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The impact of linker region between receptor and fluorescent protein on arrestin recruitment assays.

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Introduction: The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted from the gut in response to nutrient ingestion. They potentiate glucose dependent insulin secretion through their specific receptors (GLP-1R and GIPR, respectively) expressed on pancreatic β-cells. Using fluorescence resonance energy transfer (FRET) between YFP-tagged receptors and CFP-tagged arrestin3, we have previously reported that GLP-1R interacted robustly with arrestin3 in response to GLP-1 whereas GIPR showed no interaction with arrestin3 in response to GIP (1). In subsequent experiments a GIPR construct was employed that used a modified version of YFP (SYFP2). This receptor was found to be able to recruit arrestin.

Method: Arrestin3 recruitment to GIPR was investigated by monitoring FRET between YFP-labelled GIPR and CFP labelled arrestin in transiently transfected HEK-293T cells. Cells were perfused with buffer or 1 µM GIP. Arrestin recruitment was observed as an increase in FRET. The original GIPR-YFP construct contained a 10 amino acid linker between the receptor and a *XbaI* restriction site upstream of the YFP. This linker was no present in the modified GIPR-SYFP2. However, this results in the introduction of a serine residue to the end of GIPR's C-terminal tail which could potentially be a phosphorylation site. The serine/arginine (SR) coded by the *XbaI* site was then substituted with glycine/glycine (GG) by site-directed mutagenesis.

Results: Deletion of the 10 amino acid linker between GIPR and the *XbaI* restriction site results in a receptor that can recruit arrestin when stimulated with 1 µM GIP. However, substitution of SR with GG between the receptor and YFP abolishes arrestin recruitment (n=5).

Conclusion: These results highlight the importance of the linker between receptor and fluorescent protein in arrestin recruitment assays. The use of the commonly used *XbaI* restriction site may unintentionally introduce an additional phosphorylation site, potentially resulting in false positive results.

References:

1. Al-Sabah S et al., (2014) *PLoS One* **9**: e106890.