signaling can also be initiated, sustained, and terminated in the endosomes (Murphy *et al*., 2009, *PNAS*, vol. 106 no. 42, 17615-17622; Tsvetanova *et al*., 2015, *J. Biol. Chem*. 290(11), pp. 6689-6696; Vilardaga *et al*., 2014, *Nature Chemical Biology* 10, 700-706). Similarly, some G proteins have been found associated with, and activated at, the Golgi (Lo *et al*., 2015, *Dev. Cell*. **33**: 189-203) and the endoplasmic reticulum (ER)-Golgi interface (Bastin *et al*., *Front Bioeng Biotechnol*. 2015 Sep 1;3: 128). Different signals can arise from receptors at the PM and other compartments such as the endosomes and the Golgi, resulting in distinct physiological responses, and different mechanisms regulate signaling of receptors at these compartments. These distinct mechanisms of signaling and regulation raise the possibility of novel therapies based on targeting signaling at other cellular compartments (rather than PM), and thus systems and assays to monitor G protein activation at different cellular compartments are needed.

U.S. Patent No. 9,029,097 and WO/2016/058094 disclose BRET-based biosensors for monitoring G protein activation. However, these biosensors require the tagging of one or more of the G protein subunits (Gα, Gβ, and/or Gγ), and/or of the GPCR, which may influence the activity of these proteins. Also, these biosensors detect global G protein activation in the cells, but do not allow the monitoring of G protein activation at different cellular compartments and in a G protein family-selective manner.

The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY

The present disclosure provides the following items 1 to 67:

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 a domain of a protein that specifically binds to Gi; if said Gα protein subunit family is Gq, said GASIP comprises a domain of a protein that specifically binds to Gq; and if said Gα protein subunit family is G12/13, said GASIP comprises a domain of a protein that specifically binds to G12/13; 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-

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 a domain of a protein that specifically binds to Gi;

if said Gα protein subunit family is Gq, said GASIP comprises a domain of a protein that specifically binds to Gq; and

if said Gα protein subunit family is G12/13, said GASIP comprises a domain of a protein that specifically binds to G12/13; 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.

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 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 a domain of a protein that specifically binds to Gi;

if said Gα protein subunit family is Gq, said GASIP comprises a domain of a protein that specifically binds to Gq; and

if said Gα protein subunit family is G12/13, said GASIP comprises a domain of a protein that specifically binds to G12/13; 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.

2. The system of claim 1, wherein said domain of a protein that specifically binds to Gi is the G protein-binding domain of Rap1GAP or of a Regulator of G-protein signaling (RGS) protein.

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

4. The system of claim 3, wherein said G protein-binding domain of Rap1GAP comprises residues 1 to 442 of Rap1GAP (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.

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

6. The system of claim 2, wherein said GASIP comprises the G protein-binding domain of an RGS protein.

7. The system of claim 6, wherein said RGS protein is RGS17, RGS19 or RGS20.

8. The system of claim 7, wherein said G protein-binding domain comprises residues 64 to 210 of RGS17 (SEQ ID NO:17), residues 70-217 of RGS19 (SEQ ID NO:18), or residues 242-388 of RGS20 (SEQ ID NO:19).

9. The system of claim 1, wherein said domain of a protein that specifically binds to Gq is the G protein-binding domain of P63RhoGEF or GRK2.

10. The system of claim 9, wherein said G protein-binding domain of P63RhoGEF comprises residues 295 to 502 of P63RhoGEF (SEQ ID NO:25).

11. The system of claim 9, wherein said G protein-binding domain of GRK2 comprises residues 30 to 203 of GRK2 (SEQ ID NO:27).

12. The system of claim 1, wherein said domain of a protein that specifically binds to G12/13 is the G protein-binding domain of PDZRhoGEF or P115RhoGEF.

13. The system of claim 12, wherein said G protein-binding domain of PDZRhoGEF comprises residues 281 to 483 of PDZRhoGEF (SEQ ID NO:21).

14. The system of claim 12, wherein said G protein-binding domain of P115RhoGEF comprises residues 1 to 244 of P115RhoGEF (SEQ ID NO:23).

15. The system of any one of claims 1 to 14, 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.

16. The system of any one of claims 1 to 15, wherein said PM targeting moiety is a PM protein or a fragment thereof that localizes to the PM.

17. The system of claim 16, wherein said PM protein or fragment thereof comprises (a) a palmitoylation, myristoylation, and/or prenylation signal sequence and/or (b) a polybasic sequence.

18. The system of claim 16 or 17, wherein said PM targeting moiety comprises the amino acid sequence GCMSCKCVLS (SEQ ID NO:60), GCMGLPCVVM (SEQ ID NO:61), CVKIKKCIIM (SEQ ID NO:62), KKKKKKSKTKCVIM (SEQ ID NO:63), or KNGKKKRKSLAKRIRERCCIL (SEQ ID NO: 45), CMSCKCCIL (SEQ ID NO:4), or SPKKGLLQRLFKRQHQNNSKS (SEQ ID NO:5).

19. The system of any one of claims 1 to 15, wherein said endosomal targeting moiety is an endosomal protein or a fragment thereof that localizes to the endosomes.

20. The system of claim 19, wherein said endosomal protein or fragment thereof comprises a FYVE domain.

21. The system of claim 20, wherein said endosomal targeting moiety comprises residues 739 to 806 of human endofin (SEQ ID NO:39).

22. The system of any one of claims 1 to 15, wherein said Golgi targeting moiety is eNOS1 or a fragment thereof that localizes to the Golgi.

23. The system of claim 22, wherein said Golgi targeting moiety comprises residues 1 to 73 of human eNOS1 (SEQ ID NO:46).

24. The system of any one of claims 1 to 23, 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.

25. The system of claim 24, wherein said linker is a peptide linked of 5 to 25 amino acids.

26. The system of any one of claims 1 to 25, further comprising a third component that is a cell surface receptor that signals through said G protein.

27. The system of claim 26, where said cell surface receptor is a GPCR, an RTK or an integrin receptor.

28. The system of any one of claims 1 to 27, further comprising a fourth component that is a recombinant Gα subunit polypeptide.

29. The system of claim 28, 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.

30. The system of claim 29, wherein said GEF is GIV/Girdin, and wherein said GASIP comprises the G protein-binding domain of Rap1GAP as defined in any one of claims 1 to 5.

31. The system of claim 30, wherein said GEF is activated by a receptor tyrosine kinase (RTK).

32. The system of any one of claims 1 to 31, wherein said bioluminescent donor molecule is *Renilla* luciferase protein (*r*Luc) and said fluorescent acceptor molecule is a *Renilla* green fluorescent protein (*r*GFP).

33. A host cell expressing the components of the system defined any one of claims 1 to 32.

34. 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 32 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 the activation of said G protein of interest.

35. 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 29 to 31 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.