JAYCE TECHNOLOGY TO DECIPHER RECEPTOR BIOLOGY AND PHARMACOLOGY

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Introduction

G-Protein Coupled Receptor Pharmacology was following classical models assuming that agonists at a particular receptor elicit effects through a single mechanism of activation, implying a single activated conformation of the agonist-occupied receptor. This assumption led to the standard nomenclature of GPCR modulators based on affinity and efficacy, and to their classification as full agonist, partial agonist, neutral antagonist, or inverse agonist. However a receptor can engage a variety of biochemical responses and different agonists can elicit different patterns of these responses. Ligands should therefore be classified on the basis of their individual effects in the cell, instead of being either an agonist or an antagonist.

The current challenge in the GPCR drug discovery field is based on the development and the implementation of assays that would lead to the discovery of biased or pathways selective agonists in a pro-active and prospective manner.

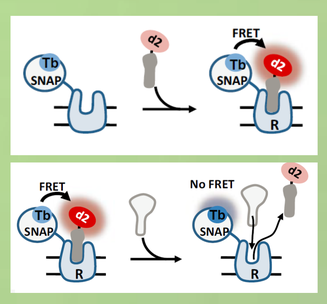
For this achievement, we have developed assays aiming at the characterization of GPCR signaling pathways. Actelion's JAYCE platform was developed to generate biological information delineating beneficial and adverse signaling pathways at specific receptors and identifies ligands that activate only the pathways associated with therapeutic benefit.

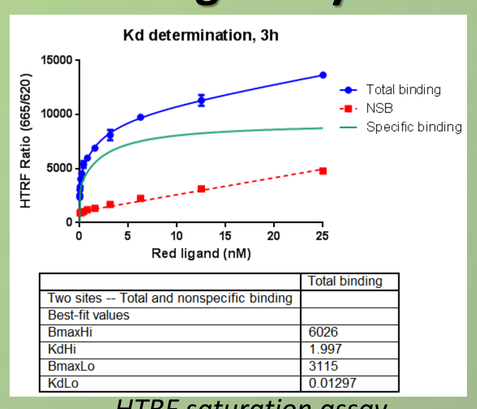
Methods

A multifunctional clone was created in HEK-293 cellular background with the a GPCR of interest tagged with a SNAP protein and linked to the N-terminal part of the Luciferase, as well as the β-arrestin 2 linked to the C-terminal part of the Luciferase. This single clone was used in ligand binding (**1**, TagLite), β-arrestin 2 recruitment (**2**, luciferase complementation) and internalization (**3**, TagLite and **4**, immunohistochemistry) assays. Two compounds are described in this study: an agonist (Compound A) and an antagonist (Compound B).

Results

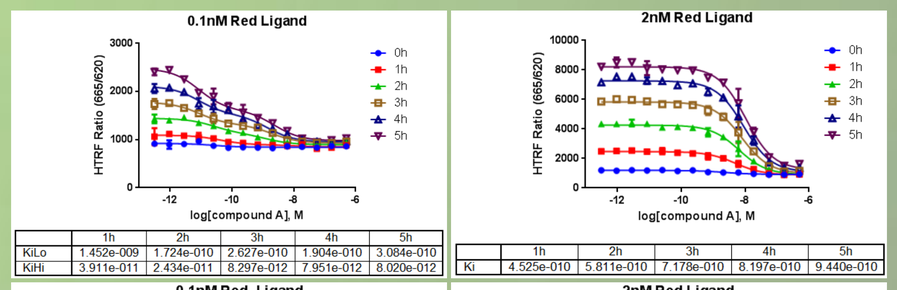
1. TagLite Ligand Binding Assay





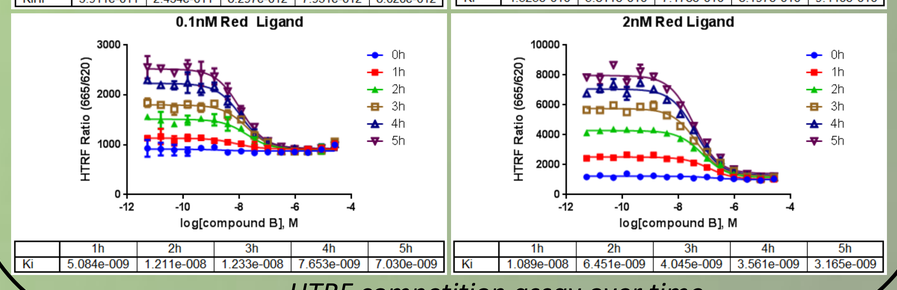
	Total binding
Two sites -- Total and nonspecific binding	
Best-fit values	
BmaxHi	6026
KdHi	1.997
BmaxLo	3115
KdLo	0.01297

HTRF saturation assay



	1h	2h	3h	4h	5h
KiLo	1.452e-009	1.724e-010	2.627e-010	1.904e-010	3.084e-010
KiHi	3.911e-011	2.434e-011	8.297e-012	7.951e-012	8.020e-012

	1h	2h	3h	4h	5h
Ki	4.525e-010	5.811e-010	7.178e-010	8.197e-010	9.440e-010



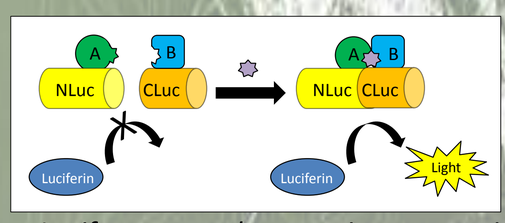
	1h	2h	3h	4h	5h
Ki	5.084e-009	1.211e-008	1.233e-008	7.653e-009	7.030e-009

	1h	2h	3h	4h	5h
Ki	1.089e-008	6.451e-009	4.045e-009	3.561e-009	3.165e-009

HTRF competition assay over time

2. Beta arrestin 2 Recruitment (Luciferase Complementation)

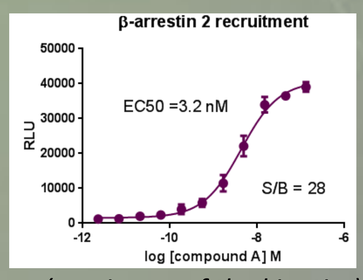
- Seeding of adherent cells in 384-well format
- Pre-incubation of the cells with D-Luciferin
- First addition (agonist or antagonist)
- 30 minutes measurement (FDSS7000)
- Second addition 10 nM agonist
- 60 minutes measurement (FDSS7000)

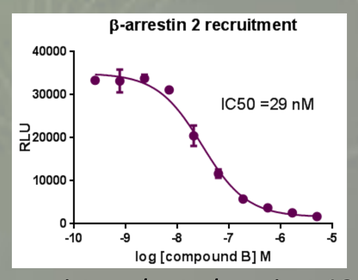


Detection of GPCR activation in real-time and in living cells
A: GPCR
B: β-arrestin 2

Luciferase complementation assay principle

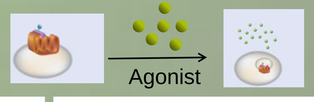
Real-time measurement of the β arrestin 2 recruitment

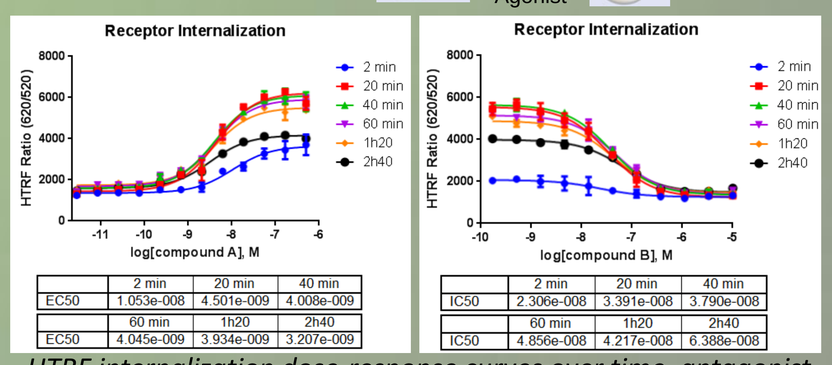




Dose-response curves (maximum of the kinetics), antagonist evaluated against 10 nM agonist

3. Internalization: HTRF Based on SNAP-tag, Real-time





	2 min	20 min	40 min
EC50	1.053e-008	4.501e-009	4.008e-009

	60 min	1h20	2h40
EC50	4.045e-009	3.934e-009	3.207e-009

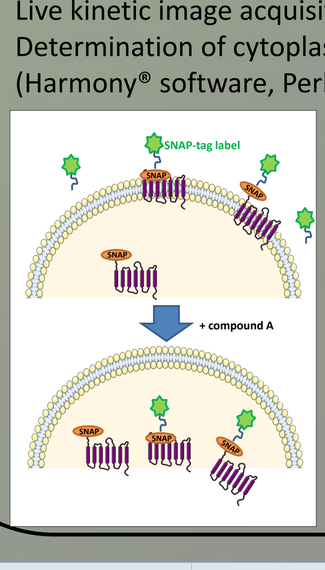
	2 min	20 min	40 min
IC50	2.306e-008	3.391e-008	3.790e-008

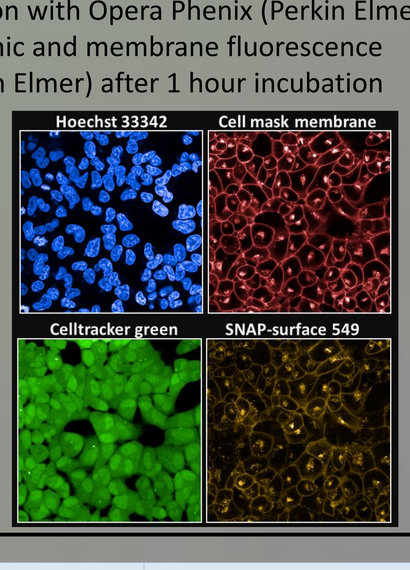
	60 min	1h20	2h40
IC50	4.856e-008	4.217e-008	6.388e-008

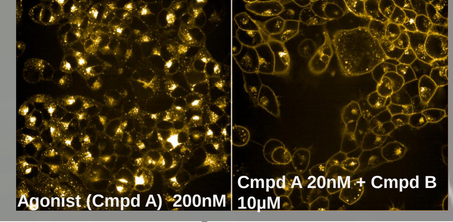
HTRF internalization dose-response curves over time, antagonist evaluated against 30 nM agonist

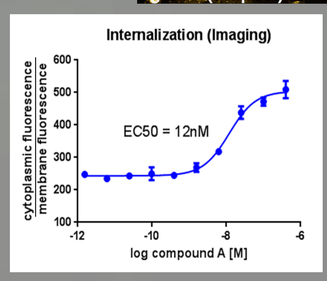
4. Internalization: Real-time Cell Imaging Based on SNAP-tag

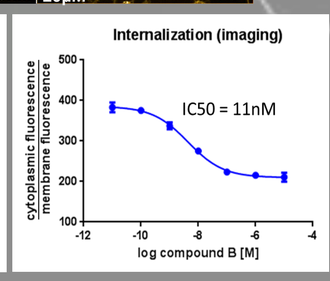
- Seeding of adherent cells in 384-well format
- Labelling of the <u>surface</u> receptor with SNAP-Surface® 549 (30 min)
- Labelling of the cellular components for imaging analysis:
  - Hoechst (nucleus)
  - Cell mask membrane deep red (membrane)
  - Cell tracker green (cytoplasm)
- Live kinetic image acquisition with Opera Phenix (Perkin Elmer)
- Determination of cytoplasmic and membrane fluorescence (Harmony® software, Perkin Elmer) after 1 hour incubation











	Calcium	Beta arrestin 2	Ligand Binding	Internalization (HTRF)	Internalization (Imaging)
Compound A EC50	5 nM	3.2 nM	0.2 nM / 6 pM	4 nM	12 nM
Compound B IC50	14 nM	29 nM	3.5 nM	48 nM	11 nM

Conclusions

Actelion's JAYCE platform is a purpose-built set of integrated tools and techniques including diverse biological assays of GPCR function and regulation allowing to predict therapeutic index and offering insights into G protein and β-arrestin-mediated mechanisms associated with pharmacological responses. These assays are aligned with biological signaling information obtained by dissecting GPCR/7TM signal transduction networks with real-time measurement.

The JAYCE platform is an effective and efficient mean for discovering meaningfully differentiated biased ligands targeting important clinical needs.

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