Electronic Supplementary Information (ESI)

Stability enhancement of fluorophores for lighting up practical application in bioimaging

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1. Nanoparticle-encapsulating fluorophores based on block copolymers

1.1 Peroxynitrite-responsive nanoprobe system

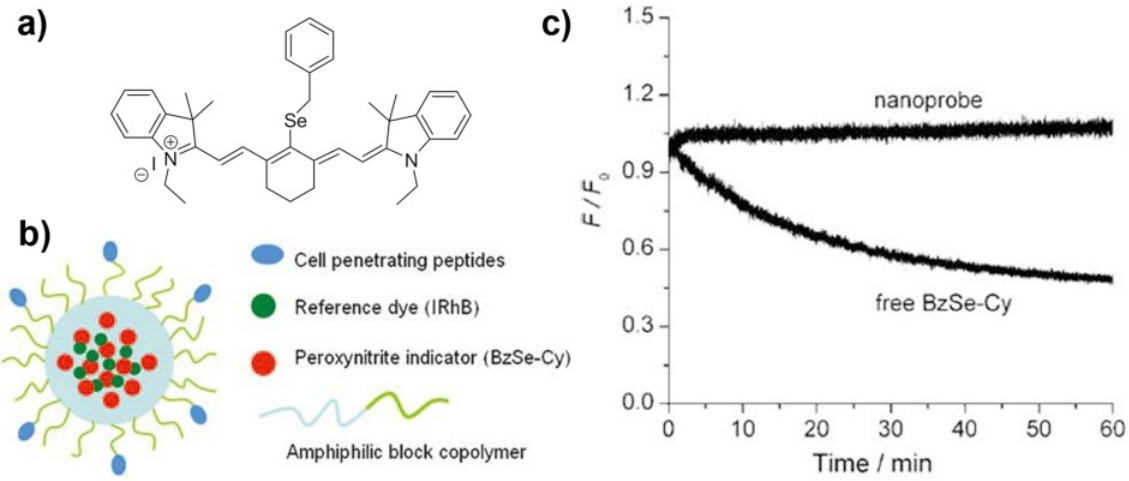

Fig. S1 The peroxynitrite-responsive nanoprobe system was consist of the NIR fluorescent peroxynitrite indicator BzSe-Cy. The nanoparticles formed by the amphiphilic block copolymer that encapsulate the dyes, and the cell penetrating peptides that is functionalized on the surface of the nanoparticles. Impressively, this nanoprobe exhibits better water solubility, biocompatibility and especially photostability than the free dye because of the encapsulation. Moreover, the response capability of the peroxynitrite indicator is retained to guarantee the detection of intracellular peroxynitrite.[S1]

1.2 Cross-linking of nanomicelles

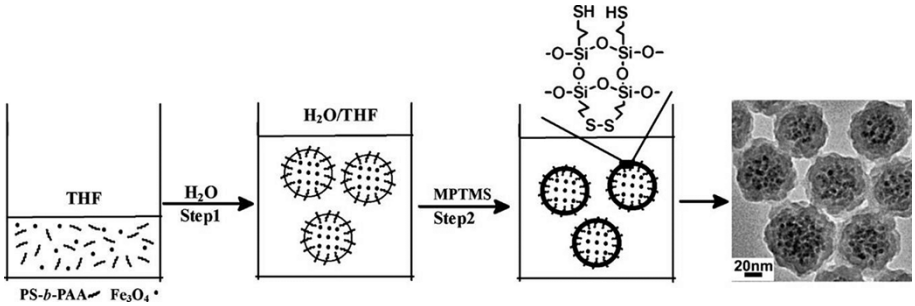

Fig. S2 The approach of the block copolymer-based nanomicelles which are covered by silica shell was initially utilized in the fabrication of magnetite nanoparticles. Hydrophobic magnetite nanocrystal (Fe_3O_4) and the amphiphilic block copolymer were dispersed in organic solvent. After the addition of water, the oil-in-water composite micelles were formed with the Fe_3O_4 in the cores. Subsequently, the magnetite micelles were coated by a silica shell using MPTMS as the silica source. The core-shell structure of the nanoparticles could be observed clearly from the TEM image.[S2]

1.3 Nanoparticle-encapsulating naphthalimide and its long-lasting stability

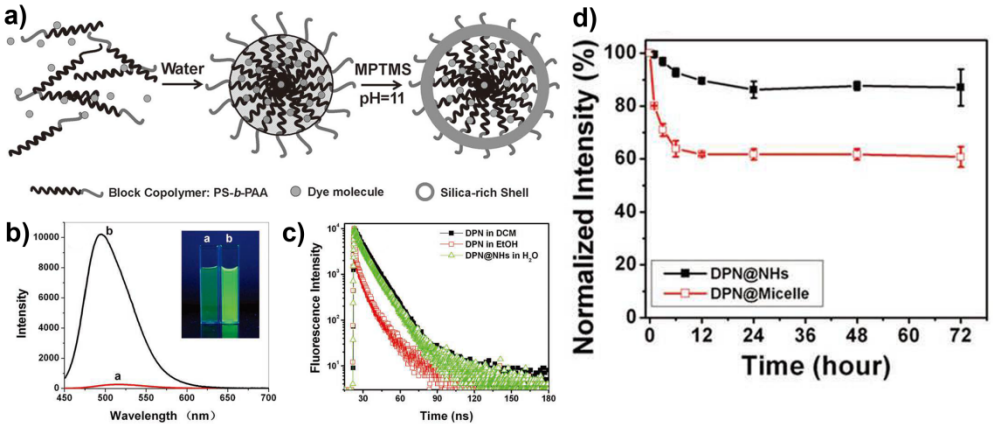

Fig. S3 The fluorescent nanoparticles were consist of the nanomicelle with the dye molecules at the core and silica shell as the coating. Owing to the isolation of the dye molecules from the solvents, the nanoparticles exhibit much stronger fluorescence even in water than the ethanol solution of the free dye, and almost the same fluorescence lift-time with the dichloromethane solution of the free dye. Fig. S3d highlights the apparently reduced leakage of the encapsulated dye molecules because of the compact silica shell, emphasizing the long-standing ability.[S3]

1.4 Nanoparticle-encapsulating cyanine dye and its application in bioimaging

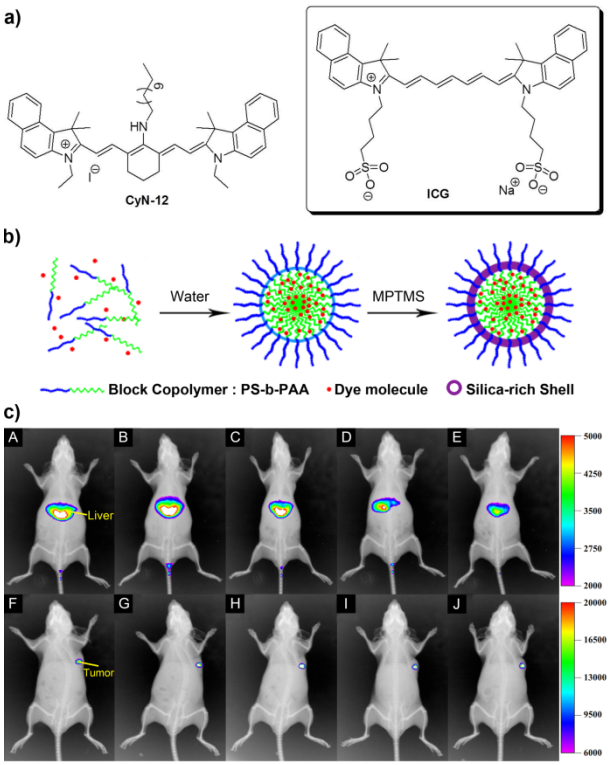

Fig. S4 The practical application of the nanoparticle-encapsulating cyanine dyes could be clearly demonstrated by the *in vivo* imaging. After the injection into the mouse, the nanoparticles basically concentrated in the liver and emitted strong NIR fluorescence whose intensity nearly decreased over time during the experiment. Moreover, the ability of the nanoparticles to retain in the tumor, which is originated from the EPR effect, could be further proved by the *in vivo* imaging experiment with the mouse tumor-injected the nanoparticles.[S4]

1.5 Nanoparticle-encapsulating pH sensor and its application in bioimaging

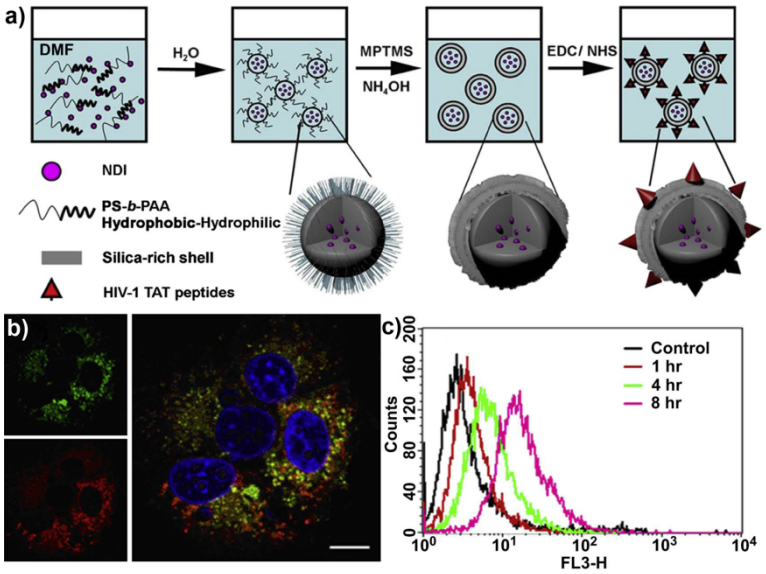

Fig. S5 The pH-responsive nanoprobe system NDI@HNPs was constructed by the surface conjugation with the high cell-penetrating peptides HIV-1 TAT based on the silica-coating nanomicelles. The fast response ability of NDI@HNPs guarantees excellent performance in the cellular pH imaging. The red fluorescence observed from NDI@HNPs matches perfectly with the green parts in the stained lysosome to yield signals in the merged images. The flow cytometry analysis also demonstrated the significant accumulation of NDI@HNPs within rather the acidic organelles of tumor cells than that of normal cells which is less acidic.[S5]

2. Fluorophore-doped nanoparticles based on inorganic matrixes

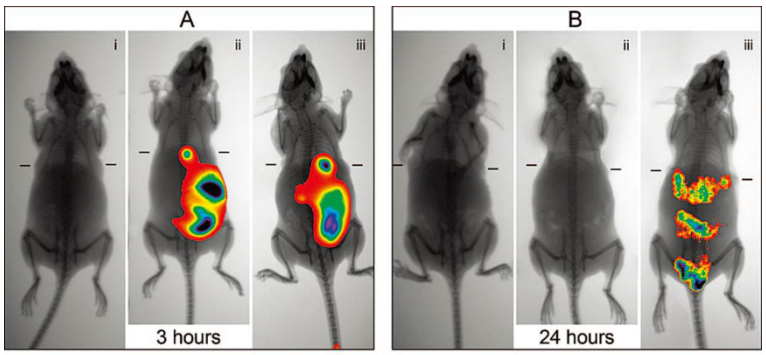

Fig. S6 The most important advantage brought by the enhanced photostability of the ICG-doped calcium phosphate could be emphasized by the time-dependent *in vivo* imaging. 3 h after the administration with ICG (ii) and ICG-CPNPs (iii) (CPNPs as the control (i)), respectively, the case of ICG and ICG-CPNPs displays similar fluorescence. However, no fluorescence signal could be detected from the case of free ICG 24 h post injection whereas the fluorescence emitted from ICG-CPNPs remains (Fig. S6B).[S6]

3. Molecular engineering on fluorescent chromophores

3.1 Dendrimer based on fluorophore core and its stability

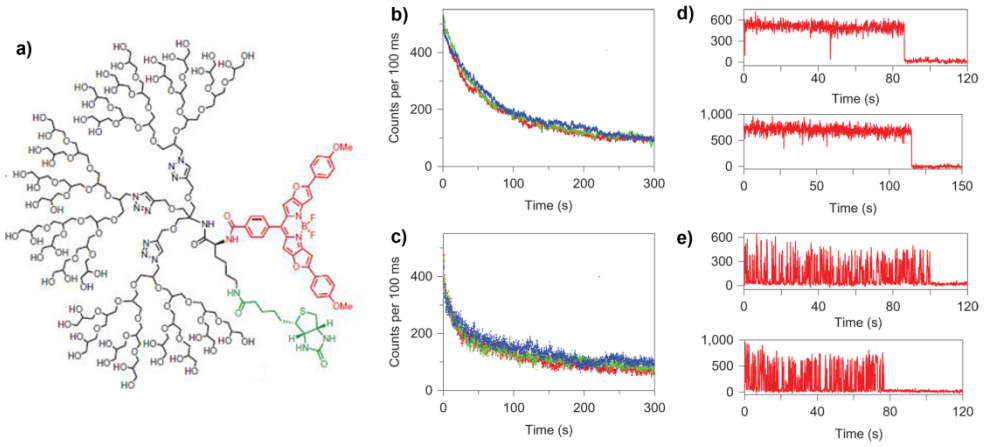

Fig. S7 The newly developed far-red fluorescent probe PGD-KFL exhibits strong fluorescence and comparable photostability with the commonly used Cy5. More importantly, the non-blinking fluorescence of PGD-KFL shows great superiority compared with the blinking behavior of Cy5.[S7]

3.2 Utilization of DCM fluorophore and its application in fluorescence-monitoring prodrug

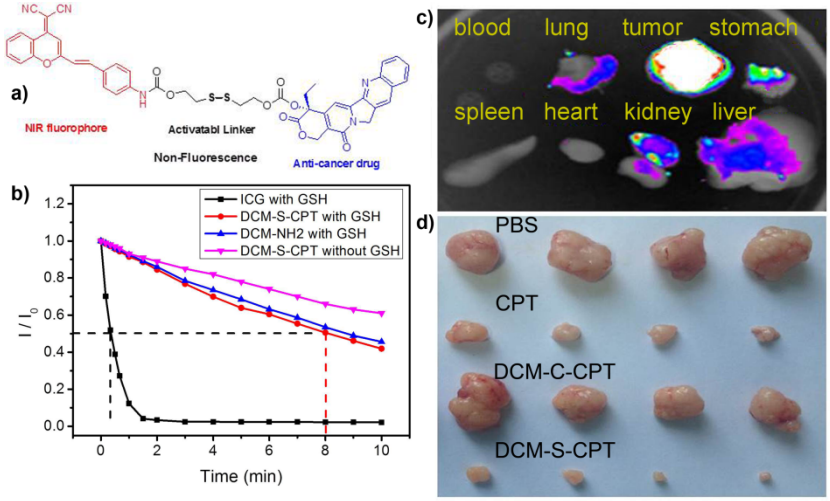

Fig. S8 The utilization of the highly photostable fluorophore DCM in the fluorescence-monitoring drug delivery system, whose photostability is 19-fold better than the commercially available ICG under the same condition, ensures the long-last *in vivo* imaging. Moreover, the GSH-response disulfide bond realize specific drug release with NIR fluorescence enhancement in tumor tissue and endow the prodrug DCM-S-CPT similar anti-tumor ability with free CPT but lower side effect.[S8]

Reference:

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