G-Protein Receptor Signaling Kinetics and Biased Agonism

Montana Molecular

Pharmechanics

About Us

Montana Molecular develops genetically encoded biosensors that monitor signaling kinetics in living cells. These biosensors change in fluorescence intensity and are easily detected on fluorescence plate readers or imaging systems. The sensors are packaged in viral vectors, including BacMam, a modified baculovirus, for optimized assays in a wide variety of cell types and tissues

Viral Delivery

- **Baculovirus**
  - BacMam = mammalian optimized
  - Large Payload (40 Kb)
  - BSL-1 = no mammalian replication
- **Lentivirus & AAV**
- **Live Cell Assay**
  - Cell Lines
  - Primary Culture
  - iPSC Derived

Experiments

- **Signaling Kinetics**
- **Dose Response**
- **Hit Prioritization**
- **Targeted Expression**
- **Live Cell Imaging**

Selected References

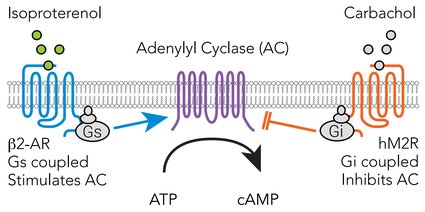
- Tewson, et al. PLoS One, Aug 2012.
- Ding, et al. Nature Methods, 2015.
- Moore, et al. PNAS, Nov 2016.
- Hilgendorf, et al. Cell, Nov 2019.
- Harlen, et al. Front. Cell. Neuro. Dec 2019.
- S. Hoare, et al. Nature Scientific Reports, Feb 2020.
- S. Hoare, et al. Nature Scientific Reports, July 2020.

Gs and Gi Signaling Pathway

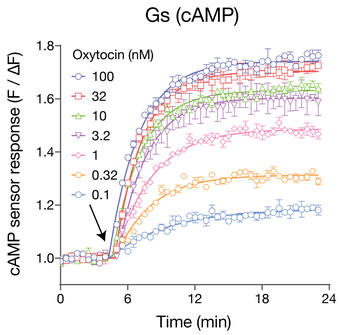
cADDis Sensors

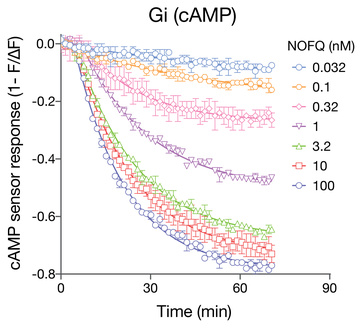
cAMP Difference Detector in-situ

Green and Red Fluorescence



- **Genetically-encoded fluorescent biosensors enable continuous recording of signaling kinetics in live cells**
- **Easy workflow - add ligand and read in plate reader**
- **Tewson et al. *Assay for detecting Gαi-mediated decreases in cAMP in living cells.* (SLAS Discovery. July 2018)**





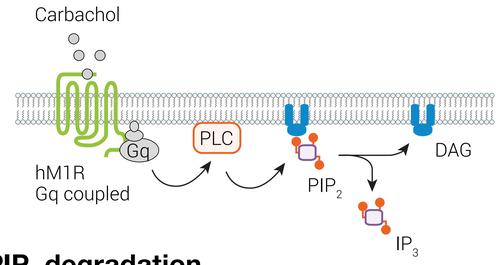
cAMP Generation by oxytocin via the V2 vasopressin receptor (top figure). cAMP Inhibition by NOFQ via the nociceptin receptor prior stimulation by forskolin (bottom figure).

Gq Signaling Pathway

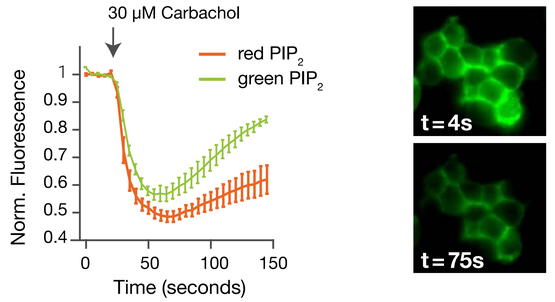
DAG, Ca^{2+}, PIP_2 Sensors

Simultaneous Measurements of Multiple Components of Gq

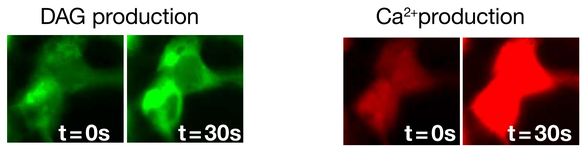
Green and Red Fluorescence

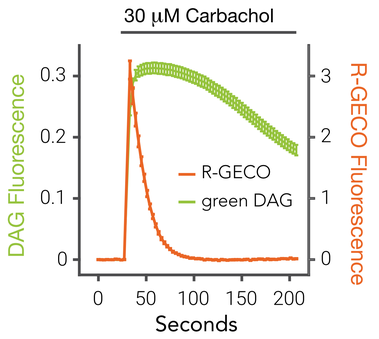


- **PIP_2 degradation**
  - **Green and red sensors of Gq mediated PIP_2 degradation in living cells**



- **Multiplexed measurement of DAG production and IP3 mediated Ca^{2+} increases**



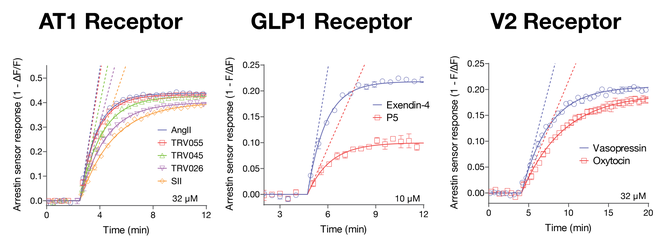


Arrestin Activity Assays

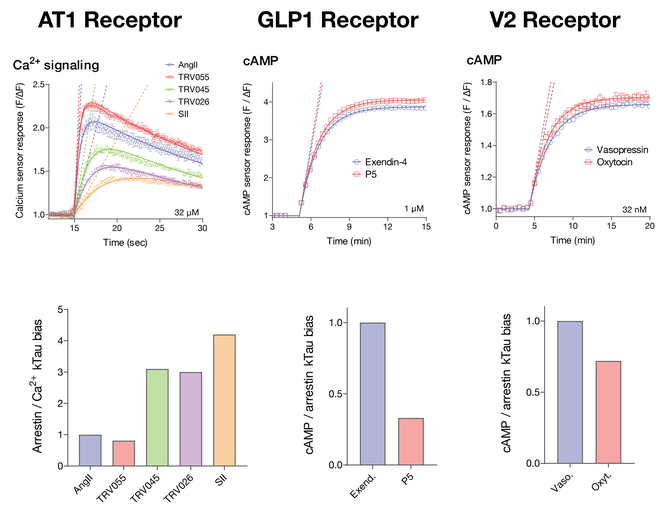
Optimized Assays for:

GLP-1R, Angiotensin, Mu Opioid, Vasopressin, β2-Adrenergic, D_1 Dopamine

- **Measuring bias using the initial rate**
  - Reproducible measurements of GPCR biased agonism
  - Hoare et al. *A kinetic method for measuring agonist efficacy and ligand bias using high resolution biosensors and a kinetic data analysis framework.* Nature Scientific Reports.



- **Quantify efficacy and bias from kinetic signaling analysis**
  - Efficacy and bias can be time-dependent
  - Solved by measuring the initial rate of signaling
  - Initial rate is extracted from curve fit parameters. kTau is the initial rate at Emax concentration.



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