Coloring β-arrestin signaling green, and G-protein signaling red, a new live cell assay for quantitatively measuring agonist bias at seven transmembrane domain receptors.

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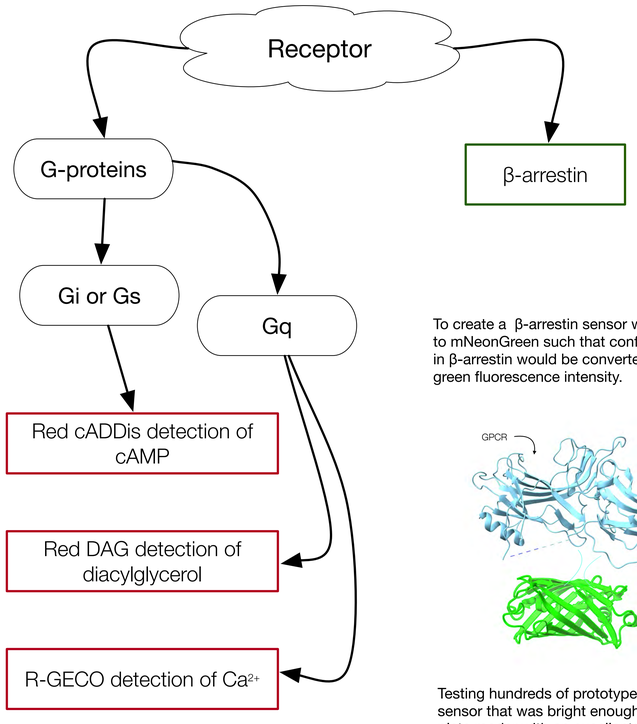
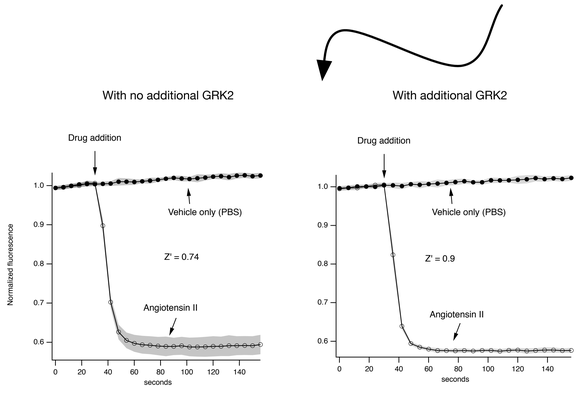
We created a green fluorescent β-arrestin sensor to pair with our red sensors for G-protein signaling in living cells.

7-transmembrane domain receptors can signal through different pathways: mediated by G-proteins and β-arrestin.



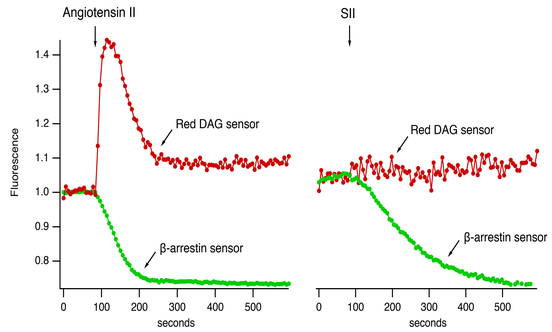
To create a β-arrestin sensor we fused β-arrestin to mNeonGreen such that conformational changes in β-arrestin would be converted into changes in green fluorescence intensity.

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.

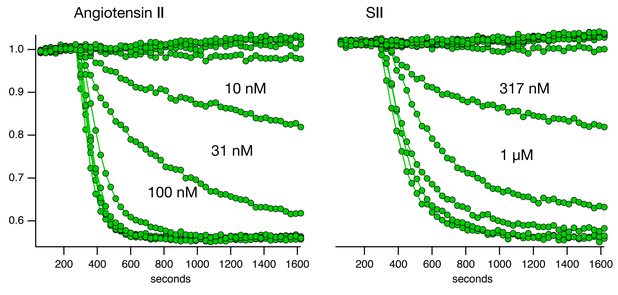


Plotted above is the average response of many wells of cells expressing the AT1R receptor as well as the β-arrestin sensor. The standard deviation is illustrated in gray. The Z' value for detecting Angiotensin II activation (30 uM) was 0.74. Additional GRK2 reduced the variability and improved the Z'.

Red and Green sensors simultaneously measure both signaling pathways in the same living cells.

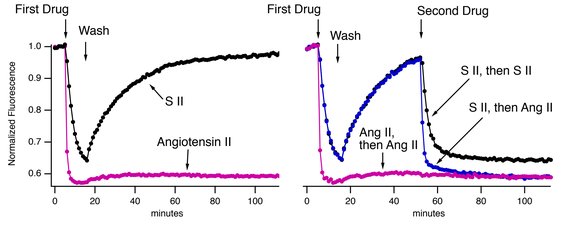


Cells expressing the AT1R receptor are activated by either Angiotensin II or SII. Angiotensin II produces a robust increase in DAG and fast β-arrestin response, while the β-arrestin biased ligand SII produces no detectable DAG response and a slow β-arrestin response.



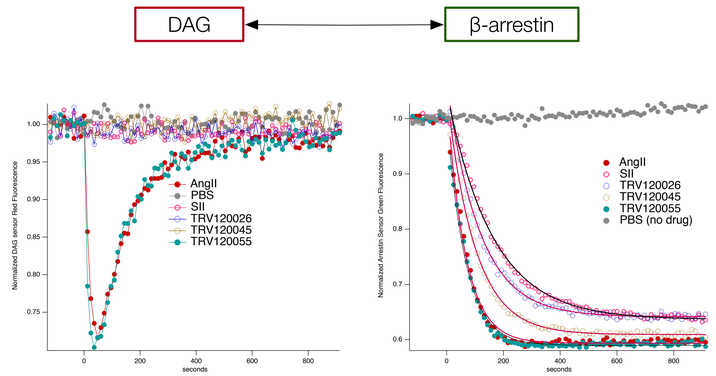
The arrestin sensor reliably reports differences in agonist concentration through a change in kinetics as well as the amplitude.

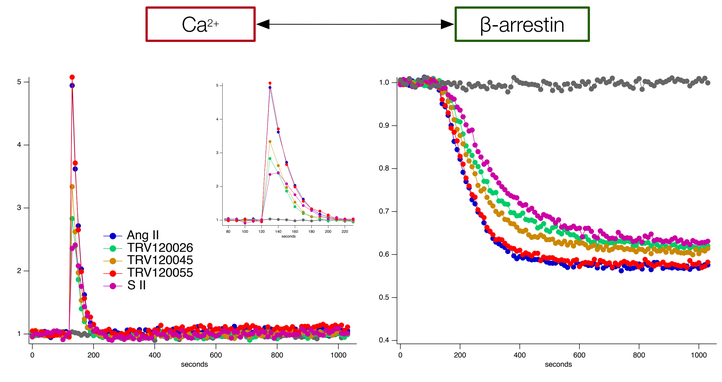
The β-arrestin sensor can follow receptor desensitization.

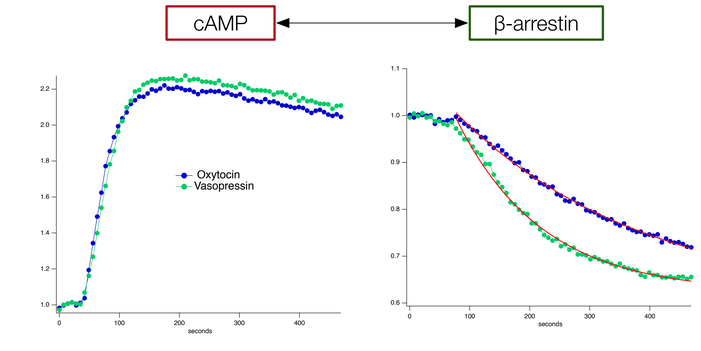


To explore receptor desensitization we first washed out the drug. Cells expressing the angiotensin receptor (AT1R) and activated with Angiotensin II did not return to baseline. However if they were treated with SII, they did recover. A second application then reactivated the β-arrestin sensor.

Real time measurements of both signaling pathways provide a new level of precision in measuring ligand bias.







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.