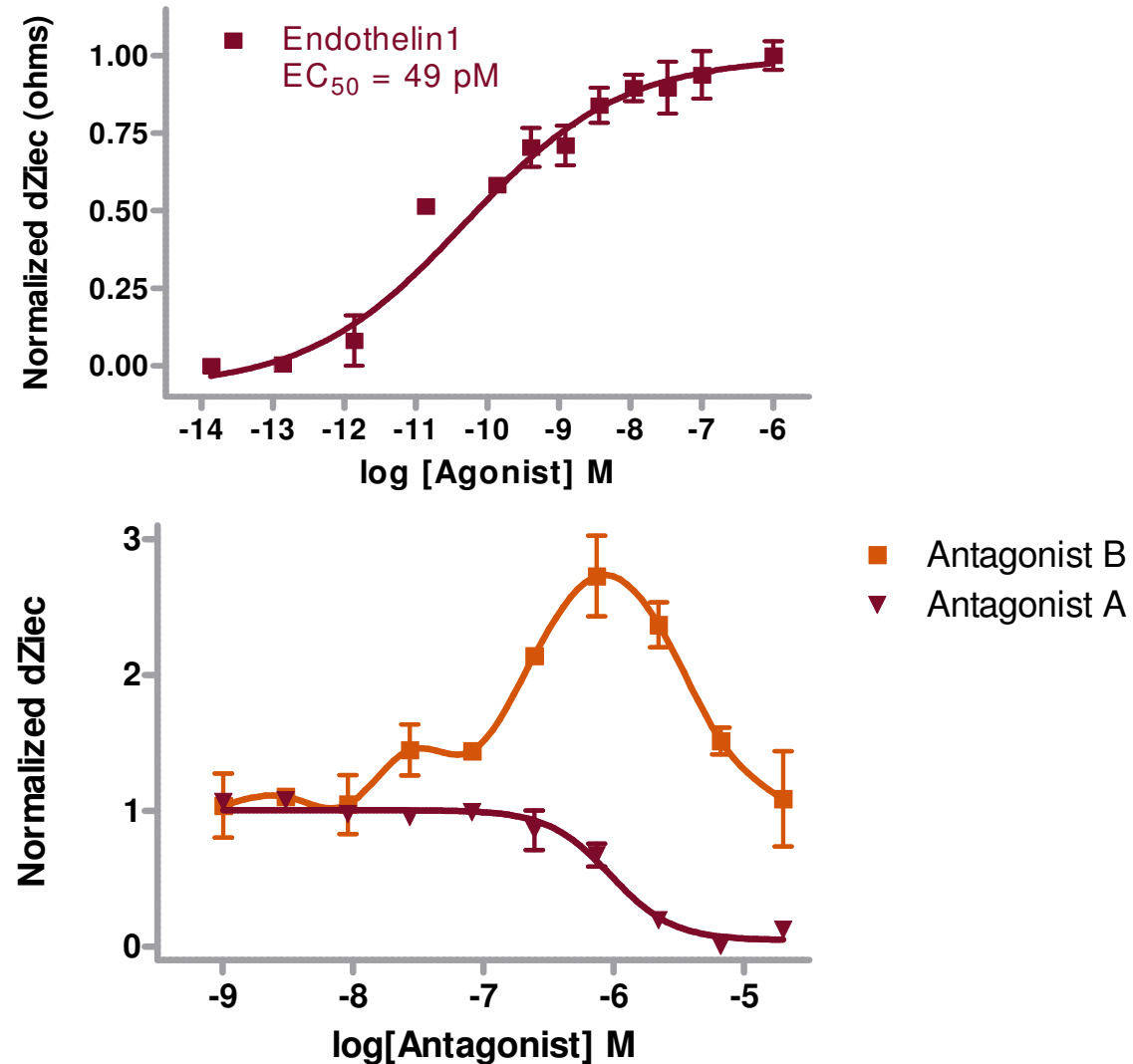
Experimental Conditions: Human Umbilical Vein Endothelial Cells

- Adapting Lonza's HUVEC for analysis on CellKey System was very simple
 - Cells cultured in optimized EGM-complete media
 - Seeded 40,000 cells/well in CellKey Standard 96W plates evenings prior to experimentation
 - No plate coating required
 - All experiments performed at 37°C on the CellKey System reader
 - Prior to each experiment, cells were washed into 135 µL assay buffer using onboard fluidics

HUVEC Summary

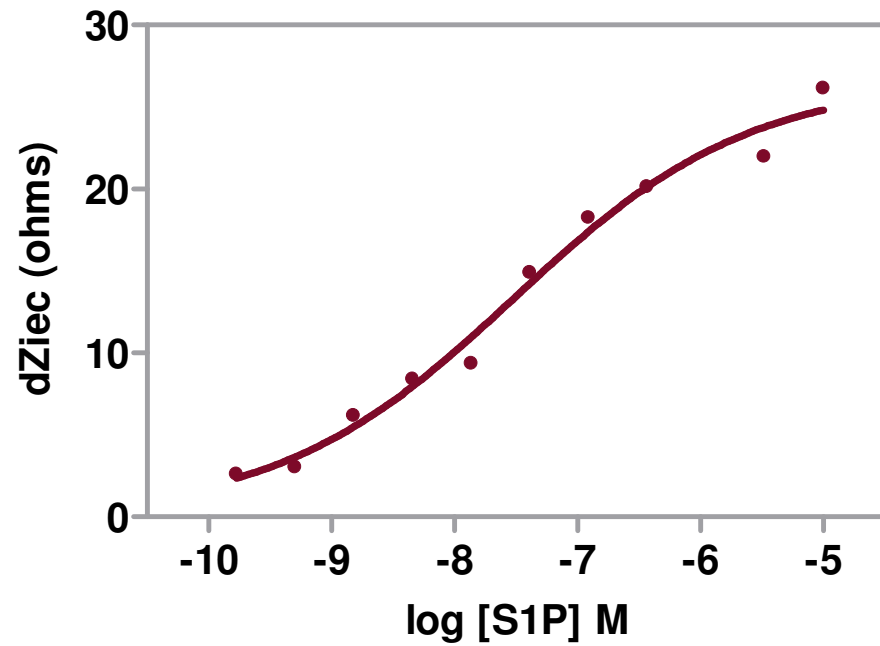
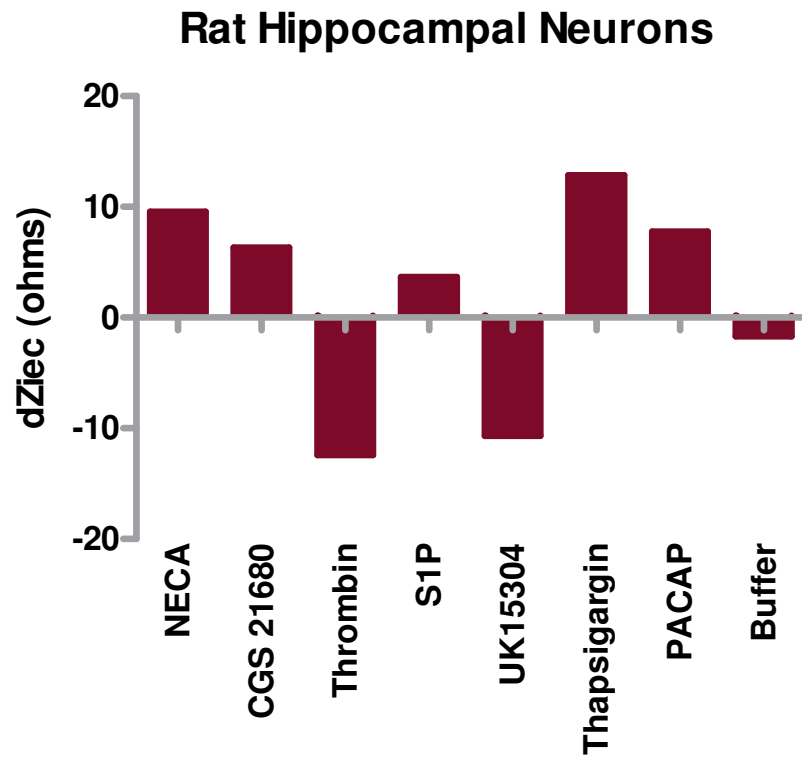
- Identified a number of interesting active endogenous receptors through receptor panning
- Analyses could be used to understand donor to donor variability
- Performed detailed pharmacology on Edg, adrenergic and purinergic receptors
- Lonza's HUVEC were easily adaptable to the CellKey System

Functional assay of ET-1 dimerization in BSMC (?)



Dimer Cooperativity Model - R. Franco et al. BJP. (2008) 153, 590-598

Preliminary Primary neuron data:



Future Work

- HUVEC cells on CellKey384 System
 - Primary cells in high density cell based assay
 - Screening of LOPAC library
- Optimization of neuronal cell types

CellKey™384 System

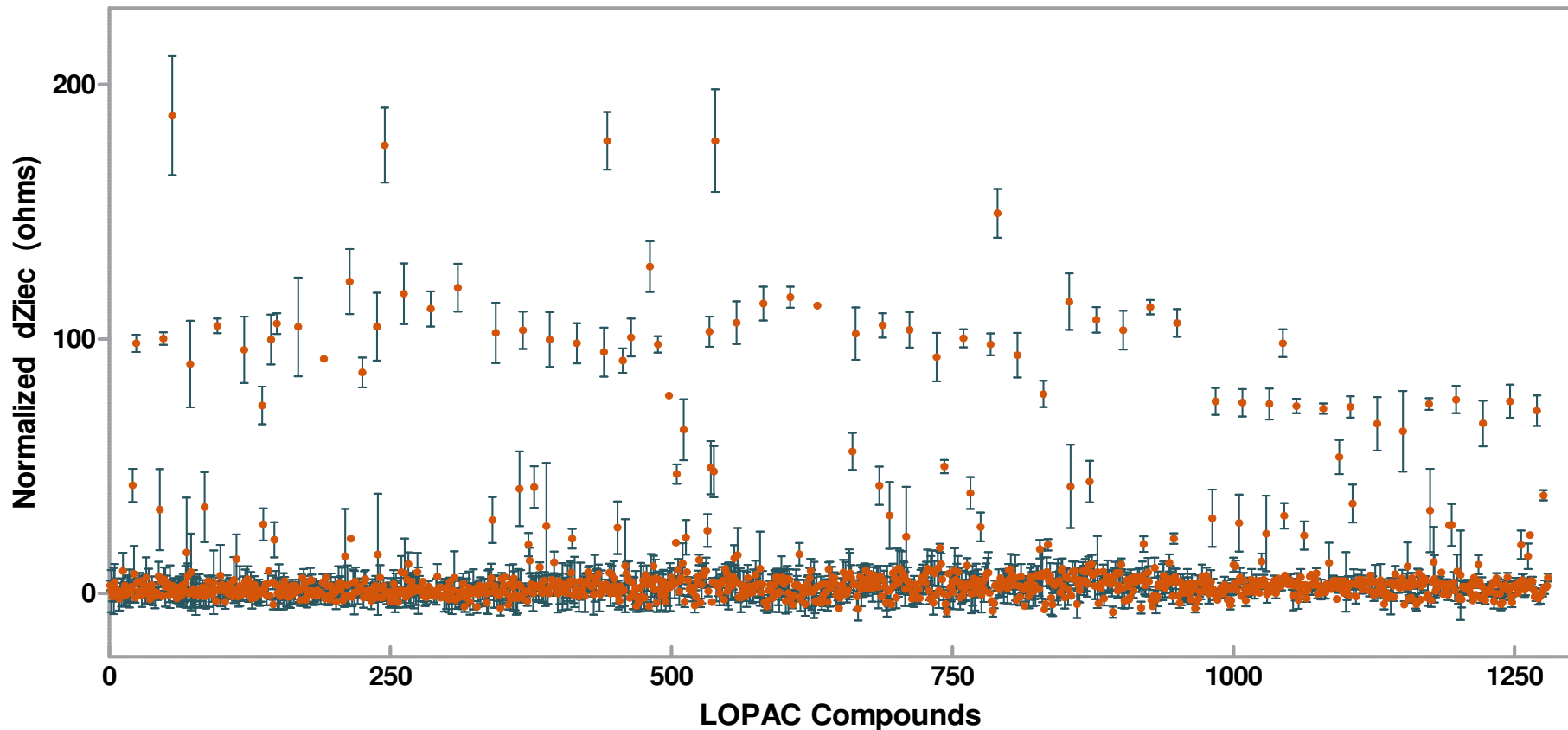


- Captures real time, cell-based kinetic measurements during simultaneous compound delivery to 384 wells
- Extends unattended throughput during automation with online tip washing
- Accelerates throughput for label-free cell-based kinetic screening, typically 24,600 wells in an 8 hour period.
- Reduces per well cost, due to high density format and reduced cell/well and reagent needs

Pilot screen of LOPAC library: Endogenous targets on CellKey384 System

- HeLa cells expressing endogenous targets
- Histamine H1 receptor used as positive control
- LOPAC1280 library of compounds at 1 μM and 10 μM
- Agonist and antagonist/modulator screens performed

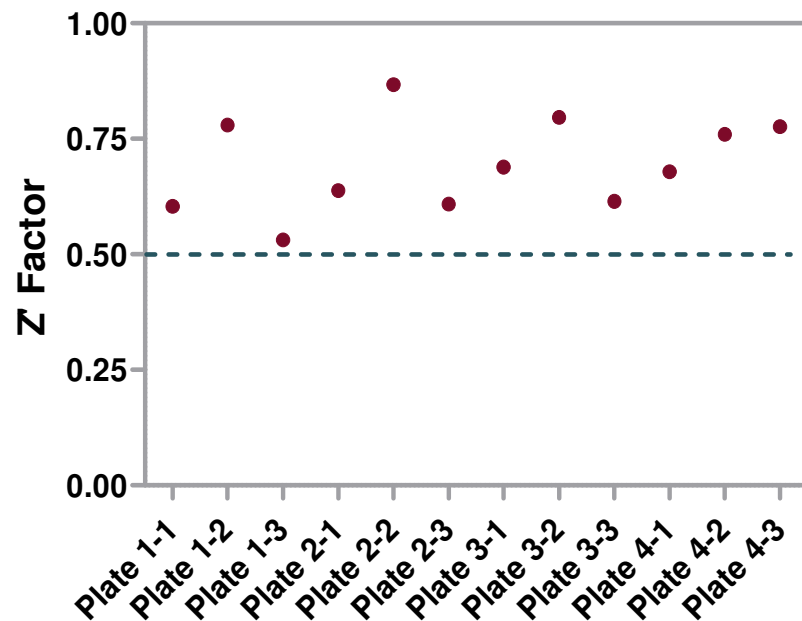
LOPAC1280 Agonist screen



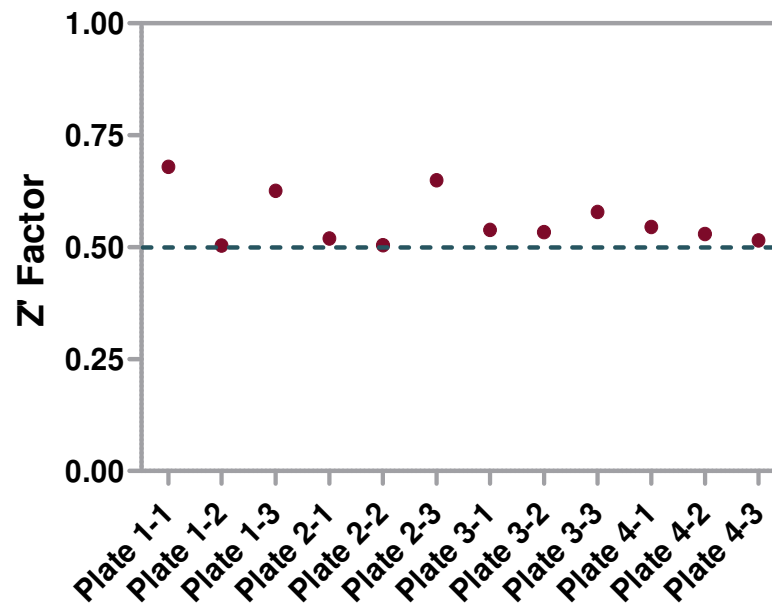
- Triplicate data: 1 well on each of 3 separate plates
- Active compounds on dopamine, histamine, adrenergic, purinergic, Edg and prostanoid receptors.

Assay statistics

- Agonist Z' Factors



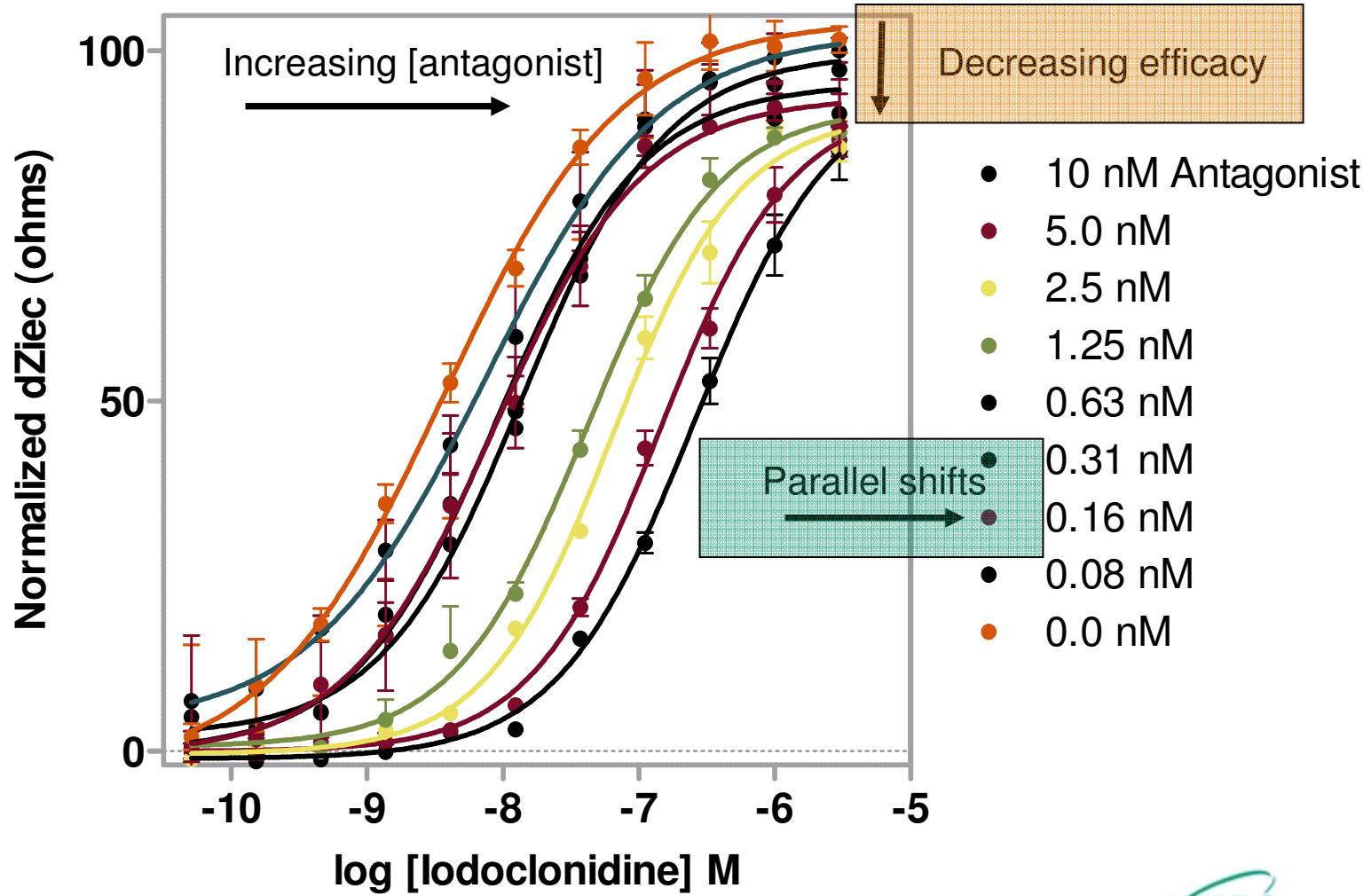
- Antagonist Z' Factors (EC80)



Compounds Screened	Average Z'	Average S:B	Hits (>50%)
Agonist Mode - 1280	0.70	12.9	67
Antagonist Mode - 1280	0.56 (EC ₈₀)	6.53 (EC ₈₀)	104

Higher order complexity - Dimerization

- Schild analysis: Endogenous α_2 Adrenergic receptor, HeLa cells



Summary

- Label-free cell based assays are powerful and flexible tools for understanding complex interactions between ligands/receptors/cellular machinery.
 - Integrated cellular responses are more informative when considering
 - Ligand biased signaling
 - Dimerization
 - Complex cellular responses
- Endogenous sensitivity opens doors to screening in primary cells

Acknowledgements

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 - Andy Keyes

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