system of any one of items 40 to 47 with a substrate for said bioluminescent donor molecule; and (b) measuring the BRET signal in the system in the presence and absence of said agent; wherein a difference in said BRET signal in the presence of said agent relative to the absence thereof is indicative that said agent modulates non-receptor GEF-mediated G protein activation.

65. The method of any one of items 54 to 64, wherein the BRET signal is measured using a plate reader or by microscopy.

66. The method of any one of items 54 to 65, wherein the substrate is a coelenterazine substrate.

67. The method of item 66, wherein the coelenterazine substrate is methoxy e-coelenterazine.

Other objects, advantages and features of the present disclosure will become more apparent upon reading of the following non-restrictive description of specific examples thereof, given by way of example only with reference to the accompanying drawings.

In one aspect, the present disclosure refers to a system for measuring modulation of G protein activation in a Gα protein subunit family-selective manner, said system comprising a cell expressing:

(i) a first component comprising a Gα subunit interacting polypeptide (GASIP) tagged with a bioluminescent donor molecule or a fluorescent acceptor molecule;

wherein:

if said Gα protein subunit family is Gi, said GASIP comprises the G protein-binding domain of RAP1 GTPase Activating Protein 1 (Rap1GAP) or of a Regulator of G-protein signaling (RGS) protein;

if said Gα protein subunit family is Gq, said GASIP comprises the G protein-binding domain of Rho guanine nucleotide exchange factor 25 (P63RhoGEF); and

if said Gα protein subunit family is G12/13, said GASIP comprises the G protein-binding domain of Rho guanine nucleotide exchange factor 11 (PDZRhoGEF) or Rho guanine nucleotide exchange factor 1 (P115RhoGEF); and

(ii) a second component comprising a plasma membrane (PM)-targeting moiety, an endosomal-targeting moiety or a Golgi-targeting moiety tagged with a bioluminescent donor molecule or a fluorescent acceptor molecule;

wherein if said GASIP is tagged with said fluorescent acceptor molecule, said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety is tagged with said bioluminescent donor molecule, and if said GASIP is tagged with said bioluminescent donor molecule, said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety is tagged with said fluorescent acceptor molecule and wherein there is no protein-protein interaction between (i) the PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety and (ii) the GASIP.

In another aspect, the present disclosure refers to a host cell expressing the components of the system as disclosed herein.

In another aspect, the present disclosure refers to a method for determining whether an agent modulates the activation of a G protein of interest, said method comprising:

(a) contacting the system as disclosed herein with a substrate for said bioluminescent donor molecule; and

(b) measuring the Bioluminescence Resonance Energy Transfer (BRET) signal in the system in the presence and absence of said agent;

wherein a difference in said BRET signal in the presence of said agent relative to the absence thereof is indicative that said agent modulates the activation of said G protein of interest.

In another aspect, the present disclosure refers to a method for determining whether an agent modulates non-receptor guanine nucleotide exchange factor (GEF)-mediated G protein activation, said method comprising

(a) contacting the system as disclosed herein with a substrate for said bioluminescent donor molecule; and

(b) measuring the BRET signal in the system in the presence and absence of said agent;

wherein a difference in said BRET signal in the presence of said agent relative to the absence thereof is indicative that said agent modulates non-receptor GEF-mediated G protein activation.

BRIEF DESCRIPTION OF DRAWINGS

In the appended drawings:

FIG. 1A depicts the principle of an effector-based sensor to monitor GPCR-mediated direct G protein activation (upper panel **i**) or guanine-nucleotide exchange factor (GEF)-mediated G protein activation (lower panel **ii**). Cells expressing a receptor, a cellular compartment (or subcellular localization) marker, such as a plasma-membrane (PM) or early endosomes (EE) marker, tagged with a suitable Bioluminescent Resonance Energy Transfer (BRET) donor or acceptor (*e.g.*, rGFP), the Gα-interaction domain of a specific G protein effector tagged with suitable BRET donor or acceptor (*e.g.*, RlucII) are exposed to an agonist to activate the coexpressed G protein. In panel **i)**, the agonist-induced GPCR stimulation activates directly G proteins, which recruits a tagged effector from the cytoplasm to the labeled membrane. In panel **ii)** G protein activation is mediated by the recruitment of a GEF such as GIV/Girdin following the activation of an RTK (*e.g.*, EGFR) or an integrin α-β complex.

FIGs 1B-1E show examples of specificity obtained with three different G protein effectors. Receptors coupled to members of Gq, Gi and G12/13 families were co-expressed with these G proteins, with an rGFP fused to a PM marker (rGFP-CAAX, GKKKKKKSKTKCVIM, SEQ ID NO: 1) and a G protein effector tagged with RlucII. **FIG. 1B** shows dose response curves (DRCs) obtained with platelet-activating factor (PAF)/PAFR-mediated G protein (Gq, G11, G14, G12, GoA, GoB, Gz, Gi1, Gi2, Gi3) activation, using Rap1GAP (SSS-AAA)-RlucII and rGFP-CAAX (PM marker). Mock response represent activation of endogenously expressed Gi1, i2 and i3 proteins. **FIG. 1C** shows DRCs obtained with U46619/TPαR-mediated G protein (Gq, G11, G14, G15, G12, G13, Gz) activation, using PDZRG-RlucII and rGFP-CAAX. Mock response represent activation of endogenously expressed G12 and G13 proteins. **FIGs. 1D and 1E** show DRCs obtained with PAF/PAFR-mediated G protein (Gq, G11, G14, G12, GoA, GoB, Gz, Gi1,

WHAT IS CLAIMED IS:

1. A system for measuring modulation of G protein activation in a Gα protein subunit family-selective manner, said system comprising a cell expressing:

(i) a first component comprising a Gα subunit interacting polypeptide (GASIP) tagged with a bioluminescent donor molecule or a fluorescent acceptor molecule;

wherein:

if said Gα protein subunit family is Gi, said GASIP comprises the G protein-binding domain of RAP1 GTPase Activating Protein 1 (Rap1GAP) or of a Regulator of G-protein signaling (RGS) protein;

if said Gα protein subunit family is Gq, said GASIP comprises the G protein-binding domain of Rho guanine nucleotide exchange factor 25 (P63RhoGEF); and

if said Gα protein subunit family is G12/13, said GASIP comprises the G protein-binding domain of Rho guanine nucleotide exchange factor 11 (PDZRhoGEF) or Rho guanine nucleotide exchange factor 1 (P115RhoGEF); and

(ii) a second component comprising a plasma membrane (PM)-targeting moiety, an endosomal-targeting moiety or a Golgi-targeting moiety tagged with a bioluminescent donor molecule or a fluorescent acceptor molecule;

wherein if said GASIP is tagged with said fluorescent acceptor molecule, said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety is tagged with said bioluminescent donor molecule, and if said GASIP is tagged with said bioluminescent donor molecule, said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety is tagged with said fluorescent acceptor molecule;

and wherein there is no protein-protein interaction between (i) the PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety and (ii) the GASIP.

2. The system of claim 1, wherein said GASIP comprises the G protein-binding domain of Rap1GAP.

3. The system of claim 2, wherein said G protein-binding domain of Rap1GAP comprises residues 1 to 442 of Rap1GAP set forth in SEQ ID NO:8, or a variant thereof in which one or more of the serine residues at positions 437, 439 and 441 are mutated or absent.

4. The system of claim 3, wherein said G protein-binding domain of Rap1GAP comprises residues 1 to 420 or 1 to 436 of Rap1GAP.

5. The system of claim 1, wherein said GASIP comprises the G protein-binding domain of an RGS protein.

6. The system of claim 5, wherein said RGS protein is Regulator of G-protein signaling 17 (RGS17), Regulator of G-protein signaling 19 (RGS19) or Regulator of G-protein signaling 20 (RGS20).

7. The system of claim 6, wherein said G protein-binding domain comprises residues 64 to 210 in the sequence of RGS17 set forth in SEQ ID NO:17, residues 70-217 in the sequence of RGS19 set forth in SEQ ID NO:18, or residues 242-388 in the sequence of RGS20 set forth in SEQ ID NO:19.

8. The system of claim 1, wherein GASIP comprises the G protein-binding domain of P63RhoGEF.

9. The system of claim 8, wherein said G protein-binding domain of P63RhoGEF comprises residues 295 to 502 in the sequence of P63RhoGEF set forth in SEQ ID NO:25.

10. The system of claim 1, wherein said GASIP comprises the G protein-binding domain of PDZRhoGEF or P115RhoGEF.

11. The system of claim 10, wherein said G protein-binding domain of PDZRhoGEF comprises residues 281 to 483 in the sequence of PDZRhoGEF set forth in SEQ ID NO:21.

12. The system of claim 10, wherein said G protein-binding domain of P115RhoGEF comprises residues 1 to 244 in the sequence of P115RhoGEF set forth in SEQ ID NO:23.

13. The system of any one of claims 1 to 12, wherein said GASIP is tagged with said bioluminescent donor molecule and said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety is tagged with said fluorescent acceptor molecule.

14. The system of any one of claims 1 to 13, wherein said PM targeting moiety is a PM protein or a peptide that localizes to the PM.

15. The system of claim 14, wherein said PM protein or peptide comprises (a) a palmitoylation, myristoylation, and/or prenylation signal sequence and/or (b) a polybasic sequence.

16. The system of claim 14 or 15, wherein said PM targeting moiety comprises the amino acid sequence GCMSCKCVLS set forth in SEQ ID NO:60, GCMGLPCVVM set forth in SEQ ID NO:61, CVKIKKCIIM set forth in SEQ ID NO:62, KKKKKKSKTKCVIM set forth in SEQ ID NO:63, KNGKKKRKSLAKRIRERCCIL set forth in SEQ ID NO: 45, CMSCKCCIL set forth in SEQ ID NO:4, or SPKKGLLQRLFKRQHQNNSKS set forth in SEQ ID NO:5.

17. The system of any one of claims 1 to 13, wherein said endosomal targeting moiety is an endosomal protein or a peptide that localizes to the endosomes.

18. The system of claim 17, wherein said endosomal protein or peptide comprises a FYVE domain.

19. The system of claim 18, wherein said endosomal targeting moiety comprises residues 739 to 806 in the sequence of human endofin set forth in SEQ ID NO:39.

20. The system of any one of claims 1 to 13, wherein said Golgi targeting moiety is Nitric oxide synthase, endothelial (eNOS1) or a fragment thereof comprising residues 1 to 73 in the sequence of human eNOS1 set forth in SEQ ID NO:46.

21. The system of claim 20, wherein said Golgi targeting moiety comprises residues 1 to 73 in the sequence of human eNOS1 set forth in SEQ ID NO:46.

22. The system of any one of claims 1 to 21, wherein (a) said first component further comprises a linker between (i) said GASIP and (ii) said bioluminescent donor molecule or fluorescent acceptor molecule; and/or said second component further comprises a linker between (i) said PM-targeting moiety, endosomal-targeting moiety or Golgi-targeting moiety and (ii) said bioluminescent donor molecule or fluorescent acceptor molecule.

23. The system of claim 22, wherein said linker is a peptide linked of 5 to 25 amino acids.

24. The system of any one of claims 1 to 23, further comprising a third component that is a cell surface receptor that signals through said G protein.

25. The system of claim 24, where said cell surface receptor is a G protein-coupled receptor (GPCR), a receptor tyrosine kinase (RTK) or an integrin receptor.

26. The system of any one of claims 1 to 25, further comprising a fourth component that is a recombinant Gα subunit polypeptide.

27. The system of claim 26, wherein said G protein activation is non-receptor guanine nucleotide exchange factor (GEF)-mediated G protein activation, wherein said recombinant Gα subunit polypeptide comprises at least one mutation in the carboxy (C)-terminal domain of said Gα subunit polypeptide, and wherein said C-terminal domain corresponds to the last seven residues of said Gα subunit polypeptide.

28. The system of claim 27, wherein said GEF is guanine nucleotide-binding (G) protein α subunit (Gα)-interacting vesicle-associated protein (GIV/Girdin), and wherein said GASIP comprises the G protein-binding domain of Rap1GAP as defined in any one of claims 1 to 4.

29. The system of claim 28, wherein said GEF is activated by a receptor tyrosine kinase (RTK).

30. The system of any one of claims 1 to 29, wherein said bioluminescent donor molecule is *Renilla* luciferase protein (*r*Luc) and said fluorescent acceptor molecule is a *Renilla* green fluorescent protein (*r*GFP).

31. A host cell expressing the components of the system defined in any one of claims 1 to 30.

32. A method for determining whether an agent modulates the activation of a G protein of interest, said method comprising:

(a) contacting the system of any one of claims 1 to 30 with a substrate for said bioluminescent donor molecule; and

(b) measuring the Bioluminescence Resonance Energy Transfer (BRET) signal in the system in the presence and absence of said agent;

wherein a difference in said BRET signal in the presence of said agent relative to the absence thereof is indicative that said agent modulates the activation of said G protein of interest.

33. A method for determining whether an agent modulates non-receptor guanine nucleotide exchange factor (GEF)-mediated G protein activation, said method comprising

(a) contacting the system of any one of claims 27 to 29 with a substrate for said bioluminescent donor molecule; and

(b) measuring the BRET signal in the system in the presence and absence of said agent;

wherein a difference in said BRET signal in the presence of said agent relative to the absence thereof is indicative that said agent modulates non-receptor GEF-mediated G protein activation.