

WHAT IS CLAIMED IS:

1. A system for measuring modulation of G protein activation in a G α protein subunit family-selective manner, said system comprising a cell expressing:
 - (i) a first component comprising a G α subunit interacting polypeptide (GASIP) tagged with a bioluminescent donor molecule or a fluorescent acceptor molecule;
5 wherein:
if said G α protein subunit family is Gi, said GASIP comprises a domain of a protein that specifically binds to Gi;
if said G α protein subunit family is Gq, said GASIP comprises a domain of a protein that
10 specifically binds to Gq; and
if said G α 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
15 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
20 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.
- 25 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.
- 30 6. The system of claim 4, wherein all three serine residues at positions 437, 439 and 441 are mutated.
7. The system of claim 4 or 6, wherein said serine residues are substituted for alanine.
8. The system of claim 2, wherein said GASIP comprises the G protein-binding domain of an RGS protein.

9. The system of claim 8, wherein said RGS protein is RGS17, RGS19 or RGS20.
10. The system of claim 9, 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).
- 5 11. 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.
12. The system of claim 11, wherein said GASIP comprises the G protein-binding domain of P63RhoGEF.
13. The system of claim 12, wherein said G protein-binding domain of P63RhoGEF comprises
10 residues 295 to 502 of P63RhoGEF (SEQ ID NO:25).
14. The system of claim 11, wherein said GASIP comprises the G protein-binding domain of GRK2.
15. The system of claim 14, wherein said G protein-binding domain of GRK2 comprises residues 30 to 203 of GRK2 (SEQ ID NO:27).
- 15 16. 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.
17. The system of claim 16, wherein said GASIP comprises the G protein-binding domain of PDZRhoGEF.
18. The system of claim 17, wherein said G protein-binding domain of PDZRhoGEF comprises
20 residues 281 to 483 of PDZRhoGEF (SEQ ID NO:21).
19. The system of claim 16, wherein said GASIP comprises the G protein-binding domain of P115RhoGEF.
20. The system of claim 19, wherein said G protein-binding domain of P115RhoGEF comprises residues 1 to 244 of P115RhoGEF (SEQ ID NO:23).
- 25 21. The system of any one of claims 1 to 20, 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.
22. The system of any one of claims 1 to 21, wherein said PM targeting moiety is a PM protein or a fragment thereof that localizes to the PM.

23. The system of claim 22, wherein said PM protein or fragment thereof comprises (a) a palmitoylation, myristoylation, and/or prenylation signal sequence and/or (b) a polybasic sequence.

24. The system of claim 22 or 23, wherein said PM targeting moiety comprises the amino acid sequence GCMSCCKCVLS (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).

25. The system of claim 24, wherein said PM targeting moiety comprises the amino acid sequence GKKKKKSKTKCVIM (SEQ ID NO:1).

26. The system of any one of claims 1 to 21, wherein said endosomal targeting moiety is an endosomal protein or a fragment thereof that localizes to the endosomes.

27. The system of claim 26, wherein said endosomal protein or fragment thereof comprises a FYVE domain.

28. The system of claim 27, wherein said endosomal targeting moiety comprises the FYVE domain of human endofin.

29. The system of claim 28, wherein said endosomal targeting moiety comprises residues 739 to 806 of human endofin (SEQ ID NO:39).

30. The system of any one of claims 1 to 21, wherein said Golgi targeting moiety is a Golgi protein or a fragment thereof that localizes to the Golgi.

31. The system of claim 30, wherein said Golgi targeting moiety is eNOS1 or a fragment thereof that localizes to the Golgi.

32. The system of claim 31, wherein said Golgi targeting moiety comprises residues 1 to 73 of human eNOS1 (SEQ ID NO:46).

33. The system of any one of claims 1 to 32, wherein said first component further comprises a linker between (i) said GASIP and (ii) said bioluminescent donor molecule or fluorescent acceptor molecule.

34. The system of any one of claims 1 to 33, wherein 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.

35. The system of claim 33 or 34, wherein said linker is a peptide linked of 5 to 25 amino acids.

36. The system of any one of claims 1 to 35, further comprising a third component that is a cell surface receptor that signals through said G protein.

37. The system of claim 36, where said cell surface receptor is a GPCR, an RTK or an integrin receptor.

5 38. The system of any one of claims 1 to 37, wherein said G protein activation is G protein-coupled receptor (GPCR)-mediated G protein activation.

39. The system of any one of claims 1 to 38, further comprising a fourth component that is a recombinant G α subunit polypeptide.

10 40. The system of claim 39, wherein said G protein activation is non-receptor guanine nucleotide exchange factor (GEF)-mediated G protein activation, wherein said recombinant G α subunit polypeptide comprises at least one mutation in the carboxy (C)-terminal domain of said G α subunit polypeptide, and wherein said C-terminal domain corresponds to the last seven residues of said G α subunit polypeptide.

15 41. The system of claim 40, wherein said mutation is a deletion or substitution of at least the last two C-terminal residues.

42. The system of claim 41, wherein said mutation is a deletion or substitution of at least the last five C-terminal residues.

43. The system of claim 40, wherein said mutation is a deletion or substitution of at least one of the conserved leucine residues in said C-terminal domain.

20 44. The system of claim 43, wherein said mutation is a substitution of the last conserved leucine residue in said C-terminal domain.

45. The system of claim 44, wherein said substitution is a leucine to aspartate, leucine to proline, or leucine to arginine substitution.

25 46. The system of any one of claims 40 to 45, 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 7.

47. The system of claim 46, wherein said GEF is activated by a receptor tyrosine kinase (RTK).

30 48. The system of any one of claims 1 to 47, wherein said bioluminescent donor molecule is a luciferase, preferably a *Renilla* luciferase protein (*rLuc*).

49. The system of any one of claims 1 to 48, wherein said fluorescent acceptor molecule is a green fluorescent protein (GFP), preferably a *Renilla* GFP (*rGFP*).

50. One or more nucleic acids encoding the first and/or second components of the system of any one of claims 1 to 49.
51. The one or more nucleic acids of claim 50, comprising a first nucleic acid encoding the first component of the system of any one of claims 1 to 49 and a second nucleic acid encoding the second component of the system of any one of claims 1 to 49.
52. One or more vectors comprising the one or more nucleic acids of claim 50 or 51.
53. A host cell expressing the components of the system defined any one of claims 1 to 49.
54. A method for determining whether an agent modulates the activation of a G protein of interest, said method comprising:
- 10 (a) contacting the system of any one of claims 1 to 49 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.
- 15 55. The method of claim 54, wherein said G protein of interest is of the Gi protein subunit family, and wherein said GASIP comprises the G protein-binding domain of Rap1GAP as defined in any one of claims 1 to 7.
56. The method of claim 49, wherein the system comprises a recombinant G α subunit polypeptide of the Gi protein subunit family.
- 20 57. The method of claim 56, wherein the method is performed using a plurality of systems, and wherein each of said systems comprises a different recombinant G α subunit polypeptide of the Gi protein subunit family.
58. The method of claim 54, wherein said G protein of interest is of the Gq protein subunit family, wherein said GASIP comprises the G protein-binding domain of P63RhoGEF or GRK2 as defined in any one of claims 1 and 11 to 15.
- 25 59. The method of claim 58, wherein the system comprises a recombinant G α subunit polypeptide of the Gq protein subunit family.
60. The method of claim 59, wherein the method is performed using a plurality of systems, and wherein each of said systems comprises a different recombinant G α subunit polypeptide of the Gq protein subunit family.
- 30 61. The method of claim 54, wherein said G protein of interest is of the G12/13 protein subunit family, wherein said GASIP comprises the G protein-binding domain of PDZRhoGEF or P115RhoGEF as defined in any one of claims 1 and 16 to 20.

62. The method of claim 61, wherein the system comprises a recombinant G α subunit polypeptide of the G12/13 protein subunit family.

63. The method of claim 62, wherein the method is performed using a plurality of systems, and wherein each of said systems comprises a different recombinant G α subunit polypeptide of the G12/13 protein subunit family.

64. 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 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 claims 54 to 64, wherein the BRET signal is measured using a plate reader or by microscopy.

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

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