

Creation of a large-spectrum GPCR biosensor for functional *in vitro* safety and systems pharmacology analysis

Mancini A^{1*}, Avet C^{3*}, Breton B^{1*}, Normand C¹, Gross F¹, Lukasheva V³, Hogue M³, Morissette S¹, Fauman E⁴, Fortin JP⁴, Leroy X², Schann S², Le Guillou C³, Bouvier M³

¹Domain Therapeutics NA Inc / ²Domain Therapeutics SA / ³Institut de Recherche en Immunologie et en Cancérologie / ⁴Pfizer Global R&D

It is now recognized that a given GPCR can engage multiple signaling pathways and that specific ligands can selectively engage different subsets of these pathways. Using 15 pathway-selective bioluminescence resonance energy transfer (BRET) biosensors monitoring live-cell activation of each Gα protein and βarrestin, we mapped the signaling signatures of over 100 therapeutically relevant human GPCRs in response to their endogenous ligands. Analysis of G protein coupling for the GPCRs studied herein revealed that over 95% of these receptors couple to Gz and/or G15 pathways (Fig. 1).

Accordingly, the creation of a biosensor capable of simultaneously measuring activation of both pathways could serve as a “large-spectrum” sensor applicable to screening and receptor deorphanization campaigns. Additionally, a Gz/15 sensor could be applied to *in vitro* safety pharmacology and systems pharmacology profiling. In such applications, compounds are screened against a range of molecular targets distinct from the intended therapeutic target (i.e., “off-target” activity) and linked to adverse drug reactions or therapeutic efficacy. We thus proceeded to develop a novel large spectrum Gz/G15 biosensor and demonstrated its use for *in vitro* safety and systems pharmacology analysis (Fig 2).

Validation of large-spectrum Gz/G15 biosensor on 24 GPCRs included in the safety target panel.

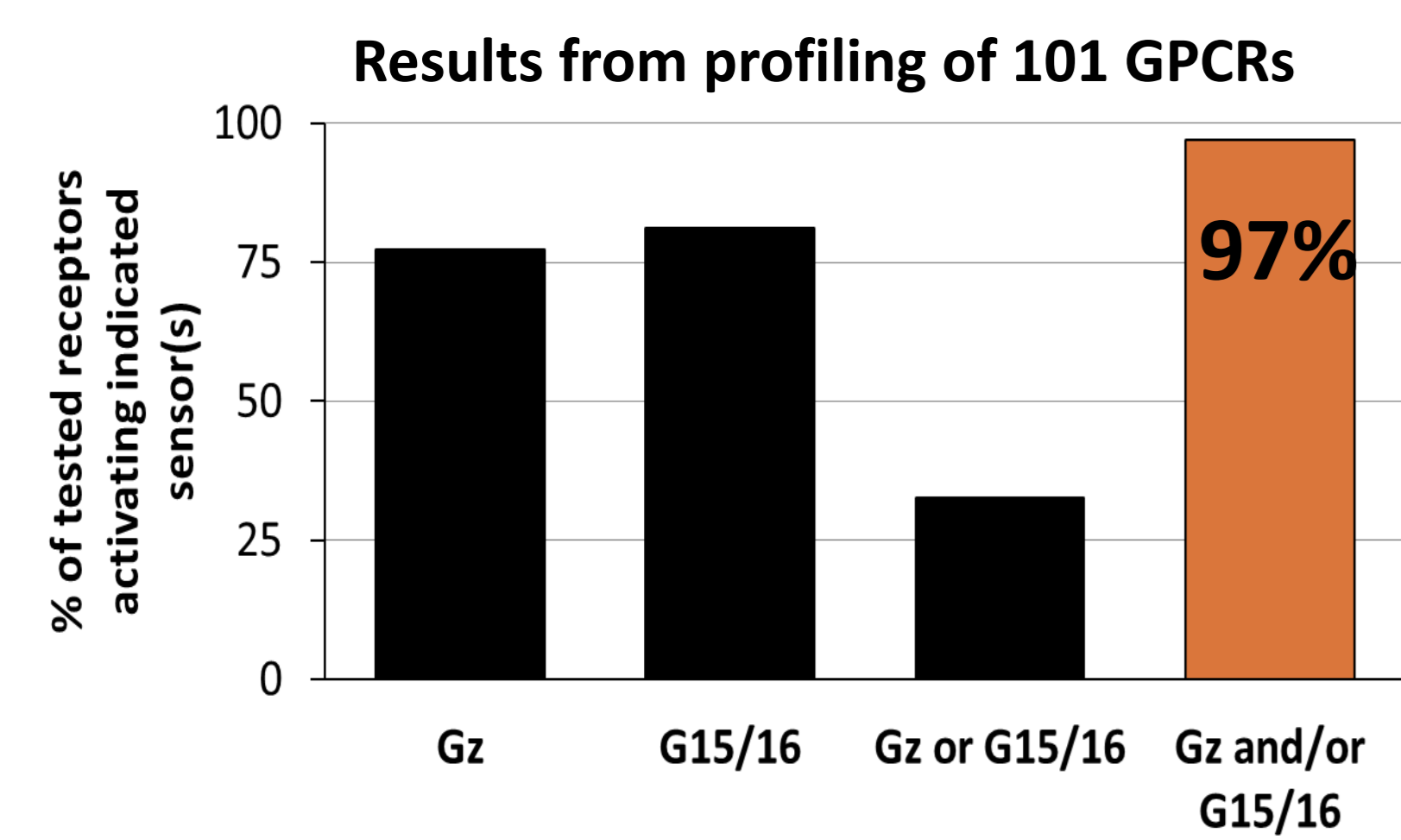


Figure 1: Relevance of the large-spectrum Gz/G15 biosensor.

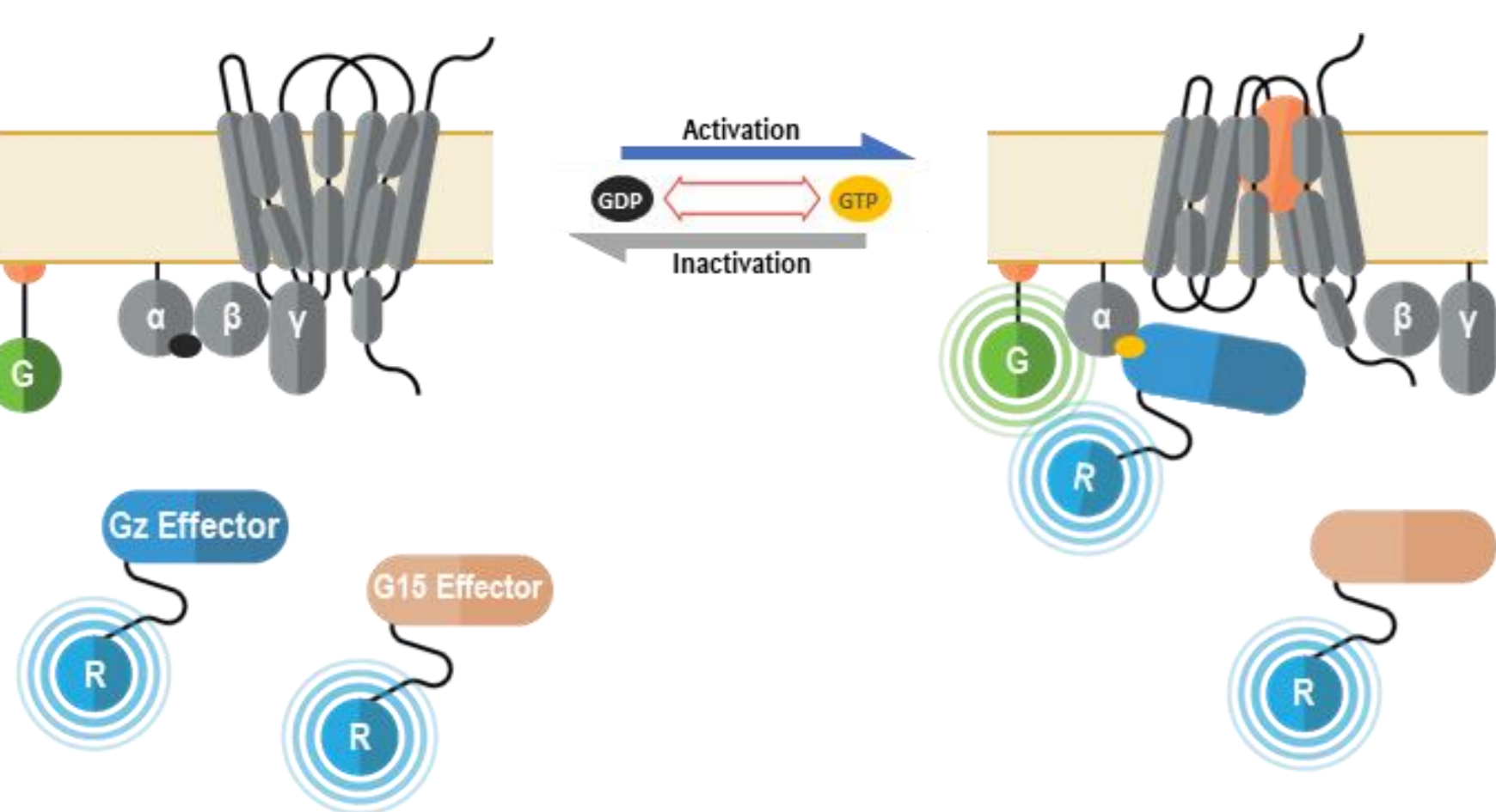


Figure 2: Large-spectrum Gz/G15 biosensor assay principle.

- More than 95% of the 101 GPCRs tested coupled Gz or G15 pathways
- Development of a biosensor depicting the recruitment of both Gz and G15 pathways:
 - Gz/G15 large-spectrum biosensor
 - BRET biosensor
 - Leaves GPCRs untagged

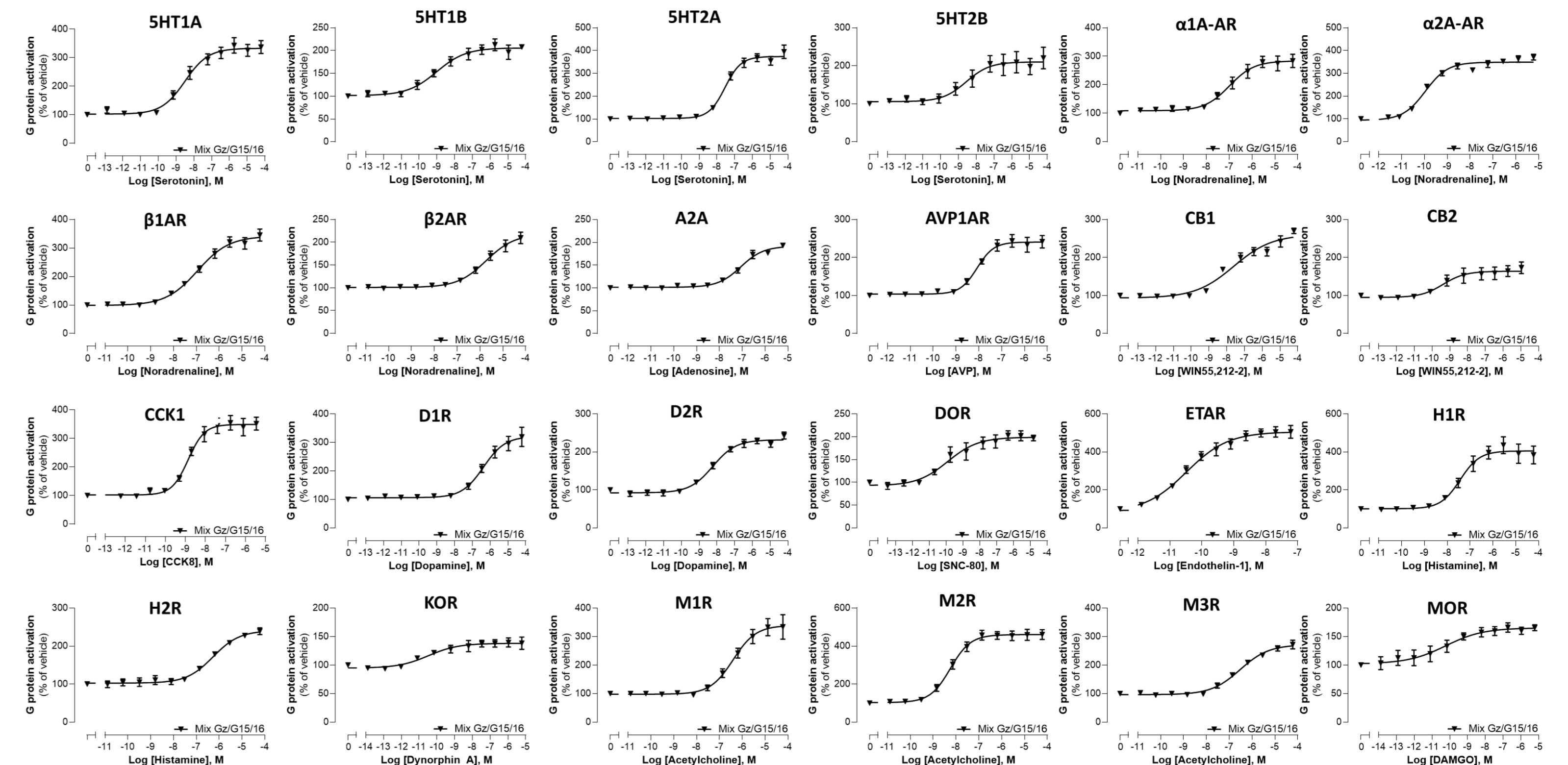


Figure 3: Dose response curves generated with the Gz/G15 biosensor on a set of 24 GPCRs (“The safety panel”).

- The large-spectrum Gz/G15 biosensor engaged all 24 GPCRs in a ligand dose-dependent fashion → **functionality** of this sensor;
- The Gz/G15 sensor detected ligand-induced activation of receptors largely or uniquely coupled to Gz (e.g., CB2) or G15 (e.g., A2A), as well as receptors coupled (to varying degrees) to both pathways;
- Data were **reproducible** across experimental replicates with robust assay windows: **HTS compatibility** of this assay;
- Data support the **suitability of the large-spectrum Gz/G15 biosensor for deorphanization campaigns.**

Applicability of the large-spectrum Gz/G15 biosensor for *in vitro* safety pharmacology and systems pharmacology profiling.

	1	2	3	4	5	6	7	8	9	10	11	12
A	TrueBRET	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR	hA2A	hAVP1AR	control
B	TrueBRET	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR	control
C	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR
D	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR
E	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR
F	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR
G	control	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	TrueBRET
H	control	hβ1AR	hβ2AR	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	TrueBRET

	1	2	3	4	5	6	7	8	9	10	11	12
A	1,01	0,94	1,02	0,93	0,95	0,96	0,94	0,93	0,93	0,97	0,89	0,98
B	1,03	1,05	1,02	4,13	0,92	0,91	1,01	0,93	0,93	0,95	1,00	0,93
C	0,97	0,91	0,97	0,94	0,94	1,02	0,92	0,86	0,93	0,95	0,91	0,91
D	1,03	0,92	1,01	1,07	4,11	0,84	0,95	1,02	0,96	0,90	0,92	1,02
E	0,98	0,90	0,90	0,99	0,92	1,01	0,98	0,84	0,92	0,96	0,96	0,95
F	1,02	0,93	1,02	1,01	4,02	0,88	0,91	0,99	0,92	0,88	0,98	1,01
G	0,99	0,93	0,90	0,91	0,90	0,93	1,02	0,89	0,89	0,89	0,91	0,94
H	0,95	0,99	0,92	0,98	0,90	1,00	1,00	4,27	0,84	0,90	0,97	1,00

Figure 5: Specificity test assay: Adding CCK-8 ligand on the GPCR safety panel. Fold response vs baseline.

	1	2	3	4	5	6	7	8	9	10	11	12
A	TrueBRET	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR	hA2A	hAVP1AR	control
B	TrueBRET	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR	control
C	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR
D	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR
E	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	hβ1AR	hβ2AR
F	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	hETAR	hH1R	hH2R	hKOR
G	control	hM1R	hM2R	hM3R	hMOR	h5HT1a	h5HT1b	h5HT2a	h5HT2b	ha1A-AR	ha2A-AR	TrueBRET
H	control	hβ1AR	hβ2AR	hA2A	hAVP1AR	hCβ1	hCβ2	hCCK1	hD1R	hD2R	hDOR	TrueBRET

Figure 6: Cross-reactivity assay: Adding Noradrenaline to the GPCR safety panel.

- Certain ligands displayed functional cross-reactivity with GPCRs other than their natural targets:
 - Noradrenaline and serotonin activated the large-spectrum sensor in hDRD2-transfected cells (Fig 6, green wells);
- Responses confirmed via ligand dose-response assays using biosensors specific for pathways known to be engaged by hDRD2 (i.e., GoB and βarrestin2) (Fig 7);
- G protein and βarrestin2 activation by dopamine, noradrenaline and serotonin in hDRD2-transfected cells was blocked by DRD2 antagonist eticlopride.
- Validation of the large-spectrum Gz/G15 biosensor for *in vitro* safety pharmacology and systems pharmacology profiling.

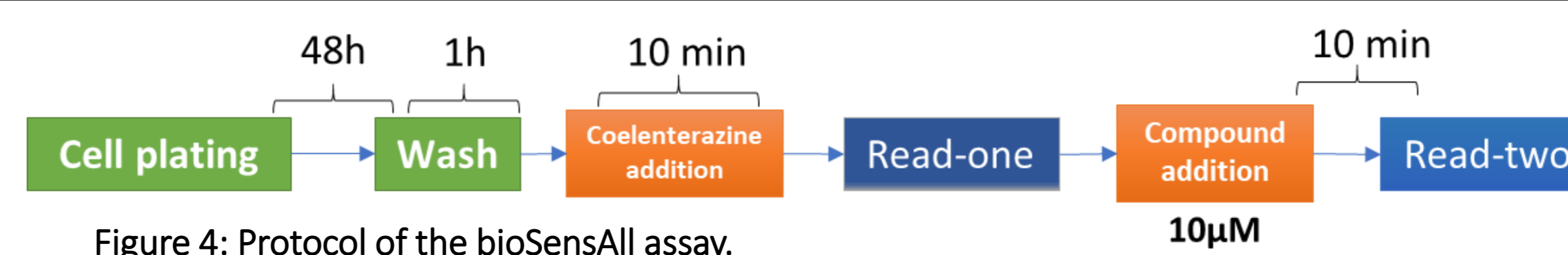


Figure 4: Protocol of the bioSensAll assay.

Plate are seeded with cells transfected with i) the Gz/G15 biosensor, and ii) one of the 24 GPCRs in the safety target panel (Fig 5). Baseline BRET values are recorded (*read-one*). Plate is then treated with ligand CCK-8 and BRET is measured (*read-two*). CCK8 agonist activity is then determined using the following ratio:

$$[\text{Read-two BRET value} / \text{Read-one BRET value}]$$

- Only CCK1-expressing cells responded to CCK-8: Validation of the **biosensor specificity** (Fig 5, green wells);
- Large assay window** detected (~4-fold stimulation vs. baseline);
- Results displayed little variability across technical quadruplicates, demonstrating **assay robustness.**

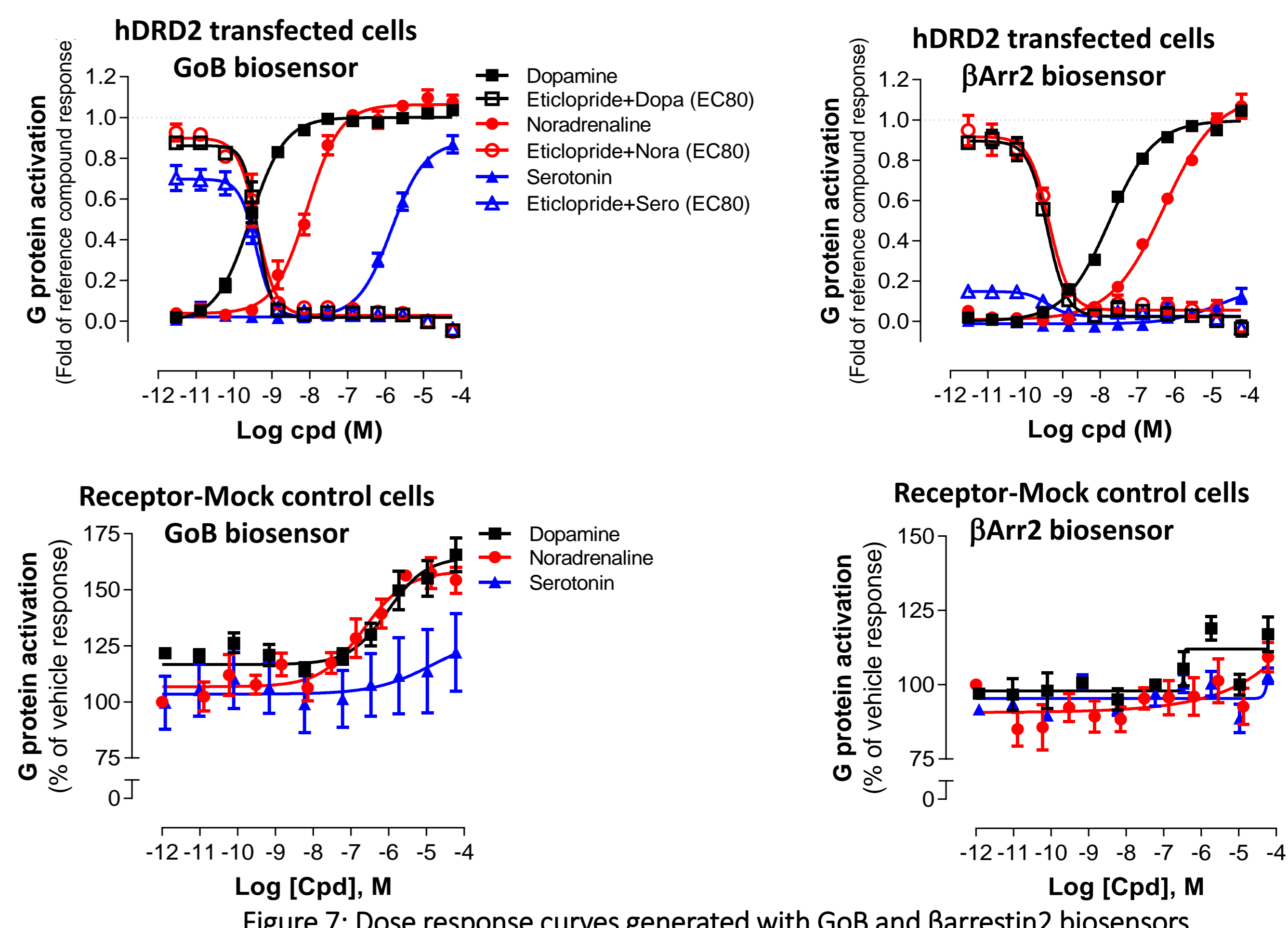


Figure 7: Dose response curves generated with GoB and βarrestin2 biosensors.

This study served as a validation of the utility of the **Gz/G15 large-spectrum biosensor for safety and systems pharmacology profiling.** Moreover, our results support the use of this cell-based functional assay in high throughput receptor **deorphanization and functional screening programs.**