MONITORING AT1R:A2C-AR RECEPTOR HETERODIMERIZATION USING BIOSENS-ALL®

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Introduction

The bioSens-All® platform is an enhanced bystander bioluminescence resonance energy transfer (ebBRET) technology primarily used for the monitoring of GPCR downstream signaling. This typically includes G protein activation, βarrestin2 engagement as well as receptor and effector intracellular trafficking. A novel application of this technology was developed to monitor receptor heterodimerization. It has been demonstrated that arterial hypertension (HT) and heart failure (HF)-like neurohumoral active state of the AT1-R–α2C-AR GPCR heterodimer could constitute a promising target for future HT and HF treatment. Using AT1R and α2C-AR receptors, we successfully detected heterodimer interaction, βarrestin2 engagement as well as internalization and intracellular trafficking of AT1R:α2C-AR heterodimer in response to stimulation with AT1R and α2C-AR ligands, Angiotensin II (AngII) and Norepinephrine (NE), respectively. Assays monitoring internalization of AT1R:α2C-AR heterodimer and its localization at the early endosome compartment in response to AngII and NE were successfully miniaturized and adapted to an HTS assay format (Z' 0.6 – 0.7). Furthermore, ligand-dependent effect of AT1R:α2C-AR heterodimer activation was confirmed by measuring an atypical cAMP signaling in response to simultaneous stimulation with AngII and NE. These findings provided the development of a drug discovery screening assay to identify cardiovascular therapeutics targeting the heterodimer for HT and HF diseases. These proof-of-principle results represent a significant technological advancement to the bioSens-All® platform capabilities, opening new opportunities to explore heterodimer complexes, unveil their unique functional properties and provide insights into disease mechanisms, paving the way for innovative therapeutic strategies.

Materials and Methods

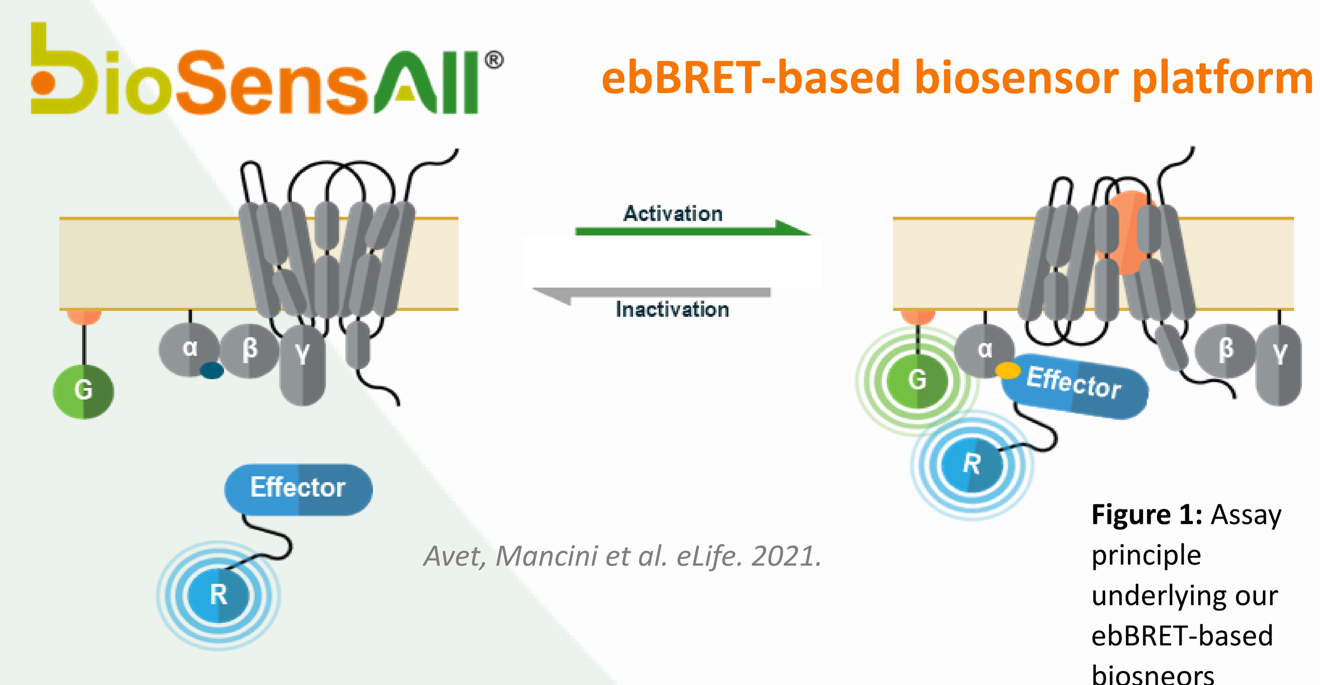

Figure 1: Assay principle underlying our ebBRET-based biosensors

Results

AT1R:α2C-AR direct interaction

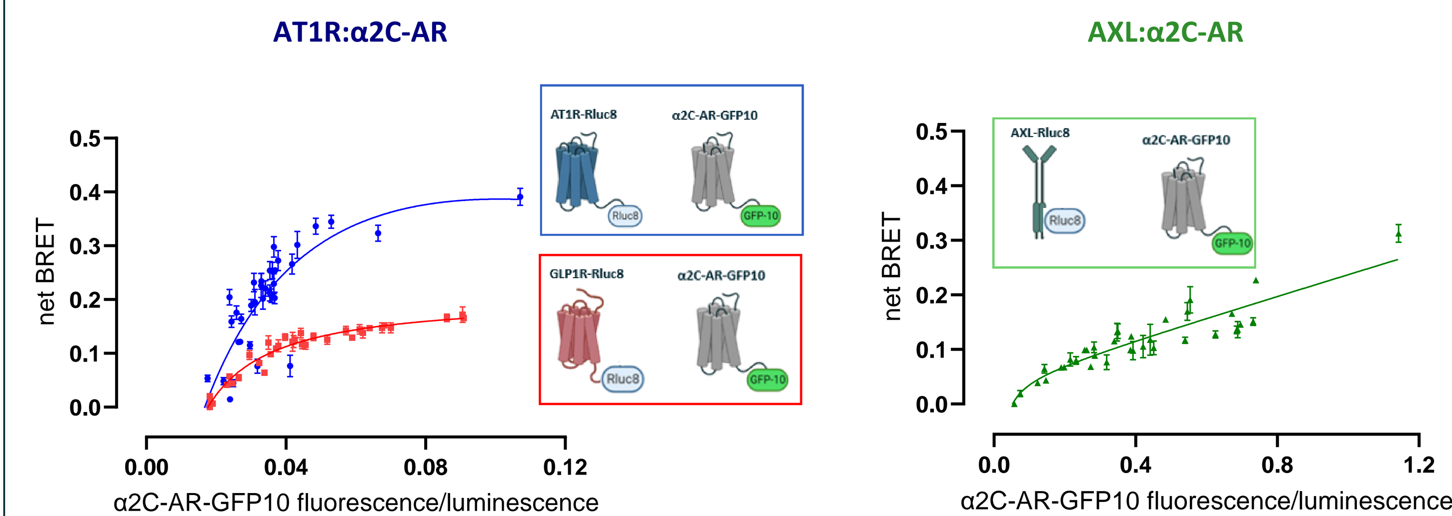

Figure 2: BRET saturation assays revealing AT1R:α2C-AR direct and specific interaction. BRET was measured in HEK293 cells co-expressing a fixed amount of the indicated Rluc8-tagged receptor and increasing amounts of GFP10-tagged α2C-AR. Saturation of the BRET signal was observed when heterodimerization was monitored between AT1R and α2C-AR as well as between GLP1R and α2C-AR receptors, suggesting a strong and specific interaction between the receptor pairs. BRET response obtained between AXL and α2C-AR was however linear, indicating a non-specific interaction. Pooled data was analyzed by nonlinear regression and assuming a one-site binding model.

AT1R:α2C-AR signaling

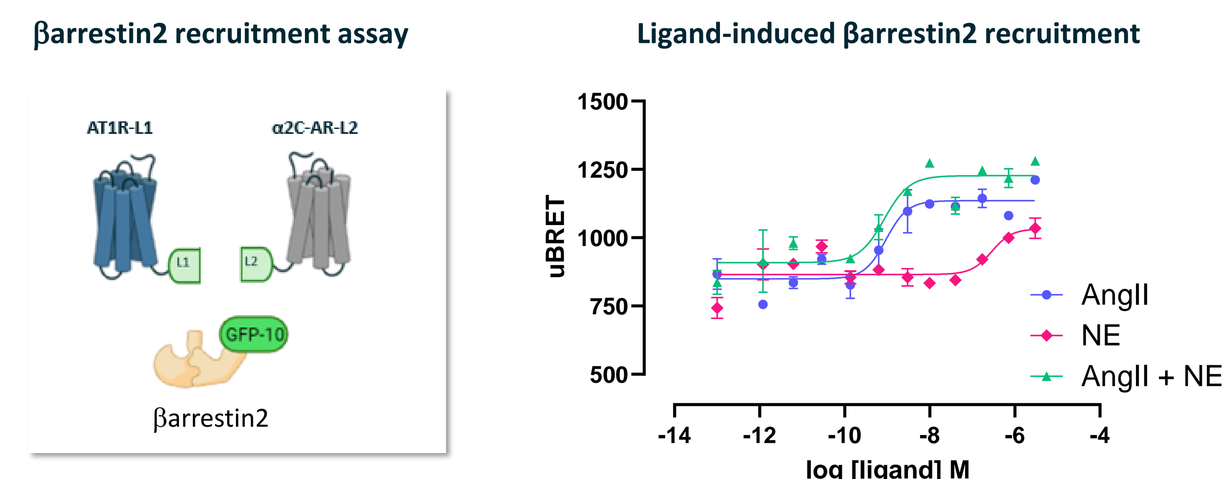

Figure 3: Ligand-induced βarrestin2 recruitment to AT1R:α2C-AR was monitored in HEK293 cells co-expressing AT1R and α2C-AR fused at their C-termini to an N-terminal (L1) or a C-terminal (L2) fragment of Rluc8, respectively. BRET was measured between the Rluc8-tagged heterodimer and a GFP10-tagged βarrestin2 in the presence of GRK2 overexpression. Both AT1R ligand (Angiotensin, AngII) and α2C-AR ligand (Norepinephrine, NE) individually induced βarrestin2 recruitment to the heterodimer with distinct potencies. Simultaneous addition of AngII and NE induced a slightly higher BRET response, yet non-significantly different from AngII response alone. These preliminary results indicate that differential signaling downstream of the heterodimer can be monitored.

AT1R:α2C-AR internalization and trafficking

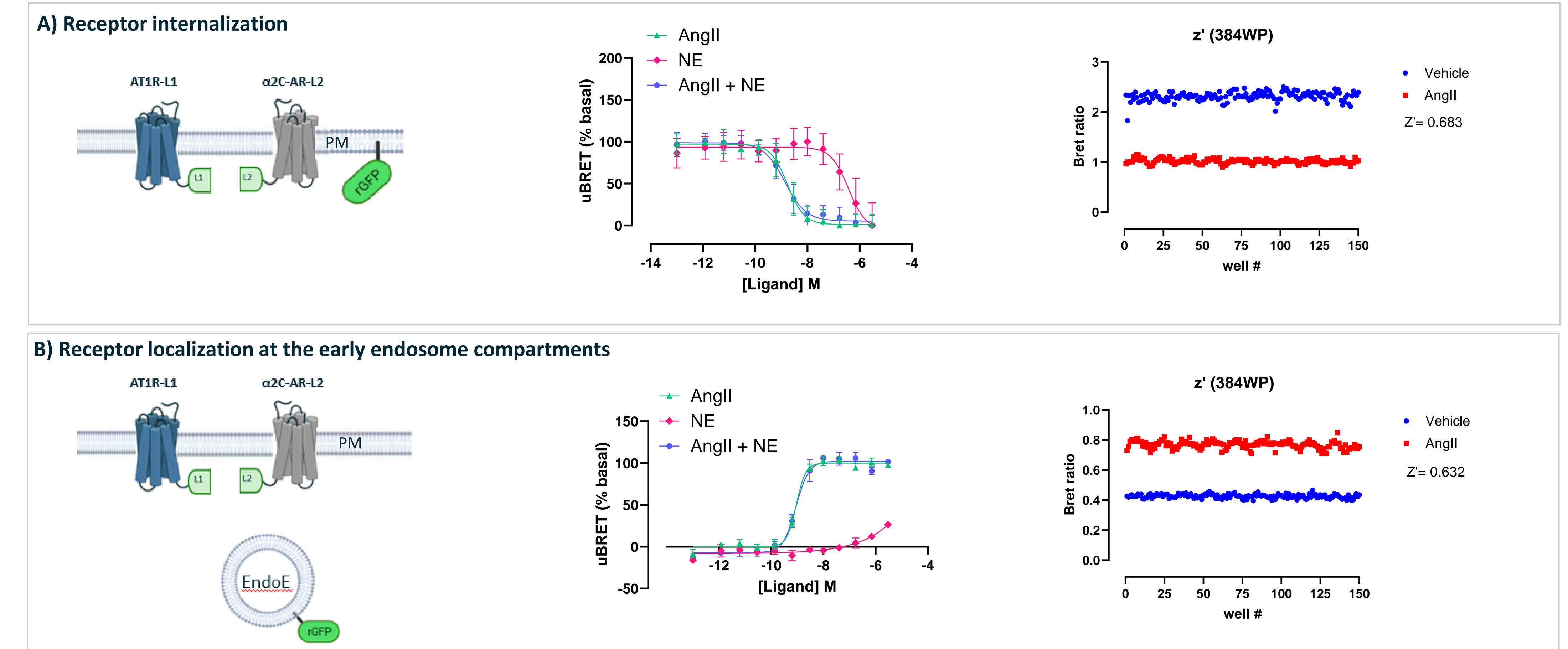

Figure 4: Ligand-induced internalization and intracellular trafficking of AT1R:α2C-AR heterodimer was monitored in HEK293 cells co-expressing AT1R and α2C-AR fused at their C-termini to an N-terminal (L1) or a C-terminal (L2) fragment of Rluc8, respectively. BRET was measured between the Rluc8-tagged heterodimer and an rGFP-tag anchored **(A)** at the plasma membrane (PM) or **(B)** at the early endosome compartments. AngII and NE individually induced AT1R:α2C-AR heterodimer internalization. NE however had a much lower potency. AngII also induced a significant increase in heterodimer localization at the early endosome compartments while NE response was quasi absent. Simultaneous addition of both compounds did not potentiate AngII effect. Internalization and trafficking of the heterodimer can be monitored and both assays were successfully miniaturized to 384 well-plate format as a tool for a drug screening program.

Atypical signaling downstream of AT1R:α2C-AR heterodimer

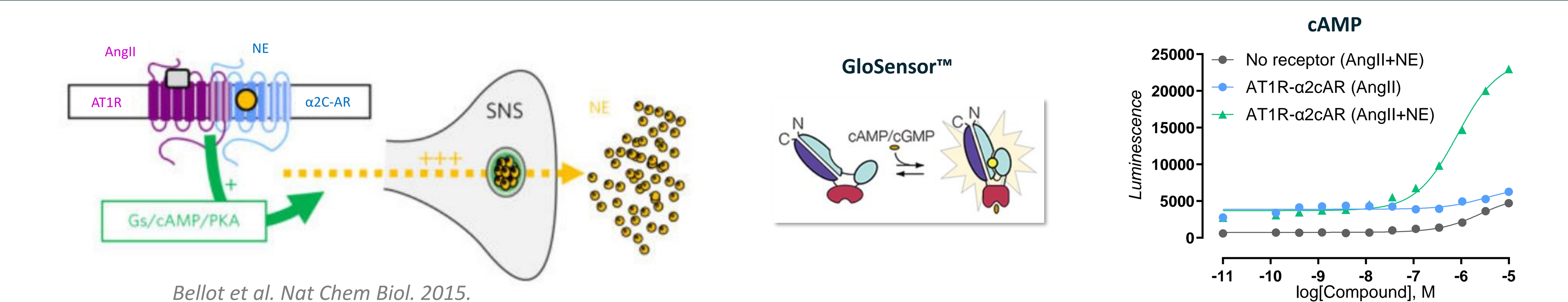

Figure 5: Simultaneous stimulation with AngII and NE induces an atypical cAMP production downstream of AT1R:α2C-AR heterodimer. cAMP production was quantified in HEK293 cells co-expressing native AT1R and α2C-AR receptors using a GloSensor™ assay. While AngII alone had no effect, co-stimulation with NE induced a significant increase in cAMP production.

Conclusion

- Using BRET-based assays, we detected constitutive interaction between AT1R and α2C-AR receptors as well as the ability of the heterodimer to recruit βarrestin2 and to internalize into early endosome compartments in response to ligand stimulation.
- Simultaneous stimulation with AngII and NE induced an atypical cAMP production downstream of AT1R-α2C-AR heterodimer, a phenomenon normally not characteristic of the individual protomers. These observations unveil a novel receptor functional entity with a distinctive signaling signature and provide insights into mechanisms underlying cardiovascular diseases.
- Methods developed in this study can be used as screening assays for the development of cardiovascular therapeutics targeting AT1R-α2C-AR.