Measuring β-Arrestin Recruitment Through Native GPCRs in High Throughput

MULTISPAN
Assay Experts in GPCRs and Beyond

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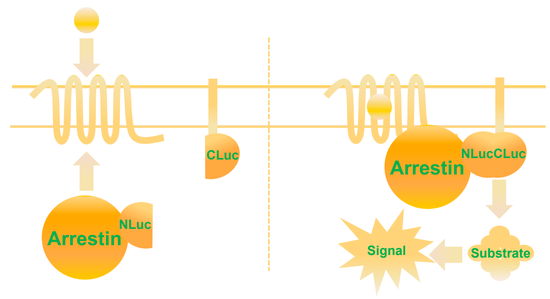
ABSTRACT

A ligand or compound that binds to a G-protein coupled receptor (GPCR) may induce or inhibit multiple signaling pathways, such as activation of G proteins and β-arrestin. Furthermore, more than one Gα protein subtype may bind to its cognate receptor. The properties of a ligand that preferentially activate or inhibit one pathway over another are described as signaling bias and screening compounds for biased signaling may lead to selective perturbation of disease-specific pathways. Measuring compound activities in assays in the same cellular environment using unmodified GPCRs, recombinant or native, is key for accurate signaling bias analysis. Our newly developed proprietary MultiScreen™ β-arrestin technology overcomes the receptor-tagging drawback of other existing technologies, enabling high-throughput detection of β-arrestin translocation induced by native GPCRs in vitro and in vivo for the first time. This presentation will highlight these novel features and the robustness of this assay among other updates of our portfolio.

MultiScreen™ β-Arrestin Technology



Benefits
- Study all signaling bias in one MultiScreen™ stable cell line or primary cells
- True phamacology in vitro and in vivo without GPCR tagging
- No GRK2 co-transfection required

Figure 1. Illustration of β-Arrestin signaling pathway mechanism and assay platform.

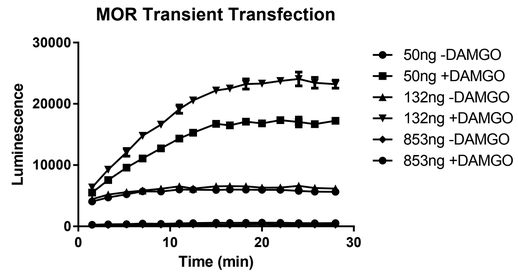
Materials and Methods

- **Stable Cell lines:** Mu Opioid Receptor (MOR, Multispan CA1350-1a), Delta Opioid Receptor (DOR, Multispan CA1351-1), Kappa Opioid Receptor (Multispan CA1352-1a), Nociceptin Receptor (NOP, Multispan CA1354-1), Beta 2 Adrenergic Receptor (Multispan CA1438-1a), Vasopressin 2 Receptor (V2A, Multispan CA1044-1), Cannabinoid 1 Receptor (CB1, Multispan CA1229-1a), Cannabinoid 2 Receptor (CB2, Multispan CA1230-1a)
- **Ligands:** DAMGO (Phoenix Pharmaceuticals 024-10), Buprenorphine (Sigma B-044-1ML), PZM21 Sulfate, Dynorphin B (Tocris 3196, Deltorphin II (Tocris 1180), OFQ (Sigma O4011), Isoproterenol (Cayman Chemicals 15592), Vassopressin (Sigma V9879), CP55940 (Sigma C1112)
- **Transfection:** Cells were stably transfected with MultiScreen™ β-Arrestin sensor using TransIT®-LT1 Transfection Reagent (Mirus MIR 2305)
- **cDNA:** MultiScreen™ β-Arrestin sensor cloned into pcDNA3.1™ (+) or pcDNA™ 3.1/Hygro (+). ARRB2 GenBank accession number NM_004313.3.

Simple Protocol for MultiScreen™ β-Arrestin

- Seed cells in 384-well plates
- Incubate overnight
- Wash with MultiScreen™ β-Arrestin Assay Buffer
- Add MultiScreen™ Substrate and incubate for 5 minutes
- Treat with compounds for 20 minutes
- Read luminescence emission
- Total assay time 30 minutes

MultiScreen™ β-Arrestin Assay Time Course



Read every 2 minutes ☆ Signal stabilizes at 20 minutes

Figure 2. Stimulation from arrestin recruitment upon treatment with and without DAMGO. Cells were transiently transfected wth 50, 132, or 853ng of MOR cDNA with a 1:1 ratio of PEI in 12-well plate format.

MultiScreen™ β-Arrestin Assays in Stable Cell Lines (Part 1)

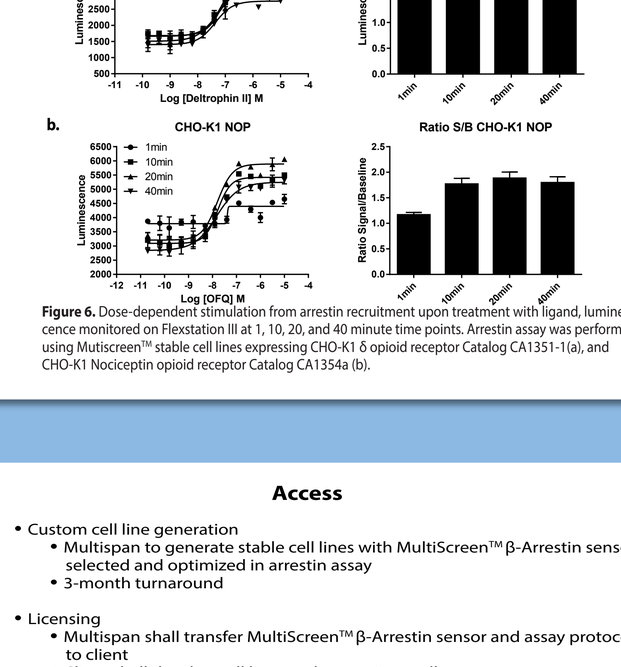


Figure 3. Dose-dependent stimulation from arrestin recruitment upon treatment with ligand, luminescence monitored on Flexstation III. Arrestin assay was performed using Mutiscreen™ stable cell lines expressing CHO-K1 μ opioid receptor Catalog CA1350-1a (a), CHO-K1 κ opioid receptor Catalog CA1352-1a (b), CHO-K1 δ opioid receptor Catalog CA1351-1(c), and CHO-K1 Nociceptin opioid receptor Catalog CA1354a (d).

MultiScreen™ β-Arrestin Assays in Stable Cell Lines (Part 2)

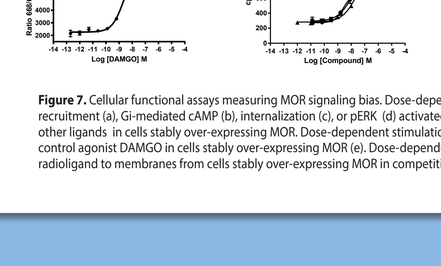


Figure 4. Dose-dependent stimulation from arrestin recruitment upon treatment with ligand, luminescence monitored on Flexstation III. Arrestin assay was performed using Mutiscreen™ stable cell lines expressing CHO-K1 β2 Adrenoceptor Catalog CA1438-1a (a), CHO-K1 V1A receptor Catalog CA1042-1 (b), and CHO-K1 CB2 receptor Catalog CA1230-1a (c).

MOR and KOR Stable Cell Line Time Course

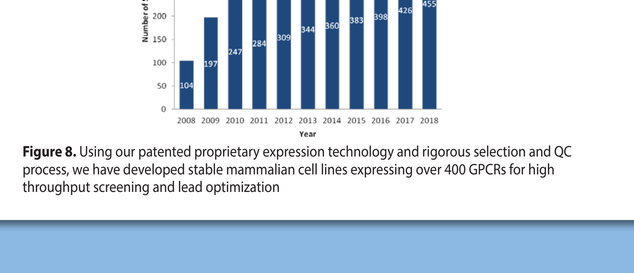


Figure 5. Dose-dependent stimulation from arrestin recruitment upon treatment with ligand, luminescence monitored.on Flexstation III at 1, 10, 20, and 40 minute time points. Arrestin assay was performed using Mutiscreen™ stable cell lines expressing CHO-K1 μ opioid receptor Catalog CA1350-1a (a), CHO-K1 κ opioid receptor Catalog CA1352-1a (b).

DOR and NOP Stable Cell Line Time Course

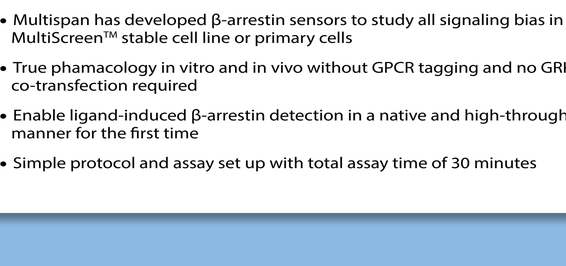


Figure 6. Dose-dependent stimulation from arrestin recruitment upon treatment with ligand, luminescence monitored on Flexstation III at 1, 10, 20, and 40 minute time points. Arrestin assay was performed using Mutiscreen™ stable cell lines expressing CHO-K1 δ opioid receptor Catalog CA1351-1(a), and CHO-K1 Nociceptin opioid receptor Catalog CA1354a (b).

True Signaling Bias-All Assays in One Cell Line

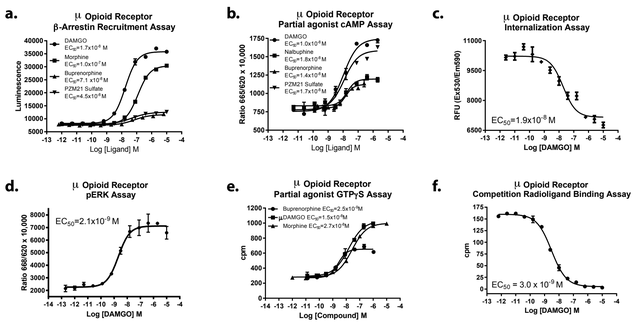


Figure 7. Cellular functional assays measuring MOR signaling bias. Dose-dependent stimulation of β-arrestin recruitment (a), Gi-mediated cAMP (b), internalization (c), or pERK (d) activated by control agonist DAMGO and other ligands in cells stably over-expressing MOR. Dose-dependent stimulation of [35S] GTP γS binding by control agonist DAMGO in cells stably over-expressing MOR (e). Dose-dependent binding of [3H] DAMGO radioligand to membranes from cells stably over-expressing MOR in competition binding (f).

Validated MultiScreen™ β-Arrestin Stable Lines

Receptor	Species	Assay Format						
		Ca^{++}	cAMP	β-arrestin	Internali-zation	Ligand Binding	pERK	GTPγS
mu	human	✓	✓	✓	✓	✓	✓	✓
kappa	human	✓	✓	✓	✓	✓	✓	✓
delta	human	✓	✓	✓			✓	✓
NOP	human	✓	✓	✓	✓		✓	✓
beta2	human	✓	✓	✓			✓	
V1A	human	✓		✓				

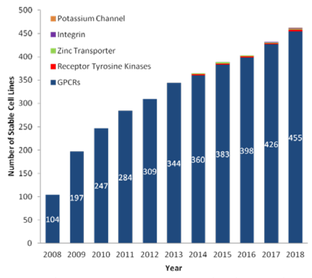


Figure 8. Using our patented proprietary expression technology and rigorous selection and QC process, we have developed stable mammalian cell lines expressing over 400 GPCRs for high throughput screening and lead optimization

Access

- Custom cell line generation
  - Multispan to generate stable cell lines with MultiScreen™ β-Arrestin sensor, selected and optimized in arrestin assay
  - 3-month turnaround
- Licensing
  - Multispan shall transfer MultiScreen™ β-Arrestin sensor and assay protocols to client
  - Client shall develop cell lines and assays internally

Summary

- Multispan has developed β-arrestin sensors to study all signaling bias in one MultiScreen™ stable cell line or primary cells
- True phamacology in vitro and in vivo without GPCR tagging and no GRK2 co-transfection required
- Enable ligand-induced β-arrestin detection in a native and high-throughput manner for the first time
- Simple protocol and assay set up with total assay time of 30 minutes