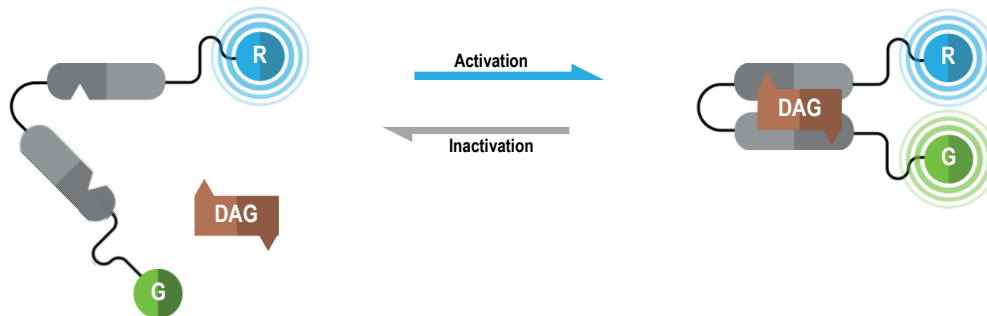


## DIACYLGLYCEROL (DAG) BIOSENSORS

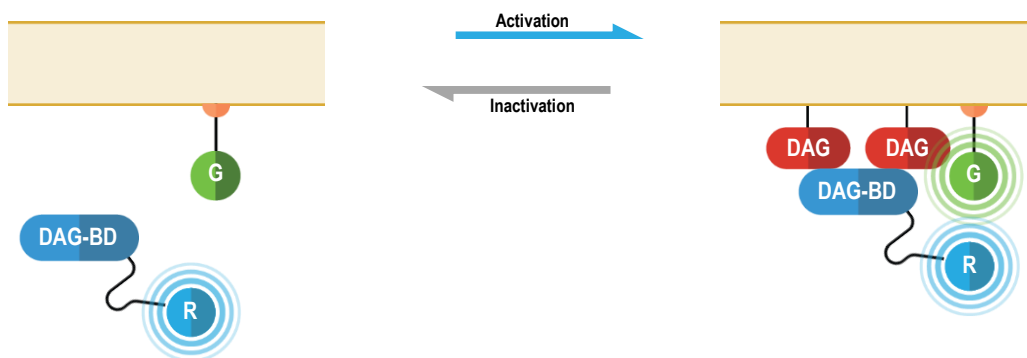
**Summary:** The principal effector of the  $G\alpha_q/11$  pathway is phospholipase C- $\beta$  (PLC $\beta$ ), which catalyzes the cleavage of membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) into the second messengers inositol (1,4,5) trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). Binding of IP<sub>3</sub> to its receptor on the endoplasmic reticulum (ER) results in the release of ER calcium stores. Increased concentrations of calcium and DAG lead to the activation of protein kinase C (PKC). Consequently, biosensors monitoring DAG levels and PKC activity have been developed as distal measures of G protein-coupled receptor (GPCR)-downstream  $G\alpha_q/11$  activation.

Two versions of DAG biosensors have been developed:

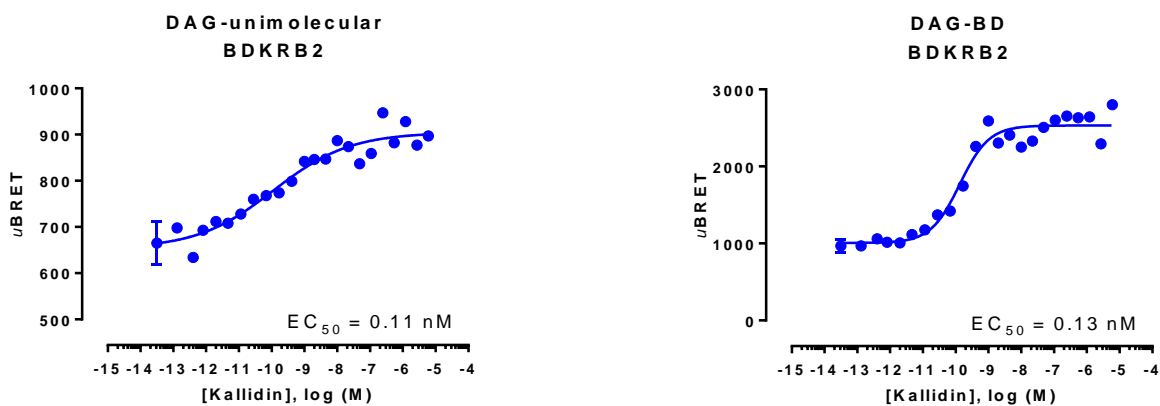
- 1) The unimolecular DAG sensor consists of “core region” containing C1b DAG-binding domains (C1b DBDs) fused via a flexible linker. This core region is tagged with *Renilla* luciferase (RLuc; R in the figure below) at its N-terminus and green fluorescent protein (GFP; G in the figure hereafter) at its C-terminus. Binding of DAG to the core region results in the interaction of C1b DBDs leading to an increase in the physical proximity between terminal RLuc and GFP tags. As a result, DAG production translates into an increased BRET efficiency, the magnitude of BRET signal being directly proportional to intracellular DAG levels.



- 2) The multimolecular DAG biosensor was designed to monitor the recruitment of an RLuc-tagged version of the previously described core region to the plasma membrane. Following GPCR activation, generation of DAG to the plasma membrane leads to an increase in BRET signal.



**Results**



HEK293 cells were transfected with a receptor coding plasmid (the human bradykinin receptor B2 (BDKRB2)) as well as plasmids coding for the unimolecular (*left*) or multimolecular (*right*) DAG biosensor. On the day of BRET, cells were rinsed with assay buffer, incubated with coelenterazine and increasing amounts of kallidin for 10 minutes and BRET subsequently measured.