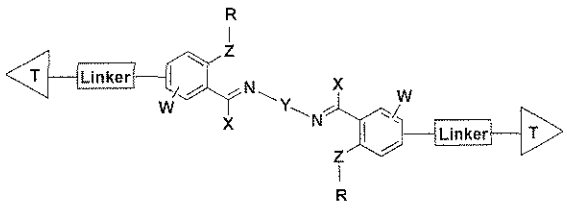
ability and excellent turn-on ratio. The probes with additional ESIPT properties provide extraordinarily large Stokes shift and a "turn-on" mechanism by forming intramolecular hydrogen bonds. Therefore, a method using fluorescent probes with both advantages of "AIE + ESIPT" mechanisms provided by the 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 ~~invention in a second aspect~~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 ~~invention~~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,

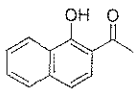
Linker is a single bond or a combination of chemical bonds linking the targeting group T to the probe molecule, and

T represents a group that has an ability to interact with an organelle. In one example there is provided the fluorescent probe precursors of formula (I)

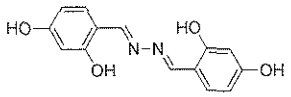
T represents a targeting group that has an ability to interact with an organelle.

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.

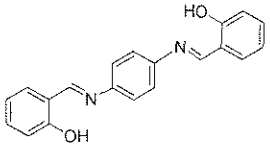
According to a third aspect of the invention, there is provided ~~the use of~~a method of using one or more of the following compounds or their derivatives for preparing a fluorescent probe or its precursor:



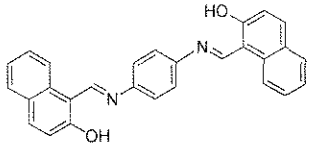
Blue-1



Green-2



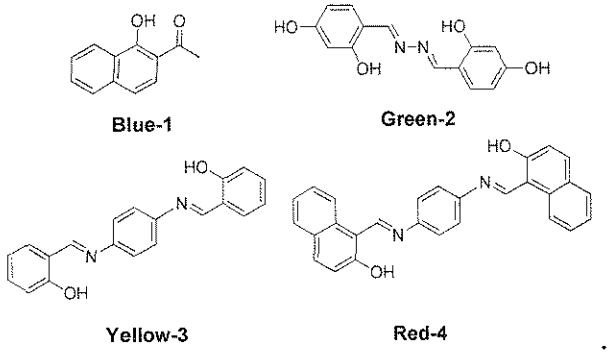
Yellow-3



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.

According to a fourth aspect of the invention the fluorophores can also be used as such to include them into nanoparticles. The invention provides nanoparticles containing at least one fluorescent probe consisting of one of the following compounds or their derivatives:



Advantageously, fluorescent nanoparticles with AIE and ESIPT properties have been obtained according to the ~~invention~~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 fifth 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

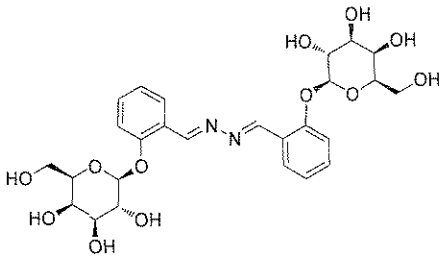
The following words and terms used herein shall have the meaning indicated:

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.

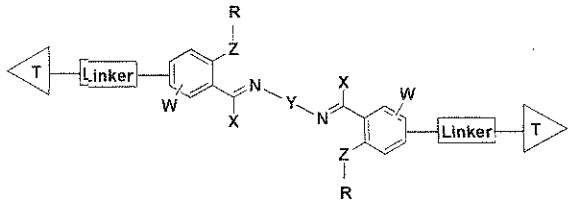
As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1,

13. The method according to claim 1, wherein the fluorescent probe is the compound of formula (IV) or a derivative thereof



(IV).

~~13~~14. 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 group that has an ability to interact with an organelle.

~~14~~15. A fluorescent probe or its precursor of claim ~~13~~14, wherein in formula(I)',

X represents hydrogen, C_1-C_6-alkyl, C_1-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_1-C_6-alkenyl, C_1-C_6-alkynyl, C_5-C_7-cycloalkyl, C_1-C_6-alkylthio, amido, amino,

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.

~~15~~16. 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 ~~13~~14.