BRET-Based Analysis of G-Protein Coupled Receptor (GPCR) Signaling

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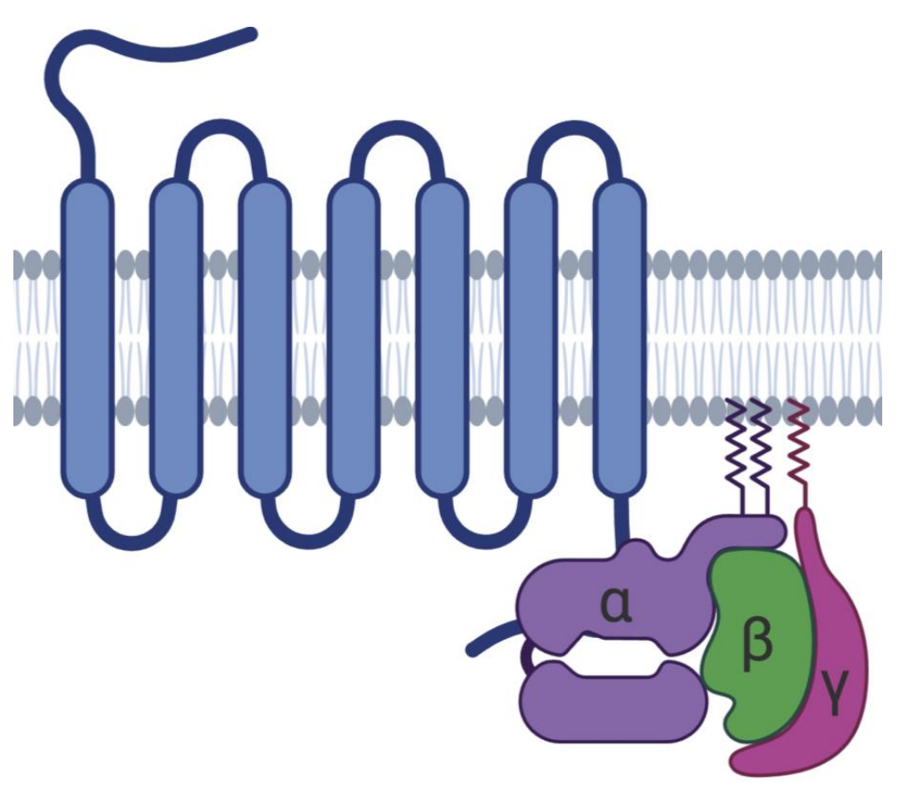
GPCR-Mediated Signaling

GPCR

G-protein-coupled receptors (GPCRs) are widely expressed cell-surface receptors that play central roles in cellular signaling.

They respond to different type of stimuli;
- Hormonal
- Peptidic
- Small molecules (smells and tastes)
- neurotransmitters

Upon ligand binding they:
- Change conformation
- Activate heteromeric G-proteins (Gα, Gβ, and Gγ)
- The G-proteins activate downstream effectors

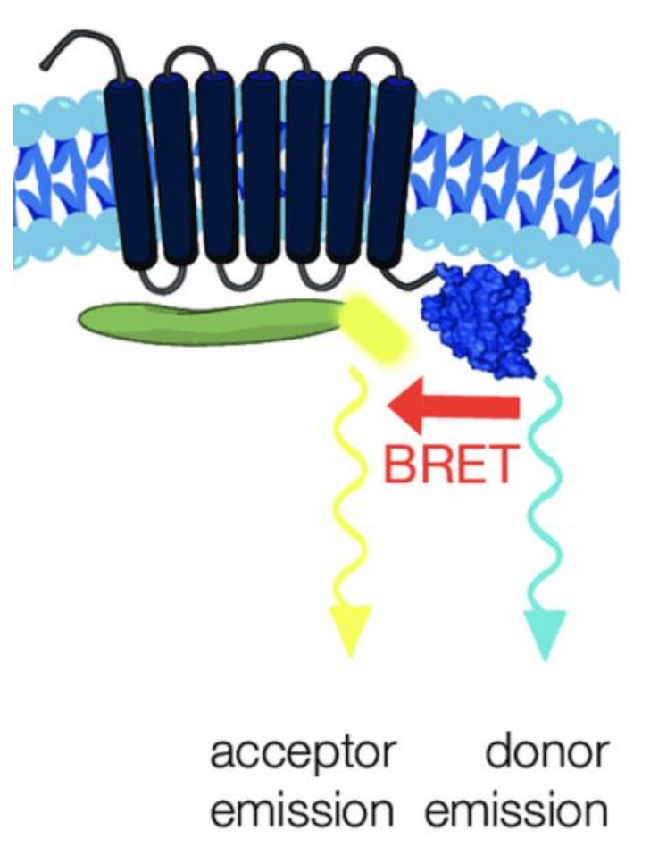


BRET

The downstream effectors can activate or shut down pathways in the cell, and these interactions can be observed through BRET.

Bioluminescene **R**esonance **E**nergy **T**ransfer is a technique that allows to track protein-protein interactions with biosensors, engineered proteins that allow us to track interactions within 10 nm. **(see methods)**

Biosensors use:
- Luciferase, a light emitting enzyme
- Green Fluorescent protein, an excitable protein that can reflect light.



M2 (Muscarinic acetylcholine receptor 2)

We sought to characterize the classical Gi-mediated regulation of cAMP signaling and to examine the interplay between the Gβγ dimer and potassium channel tetramerization domain–containing (KCTD) proteins, which promote Gβγ degradation and modulate GPCR signaling. These mechanisms are especially relevant in cardiac tissues, where M2 receptors are highly expressed and play a key role to parasympathetic regulation of heart function.

UT (Urotensin receptor)

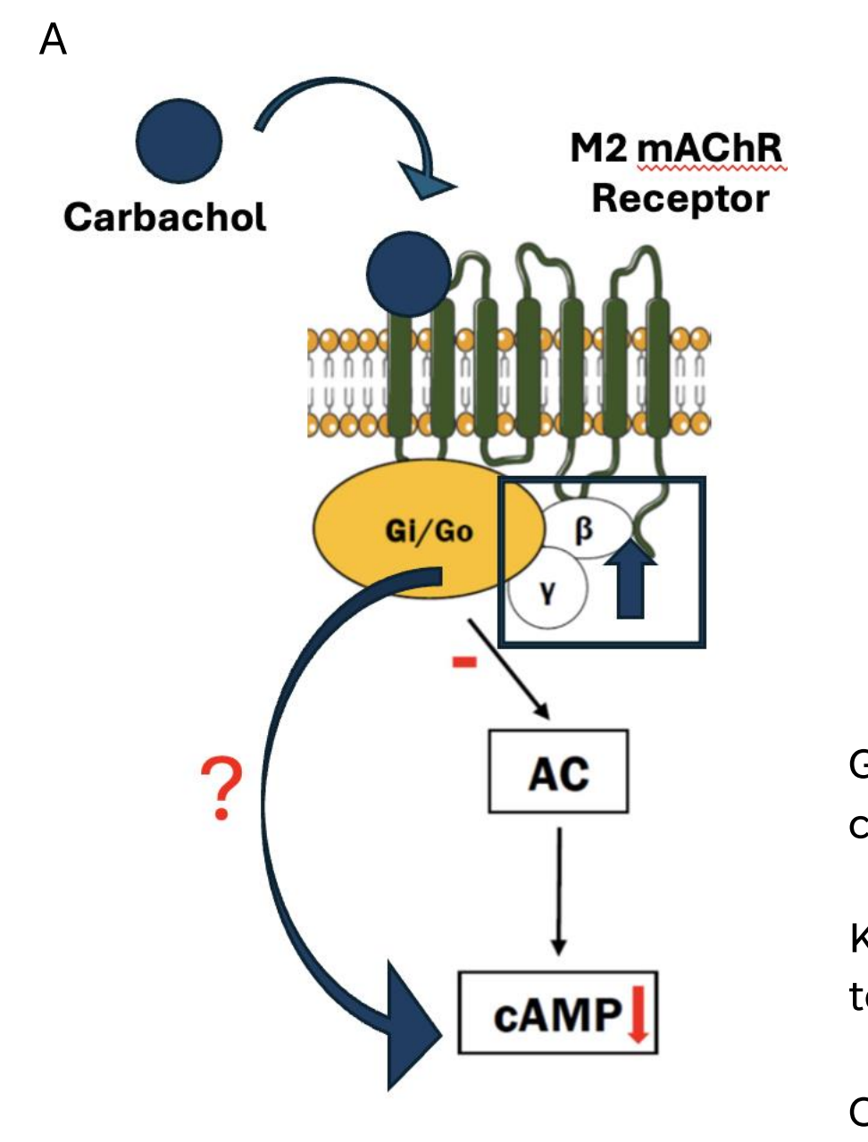
We aimed to characterize the differences between the rat UT and the human UT. The urotensinergic system is implicated in cardiovascular and inflammatory diseases. UT antagonists that were promising in preclinical studies (rodents) later failed in clinical trials (humans). This deeper insight may help explain the gap between preclinical and clinical outcomes and support the development of more effective antagonists.

Muscarinic Acetylcholine Receptor 2 (M2)

M2 Muscarinic Receptor-Mediated Signaling

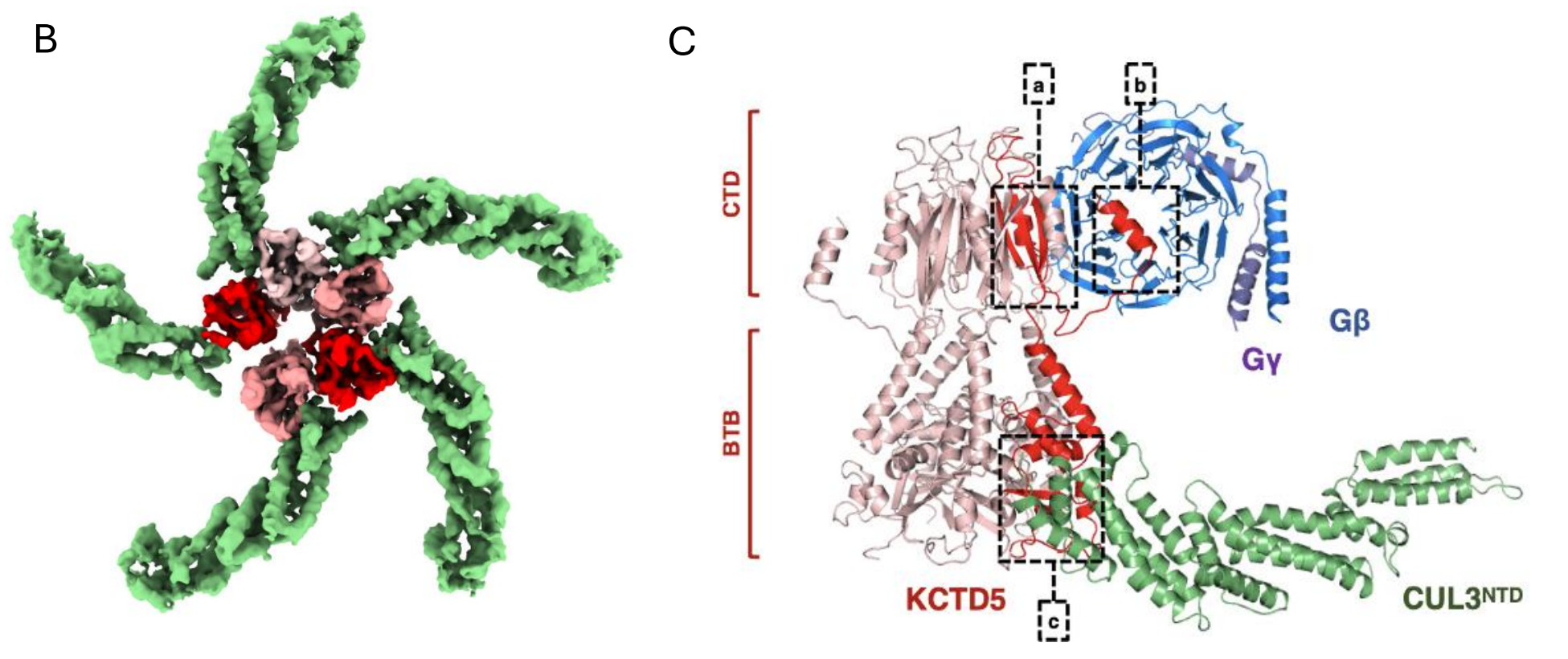
- M2 receptor (M2R) is a G protein-coupled receptor expressed in cardiac tissue that primarily couples to Gai proteins
- Activated Gai inhibits adenylyl cyclase, leading to reduced cAMP levels and decreased PKA activity, which modulates the phosphorylation state of cardiac ion channels and influences heart rate and contractility.
- KCTD protein bind Gβγ subunits and target them for proteasomal degradation.

Hypothesis :



Nguyen, D. M., et al. (2024)

Structure of the pentameric KCTD5/CUL3NTD/Gβγ complex

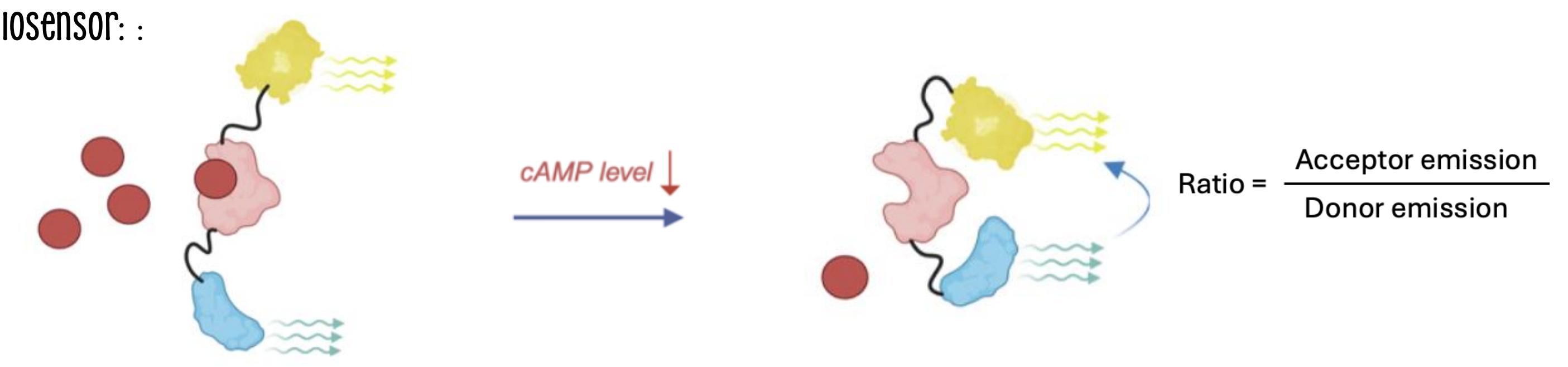


Gβγ subunits can independently regulate downstream effectors such as adenylyl cyclase and ion channels, providing an additional layer of signaling control.

KCTD5 binds these free Gβγ subunits and, through its CUL3 ubiquitin ligase domain, targets them for proteasomal degradation, limiting their independent signaling.

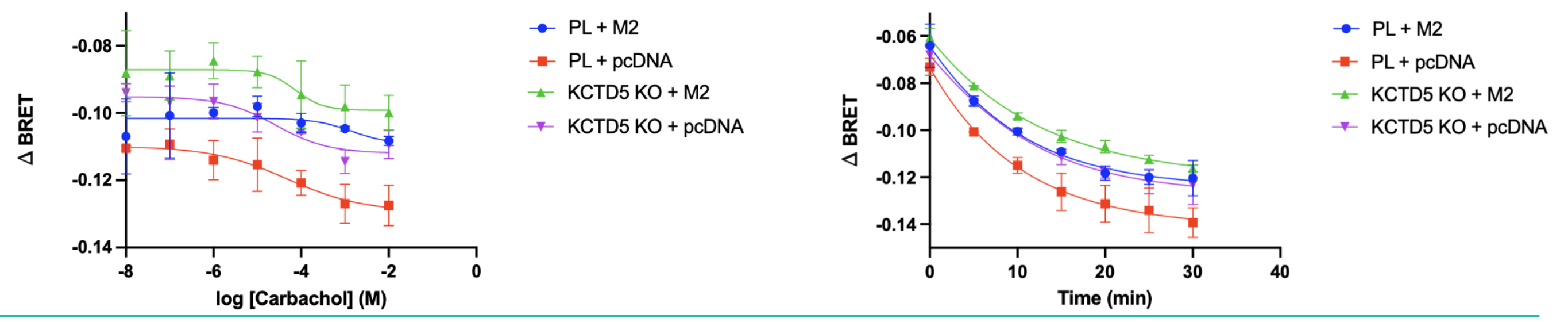
Characterizing this axis will reveal how Gβγ availability and degradation by KCTD5 modulate downstream signaling dynamics and overall pathway activity.

EPAC Biosensor: :

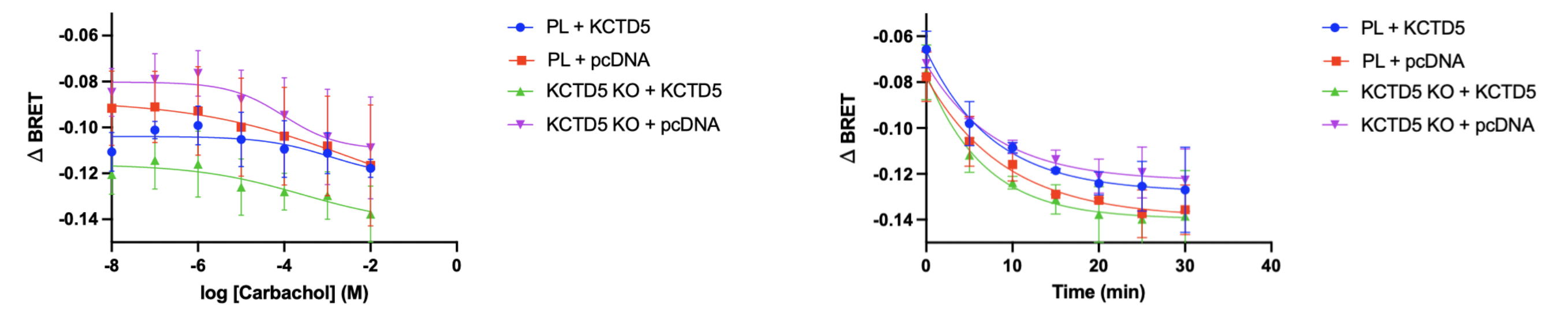


Ratio = Acceptor emission / Donor emission

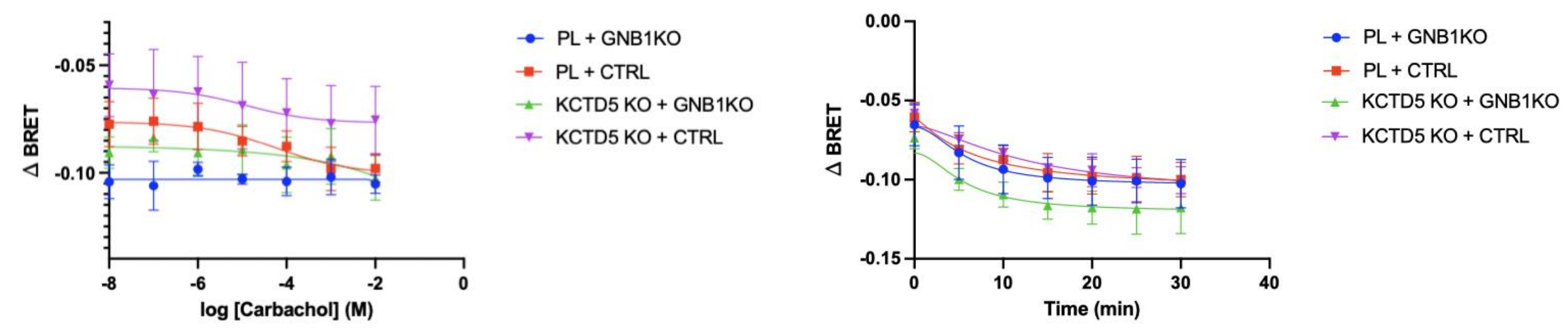
Elevated Gβγ in KCTD-KO cells sensitizes Gαi-dependent inhibition of cAMP upon M2 mAChR receptor activation



KCTD5 rescues cAMP production in KCTD5-KO cells



My goal is to determine how do the cAMP levels change when the G**βy dimers are upregulated.**



Urotensin Receptor (UT)

The Urotensinergic System

- Receptors expressed in the human cardiovascular system and nervous system, **Fig.1** shows the relevant cellular pathways. (1)
- Potent vasoconstrictor in smooth muscle, vasodilating in endothelium (1), pro-inflammatory and chemokine features (2)
- Urotensin II (UII) and Urotensin related peptide (URP, **Fig.2)** are elevated in patients with chronic kidney disease and cardiovascular disease, as well as numerous other metabolic diseases (7)

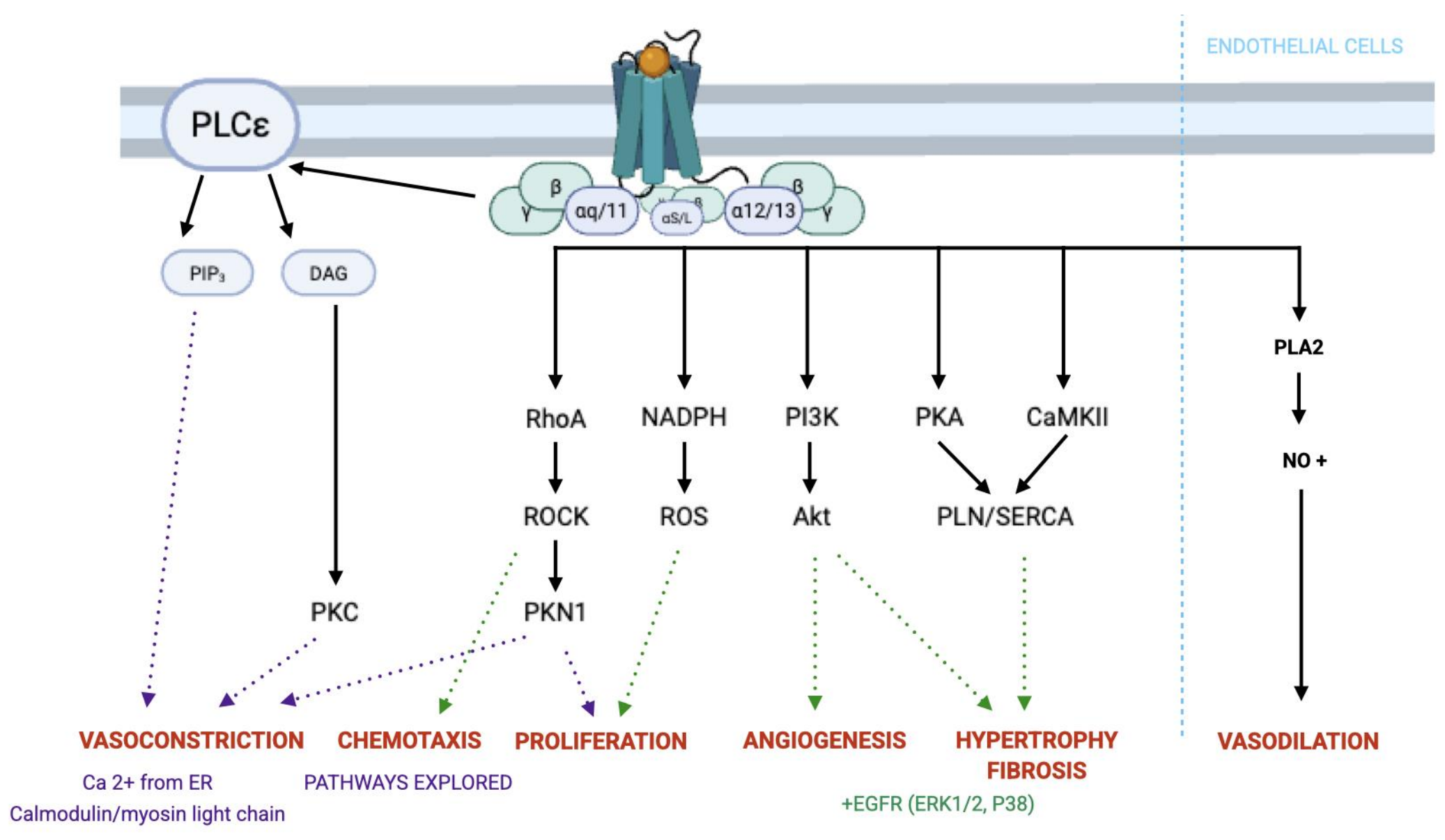


Figure 1. Major pathways activated by UT(1-2)

Created in BioRender.com bio

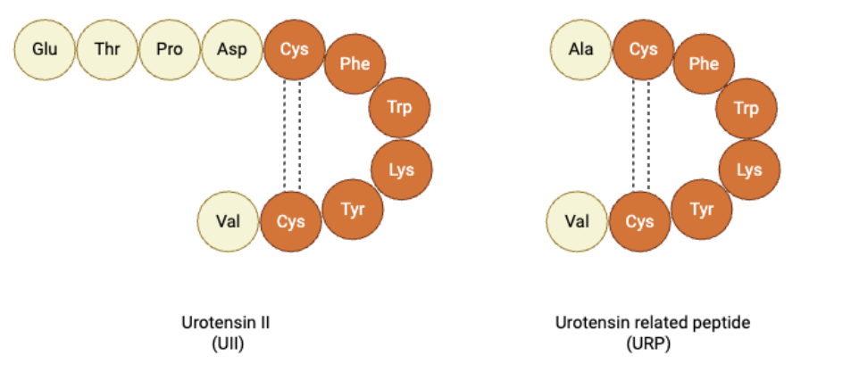


Figure 2. Human endogenous peptides (1,5)

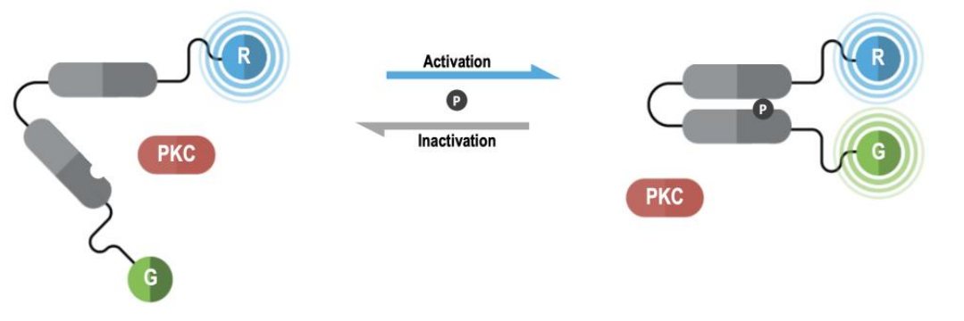


Figure 3. Protein Kinase C (PKC)(8)*, our biosensor is engineered with Luciferase (rLuc) and fluorescent green protein (rGFP), upon activation by DAG, it folds upon itself allowing rLuc to get near rGFP. This allows the BRET readings (methods).*

UT antagonists as a therapeutic

- Inhibition of hUT inhibits inflammation, showing promise to impede cardiovascular disease developement(4)
- Antagonists have been successful in rodent models in preclinical trials, yet failed in clinical trials in human. (5)

Hypothesis:

- Clinical trial failure is due to interspecies differences between humans and rodents receptors and endogenous ligands(5), characterizing their individual response will allow us to design better antagonists.
- PHYSIOLOGICAL DIFFERENCE: Rats show lower levels of circulating UII than humans (1)
- RECEPTOR DIFFERENCES: only 75% homology between human (hUT) and rat receptors (rUT)(6) **(Table.1)**
- ENDOGENOUS LIGAND DIFFERENCES: rat UII (rUII) has 14 residues, compared to 11 residues in humans (hUII)(6).

	Receptor	UII peptidic sequence
rUT	386 residues C-terminal palmitoylation	<Gln-His-Lys-Gln-Gly-Ala-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Ile-OH
hUT	389 residues	H-Glu-Thr-Pro-Asp-Cys-Phe-Trp-Lys_Tyr-Cys-Val-OH

Table 1. Interspecies differences between rat and human receptors and UII peptidic sequence (6)

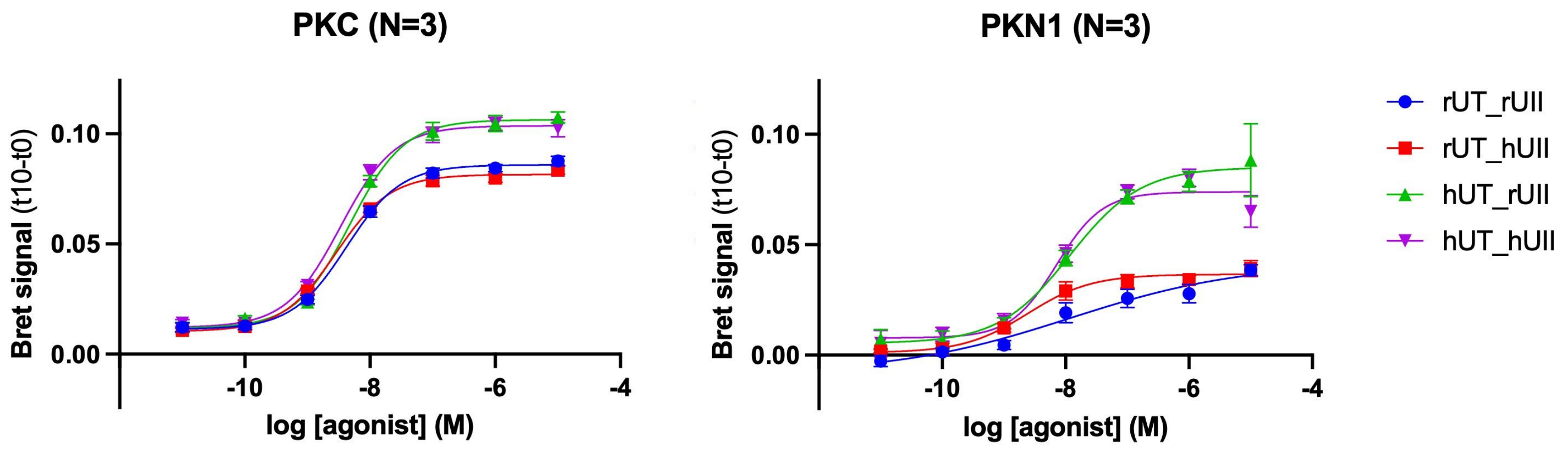
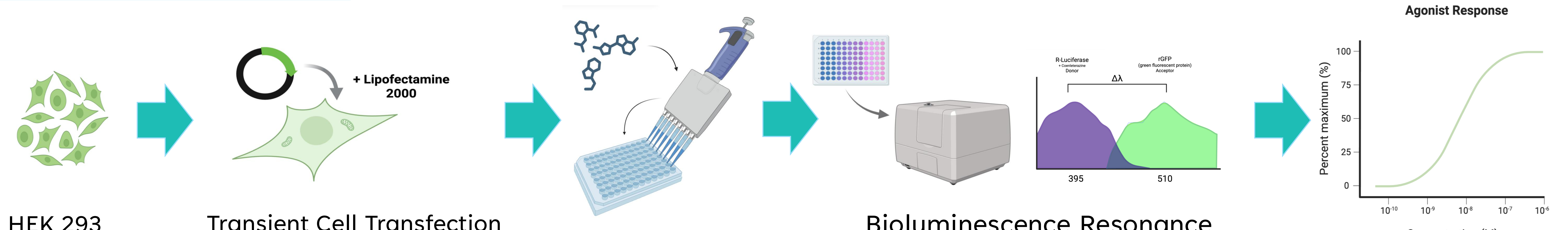


Figure 4. Agonist Dose-Response Curves using PKC and PKN1, (N=3). There is a significant difference between rUT and hUT in Emax (0.07-0.09 respectively). With PKN1, there is a significant difference in the Emax of the dose response curve (rUT; 0.042, hUT 0.073).

Demonstration of biased agonism between rat receptor (rUT) and human receptor (hUT)

- To explore biased agonism, we chose Protein Kinase C (PKC), activated through Gaq/11, and PKN1, activated through Ga12/13, visible in **Figure 1.**
- There is a significant difference in PKC and PKN1 dynamics in both rUT and hUT (p-value = 0.0001). This, coupled with interspecies physiological prevalence infers at biased agonism.
- More research with different biosensors would allow a more complete characterization of rUT and hUT response to agonist and antagonist ligands.
- **This renews the interest of antagonist drug development.**

Methods



HEK 293 Cells

Transient Cell Transfection Receptors and Biosensors

Drug Treatment

Bioluminescence Resonance Energy Transfer (BRET)

Dose-Response Curves

References

GPCR

M2

UT

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