Electronic Supplementary Material (ESI) for Analyst.


# Supplementary data

## A sensitive and fast responsive fluorescent probe for imaging hypoxic tumors

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### 1.1. The synthesis of BBP

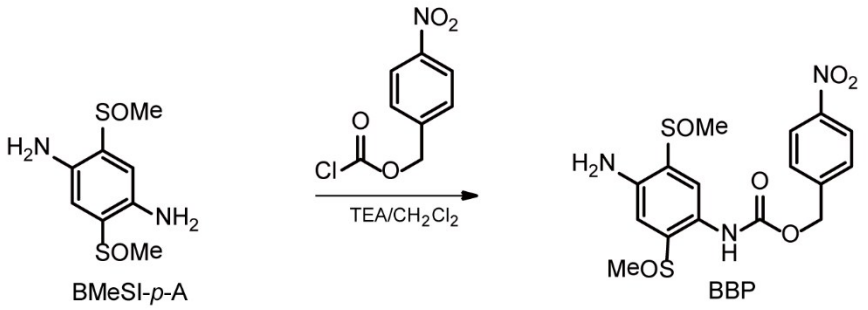



**Fig. S1.** The synthesis of BBP.

### 1.2. Identification of the release of BMeSI-*p*-A from BBP in the presence of NTRs

A mixed solution of BBP (4 μM) and NADH (100 μM) in PBS (50 μL) was added NTRs (5 μg/mL). The reaction mixture was incubated at 37 ℃ for 30 minutes, and was identified by LC-MS spectrometry.

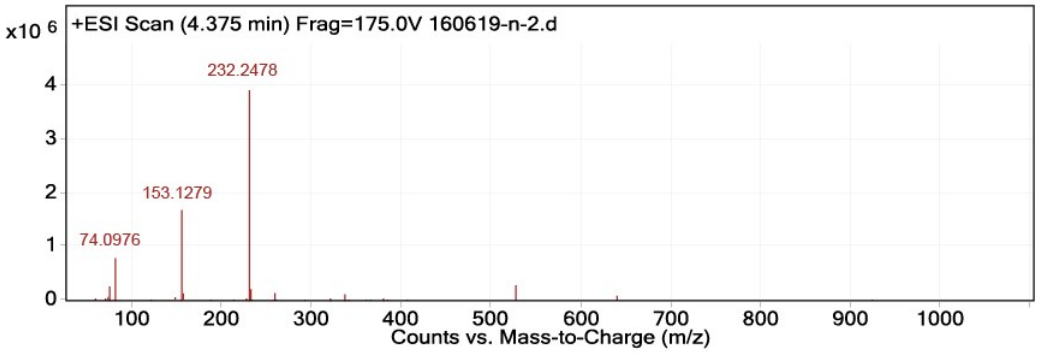



**Fig. S2.** LC-MS spectrum of BBP degradation products.

### 1.3. The serum stability of BBP

A solution of BBP (5 μM) was mixed with 50% serum, and the mixture was incubated at 37 ℃ for one week. Both absorbance (λ = 348 nm) and fluorescence intensity (Ex/Em=375/520 nm) were measured using Microplate Reader (TECAN, 200 pro, USA) at the different time points.

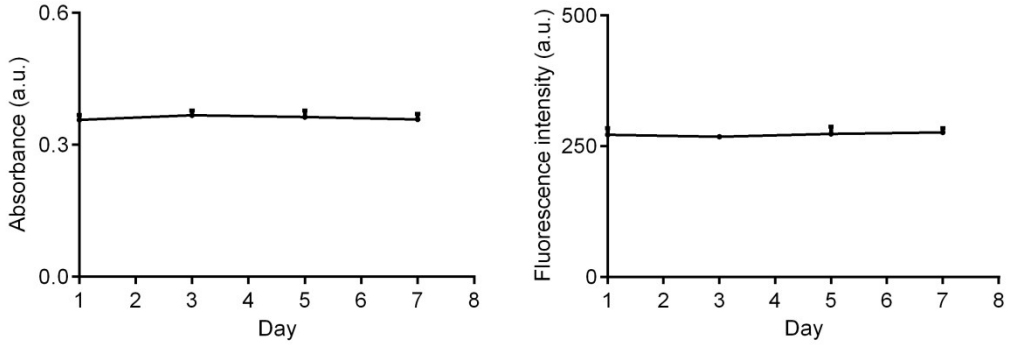



**Fig. S3.** The serum stability of BBP.

### 1.4. The effects of pH and temperature on the detection of NTR activities

A mixed solution of BBP (5 μM) and NADH (100 μM) in PBS (50 μL) was added NTRs (5 μg/mL). The mixture was incubated at different pH values (5, 6, 7 and 8) and different temperature (32, 35, 37 and 40 ℃), and the absorbance (λ = 375 nm) and fluorescence intensity (Ex/Em=375/520 nm) were measured using Microplate Reader (TECAN, 200 pro, USA). Meanwhile, a mixture of BBP and NADH in PBS without NTRs was chosen as the control.

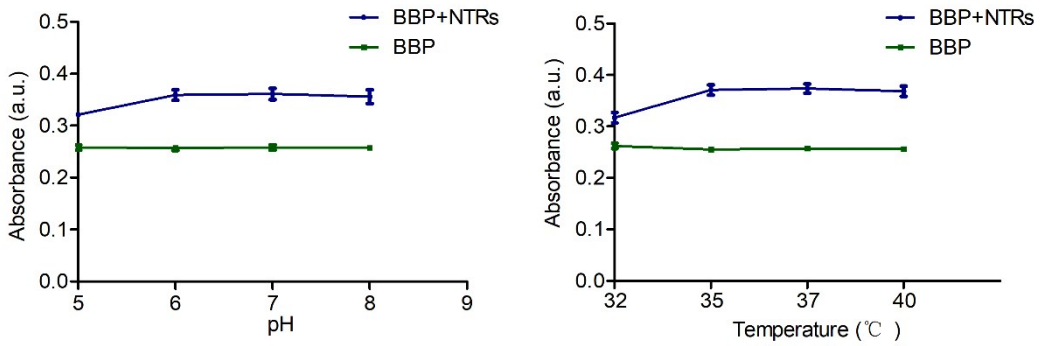



**Fig. S4.** The effects of pH and temperature on the absorbance of BBP.

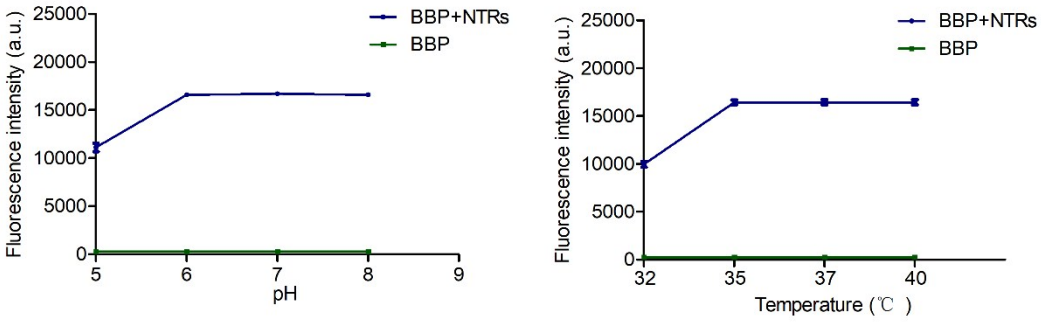



**Fig. S5.** The effects of pH and temperature on the emission of BBP.

### 1.5. The specificity of BBP toward NTRs

The selectivity of BBP was evaluated by treating BBP with various biological interfering species, such as salts (NaCl and $CaCl_2$), small biomolecules (Cys), bio-macromolecules (BSA), enzymes (Trypsin), and a mixture of NTRs (5 μg/mL) and NTR inhibitor discoumarin (0.15 mM). A mixed solution of BBP (5 μM) and NADH (100 μM) in PBS (50 μL) was added the interfering species. The mixture was incubated at 37 ℃ for 30 minutes, and the fluorescence intensity (Ex/Em=375/520 nm) was measured using Microplate Reader (TECAN, 200 pro, USA). Meanwhile, a mixture of BBP and NADH in PBS without NTRs was chosen as the control.

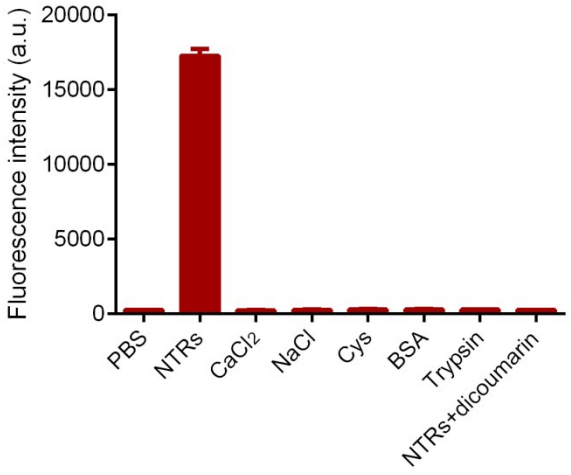



**Fig. S6.** Fluorescence intensity of BBP (5 μM) responding to various species including PBS (pH 7.4), NTR (5 μg/mL), $CaCl_2$ (10 mM), NaCl (10 mM), Cysteine

(10 mM), BSA (10 mM), Trypsin (10 mM), and a mixture of NTR (5 μg/mL) and discoumarin (0.15 mM).

### 1.6. The detection limit of BBP towards NTR

A mixed solution of BBP (5 μM) and NADH (100 μM) in PBS (50 μL) was added different concentrations of NTRs (0.01, 0.02, 0.05, 0.1, 0.5, 1, 2.5 and 5 μg/mL). The mixture was incubated at 37 ℃ for 30 minutes, and fluorescence intensity (Ex/Em=375/520 nm) was measured every one minutes using Microplate Reader (TECAN, 200 pro, USA). Meanwhile, a mixture of BBP and NADH in PBS without NTRs was chosen as the control.

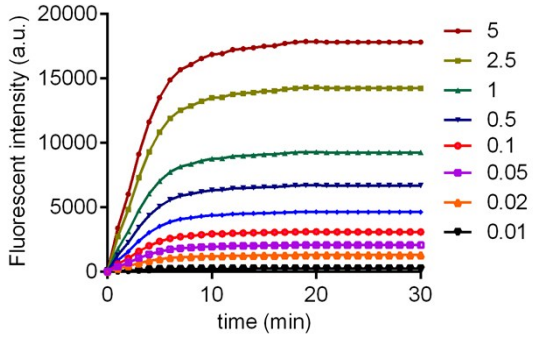
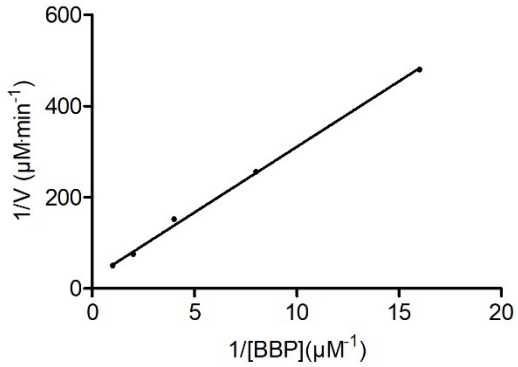



**Fig. S7.** A plot of fluorescence intensity of BBP against the reaction time in the presence of varied concentrations of NTRs (0.01, 0.02, 0.05, 0.1, 0.5, 1, 2.5 and 5 μg/mL). Fluorescence intensity (Ex/Em=375/520 nm) was measured at 37 ℃.

### 1.7. The kinetic studies of BBP reacting with NTRs

The catalytic activities of NTRs toward the reduction of BBP were assessed. The




kinetic curves of reactions between NTRs (5 μg/mL) and different concentrations of BBP (0.0625, 0.125, 0.25, 0.5 and 1 μM) were measured. The reaction mixture was incubated at 37 ℃, and fluorescence intensity (Ex/Em=375/520 nm) was measured using Microplate Reader (TECAN, 200 pro, USA).

**Fig. S8.** Lineweaver-Burk plot for the NTR-catalyzed reduction of BBP. The Michaelis-Menten equation was described as: $V=V_{max}$ [probe]/ ($K_m$+ [probe]), where V was the reaction rate, [probe] was the probe concentration (substrate), the $K_m$ was the Michaelis constant. Reaction at each concentration was repeated three times and the error bars represent standard deviations. Points were fitted using a linear regression model (V=38.469[probe] +30.288, correlation coefficient R=0.999).

### 1.8. Cytotoxicity of BBP on HepG-2 and L02 cells

MTT assay was exploited to determine the cytotoxicity of the BBP. HepG-2 and L02 cells were seeded in a 96-well plate at the density of 5 $\times 10^3$ cells per well in complete 1640 medium at 37 $^oC$ in a humidified atmosphere containing 5% $CO_2$ for 24 hours. Then the medium was replaced by 1640 supplemented with different doses of BBP. Both comparison groups were cultured for 24 hours at normoxic and hypoxia. Anaero Pack-Anaero (Mitsubishi Gas Chemical Co.Inc., Japan) were used to provide hypoxic cell culture environment with 1% oxygen concentration. 20 μL of 3-(4, 5-

dimethylthiazol -2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) solution was added to each well and incubated for 4 hours. Then, the supernatant was removed and the products were lysed with 200 μL of DMSO. The absorbance values were recorded at 570 nm using a microplate reader (TECAN 200pro, USA). The absorbance of the untreated cells was used as a control and its absorbance was set as the reference value for calculating 100% cellular viability.

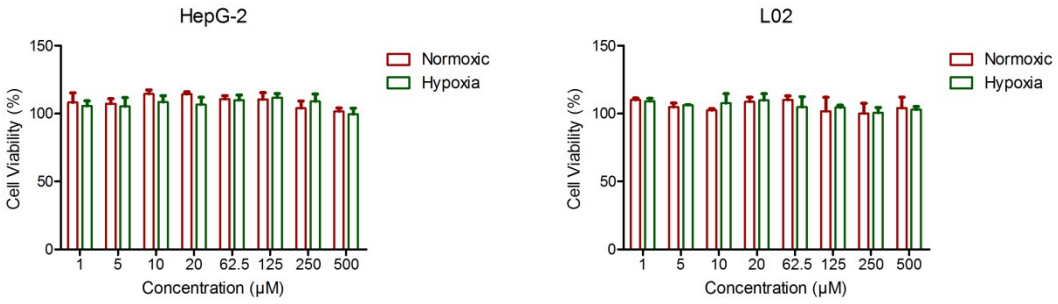



**Fig. S9.** The MTT assay of BBP after co-culture with HepG-2 and L02 cells under normoxic and hypoxia for 24 hours.

Table. S1 The quantum yield of probes

| **probe** | **Quantum Yield [%]** |
|---|---|
| BMeSI-*p*-A | 18.21±0.02 |
| BBP | 0.09±0.01 |