

## Pharmacological Profiling of Endogenous G Protein-Coupled Receptors on the Label-Free BIND<sup>®</sup> System

### Abstract

GPCR subtype (Gi, Gq, Gs, G12/13 coupled) activation is measured using a number of assay platforms based on various cellular responses most commonly calcium mobilization,  $\beta$ -arrestin localization or second messenger levels. GPCR activation is further complicated by the receptors' use of multiple G protein signaling. SRU Biosystems' label-free BIND technology allows for cell-based detection of all GPCR subtype activation using a single assay. Additionally, BIND technology is compatible with adherent and non-adherent cells and is sensitive enough for use with both over-expressed and endogenous GPCRs. This application note details BIND assays demonstrating agonist and antagonist compound activity on endogenously expressed Gi, Gq and Gs coupled receptors.

### Introduction

G protein-coupled receptors (GPCRs) are one of the most important target classes in drug discovery. There are a variety of assay technologies that are currently utilized in drug discovery to identify lead molecules in high throughput screening. Technologies such as FLIPR require the development of recombinant cell lines that over express both the target of interest and an artificial G protein to drive coupling of calcium mobilization. These artificial assay systems yield false positives, often identify leads that do not perform in the *in vivo* disease model and are incompatible with certain GPCR targets such as chemokine receptors. The current trend in pharmaceutical research to utilize more physiologically relevant assays earlier in the discovery process, has driven the need for a high throughput technology that enables use of endogenous expression of target receptors in human primary cells for lead profiling and screening. BIND<sup>®</sup> label-free technology not only provides a platform to assay any endogenous subtype (Gi, Gq, Gs coupled) GPCR but also utilizes lower cell numbers per assay.

The BIND<sup>®</sup> System provides label-free analysis of live cell receptor activation for both adherent and suspension cell types. The system shows equivalent sensitivity when measuring either recombinant or endogenous receptors. This study is focused on the endogenous GPCRs (muscarinic,

beta2 adrenergic, angiotensin and prostaglandin E2) expressed on HEK293 cells, thus providing potencies of agonists and antagonists on the native biological system.

### BIND<sup>®</sup> Technology

BIND from SRU Biosystems is a label-free universal assay system that enables detection of drug-target interactions. The system consists of the BIND<sup>®</sup> Reader and 96-, 384- and 1536-well microplate BIND<sup>®</sup> Biosensors.

The BIND System takes advantage of a novel optical effect to provide very sensitive measurements of changes in binding or adherence in the proximity of the biosensor surface. The

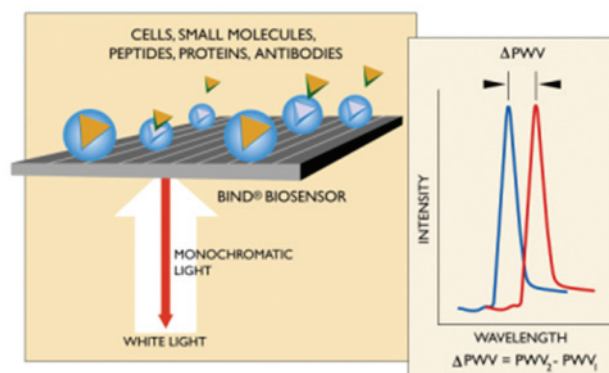


Figure 1

biosensor incorporates a proprietary nanostructured optical grating, and is incorporated into microwell plates in industry standard formats. The grating of the BIND Biosensor reflects only a single wavelength (“Peak Wavelength Value” or “PWV”).

When a cell binds to the biosensor surface, this reflected wavelength increases (Figure 1). Real time binding can be observed by measurement of the shift in PWV over time. For example, when cells are seeded onto the biosensor surface, cell attachment can be detected without the use of a label. Each addition of an assay component, such as a receptor ligand, to an assay well can be observed in real time to monitor the response of the cell.

## Materials and Methods

BIND® Reader (SRU Biosystems)

384-well BIND® Biosensor, Part No. TiO-384-M (SRU Biosystems) or CA1-384-M (SRU Biosystems)

HEK293 cells, Cat # CRL-1573 (ATCC), are grown and harvested according to the vendor recommended protocols. DMEM with 10%FBS and 1%Pen/Strep was used for growth of the cells and the starvation assay media was HBSS with 10mM HEPES, 2mM CaCl<sub>2</sub>, 2mM MgCl<sub>2</sub>, 5mM Glucose.

The compounds isoproterenol (Sigma), prostaglandin E2 (Sigma), carbachol (Sigma), angiotensin II (Sigma) and atropine (Sigma) are prepared in starvation assay media at the appropriate concentration for addition to the assay plate.

## BIND® Assay Step 1 (Optional)

### Starvation buffer only baseline

This step is helpful when developing assay conditions for cell density and seeding time. A 384-well BIND Biosensor is removed from its packaging and 25 ul of cell assay media is dispensed into each well. Let the plate sit at room temperature for 10 minutes. A starvation buffer only baseline is obtained on the BIND Reader. Acquire baseline data for approximately 5 minutes.

Note: If using a 384-well TiO BIND Biosensor, 25 ul of serum containing media should be added and removed with washing before adding 25 ul of starvation assay media.

## BIND® Assay Step 2

### Cell attachment signal

In screening mode, Step 2 would be the baseline step. HEK293 cells are harvested by transferring them from the tissue culture flask to a 50 ml tube, spinning the cells for 5 minutes at 1000 rpm, aspirate media carefully so as not to disturb cell pellet, and add 10 ml of starvation assay media.

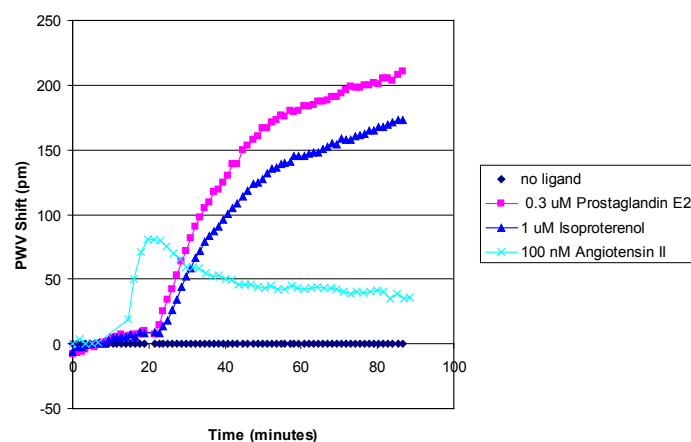
Count cells, and resuspend in starvation assay buffer to adjust cell density. Add 25ul of cells into each well (the wells already contains 25 ul of starvation assay media) at densities ranging from 10,000 to 20,000 cells per well for optimal response. Note that cell number is dependent on cell type. Incubate at 37°C for two hours and up to overnight. Let the plate sit at room temperature for 10 minutes before proceeding. Acquire data until a stable baseline is obtained (typically 5 minutes). In assay development mode baseline Step 2 to Step 1

## BIND® Assay Step 3

### Compound addition

Immediately after finishing the BIND Assay Step 2, place plate into an automated liquid handler and “stamp” compound(s) of choice (one compound per well in screening mode, or a compound serial dilution for cellular pharmacology assays). Inhibitors are allowed to incubate with cells for 30 minutes prior to addition of agonist. Acquire data for 30 to 60 minutes to record cell response to compound addition. The data acquired at this step should be baselined to the last data time point of the previous step. Export the data for analysis using EMS Export Wizard. The exported data can be analyzed with standard software or using the BIND® EC<sub>50</sub>/IC<sub>50</sub> template. All data shown here were analyzed using the BIND EC<sub>50</sub>/IC<sub>50</sub> template.

**Tip:** Final DMSO concentration is always a consideration in screening assays but it is important to maintain the same final DMSO concentration for each well to prevent bulk refractive index changes. It is recommended for dose response measurements that all concentrations are first diluted into DMSO followed by an equal volume addition to the final assay volume.

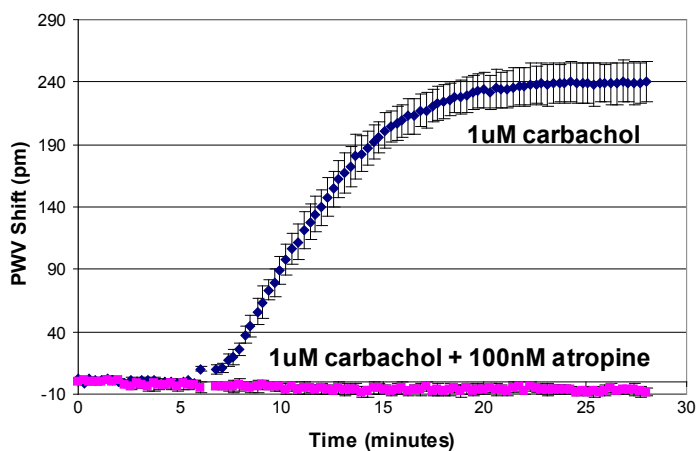


**Figure 2: Endogenous GPCR Subtype response on HEK293 cells.** HEK293 cells seeded on the serum treated 384-well TiO BIND Biosensor at 20K/well and treated with either prostaglandin E2 (0.3uM), isoproterenol (1 uM) or angiotensin II (100 nM). Cells treated with buffer alone (no ligand) were used as a background.

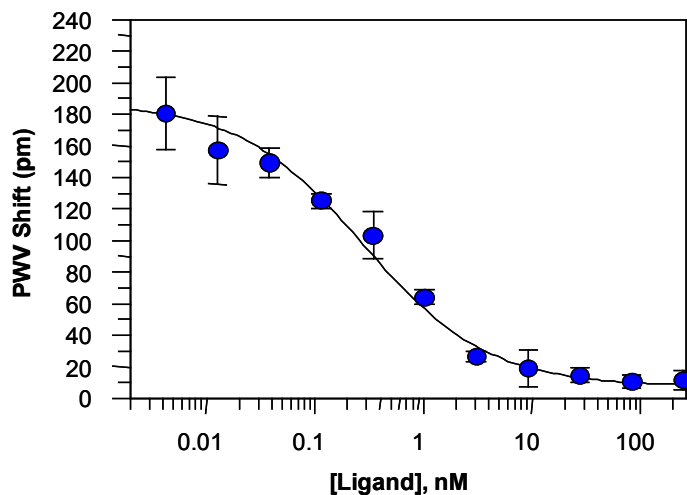
## Results and Discussion

Development of robust assay conditions for all GPCR subtypes ( $G_s$ ,  $G_i$  and  $G_q$ ) can be achieved using label-free BIND. Not only is the technology applicable to all types of GPCRs but the assay is robust enough for screening using endogenous expression levels of the receptor. This assures physiologically relevant signaling for characterization of all compounds. Figure 2 shows the response for both G protein-coupled receptors (prostaglandin E2 and beta2 adrenergic) and a G protein-coupled receptor (angiotensin II). For a given cell type a characteristic time-dependent cell response is obtained. For example, angiotensin II (100 nM) acting on a G protein-coupled receptor subtype produces an initial rapid rise to a maximum value which then decays to a stable level over 30 minutes (Figure 2). Isoproterenol (1uM) and prostaglandin E2 (0.3 uM) signaling through G protein-coupled pathways give a slower response and reach a stable maximum. The shape of the time dependent response curve is cell type dependent and must be characterized during assay development.

Both agonists and antagonists are easily characterized using BIND. HEK293 cells are incubated for 4 hours in starvation assay media on the serum treated TiO BIND Biosensor plate at 37°C. The cells are then treated with agonist or antagonist. If testing for inhibition of response, it is recommended that the cells are first incubated with antagonist for 30 minutes and then the agonist is added just prior to measurement on the BIND Reader. Data is collected for up to 60 minutes. The muscarinic receptor response to carbachol (1 uM) addition is completely inhibited by preincubation of atropine at 100 nM (Figure 3). The  $EC_{50}$  for carbachol (4.5 uM) and the  $IC_{50}$  for atropine (270 nM) can be obtained using the same assay conditions (Figure 4).



**Figure 3: Inhibition of endogenous G Protein-Coupled Receptor on HEK293 cells** HEK293 cells seeded on the 384-well CA-1 BIND Biosensor at 20K/well and treated with carbachol (1 uM) in the presence of the muscarinic receptor antagonist atropine (100 nM). Cells treated with buffer alone (no ligand) were used as a background.



**Figure 4: Titration of Atropine against 50 uM Carbachol on HEK-293 cells** HEK293 cells seeded on the 384-well CA-1 BIND Biosensor at 20K/well and treated with atropine. Cells treated with buffer alone (no ligand) were used as a background. The  $IC_{50}$  for atropine was determined to be 270 nM.

## Conclusions

- BIND is a universal platform that can be used to characterize all subtypes of GPCRs (Gi, Gs and Gq) .
- Robust assay conditions for endogenous GPCRs can be developed rapidly and used for characterization of agonists and antagonists
- The benefit of label-free cell-based assays is evident in the elimination of the cost of labeling reagents and the production of recombinant cell lines.
- The high sensitivity of label-free BIND allows measurement a more physiologically relevant assay for drug discovery.



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