HIGH-THROUGHPUT APPLICATIONS FOR SCREENING GPCR ACTIVATION USING bioSensAll®

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Abstract

G protein-coupled receptors (GPCRs) represent the largest class of membrane-bound proteins which regulate physiological processes by engaging diverse signaling pathways. Although dysregulation of GPCR activity has been linked to pathological consequences, the involvement of specific signaling pathways toward distinct clinical repercussions remain unresolved. Thus, interrogating the complex signaling behaviors of GPCRs is not only important for understanding GPCR biology, but also essential for successfully targeting these receptors as therapeutic avenues. Here, we describe the application of our bioSens-All® technology within the context of a high throughput screening platform as well as a mechanical stimulation approach to characterize GPCR signaling.

bioSens-All® consists of a panel of 16 selective enhanced bystander bioluminescence resonance energy transfer (ebBRET)-based biosensors that monitor the activation of heterotrimeric G proteins and β-arrestins upon GPCR activation. To achieve high-throughput volumes, bioSens-All® assays were miniaturized and adapted to a 384-well format, where HEK293 cells were co-transfected with receptors and biosensors. Assays were performed using a laboratory automation workflow software to coordinate the interaction between various instruments via a robotic microplate mover. Through this automated platform, we assessed signaling profiles of ~12,000 clinically relevant variants across 60 GPCRs. Analysis was performed through an automated tool to generate and compile over 120,000 concentration-response curves. Signaling profiles for a subset of variants revealed gain- or loss-of-function properties across different signaling pathways thus also allowing us to identify genetic alterations that impart biased signaling profiles for each profiled GPCR.

Using the bioSens-All® technology in a separate application, we developed a novel *in vitro* assay detecting adhesion GPCR ADGRE5 activation following mechanical stimulation. In this assay, cells co-transfected with receptors and biosensors were seeded in 96-well plates and subjected to vigorous orbital shaking prior to BRET measurements. We demonstrated that mechanostimulation of full-length human ADGRE5-transfected cells resulted in a rapid time-dependent recruitment of β-arr2 to the plasma membrane, while such mechanosensitivity was not observed with a control receptor (mu opioid receptor, MOR) despite MOR's ability to engage β-arr2 following ligand stimulation. Finally, the assay demonstrated a Z' value of 0.73, thus highlighting its compatibility for high-throughput screening of novel therapeutic agents acting to modulate physiologically relevant modes of ADGRE5 activation.

Altogether, these applications of our bioSens-All® technology allows interrogation of diverse activation mechanisms and complex signaling behaviors of GPCRs. Ultimately, such approaches can be applied to other GPCRs to facilitate the validation of novel therapeutic targets and discovery of innovative therapeutic agents.

Instrumentation


Ligand Dispenser


Microplate Reader

Robotic Microplate Mover


BRET Substrate Injector


Automated Incubator

bioSens-All®

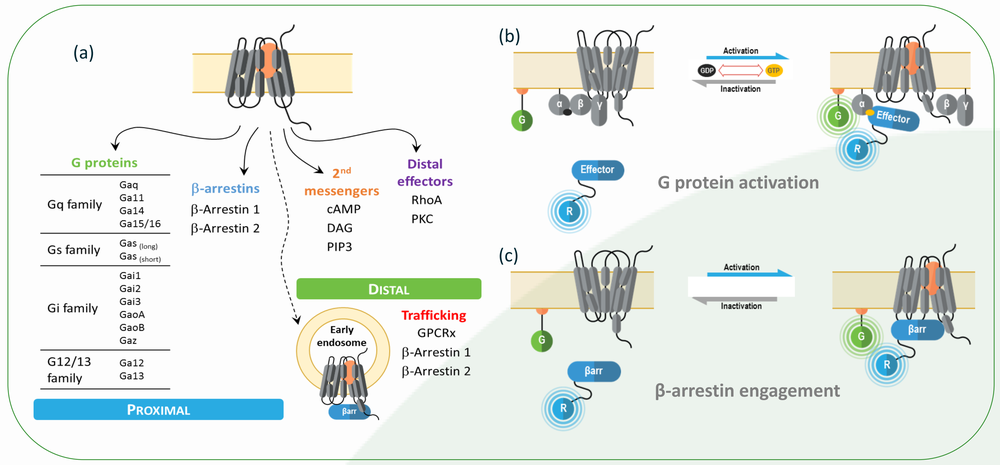

Fig. 1. BRET-based biosensors for G protein and βarr activation. (a) The bioSens-All® platform consists of 16 biosensors for monitoring activation of specific Gα proteins and βarrs downstream of GPCRs. These biosensors measure receptor proximal events engaged upon receptor activation, namely G protein activation and βarr recruitment. In addition to proximal biosensors, the technology includes sensors detecting distal effector activity and second messenger generation. **(b)** Upon receptor activation, RlucII-tagged effector proteins (Effector-RlucII) translocate towards and interact with active Gα subunits from each G protein family, leading to increased BRET. **(c)** Upon receptor activation, RlucII-tagged βarrs (βarr-RlucII) translocate to the plasma membrane, thus increasing BRET with membrane-anchored rGFP.

Automated Screening Platform

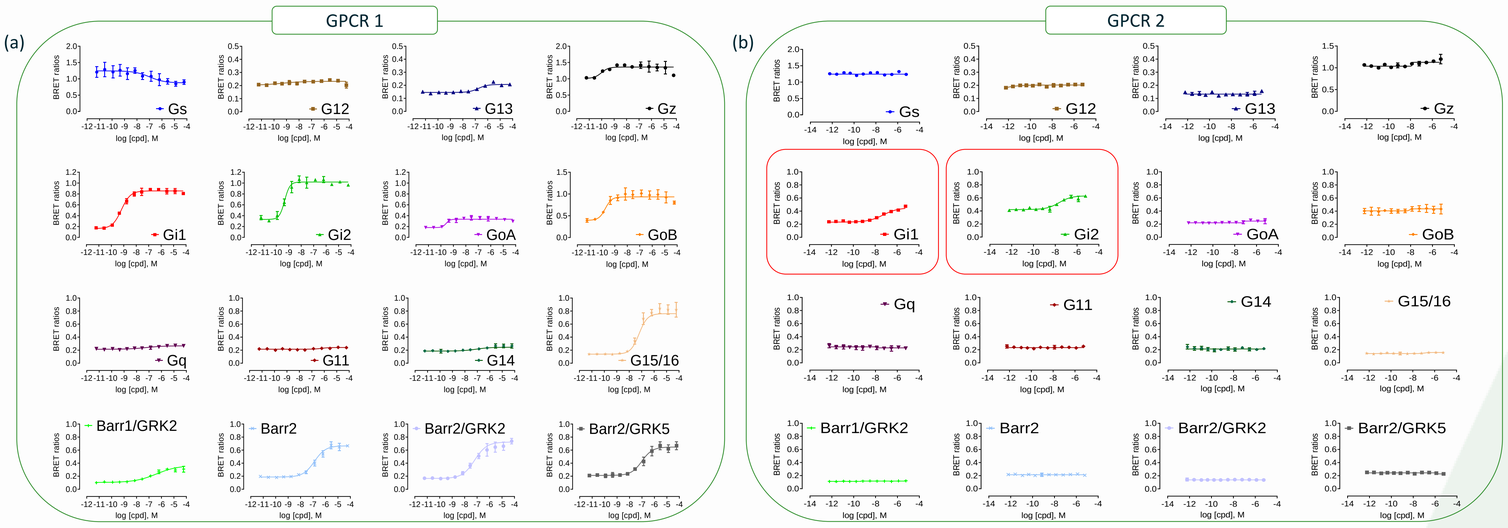

Fig. 2. Signaling profiles of WT human GPCRs. HEK293 cells were transfected with plasmids coding for a GPCR and one of 16 biosensors. Cells were treated with GPCR-specific ligand and response was recorded 30 mins later. **(a)** GPCR 1 demonstrated promiscuous signaling across many G protein and βarr pathways. **(b)** GPCR 2 demonstrated selective signaling on Gi1 and Gi2 pathways only.

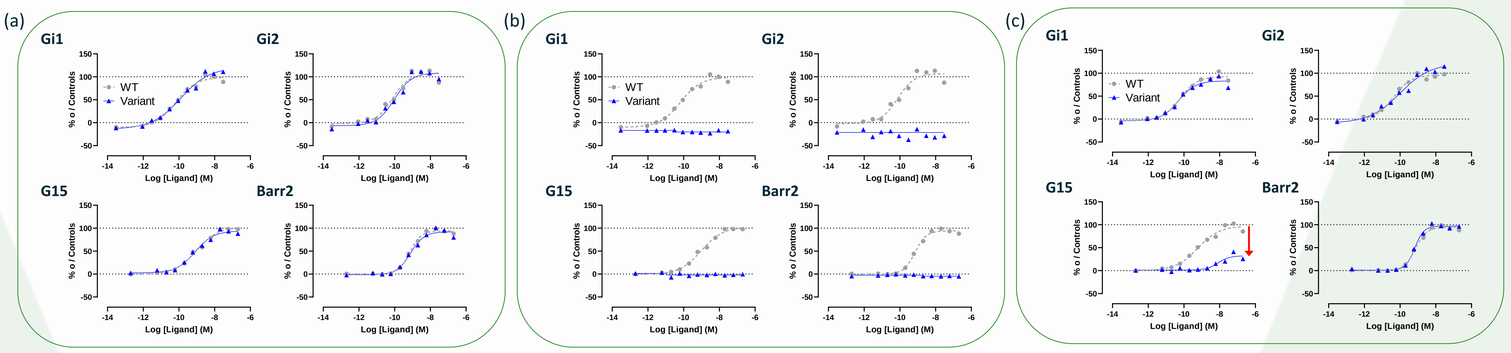

Fig. 3. Signaling profiles of variant receptors. HEK293 cells were transfected with plasmids coding for a specific GPCR and one of 16 biosensors. Cells were treated with GPCR-specific ligand and response recorded 30 mins later. **(a)** Variant displays WT-like profile on all pathways selected. **(b)** Variant displays complete loss of function profile on all pathways selected. **(c)** variant displays loss of function on G15 and WT-like profile on Gi1, Gi2, and βarr2, thus yielding an overall biased profile.

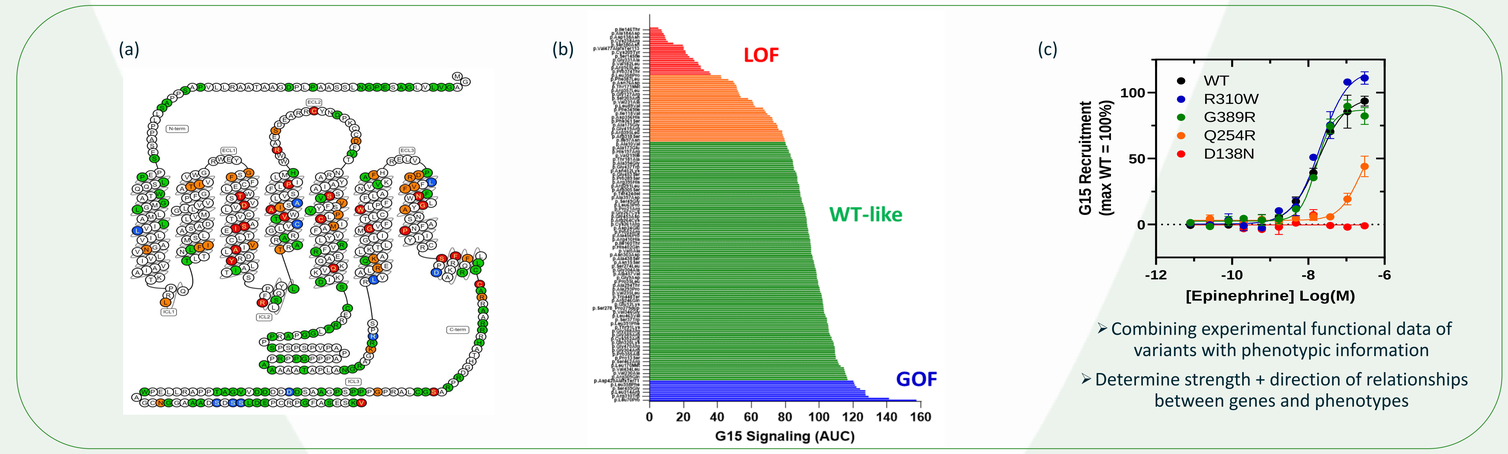

Fig. 4. Activity of 265 variants of ADRB1. (a) Location of variants on the serpentine map of ADRB1. **(b)** In vitro pharmacology of G15 area under the curve (AUC) for all profiled ADRB1 variants. **(c)** G15 signaling of four representative variants.

Mechano-Stimulation Assay

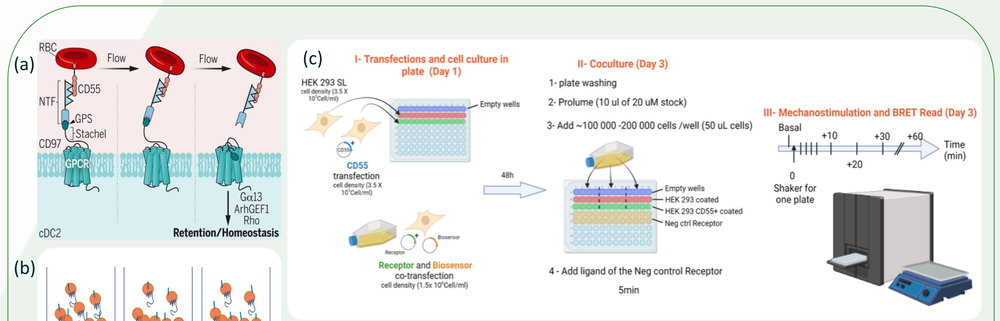

Fig. 5. Schematic representation of mechano-stimulation workflow. (a) Illustration of adhesion GPCR activation by Dan Liu. et al, Science (2022). **(b and c)** HEK293 cells co-transfected with hADGRE5 and biosensor were seeded into empty wells or pre-coated wells with HEK293 in 96-wells plates and subjected or not to vigorous orbital shaking before BRET measurements.

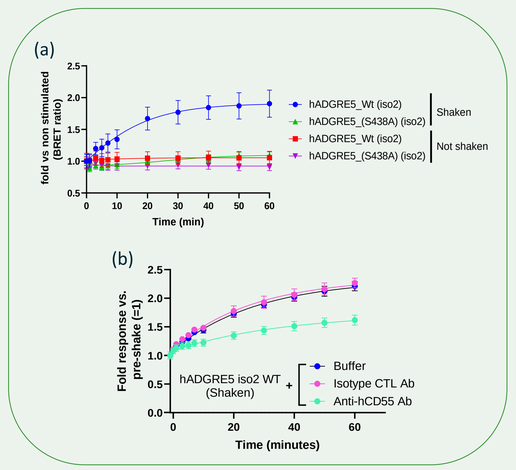

Fig. 6. Measuring mechano-stimulation of hADGRE5 through β-arr plasma membrane recruitment sensor. (a and b) HEK293 cells co-transfected with biosensor and hADGRE5 or uncleavable GPS point mutant S438A-hADGRE5 were seeded into pre-coated wells with HEK293 in 96-wells plates and subjected or not to vigorous orbital shaking before BRET measurements. **(b)** Cells were incubated with neutralizing anti-CD55 antibody, isotype control antibody, or buffer.

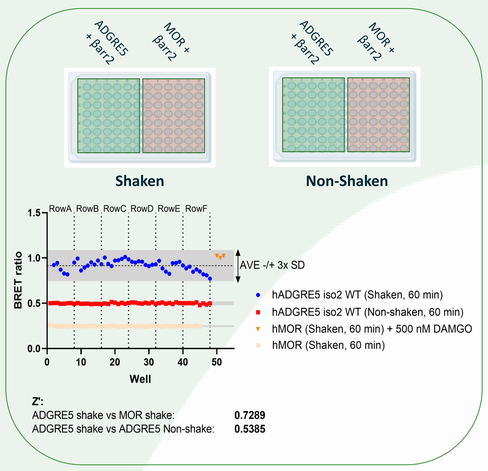

Fig. 7. Z' robustness of mechano-stimulation BRET assay. HEK293 cells co-transfected with β-arr plasma membrane recruitment sensor and hADGRE5 or MOR were subjected or not to vigorous orbital shaking before BRET measurements. Each point represents the BRET ratio value from one well belonging to the row indicated between the dotted lines.

Conclusions

➢ bioSens-All® is a robust and flexible platform to assess GPCR signaling:
- Uses unmodified receptors and G proteins
- Permits pan-pathway analysis in parallel using one protocol
- Automation of platform allows large scale GPCR variant profiling
- Adaptable to assess physiologically relevant modes of adhesion GPCR activation

➢ Pairing signaling data generated through bioSens-All® with clinical phenotypes can reveal associations between altered receptor pharmacology and disease states

➢ Facilitate the validation of novel therapeutic targets and discovery of innovative therapeutic agents