when large amounts of the fluorescent dyes are confined to a small subcellular area; (c) they can only be used in very diluted solutions and have very poor photostability; (d) they have small Stokes shifts (usually less than 30 nm), which is difficult to distinguish from the background signals and require precise emission filters; (e) their synthesis or preparation is usually difficult, which makes them hard to adjust the emission spectrum and introduce reactive sites for enzymes or bioactive molecules.

Therefore there is a need for fluorescent probes that target organelles, do not easily diffuse away from the enzyme reactive sites due to their aqueous solubility, show higher Stokes shifts and at the same time are less difficult to synthesize and functionalize. The probes should show less background noise as the known AIE based probes. The previous fluorescent probes based solely on an AIE turn-on mechanism must have good water solubility to ensure low background noise, which restricts their applications for detection of enzymes with hydrophobic ligand-binding sites.

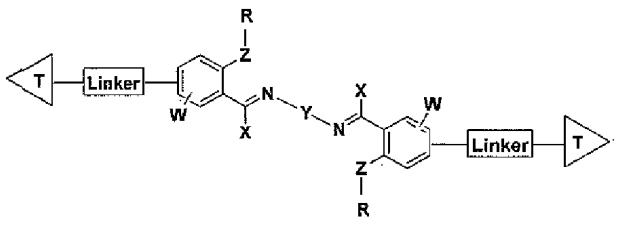
Thus, there is a need for improved imaging methods and new fluorescent probes for organelle-specific enzyme and bioactive molecule detection.

Summary

According to a first aspect of the invention, there is provided a method for imaging the activity of enzymes or bioactive molecules in cells using fluorescent probes with aggregation-induced emission ("AIE") properties and excited-state intramolecular proton transfer ("ESIPT") properties, the method comprises the steps of (a) incubating the cells with a florescent probe precursor and (b) allowing the enzymes or bioactive molecules to interact with the fluorescent probe precursor by a

cleavage of a group at a reactive site of the fluorescent probe precursor to form the fluorescent probe and (c) measuring the florescence response,

wherein the florescent probe precursor has the formula (I),



(I)

wherein

X represents hydrogen, alkyl, alkynyl or heteroaryl,

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

R' represents alkyl, aryl or heteroaryl,

R represents the reactive site of the fluorescent probe precursor for reaction with enzymes or biomolecules,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl; or represents a fused aromatic ring to the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino,

monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

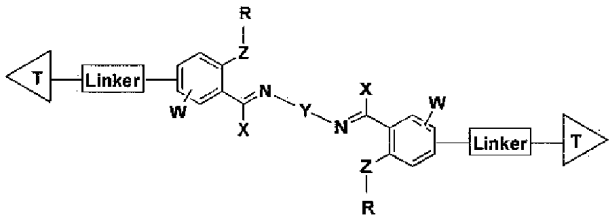
Linker is a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety or represents a moiety that is a ligand or antibody for organelles. Because of its AIE property, the probes used in the method are non-emissive in solution state but highly emissive at an aggregation state, which has advantages in high photostability, good in situ retention

ability and excellent turn-on ratio. The probes with additional ESIPT properties provide extraordinarily large Stokes shift and a "turn-on" mechanism by forming intramolecular hydrogen bonds. Therefore, a method using fluorescent probes with both advantages of "AIE + ESIPT" mechanisms provided by the method according to the invention can be used in "turn-on" imaging of organelle-specific enzymes and molecules to generate signal amplification with high contrast and sensitivity.

The disclosure also provides a method wherein the fluorescent probe has an organelle specific target group ("T") and a reactive site ("R") for reaction with enzymes and biomolecules. In this method the probes are decorated with diverse specific targeting groups for different organelles. Furthermore after reaction with enzymes or bioactive molecules with the reactive site of the probe, highly fluorescent products are yielded at aggregation state, which have excellent in situ retention ability at the reactive sites. Due to the aggregation-induced emission (AIE) properties of the probes, which are highly emissive at aggregation state but non-emissive in solution state, the method according to the disclosure is characterized by an advantageously high signal-to-noise ratio. Due to the ESIPT characteristics exceptionally large Stokes shifts (in some cases > 150 nm) are also obtained. The novel "turn-on" mechanism of the probes used in the method is based on specific reactions with enzymes or bioactive molecules to promote the formation of intramolecular bonds and aggregates in aqueous media, which can guarantee the wide detection scope and high accuracy.

The invention in a second aspect also provides a probe or precursor of a probe of the formula (I)',



(I)'

wherein

X represents hydrogen, alkyl, alkynyl or heteroaryl,

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

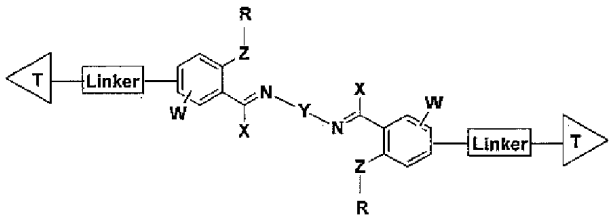
R' represents alkyl, aryl or heteroalkyl.

R is hydrogen or a reactive group for reaction with enzymes and biomolecules,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl or forms a fused aromatic ring with the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a single bond or a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety or represents a moiety that is a ligand or antibody for organelles. In one example there is provided the fluorescent probe precursors of formula (I)



(I)

wherein

X represents hydrogen, alkyl, alkynyl or heteroaryl,

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

R' represents alkyl, aryl or heteroaryl,

R is the reactive group,

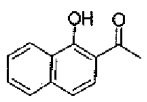
W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl; or represents a fused aromatic ring to the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a single bond or a combination of chemical bonds linking the targeting group T to the probe molecule, and

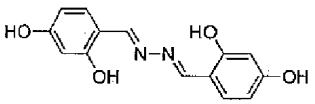
T represents a targeting group that has an ability to interact with an organelle.

These probe precursors, which are salicyladazine dyes or similar compounds, can be synthesized and functionalized with targeting groups and reactive sites by methods known in the art. It is therefore easy to tailor-make these probe precursors for the desired use of imaging of specific enzyme activities within organelles. Advantageously, the fluorescent probes have high "turn-on" ratio, large Stokes shift, excellent in situ retention ability, high photostability, and low cytotoxicity.

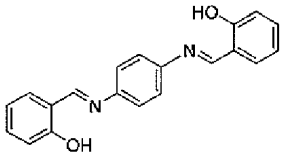
In one example, there is provided a method of using one or more of the following compounds or their derivatives for preparing a fluorescent probe or its precursor:



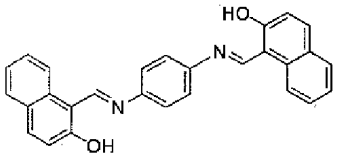
Blue-1



Green-2



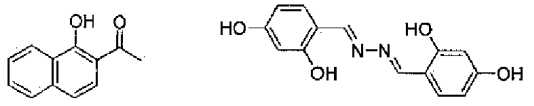
Yellow-3



Red-4

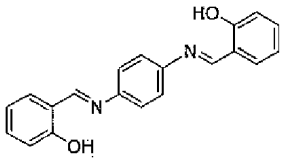
The compounds constitute the fluorophore of the probes and can be advantageously tuned to a given emission spectrum. As indicated by their naming their emission spectrum leads to different colors.

In some examples, the fluorophores can also be used as such to include them into nanoparticles. The disclosure provides nanoparticles containing at least one fluorescent probe consisting of one of the following compounds or their derivatives:

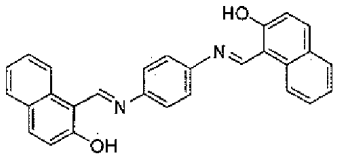


Blue-1

Green-2



Yellow-3



Red-4

Advantageously, fluorescent nanoparticles with AIE and ESIPT properties have been obtained according to the disclosure with a tunable emission color from blue to red. They can me modified to be inserted into target cells which can then be imaged for their behavior and the activity of bioactive molecules on them.

According to the third aspect of the invention, there is provided a kit for imaging the activity of biomolecules or enzymes in organelles comprising a fluorescent or its probe precursor of formula (I)' according to the present invention.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "aggregation-induced emission" (AIE) refers to a light emission of a luminogen system which is induced by aggregate formation. It is a phenomenon observed for certain molecules and is the opposite of an aggregation-caused quenching property.

The term "excited-state intramolecular proton transfer" (ESIPT) refers to a charge motion involving the hopping of a proton in a molecular framework.

As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl; or represents a fused aromatic ring to the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a targeting group that has an ability to interact with an organelle.

X preferably represents hydrogen, C_1-C_6-alkyl, C_2-C_6-alkynyl or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S. X more preferably represents hydrogen.

Y preferably represents a direct bond between the two nitrogen atoms or represents an aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl group having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S which both can be optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S. Y more

preferably represents a direct bond between the two nitrogen atoms or represents phenyl.

R preferably represents an ethyl, acetyl, sulfate, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl, 2,4-dinitrobenzenesulfonyl, 1,4-diketone, carbohydrate, amino acid, glycosyl or peptide group. R more preferably represents ethyl, acetyl, sulfate, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl, 2,4-dinitrobenzenesulfonyl, glycosyl or peptide group. The peptide group has preferably a chains of 2 to 20 amino acid monomers linked by peptide (amide) bonds, more preferably it is an oligopeptide with 2 to 10 amino acid monomers linked. As R the peptide (Asp-Glu-Val-Asp) (= DEVD), the peptide α-1,2-mannoside, the peptide (Ac-Leu-Glu-Val-Asp-), peptide (Ac-Ala-Thr-Ala-Asp-) can be especially mentioned for detecting caspase-3, α-1,2-mannosidase, Caspase-4 and caspase-12 respectively. R most preferably represents an acetyl or a galactosidyl group.

R' preferably represents C_1-C_6-alkyl, aryl having 6 to 10 carbon atoms or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S. R' more preferably represents methyl or ethyl.

W preferably represents no substituent or one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S; or W represents a fused aromatic ring to the phenyl

moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S. W more preferably represents no substituent which means that the phenyl moiety has no additional substituent.

Linker preferably represents a single bond or an ether, thioether, amine, ester, carboxamide, or sulfonamide bridge having 1 to 16 carbon atoms and which is saturated or partly saturated. Linker that has a bridge having 3 to 10 carbon atoms can be especially mentioned. Linker most preferably represents an ether group, such as an oligomethyleneoxy group having 1 to 16 carbon atoms. An oligomethyleneoxy group having 3 to 10 carbon atoms can be especially mentioned.

The group T can be preferably a lysosome, mitochondria, Golgi apparatus or endoplasmic retilculum targeting moiety.

T preferably represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety or represents a moiety that is a ligand or antibody for organelles. T most preferably represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate group. A triphenylphosphonium, pyridinium and morpholine group can be especially mentioned.

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

R' represents alkyl, aryl or heteroaryl.

R is hydrogen or reactive group reactive group for reaction with enzymes and biomolecules,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl or forms a fused aromatic ring with the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a single bond or a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a group that has an ability to interact with an organelle.

Preferred are compounds of formula (I)', wherein

X represents hydrogen, C_1-C_6-alkyl, C_2-C_6-alkynyl or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

Y represents a direct bond between the two nitrogen atoms or represents an aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl group having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S which are optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

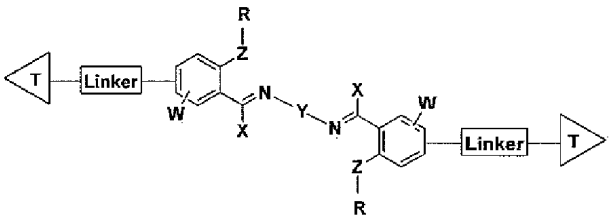
R represents hydrogen or an ethyl, acetyl, sulfate, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl, 2,4-dinitrobenzenesulfonyl, 1,4-diketone, carbohydrate, amino acid, glycosyl or peptide group,

W represents one or more substituents selected from hydrogen, halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S or forms a fused aromatic ring with the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-

CLAIMS

1. A method for imaging the activity of enzymes or bioactive molecules in cells using fluorescent probes with aggregation-induced emission properties and excited-state intramolecular proton transfer properties, the method comprises the steps of (a) incubating the cells with a florescent probe precursor and (b) allowing the enzymes or bioactive molecules to interact with the fluorescent probe precursor by a cleavage of a group at a reactive site of the fluorescent probe precursor to form the fluorescent probe and (c) measuring the florescence response,

wherein the florescent probe precursor has the formula (I),



(I)

wherein

X represents hydrogen, alkyl, alkynyl or heteroaryl,

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

R' represents alkyl, aryl or heteroaryl,

R represents the reactive site of the fluorescent probe precursor for reaction with enzymes or biomolecules,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl; or represents a fused aromatic ring to the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety or represents a moiety that is a ligand or antibody for organelles.

2. The method according to claim 1, wherein in formula (I),

X represents hydrogen, C_1-C_6-alkyl, C_2-C_6-alkynyl or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

Y represents a direct bond between the two nitrogen atoms or represents an aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl group having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S which are optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl,

C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

R represents an ethyl, acetyl, sulfate, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl, 2,4-dinitrobenzenesulfonyl, 1,4-diketone, carbohydrate, amino acid, glycosyl or peptide group,

W represents no substituent or one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S; or represents a fused aromatic ring to the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

Linker represents a single bond or an ether, thioether, amine, ester, carboxamide, or sulfonamide

bridge having 1 to 16 carbon atoms and which is satureated or partly saturated.

3. The method according to claim 1 or 2, wherein in formula (I),

X represents hydrogen,

Y represents a direct bond between the two nitrogen atoms or represents phenyl,

Z represents O or NH,

W represents no substituent,

R represents an acetyl or a galactosidyl group,

Linker represents an oligomethyleneoxy group having 1 to 16 carbon atoms, and

T represents represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety.

4. The method according to any one of claims 1 to 3, wherein in formula (I),

T represents pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, or oxazepine, and

R represents an acetoxy, sulfonate, β-galactosidaside, β-glucoside, α-glucoside, or N-acetyl-D-hexosamine group.

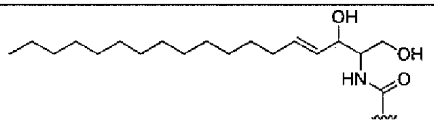
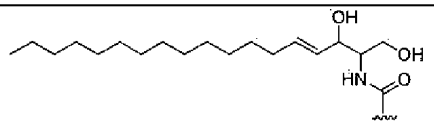
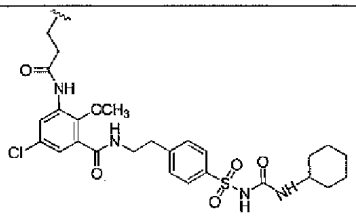
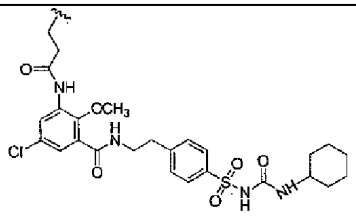
5. The method according to any one of claims 1 to 4, wherein in formula (I)

T represents a triphenylphosphonium or pyridinium group, and

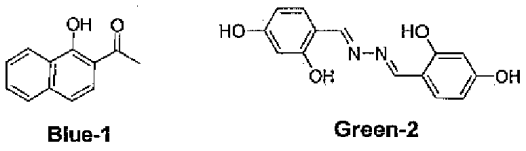
R represents an alkylamine, ethyl, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl or 2,4-dinitrobenzenesulfonyl

group; or represents a pendant disulfide, a pendant ester, a 1,4-diketone, or a diphenyl(tert-butyl)silyl group.

6. The method according to any one of claims 1 to 5, wherein in formula (I), R and T have the meaning as listed in the following table:

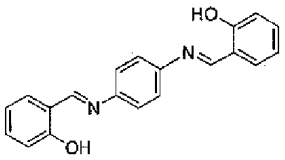
R	
Peptide (Asp-Glu-Val-Asp) (= DEVD)	"T" = 
α-1,2-mannoside	"T" = 
Peptide (Ac-Leu-Glu-Val-Asp-)	"T" = 
Peptide (Ac-Ala-Thr-Ala-Asp-)	"T" = 

7. The method according to any one of claims 1 to 6, wherein one of the following fluorophore core structures constitutes the fluorophore of the fluorescent probe:

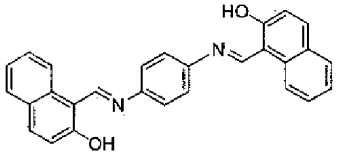


Blue-1

Green-2

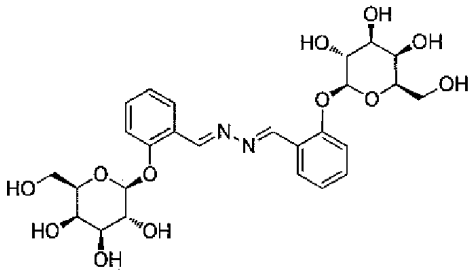


Yellow-3



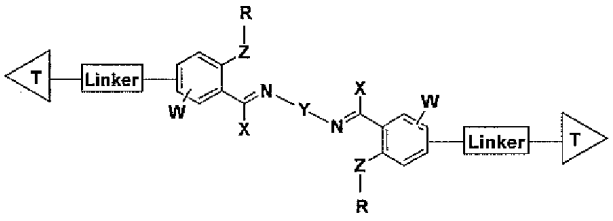
Red-4

8. The method according to any one of claims 1 to 7, wherein the fluorescent probe is the compound of formula (IV) or a derivative thereof



(IV).

9. A probe or precursor of a probe of the formula (I)',



(I)'

wherein

X represents hydrogen, alkyl, alkynyl or heteroaryl,

Y represents a direct bond between the two nitrogen atoms or represents an aryl or heteroaryl group which are optionally substituted by one or more substituents

selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl or heteroaryl,

Z represents O, NH or NR',

R' represents alkyl, aryl or heteroalkyl.

R is hydrogen or a reactive group for reaction with enzymes and biomolecules,

W represents no substituent or one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl and heteroaryl or forms a fused aromatic ring with the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, alkyl, perfluoroalkyl, alkoxy, aryl-oxy, alkenyl, alkynyl, cycloalkyl, alkylthio, amido, amino, monoalkylamino, dialkylamino, carboxamide, hydroxy, mercapto, aryl, heteroaryl,

Linker is a single bond or a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a pyrrolidine, piperidine, piperazine, morpholine, imidazole, diazepine, triazepine, oxazepine, triphenylphosphonium, pyridinium, peptide or carbohydrate moiety or represents a moiety that is a ligand or antibody for organelles.

10. The fluorescent probe or its precursor of claim 9, wherein in formula(I)',

X represents hydrogen, C_1-C_6-alkyl, C_2-C_6-alkynyl or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

Y represents a direct bond between the two nitrogen atoms or represents an aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl group having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S which are optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety or heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

R represents hydrogen or an ethyl, acetyl, sulfate, 2-ethyl-5-nitrothiophene, arylboronate, p-aminophenol, homoallyl ether, diphenylphosphinyl, 2,4-dinitrobenzenesulfonyl, 1,4-diketone, carbohydrate, amino acid, glycosyl or peptide group,

W represents one or more substituents selected from hydrogen, halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino, C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S or forms a fused aromatic ring with the phenyl moiety that it is linked to and is optionally substituted by one or more substituents selected from halogen, cyano, C_1-C_6-alkyl, C_1-C_6-perfluoroalkyl, C_1-C_6-alkoxy, aryl-oxy having 6 or 10 carbon atoms in the aryl moiety, C_2-C_6-alkenyl, C_2-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino, C_1-C_6-monoalkylamino, di-C_1-C_6-alkylamino,

C_1-C_6-carboxamide, hydroxy, mercapto, aryl having 6 or 10 carbon atoms in the aryl moiety and heteroaryl having 5 to 6 ring members and 1 to 3 hetero atoms selected from O, N or S,

Linker represents a single bond or an ether, thioether, amine, ester, carboxamide, or sulfonamide bridge having 1 to 16 carbon atoms and which is saturated or partly saturated.

11. A kit for imaging the activity of biomolecules or enzymes in organelles comprising a fluorescent probe or its precursor of formula (I)' according to claim 9 or 10.