Recent years have witnessed significant progress in molecular probes for cancer diagnosis. However, the conventional molecular probes are designed to be “always-on” by attachment of tumor-targeting ligands, which limits their abilities to diagnose tumors universally due to the variations of targeting efficiency and complex environment in different cancers. Here, it is proposed that a color-convertible, activatable probe is responding to a universal tumor microenvironment for tumor-specific diagnosis without targeting ligands. Based on the significant hallmark of up-regulated hydrogen peroxide (H$_2$O$_2$) in various tumors, a novel unimolecular micelle constructed by boronate coupling of a hydrophobic hyperbranched poly(fluorene-co-2,1,3-benzothiadiazole) core and many hydrophilic poly(ethylene glycol) arms is built as an H$_2$O$_2$-activatable fluorescent nanoprobe to delineate tumors from normal tissues through an aggregation-enhanced fluorescence resonance energy transfer strategy. This color-convertible, activatable nanoprobe is obviously blue-fluorescent in various normal cells, but becomes highly green-emissive in various cancer cells. After intravenous injection to tumor-bearing mice, green fluorescent signals are only detected in tumor tissue. These observations are further confirmed by direct in vivo and ex vivo tumor imaging and immunofluorescence analysis. Such a facile and simple methodology without targeting ligands for tumor-specific detection and imaging is worthwhile to further development.
probes that are responding to universal tumor-microenvironment stimuli, such as acid, redox, overexpression proteins, and hypoxic surroundings, allow us to gain a new perspective on tumor-specific detection and imaging without active targeting ligands.[8–10] Among these stimuli, up-regulated hydrogen peroxide (H)$_2$O$_2$ has been described as a significant hallmark of various cancers because cancer cells display an increased H$_2$O$_2$ level ($10 \times 10^6$ to $50 \times 10^6$ m), which is higher than that in other normal cells ($0.05 \times 10^6$ to $0.7 \times 10^6$ m).[11–14] Despite advances of H$_2$O$_2$ probes, the vast majority of current H$_2$O$_2$ fluorescent probes are related to intensity changes, which suffers from poor accuracy because of the influences of many parameters, including photobleaching, optical path-length, and leakage from the cells.[15–18] Moreover, the strategy to image tumors in vivo remains a critical challenge, owing in part to the fast clearance after injection (in the case of small-molecule probes).[19,20] Alternatively, an activatable strategy to image tumors in vivo remains a critical challenge, owing in part to the fast clearance after injection (in the case of small-molecule probes).[19,20] Additionally, an activatable, fluorescent, unimolecular micelle with a hydrophilic poly(ethylene glycol-co-2,1,3-benzothiadiazole) (HPFBT), H$_2$O$_2$-activatable, fluorescent, unimolecular micelle-based, activatable platform is presented in the Supporting Information. Detailed preparation information on intermediates and target polymer were given in the Supporting Information. All compounds were fully characterized by 1H, 11B, and 13C nuclear magnetic resonance spectroscopy (NMR) as well as Fourier transform infrared spectroscopy (FTIR) (Figures S1–S14, Supporting Information). In the 2. Results and Discussion

The synthetic route of HPFBT-star-PEG is shown in Scheme 1. First, HPFBT was prepared from 2,7-dibromo-9,9-dihexylfluorene, 9,9-dihexylfluorene-2,7-diboronic acid, 1,3,5-tribromobenzene, and 4,7-dibromo-2,1,3-benzothiadiazole (BT) by a classical Suzuki polycondensation using Pd(PPh$_3$)$_4$ (Adamas-beta) as a catalyst in biphasic system (toluene/aqueous Na$_2$CO$_3$) at 90 °C for 72 h.[31–33] To obtain the boronic acid terminal groups of HPFBT, the mixture was further reacted with the addition of end-capped monomer 9,9-dihexylfluorene-2,7-diboronic acid and additional Pd(PPh$_3$)$_4$ for 48 h. Subsequently, the resulting HPFBT without purification was esterified with glycerol to produce boronate-containing HPFBT with hydroxyl terminal group (HPFBT-OH). Finally, HPFBT-star-PEG was synthesized by tosyl-hydroxyl condensation reaction of poly(ethylene glycol) monomethyl ether p-toluene sulfonate (PEG-OTs) and HPFBT-OH. After precipitation in methanol, the pure HPFBT-star-PEG was obtained as a light yellow solid. Detailed preparation information on intermediates and targeted polymer are presented in the Supporting Information. All compounds were fully characterized by 1H, 11B, and 13C nuclear magnetic resonance spectroscopy (NMR) as well as Fourier transform infrared spectroscopy (FTIR) (Figures S1–S14, Supporting Information). In the 1H NMR spectra of Figure S12 (Supporting Information), the proton signals at $\delta = 7.0–9.0, 4.0$, and $0.5–2.0$ ppm are assigned to the aromatic skeletons, (1,3,2-dioxaborolan-4-yl)-methanol, and alkyl groups of HPFBT-OH, respectively. Moreover, in 11B NMR spectrum of HPFBT-OH (Figure S11, Supporting Information), the signal of boron at $\delta = 9.4$ ppm can be observed, indicating the existence of many boronate units on the surface of HPFBT-OH. With the 1H NMR spectrum in Figure S14 (Supporting Information), we found that the content of BT in the polymer was about 4.6%. After grafting with PEG units a strong proton signal of OCH$_3$CH$_2$O at $\delta = 3.3–3.8$ ppm is observed in the 1H NMR spectrum of this color-convertible, activatable nanoprobe, the fluorescent color-conversion would be activated from blue to green through an enhanced FRET effect of HPFBT core, along with cleavage of boronate bonds and deprotection of PEG arms when exposed to tumoral H$_2$O$_2$. More importantly, the value of detection ability toward H$_2$O$_2$ was $(14.0 \pm 2.93) \times 10^6$ m, which was higher than the H$_2$O$_2$ level in normal cells and, in turn, just met the requirement of selective imaging for tumoral H$_2$O$_2$, according to the blue-to-green conversion. This color-convertible, activatable nanoprobe is obviously blue-fluorescent in various normal cells, but becomes highly green-emissive in various cancer cells. After intravenous administration to tumor-bearing mice, green fluorescent signals were only detected in tumor tissue. These observations were further confirmed by direct in vivo and ex vivo tumor imaging and immunofluorescence analysis. Thereby, the tumor-specific detection and imaging could be visualized in vitro and in vivo by using our color-convertible, unimolecular, micelle-based, activatable fluorescent probes.
HPFBT-star-PEG, demonstrating the grafting of PEG arms onto the HPFBT core. FTIR results are consistent with the 1H NMR observation, further confirming the successful synthesis of HPFBT-OH and HPFBT-star-PEG. The number- and weight-average molecular weights (Mn and Mw) and polydispersities of HPFBT-OH and HPFBT-star-PEG are summarized in Table S1 (Supporting Information). After grafting of PEG onto the HPFBT-OH, the molecular weight of HPFBT-star-PEG is increased.

HPFBT-OH shows excellent solubility in a variety of organic solvents, such as tetrahydrofuran (THF), chloroform, toluene, and so on. The optical properties of HPFBT-OH in THF are depicted in Figure 1a. In UV–vis spectrum of HPFBT-OH, a maximum absorption peak appears at 374 nm, and a weak shoulder peak around at 440 nm is observed. The ultraviolet regions typically originate from the π–π* transitions of fluorene segments in HPFBT, while the small shoulder peak ranging from 421 nm to 515 nm is related to the n–π* transitions of low-content donor–acceptor units in HPFBT-OH. Upon the excitation at 365 nm, HPFBT-OH displays strong blue fluorescence emissions with emission maximum (λem) at 425 nm. The presence of a small emission band in 520–650 nm regions is attributed to the inefficient intramolecular FRET in dilute solution due to the low molar ratio of BT in HPFBT-OH, which is in agreement with that of UV–vis results. The concentration-dependent fluorescence spectra of HPFBT-OH in THF show that the emission intensity of HPFBT-OH is increased with the concentration (Figure 1b).

Furthermore, the ratio of photoluminescence (PL) intensities (inset: I540/I425, I540 represents the PL peak intensity at 540 nm; while I425 represents the PL intensity at 425 nm) is also increased with the concentration, giving raise to the emission color-change of blue-to-green. This result demonstrates the concentration-induced enhancement of intermolecular FRET in HPFBT-OH.

After grafting with PEG, both UV–vis absorption and PL spectra of HPFBT-star-PEG in THF solvent show little changes, demonstrating the weak influence of PEG on the conjugated backbone of HPFBT core (Figure S15, Supporting Information). Benefiting from its amphiphilicity, HPFBT-star-PEG could self-assemble in the aqueous medium. Figure 1c gives UV–vis absorption and PL spectra of HPFBT-star-PEG in pH 7.4 phosphate-buffered saline (PBS) buffer. Compared with those of THF solution, UV–vis absorption and PL spectra of HPFBT-star-PEG in PBS solution are slightly changed. Moreover, HPFBT-star-PEG in PBS buffer shows that the I540/I425 ratio is independent with their concentration (Figure 1d), which is different from that of linear conjugated copolymer. This unique optical properties without intermolecular interaction of the HPFBT core is related to the special architecture of unimolecular micelle, in which the HPFBT core is well wrapped in the interior of HPFBT-star-PEG, and the intermolecular interaction of the HPFBT core is prevented efficiently by the PEG shell.[31–33] Thus, HPFBT-star-PEG in PBS buffer keeps blue fluorescence over a large range of concentration. We also summarized the quantum
yield and emission lifetime of HPFBT-OH and HPFBT-star-PEG in Table S2 (Supporting Information). In addition, the influence of pH on the fluorescence spectra of HPFBT-star-PEG in PBS solution was also investigated in Figure S16 (Supporting Information). Slight changes of emission-intensity ratio of $I_{540}/I_{425}$ are observed over an extensive pH, ranging from 4.0 to 9.0, indicating that the color-convertible, unimolecular, micelle-based probe is stable in various pH environments.

Due to the existence of H$_2$O$_2$-activatable boronate groups, optical properties of HPFBT-star-PEG in PBS buffer with different amounts of H$_2$O$_2$ were investigated. UV–vis absorption spectra show that the intensity of the main absorption bands reduces gradually with the increase of H$_2$O$_2$ content (Figure S17, Supporting Information), owing to the aggregation of the hydrophobic HPFBT core. Figure 2a gives the change in the fluorescence spectra of HPFBT-star-PEG upon addition of H$_2$O$_2$ for 60 min from $0 \times 10^{-6}$ to $100 \times 10^{-6}$ M, at intervals of $10 \times 10^{-6}$ M. As H$_2$O$_2$ increases, the emission band at 540 nm progressively grows at the expense of the emission band at 425 nm. The plot of $I_{540}/I_{425}$ as a function of the concentration of H$_2$O$_2$ in Figure 2b shows that a fluorescent-color changes from blue to green with the increase of H$_2$O$_2$. This transformation in the fluorescence of HPFBT-star-PEG reveals the enhancement of intermolecular FRET from fluorophore segments to BT units after dissociation of PEG. On the basis of this $I_{540}/I_{425}$ curve, the detection ability (S/N = 3) of HPFBT-star-PEG to H$_2$O$_2$ is calculated to be $(14.01 \pm 2.93) \times 10^{-6}$ M (Figure S18, Supporting Information), which was higher than the H$_2$O$_2$ level in normal cells and very suitable to delineate cancer cells from normal cells according to the blue-to-green conversion through an enhanced FRET effect. The time-dependent $I_{540}/I_{425}$ values of HPFBT-star-PEG with the different H$_2$O$_2$ concentration are given in Figure S19 (Supporting Information). Upon the addition of H$_2$O$_2$, the $I_{540}/I_{425}$ is increased rapidly, as expected with the increase of H$_2$O$_2$ concentration in the first 30 min, and then a plateau trend is sustained over the next 30 min. This observation suggests the robust ability to respond the H$_2$O$_2$, leading to the enhancement of intermolecular FRET efficiency through the aggregation of HPFBT core. The selectivity of HPFBT-star-PEG probe toward various reactive oxygen species (ROS) analytes, including H$_2$O$_2$, nitric oxide (NO), superoxide (O$_2^-$), tert-butoxy radical (•OtBu), hydroxyl radical (HO•), tert-butylhydroperoxide, singlet oxygen (1O$_2$), and hypochlorite (OCl$^-$), was evaluated by unperturbed fluorescent ratios of $I_{540}/I_{425}$. As shown in Figure 2c, an obvious change of the value of $I_{540}/I_{425}$ is observed for H$_2$O$_2$ in comparison to other ROS analytes, indicating that HPFBT-star-PEG could be served as an activatable probe with interconvertible fluorescent-color for specific imaging for H$_2$O$_2$. The self-assembled behavior of HPFBT-star-PEG in the aqueous medium before and after addition of H$_2$O$_2$ was determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements. In Figure 2d, the DLS curve of HPFBT-star-PEG gives a size of nanoparticle around at 62.9 $\pm$ 2.5 nm, which reveals the large multimicelle aggregates from unimolecular micelles through a “multimicelle aggregates” mechanism.

Figure 1. a) Normalized UV–vis absorption and normalized emission spectra of HPFBT-OH in THF (1 $\mu$g mL$^{-1}$). b) The concentration-dependent fluorescence spectra of HPFBT-OH in THF. Inset: changes of the emission ratio of $I_{540}/I_{425}$ with the corresponding concentration, and excitation at 365 nm. c) Normalized UV–vis absorption and normalized emission spectra of HPFBT-star-PEG (1 $\mu$g mL$^{-1}$) in pH 7.4 PBS buffer. d) The concentration-dependent fluorescence spectra of HPFBT-star-PEG in pH 7.4 PBS buffer. Inset: changes of the emission ratio of $I_{540}/I_{425}$ with the corresponding concentration, and excitation at 365 nm.
In order to investigate the self-assembly ability of HPFBT-star-PEG in aqueous solution, the critical micelle concentration (CMC) was measured by using 1,6-diphenyl-1,3,5-hexatriene as a probe. As shown in Figure S21 (Supporting Information), this HPFBT-star-PEG offers a low CMC value (3.37 µg mL\(^{-1}\)), indicating the high stability of HPFBT-star-PEG micelles before responding to tumoral H\(_2\)O\(_2\). After adding H\(_2\)O\(_2\), the diameter of nanoparticles is increased to 262.5 ± 2.3 nm. This phenomenon attributes to the decrease of the polymers water solubility through cleaving PEG from HPFBT-star-PEG (confirmed by \(^1\)H NMR spectra in Figure S22, Supporting Information), which is in agreement with the thermodynamic aggregation of block copolymers.\(^{[39]}\) To confirm the DLS results, the size and morphology of HPFBT-star-PEG were further investigated by TEM. HPFBT-star-PEG forms spherical micelles with the size of 60.4 ± 2.7 nm in Figure 2e, and the size of micelles increase to 260.6 ± 2.1 nm after adding H\(_2\)O\(_2\) in Figure S23 (Supporting Information). These results are consistent with those of DLS data. Moreover, the time-dependent aggregation behavior of HPFBT-star-PEG in H\(_2\)O\(_2\)-containing PBS buffer was measured by DLS. The result in Figure 2f shows that the diameter of nanoparticles increases rapidly in the first 30 min. Above this critical point, the increase of micelles size becomes slow. This critical time is consistent with that of PL observation, indicating the effective degradation of HPFBT-star-PEG through the reaction between H\(_2\)O\(_2\) and boronate bond.
The in vitro cytotoxicities of HPFBT-star-PEG micelles against cancer cells and normal cells were evaluated by methyl tetrazolium (MTT) assay. As an example, HeLa cancer cells and L929 normal cells are chosen to study the cell viabilities after 48 h incubation with HPFBT-star-PEG micelles. Figures S24 and S25 (Supporting Information) exhibit that the cell viabilities of HeLa cells and L929 cells are over 85.7% and 84.3%, respectively, when the concentration of micelles reaches up to 1000 µg mL\(^{-1}\). It demonstrates that HPFBT-star-PEG has low cytotoxicity against cancer cells and normal cells. In addition, the cellular uptake of HPFBT-star-PEG micelles was investigated by flow cytometry analysis. Flow cytometry results in Figures S26 and S27 (Supporting Information) reveal that the relative geometrical mean fluorescence intensities (GMFI) of HeLa cells and L929 cells pretreated with HPFBT-star-PEG micelles are much higher than those of nonpretreated cells after 0.5 h incubation. With prolonged incubation time, the intensity of GMFI increases obviously. These prominent fluorescence signals are associated with the cellular uptake of HPFBT-star-PEG micelles into HeLa cells and L929 cells, respectively.

Since the excellent H\(_2\)O\(_2\)-activatable ability and highly efficient cellular uptake of HPFBT-star-PEG micelles, the imaging of intracellular H\(_2\)O\(_2\) was further investigated by confocal laser scanning microscopy (CLSM) measurements. As shown in Figure 3a, the bright blue emission is visualized clearly in the cytoplasmic region of L929 normal cells after culturing with HPFBT-star-PEG micelles for 4 h at 37 °C. This result indicates that HPFBT-star-PEG keeps its core–shell unimolecular topological structure due to its low H\(_2\)O\(_2\) microenvironment in the normal cells, which leads to an inefficient intramolecular FRET. By contrast, HeLa cells containing up-regulated H\(_2\)O\(_2\) display a strong green-emission with the incubation of HPFBT-star-PEG (Figure 3b). This change of fluorescence color is activated by the enhanced intermolecular FRET in response to tumoral H\(_2\)O\(_2\). To further confirm the influence of intracellular H\(_2\)O\(_2\) on the fluorescent color transition, CLSM of HPFBT-star-PEG was investigated by culturing L929 cells pretreated with \(50 \times 10^{-6} \text{m} \) \(\text{H}_2\text{O}_2\) to reach up to the same oxidation level as HeLa cancer cell (Figure S28, Supporting Information). Interestingly, L929 cells pretreated with \(\text{H}_2\text{O}_2\) could also emit a strong green-fluorescence in Figure 3c, which is similar to the emission phenomenon in HeLa cancer cells. Furthermore, the intracellular imaging of \(\text{H}_2\text{O}_2\) against various cancer cells and normal cells were further studied by CLSM. As shown in Figures S29 and S30 (Supporting Information), the blue fluorescence signals are visualized in the various normal cells, while green-emission color is activated in the tumor cells. These observations of the different imaging colors in living cells are attributed to the variations of intracellular H\(_2\)O\(_2\), which holds great promise to identify the cancer cells from normal cells.

Being aware of the highly specific detection and imaging of cancer cells and normal cells with different fluorescent color by HPFBT-star-PEG micelles, the diagnosis of cancer cells was further carried out in cell mixtures by flow cytometry and CLSM.\(^{[41,42]}\) Firstly, HeLa and L929 cells were treated with HPFBT-star-PEG micelles for 4 h, respectively, and then mixed in equal proportion (1:1, count per count). The untreated cell mixtures were used as control (Figure 4a). Results show that the single-stained cells (either HeLa cancer cells were stained by HPFBT-star-PEG micelles or L929 normal cells were stained) enable to be detected from the

\[\text{DIC} \quad \text{Blue} \quad \text{Green} \quad \text{Overlay}\]

**Figure 3.** CLSM images of a) L929 cells incubated with HPFBT-star-PEG micelles for 4 h. b) HeLa cells incubated with HPFBT-star-PEG micelles for 4 h. c) L929 cells pretreated with \(50 \times 10^{-6} \text{m} \) \(\text{H}_2\text{O}_2\) and incubated with HPFBT-star-PEG micelles for 4 h. The scale bars represent 20 µm.
cell mixtures through the change of fluorescence intensity (Figure 4b,c). More importantly, with respect to double-stained cells (both HeLa cancer cells and L929 normal cells were stained by HPFBT-star-PEG micelles), tumor cells and normal cells can be delineated clearly through the isolation of green and blue tunnel in Figure 4d, due to the entirely different emitting color. All the ratios of staining cells in whole test are consistent well with the initially mixing ratio of 1:1. The diagnosis of cancer cells in cell mixtures with HPFBT-star-PEG staining was further studied by CLSM. The untreated cell mixtures as a control (Figure S31a, Supporting Information), single-stained cell mixtures and double-stained cell mixtures were photographed for statistical analysis. For the single-stained cell mixtures of HeLa (50%)/L929 (50%), the stained HeLa cells (Figure S31b, Supporting Information) and L929 cells (Figure S31c, Supporting Information) can emit green and blue fluorescence, respectively. As shown Figure 4e, the corresponding percentage of the captured fluorescent cells is 50.57% ± 1.14% and 51.18% ± 1.62%, which is similar to the original ratio. With regard to the double-stained cell mixture (Figure S31d, Supporting Information), two kinds of cells with different color are visualized clearly with percentage of 51.29% ± 1.48% (green HeLa cells) and 48.71% ± 1.31% (blue L929 cells), which is in good agreement with initially feed ratio.

Cytochrome c, keeping intracellular redox balance, is a key signal transduction indicator for cell apoptosis through mitochondrial pathway.[43,44] The immunocytochemistry of cytochrome c is efficient strategy to evaluate the apoptosis of cells. Aberrant production and accumulation of ROS (e.g., H$_2$O$_2$) would induce the decrease of cytochrome c, which leads to the abnormal apoptosis and subsequent tumor generation. Based on the close relationship between cytochrome c expression and cancerous cells, HPFBT-star-PEG could be employed as an indirect signal transducer for monitoring the canceration of living cells. Figure 5 gives the CLSM images of HeLa cells and L929 cells incubated with P-phycoerythrin (PE)-conjugated cytochrome c antibody (“always-on” probe) and HPFBT-star-PEG micelles (activatable nanoprobe), respectively. As shown in Figure 5a,b, strong red-fluorescent signals from traditional “always-on” probes were observed mainly in the cytoplasm of normal L929 cells, while the blue fluorescence of HPFBT-star-PEG could be detected in L929 cells. It indicates that the cytochrome c is adequately expressed and released to keep ROS level in normal level. For HeLa cells, a robust red fluorescence was still observed in cancer cells (Figure 5c). Actually, the expression of...
cytochrome c should be inhibited by the up-regulated H$_2$O$_2$ in cancer cells. Thus in this regard, the “always-on” probe suffers from poor accuracy in response to the cytochrome c due in part to serious photobleaching. Differently, our color-convertible activatable nanoprobes of HPFBT-star-PEG could generate a bright-green emission in cancer cells (Figure 5d), which is much more accurate and intuitive than that of “always-on” probes because of the obvious blue-to-green conversion. To further confirm the inhibition of cytochrome c by up-regulated H$_2$O$_2$, fluorescence imaging of L929 cells pretreated with H$_2$O$_2$ were investigated. The emission observation in H$_2$O$_2$-pretreated L929 cells (Figure 5c,f) is very similar to HeLa cancer cells. Therefore, compared with the commercial “always-on” probes, our color-convertible activatable nanoprobes with inter-convertible emission color of blue-to-green are much more accurate and intuitive to detect the slight difference of the subjects.

The outstanding diagnosis ability of our color-convertible activatable nanoprobes in vitro studies motivates us to further investigate the tumor diagnosis in vivo. At first, the pharmacokinetic evaluation was undertaken by intravenous injection with HPFBT-star-PEG micelles and traditional small molecular dye of Cy5.5 (as a control) to Sprague-Dawley (SD) rats via tail vein. Figure 6a gives the time profiles of HPFBT-star-PEG micelles and Cy5.5 dye in serum. It can be seen that the nanoprobe has longer blood retention time than that of Cy5.5 dye, ascribing to its nanocharacteristics, which provides the possibility of enhanced probe accumulation in the tumor tissues via EPR effect. Besides, the distribution of HPFBT-star-PEG micelles in vivo, especially in the tumor region, was further studied by a ZKKS-MI in vivo imager with a 425-nm excitation laser (the same photon as the emission of donors) and a 525-nm (green fluorescence from the acceptors) emission filter over the course of 12 h. Figure 6b shows that the green fluorescent signals of micelles are only observed clearly from the epidermis implanted tumor at 2 h after both intravenous and intratumoral injection, due to the tumor accumulation and the low penetration depth of the green fluorescence. The tumor accumulation of HPFBT-star-PEG micelles via intravenous administration should be caused by passive targeting through EPR effect. As a control, no green fluorescent signals are detected in whole body preinjected with PBS (Figure S32, Supporting Information). The amount of micelles in the tumors and other organs from HeLa tumor-bearing mice was further evaluated by fluorescence spectroscopy after preinjection HPFBT-star-PEG with different time intervals. Figure 6c gives the biodistribution of the HPFBT-star-PEG micelles in tumors and normal organs, in which the concentration of micelles is quantified as a percent of injected dose. A large amount of HPFBT-star-PEG is accumulated in the liver and kidney at the first 2 h. After 12 h postinjection, the content of micelles in these organs decreases. The contents of HPFBT-star-PEG in heart, spleen, and lung hold a low level due to the residue of micelles in the blood. In the tumors, the contents of HPFBT-star-PEG are respectively calculated to be 5.3% ± 0.8%, 10.4% ± 0.6%, and 8.5% ± 0.9% after 2, 6, and 12 h postinjection, which suggests that the accumulated HPFBT-star-PEG micelles can gain a plateau during the postinjection of 12 h. These results demonstrate that the HPFBT-star-PEG micelles can be accumulated in tumors, which is beneficial to cancer diagnosis by the color-convertible, activatable nanoprobes. Finally, tissues including tumor, heart, liver, spleen, lung, and kidney were isolated to evaluate the ex vivo fluorescent imaging at 6 h postinjection. In Figure 6d, strong green-fluorescent signal is detected in tumor, while a nongreen-fluorescent signal is activated in other normal organs, indicating the enhanced FRET effect from HPFBT-star-PEG micelles along with green-fluorescence emitting is activated by tumoral H$_2$O$_2$.

The tumor-specific imaging of HPFBT-star-PEG micelles was further investigated by immunofluorescence analysis. First, the tumors and major organs were collected from HeLa tumor-bearing mice with intravenously injected with

![Image](https://www.advancedsciencenews.com/pdf/1604062.pdf)
HPFBT-star-PEG micelles for 6 h, sectioned and then imaged by confocal microscope. As shown in Figure 7a, strong green-fluorescence color is only observed in the tumor slice, due to the highly accumulation in tumor region and the enhanced FRET process in the high H₂O₂ tumoral microenvironment. The detectable blue fluorescence signal is generated in normal liver and kidney, according to the inefficient FRET process in the low H₂O₂ normal tissue microenvironment. In addition, almost nonfluorescence was detected in heart, spleen, and lung, presumably attributing to a small amount of probe accumulation in these organs. On the other hand, with respect to the mice with intratumoral injection of HPFBT-star-PEG micelles, the images in Figure S33 (Supporting Information) also demonstrate that the bright green fluorescence only exists from tumor slice. In short, HPFBT-star-PEG micelles could yield strong green fluorescence selectively both in vitro cancer cells and even in vivo tumor tissue, indicating its potential application for valuable cancer diagnosis.

The biocompatibility of the probe is crucial for biomedical applications. Thus, the potential toxic side effect of HPFBT-star-PEG micelles during the in vivo imaging process was studied. Body-weight loss is one of the important indicators to evaluate in vivo toxicity. After injecting HPFBT-star-PEG micelles to healthy, tumor-free, nude mice, no noticeable body-weight losses are detected during one week, which is similar to PBS pretreated healthy mice (Figure 7b).

We also assessed the in vivo toxicity of HPFBT-star-PEG by histologic analyses. In Figure 8, All hematoxylin and eosin (H&E) stainings of normal tissue slices collected from the healthy, tumor-free, nude mice pretreated HPFBT-star-PEG micelles show no appreciable abnormality or noticeable damage in comparison with the controls (PBS group). Therefore, combined with the MTT results in vitro cytotoxicity above, all results suggest that our nanoprobe has no obvious toxicity, which preliminarily proves that HPFBT-star-PEG micelles can be used as a safe and effective fluorescent probe for cancer diagnosis.

3. Conclusion
In summary, we reported a novel unimolecular micelle used as a color-convertible H₂O₂-activatable probe for tumor-specific detection and imaging in vitro and in vivo. This H₂O₂-activatable nanoprobe displays obvious color-conversion of blue-to-green via an aggregation-enhanced FRET strategy. This probe is obviously blue-fluorescent in various normal cells but becomes highly green-emissive in various cancer cells. After intravenous injection to tumor-bearing mice, green fluorescence signals were only detected in tumor tissue. This observation was further confirmed by direct in vivo and ex vivo tumor imaging and immunofluorescence analysis. We believe that the H₂O₂-activatable nanoprobe...
with interconvertible emission color to detect tumors from normal tissues might contribute an accurate and readily observable strategy for noninvasive cancer-specific diagnosis. Besides, such a facile and simple methodology without targeting ligands for tumor-specific detection and imaging is worthwhile to further extend its potential application in response to other tumor microenvironment stimuli via simply changing the activatable bonds, and synchronously holds a great promise for stimuli-responsive drug delivery in cancer therapy.

Figure 7. a) CLSM images of tumor and major normal tissues slices after intravenous injection of HPFBT-star-PEG micelles at 6 h postinjection. Gray color is PI staining in nucleus. Magnification × 200. b) Body weight changes of the healthy tumor-free nude mice with PBS and HPFBT-star-PEG micelles treatment (n = 5 mice per group) monitored for 7 d.

Figure 8. Representative histological features of organs (heart, liver, spleen, lung, and kidney) stained with H&E after the treatment of PBS and HPFBT-star-PEG micelles in the 7 d evaluation period (n = 5 mice per group and magnification × 400). The scale bars represent 50 µm.
Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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