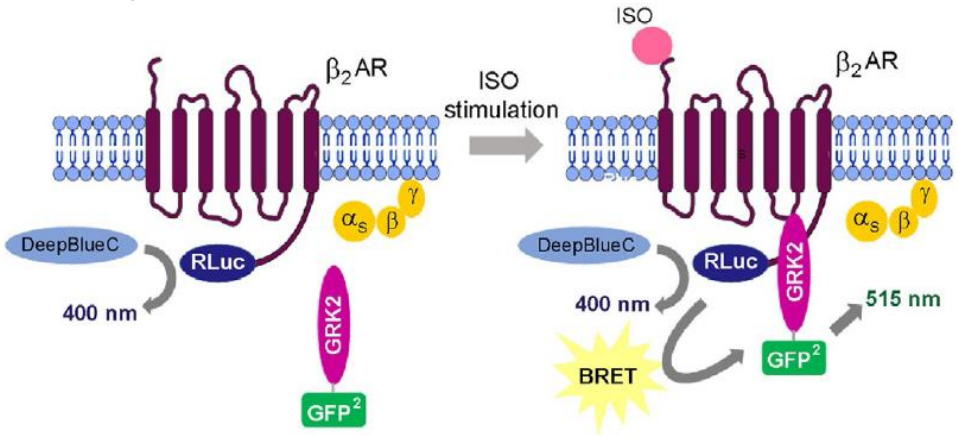
BRET-based assay to specifically monitor β_2AR/GRK2 interaction and β-arrestin2 conformational change upon βAR stimulation

The β-adrenergic receptors (βARs) are members of G protein-coupled receptor (GPCR) family and have been one of themost important GPCRs for studying receptor endocytosis and signaling pathway. Agonist binding of βARs leads to an activation of G proteins and their canonical effectors. In a parallel way, βAR stimulation triggers the termination of its signals by receptor desensitization. This termination process is initiated by G protein-coupled receptor kinase (GRK)-induced βAR phosphorylation that promotes the recruitment of β-arrestins to phosphorylated βAR. The uncoupled βARs which formed a complex with GRK and β-arrestin subsequently internalize into the cytosol. In addition, GRKs and β-arrestins also act as scaffolding proteins and signal transducers in their own functions tomodulate various downstream effectors. Upon translocation to the βAR, β-arrestin is believed to undergo an important conformational change in the structure that is necessary for its signal transduction.

The bioluminescence resonance energy transfer (BRET) technique involves the fusion of donor (luciferase) and acceptor (fluorescent) molecules to the interested proteins. Co-expression of these fusion proteins enables direct detection of their interactions in living cells.

Here we describe the use of our established BRET technique to track the interaction of βAR with both GRK and β-arrestin. The assay described here allows the measurement of the BRET signal for detecting the interaction of β2AR with GRK2 and the conformational change of β-arrestin2 following βAR stimulation.



The BRET^2 detection of β_2AR-RLuc and GRK2-GFP^2 interaction

The advanced BRET (BRET^2) technique is used for monitoring the interaction between β_2AR-RLuc and GFP^2-GRK2. Stimulation of β_2AR with isoproterenol (ISO) induced the interaction of β_2AR with GRK2 that brings the acceptor (GFP^2) closed proximity to the donor (RLuc). The energy is transferred from a donor to an acceptor, leading to the detection of BRET signal.

Reference:

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