# Mechanistic model of oxytocin/vasopressin-stimulated G protein-coupled receptor (GPCR).

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## Context

Around 30% of commercialised drugs interacts at cell membrane with a G protein-coupled receptor (GPCR). Oxytocin (OT) and vasopressin (AVP) are hypothalamic peptides that bind to four GPCR: oxytocin receptor OTR and vasopressin receptors V1A, V1B and V2. These binding lead to different interactions with downstream signalling transducers. Known for their prosocial effect, these pathways are an active area of research in autism spectrum disorders.
Understanding downstream intracellular signal transduction dynamics is mandatory to ensure drugs safety and efficacy.

## Objectives

- Building a mechanistic model of oxytocin and vasopressin-stimulated GPCR using population compartmental analysis
- Proposes new quantifications of the interaction of oxytocin or vasopressin-stimulated GPCR with downstream G proteins.

## Materials

Bioluminescence resonance energy transfer (BRET) assays the proximity of a bioluminescent donor and a fluorescent acceptor. Coupling donor and acceptor with two proteins of interest gives a unique insight in these protein-protein interactions in real time. Thus, it is a well-established technique for quantitative monitoring of these actors and their dynamics in signal transduction.

A total of 480 BRET assays were performed to quantify the interaction of murine mOTR, mV1A mV1B, and mV2 or human hV2 receptors tagged with the Renilla luciferase (Rluc8) with downstream Gs, Gi or Gq tagged with enhanced yellow fluorescent protein (Venus), after stimulation with OT or AVP. OT and AVP doses were ranging from 0 to $10^{-4}$ M. Upon substrate coelenterazine addition Rluc8 emits a 480nm signal (BRET1). If Venus is present within a 100 Å distance, it is excited and emits a 530nm signal (BRET2). The intensities of BRET1 and BRET2 are monitored and used for analysis. BRET assays were performed in murine neuroblastoma cell (mNeuro2a).

## Model

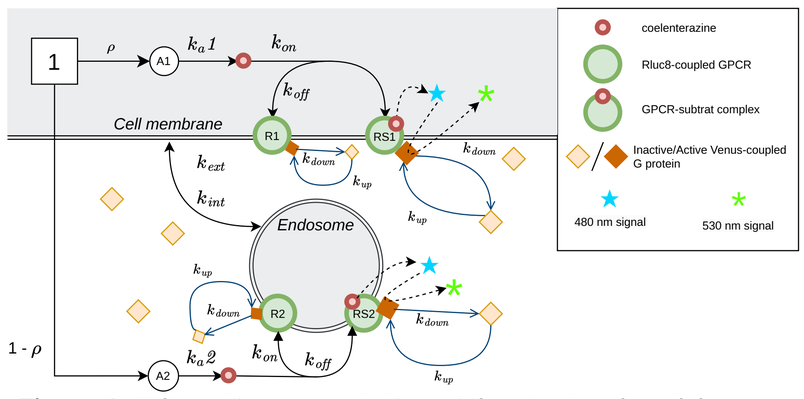


**Figure 1.** *Schematic representation of the structural model.*

Unity dose of coelenterazine diffuses to cell membrane ($\rho$) or endosomes ($1 - \rho$) . The absorption of the coelenterazine is described with a one-order rate $k_a1$ to reach Rluc8 on membrane GPCR and $k_a2$ on endosomal GPCR. The substrate cant bind and be consumed by the GPCR to emit at 480 nm.

Intracellular Venus-coupled G proteins can distribute to the proximity of the late 480 nm signal ($k_{up}$) to emite a 530 nm signal. dissociation rate $k_{down}$ is reduced by the dose of OT or AVP ($k_{down}max$, $C_{50}$, $\sigma$).

A scale correction is assured by the estimation of a global gain G defined as $BRET = signal \times 10^{gain/10}$. Parameters were estimated using non-linear mixed effect model (Monolixsuite 2023R1, lixoft, Antony, Frannce).

## Results

### Fitting

| parameter (unit) | $\theta$ (r.s.e. %) | $\omega$ (r.s.e. %) |
|---|---|---|
| $T_{ini}$ (min) * | -2.04 (20) | 2.25 (4) |
| $k_a1$ ($min^{-1}$) | 0.024 (6) | 1.08 (4) |
| $k_a2$ ($min^{-1}$) | 0.26 (7) | 1.24 (4) |
| $\rho$ (-) ** | 0.53 (1) | 0.27 (4) |
| *gain* (dB) * | 76.79 (<1) | 1.19 (4) |
| $k_{on}$ ($min^{-2}$) | 0.05 (7) | 1.11 (4) |
| $k_{off}$ ($min^{-1}$) | 0.25 (5) | 0.78 (4) |
| $R_{ini}$ | 1.68 (28) | 1.11 (4) |
| $\beta_{mV2}$ | -0.68 (44) | |
| $k_{int}$ ($min^{-1}$) | 3.79 (6 ) | 1.05 (4) |
| $k_{ext}$ ($min^{-1}$) | 0.76 (6 ) | 1.05 (4) |
| $G_{ini}$ | 0.35 (6 ) | 0.83 (4) |
| $k_{up}$ ($min^{-2}$) | 4.98 (5) | 0.83 (4) |
| $k_{down}$ ($min^{-1}$) | 9.31 (5) | 0.83 (4 ) |
| $k_{down}max$ ($min^{-1}$) | 0.015 (39 ) | 0.36 (18) |
| $\beta_{miniGq}$ | 3.47 (10) | |
| $\beta_{miniGs}$ | 1.21 (26) | |
| $\beta_{mV1B}$ | 1.39 (21) | |
| $\beta_{mV2}$ | 1.9 (20) | |
| $C_{50}$ | $2.2 \times 10^{-6}$ (69) | 1.01 (12) |
| $\beta_{miniGs}$ | -0.72 (46) | |
| $\beta_{mV1B}$ | 2.88 (21) | |
| $\beta_{mV2}$ | 2.81 (24) | |
| $\sigma$ | 8.06 (<1) | 0.00036 (22) |
| $\beta_{miniGq}$ | -0.93 (<1) | |
| $\beta_{miniGs}$ | -0.98 (<1) | |
| $\beta_{OXT}$ | -0.0034 (7) | |
| $\beta_{mV1B}$ | -0.098 (<1) | |

**Table 1.** *Population parameters: All parameters were correctly estimated. *Normal and **Probit distributions.*

BRET data were correctly described. All the parameters values and their variabilities can be estimates with r.s.e. < 10%.

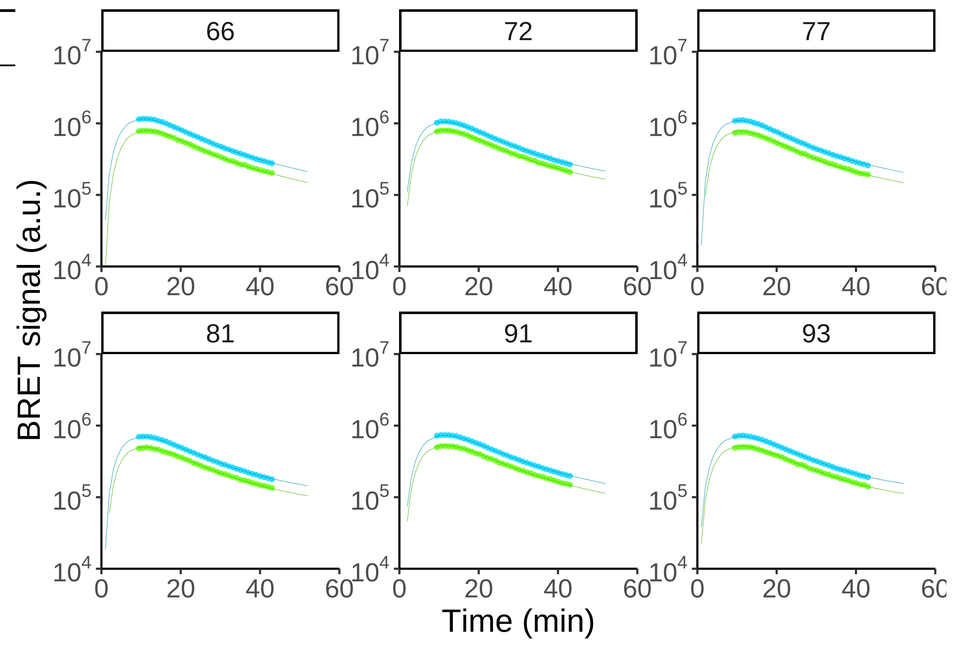


**Figure 2.** *Observed (dots) and model predicted (lines) 480 nm (blue) and 530 nm (green) BRET signals over time for 6 conditions.*

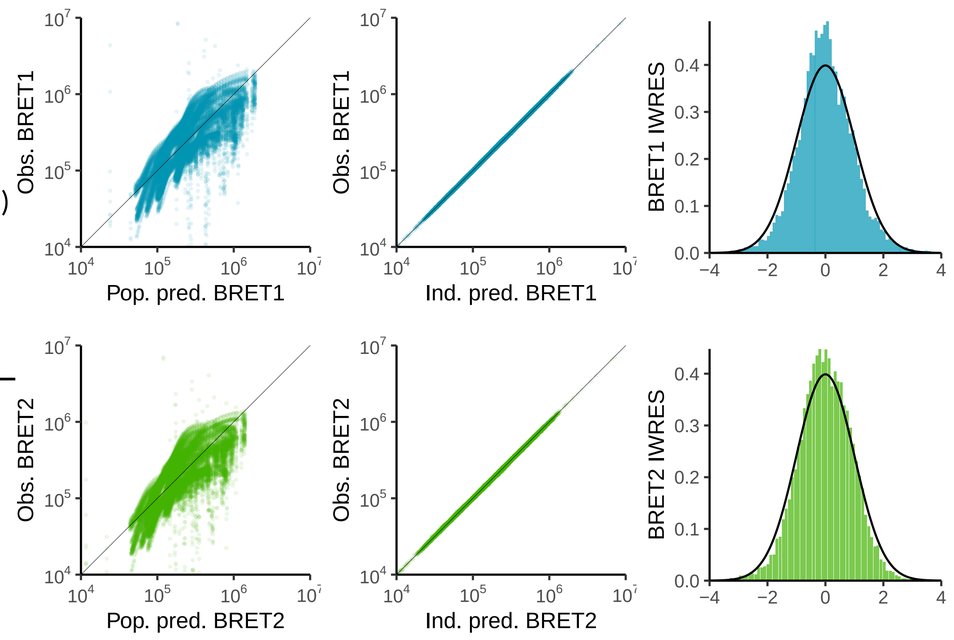


**Figure 3.** *Observed vs. population (left) or individual (center) predictions and individual weigth residue distribution of 480 nm (blue; top) and 530 nm (green bottom) BRET signals.*

## Conclusions and perspectives

After cautious scaling of the data, BRET data can be modelled to extract information on RCPG dynamics after ligand binding.

This modelling approach gives robust description of BRET data. Estimation of a global gain informs about cell state and level of receptor expression variabilities. Mixed effect modelling allows to quantify the potential influences of the different conditions assays on signals.

Despite OT and AVP share similar molecular characteristics they display important differences in signalling transduction at the four receptors. The same modelling approach can be used for flowing downstream protein-protein interactions of the signal transduction cascade.

## References

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[3] Reiter *et al.* 2017. β-arrestin signalling and bias in hormone-responsive GPCRs. Molecular and Cellular Endocrinology.