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(54) Title: PHOTOSTABLE FLUORESCENT COMPOUNDS FOR ORGANELLE IMAGING IN LIVE CELLS AND DEEP TISSUES

(57) Abstract: The present subject matter contemplates small molecule, fluorescent compounds having aggregation-induced emission (AIE) characteristics. The compounds exhibit near-infrared solid-state emission, large Stokes shift (>180 nm), high fluorescence quantum yield (12.8%-13.7%) and good two-photon absorption cross section (up to 88 GM). The compounds can provide membrane and specific organelle staining in live cells and deep tissues. The present compounds also exhibit high biocompatibility and high photostability under one-photon and two-photon continuous irradiation.



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Photostable Fluorescent Compounds for Organelle Imaging in Live Cells and Deep Tissues

FIELD

The present subject matter relates generally to use of a series of photostable fluorescent compounds having aggregation-induced emission (AIE) characteristics for specific organelle staining, and particularly, for imaging of organelles in live cells and deep tissues.

BACKGROUND

Fluorescence imaging has received considerable attention in real-time tracking, visualization of dynamic change and imaging-guided therapy in live samples, due to its remarkable sensitivity, high selectivity, fast acquisition and easy operation. The performance of fluorescence imaging is highly dependent on the fluorophore used. As a particularly important parameter of the fluorophore, photostability is carefully considered for long-term tracking of dynamic changes associated with biological events. However, photostability is a common concern for traditional fluorophores especially commercial dyes like MitoTracker Green FM. This drawback of conventional fluorophores often makes it difficult to capture the optimal fluorescence image, resulting in inefficiency and false biological signals. In addition, some photo-oxidation products resulting from photobleaching can cause severe damage to live samples. The unstable resistance to photobleaching of these fluorophores can generally be attributed to a low concentration of these fluorophores. Increasing the concentration of these fluorophores, however, often results in aggregation-caused quenching (ACQ). Therefore, development of novel fluorophores with enhanced photostability as well as inhibited ACQ effect is of particular importance.

Tang and co-workers discovered unique organic fluorophores that show no or faint emission in organic solvent but highly boosted emission in aggregate or solid state. This phenomenon was first termed as aggregation-induced emission (AIE). The concept of restriction of intramolecular motion (RIM) was proposed to explain this unique phenomenon. Based on RIM, aggregation-induced emission luminogens (AIEgens) can be used in biomedical imaging applications with increased concentration, leading to high photostability as well as bright emission. Indeed, many AIEgens exhibit high resistance to photobleaching in biological imaging.

Compared with short-wavelength emissive fluorophores, long-wavelength emissive AIEgens, especially near-infrared (NIR) AIEgens, hold tremendous advantages for bioimaging

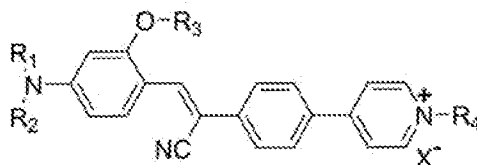
because of deep tissue penetration, minimal photodamage and high signal-to-noise ratio in live biological samples. Until now, the majority of NIR AIEgens are organic nanoparticles (NPs) and amphiphilic surfactants are generally used to achieve internalization of these NIR AIEgen NPs in live samples. However, fabricating these NIR AIEgen NPs is complicated and time-consuming, and use of commercial amphiphilic surfactants, such as DSPE-PEG, is very expensive. Therefore, inherent NIR AIEgens with excellent penetrability in live cells and tissues are needed.

Cyanostilbenes, have been extensively investigated and applied in many fields such as self-assembly, chemosensor and bioimaging, due to their facile synthesis and easy purification. Previous studies have demonstrated that introduction of strong electron withdrawing groups like -F and -CN can improve the fluorophores' resistance to photobleaching. Though some achievements have been made to develop NIR cyanostilbenes, these AIEgens basically showed very low cell penetrability.

SUMMARY

The present subject matter contemplates small molecule, fluorescent compounds having aggregation-induced emission (AIE) characteristics. The compounds exhibit near-infrared solid-state emission, large Stokes shift (>180 nm), high fluorescence quantum yield (12.8%-13.7%) and good two-photon absorption cross section (up to 88 GM). The compounds can provide membrane and specific organelle staining in live cells. The present compounds also exhibit high biocompatibility and high photostability under one-photon and two-photon continuous irradiation.

In an embodiment, the fluorescent compounds can include a compound having the following backbone structural formula:



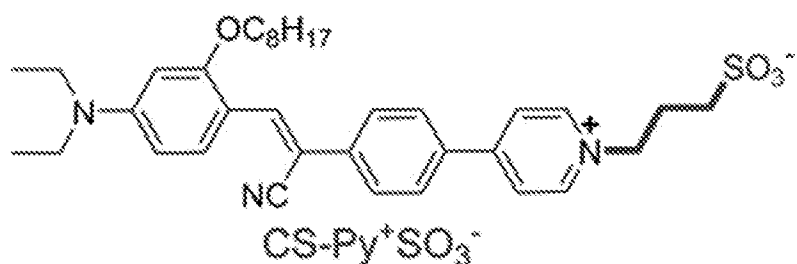
wherein each R_1 , R_2 , R_3 , and R_4 is substituted or unsubstituted and is independently selected from the group consisting of C_nH_{2n+1} , C_6H_5 , C_7H_7 , $C_{10}H_7$, $C_nH_{2n}SO_3^-$, $C_nH_{2n}COOH$, $C_nH_{2n}NCS$, $C_nH_{2n}N_3$, $C_nH_{2n}NH_2$, $C_nH_{2n}Cl$, $C_nH_{2n}Br$, and $C_nH_{2n}I$;

wherein each X is substituted or unsubstituted and is independently selected from the

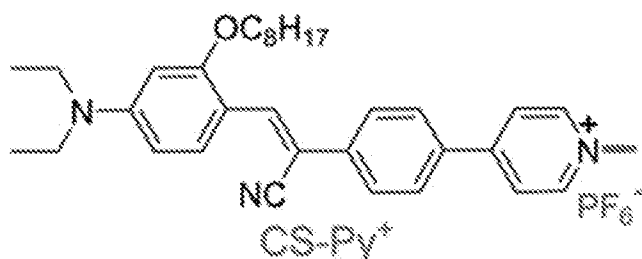
group consisting of Cl, Br, I, NO₃, ClO₄, BF₄, PF₆, CF₃CO₂ and CF₃SO₃; and

wherein each n is independently an integer ranging from 0 to 16.

In an embodiment, the compound is selected from the group consisting of



and



In an embodiment, a method of cellular imaging is contemplated, including contacting a target cell with the present compound and identifying a target of interest in the target cell using an imaging method. The imaging method can include one-photon fluorescence microscopy or two-photon fluorescence microscopy. In an embodiment, the imaging method comprises irradiating the compound for about 310 seconds. In an embodiment, identifying a target of interest can include visualizing organelle-associated changes in live cells.

In an embodiment, the target of interest is selected from the group consisting of a cellular membrane and a cellular organelle. In an embodiment, the cellular organelle is mitochondria. In an embodiment, the target cell is a live cell. In an embodiment, the target cell is in live tissue. In an embodiment, the target cell is at a depth of about 50 μm to about 100 μm in the live tissue.

BRIEF DESCRIPTION OF DRAWINGS

Various embodiments will now be described in detail with reference to the accompanying drawings.

Fig. 1 depicts ^1H NMR spectrum of compound **2** in CDCl_3 .

Fig. 2 depicts ^{13}C NMR spectrum of compound **2** in CDCl_3 .

Fig. 3 depicts ^1H NMR spectrum of compound **3** in CDCl_3 .

Fig. 4 depicts ^{13}C NMR spectrum of compound **3** in CDCl_3 .

Fig. 5 depicts ^1H NMR spectrum of compound **4** in CDCl_3 .

Fig. 6 depicts ^{13}C NMR spectrum of compound **4** in CDCl_3 .

Fig. 7 depicts ^1H NMR spectrum of $\text{CS-Py}^+\text{SO}_3^-$ in DMSO-d_6 .

Fig. 8 depicts ^{13}C NMR spectrum of $\text{CS-Py}^+\text{SO}_3^-$ in $\text{CDCl}_3/\text{CD}_3\text{OD} = 2:1$.

Fig. 9 depicts ^1H NMR spectrum of CS-Py^+ in DMSO-d_6 .

Fig. 10 depicts ^{13}C NMR spectrum of CS-Py^+ in DMSO-d_6 .

Fig. 11 depicts ^{19}F NMR spectrum of CS-Py^+ in DMSO-d_6 .

Fig. 12 depicts HRMS spectrum of $\text{CS-Py}^+\text{SO}_3^-$.

Fig. 13 depicts HRMS spectrum of CS-Py^+ .

Fig. 14 depicts (A) a single crystal structure of $\text{CS-Py}^+\text{SO}_3^-$; (B) a single crystal structure of CS-Py^+ ; (C) molecular packing in the crystal of $\text{CS-Py}^+\text{SO}_3^-$; and (D) molecular packing in the crystal of CS-Py^+ (distances in Å).

Fig. 15 depicts (A) the principal intermolecular packing interactions of $\text{CS-Py}^+\text{SO}_3^-$; and (B) the principal intermolecular packing interactions of CS-Py^+ (distances in Å).

Fig. 16 depicts (A) normalized absorption spectra of $\text{CS-Py}^+\text{SO}_3^-$ (5 μM) and CS-Py^+ (5 μM) in DMSO; (B) fluorescence spectra of $\text{CS-Py}^+\text{SO}_3^-$ (5 μM); (C) fluorescence spectra of CS-Py^+ (5 μM) in DMSO and DMSO/water mixture with different water fractions; (D) plots of α_{AIE} (fluorescence intensity I/I_0) versus the composition of the DMSO/water mixtures of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ ; (E) dynamic light scattering data of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ in water containing 5% DMSO; (F) normalized fluorescence spectra of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ in solid state, (inset: fluorescent photos of solids of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ taken under 365 nm UV irradiation from a handheld UV lamp; (G) fluorescence spectra of $\text{CS-Py}^+\text{SO}_3^-$; (H) fluorescence spectra of CS-Py^+ in different polar solvents; and (I) two-photon absorption (TPA) cross sections of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ in THF. $1 \text{ GM} \equiv 10^{-50} \text{ cm}^4 \text{ s/photon}$.

Fig. 17 depicts the cytotoxicity of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ in HeLa cells.

Fig. 18 depicts confocal laser scanning microscopy images of HeLa cells incubated with $\text{CS-Py}^+\text{SO}_3^-$ (1 μM) and CS-Py^+ (1 μM) (scale bar: 20 μm).

Fig. 19 depicts *in situ* fluorescence (FL) spectra of CS-Py⁺SO₃⁻ and CS-Py⁺ in HeLa cells.

Fig. 20 depicts (A) confocal laser scanning microscopy images of live HeLa cells incubated with CS-Py⁺SO₃⁻ (1 μM) and DiI (0.2 μM); and (B) confocal laser scanning microscopy images of live HeLa cells incubated with CS-Py⁺ (1 μM) and MTDR (0.2 μM) (scale bar: 20 μm).

Fig. 21 depicts (A) one-photon fluorescent microscopic images of rat skeletal muscle tissues incubated only with CS-Py⁺ (1 μM); (B) one-photon fluorescent microscopic images of rat skeletal muscle tissues incubated with CS-Py⁺ (1 μM) and MTDR (0.5 μM); and (C) one-photon ($\lambda_{\text{ex}} = 488 \text{ nm}$) and two-photon ($\lambda_{\text{ex}} = 900 \text{ nm}$) fluorescent microscopic images of rat skeletal muscle tissues incubated with CS-Py⁺ (1 μM) (scale bar: 20 μm).

Fig. 22 depicts (A) one-photon ($\lambda_{\text{ex}} = 488 \text{ nm}$) fluorescent microscopic images of the mouse skeletal muscle tissue stained with CS-Py⁺ (1 μM) at different penetration depths along z-axis (scale bar: 20 μm); (B) reconstructed 3D one-photon fluorescent microscopic images of the image the mouse skeletal muscle tissue stained with CS-Py⁺ (1 μM); (C) two-photon ($\lambda_{\text{ex}} = 900 \text{ nm}$) fluorescent microscopic images of the mouse skeletal muscle tissue stained with CS-Py⁺ (1 μM) at different penetration depths along z-axis (scale bar: 20 μm); reconstructed 3D two-photon fluorescent microscopic image of the image the mouse skeletal muscle tissue stained with CS-Py⁺ (1 μM).

Figs. 23 depicts (A) normalized fluorescence intensity of CS-Py⁺SO₃⁻, CS-Py⁺, DiI and MTDR in HeLa cells by continuous irradiation with confocal lasers (irradiation conditions: for CS-Py⁺SO₃⁻ and CS-Py⁺, 488 nm laser, laser power 12%; for DiI, 543 nm laser, laser power 12%; for MTDR, 635 nm laser, laser power 12%; image was scanned about every 2.1 s); (B) two-photon fluorescent images of CS-Py⁺ by continuous irradiation with two-photon NIR pulsed laser (900 nm, intensity of 2476 mW) at different scans (the image was scanned about every 5.2 s, scale bar: 20 μm); and (C) normalized fluorescence intensity of CS-Py⁺ by continuous irradiation with two-photon NIR pulsed laser (900 nm, intensity of 2476 mW) at different scans (the image was scanned about every 5.2 s, scale bar: 20 μm).

DETAILED DESCRIPTION

Definitions

The following definitions are provided for the purpose of understanding the present subject matter and for constructing the appended patent claims.

It is noted that, as used in this specification and the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise.

The term “ λ_{ex} ” as used herein refers to excitation wavelength.

The phrase “aggregation caused quenching” or “ACQ” as used herein refers to the phenomenon wherein the aggregation of π -conjugated fluorophores significantly decreases the fluorescence intensity of the fluorophores. The aggregate formation is said to “quench” light emission of the fluorophores.

The phrase “aggregation induced emission” or “AIE” as used herein refers to the phenomenon manifested by compounds exhibiting significant enhancement of light-emission upon aggregation in the amorphous or crystalline (solid) states whereas they exhibit weak or almost no emission in dilute solutions.

“Emission intensity” as used herein refers to the magnitude of fluorescence/phosphorescence normally obtained from a fluorescence spectrometer or fluorescence microscopy measurement; “fluorophore” or “fluorogen” as used herein refer to a molecule which exhibits fluorescence; “luminogen” or “luminophore” as used herein refer to a molecule which exhibits luminescence; and “AIEgen” as used herein refers to a molecule exhibiting AIE characteristics.

As used herein, “halo” or “halogen” refers to fluoro, chloro, bromo, and iodo.

As used herein, “alkyl” refers to a straight-chain or branched saturated hydrocarbon group. Examples of alkyl groups include methyl (Me), ethyl (Et), propyl (e.g., n-propyl and z'-propyl), butyl (e.g., n-butyl, z'-butyl, sec-butyl, tert-butyl), pentyl groups (e.g., n-pentyl, z'-pentyl, -pentyl), hexyl groups, and the like. In various embodiments, an alkyl group can have 1 to 40 carbon atoms (i.e., C1-40 alkyl group), for example, 1-30 carbon atoms (i.e., C1-30 alkyl group). In some embodiments, an alkyl group can have 1 to 6 carbon atoms, and can be referred to as a “lower alkyl group”. Examples of lower alkyl groups include methyl, ethyl, propyl (e.g., n-propyl and z'-propyl), and butyl groups (e.g., n-butyl, z'-butyl, sec-butyl, tert-butyl). In some embodiments, alkyl groups can be substituted as described herein. An alkyl group is generally not substituted with another alkyl group, an alkenyl group, or an alkynyl group.

As used herein, “alkenyl” refers to a straight-chain or branched alkyl group having one or

more carbon-carbon double bonds. Examples of alkenyl groups include ethenyl, propenyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl groups, and the like. The one or more carbon-carbon double bonds can be internal (such as in 2-butene) or terminal (such as in 1-butene). In various embodiments, an alkenyl group can have 2 to 40 carbon atoms (i.e., C2-40 alkenyl group), for example, 2 to 20 carbon atoms (i.e., C2-20 alkenyl group). In some embodiments, alkenyl groups can be substituted as described herein. An alkenyl group is generally not substituted with another alkenyl group, an alkyl group, or an alkynyl group.

As used herein, “heteroatom” refers to an atom of any element other than carbon or hydrogen and includes, for example, nitrogen, oxygen, silicon, sulfur, phosphorus, and selenium.

As used herein, “aryl” refers to an aromatic monocyclic hydrocarbon ring system or a polycyclic ring system in which two or more aromatic hydrocarbon rings are fused (i.e., having a bond in common with) together or at least one aromatic monocyclic hydrocarbon ring is fused to one or more cycloalkyl and/or cycloheteroalkyl rings. An aryl group can have 6 to 24 carbon atoms in its ring system (e.g., C6-24 aryl group), which can include multiple fused rings. In some embodiments, a polycyclic aryl group can have 8 to 24 carbon atoms. Any suitable ring position of the aryl group can be covalently linked to the defined chemical structure. Examples of aryl groups having only aromatic carbocyclic ring(s) include phenyl, 1-naphthyl (bicyclic), 2-naphthyl (bicyclic), anthracenyl (tricyclic), phenanthrenyl (tricyclic), pentacenyl (pentacyclic), and like groups. Examples of polycyclic ring systems in which at least one aromatic carbocyclic ring is fused to one or more cycloalkyl and/or cycloheteroalkyl rings include, among others, benzo derivatives of cyclopentane (i.e., an indanyl group, which is a 5,6-bicyclic cycloalkyl/aromatic ring system), cyclohexane (i.e., a tetrahydronaphthyl group, which is a 6,6-bicyclic cycloalkyl/aromatic ring system), imidazoline (i.e., a benzimidazolyl group, which is a 5,6-bicyclic cycloheteroalkyl/aromatic ring system), and pyran (i.e., a chromenyl group, which is a 6,6-bicyclic cycloheteroalkyl/aromatic ring system). Other examples of aryl groups include benzodioxanyl, benzodioxolyl, chromanyl, indolyl groups, and the like. In some embodiments, aryl groups can be substituted as described herein. In some embodiments, an aryl group can have one or more halogen substituents, and can be referred to as a “haloaryl” group. Perhaloaryl groups, i.e., aryl groups where all of the hydrogen atoms are replaced with halogen atoms (e.g., -C₆F₅), are included within the definition of “haloaryl”. In certain embodiments, an aryl group is substituted with another aryl group and can be referred to as a biaryl group. Each of the aryl

groups in the biaryl group can be substituted as disclosed herein.

As used herein, "heteroaryl" refers to an aromatic monocyclic ring system containing at least one ring heteroatom selected from oxygen (O), nitrogen (N), sulfur (S), silicon (Si), and selenium (Se) or a polycyclic ring system where at least one of the rings present in the ring system is aromatic and contains at least one ring heteroatom. Polycyclic heteroaryl groups include those having two or more heteroaryl rings fused together, as well as those having at least one monocyclic heteroaryl ring fused to one or more aromatic carbocyclic rings, non-aromatic carbocyclic rings, and/or non-aromatic cycloheteroalkyl rings. A heteroaryl group, as a whole, can have, for example, 5 to 24 ring atoms and contain 1-5 ring heteroatoms (i.e., 5-20 membered heteroaryl group). The heteroaryl group can be attached to the defined chemical structure at any heteroatom or carbon atom that results in a stable structure. Generally, heteroaryl rings do not contain O-O, S-S, or S-O bonds. However, one or more N or S atoms in a heteroaryl group can be oxidized (e.g., pyridine N-oxide thiophene S-oxide, thiophene S,S-dioxide). Examples of heteroaryl groups include, for example, the 5- or 6-membered monocyclic and 5-6 bicyclic ring systems shown below: where T is O, S, NH, N-alkyl, N-aryl, N-(arylalkyl) (e.g., N-benzyl), SiH₂, SiH(alkyl), Si(alkyl)₂, SiH(arylalkyl), Si(arylalkyl)₂, or Si(alkyl)(arylalkyl). Examples of such heteroaryl rings include pyrrolyl, furyl, thienyl, pyridyl, pyrimidyl, pyridazinyl, pyrazinyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, isothiazolyl, thiazolyl, thiadiazolyl, isoxazolyl, oxazolyl, oxadiazolyl, indolyl, isoindolyl, benzofuryl, benzothienyl, quinolyl, 2-methylquinolyl, isoquinolyl, quinoxalyl, quinazolyl, benzotriazolyl, benzimidazolyl, benzothiazolyl, benzisothiazolyl, benzisoxazolyl, benzoxadiazolyl, benzoxazolyl, cinnolyl, 1H-indazolyl, 2H-indazolyl, indolizyl, isobenzofuryl, naphthyridinyl, phthalazinyl, pteridinyl, purinyl, oxazolopyridinyl, thiazolopyridinyl, imidazopyridinyl, furopyridinyl, thienopyridinyl, pyridopyrimidinyl, pyridopyrazinyl, pyridopyridazinyl, thienothiazolyl, thienoxazolyl, thienoimidazolyl groups, and the like. Further examples of heteroaryl groups include 4,5,6,7-tetrahydroindolyl, tetrahydroquinolyl, benzothienopyridinyl, benzofuropyridinyl groups, and the like. In some embodiments, heteroaryl groups can be substituted as described herein.

As used herein, a "donor" material refers to an organic material, for example, an organic nanoparticle material, having holes as the majority current or charge carriers.

As used herein, an "acceptor" material refers to an organic material, for example, an organic nanoparticle material, having electrons as the majority current or charge carriers.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the presently described subject matter pertains.

Where a range of values is provided, for example, concentration ranges, percentage ranges, or ratio ranges, it is understood that each intervening value, to the tenth of the unit of the lower limit, unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the described subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and such embodiments are also encompassed within the described subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the described subject matter.

Throughout the application, descriptions of various embodiments use “comprising” language. However, it will be understood by one of skill in the art, that in some specific instances, an embodiment can alternatively be described using the language “consisting essentially of” or “consisting of”.

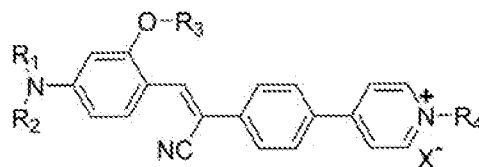
For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about”. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Fluorescent Compounds

The present subject matter contemplates small molecule, fluorescent compounds having aggregation-induced emission (AIE) characteristics. The present compounds include cyanostilbene based AIEgens (CS AIEgens). The compounds can include a long alkyl chain substituent and a donor- π -acceptor (D- π -A) structure. The present AIEgens can exhibit near-infrared (NIR) solid-state emission, a large Stokes shift (e.g., >180 nm), a high fluorescence quantum yield (e.g., about 12.8% to about 13.7%) and good two-photon absorption cross section

(e.g., up to about 88 GM). One or more of the compounds can provide specific staining in the cell membrane of a live cell. One or more of the compounds can provide specific staining in a cellular organelle, e.g., mitochondria, of a live cell. The present compounds can exhibit high biocompatibility and high photostability under one-photon and two-photon continuous irradiation.

In an embodiment, the fluorescent compounds can include a compound having the following backbone structural formula:

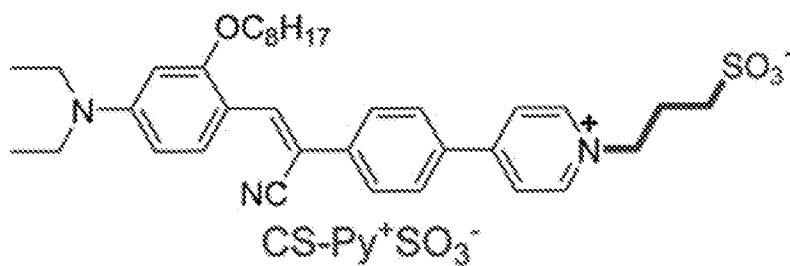


wherein each R_1 , R_2 , R_3 , and R_4 is substituted or unsubstituted and is independently selected from the group consisting of C_nH_{2n+1} , C_6H_5 , C_7H_7 , $C_{10}H_7$, $C_nH_{2n}SO_3^-$, $C_nH_{2n}COOH$, $C_nH_{2n}NCS$, $C_nH_{2n}N_3$, $C_nH_{2n}NH_2$, $C_nH_{2n}Cl$, $C_nH_{2n}Br$, and $C_nH_{2n}I$;

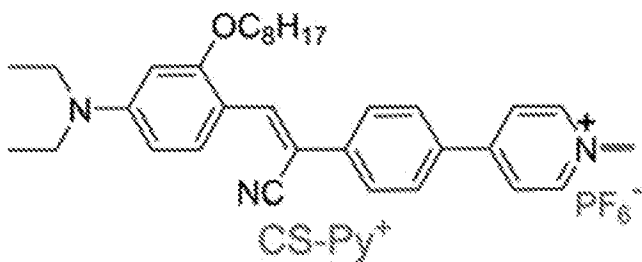
wherein each X is substituted or unsubstituted and is independently selected from the group consisting of Cl , Br , I , NO_3 , ClO_4 , BF_4 , PF_6 , CF_3CO_2 and CF_3SO_3 ; and

wherein each n is independently an integer ranging from 0 to 16.

In an embodiment, the compound is selected from the group consisting of



and



Bio-imaging Applications

The present compounds can be used for *in vitro* and *ex vivo* cellular imaging. In an embodiment, a method of cellular imaging can include contacting a target cell with one or more of the present compounds and identifying a target of interest in the target cell using an imaging method. In an embodiment, the target of interest includes a cellular membrane. In an embodiment, the target of interest includes a cellular organelle. In an embodiment, the cellular organelle includes mitochondria. The target cell can be a live cell. In an embodiment, the live cell is in live tissue. The target cell can be at a depth of about 50 μm to about 100 μm in the live tissue.

The imaging method can include one-photon fluorescence microscopy (confocal laser scanning microscopy) or two-photon fluorescence microscopy. One-photon fluorescence microscopy uses a single photon to excite fluorescent dyes using mainly visible excitation wavelengths (390-700 nm). Two-photon fluorescence imaging technology has been widely used for bio-imaging applications due to its high penetration depth with near-infrared (NIR) excitation, high spatial resolution and signal-to-noise ratio, and low tendency for photobleaching. Two-photon absorption (2PA) cross section ($\delta_{2\text{PA}}$) is used to predict whether a luminogen is suitable for 2PM.

In an embodiment, the imaging method can include continuous irradiation of the compound or irradiating the compound for about 310 seconds. In an embodiment, the imaging method can provide long-term tracking of organelle-associated changes in live cells. For example, the imaging method can be used to track changes in the mitochondria that are associated with mitochondria-related diseases, such as mitochondrial myopathy.

Generally, extending the π -conjugation of the skeleton of AIEgens is an effective method to construct NIR AIEgens. However, with increased π -conjugation, the cell penetration of such

AIEgens can significantly decrease. Construction of a donor- π -acceptor (D- π -A) structure is another effective method to develop NIR AIEgens, but a strong twisted intramolecular charge transfer (TICT) effect can sometimes result in AIEgens with very low fluorescence quantum yield. Synthesis of the present compounds successfully incorporates both of these two strategies such that optimal penetration and fluorescence are achieved. Thus, the resulting modified cyanostilbenes are inherent photostable AIEgens. The presence of an electron withdrawing group like pyridinium in the D- π -A based cyanostilbenes enhances the TICT effect, resulting in a narrow energy gap and NIR emissive AIEgens. The present compounds can exhibit solid-state NIR emission and TICT effect due to strong D- π -A effects.

The present compounds exhibit specific cell membrane and organelle staining with high biocompatibility. As eukaryotic cells include a cell membrane and numerous membrane-enclosed organelles (e.g., mitochondria and Golgi apparatus), it is believed that the electrical charge of the compounds can interact with the amphipathic phospholipid of the cell membrane, leading to increased penetrability and location in specific organelles in live cells. The present compounds balance hydrophobicity and electrical charge distributions. In an embodiment, one or more of the present compounds can be used to stain the cell membrane of live cells. In an embodiment, CS-Py⁺SO₃⁻ can be used to stain the cell membrane of a live cell. In an embodiment, one or more of the present compounds can be used to stain mitochondria of a live cell. In an embodiment, CS-Py⁺ can be used for staining mitochondria.

In an embodiment, the present compounds can stain cellular organelles in live tissue with deep tissue penetration. Deep tissue penetration can include a depth ranging from about 50 μ m to about 100 μ m in live tissue. For example, CS-Py⁺ was successfully used for staining mitochondria in live rat skeletal muscle tissues with deep-tissue penetration (e.g., 100 μ m depth) under two-photon excited imaging mode. The present compounds exhibit remarkable resistance to photobleaching under continuous irradiation with one-photon and two-photon lasers.

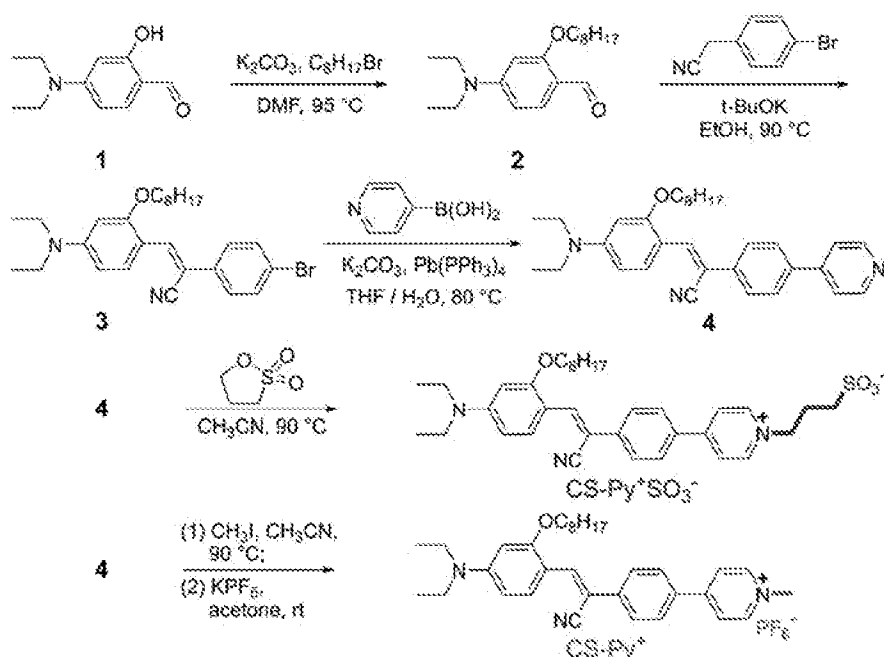
The present teachings are illustrated by the following examples.

EXAMPLE 1

Synthesis

In synthesizing the present compounds, cyanostilbene with a long alkyl chain substituent was adopted as the AIEgen core and D- π -A structure was further introduced in the cyanostilbene skeleton to red shift the fluorescence as well as enhance the TICT effect. Exemplary reaction

schemes for synthesizing CS-Py⁺SO₃⁻ and CS-Py⁺ is provided below:



The ¹H NMR spectrum of compound 2 in CDCl₃ is provided in Fig. 1. The ¹³C NMR spectrum of compound 2 in CDCl₃ is provided in Fig. 2. The ¹H NMR spectrum of compound 3 in CDCl₃ is provided in Fig. 3. The ¹³C NMR spectrum of compound 3 in CDCl₃ is provided in Fig. 4. The ¹H NMR spectrum of compound 4 in CDCl₃ is provided in Fig. 5. The ¹³C NMR spectrum of compound 4 in CDCl₃ is provided in Fig. 6. The ¹H NMR spectrum of CS-Py⁺SO₃⁻ in DMSO-d₆ is provided in Fig. 7. The ¹³C NMR spectrum of CS-Py⁺SO₃⁻ in CDCl₃/CD₃OD = 2:1 is provided in Fig. 8. The ¹H NMR spectrum of CS-Py⁺ in DMSO-d₆ is provided in Fig. 9. The ¹³C NMR spectrum of CS-Py⁺ in DMSO-d₆ is provided in Fig. 10. The ¹⁹F NMR spectrum of CS-Py⁺ in DMSO-d₆ is provided in Fig. 11. The HRMS spectrum of CS-Py⁺SO₃⁻ is provided in Fig. 12. The HRMS spectrum of CS-Py⁺ is provided in Fig. 13.

EXAMPLE 2

Crystal Structure Analysis

The structures of CS-Py⁺SO₃⁻ and CS-Py⁺ were further confirmed by X-ray crystal structure analysis (Figs. 14A-14C and 15A-15B). Single crystals of CS-Py⁺SO₃⁻ and CS-Py⁺ suitable for X-ray structure analysis were obtained by slow evaporation of mixed solvent of CH₂Cl₂ and MeOH (CH₂Cl₂/MeOH = 2:1, v/v) at ambient temperature. CS-Py⁺SO₃⁻ and CS-Py⁺

exhibited intramolecular π - π interaction and C-H $\cdots\pi$ interaction (Fig. 14C and 14D). The molecules of CS-Py⁺SO₃⁻ in the crystal lattice are arranged in a head-to-tail antiparallel arrangement, resulting in strong intermolecular donor-acceptor interaction, while those of CS-Py⁺ in the crystal lattice showed head-to-head arrangement. It is believed that multiple intramolecular interactions, such as C-H $\cdots$ O, C-H $\cdots$ N, C-H $\cdots\pi$, C-H $\cdots$ F and P-F $\cdots\pi$ interactions, stabilize these different packing modes of CS-Py⁺SO₃⁻ and CS-Py⁺ (Figs. 15A-15B), which could contribute to restriction of intramolecular motion (RIM) and blocking of non-radiative processes in the aggregated state.

Example 3

Absorption and Fluorescence

The absorption and fluorescence (FL) data of CS-Py⁺SO₃⁻ and CS-Py⁺ are summarized in Table 1, and corresponding spectra are provided in Figs. 16A-16I.

Table 1. Photophysical properties of CS-Py⁺SO₃⁻ and CS-Py⁺

	DMSO				Solid		α_{AIE}
	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)	Stokes shift (nm)	$\Phi_{F,S}$ (%)	λ_{em}^{max} (nm)	$\Phi_{F,P}$ (%)	
CS-Py ⁺ SO ₃ ⁻	478	658	180	1.4	679	12.8	9.14
CS-Py ⁺	477	660	183	1.3	685	13.7	10.54

λ_{abs}^{max} = absorption maximum; λ_{em}^{max} = emission maximum; $\Phi_{F,S}$ and $\Phi_{F,P}$ = fluorescence quantum yield in solution and solid powder, respectively; $\alpha_{AIE} = \Phi_{F,S}/\Phi_{F,P}$.

As shown in Fig. 16A, the AIEgens CS-Py⁺SO₃⁻ (5 μ m) and CS-Py⁺ (5 μ m) exhibit very similar absorption, with absorption peaks (λ_{abs}^{max}) at 478 nm and 477 nm for CS-Py⁺SO₃⁻ and CS-Py⁺, respectively. CS-Py⁺SO₃⁻ showed very low near-infrared (NIR) fluorescence (NIR emission peak λ_{em}^{max} of about 658 nm) in dilute DMSO. With an increased water fraction (f_w) in the DMSO/water mixtures, the emission intensity of CS-Py⁺SO₃⁻ showed a slow increase while the wavelength peak showed a very slight change (Fig. 16B). CS-Py⁺ also showed typical aggregation-enhanced emission (AEE) properties. It was noted, however, that the aggregates (f_w = 95% in the DMSO/water mixture) of CS-Py⁺ showed enhanced and blue-shifted emission (Fig. 16C), which is probably the result of the twisted structure and intramolecular charge transfer

(TICT) of the compound. It was noted that the FL of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ in a high water fraction ($f_w = 95\%$) increased only several-fold compared with that in DMSO (Fig. 16D), probably because of the formation of loosely packed aggregates. In addition, the existence of aggregates in water solution with 5% DMSO were confirmed by dynamic light scattering data with hydrated diameters of 148 and 156 nm for $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ , respectively (Fig. 16E). The solid FL of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ displayed NIR emissions of 679 nm and 685 nm for $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ , respectively (Fig. 16F). The absolute FL quantum yields of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ were measured to be 12.8% and 13.7%, respectively, which were advantageous for bio-imaging, especially for *in vivo* imaging. The maximal emission wavelengths of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ basically increased from toluene, THF, acetone, MeOH to DMSO (Fig. 16G and 16H), indicating the phenomenon of positive solvatochromism. However, the fluorescence intensity of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ greatly decreased in high polar solvent compared with that in low polar solvent, due to their TICT properties. These data demonstrated that $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ are typical donor-acceptor molecules. Furthermore, the two-photon excited fluorescence of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ was investigated in THF using a femtosecond pulsed laser as excitation source (800–980 nm). Using rhodamine in MeOH as the standard, the two-photon absorption cross sections of these AIEgens were calculated at different excitation wavelengths (Fig. 16I). $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ exhibit good two-photon absorption cross sections (about 30–88 GM at 860–900 nm), which are comparable to those of the standard rhodamine B.

Example 4

Cytotoxicity

The cytotoxicity of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ by standard MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay were examined. After incubation in HeLa cells for 24 h, the cell viabilities of $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ were generally over 85% (Fig. 17). $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ exhibited negligible cytotoxicity within the concentration range depicted.

Example 5

Biological Imaging

To demonstrate the biological applications of AIEgens, $\text{CS-Py}^+\text{SO}_3^-$ and CS-Py^+ , fluorescence imaging was conducted of live HeLa cells by confocal laser scanning microscopy. A low concentration of 1 μM was used in the imaging experiments. After incubation for 20 min,

bright fluorescence of CS-Py⁺SO₃⁻ and CS-Py⁺ in HeLa cells could be observed (Fig. 18), indicating their excellent cell staining property. It is believed that AIEgen CS-Py⁺SO₃⁻ stained the cell membrane, while CS-Py⁺ stained the subcellular organelle in the cytoplasm. *In situ* fluorescence spectra of CS-Py⁺SO₃⁻ and CS-Py⁺ in HeLa cells were acquired by using the Lambda mode (Fig. 19). The *in situ* fluorescence data of CS-Py⁺SO₃⁻ and CS-Py⁺ in live cells showed the blue-shift feature compared with that in THF, probably due to their TICT effect. Considering their AIE property and TICT effect, it was anticipated that CS-Py⁺SO₃⁻ and CS-Py⁺ showed very faint emission at low incubation concentration (1 μM) due to molecular motion, but the compounds could boost their blue-shifted emission in a low polar (TICT effect) and restricted environment, resulting in a “wash-free” imaging property.

Then, co-staining imaging experiments were conducted to confirm the location of CS-Py⁺SO₃⁻ and CS-Py⁺ in live HeLa cells. As predicted, CS-Py⁺SO₃⁻ showed good overlap (Pearson's coefficient of 0.82) with commercial membrane dye DiI (Fig. 20A), indicating CS-Py⁺SO₃⁻ mainly stains cell membrane. Normally, positive-charged dyes mainly stain mitochondria due to the high negative membrane potential of mitochondria. Hence, CS-Py⁺ exhibited distributions in HeLa cells very similar to that of commercial mitochondria dye MitoTracker Deep-Red FM (MTDR) (Fig. 20B), and the corresponding Pearson's co-efficient was 0.84, which revealed that AIEgen CS-Py⁺ is mainly located in mitochondria.

To test whether these AIEgens could show specific imaging in live tissues, *ex vivo* imaging in live rat skeletal muscle tissues were carried out using CS-Py⁺. Fluorescence imaging data indicated that mitochondria are regularly arranged and formed reticulum in muscle with a high signal-to-noise ratio (Fig. 21A), while the tubular morphology could be shown in the transverse plane. This observation is in good agreement with previous data in skeletal muscle tissues obtained by scanning electron microscope (SEM). Additionally, co-staining imaging experiments with commercial mitochondria dye MTDR were carried out to confirm the location in live tissues. As shown in Fig. 21B, the staining pattern of CS-Py⁺ shows a good overlap with that of MTDR and the corresponding Pearson's coefficient is 0.84, further demonstrating excellent mitochondrial staining of CS-Py⁺ in live tissues.

Considering the good two-photon absorption cross-section, high two-photon excited fluorescence of CS-Py⁺ and its impressive live-tissue staining pattern, further imaging experiments were carried out to evaluate its two-photon imaging ability. After incubation in live

tissues for 1 h, two-photon imaging was performed using a NIR pulsed laser (900 nm). Bright fluorescence from mitochondria could be captured and this two-photon excited fluorescence was almost identical to that observed with one-photon excitation (Fig. 21C), indicating the great potential of CS-Py⁺ in two-photon fluorescence imaging. Compared with one-photon imaging, two-photon imaging shows much better performance, especially in deep-tissue imaging. To verify this, the fluorescent images at different depths along the z-axis were scanned. For one-photon imaging, the fluorescent images of regularly arranged mitochondria could be captured with satisfied signal-to-noise ratio at a depth of 50 μm and 3D one-photon fluorescent image was successfully reconstructed (Fig. 22A and 22B). However, two-photon excited fluorescence signals of CS-Py⁺ in muscle tissue could even be obtained at the depth of about 100 μm (Fig. 22C). Likewise, reconstructed 3D two-photon fluorescent image was also realized (Fig. 22D). Interestingly, such depth obtained by two-photon excitation is deeper than other two-photon probes. Taken together, the remarkable *ex vivo* two-photon imaging performance renders CS-Py⁺ an excellent candidate probe for biomedical imaging of mitochondria in live deep tissues.

Example 6

Photostability

The photostability of CS-Py⁺SO₃⁻ and CS-Py⁺ is a crucial parameter for their remarkable live cell stain properties. Photostability was first evaluated by continuous irradiation with confocal lasers. As seen in Fig. 23A, the fluorescence intensities of CS-Py⁺SO₃⁻ and CS-Py⁺ showed nominal signal loss after 180 scans, while the fluorescence signals of DiI and MTDR obviously decreased. Given the impressive performance of two-photon fluorescence imaging in live tissues, the photostability of CS-Py⁺ was further investigated by continuous irradiation with two-photon NIR pulsed laser (900 nm, output intensity of 2476 mW). The fluorescent images were scanned about every 5.2 s. After scanning 60 times, the two-photon fluorescent image was clearly obtained with good signal-to-noise ratio (Fig. 23B). In addition, normalized two-photon fluorescence intensity data in Fig. 23C revealed that CS-Py⁺ only suffered a low extent of emission drop under exposure to strong two-photon NIR pulsed laser and over 70% of the initial intensity still remained after the 60th scan (about 310 s). These data demonstrated that CS-Py⁺SO₃⁻ and CS-Py⁺ exhibit high photobleaching resistance and CS-Py⁺ can be applied for long-term one-photon and two-photon mitochondrial tracking.

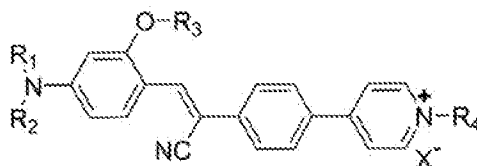
The present subject matter being thus described, it will be apparent that the same may be

modified or varied in many ways. Such modifications and variations are not to be regarded as a departure from the spirit and scope of the present subject matter, and all such modifications and variations are intended to be included within the scope of the following claims.

CLAIMS

We Claim:

1. A fluorescent compound exhibiting aggregation induced emission properties, the compound having the following backbone structural formula:

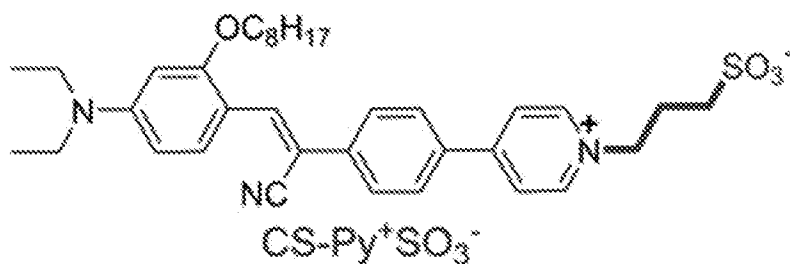


wherein each R_1 , R_2 , R_3 , and R_4 is substituted or unsubstituted and is independently selected from the group consisting of C_nH_{2n+1} , C_6H_5 , C_7H_7 , $C_{10}H_7$, $C_nH_{2n}SO_3^-$, $C_nH_{2n}COOH$, $C_nH_{2n}NCS$, $C_nH_{2n}N_3$, $C_nH_{2n}NH_2$, $C_nH_{2n}Cl$, $C_nH_{2n}Br$, and $C_nH_{2n}I$;

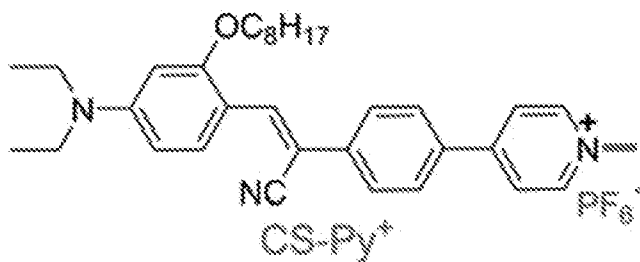
wherein each X is substituted or unsubstituted and is independently selected from the group consisting of Cl , Br , I , NO_3 , ClO_4 , BF_4 , PF_6 , CF_3CO_2 and CF_3SO_3 ; and

wherein each n is independently an integer ranging from 0 to 16.

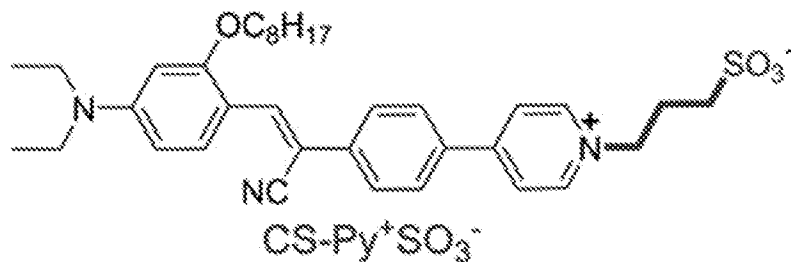
2. The compound according to claim 1, wherein the compound comprises one or more compounds selected from the group consisting of:



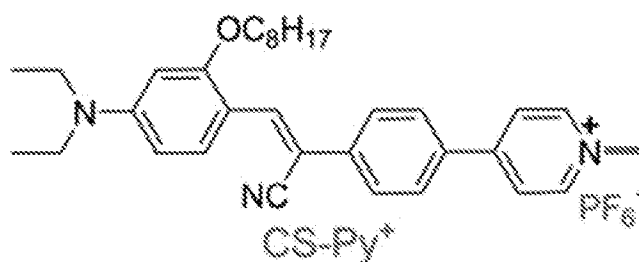
and



3. A method of cellular imaging, comprising:
contacting a target cell with the compound of claim 1; and
identifying a target of interest in the target cell using an imaging method.
4. The method of claim 3, wherein the target of interest is selected from the group consisting of a cellular membrane and a cellular organelle.
5. The method of claim 4, wherein the target of interest is a cellular organelle and the cellular organelle comprises mitochondria.
6. The method of claim 4, wherein the target cell is a live cell.
7. The method of claim 4, wherein the target cell is in live tissue.
8. The method of claim 7, wherein the target cell is at a depth of about 50 μm to about 100 μm in the live tissue.
9. The method of claim 3, wherein the imaging method is selected from the group consisting of one-photon fluorescence microscopy and two-photon fluorescence microscopy.
10. The method of claim 3, wherein identifying the target of interest comprises visualizing organelle-associated changes in live cells.
11. The method of claim 3, wherein the imaging method comprises irradiating the compound for about 310 seconds.
12. A fluorescent compound exhibiting aggregation induced emission properties, the compound comprising one or more compounds selected from the group consisting of:



and



13. A method of cellular imaging, comprising contacting a target cell with the compound of claim 12; and identifying a target of interest in the target cell using an imaging method.
14. The method of claim 13, wherein the target of interest is selected from the group consisting of a cellular membrane and a cellular organelle.
15. The method of claim 14, wherein the target of interest comprises a cellular organelle and the cellular organelle comprises mitochondria.
16. The method of claim 13, wherein the target cell is a live cell.
17. The method of claim 13, wherein the target cell is in live tissue.
18. The method of claim 17, wherein the target cell is at a depth of about 50 μm to about 100 μm in the live tissue.
19. The method of claim 13, wherein the imaging method is selected from the group consisting of one-photon fluorescence microscopy and two-photon fluorescence microscopy.
20. The method of claim 13, wherein identifying the target of interest comprises visualizing organelle-associated changes in live cells.

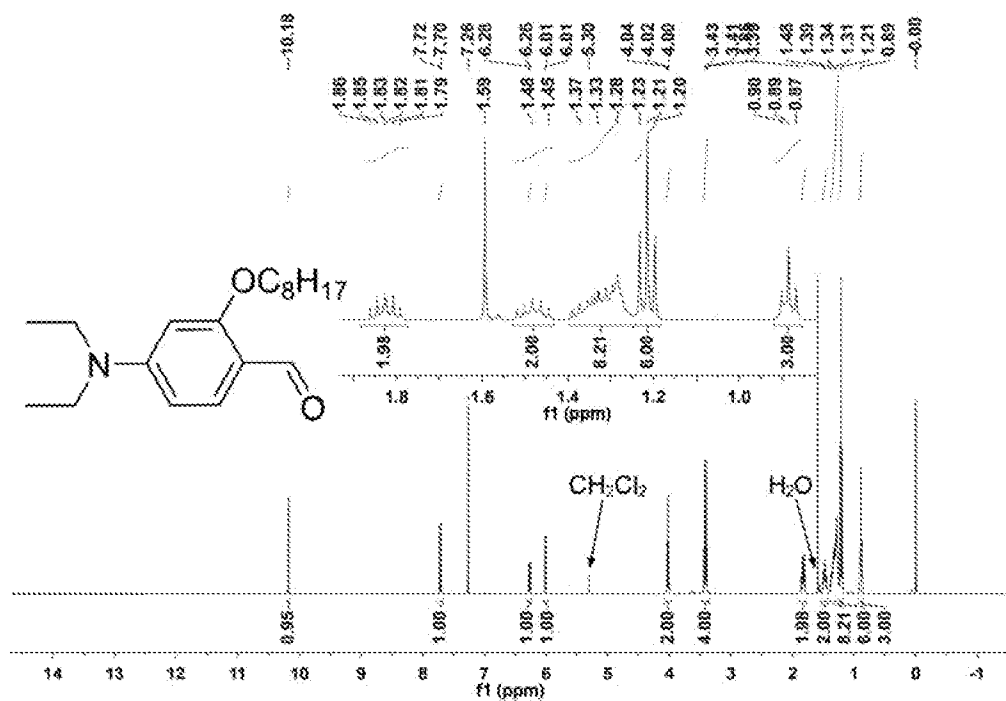


Fig. 1

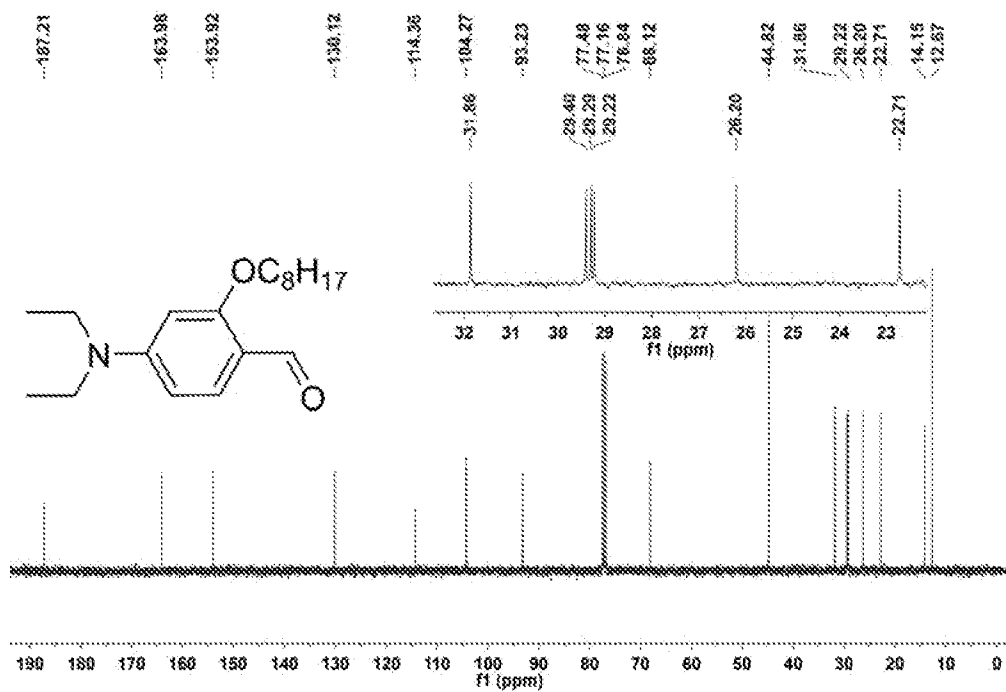


Fig. 2

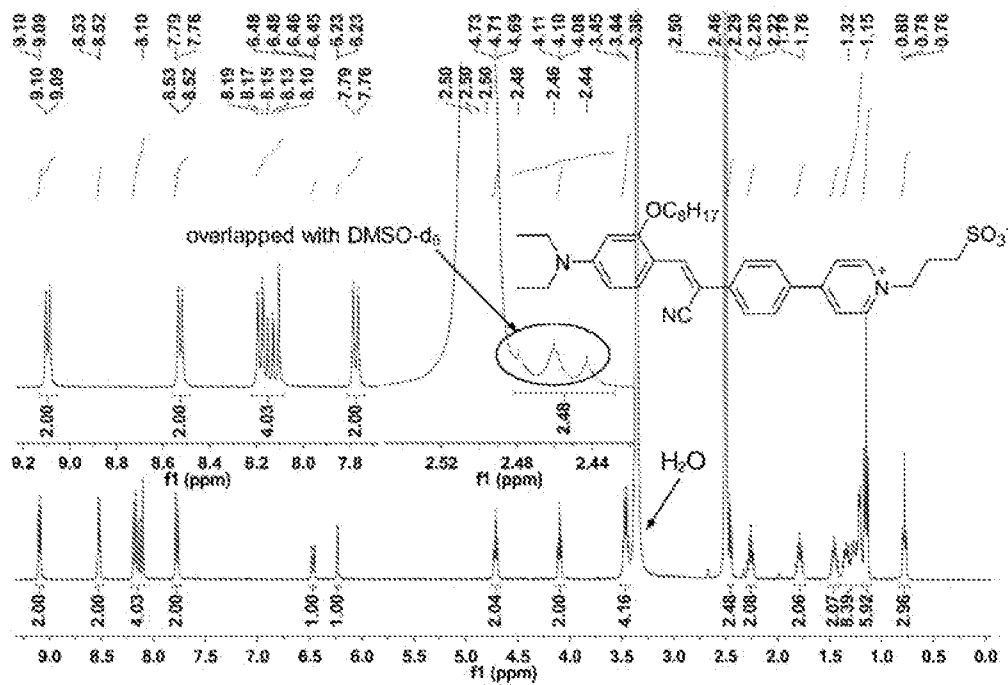


Fig. 7

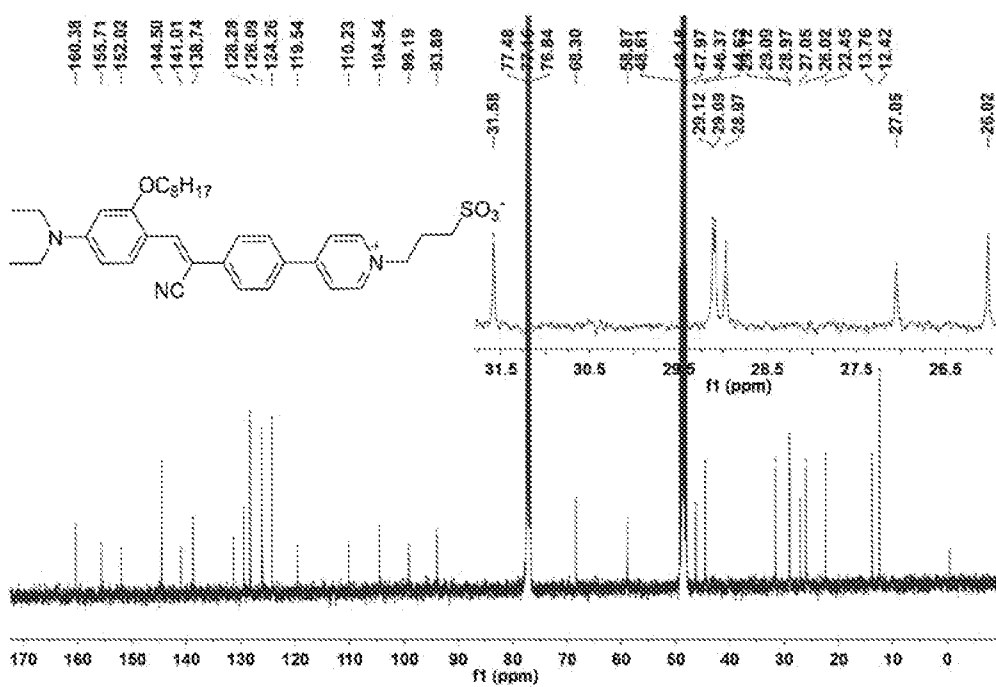


Fig. 8

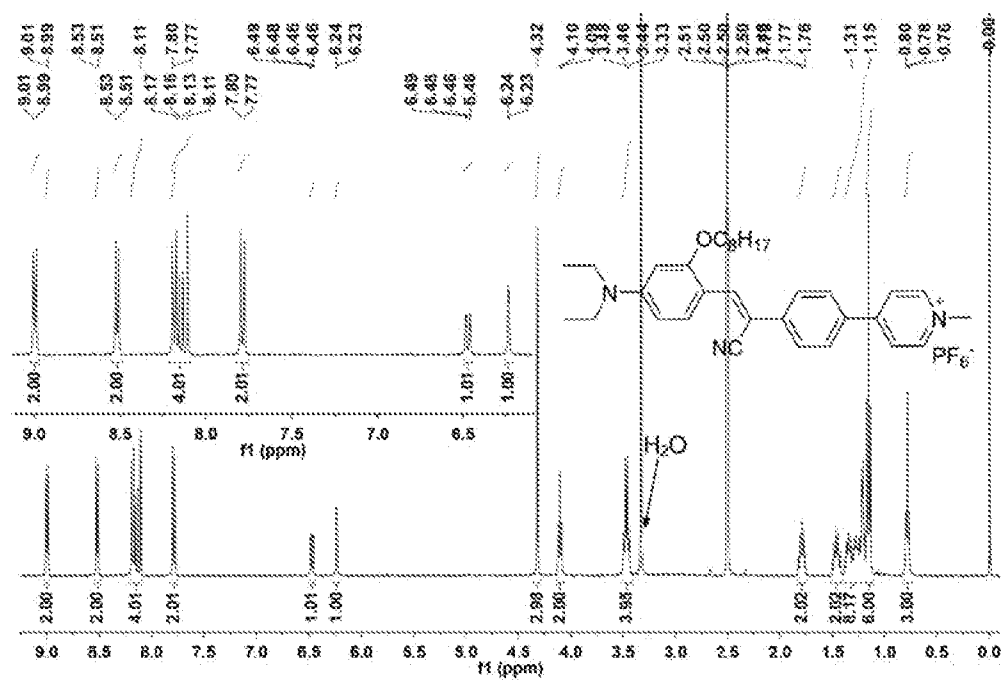


Fig. 9

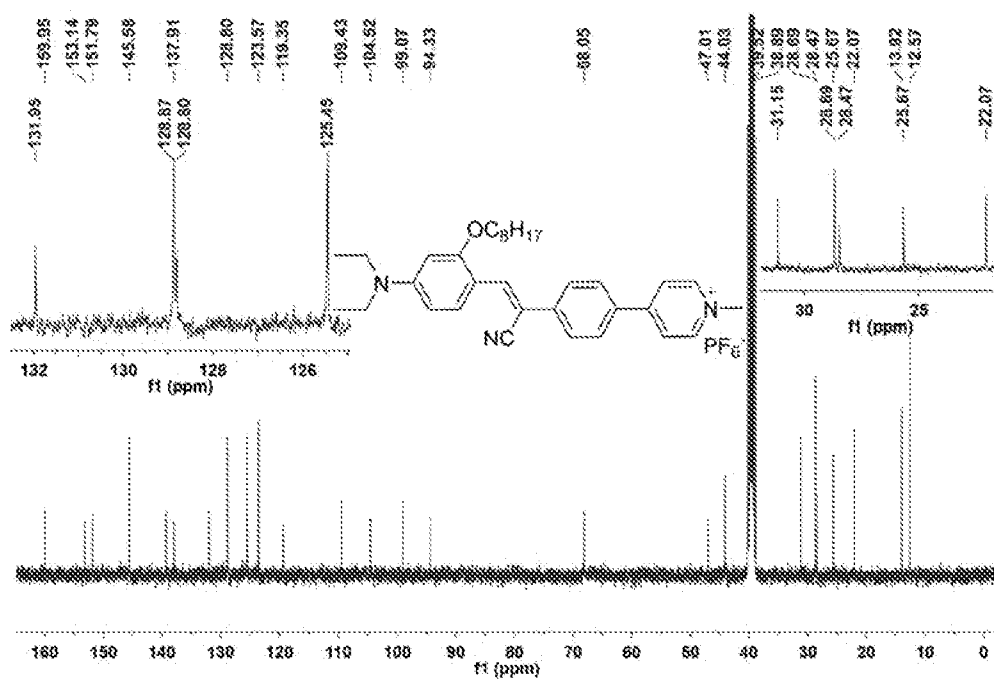


Fig. 10

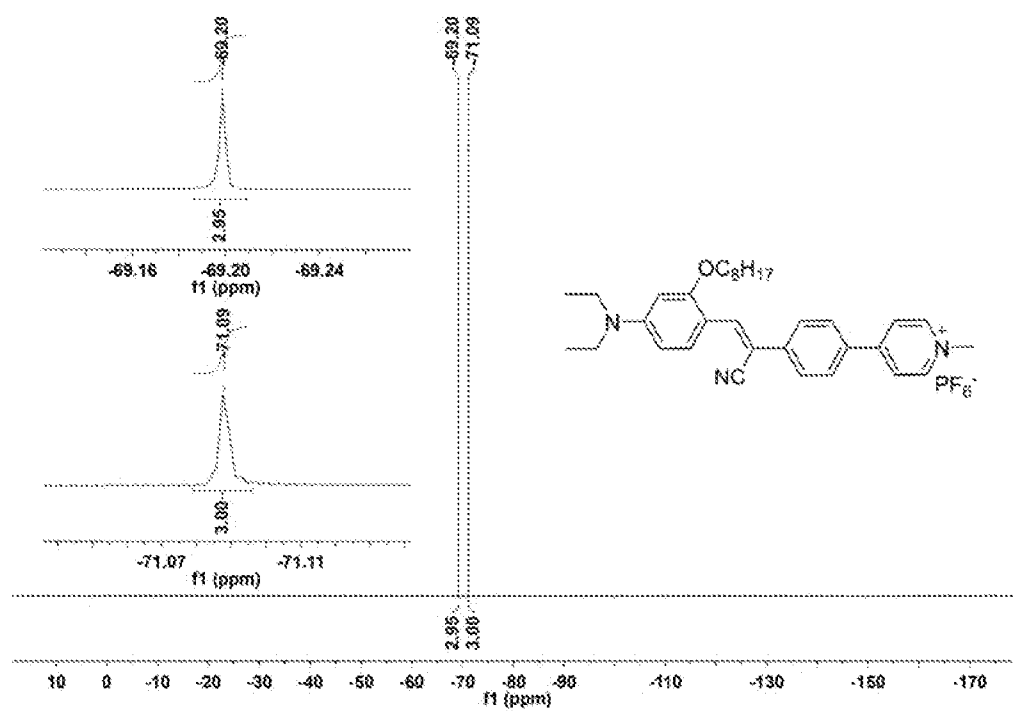


Fig. 11

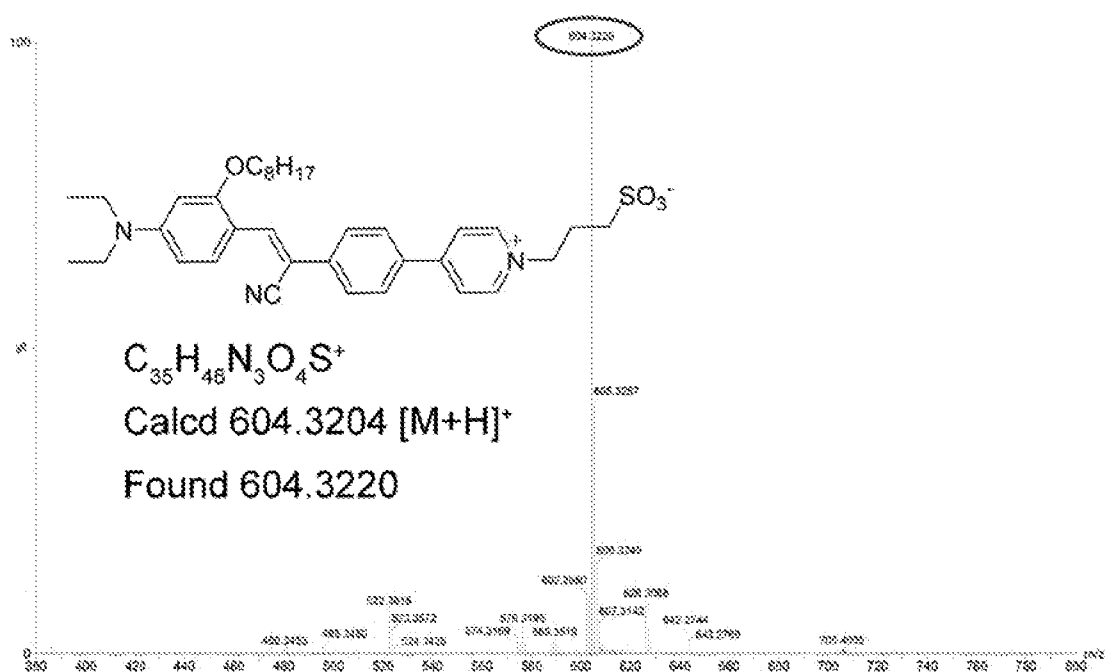


Fig. 12

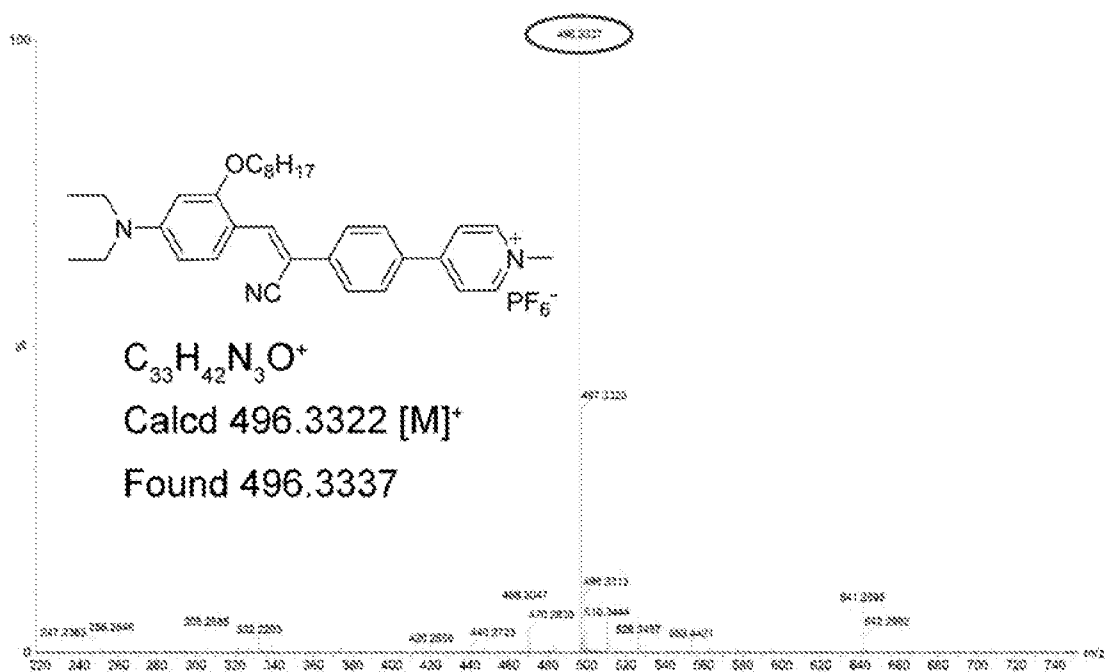


Fig. 13

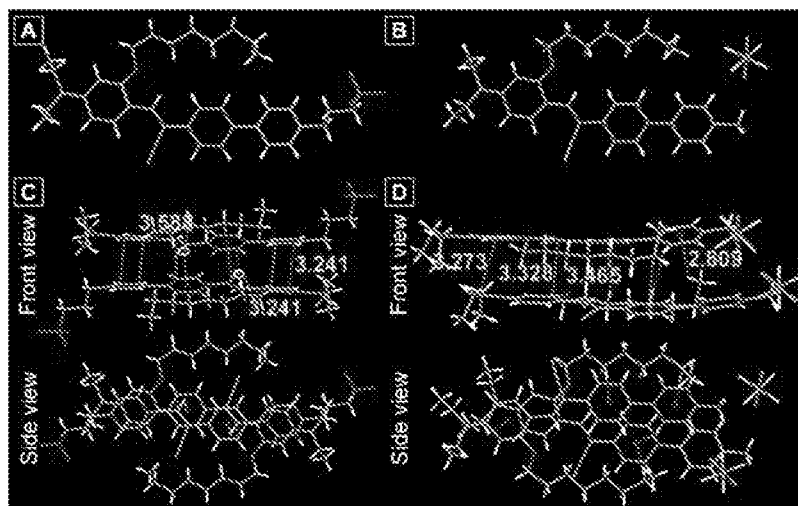


Fig. 14

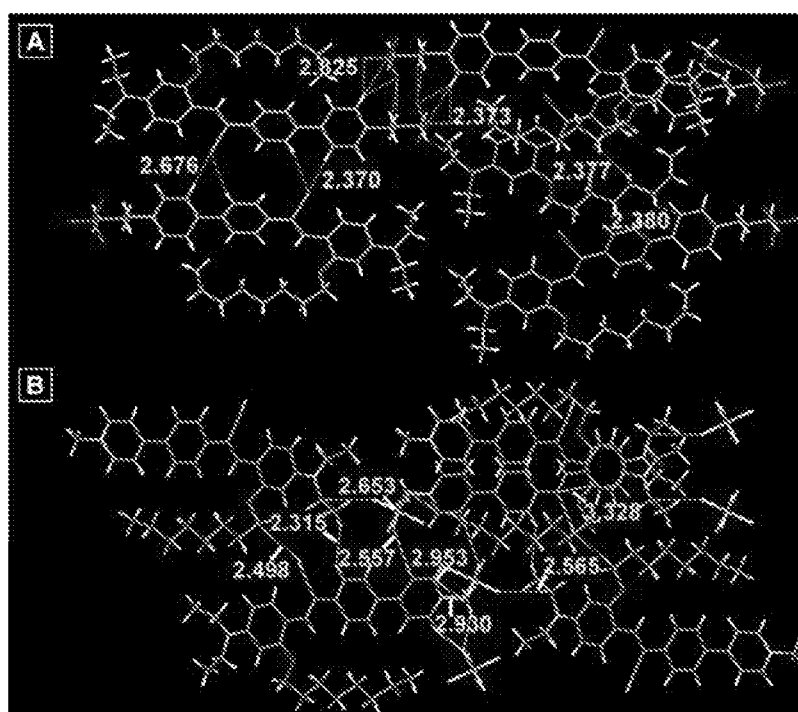
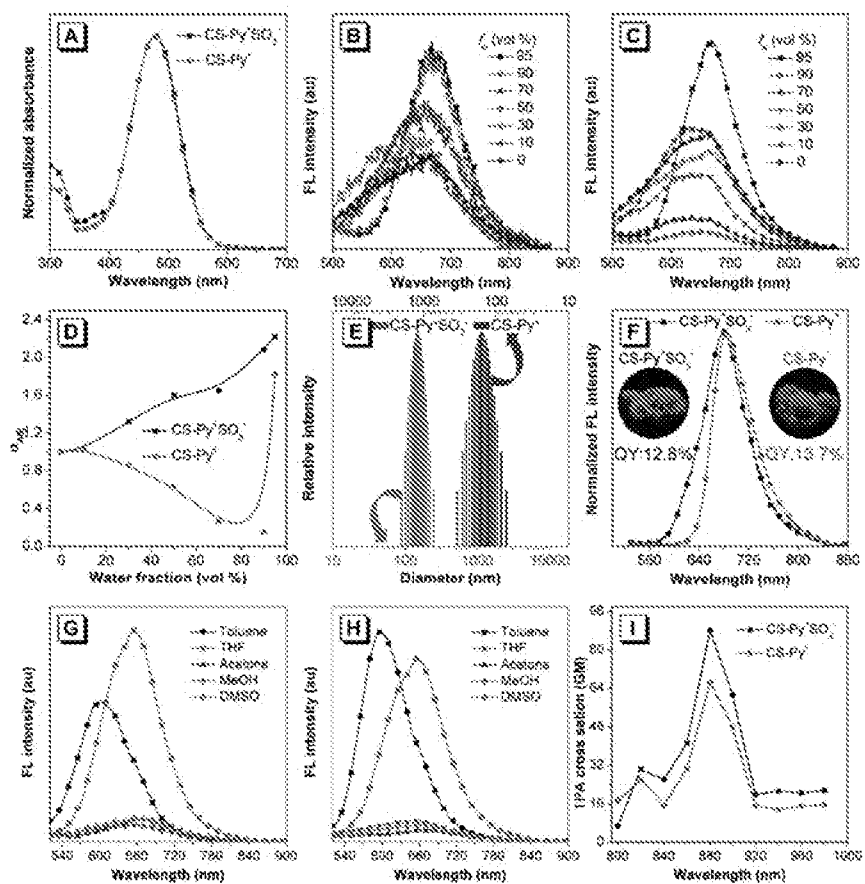


Fig. 15



Figs. 16A-16I

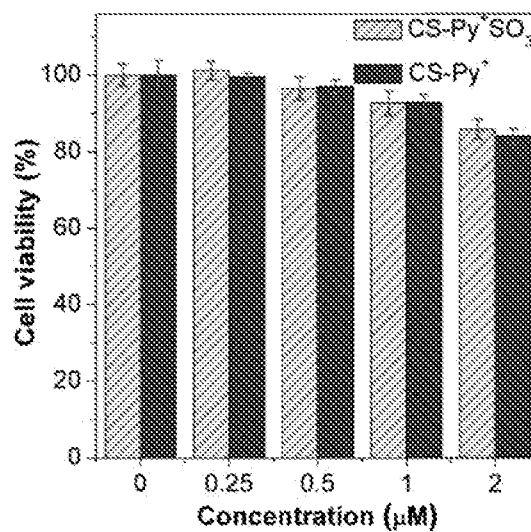


Fig. 17

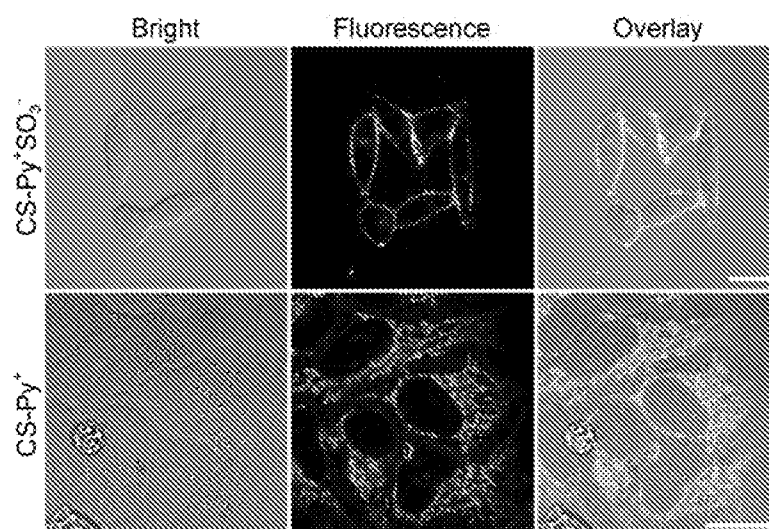


Fig. 18

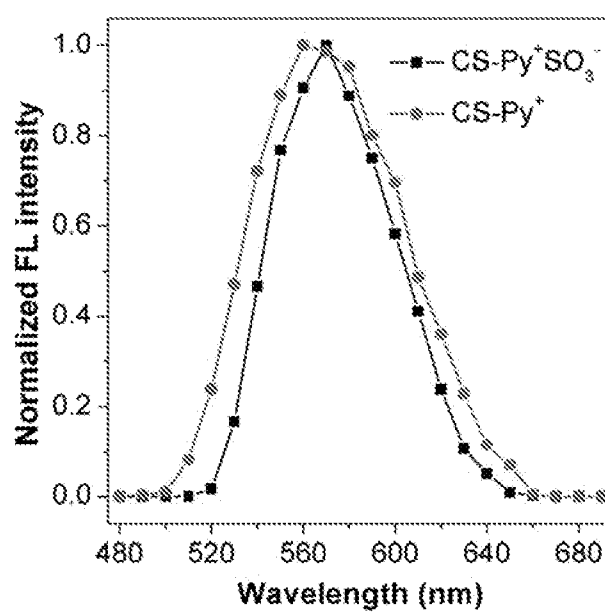
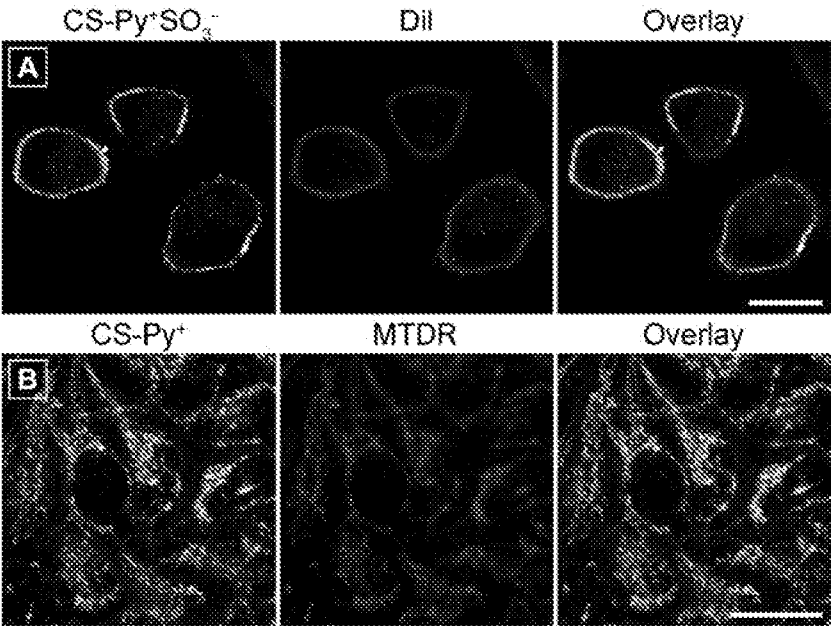
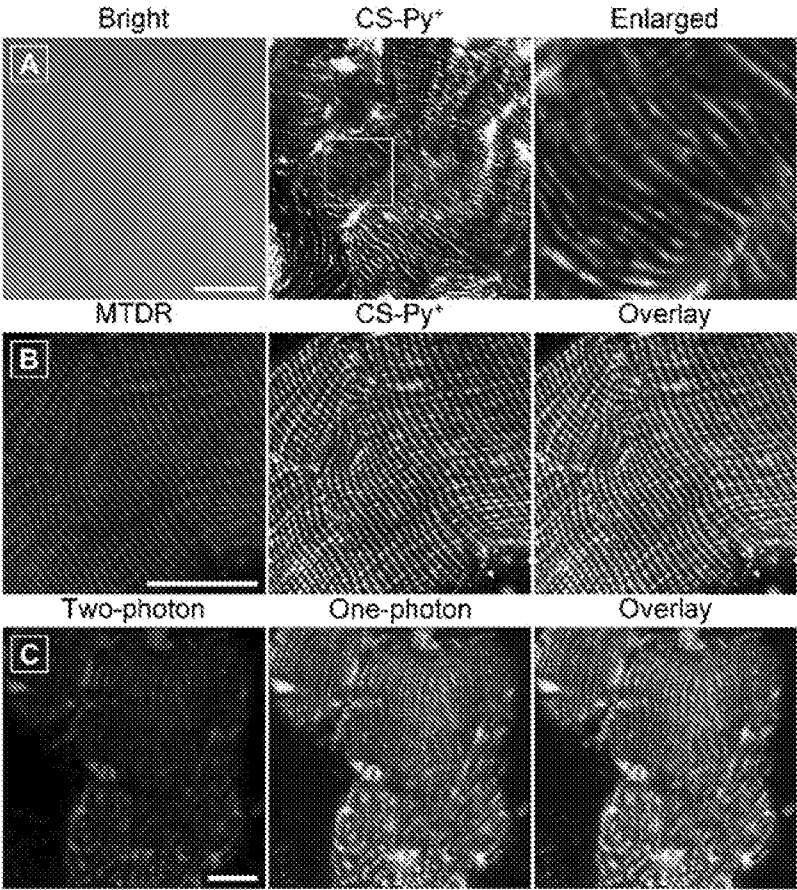


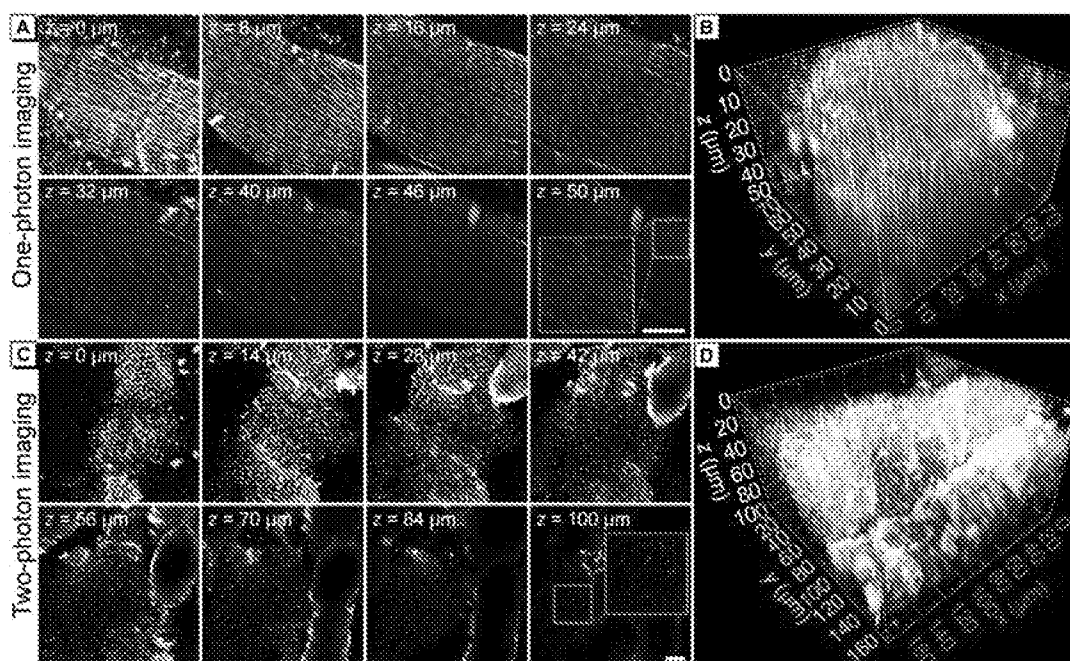
Fig. 19



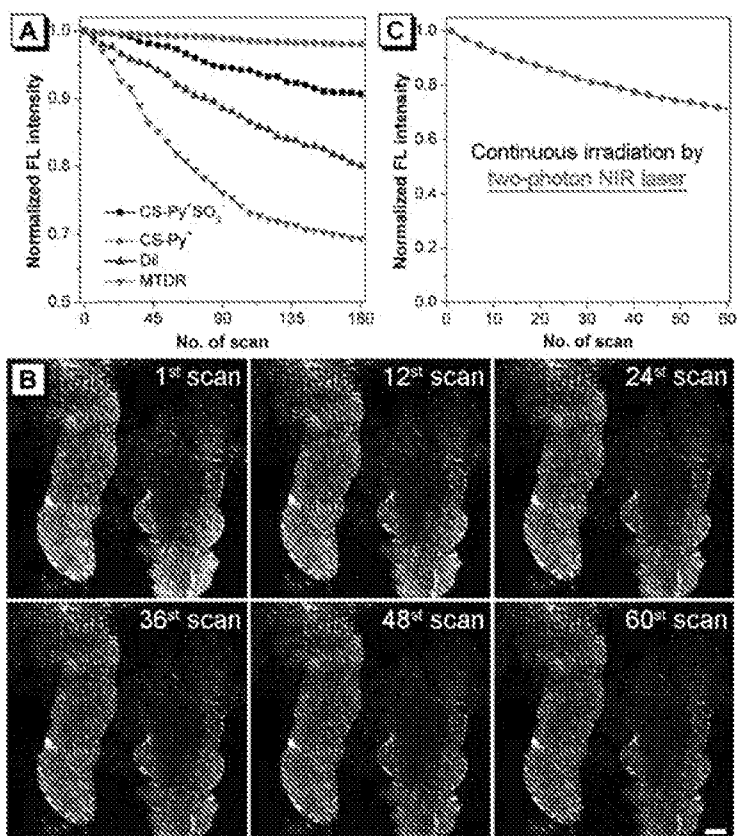
Figs. 20A-20B



Figs. 21A-21C



Figs. 22A-22C



Figs. 23A-23C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/071328

A. CLASSIFICATION OF SUBJECT MATTER

C07D 213/04(2006.01)i; C09K 11/06(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D; C09K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI; SIPOABS; CNKI; CNABS; STNext: The HongKong University of Science and Technology, fluorescent, aggregation, induc+, emission, AIE, AIEgen, cellular imaging

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	WO 2019137449 A1 (UNIV. HONG KONG SCIENCE & TECHNOLOGY) 18 July 2019 (2019-07-18) the abstract, claims 1-20, Fig.35-38	1-20
PX	NIU G.et al. "Highly photostable two-photon NIR AIEgens with tunable organelle specificity and deep tissue penetration" <i>Biomaterials</i> , Vol. 208, 09 April 2019 (2019-04-09), pages 72-82	1-20
X	NIU G.et al. "Specific Two-Photon Imaging of Live Cellular and Deep-Tissue Lipid Droplets by Lipophilic AIEgens at Ultralow Concentration." <i>Chem. Mater.</i> , Vol. 30, 02 July 2018 (2018-07-02), pages 4778-4787	1-20
A	CN 107001927 A (UNIV HONG KONG SCIENCE & TECHNOLOGY) 01 August 2017 (2017-08-01) the abstract, paragraphs 165-169	1-20
A	CN 106461641 A (UNIV. SINGAPORE NAT.et al.) 22 February 2017 (2017-02-22) the abstract, claims 1-7, page 6 lines 12-14	1-20



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 March 2020

Date of mailing of the international search report

10 April 2020

Name and mailing address of the ISA/CN

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CUI,Chuanming

Telephone No. (86-10)53961869

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/071328

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAO Y. et al. "2D amphiphilic organoplatinum(II) metallacycles:their syntheses, self-assembly in water and potential application in photodynamic therapy" <i>Chem. Commun.</i> , Vol. 54, 31 December 2018 (2018-12-31), pages 8068-8071	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/071328

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **3-11,13-20**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Claims 3-11 and 13-20 direct to a method of cellular imaging, and do not meet the criteria set out in PCT Rules 39.1(iv). The search has been carried out and based on the use of the compounds for the manufacturing of an agent for cellular imaging.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2020/071328

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO	2019137449	A1	18 July 2019	None	
CN	107001927	A	01 August 2017	CN 107001927 B	17 September 2019
				WO 2016078603 A1	26 May 2016
CN	106461641	A	22 February 2017	WO 2015112092 A8	18 February 2016
				US 2016356723 A1	08 December 2016
				WO 2015112092 A3	26 November 2015
				SG 11201606147S A	30 August 2016
				WO 2015112092 A2	30 July 2015