Ultrabright and ultrastable near-infrared dye nanoparticles for in vitro and in vivo bioimaging

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ABSTRACT

We report a new strategy of using carrier-free pure near-infrared (NIR) dye nanoparticles (NPs) to achieve highly luminescent NIR fluorescent probes for in vitro and in vivo imaging. Bis(4-(N-(2-naphthyl) phenylamino) phenyl)-fumaronitrile (NAPFP) NPs are shown to exhibit favorable biocompatibility, wide-range pH stability (pH 4–10) and much more superior photostability than conventional dyes. Importantly, the combined merits of high dye loading content and aggregation-induced emission enhancement properties, endow the NIR probes with high brightness and a high quantum yield up to 14.9%. The NAPFP NPs can be readily conjugated with folic acid for targeted in vitro cell imaging. Applications of the NPs probes in high efficiency in vivo and ex vivo imaging were successfully demonstrated. Intense fluorescent signals of NAPFP NPs can be distinctly, selectively and spatially resolved in tumor sites with ultrahigh sensitivity, even with 5 ms exposure time, due to the preferentially accumulation of NPs in tumor sites through passive enhanced permeability and retention effect. The totality of results clearly demonstrate the exciting potential of the functionalized NAPFP NPs as a NIR fluorescent probe for in vitro and in vivo imaging and diagnostics.

1. Introduction

Fluorescence cellular probes with near-infrared (NIR, 650–900 nm) emission are highly desirable for sensitive early cancer detection, because biological media show very low absorption and autofluorescence background in the NIR region [1–4]. Typical materials used for these NIR probes mainly include inorganic nanoparticles (NPs)/quantum dots (QDs), upconversion nanoparticles (UCNP) and organic dyes [5]. Usage of inorganic NPs/ QDs are often limited due to the possible release of cytotoxic heavy metals to biological systems [6]. Besides the concern of long-term toxicity, the low quantum yield and thus limited brightness of UCNP further restricted their applications in sensitive imaging [7]. Organic dyes are the most widely used NIR fluorescent markers for biological imaging, however, the problems of intrinsic hydrophobicity and instability in bio-environment place great restrictions for utilization [8]. To cope with these problems, various nanocarriers such as polymer or inorganic matrix-based particles have been used, where NIR dyes are covalently linked or physically entrapped [9]. While these carriers do offer effective solutions for the dispersibility and the stability problems, such carrier-based systems however often face the dilemma of either low brightness at low dye concentrations versus low fluorescence quantum yield (QY) at high dye concentrations due to aggregation-induced quenching (AIQ) [10]. Such AIQ effect is especially serious for NIR dyes, because they usually have much larger π–π conjugation systems, which makes the dyes more flopply and enhance AIQ. Thus the NIR dye loading content is usually very limited in conventional nanocarriers. However, it should be noted that useful fluorescence brightness depends not only on the QY but also on the absorptivity. High QY but low absorption cannot yield optimum brightness. Since, absorption is normally proportional to the number of the dyes, thus high dye loading content would definitely be rewarding for developing high-brightness probes, provided that the QY is not seriously affected by the AIQ effect.

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Herein, we report a new strategy of using carrier-free pure NIR dye NPs with the characteristics of aggregation-induced emission enhancement (AIEE) to achieve highly bright NIR fluorescent probes. NIR dye bis(4-(N-(2-naphthyl)phenylamino)phenyl)fumaronitrile (NPAPF) were first prepared into NPs and then followed by surface modification with poly(ethylene glycol) (PEG) (Fig. 1a). The modified NPs possess favorable biocompatibility, robust pH stability covering wide pH 4–10, high brightness with high QY up to 14.9%, as well as much more superior photostability than conventional dyes. Applications of the excellent NIR NPs for targeted in vitro and in vivo imaging were demonstrated. The fluorescent signals of NPAPF-PEG NPs can be distinctly, selectively and spatially resolved in tumor site with ultrahigh sensitivity even with exposure time reduced to 5 ms. It is noted that images with such high sensitivity have seldom been reported before for traditional fluorescence probes with such emission wavelength for in vivo imaging. These results clearly demonstrate the exciting potential of the functionalized NPAPF NPs as a NIR fluorescent probe for in vitro targeting and in vivo imaging and diagnostics.

2. Experimental section

2.1. Materials

4-bromophenylacetonitrile, N-phenyl-substituted amine, Pd(OAc)2, Cs2CO3 and P(t-Bu)3 were purchased from J&K Scientific Ltd. Triethylamine, toluene, iodine, diethyl ether, sodium, methanol, chloroform, methanol, dichloromethane and petroleum were purchased from Sinopharm Chemical Reagent Co., Ltd. Fetal bovine serum (FBS), Roswell Park Memorial Institute-1640 (RPMI-1640) medium, folic acid (FA)-free RPMI-1640 and Penicillin-streptomycin solution were purchased from

![Diagram](image-url)

**Fig. 1.** (a) Schematic illustration of preparation and functionalization of NPAPF NPs, and the concept of in vivo imaging (b) SEM image (Scale bar 1 μm) and (c) UV–vis absorption spectrum of NPAPF NPs and photoluminescence (PL) spectra of NPAPF NPs and in THF solution, respectively (d) Stability studies of NPAPF-PEG NPs treated with PBS, serum, and pH 4 to 10 solutions (e) Cell viability of cells incubated with different concentration of NPAPF NPs for 24 h using MTT assay.
invitrogen (San Diego, CA). 1-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma Aldrich (Milwaukee, WI). Distilled water was obtained from a Milli-Q Biocel (Millipore Corporation, Breford, USA) water purification system (18.2 MΩ cm resistivity). A human nasopharyngeal epidermal carcinoma cell line (KB cell) was provided by American Type Culture Collection (ATCC). Scanning electron microscopic (SEM) images were obtained on a FEI Quanta 200 FEG field emission scanning electron microscope operated at an accelerating voltage of 30 kV. Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) with a 633 nm He-Ne laser.

2.2. Synthesis of NPAPF

Bis(4-bromophenyl) fumaronitrile was synthesized following the literature procedure [11]. A mixture of 4-bromophenylacetonitrile (4.86 g, 24.8 mmol) and iodine (6.35 g, 25 mmol) was purged with N2 and dry diethyl ether (100 mL) was added. A mixture of bis(4-bromophenyl)fumaronitrile (0.787 g, 2.03 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) (1.4 g, 7.5 mmol) and 4-dimethylaminopyridine (NHS) (1.3 mg, 13.6 mmol) was dissolved in water, which was dialyzed for 2 days in a dialysis bag with molecular weight cut-off (MWCO) of 14 kDa to remove unreacted EDC/NHS. After lyophilization, the final product in a white solid was stored at 20 °C for future use.

2.3. Synthesis of poly(maleic anhydride-alt-1-octadecene)-poly(ethylene glycol) (C18PMH-PEG)

C18PMH-PEG was synthesized following the literature procedure [12]. Briefly, 10 mg (1 eq) of Poly(maleic anhydride-alt-1-octadecene) (C18PMH, Sigma Aldrich) and 143 mg (1 eq) of mPEG-NH2 (5 K) (PegBio, Suzhou, China) were dissolved in 5 mL of dichloromethane with 6 mL triethylamine (TEA, Sinopharm Chemical Reagent Co.) were mixed in dichloromethane under agitation to form a homogeneous solution. EDC (2eq) and TFA (3eq) were then added under magnetic stirring. After stirring for 24 h at room temperature, the dichloromethane solvent was blown dry by N2, the leftover solid was dissolved in water, forming a transparent clear solution, which was dialyzed against distilled water for 2 days in a dialysis bag with molecular weight cut-off (MWCO) of 14 kDa to remove unreacted mPEG-NH2. After lyophilization, the final product in a white solid was stored at 20 °C for future use.

2.4. Synthesis of folic acid conjugated C18PMH-PEG (C18PMH-PEG-FA)

C18PMH (1eq), mPEG-NH2 (5k) (1eq) and NH2-mPEG(5k)-BOC (Polymer, Germany) (0.5eq) were mixed in dichloromethane under agitation to form a homogeneous solution. EDC (2eq) and TFA (3eq) were then added under magnetic stirring. After stirring for 24 h at room temperature, the dichloromethane solvent was blown dry by N2. Subsequently, 2 mL of trifluoroacetic acid (TFA, Sinopharm Chemical Reagent Co.) was added under magnetic stirring for 3 h at room temperature to protect the Boc group. After evaporating the TFA solvent, the leftover solid was dissolved in water, which was dialyzed for 2 days in a dialysis bag (MWCO – 14 kDa) to remove unreacted PEG polymers and other reagents. After lyophilization, the final product (C18PMH-PEG-NH2) in white solid was stored at -20 °C for future use.

The folic acid conjugated C18PMH-PEG was prepared by conjugating the amine-functionalized C18PMH-PEG-NH2 with activated FA. Briefly, 35 mg of FA was mixed with 15 mg EDC and 23 mg NHS in 5 mL anhydrous dimethyl sulfoxide (DMSO, Sigma–Aldrich) for 15 min at room temperature. 20 mg of C18PMH-PEG-NH2 in 5 mL DMSO was added afterwards (molar ratio of NH2/FA/EDC/NHS = 1:2:2.5). After reaction at room temperature under stirring for 8 h, water was added and the product was purified by dialysis. The final product was lyophilized and stored at -20 °C until use.

2.5. Preparation and functionalization of NPAPF NPs

NPAPF NPs were prepared by a solvent exchange method. Briefly, 200 mL of 1 x 10-8 M NPAPF/THF solution was dropped into 10 mL of aqueous solution by microsyringe at room temperature under vigorous stirring at 1000 rpm. After mixing for 5 min, the sample was stabilized for 72 h. 1 mg C18PMH-PEG or C18PMH-PEG-FA polymer was dispersed completely in 10 mL distilled water for further use. PEG is an effective building block that has been widely integrated into biomaterials to suppress nonspecific adsorption of biological substances and to provide excellent long-term stability in high salt concentrations and pH extremes. Conjugation and alteration of the head groups of PEG derivatives allows selective attachment to nanoparticles surfaces, and enables further bio-functionalization. For functionalization of NPAPF NPs, 300 mL of C18PMH-PEG/H2O (C18PMH-PEG-FA/H2O) was added to 10 mL of NPAPF NPs solutions, and the mixture was then subjected to ultrasonic treatment for 5 min, and then stored for 2 h. The nanoparticles size was measured by DLS at 25 °C. The data reported represented an average of five measurements with ten scans each.

2.6. Cell culture

A549 murine breast cancer cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured at 37 °C, 5% CO2 in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin solution. The cells were pre-cultured prior to experiments until confluence was reached. KB cells were cultured in a normal RPMI-1640 and FA-free RPMI-1640 medium supplemented with 10% FBS and 1% (v/v) penicillin/streptomycin solution at 37 °C in a humidified atmosphere containing 5% CO2. The concentration of FA in serum-containing FA-free medium was only 3 nM as opposed to 2.26 μM under normal culture conditions. Cells were routinely passaged by treatment with trypsin (0.05%)/EDTA.

2.7. In vitro cytotoxicity

0.1 mL complete medium with A549 cells was seeded in 96-well plates (60,000 wells) and incubated for 24 h. Then, 100 μL sterilized NPAPF NPs with different concentrations in phosphate buffered saline (PBS) buffer were added to each well for incubation (37 °C, 5% CO2) for 24 h. The cells were then treated with 20 μL of MTT solution (5 mg/mL in PBS) and incubated for 5 h. The medium was removed and the cells were lysed by adding 150 μL of DMSO, cell viabilities were then measured by MTT assay.

2.8. Blood circulation

Blood circulation was measured from approximately 10 μL blood from the tail vein of nude mice post injection of NPAPF NPs-PEG. Each blood sample was dissolved in 1 mL of lysis buffer (1%SDS, 1% Triton X-100, 40 mM Tris Acetate). The concentration of NPAPF NPs-PEG in the blood was determined by the fluorescence spectrum of each solubilized blood sample using a FluoroMax 4 fluorometer (HORIBA Jobin Yvon, France). A series of dilution of the NPAPF NPs-PEG solution was measured to obtain a standard calibration curve. Blank blood sample without NPAPF NPs-PEG injection was measured to determine the blood autofluorescence level, which was subtracted from the fluorescence intensities of injected samples for the concentration calculation.

2.9. In vivo NIR fluorescence imaging

Tumor-bearing mice were intravenously injected with 200 μL of 93 μM NPAPF NPs-PEG and imaged using a Maestro in vivo fluorescence imaging system (CRi Inc.). Blue light with a peak wavelength at 455 nm was used as the excitation source. In vivo spectral imaging from 600 nm to 750 nm (10 nm step) was carried out with various exposure times for each image frame. Autofluorescence (particularly from food residues in the stomach and intestine) was decreased by exposure time.

2.10. Biodistribution

We sacrificed A549 bearing Balb/c mice after injection and at 1, 2, 6, 12, 24 and 48 h post injection. Various organs and tissues were spectrally imaged by the Maestro system. The concentration of NPAPF NPs-PEG fluorescent intensity of each imaged organ (after removing the tissue autofluorescence and subtracting the background, if any, of each organ before NPAPF NPs-PEG injection) was calculated for a semi-quantitative biodistribution analysis.

3. Results and discussion

3.1. Synthesis and characterization of NPAPF NPs

NPAPF NPs were prepared by a solvent exchange method. Fig. 1b is a typical SEM image of the as-prepared particles, which shows roughly spherical shape and the size distribution in the range of 60–100 nm. DLS studies show that the particles have good dispersibility with an average diameter of about 70 nm. It should be noted that such nanoparticles with size between 30 and 150 nm are particularly useful owing to enhanced bioavailability and more effective EPR effect [13,14].
3.2. Optical properties of NPAPF NPs

We studied the optical properties of the as-prepared NPs, as shown in Fig. 1c. NPAPF was weakly fluorescent when dissolved in THF, whereas the NPs show almost 10 times enhancement in fluorescence intensity, due to the aggregation-induced restriction of intramolecular rotation and consequential suppression of non-radiative pathways [15]. NPAPF NPs exhibit intense near-infrared (NIR) fluorescence with emission peak at 650 nm, which would have deeper penetration into biological tissues and reduce optical interference like autofluorescence and light scattering [16]. The QY of NPAPF NPs in water was measured to be as high as 14.9%, the remarkably high QY should benefit from the close integration of molecules in the NPs. It should be noted that no other additional inert agents were added as the dye-carriers, which substantially increases dyes loading content. Combined with the high dye loading capacity and high QY, NPAPF NPs would exhibit heavy NIR emission, which are quite desirable for high sensitive in vivo imaging. Another merit of the NPs is the large Stokes shift of 175 nm, as shown in Fig. 1c, i.e. absorption/excitation at 475 nm versus emission at 650 nm. The large Stokes shift would increase the signal-to-background ratios (sensitivity) via reducing the autofluorescence of the bio-substrate and interferences between excitation and emission [17]. All these features endow the NPs with superior advantages as a potential probe especially for in vivo imaging.

3.3. Surface functionalization of NPAPF NPs

An essential requirement for a qualified bioprobes for in vivo applications is water-dispersibility and bio-environmental stability [18,19]. Surface functionalization was then performed by putting a small amount of C18PMH-PEG/H2O solution into above-prepared NPAPF NPs solution, followed by ultrasonication for further mixing. Since surface of the organic NPs is quite hydrophobic, it is possible that C18PMH-PEG would be readily anchored to the surface of the NPAPF NPs through hydrophobic interactions [20]. As expected, the functionalized NPs show a small swelling in diameter as indicated by DLS measurement. Stability of the functionalized NPs (hereafter referred as NPAPF-PEG NPs) in various bio-environments was also studied. Size and fluorescence intensity change of the NPAPF-PEG NPs in different environments such as PBS, Serum and pH 4 to 10 solutions were examined. As shown in Fig. 1d, the NPAPF-PEG NPs preserve very stable size over wide pH range of 4–10. Even after 48 h, no obvious changes could be detected. In striking contrast, non-functionalized NPs show continuous size increase with time in solutions with different pH values, due to destruction of surface

![Fig. 2. Photostability comparison of fluorescence signals of KB cells labeled by FITC and NPAPF NPs, captured by laser-scanning confocal microscopy. KB cells are distinctively labeled by the NPAPF (red) and FITC (green). The concentration of NPs and FITC were both 2 μM; Scale bars are 5 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)

![Fig. 3. Confocal laser-scanning fluorescent microscopy images of (a) KB positive cells incubated with NPAPF-PEG-FA NPs; (b) KB positive cells incubated with NPAPF-PEG NPs; (c) KB negative cells incubated with NPAPF-PEG-FA NPs; (d) KB negative cells incubated with NPAPF-PEG NPs; (e) KB positive cells incubation FA, then incubated with NPAPF-PEG-FA NPs. The concentration of NPs was 1 μM in all samples. Scale bars are 20 μm.](image)
charge by zwitterions. Such great improvement of bio-environmental stability is attributable to the successful surface modification, which makes the in vitro and in vivo bioimaging possible.

3.4. Cytotoxicity of NPAPF NPs

Biocompatibility of the NPs is a critical requirement for future biomedical applications, especially in clinic [21]. We therefore performed cytotoxicity analysis by measuring cell viability with a colorimetric MTT assay. When KB cells were incubated with the NPs, low cytotoxicity with high cell viability (>85%) was measured after 48 h incubation with NPs of different concentrations from 2.5 to 20 μM as shown in Fig. 1e, indicating that the NPs had little interference with the cell physiology and proliferation.

3.5. Photostability of the NPAPF-PEG NPs

We further evaluated the photostability of the NPAPF-PEG NPs. It was found that the NPAPF-PEG NPs show superior photostability compared to fluorescein isothiocyanate (FITC) dye, which is one of the most stable organic dyes commonly used in bioimaging [22]. Because FITC and NPAPF-PEG NPs have the same excitation wavelength, we can compare their photostability under the same conditions. As shown in Fig. 2, when KB cells were labeled with the same concentration of FITC and NPAPF-PEG NPs, under illumination at 488 nm, the fluorescence of FITC rapidly diminished and became negligible after 2 min, due to severe photo-bleaching. In sharp contrast, the fluorescence of the NPs was remarkably stable and maintained less than 10% intensity decrease even after 30 min. We attribute such remarkable photostability to the combination effects of the unique PL properties of the NPAPF NPs and protection from the anchored surfactants and outer organic molecules on the NPs [23]. The above comparison shows the superior photostability of the red-emitting NPAPF-PEG NPs, which enables long-term and real-time bioimaging applications.

3.6. NPAPF-PEG NPs for targeted in vitro cell imaging

The water-dispersible, highly photo- and pH-stable, strongly NIR fluorescent NPAPF NPs are further explored as biological fluorescent probes for in vitro and in vivo bioimaging. It is well known that targeted cell imaging is highly desirable for early stage cancer diagnosis [24]. To realize FA-mediated targeted cell imaging, we conjugated FA to PEG terminals of C18PMH-PEG polymer and used the obtained C18PMH-PEG-FA to functionalize NPAPF NPs (hereafter referred as NPAPF-PEG-FA NPs). As expected, C18PMH-PEG-FA modified NPs also showed good water dispersibility and bio-environmental stability. Folate receptor (FR) is an attractive cancer cell probe that is over-expressed in many types of cancer cells [25]. KB cells cultured in FA-free medium with high FR expression were used as FR-positive [FR(+)] cells, while cells cultured in normal medium with low FR expression were used as FR-negative [FR(−)] cells. Both FR-negative and FR-positive cells were incubated with NPAPF-PEG NPs and NPAPF-PEG-FA NPs respectively at 37 °C for 2 h, and then stained with Hoechst (33258) for confocal imaging. It was found that intense red fluorescence only appeared in FR(+) KB cells cultured with NPAPF-PEG-FA NPs (Fig. 3a) and most of the NPs were effectively taken up by cells surrounding the cellular nucleus. No obvious signal was detected for FR(−) KB after incubation with NPAPF-PEG NPs (Fig. 3b), or for FR(−) KB cells after incubation with NPAPF-PEG NPs (Fig. 3c) or NPAPF-PEG-FA NPs (Fig. 3d). To further demonstrate the specificity of FR-mediated targeting, we first blocked FR receptors on KB cells.

![Fig. 4](image-url)
by adding excessive free FA prior to cell incubation with NPAPF-PEG-FA NPs, and observed indeed only rather weak fluorescence signals from the blocked cells (Fig. 3e). The above results clearly demonstrate highly specific FR targeting of NPAPF-PEG-FA NPs.

3.7. NPAPF-PEG NPs for in vivo imaging, blood circulation and biodistribution

To verify the applicability of the NPAPF-PEG NPs for in vivo tumor targeting imaging, we first intravenously injected NPAPF-PEG NPs into Balb/c mice and studied NPAPF-PEG NPs levels in the blood over time. Before injection, NPAPF-PEG NPs were enriched by centrifugation and followed by ultrasonic dispersion. Blood was drawn at different time after injection and solubilized by a lysis buffer, and the fluorescence of the blood sample was then measured to determine the concentration of NPs. Blood auto-fluorescence was subtracted from the measured blood fluorescence intensity. A blood circulation half-life of ~3 h was observed for NPAPF-PEG NPs as shown in Fig. 4a. The favorable blood circulation time also consistently witnessed the robust surface modification offered by C18PMH-PEG through noncovalent interactions.

It can be seen that C18PMH-PEG would improve circulation time of NPs in the living animals, thus NPAPF-PEG NPs were expected to be preferentially taken up by tumors for targeted imaging, due to the passive EPR effect in the tumor issues. We then intravenously injected NPAPF-PEG NPs (200 µL of 93 µM solution for each mouse) to nude mice bearing non-small-cell carcinoma tumor (A549). In vivo fluorescence imaging of the tumor-bearing nude mice was carried out by a Maestro EX in vivo fluorescence imaging system (CRi, Inc.) and the autofluorescence was removed using the Maestro software, leaving behind the pure fluorescence of NPs. In the beginning 30 min after injection, red fluorescence dispersed over the whole mouse, but with increasing time, high accumulation of the NPs in the tumor site with intense fluorescence was observed. Significantly, even with exposure time reduced to 5 ms, after 12 h post injection the fluorescent signals of NPAPF-PEG NPs were still distinctively bright and highly spatially resolved, in sharp contrast to the extremely low signals in other parts of the mouse body and almost no autofluorescence background detected, as shown in Fig. 4b, c and d. Here, we point out that images with such high signal-to-noise ratio (sensitivity) have seldom been reported before, and autofluorescence background is often observed for traditional fluorescence cellular probes with such emission wavelength for in vivo imaging [26]. The high contrast images are attributed to the combined effects of the high brightness and passive tumor targeting ability of the NPs in the tumor site by EPR effect, and are particularly beneficial for NIR probes for cancer diagnosis.

We further examined the biodistribution of NPs by imaging the major organs and the tumor tissues of the mouse at different post injection time points (Fig. 5a). The average fluorescence intensity of each imaged organ was calculated by a semi-quantitative biodistribution analysis. Fig. 5b shows the liver and tumor are the initial targets upon PNANF-PEG NPs injection. Consistent with the in vivo imaging results, the fluorescence of NPs in the tumor tissue increased with time. After 1 h intravenous injection, 17.4% and 17.2% injected dose (ID) g⁻¹ of the PNANF-PEG NPs were accumulated in the liver and tumor, respectively. Residual PNANF-PEG NPs were mainly distributed in spleen (4% ID g⁻¹), kidney (8% ID g⁻¹), heart (4% ID g⁻¹), lung (7% ID g⁻¹), stomach (13% ID g⁻¹) and intestine (16% ID g⁻¹), respectively. After 12 h post injection, prominent uptake of NPs with 25.2% and 29.5% ID g⁻¹, was only observed in tumor site and liver as expected, suggesting high tumor accumulation of NPs. The results clearly evidence the advantages of the NIR dye NPs for in vivo imaging.

4. Conclusions

In summary, we have demonstrated pure NPAPF NPs as a highly luminescent NIR fluorescent probe for targeted in vitro and in vivo imaging. The modified NPAPF NPs show intense NIR emission with large Stokes shifts, low cytotoxicity, robust pH- and photostability, excellent water-dispersibility and favorable blood circulation half-life. Application of functionalized NPs for in vitro and in vivo targeted imaging were successfully demonstrated, showing excellent imaging efficacy to specific receptor-positive KB cells and prominent tumor targeting with ultrahigh selectivity. Pure NPAPF NPs obtained via the present strategy should have great application potentials for in vitro and in vivo bioimaging.

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References