

Summary of the Invention

In one aspect, the present invention provides an optical probe comprising at least one fluorophore connected to at least one quencher by an enzyme cleavable peptide sequence; the or each fluorophore being substantially fluorescently quenched by the at least one quencher when connected to the enzyme cleavable peptide sequence; the or each fluorophore is separated from the at least one quencher when the enzyme cleavable peptide sequence of the at least one probe element is cleaved; wherein the enzyme cleavable peptide sequence comprises one of the list: SEQ ID NO.7; SEQ ID NO.5; SEQ ID NO.3; SEQ ID NO.4; SEQ ID NO.2; SEQ ID NO.6; SEQ ID NO. 8 to SEQ ID NO.14; and is selectively cleavable by one or more matrix metalloproteinase (MMP), wherein the unit of the at least one fluorophore connected to the enzyme cleavable peptide sequence corresponds to a probe element; the probe comprises a plurality of probe elements, each probe element being connected to a core, and wherein the core comprises the at least one quencher or each probe element comprises at least one quencher at the proximal end of the probe element to the core.

In another aspect, the present invention provides a method of detecting MMP in a target zone where the target zone is *in vitro* or *ex vivo*, the method comprising the steps:

- a. providing a probe according to any preceding claim;
- b. applying the probe to the target zone;
- c. illuminating the target area with an appropriate wavelength of light to excite the or each fluorophore of the probe; and
- d. determining the fluorescence intensity of the or each fluorophore,

wherein significant fluorescence of the or each fluorophore of the probe is indicative of the presence of MMP in the target zone.

Statements

According to a first aspect of the invention, there is provided an optical probe comprising at least one fluorophore connected to at least one quencher by an enzyme cleavable peptide sequence; the or each fluorophore being substantially fluorescently quenched by the at least one quencher when connected to the enzyme cleavable peptide sequence; the or each fluorophore is separated from the at least one quencher when the enzyme cleavable peptide sequence of the at least one probe element is cleaved; wherein the enzyme cleavable peptide sequence comprises one of SEQ ID NO.1 to SEQ ID NO.14 (Table 1) and is selectively cleavable by one or more matrix metalloproteinase (MMP).

SEQ ID NO.	Sequence
1	G-P-K-G-L-K-G
2	G-P-K-G-I-K-G
3	G-P-K-G-Nle-K-G
4	P-F-G-M-K-βA
5	P-F-G-L-K-βA
6	P-F-G-I-K-βA
7	P-F-G-Nle-K-βA
8	P-Cha-G-M-F-G
9	P-Cha-G-M-W-G
10	P-Cha-G-M-Y(Me)-G
11	P-Cha-G-M-Y-G
12	P-Cha-G-M-K-βA-G
13	P-Cha-G-M-H-G
14	P-Cha-G-M-K-G

(wherein Nle=norleucine; Cha=3-cyclohexylalanine; βA= β-alanine; Y(Me)=4-methoxyphenyl alanine)

Table 1

SEQ ID NO.1 corresponds to a known peptide sequence. However, such a sequence is not known in the type of optical probes described herein.

In one embodiment, there is provided an optical probe as described herein wherein the enzyme cleavable peptide sequence comprises one of SEQ ID NO.2 to SEQ ID NO.14 (Table 1).

Claims

1. An optical probe comprising at least one fluorophore connected to at least one quencher by an enzyme cleavable peptide sequence; the or each fluorophore being substantially fluorescently quenched by the at least one quencher when connected to the enzyme cleavable peptide sequence; the or each fluorophore is separated from the at least one quencher when the enzyme cleavable peptide sequence of the at least one probe element is cleaved; wherein the enzyme cleavable peptide sequence comprises one of the list: SEQ ID NO.7; SEQ ID NO.5; SEQ ID NO.3; SEQ ID NO.4; SEQ ID NO.2; SEQ ID NO.6; SEQ ID NO. 8 to SEQ ID NO.14; and is selectively cleavable by one or more matrix metalloproteinase (MMP), wherein the unit of the at least one fluorophore connected to the enzyme cleavable peptide sequence corresponds to a probe element; the probe comprises a plurality of probe elements, each probe element being connected to a core, and wherein the core comprises the at least one quencher or each probe element comprises at least one quencher at the proximal end of the probe element to the core.
2. The probe according to claim 1, wherein the enzyme cleavable peptide sequence is selectively cleavable by MMP-2 and/or MMP-9 and/or MMP-13.
3. The probe of either claim 1 or claim 2, wherein the enzyme cleavable peptide sequence comprises one of SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5 and SEQ ID NO. 7 to SEQ ID NO.14, preferably comprises one of SEQ ID NO.3, SEQ ID NO.5 and SEQ ID NO.7, preferably wherein the enzyme cleavable peptide sequence comprises SEQ ID NO.7.
4. The probe according to any preceding claim, wherein the at least one quencher is the same type of fluorophore as the at least one fluorophore, and the at least one quencher and the at least one fluorophore self-quench, or wherein the at least one quencher is a different type of fluorophore to the at least one fluorophore, and is a fluorescent quencher.
5. The probe according to any preceding claim, wherein the at least one fluorophore is selected from fluorescein, or a derivative thereof, a cyanine fluorophore, such as Cy2, Cy3, Cy5, or Cy7, rhodamine or derivative thereof, a fluorescent protein, such as green fluorescent protein (GFP), yellow fluorescent protein (YFP), or cyan fluorescent protein (CFP), or 7-nitrobenz-2-oxa-1,3-diazole (NBD).

6. The probe according to claim 4, wherein the at least one fluorophore and the at least one quencher form a FRET pair and are chosen from the list (fluorophore/quencher): Cy3/Cy5, Cy3/QSY21, Cy5/QSY21, Cy5/BHQ-3, fluorescein/tetramethylrhodamine, fluorescein/methyl red, NBD/methyl red, cyan fluorescent protein (CFP)/yellow fluorescent protein (YFP), and carboxy fluorescein/methyl red.
7. The probe according to any preceding claim, wherein the at least one fluorophore is connected to the enzyme cleavable peptide sequence by a spacer, optionally wherein the spacer comprises 6-aminohexanoic acid (Ahx), polyethyleneglycol (PEG).
8. The probe according to any one preceding claim, wherein each of the plurality of probe elements are independently connected indirectly to the core via a linker.
9. The probe according to claim 8, wherein the linker comprises a D-amino acid.
10. The probe according to either one of claim 8 or claim 9, wherein the at least one fluorophore of each probe element within the plurality of probe elements self-quench, such that the at least one fluorophore of a first probe element substantially fluorescently quenches the at least one fluorophore of a second probe element.
11. The probe according to any preceding claim, wherein the probe comprises at least one reporter fluorophore that is not substantially fluorescently quenched, optionally wherein the at least one reporter fluorophore fluoresces at a wavelength that is different to the wavelength of light at which the at least one fluorophore of the plurality of probe elements fluoresces.
12. A method of detecting MMP in a target zone where the target zone is *in vitro* or *ex vivo*, the method comprising the steps:
- providing a probe according to any preceding claim;
 - applying the probe to the target zone;
 - illuminating the target area with an appropriate wavelength of light to excite the or each fluorophore of the probe; and
 - determining the fluorescence intensity of the or each fluorophore,
- wherein significant fluorescence of the or each fluorophore of the probe is indicative of the presence of MMP in the target zone.

13. A method according to claim 12, wherein the target zone is a portion of tissue that has been taken from a human or non-human animal, preferably wherein the tissue type is the heart, lung, liver, connective tissue, skin, or intestine.

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14. A method according to either one of claim 12 to claim 13, wherein the presence of MMP in the target zone is indicative of active fibroproliferation within the target zone, and/or wherein the presence of MMP in the target zone is indicative of cancer within the target zone.

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15. A method according to claim 12, wherein the target zone is a portion of tissue removed from a joint of a subject, wherein the presence of MMP in the target zone is indicative of active arthritis in the target zone.

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