USE OF NOVEL ebBRET BIOSENSORS FOR COMPREHENSIVE SIGNALING PROFILING OF ONE HUNDRED THERAPEUTICALLY RELEVANT HUMAN GPCRs

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Introduction

Functional selectivity is the ability of a given GPCR to engage multiple signaling pathways, with distinct ligands of the given receptor displaying different efficacies in engaging receptor-coupled pathways. Exploitation of functional selectivity in drug development will require an exhaustive description of the effectors that can be engaged by a given receptor, thus revealing receptor- and ligand-specific signaling signatures. Here, we describe a novel suite of enhanced bystander bioluminescence resonance energy transfer (ebBRET)-based biosensors that were used to define the signaling profiles of 100 therapeutically relevant human GPCRs in response to endogenous (or prototypical) ligands.

Material and Methods

GPCR signaling profiling was performed with 15 pathway-selective ebBRET biosensors monitoring the activation of specific Gα proteins and βarrestins 1 and 2. The G protein biosensors represent a new generation of BRET-based sensors that measure the translocation of G protein effectors to the plasma membrane with no need for modifying the G proteins (except for Gαs) or the receptors.

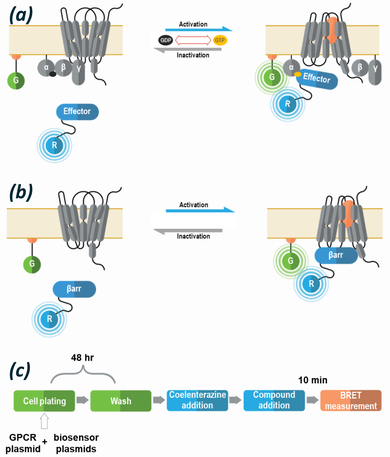

Fig. 1. ebBRET-based effector membrane translocation assay for G protein and βarrestin activation - *assay principle and workflow.* ***(a)*** Upon receptor activation, RlucII-tagged effector proteins (Effector-R) translocate towards and interact with active Gα subunits from each G protein family, thus increasing ebBRET with membrane-anchored rGFP (G). ***(b)*** Upon receptor activation, RlucII-tagged βarrestins (βarrestin-R) translocate to the plasma membrane, leading to increased ebBRET. ***(c)*** Effector membrane translocation assay workflow. The same workflow is used for all assays.

Results

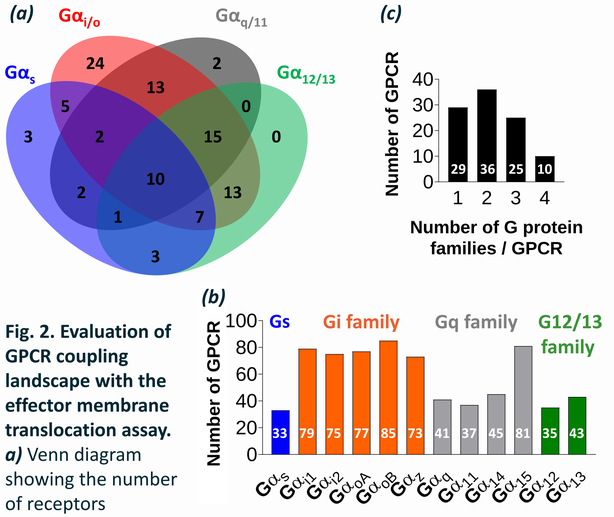

Fig. 2. Evaluation of GPCR coupling landscape with the effector membrane translocation assay. ***a)*** Venn diagram showing the number of receptors coupled to each G protein family. ***(b)*** Number of receptors that activate each Gα subunit. ***(c)*** Number of receptors that couple to members of 1, 2, 3 or 4 G protein families.

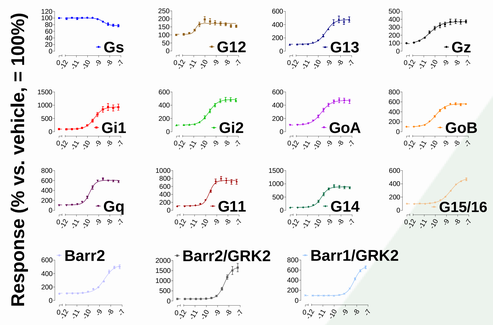

Fig. 3. Signaling profiling of the human endothelin receptor type A. HEK293 cells were transfected with plasmids coding for hET-AR and one of 15 pathway-specific biosensors. Cells were treated with endothelin 1 and the response recorded 10 mins later.

Results

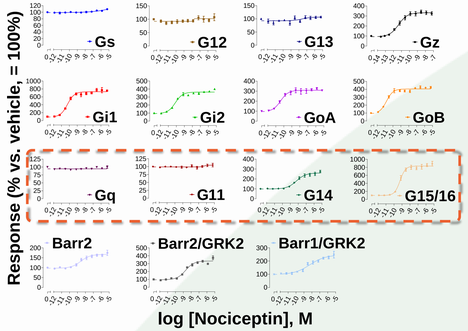

Fig. 4. Intra G protein family coupling selectivity revealed using the effector membrane translocation assay. In response to nociceptin, the human nociceptin receptor engages G14 and G15/16, but not other members of the Gq family of G proteins.

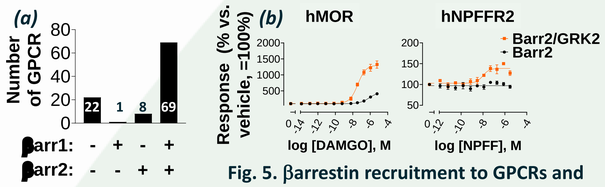

Fig. 5. βarrestin recruitment to GPCRs and its modulation by GRK2. ***(a)*** Number of GPCRs coupling to βarrestin 1 and/or βarrestin 2. ***(b)*** Co-expression of GRK2 enhances (for human mu opioid receptor (hMOR)) and even exposes (for neuropeptide FF receptor 2 (hNPFFR2)) receptor engagement of βarrestin 2.

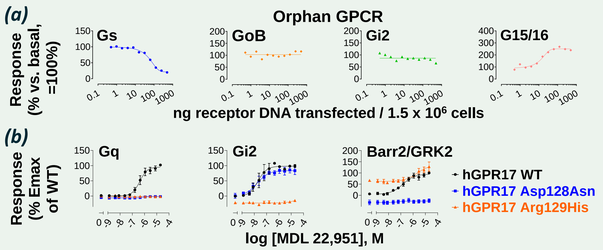

Fig. 6. Detection of constitutive activity and SNP-induced biased signaling. ***(a)*** Pathway-selective receptor dose-dependent and ligand-independent (constitutive) activity of an orphan GPCR. ***(b)*** Biased signaling of naturally occurring E/DRY motif missense variants of hGPR17.

Results

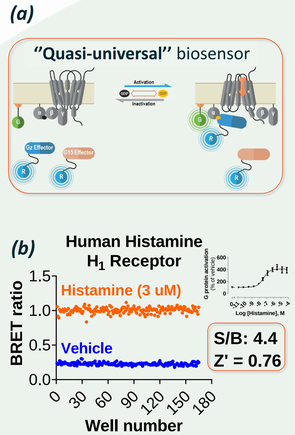

Fig. 7. Development of a "quasi-universal" biosensor for *in vitro* polypharmacology profiling. ***(a)*** Illustration of the assay principle for the quasi-universal biosensor. ***(b)*** Determination of quasi-universal biosensor robustness (Z'-factor) in 384-well format. ***(c)*** Assessment of ligand polypharmacology by screening ligands (1 or 10 μM) against various GPCRs.

Conclusion

- **The effector membrane translocation assay** is a robust and flexible ebBRET-based technology to assess GPCR signaling
  - uses unmodified receptors and G proteins (except for Gαs)
  - permits pan-pathway analysis in parallel using one protocol
  - allows for the detection of biased signaling and constitutive activity
- The data herein expose:
  - **an appreciable range of signaling plasticity** across the GPCRs analyzed
  - underappreciated coupling selectivity **within G protein families**
  - GPCR polypharmacology and transactivation
- Technology and data serve as a **key resource** for interrogating the **signaling repertoire of GPCRs,** helping deconvolute the complexities of GPCR biology and pharmacology and lead to innovative therapeutic exploitation of GPCRs