

CORNING

Epic[®]
system

Physiologically Relevant Cell-Based Assays with the Corning[®] Epic[®] System

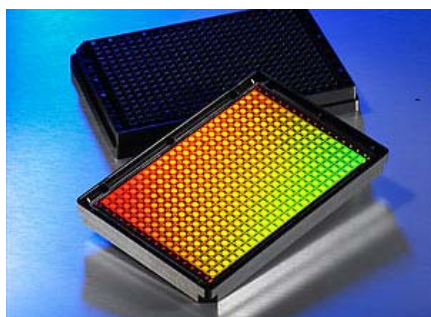
Guangshan Li and Ye Fang

Abstract

Corning® Epic® cell assay technology is a non-invasive and label-free cell assay, based on the measurements of ligand-induced dynamic mass redistribution (DMR) within the bottom portion of attached cells. This technology not only allows one to map out the signaling pathway and its network interaction(s) mediated through a particular target, but also is capable of assaying endogenous G protein-coupled receptors (GPCRs) in a high throughput manner. We first demonstrated receptor panning at both cell system- and receptor family-levels, then the performance of HT screening using endogenous bradykinin B₂ and β₂ adrenergic receptors in A431 cells, as well as over-expressed rat muscarinic M₁ receptors in Chinese hamster ovary (CHO) cells. Results suggest that Epic cell assays can be applied at many stages of the drug discovery and development processes for GPCRs.

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 and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



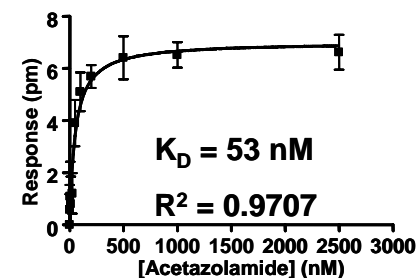
Microplate

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



Microplate Reader

- Compatible w/ HTS automation
- $\geq 40,000$ wells/8hrs
- Sensitivity of $5\text{pg}/\text{mm}^2$
(300Da drug to 75kDa target)

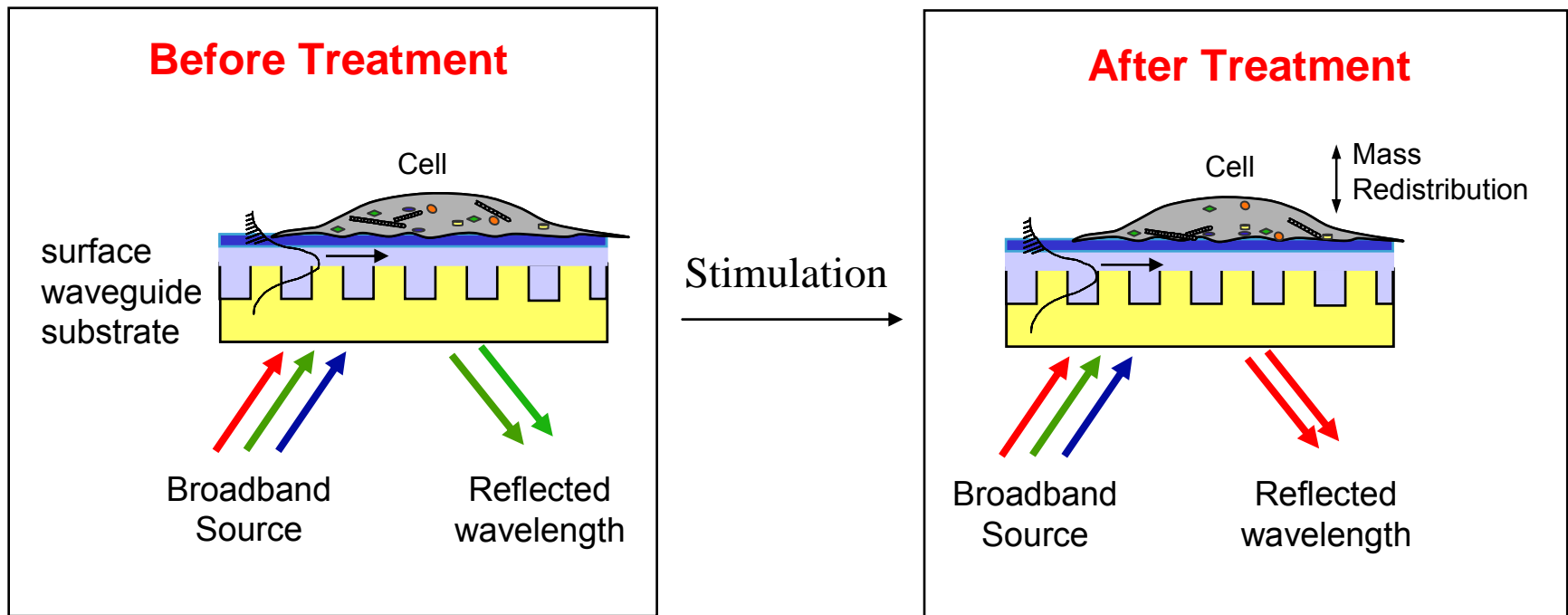


Binding Data

- Manipulated and analyzed by customer

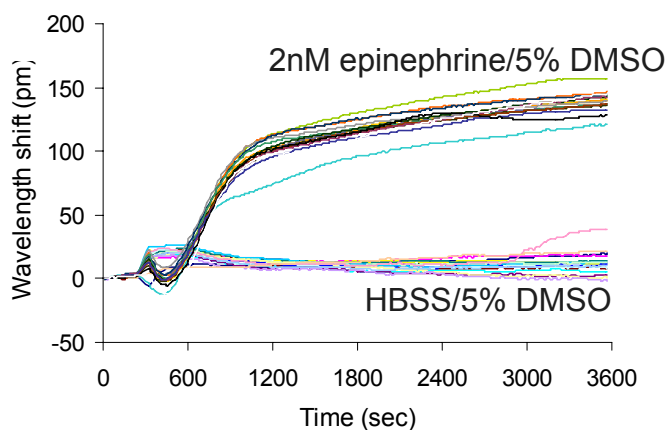
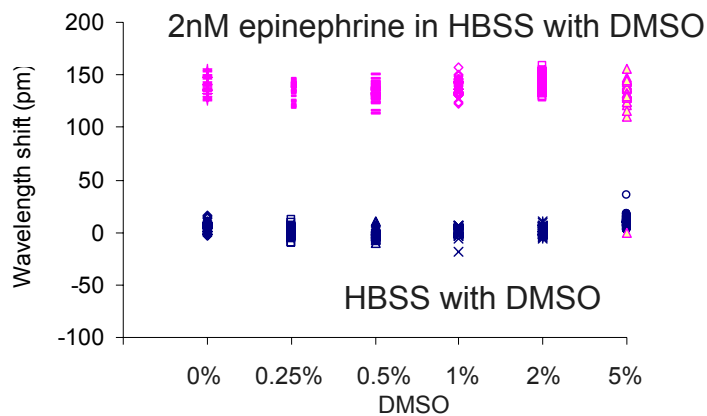
Mass Redistribution Cell Assay Technologies Using the Epic[®] System

- Measures changes in local refractive index, resulting from dynamic mass redistribution within a bottom portion (~200 nm) of the cells
- Change in index is manifested by a shift in resonant wavelength

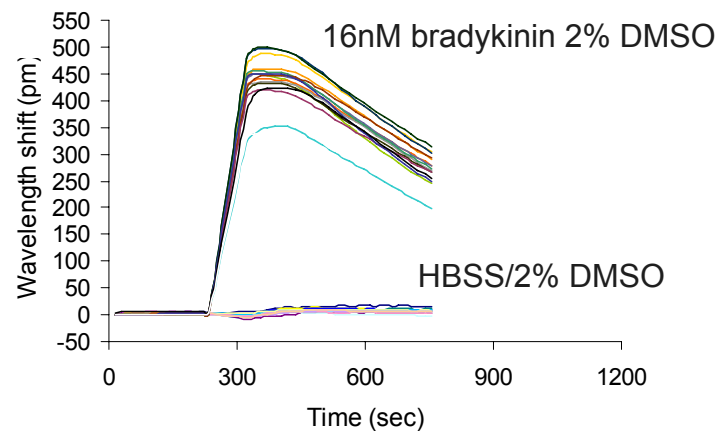
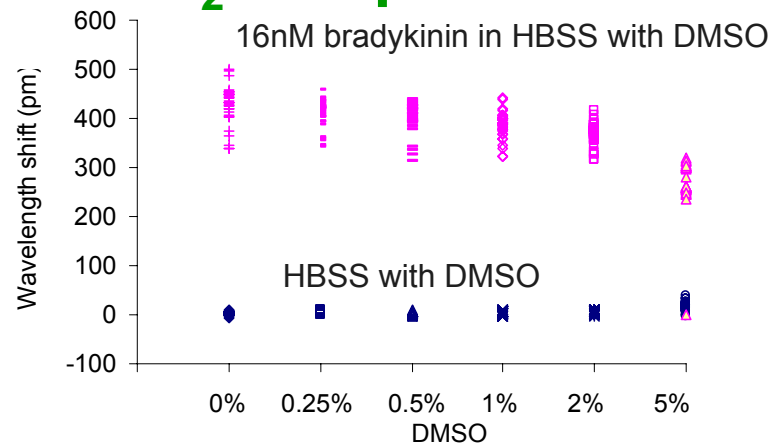


Epic[®] Cell Assays Are Tolerant to DMSO

β_2 AR in A431

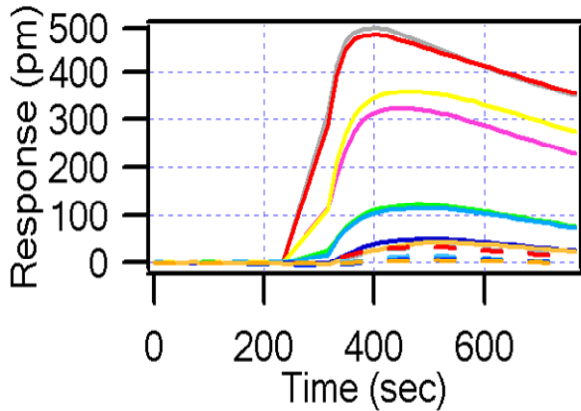


B₂ receptor in A431

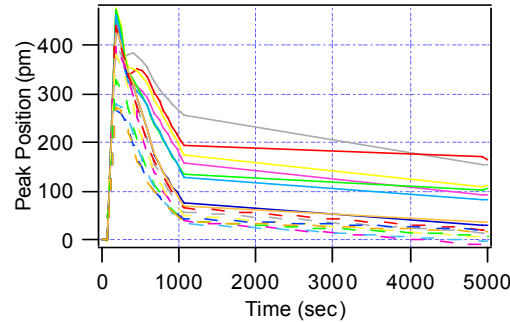


Panning Endogenous G_q-Coupled Receptors in A431

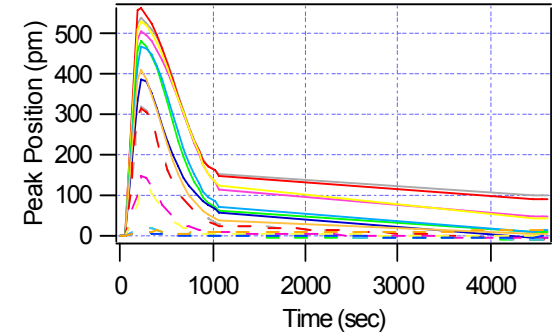
B2 Receptor



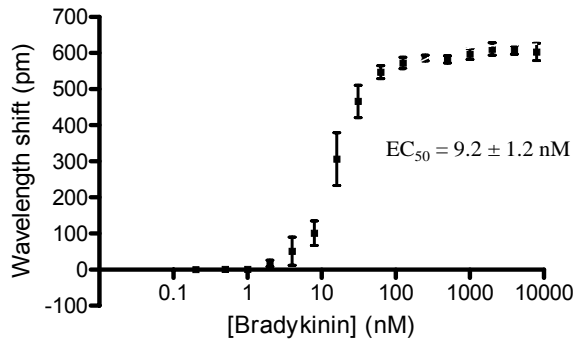
P2Y receptor



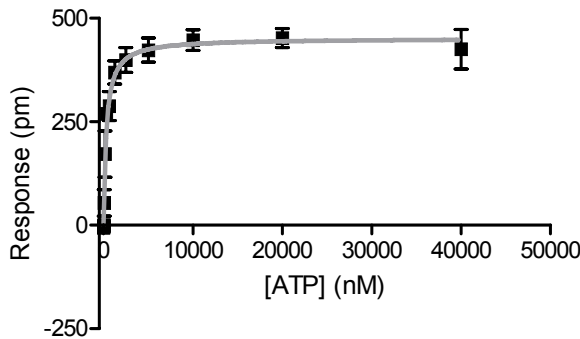
PAR1 receptor



Bradykinin Dose in A431

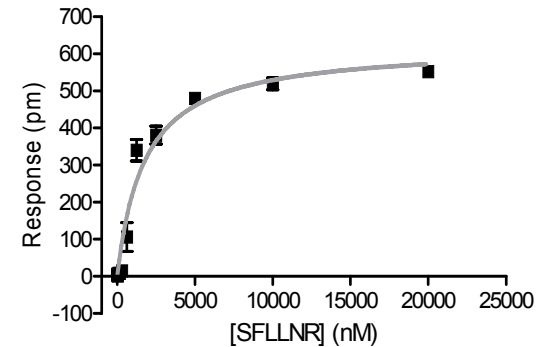


ATP Doses in A431



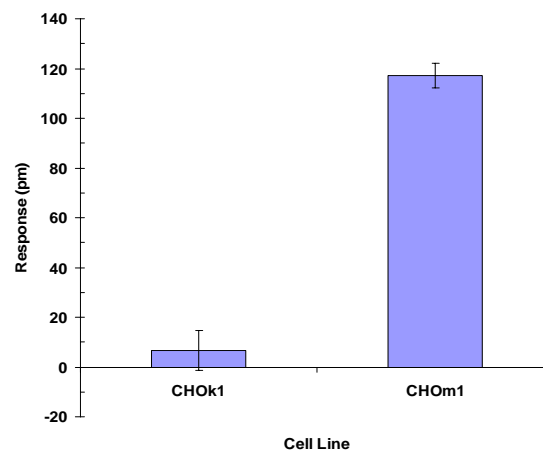
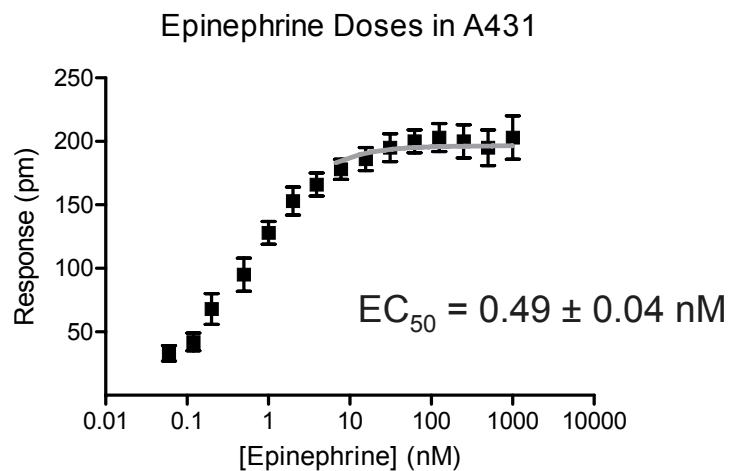
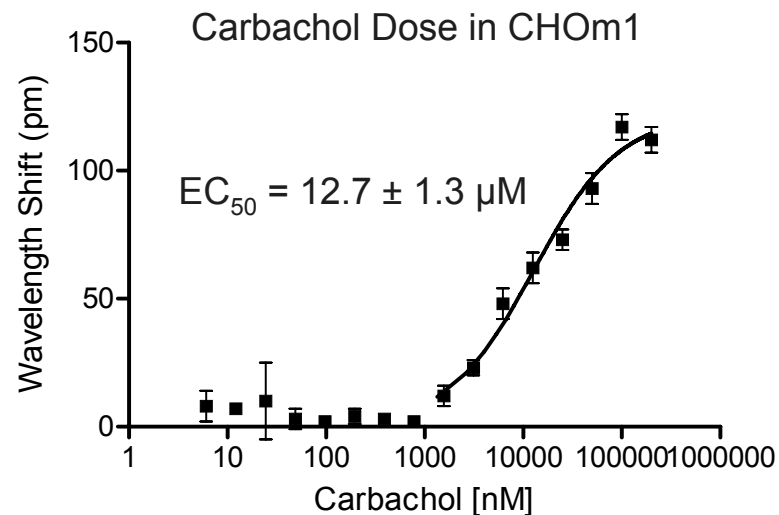
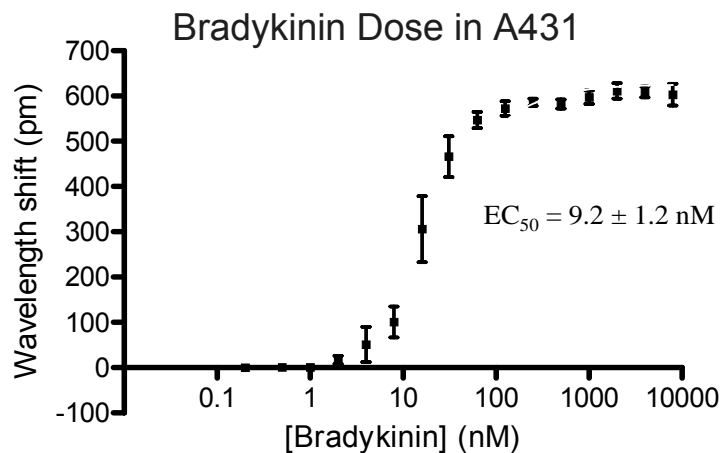
BMAX | 451.2
KD | 310.0

SFLLNR doses in A431

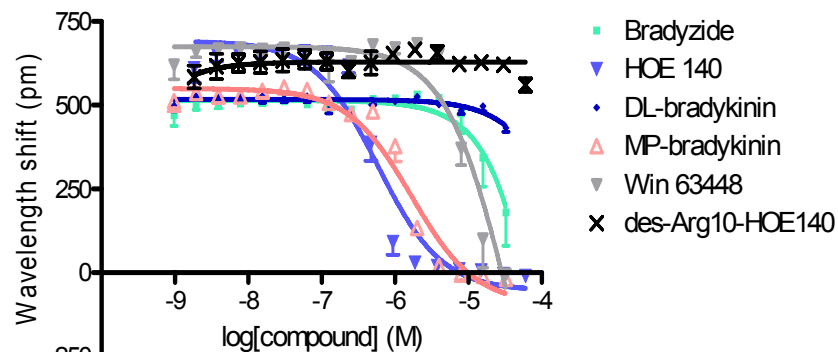
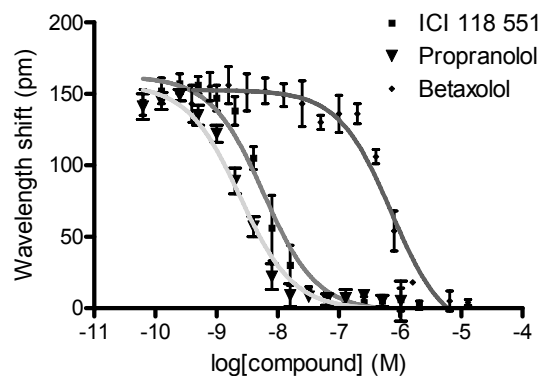


BMAX | 623.2
KD | 1774

GPCR Agonist Efficacy and Specificity



GPCR Antagonist Potency Ranking Order



Best-fit values	ICI 118 551	Propranolol	Betaxolol
BOTTOM	-1.836	-0.8441	-18.25
TOP	162.3	156.7	152.7
Log IC50	-8.230	-8.615	-6.145
IC50	5.8843e-009	2.4277e-009	7.1538e-007

	Bradyzide	HOE 140	DL-bradykinin	MP-bradykinin	Win 63448	des-Arg10-HOE140
BOTTOM	-40170	-53.36	Inactive	-93.66	-867.3	Inactive
TOP	514.2	691.0	Inactive	549.6	674.6	Inactive
Log IC50	-2.388	-6.234		-5.774	-4.435	
IC50	0.004094	5.8408e-007		1.6830e-006	3.6747e-005	

Potency ranking order

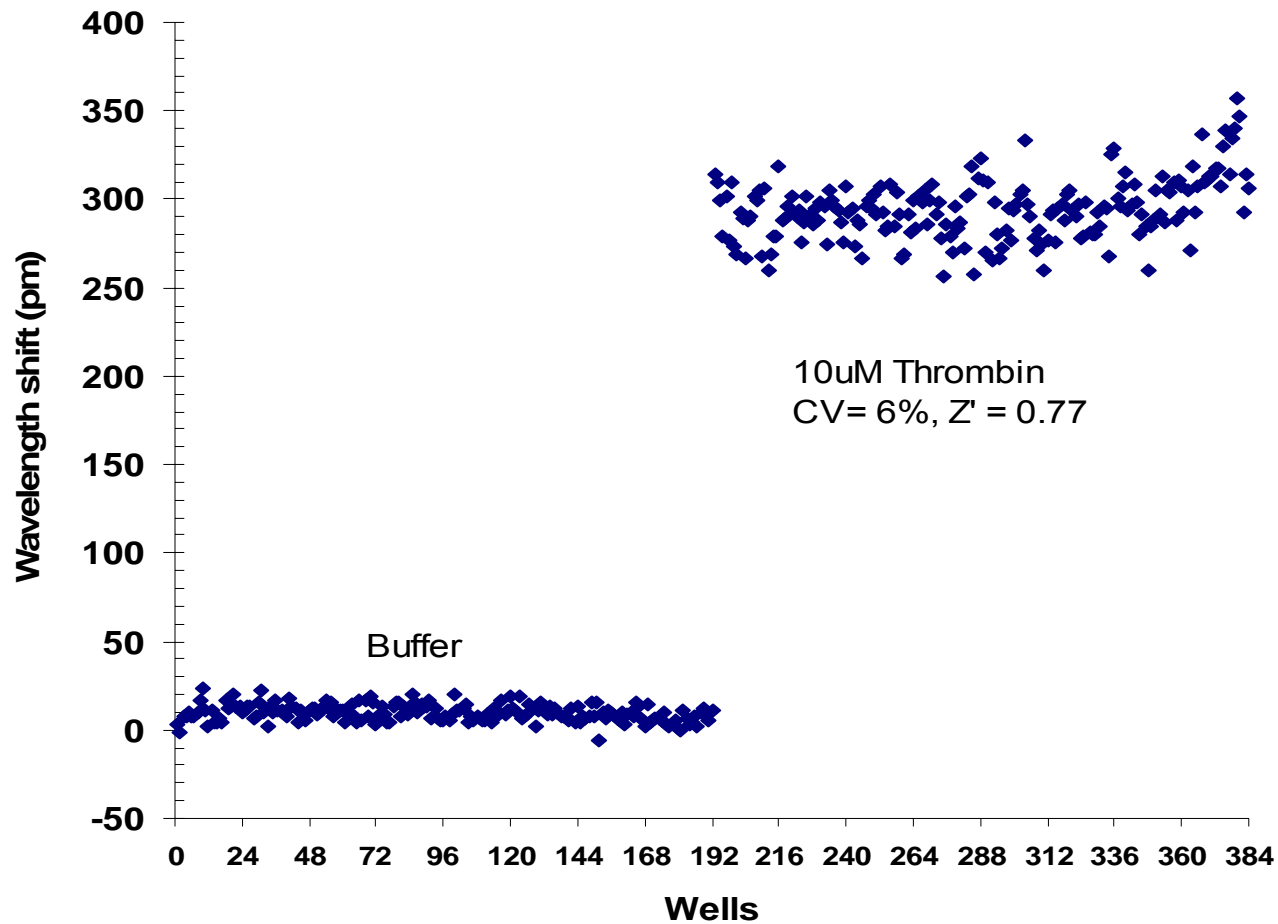
Epic®

β₂AR ICI 118551 ≈ Propranolol > Betaxolol
B₂ receptor HOE140 > MP-bradykinin > Win63448 > Bradyzide;
 DL-bradykinin and des-Arg10-bradykinin (inactive)

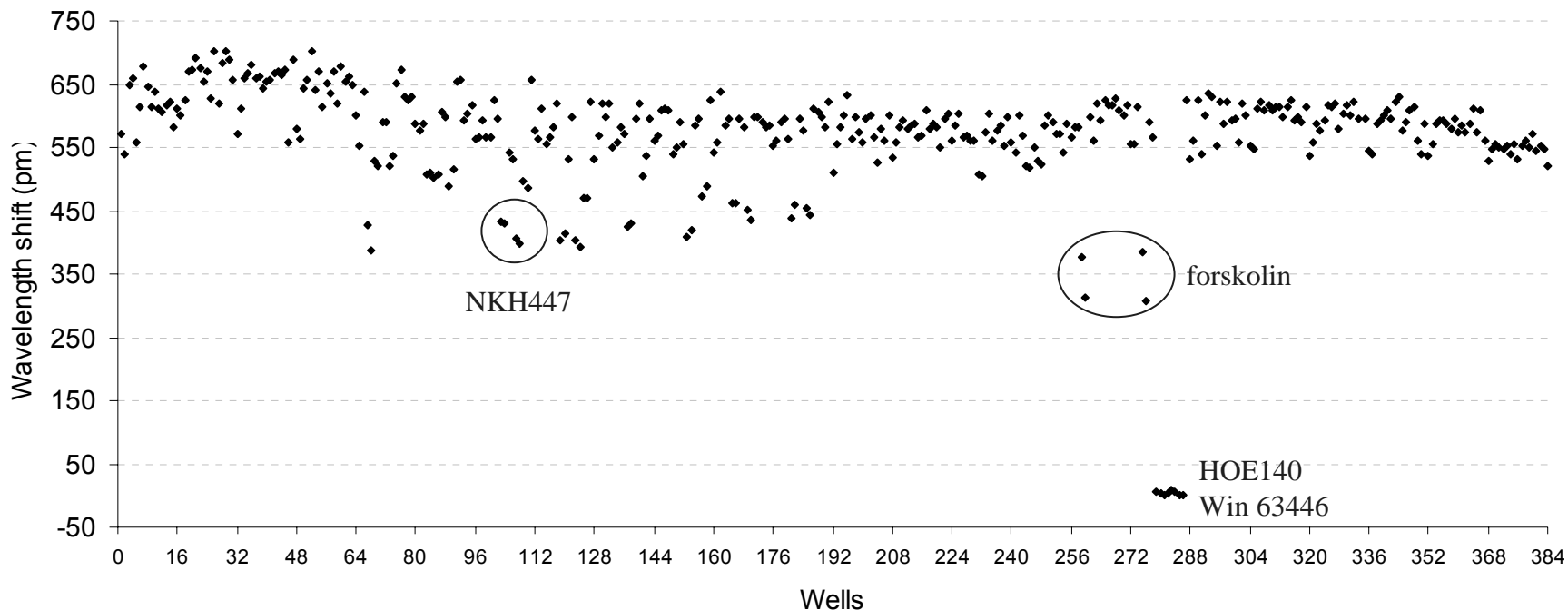
Literatures

ICI 118551 ≈ Propranolol > Betaxolol
 HOE140 ~ Win 63488 > MP-bradykinin ~Bradyzide >> Win64338;
 DL-bradykinin and des-Arg10-bradykinin (inactive)

HTS Profiling of Ligands for Endogenous Thrombin Receptors in CHOm1



HTS Profiling of Ligands for Bradykinin B₂ Receptors in A431



- Screening using a small compound library (30 GPCR signaling pathway modulators, 6 other GPCR ligands, and 2 B₂-specific antagonists) against 16nM bradykinin
- Z' factor ~ 0.65
- The modulation profiles suggest that the B₂ signaling in fully quiescent A431 is mediated through G_q (primary) and G_i (secondary)

Conclusions

- The Epic[®] System is an HTS platform that can be utilized for cell-based drug discovery and development.
- The Epic System provides novel and unique signatures for all G_q-, G_s-, and G_i-coupled receptors.
- Key Features of the Epic System :
 - Label-free
 - Standard SBS 384-well microplate format
 - Endogenous GPCRs
 - Real physiological conditions
 - Multiplexing
 - High information content:
 - Kinetics, efficacy/potency, signaling pathways, network interactions, signaling regulation, oligomerization, cross-communications ...