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Scavenger receptor-recognized and enzyme-responsive nanoprobe for fluorescent labeling of lysosomes in live cells

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ABSTRACT

Lysosomal imaging represents a potent tool for investigating the organization of related cellular events and their modulation *via* diagnostic and therapeutic approaches. However, specific labeling of the lysosome in live cells is a significant challenge. Taking advantage of the inherent lysosomal entry of nanoparticles and unique digestive inclusions in the lysosome, we developed a nanoparticle-based, enzyme-switchable fluorescence OFF-ON strategy for specific labeling of the lysosome and further imaging of extracellular acidification-induced lysosome trafficking in living cells. The nanoprobe comprised a 16 nm spherical gold nanoparticle as the core and an enzyme-responsive oligomer of fluorescein-conjugated oligo(4-vinyl-phenyl phosphate) as the shell. Due to quenching of the core gold nanoparticle, the nanoprobe was non-fluorescent. After incubation with cancer cells, the nanoprobe was rapidly internalized *via* scavenger receptor-mediated endocytosis and significantly shuffled into the lysosome. The nanoprobe specifically lighted up the lysosome owing to lysosome-induced fluorescence enhancement. Specifically, digestive inclusions in the lysosome hydrolyzed and released gold-quenched fluorescein molecules, leading to significant augmentation of fluorescence. On account of specific lysosomal labeling, the nanoprobe effectively facilitated imaging of a 4–6 μm anterograde trafficking event of the lysosome from the perinuclear region to the cell surface when an acidic extracellular environment developed. Our findings collectively highlight the use of nanoprobes for lysosomal imaging.

1. Introduction

Lysosomes are key intracellular organelles that comprise single membrane-bound vesicles containing multiple digestive enzymes for degradation of both exogenic and endogenic molecules [1]. Abnormal regulation of the lysosome has significant implications in human diseases, including tumors, inflammation, neurodegeneration and various lysosomal storage disorders [2,3]. In particular, during tumor development, the lysosome travels to membrane protrusions, which is believed to facilitate the invasion and metastasis of cancer cells [4–7]. Therefore, visualization of the lysosome and lysosomal trafficking events may represent a potential methodology for effective diagnosis of human diseases and evaluation of therapeutic outcomes.

To date, several chemical and biological probes have been used for lysosomal labeling and trafficking studies. Typically, lysosomal labels, including the popular Lyso Tracker® probes, are weak amines that selectively accumulate in acidic organelles [8–10]. However, these recognition and labeling processes are significantly disrupted by the endosome, another prevalent acidic cellular compartment in the cytosol, resulting in strong background labeling [11]. An alternative strategy for lysosomal labeling is to use biological probes targeted to the lysosomal-associated membrane protein (LAMP). For example, LAMP antibodies tagged with fluorophores bind to and label the lysosome based on the immunoreaction between the probes and protein markers [12–14]. However, LAMP is reported to localize in both endosomes and lysosomes, resulting in non-specific labeling [15,16]. More recently, luminescent nanoparticles have been exploited as potential lysosomal staining agents, based on the finding that nanoparticles can be endocytosed by live cells, usually culminating in endo/lysosomal internalization [17,18]. Due to permanent luminescence, however, luminescent nanoparticles lead to visualization of all the cellular compartments associated with endocytosis, including primary cellular vacuoles and endosomes, and thus provide non-specific labeling [18]. Specific labeling of the lysosome in live cells remains a significant challenge.

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In the present study, we aim at designing and preparing a lysosome-specific probe for lysosomal labeling and further imaging of extracellular acidification-induced lysosome trafficking in live cells. The probe refers to a nanoparticle-based and enzyme-responsive strategy exploiting the inherent lysosomal entry trend of nanoparticles and unique digestive inclusions in the lysosome. On the basis of specific lysosomal labeling, lysosome-to-nuclear center distances will be measured for quantification of the extracellular acidification-induced lysosome trafficking.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$, 99.9%), sodium citrate tribasic dihydrate (99.9%), anhydrous acetonitrile (99.8%), 4-acetoxystyrene (96%), porcine liver easterase (10 mg/mL in 3.2 M $(NH_4)_2SO_4$ stock solution), bromotrimethylsilane (97%), carbon tetrachloride (99.9%), anhydrous $CDCl_3$ (99.8%), 2, 4, 6-collidine (99%), dimethyl aminopyridine (99%), dibenzyl phosphite (95%), 5(6)-carboxyl fluorescein (>95%), N,N-diisopropylethylamine (98%), polyinosinic acid (Poly-I), polycytidylic acid (poly-C), chlorpromazine hydrochloride (CPM, 98%), sodium azide (BioXtra), 3-methyladenine (3-MA), filipin (FPN, form *Streptomyces filipinensis*, >85%) and cytochalasin D (Cyto D, from *Zygosporium mansonii*, >98%) were purchased from Aldrich. The cell lines DU145 (human prostate cancer), MCF-7 (human breast cancer) and Hela (human cervical cancer) were purchased from Shanghai Institutes for Biological Sciences (SIBS) of Chinese Academy of Sciences (CAS). Lyso-tracker Deep Red (Life Technologies, China), pHrodo™ Red Transferrin Conjugate (Life Technologies, China) and Hoechst 33258 (Beyotime Institute of Biotechnology, China) were used as purchased.

2.2. Monomer and oligomer synthesis

2.2.1. Monomer synthesis

A flask was charged with 4-acetoxystyrene (10 g, 61.73 mmol), tetrahydrofuran (THF, 25 mL), and KOH (0.125 g, 2.23 mmol) in distilled water (100 μL). After the mixture was stirred for 5 min under N_2, the temperature was raised to 65 °C. The mixture was stirred for a further 4 h and cooled to room temperature. Acetic acid (0.144 g, 2.44 mmol) in tetrahydrofuran (0.5 mL) was added slowly over 5 min, followed by stirring for another 5 min and concentration of the mixture using a rotary evaporator [19]. The residue was dissolved in toluene and filtered, followed by cooling to −78 °C. Finally, 4-hydroxystyrene was precipitated, filtered and dried to generate ca. 4.0 g product with a yield of 53%. ^{1}H NMR, δ (500 MHz, CD_4O, ppm): 7.36–7.17 (m), 6.81–6.69 (m), 6.63 (dd, J = 17.6, 10.9 Hz), 5.56 (dd, J = 17.6, 1.1 Hz), 5.03 (dd, J = 10.9, 1.1 Hz).

In the second step, dimethyl aminopyridine (DMAP, 148 mg, 1.21 mmol) was added to 4-hydroxystyrene (1.45 g, 12.1 mmol) in anhydrous acetonitrile (25 mL). The solution was cooled to −10 °C, followed by the addition of CCl_4 (5.86 mL, 60.35 mmol) and N,N-diisopropylethylamine (DIPEA, 4.42 mL, 25.4 mmol). The cold solution became cloudy and was further stirred for 30 min. After the addition of dibenzyl phosphite (4.00 mL, 18.1 mmol), the reaction became clear and was allowed to slowly warm to room temperature. The reaction mixture displayed a slightly yellow color and was stirred overnight [20]. Following quenching with Na_2HPO_4 (50 mL, 0.5 M) and extraction with ethyl acetate (EA, 3 × 30 mL), the organic phase was dried with magnesium sulfate and the solvent was removed under reduced pressure, yielding dibenzyl-4-vinylphenyl phosphate as a colorless oil (yield was 2.3 g, 56%). ^{1}H NMR, δ (500 MHz, $CDCl_3$, ppm): 7.24 (s), 7.09 (s), 6.65 (d, J = 28.5 Hz), 5.66 (d, J = 17.6 Hz), 5.22 (d, J = 10.9 Hz), 5.11 (d, J = 8.4 Hz). ^{13}C NMR, δ (500 MHz, $CDCl_3$, ppm): 150.07 (s), 135.68 (s), 134.63 (s), 128.64 (s), 127.98 (d, J = 10.4 Hz), 127.36 (s), 120.05 (s), 113.78 (s), 69.94 (s). ^{31}P NMR, δ (500 MHz, $CDCl_3$, ppm): −5.56 (p, J = 8.3 Hz).

2.2.2. Oligomer synthesis

2.2.2.1. Synthesis of oligomer 3. Oligomer **3** in Fig. 2 was synthesized as follows: a 2 mL ampoule with a stir bar was filled with synthetic RAFT CTA **2** (6.6 mg, 29.25 μmol), azodiisobutyronitrile (AIBN, 1.0 mg, 6 μmol) and dibenzyl-4-vinylphenyl phosphate (1.1119 g, 2.925 mmol) in dioxane (1.2 mL). Oxygen was removed *via* three freeze-pump-thaw cycles, and the ampoule was flame-sealed and heated at 70 °C for 8 h. The ampoule was opened, and the reaction mixture diluted with dichloromethane (2 mL) and precipitated in cold ether (20 mL). Precipitation was repeated three times, yielding Oligomer **3** as a white powder (yield: 112 mg, 10%). ^{1}H NMR, δ (500 MHz, $CDCl_3$, ppm): 7.19 (s), 6.85 (s), 6.37 (s), 4.98 (s), 3.48 (q,

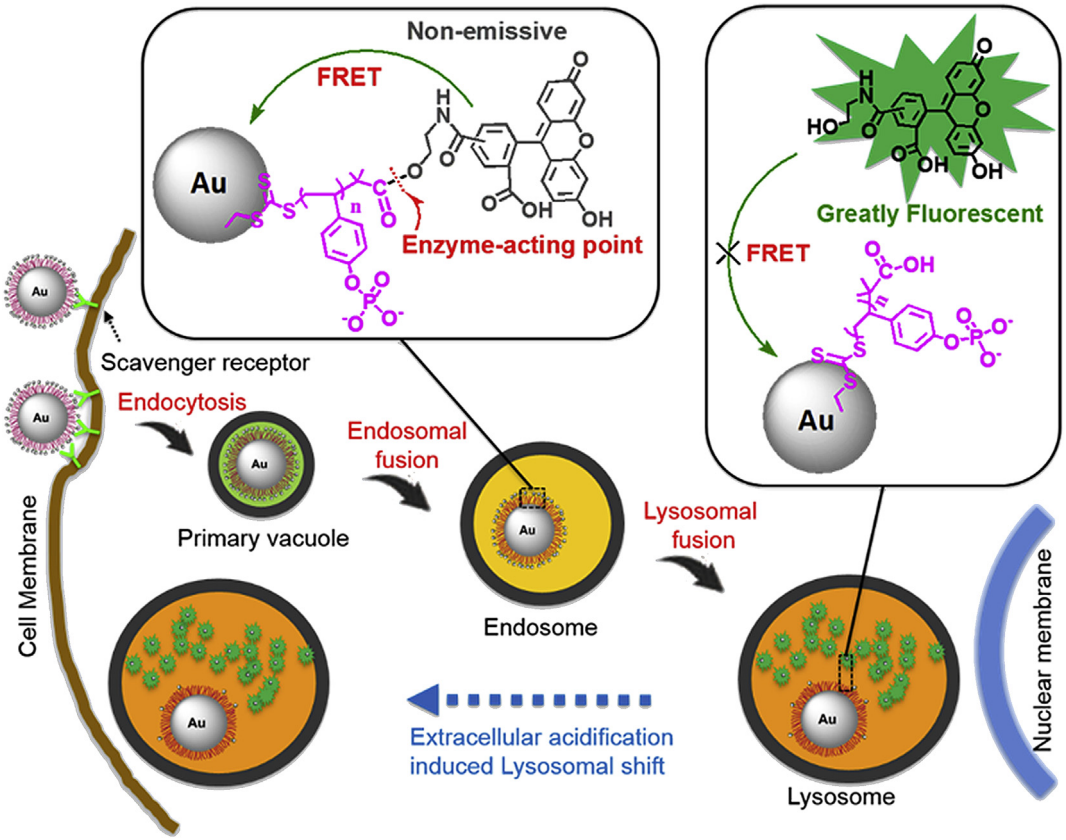

Fig. 1. Illustration of the scavenger receptor-recognized, enzyme-switchable fluorescence OFF-ON nanoprobe entering a cell and specifically lighting up the lysosome (indicated by solid black arrows). The nanoprobe facilitated imaging of an extracellular acidification-induced lysosomal trafficking event from the perinuclear region to the cell periphery (indicated by the blue dashed arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)