**Supporting Information**

**Photoswitchable probe with distinctive characteristics for selective fluorescence imaging and long-term tracing**

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**Experimental Section**

**General experimental information:** 1H NMR spectra were taken on a Bruker Ultra Shield Plus using CDCl₃ as solvent. The UV-vis absorption and fluorescence spectra were carried out on a Shimadzu UV-3600, UV-vis spectrophotometer and RF-S301PC spectrofluorophotometer, respectively. Mass spectra were recorded on a Shimadzu LC-MS/MS-2020 liquid chromatography tandem mass spectrometry. Confocal luminescence images were obtained on an Olympus FV1000 laser scanning confocal microscope. THF (Labscan) was distilled under dry nitrogen from sodium benzophenone ketyl prior to use. PBS buffer (pH 7.4) was prepared using double distilled water. Other chemicals, reagents and solvents were all purchased from Aldrich or Invitrogen and used as received.

**Synthesis and characterizations:** A 250 mL round-bottom flask was first charged with 0.84 g (3.46 mmol) 5-amino-2-(benzo[d]thiazol-2-yl)phenol and 0.55 g (1.74 mmol) 4, 4-(cyclopentene-1,2-yl)-bis(5-methyl-thiophene-2-formaldehyde), 150 ml ethanol and refluxed without light at 95 °C for 24 h. After reaction, removing the solvent by suction filtration, the residue was washed by solution dimethyl sulfoxide (DMSO). ELISA reader (Sunrise, Tecon) was used to test the optical density of each nominally identical measurement. Cultures were used to indicate the rate of cell proliferation. The results are expressed as an average over three well at a wavelength of 590 nm according to the manufacturer′s instructions. Absorbance values of experimental cultures were used to indicate the rate of cell proliferation. The results are expressed as an average over three nominally identical measurement.

**Cell culture and MTT assay:** HeLa cells were obtained from the Cell Bank (Cell Institute, Sinica Academica Shanghai, Shanghai, China) and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with fetal bovine serum (10%), penicillin (100 units/mL) and streptomycin (50 units/mL). Cells were cultured with 5% CO₂ at 37 °C in a humidified incubator. Prior to the imaging experiments, the cells were cultured until confluence was reached. Cytotoxicity of BMBT was assayed on HeLa cells by using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, from Sigma-Aldrich) to test the biocompatibility of BMBT for imaging in living cells [1]. Briefly, cells growing in log phase were plated in a 96-well microassay culture plate (1 × 10⁴ cells per well). After 24 h incubation, THF/DMEM (1:200, vol:vol) solution of BMBT (20 μM) were added to the cells at final concentrations of 5, 10, 20, 35, 50 μM. Cells were then further incubated for another 24 h. 20 μl MTT solution (5 mg mL⁻¹) was added to each well. After 4 h incubation, the medium was removed and Formazan was dissolved by solution dimethyl sulfoxide (DMSO). ELISA reader (Sunrise, Tecon) was used to test the optical density of each well at a wavelength of 590 nm according to the manufacturer’s instructions. Absorbance values of experimental cultures were used to indicate the rate of cell proliferation. The results are expressed as an average over three nominally identical measurement.

**Confocal luminescence imaging** The HeLa cells were cultured in cell plates specially for observation under CLSM at 37 °C. The BMBT was dispersed in THF buffer and were added to the plate at a final concentration of 20 μM. After incubation for 30 min, the cells were washed three times with 1 × PBS buffer and imaged by CLSM (Olympus FV1000, Tokyo, Japan) using the Olympus Fluoview FV1000 imaging software. Organisms containing the BMBT were excited at 405 nm with a semiconductor laser, and the emission was measured according to the spectral data. To better understand the intracellular distribution of BMBT in the cell, luminescence intensity profile corresponding to the extracellular region, nuclear region and cytoplasm was
investigated, respectively. After each temperature change, a 15 min interval was set before starting a new measurement to ensure thermal equilibration.

![Reaction Scheme](image)

**Fig. S1** Synthetic route of BMBT.

![Absorption Spectrum](image)

**Fig. S2** Absorption spectrum changes of BMBT in THF/DMEM mixture upon irradiation with 365 nm light and visible light ($\lambda_{ex}=520$ nm).

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Fig. S3 Confocal luminescence images of living HeLa cells incubated with 20 μM of BMBT for 30 min at 37 °C and co-stained with Hoechst and MitoTracker Red.

Fig. S4 Confocal luminescence intensity profile and luminescence image (across the line) of HeLa cells incubated with 20 μM BMBT.
**Fig. S5** Cell viability values (%) assessed by MTT proliferation test vs. incubation concentrations of 0-50µM BMBT in DMSO/DMEM mixture buffer at 37 °C for 24 h.

**Fig. S6** Maldi-tof mass spectra of BMBT in CDCl₃.
Fig. S7 $^1$H NMR spectra of BMBT

References: