Label-Free Cell-Based Screening of an Endogenous GPCR Using the Corning® Epic® System

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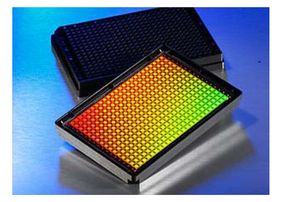
Abstract

Heterotrimeric GTP-binding protein (G protein)-coupled receptors (GPCRs) constitute the largest class of drug targets currently under therapeuticinvestigation. Receptor stimulation by cognate ligands results in rapid translocation of intracellular components involved in signal propagation and cytoskeletal reorganization. Monitoring of the resultant dynamic mass redistribution (DMR) in living cells following receptor activation is accomplished using the Corning® Epic® System – a label-free and non-invasive system, incorporating resonant waveguide grating biosensors into a 384-well microplate. The human epidermoid carcinoma cell line, A431, was used as a model system for screening the response of an endogenously expressed GPCR. A431 cells express high levels of the beta2-adrenoceptor, which is a prototypical member of the Gαs-coupled class of GPCRs. A431 cells were screened for known GPCR agonists and antagonists using the commercially available LOPAC1280 compound library. The Epic System was able to successfully identify all of the beta2-adrenoceptor agonists (7) and antagonists (1) present in the library. Further, assay robustness on the Epic System was demonstrated by reproducible identification of hits when the results from several screens were compared. Several of the compounds identified as agonist hits had no known specificity for adrenergic receptors. Additional analysis was conducted with these compounds to address the specificity of their response. This study demonstrates the utility of the Epic System for cell-based high-throughput screening.

Corning® Epic® System Concept

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.



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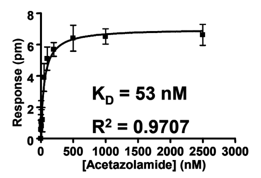


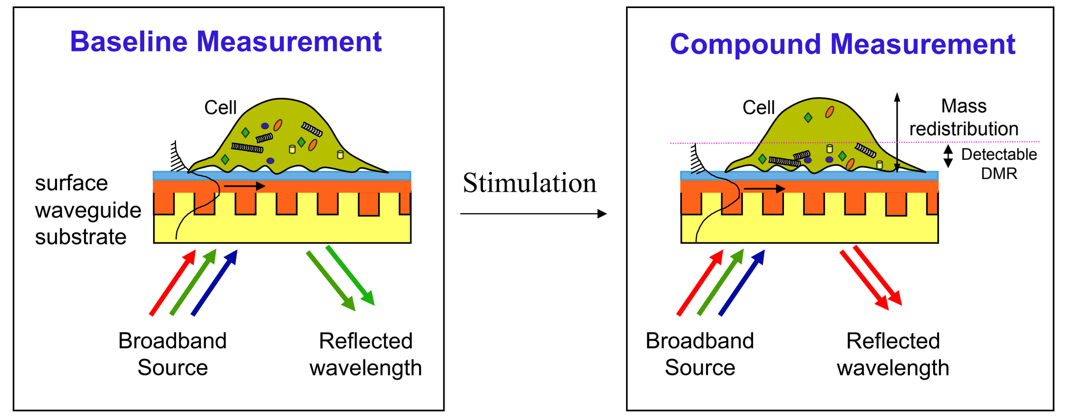
Microplate
- 384-well format
- Optical biosensor in each well
- Surface chemistry

Plate Reader
- Compatible w/ HTS automation
- ≥ 40,000 wells/8hrs
- Sensitivity of 5pg/mm² (300Da drug to 75kDa target)

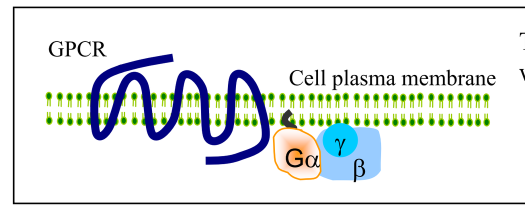
Binding Data
- Manipulated and analyzed by customer

Principle of Detection: Cell-based Assays on the Epic® System

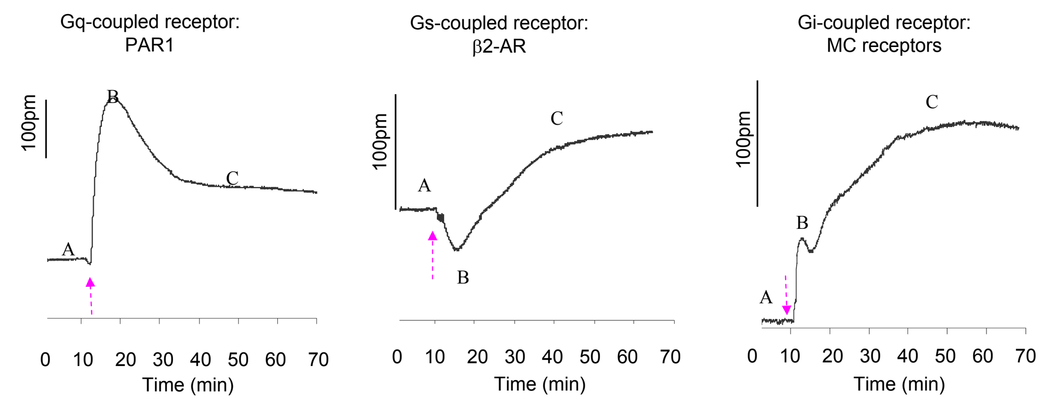
- Measures changes in local index of refraction resulting from the ligand-induced dynamic mass redistribution (DMR) within the bottom region (~150nm) of the cell monolayer.
- Change in index is manifested by a shift in resonant wavelength



Cell Assay Technologies with Epic® System Are Applicable to All Three Classes of GPCRs



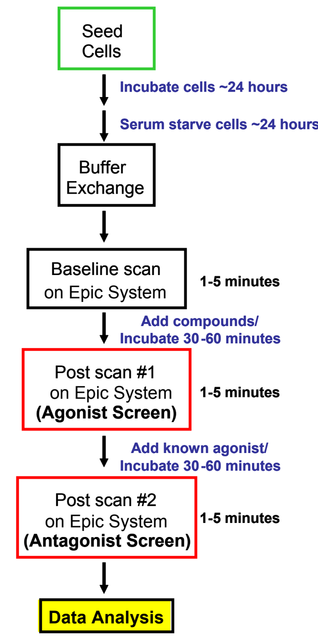
Three classes of GPCRs, depending the G protein with which the receptor is coupled:
- Gαq – release of Ca2+
- Gαs – accumulation of cAMP
- Gαi – inhibiting cAMP generation



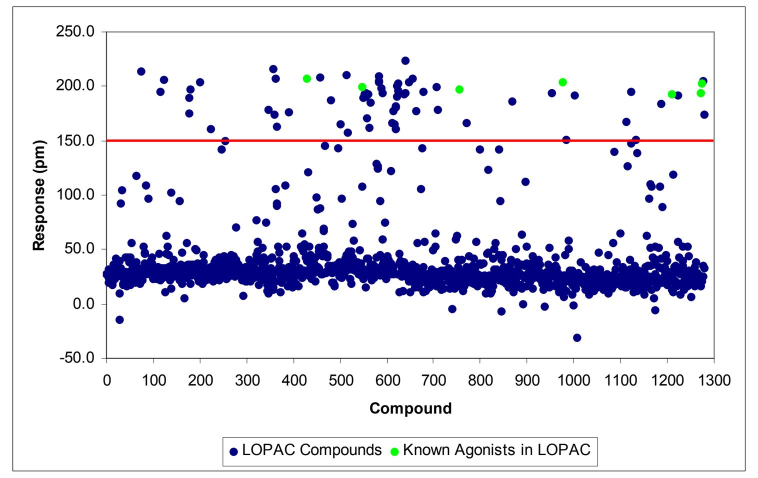
Cell-based Screen Assay Overview

Cell Culture in Epic® Microplates:
1. Seed A431 cells (10,000 cells/well) and incubate overnight.
2. Serum starve for an additional 18-20 hours.
3. Incubate cells in assay buffer (HBSS/20mM HEPES/0.6% DMSO) for 2 hours.

Epic System Procedure:
1. Take a baseline scan (~5 min).
2. Add LOPAC 1280™ compounds to the Epic microplate. Assay concentration of each compound is 1μM/0.6% DMSO.
3. Incubate Epic microplate for 1 hour (Agonist screen).
4. Take a final scan (~5 min).
5. Add 10μL of metaproterenol. Assay concentration is 50nM/0.6% DMSO.
6. Incubate Epic microplate for 1 hour (Antagonist screen).
7. Take final scan (~5 min).

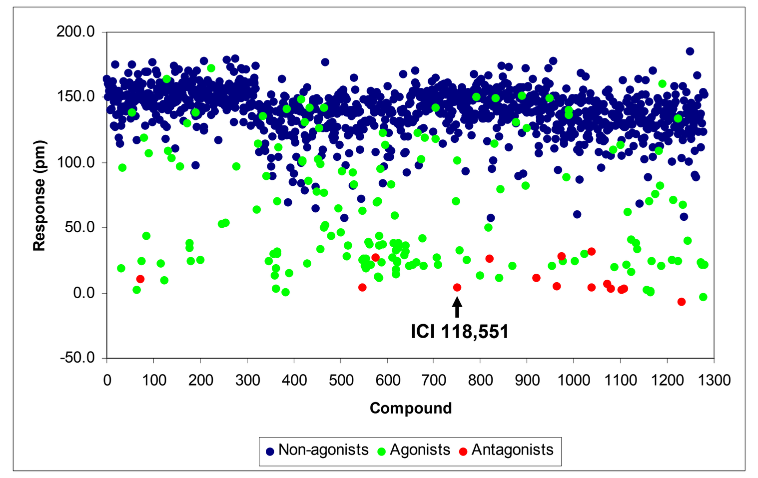


Agonist Screen on the Epic® System



- Threshold for hits was set at 150pm.
- 72 hits were identified, representing 5.6% of the LOPAC library.
- Green data points represent compounds in the LOPAC library that are known β_2AR agonists.
- All 7 known β_2AR agonists in the LOPAC library were correctly identified.

Antagonist Screen on the Epic® System



- Antagonist - Blocks metaproterenol response in antagonist screen (<50pm response), but non-responder in agonist screen (< 50pm response).
- Agonist - Blocks metaproterenol response in antagonist screen due to receptor desensitization; these compounds elicited a response > 50pm in the agonist screen.
- Non-agonist – Compounds with a response < 50pm in the agonist screen. Most of these compounds do not inhibit the response of metaproterenol in the antagonist screen.

Screening Controls

EC$_{50}$ Analysis

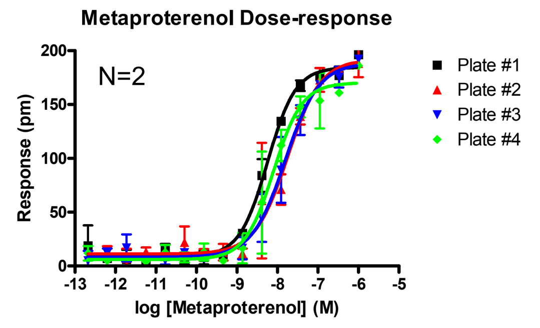


	Plate 1	Plate 2	Plate 3	Plate 4
EC$_{50}$	5.7 nM	17.9 nM	15.7 nM	8.0 nM

Z' Analysis

Agonist Screen
Positive control:
- 1μM Metaproterenol (N=16)

Negative control:
- Buffer (N=16)

Antagonist Screen
Positive control:
- 50nM Metaproterenol (N=8)

Negative control:
- Buffer (N=8)

Z'

	Plate 1	Plate 2	Plate 3	Plate 4
Agonist Screen	0.84	0.85	0.81	0.76
Antagonist Screen	0.76	0.77	0.80	0.76

- Good assay robustness was observed based on Z' and EC$_{50}$ analysis.

Additional Analysis of Non-adrenergic Agonist Hits

- Several of the compounds that were identified as hits in the agonist screen have no known specificity for adrenergic receptors.
- Additional experiments were performed with a subset of these non-adrenergic compounds.
  - The compounds were added to A431 cells in a dose-dependent manner at concentrations up to 10μM.
  - Metaproterenol was then added to the cells to determine if the compounds inhibited the response of a β_2AR specific agonist.
- The following compounds were tested:
  - 6-Chloro-PB hydrobromide
    - D1 dopamine receptor agonist
  - 5'-(N-cyclopropyl) carboxamidoadenosine
    - Potent A2 adenosine receptor agonist
  - 5'-(N-ethylcarboxamide) adenosine
    - Potent A1 and A2 adenosine receptor agonist

Additional Analysis of Non-adrenergic Agonist Hits

- Each compound exhibited dose-dependent response when added to A431 cells (Left graph).
- These compounds also inhibited the response of metaproterenol in a dose-dependent manner (Right graph).
- These compounds are agonists for dopamine and adenosine receptors, which are known to be expressed by A431 cells.
- Additional experiments are being planned to further evaluate several other "non-adrenergic" compounds that were identified as hits in the agonist screen.

Agonist Hit Summary

LOPAC Agonist Hit Summary		
Class	**Hits**	**% of Hits**
Adrenoceptor	27	38.6%
Adenosine	7	10.0%
Dopamine	7	10.0%
Histamine	2	2.9%
Glutamate	2	2.9%
Phosphorylation	8	11.4%
Nitric Oxide	3	4.3%
Cyclic Nucleotides	2	2.9%
Transcription	2	2.9%
Other	12	17.1%

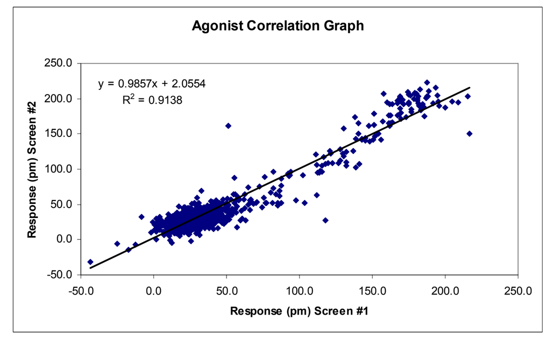
- 72 hits were identified.
- 7 known β_2AR agonists were correctly identified.
  - No false negatives
- 27 compounds are known to target adrenergic receptors.
- Compounds were also identified that target other receptors known to be expressed by A431 cells:
  - Adenosine, Dopamine, Histamine, and Glutamate

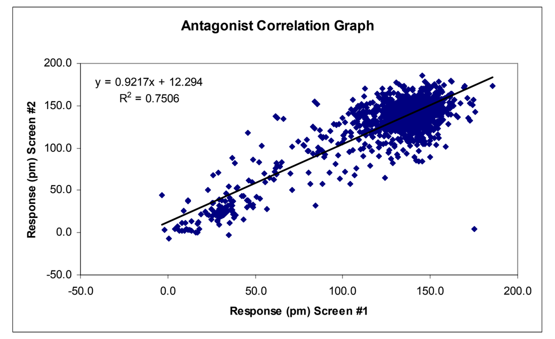
Antagonist Hit Summary

Name	Class	Enzyme	Action	Selectivity	Description
Betaxolol hydrochloride	Adrenoceptor		**Antagonist**	beta1	Selective beta1 adrenoceptor antagonist
(±)-Propranolol hydrochloride	Adrenoceptor		**Antagonist**	beta	beta Adrenoceptor antagonist; cardiac depressant (anti-arrhythmic)
ICI 118,551 hydrochloride	Adrenoceptor		**Antagonist**	beta2	Highly selective beta2 adrenoceptor antagonist
S(-)-Timolol maleate	Adrenoceptor		**Antagonist**	beta	beta Adrenoceptor antagonist; antihypertensive; antiarrhythmic; antiglaucoma agent
(±)-Sotalol hydrochloride	Adrenoceptor		**Antagonist**	beta	Potent beta-adrenoceptor antagonist, a class III antiarrythmic; prolongs the action potential and increases the refractory period
SR 59230A oxalate	Adrenoceptor		**Antagonist**	beta3	beta3-Adrenoceptor antagonist
(S)-Propranolol hydrochloride	Adrenoceptor		Blocker	beta	Active beta-adrenoceptor receptor blocking enantiomer; 5-HT1 serotonin receptor antagonist
(S)-(-)-propafenone hydrochloride	Adrenoceptor		Blocker	beta	beta-Adrenoceptor blocker; class 1C antiarrythmic agent
Prochlorperazine dimaleate	Dopamine		Antagonist		Antipsychotic agent; used in the treatment of spastic gastrointestinal disorders
R(+)-Terguride	Dopamine		Agonist		Ergot derivative; dehydrogenated analog of Lisuride; partial dopamine receptor agonist.
1-(1-Naphthyl)piperazine hydrochloride	Serotonin		Antagonist	5-HT2	5-HT2 serotonin receptor antagonist
(R)-(-)-DOI hydrochloride	Serotonin		Agonist	5-HT2	Selective 5-HT2 serotonin receptor agonist
Dequalinium dichloride	K+ Channel		Blocker		Selective blocker of apamin-sensitive K+ channels
Propafenone hydrochloride	K+ Channel		Blocker	hKv1.5	Blocks hKv1.5 and ATP-sensitive K+ channels; **beta-adrenoceptor antagonist**
Ammonium pyrrolidinedithiocarbamate	Nitric Oxide	Enzyme	Modulator	NOS	Prevents induction of nitric oxide synthase (NOS) by inhibiting translation of NOS mRNA

- 15 compounds were identified as antagonists (1.2% of library)
- 8 compounds targeted adrenergic receptors.
  - The one known β_2AR antagonist was correctly identified.
    - ICI 118,551
- Compounds that target other receptors (dopamine and serotonin) were also identified as antagonists.
- 3 additional compounds were identified as antagonists.
  - Additional experiments will be performed on a subset of these compounds to further evaluate their properties.

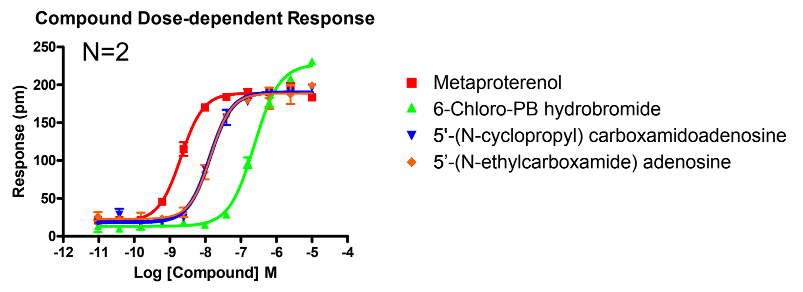
Correlation Graphs

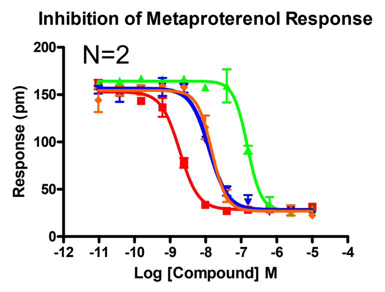




- The agonist and antagonist screens were run in duplicate.
- There was good correlation of hits in both screens.

Additional Analysis of Non-adrenergic Agonist Hits





- Left graph: A dilution series of each compound was added to A431 cells and the cells were allowed to incubate for 1 hour. Highest concentration tested: 10μM.
- Right graph: Following the 1 hour incubation in each compound, a specific β_2AR agonist, metaproterenol, was added to the A431 cells. Final assay concentration of metaproterenol: 50nM.

Summary

- The Epic® System was used to screen the LOPAC library for agonists and antagonists of the endogenously expressed β_2AR in A431 cells.
- The Epic System correctly identified the 7 known β_2AR agonists and 1 β_2AR antagonist in the LOPAC library.
- Good assay robustness was observed based on Z' and EC$_{50}$ analysis.
- There was good correlation of the agonist and antagonist hits when the screens were repeated.
- Additional experiments were performed with a subset of the "non-adrenergic" compounds previously identified as agonists.
  - These compounds were confirmed to elicit a dose-dependent response in A431 cells.
    - These compounds also inhibited the response of a known β_2AR agonist (metaproterenol).
- Additional experiments are being planned to further evaluate the "non-adrenergic" compounds identified as either agonists or antagonists.

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