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

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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.

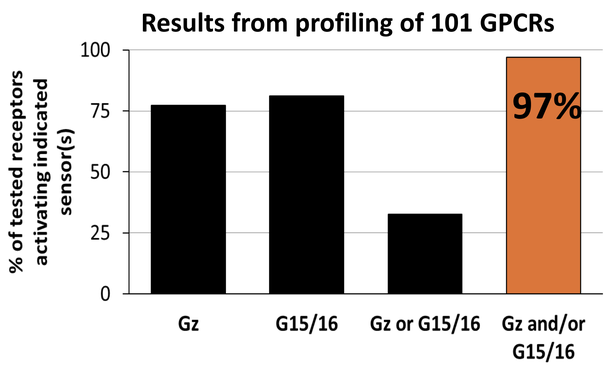

Figure 1: Relevance of the large-spectrum Gz/G15 biosensor.

- ❑ 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**

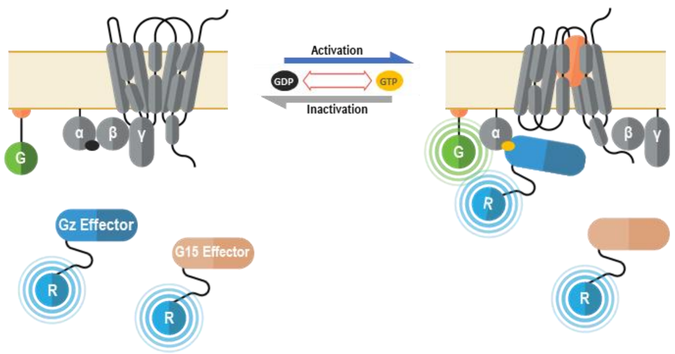

Figure 2: Large-spectrum Gz/G15 biosensor assay principle.

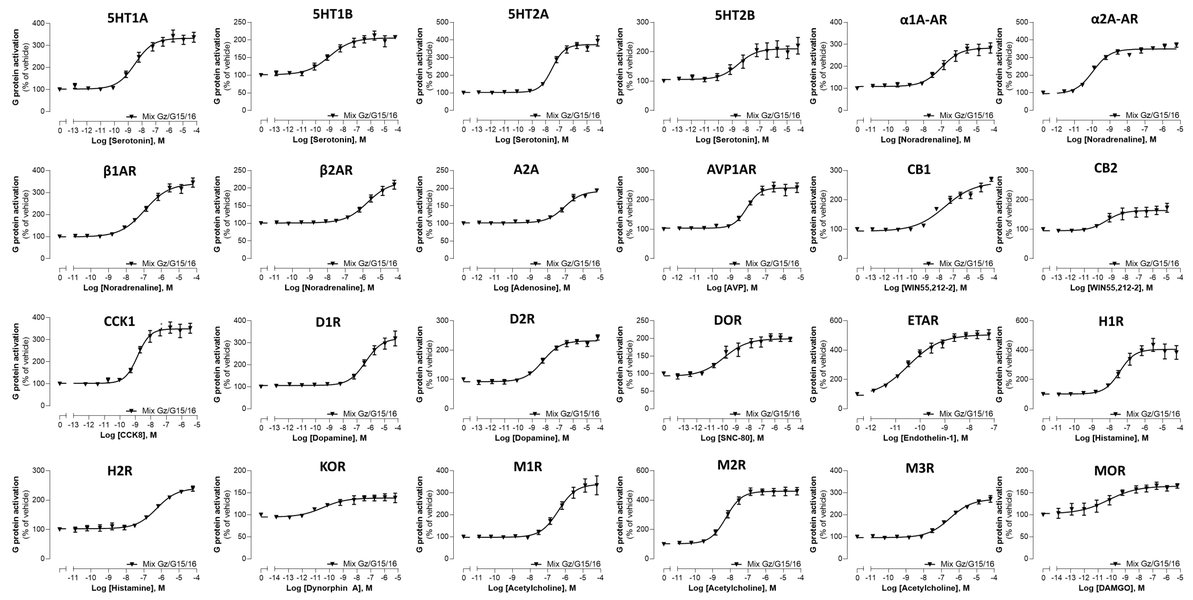

Figure 3: Dose response curves generated with the Gz/G15 biosensor on a set of 24 GPCRs (``The safety panel``).

- ❑ 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	hα1A-AR	hα2A-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	hα1A-AR	hα2A-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	hα1A-AR	hα2A-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	hα1A-AR	hα2A-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	**hα1A-AR**	**hα2A-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	**hα1A-AR**	**hα2A-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	**hα1A-AR**	**hα2A-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	**hα1A-AR**	**hα2A-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:

[Read-two BRET value / 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.**

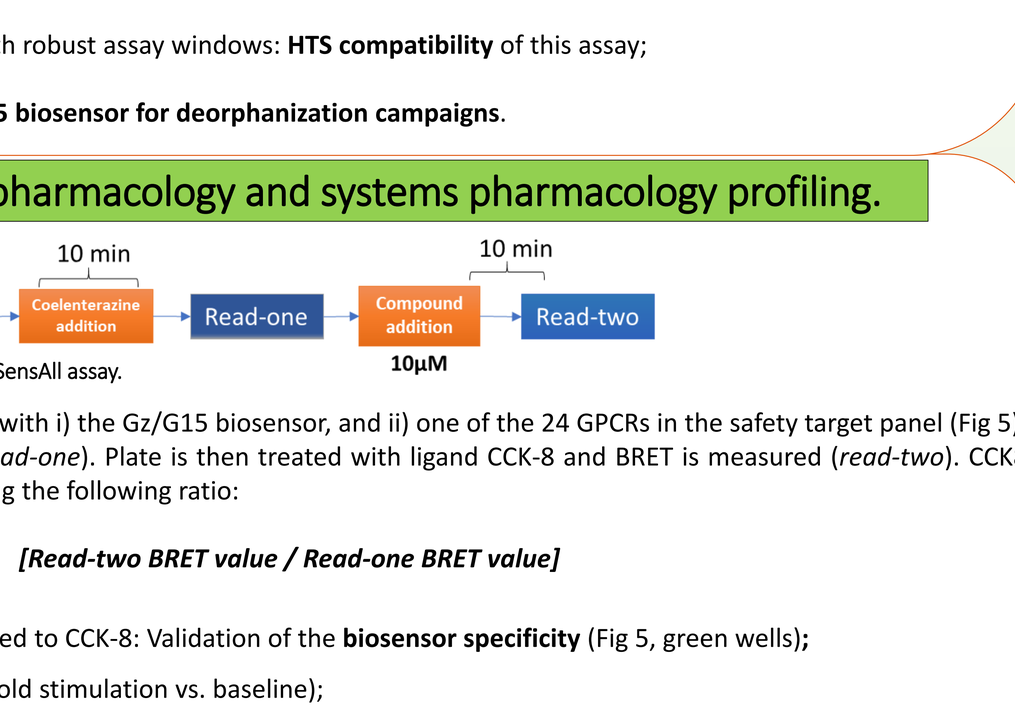

Figure 7: Dose response curves generated with GoB and βarrestin2 biosensors .

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.