CLAIMS

1. A method of detecting an analyte in a sample, the method comprising

i) flowing through a microfluidic device comprising one or more microchannels,

a) the sample,

b) a sensor molecule comprising a domain that binds the analyte, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance,

c) a substrate of the chemiluminescent donor,

ii) mixing the sensor molecule, sample and substrate in the device, and

iii) detecting modification of the substrate by the chemiluminescent donor using an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the analyte binds the sensor molecule.

2. The method of claim 1, wherein the sensor molecule is not fixed to the device.

3. The method of claim 1 or claim 2, wherein the method can be used to detect the analyte in real time.

4. The method according to any one of claims 1 to 3, wherein the sensor molecule and substrate enter the device through different microchannels.

5. The method according to any one of claims 1 to 4, wherein the Förster distance of the chemiluminescent donor domain and the acceptor domain is at least 5.6nm.

6. The method of claim 5, wherein the Förster distance of the chemiluminescent donor domain and the acceptor domain is between about 5.6nm and about 10nm.

7. The method according to any one of claims 1 to 6, wherein the analyte binding or releasing from the sensor molecule results in a change in BRET ratio which is ≥15% of the maximum observed BRET ratio.

8. The method according to any one of claims 1 to 7, wherein the quantum yield detected by the electro-optical sensing device is less than about 8%, or less than about 5%, or less than about 2%.

9. The method according to any one of claims 1 to 8, wherein the acceptor domain has a Stokes Shift of between about 50nm and about 150nm.

10. The method of claim 9, wherein the acceptor domain has a Stokes Shift of about 100nm.

11. The method according to any one of claims 1 to 10 which is performed within about 1s to about 100s.

12. The method according to any one of claims 1 to 11, wherein the sample is a liquid, gas, emulsion or suspension.

13. The method of claim 12, wherein the sample is a liquid which has been pre-equilibrated with a gas.

14. The method of claim 12, wherein the suspension is, or comprises, a cell-free extract.

15. The method of claim 12, wherein the suspension comprises cells.

16. The method according to any one of claims 1 to 15, wherein the flow rate through the microfluidic device is between about 1µl/hour to about 1.5ml/hour.

17. The method of claim 16, wherein the flow rate through the microfluidic device is between about 200µl/hour to about 1ml/hour.

18. The method according to any one of claims 1 to 17, wherein the sensor molecule comprises a protein receptor such as a G coupled protein receptor and the concentration of the sensor molecule following step ii) is between about 1nM to about 10µM.

19. The method according to any one of claims 1 to 18, wherein flowing through the microfluidic device is continuous flow, batch flow or stop flow.

20. The method according to any one of claims 1 to 19, wherein the flowing is performed by one or more of pumping, vacuum, hydraulics, suction, electrokinesis, chemiosmosis, capillary force, acoustics, electromagnetics, piezoelectrics.

21. The method according to any one of claims 1 to 20, wherein the mixing is achieved by diffusion over dimensions perpendicular to the direction of flow through a microchannel comprising the sample, sensor molecule and substrate.

22. The method of claim 21, wherein the section of the microchannel comprising the sample, sensor molecule and substrate is between 5mm and 100 mm.

23. The method according to any one of claims 1 to 22, wherein each microchannel has a cross-sectional area of about 1μm^2 to about 1mm^2.

24. The method according to any one of claims 1 to 23, wherein step iii) is performed in a reaction chamber with a volume of about 1 pl to about 200 µl.

25. The method according to any one of claims 1 to 24, wherein step iii) comprises processing at least one signal from the electro-optical sensing device to determine whether the analyte is absent or present in the sample, and if present optionally determining the concentration of the analyte in the sample.

26. The method according to any one of claims 1 to 25, wherein the domain that binds the analyte is a protein or a nucleic acid.

27. The method of claim 26, wherein the protein is a receptor, odorant binding protein, pheromone-binding protein, enzyme, ligand carrier or bacterial periplasmic binding protein.

28. The method of claim 27, wherein the receptor is a G protein coupled receptor.

29. The method according to any one of claims 1 to 28, wherein the chemiluminescent donor domain is a bioluminescent protein.

30. The method of claim 29, wherein the bioluminescent protein is a luciferase, a β-galactosidase, a lactamase, a horseradish peroxidase, an alkaline phosphatase, a β-glucuronidase or a β-glucosidase.

31. The method of claim 30, wherein the luciferase is a *Renilla* luciferase, a Firefly luciferase, a Coelenterate luciferase, a North American glow worm luciferase, a click beetle luciferase, a railroad worm luciferase, a bacterial luciferase, a *Gaussia* luciferase, Aequorin, an *Arachnocampa* luciferase, or a biologically active variant or fragment of any one, or chimera of two or more, thereof.

32. The method according to any one of claims 1 to 31, wherein the substrate is luciferin, calcium, coelenterazine, or a derivative or analogue of coelenterazine.

33. The method according to any one of claims 1 to 32, wherein the acceptor domain is a fluorescent acceptor domain.

34. The method of claim 33, wherein the fluorescent acceptor domain is a protein.

35. The method of claim 34, wherein the fluorescent acceptor domain is green fluorescent protein (GFP), blue fluorescent variant of GFP (BFP), cyan fluorescent variant of GFP (CFP), yellow fluorescent variant of GFP (YFP), enhanced GFP (EGFP), enhanced CFP (ECFP), enhanced YFP (EYFP), GFPS65T, Emerald, Venus, mOrange, Topaz, GFPuv, destabilised EGFP (dEGFP), destabilised ECFP (dECFP), destabilised EYFP (dEYFP), HcRed, t-HcRed, DsRed, DsRed2, t-dimer2, t-dimer2(12), mRFP1, pocilloporin, Renilla GFP, Monster GFP, paGFP, Kaede protein or a Phycobiliprotein, or a biologically active variant or fragment of any one thereof.

36. The method of claim 33, wherein the fluorescent acceptor domain is a non-protein.

37. The method of claim 36, wherein the fluorescent acceptor domain is an Alexa Fluor dye, Bodipy dye, Cy dye, fluorescein, dansyl, umbelliferone, fluorescent microsphere, luminescent microsphere, fluorescent nanocrystal, Marina Blue, Cascade Blue, Cascade Yellow, Pacific Blue, Oregon Green, Tetramethylrhodamine, Rhodamine, Texas Red, rare earth element chelates, or any combination or derivatives thereof.

38. The method according to any one of claims 29 to 37 which further comprises providing a co-factor of the bioluminescent protein.

39. The method of claim 38, wherein the co-factor is ATP, magnesium, oxygen, $FMNH_2$, calcium, or a combination of any two or more thereof.

40. The method according to any one of claims 29 to 39, wherein

i) the bioluminescent protein is a luciferase or a biologically active variant or fragment, and/or

ii) the substrate is luciferin, coelenterazine, or a derivative or analogue of coelenterazine, and/or

iii) the acceptor domain is green fluorescent protein (GFP), Venus, mOrange, or a biologically active variant or fragment of any one thereof.

41. The method of claim 40, wherein

i) the luciferase is a *Renilla* luciferase, the acceptor domain is GFP^2, and the substrate is coelenterazine 400a,

ii) the luciferase is a *Renilla* luciferase 2, the acceptor domain is GFP^2, and the substrate is coelenterazine 400a,

iii) the luciferase is a *Renilla* luciferase 8, the acceptor domain is GFP^2, and the substrate is coelenterazine 400a,

iv) the luciferase is a *Renilla* luciferase 2, the acceptor domain is Venus, and the substrate is coelenterazine,

v) the luciferase is a *Renilla* luciferase 8, the acceptor domain is Venus, and the substrate is coelenterazine,

vi) the luciferase is a *Renilla* luciferase 8.6-535, the acceptor domain is mOrange, and the substrate is coelenterazine, or

vii) the luciferase is a *Renilla* luciferase 8, the acceptor domain is mOrange, and the substrate is coelenterazine.

42. The method according to any one of claims 1 to 41 which comprises simultaneously or sequentially detecting two or more different analytes using the same microfluidic device.

43. The method according to any one of claims 1 to 42, wherein the microfluidic device comprises one or more sets of

a) three input microchannels, one each for the sensor molecule, substrate and sample, or

b) two input microchannels, one for the substrate and the other for a pre-mixture of the sensor molecule and sample, or

c) two input microchannels, one for the sensor molecule and the other for a pre-mixture of the substrate and sample.

44. The method according to any one of claims 1 to 43, wherein at least one microchannel comprises a reaction chamber which has a different volume to at least one other microchannel.

45. The method according to any one of claims 1 to 44, wherein at least one microchannel comprises two or more reaction chambers of the same or different volume.

46. The method according to any one of claims 1 to 45, wherein the electro-optical sensing device has at least two different wavelength channels.

47. The method according to any one of claims 1 to 46, wherein the sensor molecule is present in a cell-free extract.

48. The method according to any one of claims 1 to 47, wherein the analyte is exposed on the surface of a cell and the method further comprises diverting cells comprising the analyte through a different microchannel than cells in the sample lacking the analyte.

49. A microfluidic system for detecting an analyte in a sample, the system comprising

i) at least one reservoir suitable for containing a sensor molecule comprising a domain that binds the analyte, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance,

ii) a microfluidic device comprising one or more microchannels,

iii) means for mixing the sensor molecule, the sample and a substrate of the chemiluminescent donor domain in the device,

iv) a reaction chamber for detecting binding of the analyte to the sensor molecule, and

v) an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the analyte binds the sensor molecule.

50. The system of claim 49, wherein the sensor molecule is not fixed to the device.

51. The system of claim 49 or claim 50 which can be used to detect the analyte in real time.

52. The system according to any one of claims 49 to 51, wherein the sensor molecule and substrate enter the device through different microchannels.

53. The system according to any one of claims 49 to 52, wherein the microfluidic device comprises at least two input microchannels, wherein one of the input microchannels is for flowing the sensor molecule into the device.

54. The system according to any one of claims 49 to 53, wherein the electro-optical sensing device comprises at least two different wavelength channels.

55. The system of claim 54, wherein the electro-optical sensing device is capable of simultaneously, or in rapid succession, detecting two different wavelength channels.

56. The system of claim 55, wherein the electro-optical sensing device is capable of detecting two different wavelength channels in less than 1 second.

57. The system according to any one of claims 49 to 56, wherein the microfluidic device is designed to enable the detection of two or more analytes.

58. A method of classifying a sample, the method comprising

i) flowing through a microfluidic device comprising one or more microchannels,

a) the sample,

b) a sensor molecule comprising a domain that binds one or more analytes, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte(s), is within ± 50% of the Forster distance,

c) a substrate of the chemiluminescent donor,

ii) mixing the sensor molecule, sample and substrate in the device,

iii) detecting modification of the substrate by the chemiluminescent donor using an electro-optical sensing device,

iv) processing at least one signal from the electro-optical sensing device and correlating the pattern of electro-optical responses with one or more pre-determined characteristics of one or more samples of interest, and

v) classifying the sample based on the correlation of the pattern of responses,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the one or more analytes binds the sensor molecule.

59. The method of claim 58 which comprises two or more different sensor molecules each of which binds a different analyte or range of analytes, and step v) comprises classifying the sample based on the presence, absence or concentration of each of the analytes or range of analytes.

60. The method of claim 58 or claim 59, wherein one or more of the analytes are unknown.

61. The method according to any one of claims 58 to 60 which can be used to classify the sample in real time.

62. The method according to any one of claims 58 to 61, wherein the sensor molecule is not fixed to the device.

63. The method according to any one of claims 58 to 62, wherein the sensor molecule and substrate enter the device through different microchannels.

64. A microfluidic system for classifying a sample, the system comprising

i) at least one reservoir suitable for containing a sensor molecule comprising a domain that binds one or more analytes, a chemiluminescent donor domain and an

acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance,

ii) a microfluidic device comprising one or more microchannels,

iii) means for mixing the sensor molecule, the sample and a substrate of the chemiluminescent donor domain in the device,

iv) a reaction chamber for detecting binding of the analyte to the sensor molecule, and

v) an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the one or more analytes binds the sensor molecule.

65. The system of claim 64 which comprises two or more different sensor molecules each of which binds a different analyte or range of analytes.

66. The system of claim 64 or claim 65, wherein one or more of the analytes, or range of analytes, are unknown.

67. The system according to any one of claims 64 to 66 which can be used to classify samples in real time.

68. The system according to any one of claims 64 to 67, wherein the sensor molecule is not fixed to the device.

69. The system according to any one of claims 64 to 68, wherein the sensor molecule and substrate enter the device through different microchannels.

70. The system according to any one of claims 64 to 69, wherein the microfluidic device comprises at least two input microchannels, wherein one of the input microchannels is for flowing the sensor molecule into the device.

71. A method of screening for a compound that binds a molecule of interest, the method comprising

i) flowing through a microfluidic device comprising one or more microchannels,

a) a candidate compound,

b) a sensor molecule comprising the molecule of interest, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence the candidate compound, is within ± 50% of the Forster distance,

c) a substrate of the chemiluminescent donor,

ii) mixing the sensor molecule, the candidate compound and substrate in the device,

iii) detecting modification of the substrate by the chemiluminescent donor using an electro-optical sensing device,

v) processing at least one signal from the electro-optical sensing device to determine whether the candidate compound binds the sensor molecule, and

vi) selecting the compound if it binds the sensor molecule,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the candidate compound binds the sensor molecule.

72. The method of claim 71 which can be used to detect binding of the candidate compound to the sensor molecule in real time.

73. The method of claim 71 or claim 72, wherein the sensor molecule is not fixed to the device.

74. The method according to any one of claims 71 to 73, wherein the sensor molecule and substrate enter the device through different microchannels.

75. A microfluidic system for screening for a compound that binds a molecule of interest, the system comprising

i) at least one reservoir suitable for containing a sensor molecule comprising the molecule of interest, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of a candidate compound, is within ± 50% of the Forster distance,

ii) a microfluidic device comprising one or more microchannels,

iii) means for mixing the sensor molecule, the candidate compound and a substrate of the chemiluminescent donor domain in the device,

iv) a reaction chamber for detecting binding of the candidate compound to the sensor molecule,

v) an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the candidate compound binds the sensor molecule.

76. The system of claim 75 which can be used to detect binding of the candidate compound to the sensor molecule in real time.

77. The system of claim 75 or claim 76, wherein the sensor molecule is not fixed to the device.

78. The system according to any one of claims 75 to 77, wherein the sensor molecule and substrate enter the device through different microchannels.

79. The system according to any one of claims 75 to 78, wherein the microfluidic device comprises at least two input microchannels, wherein one of the input microchannels is for flowing the sensor molecule into the device.

80. A method of detecting an analyte in a sample, the method comprising

i) contacting the sample, in the presence of coelenterazine, with a sensor molecule comprising

a) a domain that binds the analyte,

b) *Renilla* luciferase, and

c) green fluorescent protein 2, and

ii) determining whether bioluminescent resonance energy transfer (BRET) between the bioluminescent protein and the acceptor molecule is modified,

wherein the spatial location and/or dipole orientation of the bioluminescent protein relative to the acceptor molecule is altered when the analyte binds the domain.

81. An isolated sensor molecule comprising a domain that binds one or more analytes, *Renilla* luciferase, and green fluorescent protein 2.

82. A method of detecting 2-pentanone in a sample, the method comprising

i) contacting the sample with a polypeptide which is *C. elegans* str-112 (SEQ ID NO:41) or str-113 (SEQ ID NO:42), or a variant thereof which binds 2-pentanone, and

ii) detecting whether any of the polypeptide is bound to 2-pentanone.

83. The method of claim 82, wherein the variant of str-113 is a str-114/str-113 fusion (SEQ ID NO:43).

84. The method of claim 83, wherein the polypeptide is detectably labelled.

85. A method of detecting bacteria in a sample comprising detecting 2-pentanone using the method according to any one of claims 82 to 84.

CLAIMS

1. A method of detecting an analyte in a sample, the method comprising

i) flowing through a microfluidic device comprising one or more microchannels,

a) the sample,

b) a sensor molecule comprising a domain that binds the analyte, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance, and wherein the sensor molecule is not fixed to the device,

c) a substrate of the chemiluminescent donor,

ii) mixing the sensor molecule, sample and substrate in the device, and

iii) detecting modification of the substrate by the chemiluminescent donor using an electro-optical sensing device and calculating as a ratio the energy transfer occurring between the chemiluminescent donor domain and the acceptor domain,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the analyte binds the sensor molecule.

2. A method of classifying a sample, the method comprising

i) flowing through a microfluidic device comprising one or more microchannels,

a) the sample,

b) a sensor molecule comprising a domain that binds one or more analytes, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte(s), is within ± 50% of the Forster distance, and wherein the sensor molecule is not fixed to the device,

c) a substrate of the chemiluminescent donor,

ii) mixing the sensor molecule, sample and substrate in the device,

iii) detecting modification of the substrate by the chemiluminescent donor using an electro-optical sensing device and calculating as a ratio the energy transfer occurring between the chemiluminescent donor domain and the acceptor domain,

iv) processing at least one signal from the electro-optical sensing device and correlating the pattern of electro-optical responses with one or more pre-determined characteristics of one or more samples of interest, and

v) classifying the sample based on the correlation of the pattern of responses,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the one or more analytes binds the sensor molecule.

3. The method of claim 2, wherein one or more of the analytes are unknown.

4. The method according to any one of claims 1 to 3, wherein the method can be used to detect the analyte in real time and/or which is performed within about 1s to about 100s.

5. The method according to any one of claims 1 to 4, wherein the sensor molecule and substrate enter the device through different microchannels.

6. The method according to any one of claims 1 to 5, wherein one or more or all of the following features apply;

i) the Förster distance of the chemiluminescent donor domain and the acceptor domain is at least 5.6nm,

ii) the analyte binding or releasing from the sensor molecule results in a change in BRET ratio which is ≥15% of the maximum observed BRET ratio, or

iii) the acceptor domain has a Stokes Shift of between about 50nm and about 150nm.

7. The method according to any one of claims 1 to 6, wherein the quantum yield detected by the electro-optical sensing device is less than about 8%, or less than about 5%, or less than about 2%.

8. The method according to any one of claims 1 to 7, wherein the sample is a liquid, gas, liquid which has been pre-equilibrated with a gas, emulsion or suspension.

9. The method according to any one of claims 1 to 8, wherein

i) the flow rate through the microfluidic device is between about 1μl/hour to about 1.5ml/hour, and/or

ii) flowing through the microfluidic device is continuous flow, batch flow or stop flow.

10. The method according to any one of claims 1 to 9, wherein step iii) is performed in a reaction chamber with a volume of about 1 pl to about 200 µl.

11. The method according to any one of claims 1 to 10, wherein the domain that binds the analyte is a protein or a nucleic acid.

12. The method of claim 11, wherein the protein is a receptor, odorant binding protein, pheromone-binding protein, enzyme, ligand carrier, bacterial periplasmic binding protein or a G protein coupled receptor.

13. The method according to any one of claims 1 to 12, wherein one or more or all of the following features apply;

i) the chemiluminescent donor domain is a bioluminescent protein,

ii) the substrate is luciferin, calcium, coelenterazine, or a derivative or analogue of coelenterazine, or

iii) the acceptor domain is a fluorescent acceptor domain.

14. The method of claim 13, wherein

i) the bioluminescent protein is a luciferase or a biologically active variant or fragment, and/or

ii) the substrate is luciferin, coelenterazine, or a derivative or analogue of coelenterazine, and/or

iii) the acceptor domain is green fluorescent protein (GFP), Venus, mOrange, or a biologically active variant or fragment of any one thereof.

15. The method according to any one of claims 1 to 14 which comprises simultaneously or sequentially detecting two or more different analytes using the same microfluidic device.

16. The method according to any one of claims 1 to 15, wherein the microfluidic device comprises one or more sets of

a) three input microchannels, one each for the sensor molecule, substrate and sample, or

b) two input microchannels, one for the substrate and the other for a pre-mixture of the sensor molecule and sample, or

c) two input microchannels, one for the sensor molecule and the other for a pre-mixture of the substrate and sample.

17. The method according to any one of claims 1 to 16, wherein one or more or all of the following features apply;

i) at least one microchannel comprises a reaction chamber which has a different volume to at least one other microchannel,

ii) at least one microchannel comprises two or more reaction chambers of the same or different volume, or

iii) the electro-optical sensing device has at least two different wavelength channels.

18. A microfluidic system for detecting an analyte in a sample, the system comprising

i) at least one reservoir suitable for containing a sensor molecule comprising a domain that binds the analyte, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance, and wherein the sensor molecule is not fixed to the device,

ii) a microfluidic device comprising one or more microchannels,

iii) means for mixing the sensor molecule, the sample and a substrate of the chemiluminescent donor domain in the device,

iv) a reaction chamber for detecting binding of the analyte to the sensor molecule, and

v) an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the analyte binds the sensor molecule.

19. The system of claim 18

i) which can be used to detect the analyte in real time, and/or

ii) wherein the microfluidic device is designed to enable the detection of two or more analytes.

20. A microfluidic system for classifying a sample, the system comprising

i) at least one reservoir suitable for containing a sensor molecule comprising a domain that binds one or more analytes, a chemiluminescent donor domain and an acceptor domain, wherein the separation and relative orientation of the chemiluminescent donor domain and the acceptor domain, in the presence and/or the absence of analyte, is within ± 50% of the Forster distance, and wherein the sensor molecule is not fixed to the device,

ii) a microfluidic device comprising one or more microchannels,

iii) means for mixing the sensor molecule, the sample and a substrate of the chemiluminescent donor domain in the device,

iv) a reaction chamber for detecting binding of the analyte to the sensor molecule, and

v) an electro-optical sensing device,

wherein the spatial location and/or dipole orientation of the chemiluminescent donor domain relative to the acceptor domain is altered when the one or more analytes binds the sensor molecule.