Measuring biased agonism with a kinetic analysis platform for real time data from fluorescent biosensors

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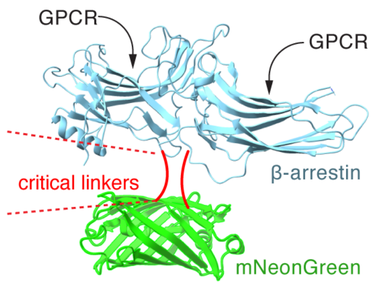
Overview

- Presently, biased agonism analysis requires specialist expertise.
- Here a new simpler method is described based on signaling kinetics (the initial rate).
- Robust biosensors from Montana Molecular provide biochemical-quality real-time signaling data in live cells.
- The model is applied to these data to measure biased agonism for angiotensin A1 and vasopressin V2 receptors.

Data analysis

- The initial rate of signaling is a measure of ligand efficacy. This parameter is called kTau[1].
- kTau is a direct measure of efficacy[1]. Consequently, bias can be quantified by dividing kTau for one pathway by kTau for another.
- Equations are applied to biosensor time course data to measure kTau. This is done for a maximally-stimulating agonist concentration[1].

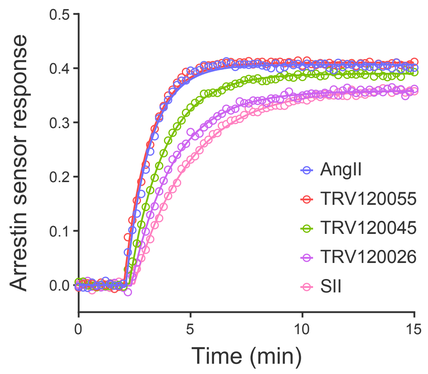
Biosensors

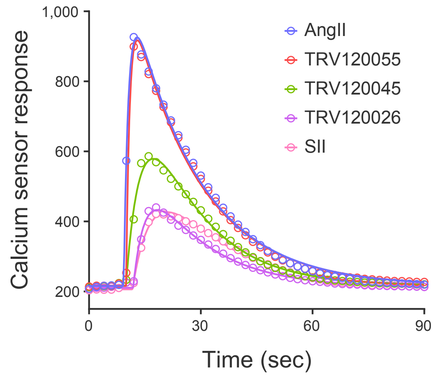


- Genetically-encoded fluorescent sensors (Montana Molecular) were used to measure arrestin recruitment, Ca^{2+} signaling (Red GECO)[2] and cAMP generation (Red-cADDis)[3,4].
- The BioTek Synergy MX plate reader was used to obtain high temporal resolution data for the arrestin signal. Short read times were enabled by the brightness (high quantum yield) of the sensors.

References
1. J. Theor. Biol. 2018, 446: 168-204
2. PLOS One 2012, 7: e42791
3. SLAS Discovery 2016, 21: 298-305
4. SLAS Discovery 2018, 23: 898-906

Angiotensin AT1 receptor





$$Y = Plateau \times (1 - e^{-K.T})$$

$$kTau = Plateau \times K$$

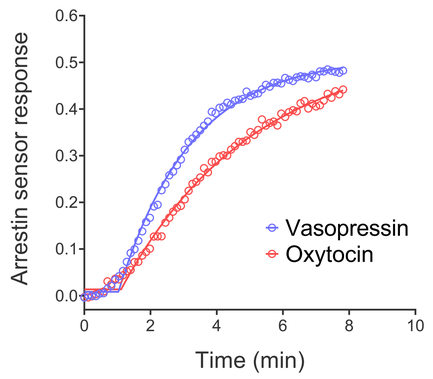
$$Y = \frac{C}{K1 - K2}(e^{-K2.T} - e^{-K1.T})$$

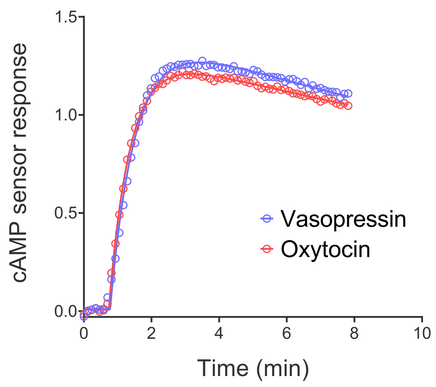
$$kTau = C$$

	kTau arrestin	kTau Ca^{2+}	Bias ratio
Ang II	100	100	1.0
TRV120055	99	120	0.8
TRV120046	66	21	3.1
TRV120026	50	15	3.3
SII	38	10	3.8

kTau values – % of Ang II
Bias ratio – kTau arrestin / kTau Ca^{2+}

V2 Vasopressin receptor





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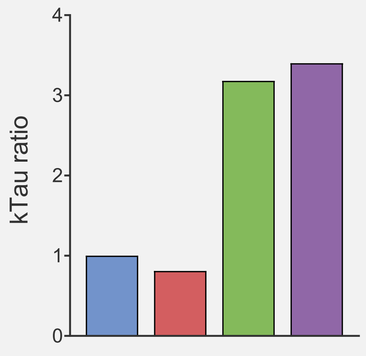
$$kTau = C$$

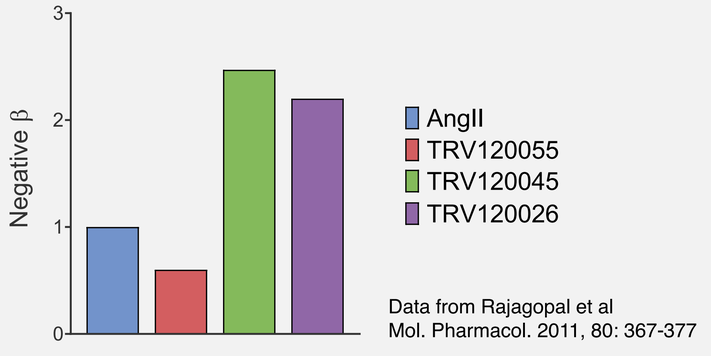
	kTau arrestin	kTau cAMP	Bias ratio
Vasopressin	100	100	1.0
Oxytocin	57	109	0.5

kTau values – % of vasopressin
Bias ratio – kTau arrestin / kTau cAMP

kTau method



Operational model



Data from Rajagopal et al
Mol. Pharmacol. 2011, 80: 367-377

Conclusions

- Quantifying biased agonism can be complex.
- We have developed a new simpler method based on initial rates (kTau).
- Robust biosensors enable the analysis platform to be applied.
- The new analysis gives similar estimates of bias to existing methods.
- This approach will improve the efficiency of biased agonism drug discovery.