# Measuring partial and biased agonism at GPCRs on the FDSS/μCell

Montana Molecular

Pharmechanics

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## Overview

- The FDSS/μCell 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, $PIP_2$, $Ca^{2+}$, cAMP and β-arrestin can be effectively used on the FDSS/μCell 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.

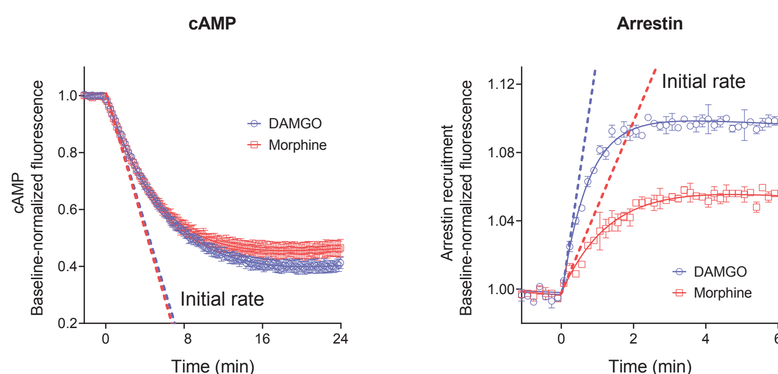
Hoare, S.R.J., Tewson, P.H., Quinn, A.M., Hughes, T.E., and Bridge, L.J. (2020). Analyzing kinetic signaling data for G-protein-coupled receptors. Sci. Rep. 10, 12263.

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 Gαi-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.




HEK 293T cells expressing the mu-opioid receptor were activated with 10μM DAMGO or morphine, resulting in a decrease in cAMP and increase in arrestin recruitment that can be detected with the cADDis and β-arrestin sensors.

- 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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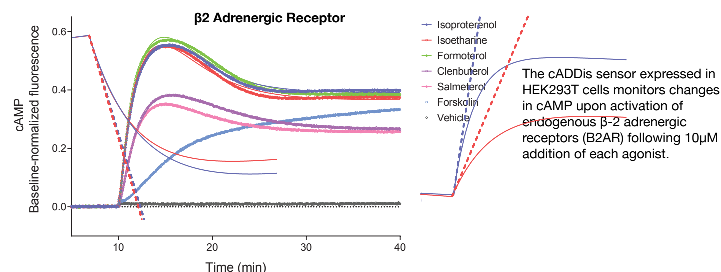
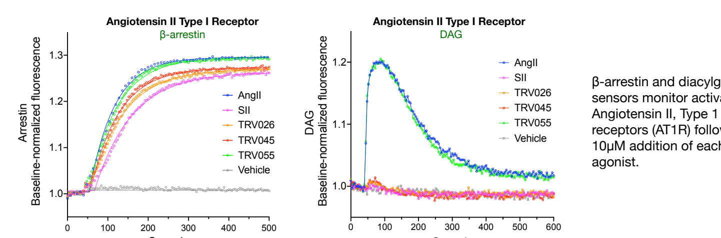
## FDSS/μCell collects high quality kinetic data from fluorescent biosensors to measure the initial rate.




The cADDis sensor expressed in HEK293T cells monitors changes in cAMP upon activation of endogenous β-2 adrenergic receptors (B2AR) following 10μM addition of each agonist.




β-arrestin and diacylglycerol sensors monitor activation of Angiotensin II, Type 1 receptors (AT1R) following 10μM addition of each agonist.

- 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).

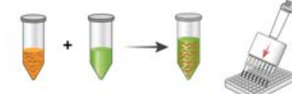
## Simple Workflow

### 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.

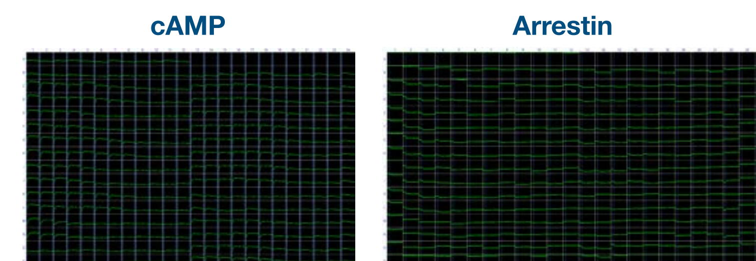


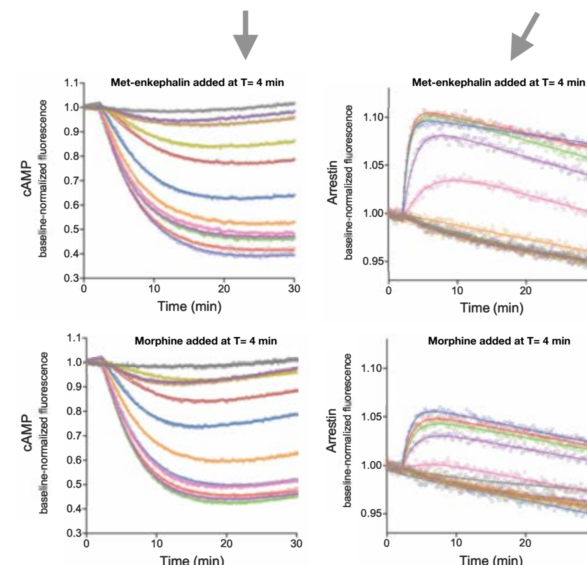
### Day 2: Measure Fluorescence

**STEP 4**

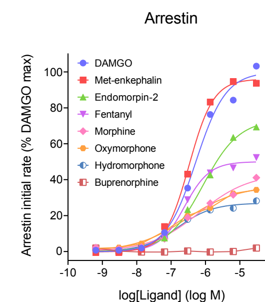
- FDSS/μCell captures biosensor data with high throughput and high fidelity.
- On-board dispense system adds compounds to 384 wells while simultaneously collecting kinetic responses from fluorescent biosensors.

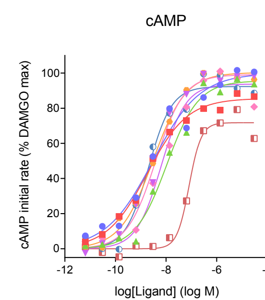
## Measuring Biased Agonism at the Mu Opioid Receptor on the FDSS/μCell




cAMP and β-arrestin activity is measured in parallel with the cADDis and β-arrestin sensors, generating high-quality time course data at a read frequency of 2 seconds. Up to 16 compounds can be run in a 12-point dose-response series on a single plate, with easy addition of compounds using the on-board 384-tip dispense head.




| 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.