Real-Time Kinetic Analysis of GPCR Signaling in Stable PathHunter® Cell Lines

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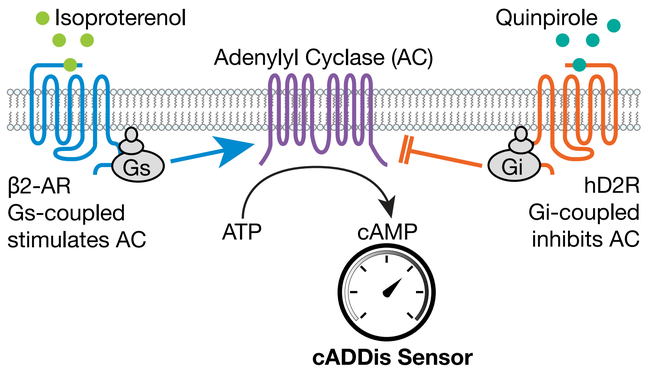
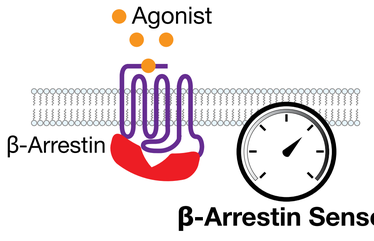
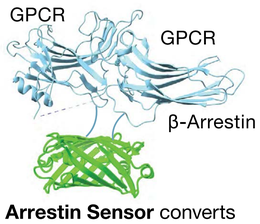
Overview

- Eurofins DiscoverX stable cell lines are widely used tools in GPCR drug discovery, providing endpoint assays for G-protein signaling, GPCR internalization, and β-arrestin recruitment.
- Montana Molecular offers genetically-fluorescent biosensors to detect cAMP, DAG, PIP_2, Ca^{2+}, cGMP, β-arrestin, and cell stress.
- The fluorescent sensors can be used effectively in both PathHunter® and cAMP Hunter® CHO-K1 cell lines to monitor signaling kinetics of G-protein and β-arrestin pathways in live cells, in real time.
- A new method of kinetic analysis provides a robust kinetic parameter (kTau), which simplifies the quantification of agonist activity and bias at particular GPCRs, and could provide a new method for studying G-protein and/or β-arrestin recruitment in opioid, angiotensin II, and vasopressin receptor cell lines.

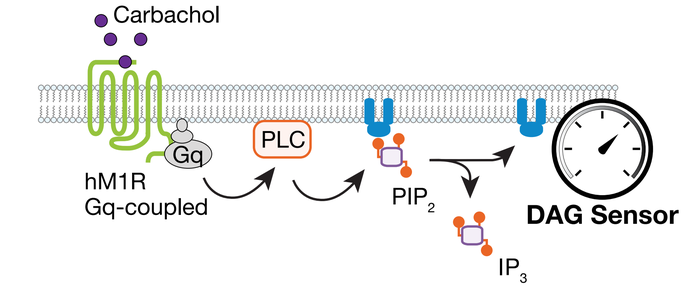
Genetically-encoded, fluorescent sensors for detecting G-protein and β-arrestin signaling







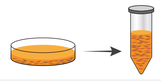
Arrestin Sensor converts GPCR binding to changes in fluorescence



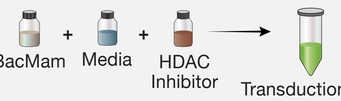
Investigate GPCR biology with a simple protocol that combines DiscoverX cells and Montana Molecular biosensors

DAY 1 TRANSDUCE AND PLATE CELLS

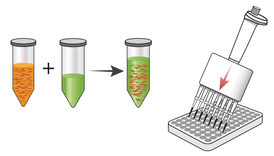
STEP 1
Prepare cells



STEP 2
Prepare viral transduction reaction

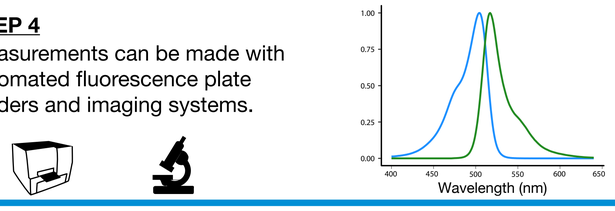


STEP 3
Mix cells and transduction reaction

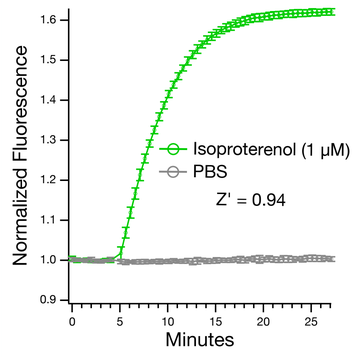


DAY 2 MEASURE FLUORESCENCE

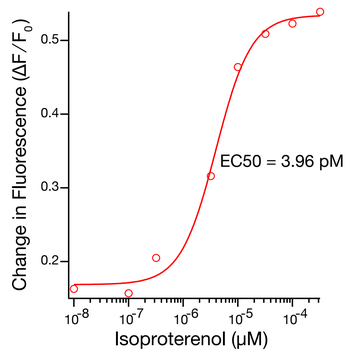
STEP 4
Measurements can be made with automated fluorescence plate readers and imaging systems.



High-resolution kinetic measurements of cAMP signaling in Eurofins DiscoverX cell lines (β-2 adrenergic receptor, ADRB2)

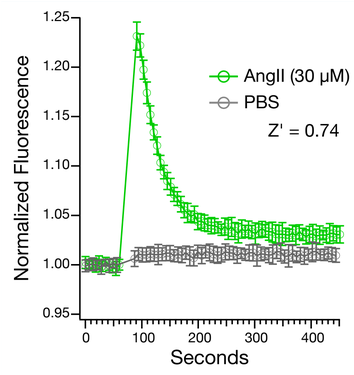


PathHunter® CHO-K1 ADRB2 cells expressing the green **cADDis Sensor**, responding to the addition of 1 μM isoproterenol. The change in fluorescence indicates an increase in cAMP production upon activation of the β-2 adrenergic receptor.

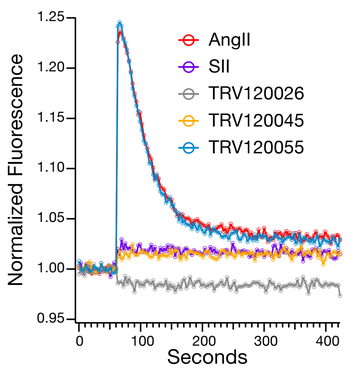


The **cADDis Sensor** can be used to generate reliable concentration response curves in PathHunter® cells.

High-resolution kinetic measurements of diacylglycerol signaling in Eurofins DiscoverX cell lines (Angiotensin II, type 1 receptor, AGTR1)

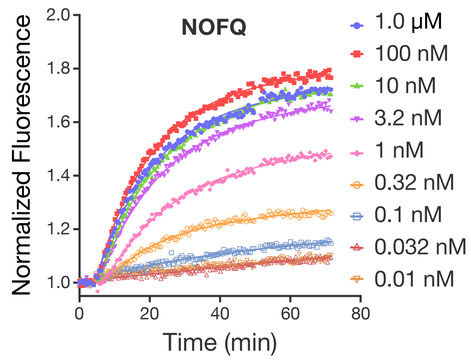


Cells expressing the Angiotensin II receptor (PathHunter® CHO-K1 AGTR1) are activated by angiotensin II, producing an increase in diacylglycerol that can be detected with the green **DAG Sensor**.

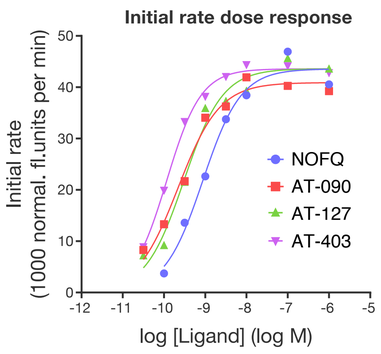


The **DAG Sensor** was used to measure the activity of 5 ligands at the AT1 receptor, three of which are known to be biased toward the β-arrestin pathway.

Kinetic measurements make it possible to extract the initial rate parameter (kTau), which can be used to quantify agonist activity at GPCRs such as the Nociceptin Opioid receptor (OPRL1)



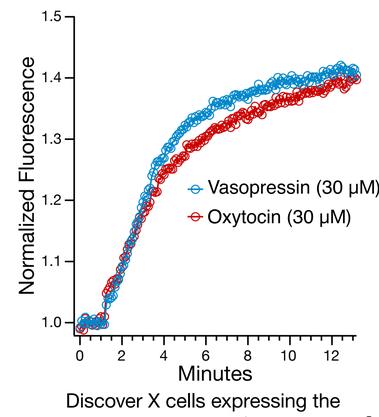
The green **cADDis Sensor** can be used in DiscoverX cells expressing the Nociceptin Opioid receptor to detect Gi signaling. Dose response measurements to the agonist nociceptin/orphanin FQ were made, and the kinetic data was used use determine the EC50 by calculating the initial rate of activity (kTau) at each dose. The initial rate of signaling (kTau) is a direct measure of ligand efficacy.



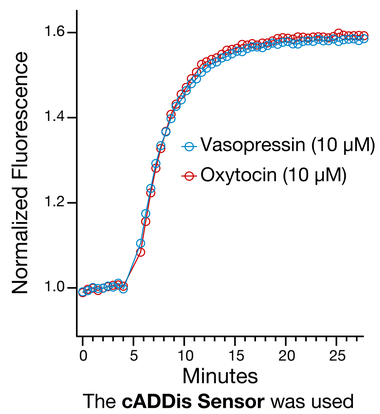
The kinetic method described above was used to evaluate the activity of three additional agonists at the OPRL1 receptor: AT-090, AT-127, and AT-403, developed by Astraea Therapeutics

Ligand	Emax/kTau %NOFQ	EC50 (pM)
NOFQ	100	870
AT-090	94	220
AT-127	100	300
AT-403	100	120

The signaling kinetics of the G-protein and β-arrestin pathways can be compared to measure agonist bias in a simple, easy to implement approach.



Discover X cells expressing the Vasopressin receptor 2 (cAMP Hunter® CHO-K1 AVPR2) were activated by vasopressin or oxytocin. β-arrestin recruitment was monitored with the green **β-Arrestin Sensor,** which revealed a difference in signaling kinetics between the two ligands.



The **cADDis Sensor** was used to measure the cAMP signal produced when the V2R receptor was activated with vasopressin or oxytocin.

	Vasopressin	Oxytocin
Arrestin response kTau (% vaso.)	100	66
cAMP response kTau (% vaso.)	100	108
cAMP : Arrestin Bias Factor	1	1.64

The β-arrestin and cAMP responses produced by each agonist, reported as kTau (% vaso.), were used to generate a bias factor, which indicated that oxytocin is biased toward the Gs pathway.