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A Photostable Near-Infrared Fluorescent Tracker with pH-Independent Specificity to Lysosomes for Long Time and Multicolor Imaging

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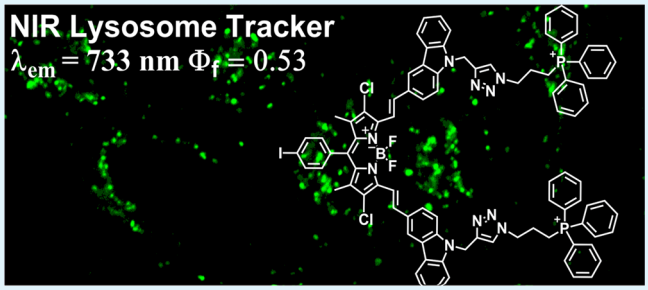
Supporting Information

ABSTRACT: A new boron−dipyrromethene-based lysosome tracker, **Lyso-NIR**, is facilely synthesized. Besides the intensive near-infrared (NIR) fluorescence and high photostability, **Lyso-NIR** shows the capability to stably localize in lysosomes, which is independent of the local pH. **Lyso-NIR** does not have the problematic alkalization effect suffered by the commonly used lysotrackers; thus, it shows ignorable cytotoxicity and slightly affects normal physiological functions of lysosomes. The above advantages of **Lyso-NIR** make it feasible to track lysosomes' dynamic changes in a relatively long time during the full cellular processes such as apoptosis, heavy metal stimulation, and endocytosis, as is demonstrated in this work. Moreover, **Lyso-NIR**'s narrow NIR emission at 740 nm with a full width at half-maximum smaller than 50 nm makes it easy to avoid the crosstalk with the emissions from other common fluorescent probes, which strengthens **Lyso-NIR**'s competitiveness as a standard lysosome tracker for multicolor bioimaging.



KEYWORDS: *lysosome, probe, near-infrared, fluorescence imaging*

INTRODUCTION

Lysosomes, described as the stomach of the cell, play critical roles in the digestion of macromolecules that they take in during the processes of phagocytosis, endocytosis, and autophagy.[1−3] To achieve their functions, lysosomes are of a highly dynamic nature, and they are constantly changing their morphology and spatial distribution. Noninvasive and real-time tracking of their movements and morphologic changes will help to understand their working status, which calls for efficient fluorescent probes of high specificity but low disturbance to lysosomes.

However, most currently used small-molecule lysotrackers, such as the widely applied and commercially available **Neutral Red** (**NR**), **DND-189**, and **DND-99**, are pH-dependent to different degrees, which results in their nonspecific staining and low localizing stability against pH change. Once lysosomal pH increases, they may leave lysosomes to go elsewhere and/or their fluorescence is quenched.[4−9] Worse still, these lysosomal probes exhibit alkalinizing effect on lysosomes, such that longer incubation with these probes can induce an increase in lysosomal pH.[9] Moreover, for some of them, the relatively low photostability is also a limitation for long time tracking under a laser confocal microscopy. Also noticeably, some commercial lysotrackers' N-containing groups gain them strong intramolecular charge transfer (ICT) effect broadening the emission band remarkably; for example, emission of **DND-99** extends from 560 to 760 nm.[9] In the cases of multicolor imaging (actually, very common), a broad emission from one single fluorophore is undesirable, because this will make the signal crosstalk between different probes hardly avoidable to lower the image quality.

Dextran conjugated fluorophores represent another type of lysosomal probes, which is used as a tool for studying phagocytosis. However, these macromolecule-based probes may not be recommended for long-time tracking, as dextran itself proves to have the activity to interrupt normal physiological function of lysosomes.[10] Similarly, the newly emerging inorganic or hybrid fluorescent nanoparticles that usually are uptaken into lysosomes may also have the problems of toxicity.[11] For example, the damage of lysosomal membranes by the SiO_2 particles has been confirmed for long time.[12]

Hence, there still remains scope to improve the applicability of lysosomal trackers, in terms of their localization specificity as well as stability, spectral properties, and cytotoxicity. In this work, a near-infrared (NIR) lysosomal probe **Lyso-NIR** (Scheme 1) is developed from our new fluorophore **DC-DPC**.[13] Interestingly, two triphenylphosphonium (TPP) moieties gain the probe an unusual lysosome-targeting capability; this discovery deviates from the common knowledge that the positively charged TPP always leads to the accumulation in mitochondria of negative potential. In lysosomes, **Lyso-NIR** shows almost the same and

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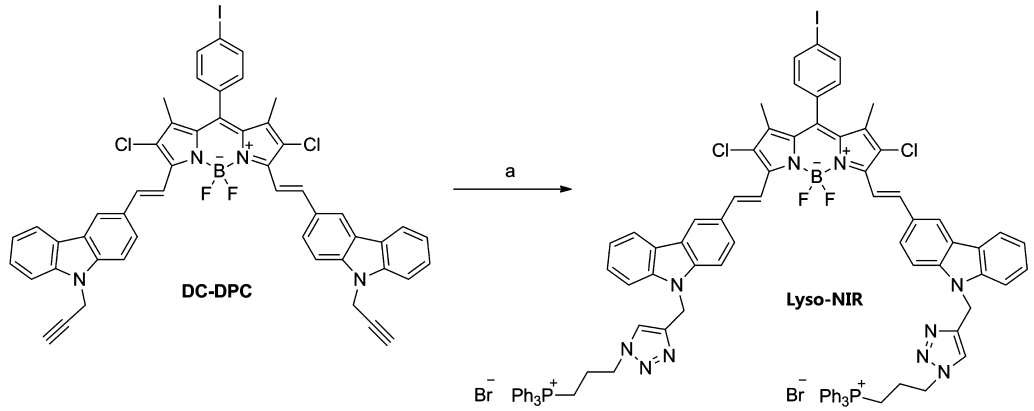
Scheme 1. Synthetic Procedure for Lyso-NIR[a]



[a]Reaction conditions: (a) (3-azidopropyl)-triphenylphosphonium bromide, DIPEA, $CuSO_4$, sodium ascorbate, alcohol, H_2O, toluene, RT, overnight, yield 70%.

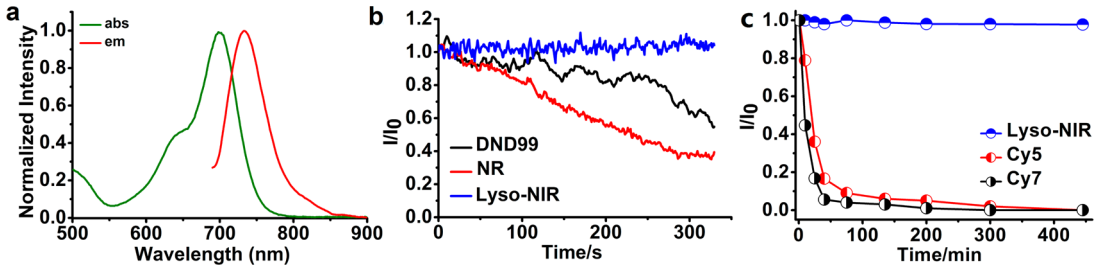


Figure 1. (a) Normalized absorption spectra and fluorescence spectra of **Lyso-NIR** in ethanol. (b) Fluorescence intensity decay of **Lyso-NIR**, **Neutral Red** (**NR**), and **DND-99** in living cells with irradiation by semiconductor laser in a confocal fluorescence microscope. Excitation wavelength is 635 nm for **Lyso-NIR**, 559 nm for **NR** and **DND-99**, and the intensity at the sample is approximately 0.5 mW. (c) Fluorescence intensity decay of **Lyso-NIR**, Cy5, and Cy7 in in CH_2Cl_2 with irradiation by a 500 W iodine−tungsten lamp. I_0 is the fluorescence intensity before the irradiation. I is the fluorescence intensity of the above compounds after a fixed time of irradiation.

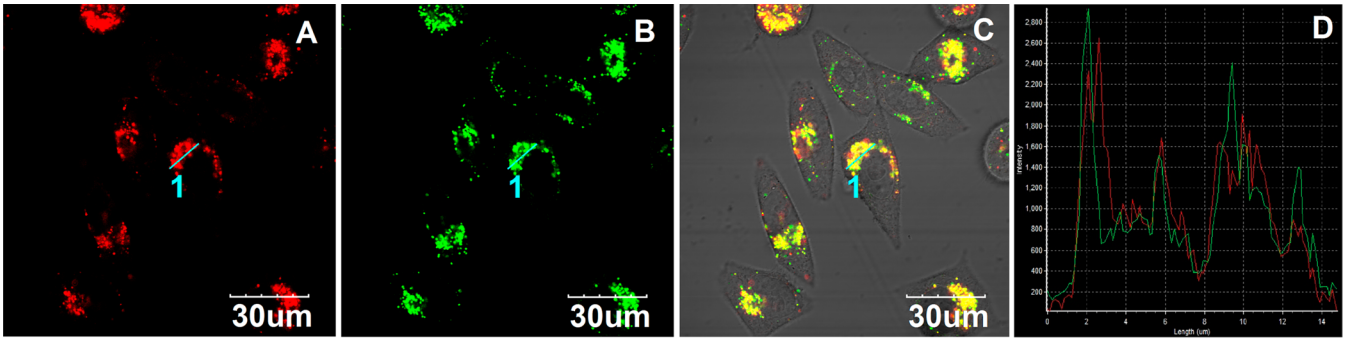


Figure 2. MCF-7 cells are costained with (A) 25 nM **NR** (λ_{ex} = 559 nm, λ_{em} = 575−640 nm, pseudo red) and (B) 30 nM **Lyso-NIR** (λ_{ex} = 635 nm, λ_{em} = 655−755 nm, pseudo green) (2 h at 37 °C 5% CO_2) in PBS. (C) Overlay of (A), (B) and the brightfield image. (D) Intensity profiles of region of interest (ROI) cross MCF-7 cell in (C).

strong fluorescence even after 24 h of incubation. And also it has the ignorable cytotoxicity confirmed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To the best of our knowledge, **Lyso-NIR** is the longest wavelength (~740 nm) lysosomal tracker.[9] Additionally, a small full width at half-maximum (fwhm) of spectrum (~48 nm) makes it an ideal choice for multicolor imaging. In a word, **Lyso-NIR** possesses the advantages of including its specifically targeting lysosomes, stably emitting strong and narrow-band NIR fluorescence there, and being free from the affection by or causing an increase in lysosomal pH, which qualifies it as a reliable tracker for lysosome under physiological or toxicological processes.

RESULTS AND DISCUSSION

Design and Synthesis of Probe. **DC-DPC** is our newly reported fluorophore that bears good photophysical properties. Two kinds of convenient functionalization sites have been reserved in the molecule: the aryl iodide for organometallic couplings and the terminal alkyne groups for click reactions. Here, **DC-DPC** is facilely functionalized via click chemistry into **Lyso-NIR**. The reaction is conducted in mild condition and with high yield. The introduction of two TPP moieties mediates its hydrophilic−lipophilic balance and gains the probe this unusual lysosome-targeting capability. The detailed synthetic route is described in Experimental Section.

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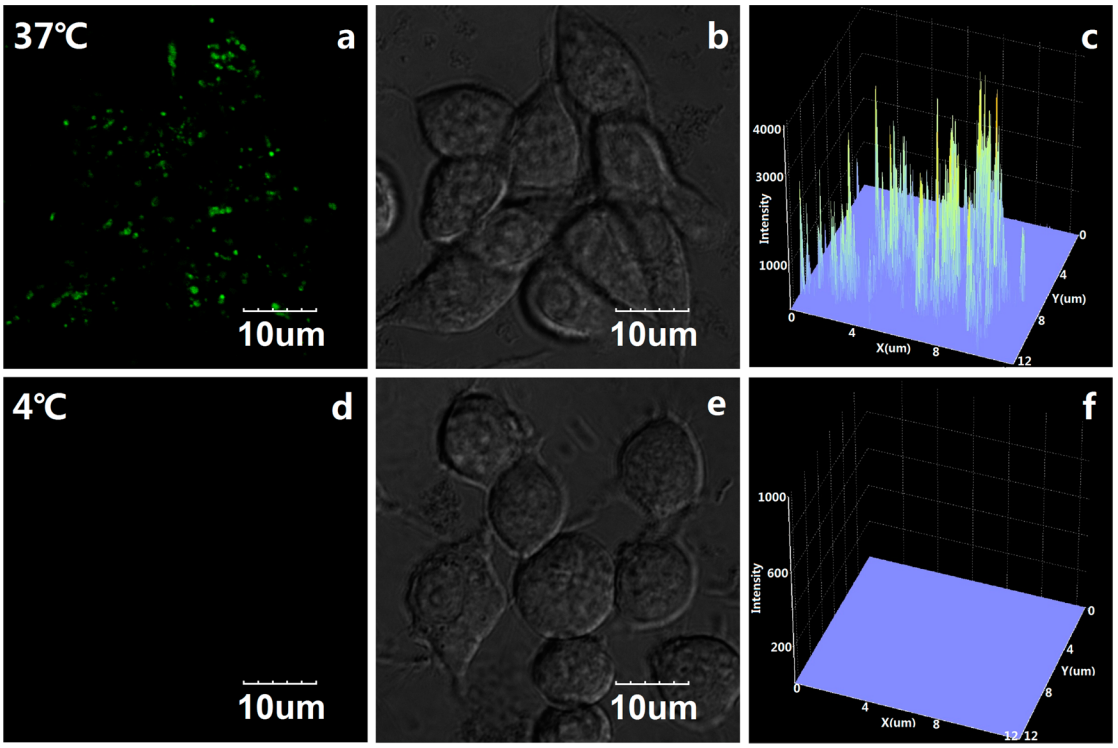

Figure 3. Fluorescence images of macrophage cells stained with **Lyso-NIR** for 2 h under 37 or 4 °C. (b, e) Brightfield and (c, f) 3D intensity profile of (a, d).

Fundamental Optical Properties and Photostability. **Lyso-NIR** has satisfactory optical characteristics for biological imaging. The normalized absorption and emission spectra in ethanol are recorded in Figure 1a, and the spectral data in different solvents are listed in Supporting Information, Table S1. **Lyso-NIR** exhibits strong absorption and emission in the NIR region (λ_{abs} = 700 nm, λ_{em} = 733 nm) with quantum yield of 0.53. Also noticeably, **Lyso-NIR** is environmental factor-independent fluorophores that exhibits stable optical characteristics (Supporting Information, Table S1).

Photobleaching is an unavoidable problem for most organic dyes that will compromise the temporal monitoring of dynamic events inside cells. Here, photodecomposition experiments prove that **Lyso-NIR** has better photostability compared with commercial lysosomal tracker **NR** and **DND-99** in living cells. After irradiation by semiconductor laser in a confocal fluorescence microscope, the fluorescence intensity of **Lyso-NIR** remains at its original level, while that of **NR** and **DND-99** decrease to 40% and 55%, respectively (Figure 1b). Another group of photodecomposition experiments compared with that of the well-known NIR fluorophore Cy5 and Cy7 were conducted in solution. After irradiation by a 500 W iodine tungsten lamp for 450 min, the fluorescence of **Lyso-NIR** remains at its original level, while Cy5 and Cy7 are almost bleached within 100 min. Enhanced photostability demonstrates the advantage of **Lyso-NIR** for bioapplications.

Colocalization Imaging Studies. Colocalization imaging experiment unambiguously proves that **Lyso-NIR** could localize specifically in lysosomes. As shown in Figure 2, colocalization experiment of **Lyso-NIR** and **NR** (one of the typical lysotrackers) displays high overlap within lysosomes (Figure 2). Intensity profiles of region of interest (ROI) across MCF-7 cells costained with **NR** vary in close synchrony (Figure 2d). One may argue that there have been a lot of TPP-containing molecular probes that selectively accumulate in mitochondria, for their negative potential of the inner membranes attract the positively charged molecules.[14,15] To answer this question, we carefully carried out another colocalization experiment by staining cells with **Lyso-NIR** and the Rh-123 (commercial mitochondrial probe); the result indicated that **Lyso-NIR** and the Rh-123 distribute in the different intracellular regions (Supporting Information, Figure S4) indeed, which, without a doubt, ruled out any possibility for **Lyso-NIR** to stain mitochondria.

Since the discovery that **Lyso-NIR**'s specificity toward lysosomes rather than mitochondria deviates from the common knowledge, it deserves a detailed clarification on the origin of such an unusual subcellular localization. Here, we provide explanations from two perspectives, both of which are supported by the results of experiments.

From the perspective of molecular structure, it is the combined effect of several factors including the number of charge, the molar/ionic weight, and the balance between hydrophilicity and lipophilicity, but not just a single one of them, that decides the molecular probes' selectivity to certain subcellular organelles.[16,17] Horobin et al. suggested to predict the molecular selectivity to organelles by mainly analyzing their log *P* value and ionic number.[18,19] According to their theory, a molecule that has a log *P* value between −5 and 0 and cation number more than 0 shows high probability to localize in lysosomes. Here, we tested the log *P* values of **Lyso-NIR** as −2.89 (detailed method see Experimental Section), indicating its higher hydrophilicity. Given that **Lyso-NIR** has two cations, it is reasonable that **Lyso-NIR** prefers to localize in lysosomes. To our knowledge, quite a few TPP-containing probes are reported to be mitochondria-targeted, but all of them have only one TPP moiety; they have only one positive charge, and thus they are

actually more lipophilic than our **Lyso-NIR**, which contains two positively charged TPPs.[14,15]

From the perspective of cell uptake, because cell membranes' phospholipid bilayers are of a strongly lipophilic nature, the way for lipophilic probes to enter cells is to diffuse freely and quickly, but that for hydrophilic molecules, especially the multiply charged ones, is through endocytosis (i.e., to be engulfed by cells). Notice that the destinations in the route of the endocytosis are, exactly, lysosomes. With these in our mind, we carried out a comparison study of macrophage cell uptake experiments at 37 and 4 °C, which verified that **Lyso-NIR** indeed enter into cells through endocytosis, as demonstrated in Figure 3. At the normal incubation temperature (37 °C), it took ∼2 h for **Lyso-NIR**'s staining to generate a clear NIR fluorescent image; however, other common lysosome probes, for example, **Neutral Red**, **DND-189**, and **DND-99**, only need several minutes to diffuse into cells; thus, the longer incubation time, or the lower speed to stain cells, indicated that **Lyso-NIR** cannot diffuse into cell freely but can only undergo the slow endocytosis. At 4 °C, after 2 h of incubation with **Lyso-NIR**, no fluorescence signals can be observed under microscope, which means the uptake of **Lyso-NIR** by macrophage cells is blocked under low temperature. In addition, a similar experiment by using MCF-7 cells gave the same result. The uptake of **Lyso-NIR** by MCF-7 cells could also be blocked under low temperature (Supporting Information, Figure S2). This phenomenon is also clear evidence for endocytosis that can be efficiently inhibited by incubating the cells at low temperature.

In addition, stained MCF-7 cells by **Lyso-NIR** are further incubated for 24 h. Fluorescence images demonstrate that these stained MCF-7 cells demonstrate the same fluorescence intensity as the moment they are just stained (Supporting Information, Figure S3). Stable localization in lysosomes enables **Lyso-NIR** to trace lysosome movements and morphological changes in long-time processes.

Effect of pH Changes on the Fluorescence Properties of Lyso-NIR in Solution and in Live Cells. Most currently used lysotrackers are pH sensitive, and their targeting-ability is based on this alkalization effect. However, **Lyso-NIR** shows stable targeting ability against lysosomal pH changes. In Figure 4, pH titration experiment reveals that fluorescence of **Lyso-NIR** is insensitive to the change of pH. In vivo, **Lyso-NIR** also shows stable localization and stable fluorescence against drug-induced lysosomal pH changes. In this experiment, chloroquine that can cause the leakage of protons out of lysosomes is used to increase lysosomal pH.[20] Under costaining (with **NR**) condition, the addition of 20 μM chloroquine induces a clear decrease of fluorescence intensities from **NR** within 20 min, while fluorescence from **Lyso-NIR** only shows a slight fluctuation, possibly due to the migration of lysosomes (Figure 5). Such stable fluorescence intensity independent of lysosome pH change is important for correctly indicating the location and morphology of lysosomes.

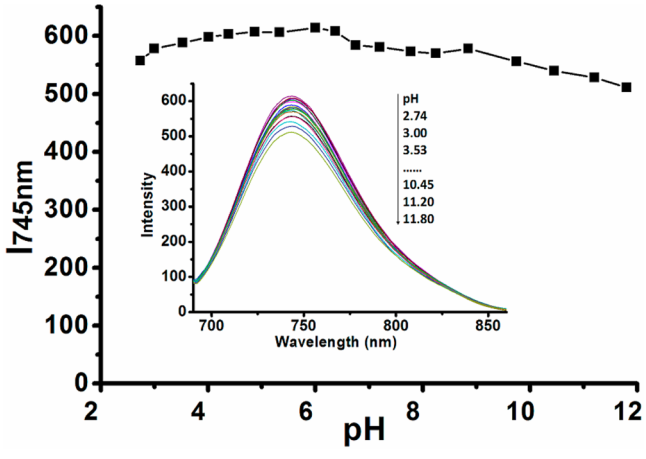

Figure 4. Change of intensity plot of **Lyso-NIR** (3 μM) at 745 nm vs different pH values. (inset) Changes in fluorescence spectra of **Lyso-NIR** in aqueous solution containing 30% acetronitile as cosolvent vs different pH values. (λ_{ex}: 690 nm slit: 3 nm).

Monitoring Morphology Variation of Lysosomes under Toxicological or Physiological Conditions. As we should know, during a long time range under physiologic conditions, pH values of the highly inhomogeneous lysosomes are always changing, and for lysosomes in different physiological status the pH values are also different. However, for currently used lysosomal probes, once the pH rises, they will lose their fluorescence or will leave lysosomes. These features are only suitable for the "healthy" lysosomes with low pH. Actually, in further biological study, we treated stained cells with heavy metal or induce cell apoptosis to simulate pathologic status. **Lyso-NIR** is proved to be stably localized in lysosome during these two processes. However, the commercial lyso-tracker **DND-99** shows evident change in fluorescence intensity in the same experiments. As is shown in Figure 6a, after incubated in 100 μM Hg^{2+} for 30 min, MCF-7 cells stained with **Lyso-NIR** maintain their original intensity.[21] But cells stained with **DND-99** almost lost their fluorescence completely (Figure 6b). During the other process, cell apoptosis induced by 10 μM dexamethasone for 30 min (Figure 6c), cells stained by **Lyso-NIR** keep their original fluorescence.[22] However, those stained by **DND-99** show a clear intensity decrease after the stimulation (Figure 6d). Therefore, under pathologic conditions, for example, when lysosomes are stimulated by various external stimulants, a pH-insensitive and stable targeting tracker is recommended for monitoring the lysosomal migrations.

Tracking Endocytosis Stimulated by Bacterium or Fluorescence Microsphere. Since **Lyso-NIR** could be stably localized in lysosome and maintain stable fluorescence, its tracking performance was further examined in endocytosis, one of the relatively long-term cellular processes. Changes of lysosome morphology during RAW 264.7 cells' endocytosing different bacteria and fluorescence microspheres are recorded and compared. One Gram-positive bacterium *Staphyloccocus aureus* (GFP expressed) and two Gram-negative bacteria, namely, *Escherichia coli* (*E. coli*) and *Salmonella* (prestain by FITC), are incubated with prestained RAW 264.7 cells for 7 h. First of all, lysosome in these RAW 264.7 cells exhibited bright fluorescence after 6 h of incubation. And we found bacteria swallowed by RAW 264.7 cells showed a different status: for *E. coli* and *Salmonella*, swallowed bacterium showed clear fluorescence overlay as yellow (Figure 7b,c), which means they were further swallowed by lysosome and were going to be degraded by RAW 264.7 cells. As for *S. aureus*, they could be easily swallowed by RAW 264.7 cells; however, all of those swallowed *S. aureus* distributed in cytoplasm other than lysosome. This can be determined by three-dimensional (3D) image in Figure 7. This is in accordance with former knowledge that *S. aureus* has a self-protecting ability that could get away with degradation by lysosome. As for fluorescence microspheres, they also can be swallowed by RAW 264.7 cells after 8 h of incubation.

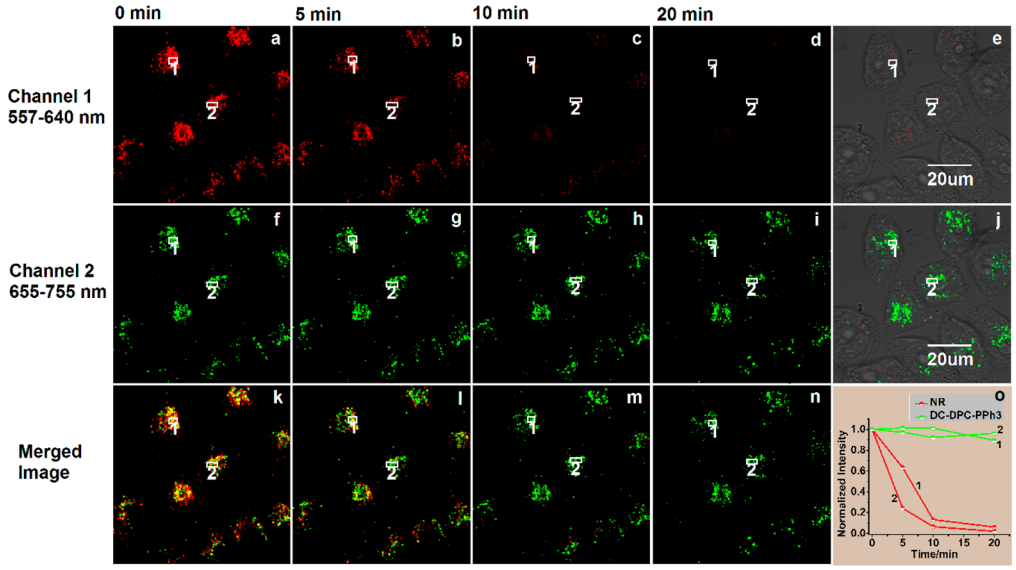

Figure 5. Fluorescence images of MCF-7 stained with **NR** (channel 1, pseudocolor red) and **Lyso-NIR** (channel 2, pseudocolor green) stimulated with chloroquine. (a–d) Images from channel 1 after chloroquine stimulation for 0, 5, 10, and 20 min; (e) overlay of d and brightfield. (f–i) Images from channel 2 after chloroquine stimulation for 0, 5, 10, and 20 min; (j) overlay of (i) and brightfield. (k–n) Merged images of channel 1 and channel 2. (o) Normalized fluorescence intensity change of ROIs from two channels, respectively.

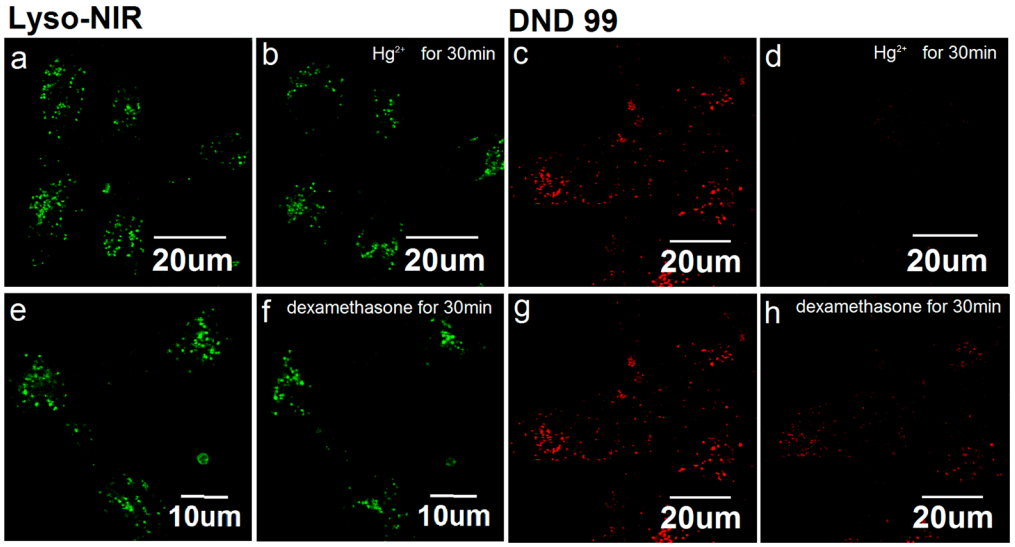

Figure 6. Fluorescence images of MCF-7 stained with **Lyso-NIR** (pseudo green) and **DND-99** (pseudo red) stimulated with 100 μM Hg^{2+} [(a, b) **Lyso-NIR**; (c, d) **DND-99**] and 10 μM dexamethasone [(e, f) **Lyso-NIR**; (g, h) **DND-99**].

This prestained polystyrene microsphere (green, λ_{em} 500–550 nm) has a diameter of ~2 μm, which is bigger than three bacteria. However, it is still swallowed by lysosome as shown in Figure 7d. Though we cannot distinguish whether it is swallowed by one single lysosome or by several lysosomes together, this foreign matter is on its way to degradation.

Muticolour Imaging Study. Further muticolour imaging study highlights the optical advantages: NIR emission and small fwhm. As shown in Figure 8, MCF-7 cells were costained with nucleic acid stain **SYTO Green** (Ex/Em 508/527 nm), mitochondrial tracker **TMRM** (Ex/Em 559/590 nm), and **Lyso-NIR**. Clear overlay images of nucleic acid, mitochondria, and lysosomes prove that **Lyso-NIR** can avoid the crosstalk with the emissions from the other two fluorescent probes.

The Cytotoxicity Study. Since low cell toxicity a key feature for living cell imaging, the cytotoxicity of **Lyso-NIR** is further determined by using the MTT test. Figure 9 has revealed that even incubated within a much higher concentration (10 μM) than optimized level for staining, 91% of MCF-7 cells survive after being incubated for 24 h. Most previous lysosomal probes are not recommended to incubate cells for longer time because of their strong cytotoxicity caused by the alkalizing effect from their various amino groups that help to target the acidic environment within lysosomes.[9] **Lyso-NIR** possesses no such basic groups, which partly explains its low cell toxicity. This is a favorable characteristic of a practical lysosomal probe for long-time physiological processes.

CONCLUSION

In summary, the NIR boron–dipyrromethene derivative **Lyso-NIR** specifically localizes in lysosomes through endocytosis pathway, and its strong NIR fluorescence in lysosomes is pH

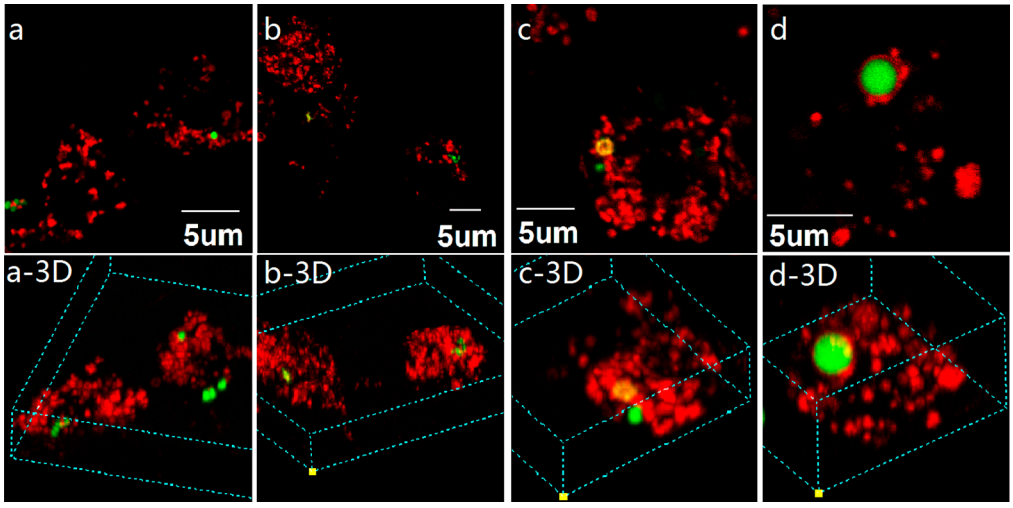

Figure 7. (a–d) Fluorescence images *S. aureus* (GFP expressed), *E. coli* (prestained by FITC), *Salmonella* (prestained by FITC), and fluorescence microspheres endocytosed by stained RAW 264.7 cells (**Lyso-NIR**, pseudocolor red). (lower) The 3D images corresponding to a–d.

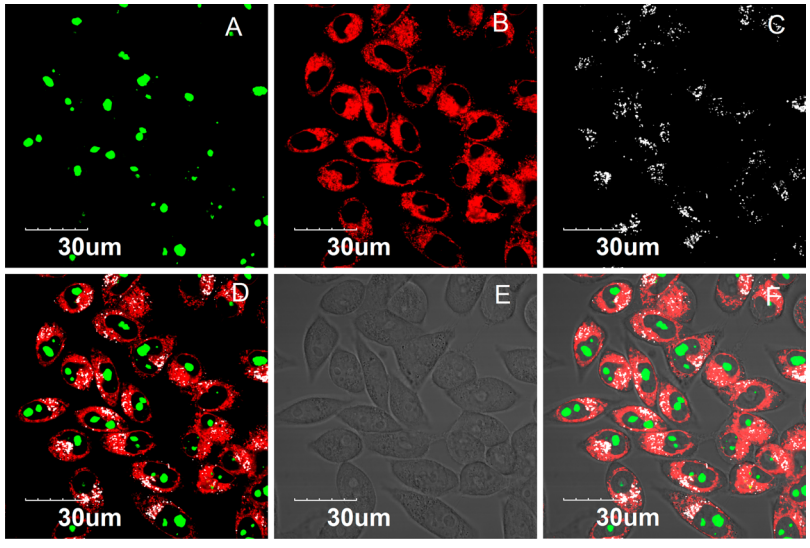

Figure 8. Multicolor imaging of living MC90 cells costained with **SYTO Green** (A, λ_{ex} = 488 nm, λ_{em} = 500–540 nm, pseudogreen), **TMRM** (B, λ_{ex} = 559 nm, λ_{em} = 560–610 nm, pseudored), and **Lyso-NIR** (C, λ_{ex} = 635 nm, λ_{em} = 655–755 nm, pseudowhite). (D) Overlay of (A–C). (E) Bright field. (F) Overlay of (D, E).

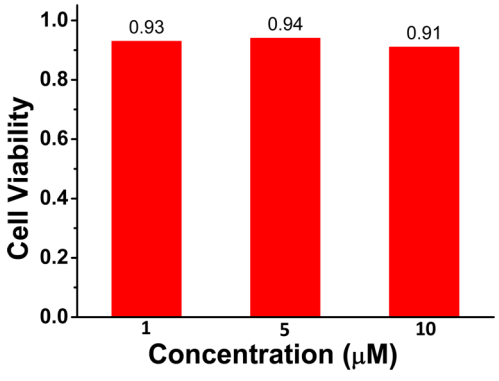

Figure 9. Viability of MCF-7 cells toward **Lyso-NIR** after treatment in different concentrations for 24 h.

independent. Regardless of the pH changes due to pathology or toxicology processes, **Lyso-NIR** firmly retains and intensively emits exclusively in lysosomes. This is completely different from the staining character of other common lysotrackers (e.g., **Neutral Red**, **DND 99**) that usually are driven out of lysosomes and have their fluorescence intensity significantly quenched, once the local pH raises. Thus, **Lyso-NIR** is recommendable for tracking lysosomes under different physiological status, apoptosis, or poisoning. Moreover, free from alkalizing effect suffered by most previous lysotrackers, **Lyso-NIR** shows low cytotoxicity, which is quite important to qualify a lysosomal tracker, especially for the monitoring of living cells for a long time. We have proved the applicability of **Lyso-NIR** through tracking the full processes of endocytosis stimulated by bacterium or fluorescence microsphere. These application studies demonstrated that its long-wavelength and narrow-band emission peaked at ~740 nm will avoid the spectral crosstalk with other probes emitting in the visible light region, qualifying **Lyso-NIR** as a standard colocalizing agent to estimate the other probes' location distributions. We expect that **Lyso-NIR** will become a valuable tool for fluorescent imaging and sensing in cell biology.

EXPERIMENTAL SECTION

Effects on Cell Growth/Viability. Cells are obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS). All cell lines are maintained under standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37 °C) in RPMI 1640 medium, supplemented with 10% fetal calf serum (FBS). The cytotoxic effects of **Lyso-NIR** are assessed using the MTT assay. Briefly, the cells in the exponential phase of growth are used in the experimentation. 1.5 × 10^3 cells/well are seeded onto 96-well plates and allowed to grow for 24 h prior to treatment with **Lyso-NIR**. The incubation time of **Lyso-NIR** is 24 h. At the end of this time, the **Lyso-NIR**-containing medium is replaced with phosphate-buffered saline (PBS), and MTT is then added to each well (final concentration = 0.5 mg/mL) for 4 h at 37 °C, and formazan crystals formed through MTT metabolism by viable cells are dissolved in dimethyl sulfoxide. Optical densities are measured at 570 nm using a Microplate Reader AC100−120 (TECAN). IC50 values (concentrations reducing the cell survival fraction by 50%) are obtained by nonlinear regression analysis, using Origin 8.5.

Culture of Cells and Fluorescent Imaging. Cells grew in the exponential phase of growth on 35 mm glass bottom culture dishes (Φ 20 mm) for 1−2 d to reach 70−90% confluency. These cells were used in colocalization experimentation. The cells were washed three times with RPMI 1640 and then were incubated with 2 mL of RPMI 1640 containing **Lyso-NIR** (37.5 nM) and **Rh-123**, **DND-99**, or **Neutral Red** (25 nM) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Cells were washed twice with 1 mL of PBS at room temperature (RT), then 1 mL of RPMI 1640 culture medium was added; observations were made under a confocal microscopy (Olympus FV1000).

Photostability. Photofading of dyes (**Lyso-NIR**, **NR**, and **DND-99**) in living cells with irradiation by semiconductor laser in a confocal fluorescence microscope. Excitation wavelength is 635 nm for **Lyso-NIR** and 559 nm for **NR** and **DND-99**, and the intensity at the sample is approximately 0.5 mW. Another group of photodecomposition experiments compared with that of the well-known NIR fluorophores Cy5 and Cy7 was conducted in solution. The solutions are irradiated under a 500 W iodine−tungsten lamp for 450 min at a distance of 250 mm. An aqueous solution of sodium nitrite (50.0 g/L) is placed between the samples and the lamp as a light filter (to cut off the light shorter than 400 nm) and heat filter. The photostabilities are expressed in the terms of remaining fluorescence calculated from the changes of fluorescence at the fluorescence maximum before and after irradiation by iodine−tungsten lamp.

Determination of the Partition Coefficient. A shake flask ultraviolan spectrophotometry method is used to determine *n*-octanol/water partition coeffiecent of **Lyso-NIR**. First, shake the mixed solution of 100 mL of *n*-octanol and 100 mL of water at 37 °C for 48 h. Prepare **Lyso-NIR** standard solution (C = 5 μM) by using the water phase and organic phase. Second, prepare **Lyso-NIR** detecting solution (C = 5 μM) with mixed solvent of 50 mL of *n*-octanol and 50 mL of water, and shake it for 48 h. Third, determine the concentration of **Lyso-NIR** in the water phase (C_w) and organic phase (C_o) of the detecting solution separately by using ultraviolan spectrophotometry. The *n*-octanol/water partition coeffiecent, namely, log *P*, is caculated by the following equation:

$$\log P = C_o/C_w$$

The Synthesis of Lyso-NIR. Under a nitrogen atmosphere, $CuSO_4 \cdot 5H_2O$ (5.6 mg, 0.024 mmol) in H_2O (2 mL) and Na ascorbate (8.4 mg, 0.044 mmol) in H_2O (2 mL) followed by *N*,*N*-diisopropylethylamine (DIPEA) (60 μL, 0.35 mmol) were added to a solution of (3-azidopropyl)triphenylphosphonium bromide (90 mg, 0.21 mmol) in EtOH (10 mL). Then a solution of **DC-DPC** (82 mg, 0.086 mmol) in toluene (30 mL) was slowly added dropwise to the mixture in the absence of light. The reaction mixture was stirred in the dark for 18 h at RT. Still in the absence of light, the solvent was evaporated under vacuum, and the product was purified by column chromatography. (SiO_2, alcohol/CH_2Cl_2, 1:20). After evaporation of the solvent, **Lyso-NIR** (108.3 mg, 70%) was obtained as dark brown solid. mp 174−176 °C. ^{1}H NMR (400 MHz, CDCl3) δ 8.43 (s, 1H), 8.24 (d, *J* = 16.0 Hz, 1H), 8.16 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.30−7.82 (m, 20H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.09 (d, 1H), 5.56 (s, 2H), 4.80 (s, 2H), 3.77 (s, 2H), 2.15 (s, 2H), 1.47 (s, 3H). ^{13}C NMR (100 MHz, CDCl3) δ 141.0, 140.7, 140.6, 138.5, 137.8, 135.1, 133.7, 133.6, 131.1, 130.6, 130.5, 128.5, 126.6, 123.5, 122.9, 121,4, 120.4, 120.1, 118.1, 117.3, 95.3, 38.57, 29.7, 24.3, 12.1. *m*/*z* (time-of-flight electrospray mass spectrometry): Calcd [M-2Br]+ for $C_{93}H_{76}BN_{10}F_2P_2Cl_2I$: 1640.4213, found: 1640.4224.

ASSOCIATED CONTENT

Supporting Information

Experimental methods, polar sensitivity measurements, fluorescence imaging, biological study of **Lyso-NIR**, ^{1}H and ^{13}C spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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