

Measuring partial and biased agonism at GPCRs on the FDSS/ μ Cell

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Overview

- The FDSS/ μ Cell functional drug screening system produces high-resolution kinetic signaling data needed to capture important pharmacological parameters including agonist efficacy, potency, partial and biased agonism (Hoare *et.al* 2020).
- Montana Molecular's genetically-encoded, fluorescent biosensors for DAG, PIP₂, Ca²⁺, cAMP and β -arrestin can be effectively used on the FDSS/ μ Cell to measure and compare the activity of GPCR agonists in real time. (Tewson 2012, Ding 2015, Tewson 2018).
- Biosensors and receptors are packaged in the BacMam vector for delivery to practically any cell type (Tewson 2016).

Ding, Y., Li, J., Enterina, J.R., Shen, Y., Zhang, I., Tewson, P.H., Mo, G.C.H., Zhang, J., Quinn, A.M., Hughes, T.E., et al. (2015). Ratiometric biosensors based on dimerization-dependent fluorescent protein exchange. *Nat. Methods* 12, 195–198.

Hoare, S.R.J., Tewson, P.H., Quinn, A.M., and Hughes, T.E. (2020). A kinetic method for measuring agonist efficacy and ligand bias using high resolution biosensors and a kinetic data analysis framework. *Sci. Rep.* 10, 1766.

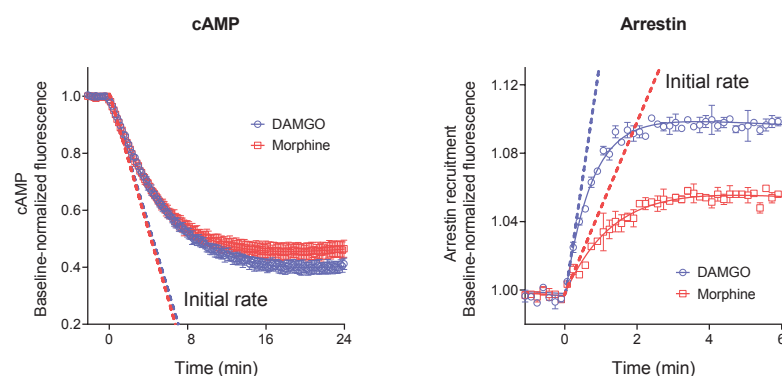
Hoare, S.R.J., Tewson, P.H., Quinn, A.M., Hughes, T.E., and Bridge, L.J. (2020). Analyzing kinetic signaling data for G-protein-coupled receptors. *Sci. Rep.* 10, 12263.

Tewson, P., Westenberg, M., Zhao, Y., Campbell, R.E., Quinn, A.M., and Hughes, T.E. (2012). Simultaneous detection of Ca²⁺ and diacylglycerol signaling in living cells. *PLoS One* 7, e42791.

Tewson, P.H., Martinka, S., Shaner, N.C., Hughes, T.E., and Quinn, A.M. (2016). New DAG and cAMP Sensors Optimized for Live-Cell Assays in Automated Laboratories. *J. Biomol. Screen.* 21, 298–305.

Tewson, P., Martinka, S., Shaner, N., Berlot, C., Quinn, A.M., and Hughes, T. (2018). Assay for Detecting Gai-Mediated Decreases in cAMP in Living Cells. *SLAS Discov* 23, 898–906.

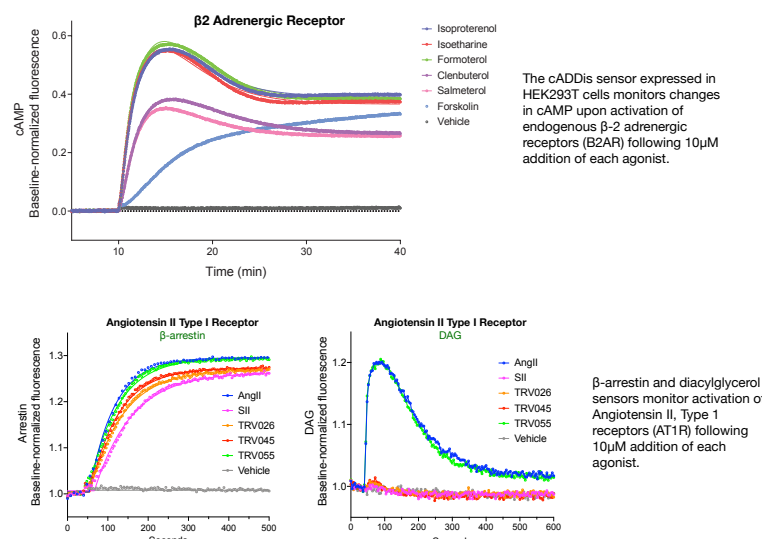
The Initial Rate of second messenger signaling is a key parameter for agonist efficacy and bias.



- The initial rate parameter kTau is extracted from time course measurements.
- kTau can be used to address the time-dependency of efficacy and bias.
- Use kTau to arrive at the bias ratio: bias is the ratio of the Emax concentration initial rate (kTau, normalized to a reference ligand). (See Hoare 2020)

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FDSS/ μ Cell collects high quality kinetic data from fluorescent biosensors to measure the initial rate.



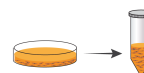
- Discover pharmacological differences between receptor agonists that are undetectable with end-point assays.
- Capture fast signaling events, generate waveforms and high-quality curve fits.
- Monitor regulation of signaling (e.g. desensitization, degradation).

Simple Workflow

Day 1: Transduce and Plate Cells in 384-Well Format

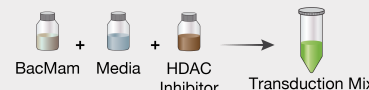
STEP 1

Prepare cells.



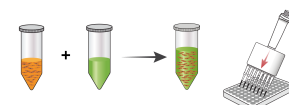
STEP 2

Prepare viral transduction reaction.



STEP 3

Mix cells and transduction reaction.



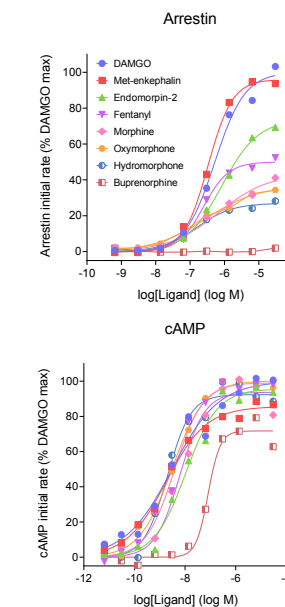
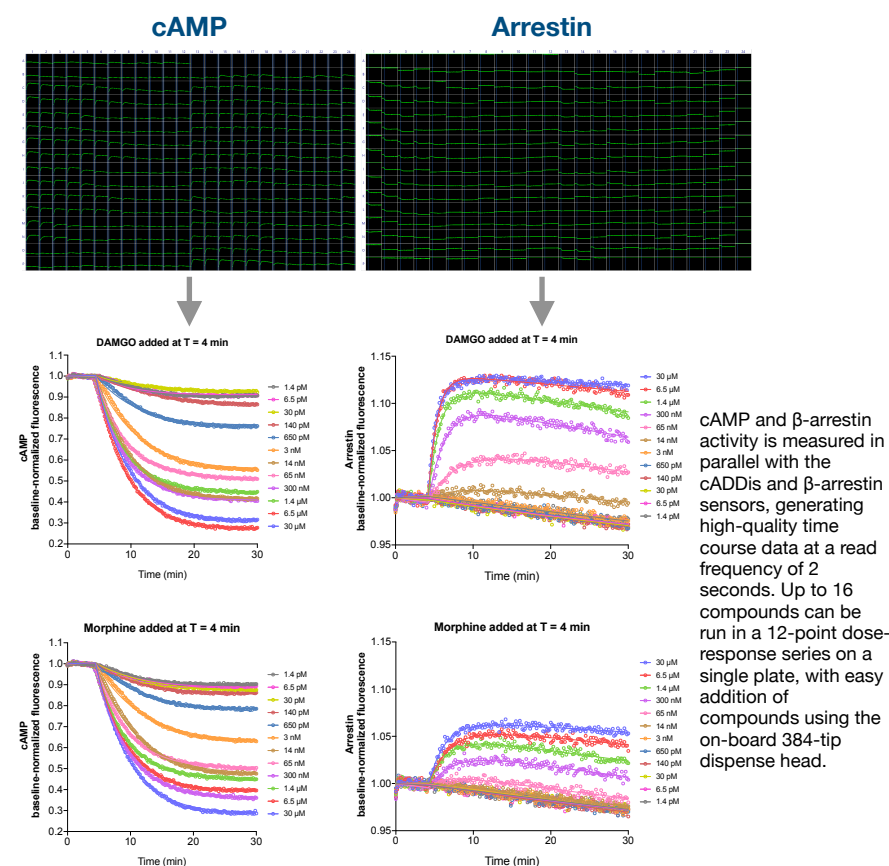
Day 2: Measure Fluorescence

STEP 4

- FDSS/ μ Cell captures biosensor data with high throughput and high fidelity.
- On-board dispense system adds compounds to 384 wells while simultaneously collecting kinetic responses from fluorescent biosensors.



Measuring Biased Agonism at the Mu Opioid Receptor on the FDSS/ μ Cell



Ligand	Arrestin kTau	cAMP kTau	Bias (cAMP / Arrestin)
DAMGO	100	100	1.0
Met-enkephalin	96	86	0.9
Endomorphin-2	74	96	1.3
Fentanyl	50	100	2.0
Morphine	43	94	2.2
Oxymorphone	37	100	2.7
Hydromorphone	27	92	3.4
Buprenorphine	0	71	> 10

β -arrestin and cAMP kinetic response data for eight agonists was analyzed, and used to calculate the initial rate of signaling (kTau) at each dose. Dose-response curves indicate differences in potency and efficacy. The Emax concentration initial rate (kTau, normalized to DAMGO) for arrestin and cAMP was used to generate bias ratios.