Using receptor kinetics to quantitatively measure agonist bias at G-protein coupled receptors

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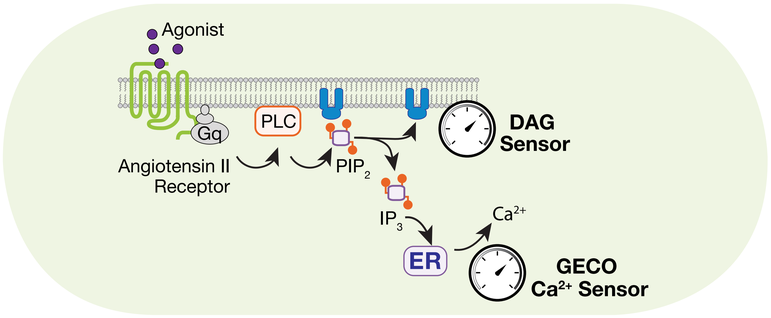
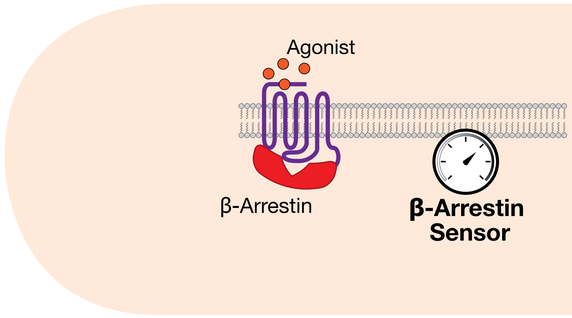
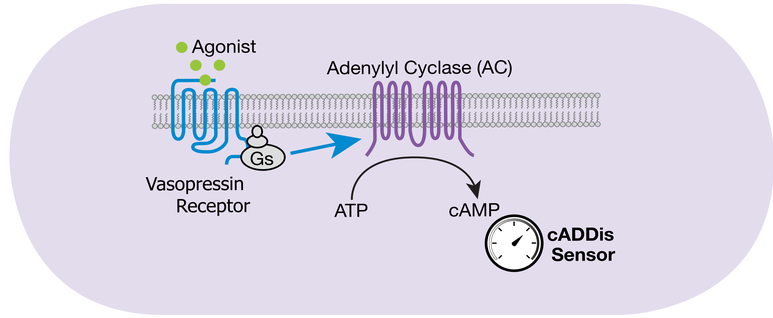
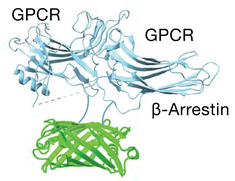


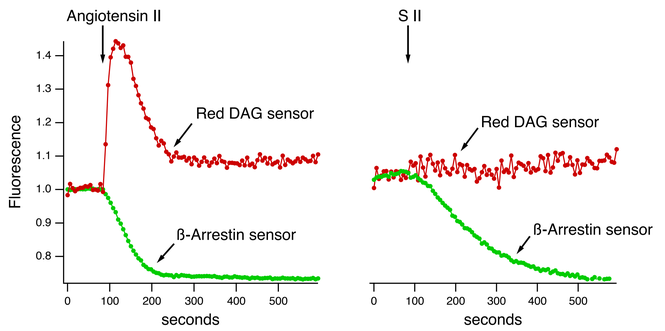




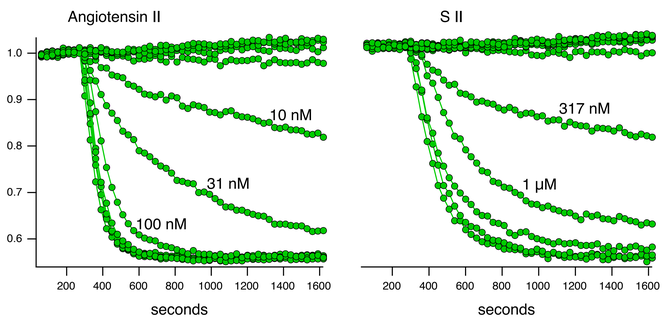
We created a green fluorescent β-arrestin biosensor.



By testing hundreds of prototypes, we found a sensor that was bright enough to be used on a plate reader with an excellent Z' value for detection of AT1R activation with angiotensin II.

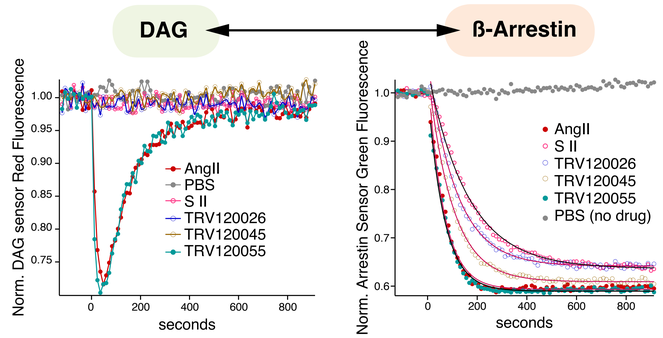


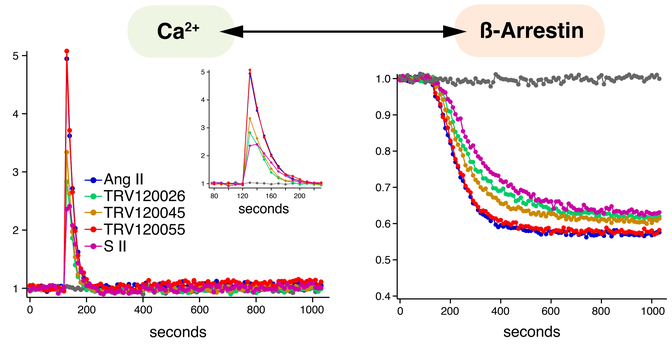
Cells expressing the AT1R receptor are activated by either angiotensin II or S II. Angiotensin II produces a robust increase in DAG and fast β-arrestin response, while the β-arrestin biased ligand S II produces no detectable DAG response and a slow β-arrestin response.



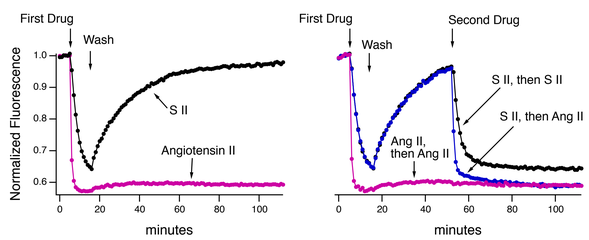
The arrestin sensor reliably reports differences in agonist concentration through a change in kinetics as well as the amplitude.

Different biased compounds at the Angiotensin II receptor (AT1R) produce responses with different kinetics.



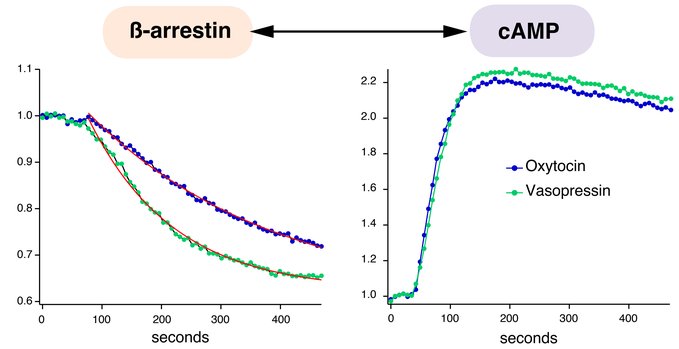


β-arrestin sensor can follow receptor desensitization



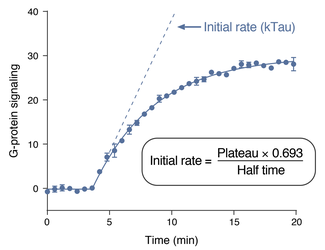
To explore receptor desensitization, we first washed out the drug. Cells expressing the angiotensin receptor (AT1R) and activated with angiotensin II did not return to baseline. However if they were treated with S II, they did recover. A second application then reactivated the β-arrestin sensor.

Vasopressin and Oxytocin produce different response kinetics at the Vasopressin receptor.



The human vasopressin receptor (human AVPR2) signals through cAMP and β-arrestin. Oxytocin and Vasopressin both activate the receptor and elevate cAMP. The β-arrestin responses are consistently different.

Kinetics Can be Used to Measure Bias



Quantifying bias involves comparing the responses of the G-protein based second messengers with the β-arrestin sensor. The kinetic data that all of the sensors produce can be used to accurately determine the initial rate of the reaction at saturating concentrations of the agonist. These initial rates can then be compared to arrive at the bias ratio. A full description of this approach can be found at:

Hoare, S.R.J., Tewson, P.H., Quinn, A.M., and Hughes, T.E. (2019).
A new kinetic method for measuring agonist efficacy and ligand bias using high resolution biosensors and a kinetic data analysis framework.
bioRxiv doi: https://doi.org/10.1101/772293

Ligand	Arrestin k_r (NFU.min^{-1}) [1]	Arrestin k_r (% AngII)	Diacylglycerol k_r (NFU.min^{-1}) [1]	Diacylglycerol k_r (% AngII)	Arrestin/DAG k_r ratio (% AngII ratio)	Calcium k_r (NFU.min^{-1}) [1]	Calcium k_r (% AngII)	Arrestin/Ca^{2+} k_r ratio (% AngII ratio)
AngII	0.40 ± 0.03	100	1.7 ± 0.2	100	1.00	2.2 ± 0.2	100	1.0
TRV120055	0.37 ± 0.02	92	2.1 ± 0.1	120	0.74	2.5 ± 0.2	110	0.81
TRV120045	0.38 ± 0.06	93				0.66 ± 0.02	30	3.1
TRV120026	0.25 ± 0.03	62				0.46 ± 0.08	21	3.0
SII	0.20 ± 0.04	49				0.25 ± 0.06	12	4.2

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