*Supporting Information for*

# A novel fluorescent probe with high photostability for imaging distribution of RNA in the living cells and tissues

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## 1. Materials

Firstly, for all experiment, all reagents of synthesis and analysis experiment were obtained by commercial suppliers. These reagents do not further purification before experiment.

Secondly, for synthesis experiment, all separation and purification of compounds were determined by thin-layer chromatography analysis. This method was performed on silica gel plates; In addition, column chromatography was carried out by silica gel (mesh 200–300); Silica gel was obtained from the Qingdao Ocean Chemicals.

Thirdly, for characterization of compounds, mass spectra were demonstrated by an LCQ advantage ion trap mass spectrometer. It models is Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer; NMR spectra were obtained by the AVANCE III 400 MHz Digital NMR spectrometer.

Fourthly, for analysis experiment, ultraviolet absorption spectra were measured by a Labtech UV Power PC spectrometer; Fluorescence emission spectra were recorded with the HITACHI F4600 fluorescence spectrophotometer.

Fifthly, for biological imaging, fluorescence imaging of the cells and tissues slices was obtained with Nikon A1MP two-photon confocal microscopy. Two-photon imaging was conducted on with Nikon A1MP two-photon confocal microscopy (a Chameleon Vision II: Range 680~1080nm, a repetition rate of 80 MHz.).

## 2. Quantum Yields

The fluorescence quantum yields are calculated by the following equation:

$$\Phi_s = \Phi_r \left( \frac{A_r(\lambda_r)}{A_s(\lambda_s)} \right) \left( \frac{n_s^2}{n_r^2} \right) \frac{F_s}{F_r}$$

In the above equation, **s** and **r** stand for sample and the reference, respectively; **φ** and **F** stand for quantum yield and integrated emission intensity, respectively. **A** and **n** stand for absorbance and refractive index, respectively.

**3. Calculation method of RNA and DNA concentration**

RNA and DNA concentration were calculated by follow equation. The ultraviolet absorption intensity stranded for size of the electron energy level transition probability and abides by the lambert-beer's law (1).

$$A = -\log\frac{I}{I_0} = \varepsilon cl \quad (1)$$

**A** stand for absorbance; **ε** stand for extinction coefficient, extinction coefficient of RNA is 7700, extinction coefficient of DNA is 6600; c stand for molar concentration; **l** stand for length of sample pool; $\mathbf{I_0}$ and **I** stand for intensity of the incident light and transmission light, respectively.

**4.**

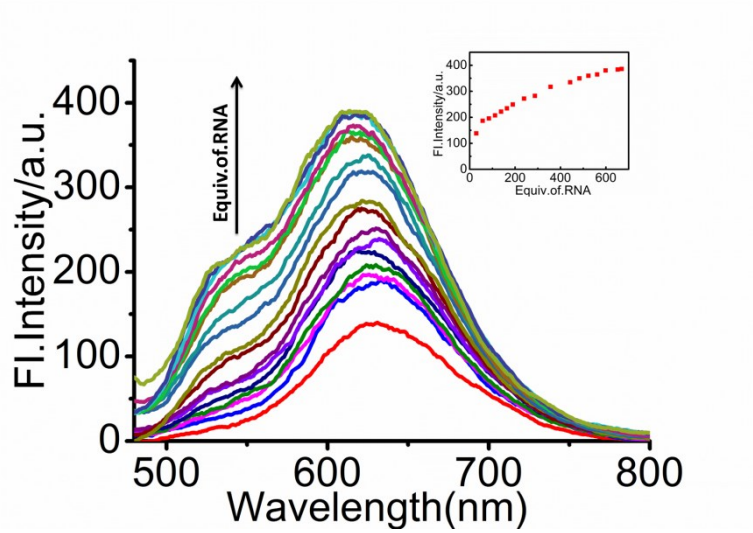


**Figure S1.** Fluorescence spectra of **BT-PHIT** upon addition of RNA in buffer solution. [**BT-PHIT**]: 1 μM. Buffer (10 mM Tris–HCl, 100 mM KCl, pH = 7.2). Inset: Fluorescence intensity changes at 630 nm of **BT-PHIT** (1 μM) with the amount of RNA. $\lambda_{ex}$ = 460 nm.

**5.**

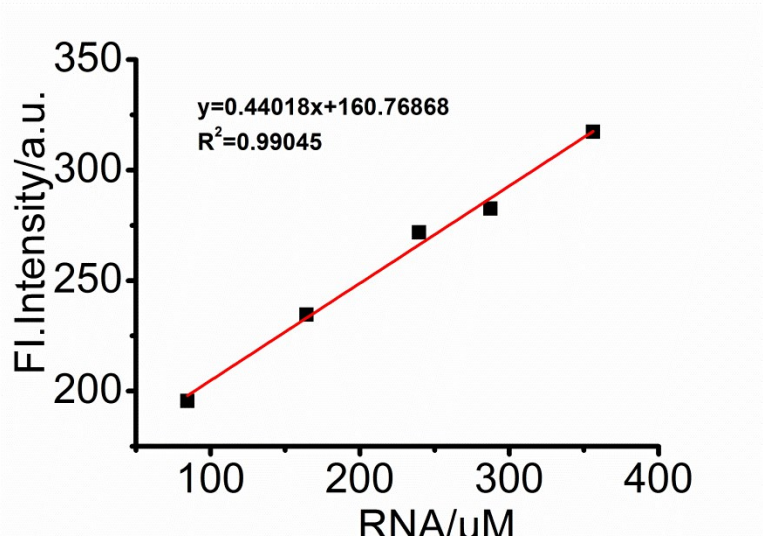


**Figure S2.** The limit of detection for RNA.

**6.**

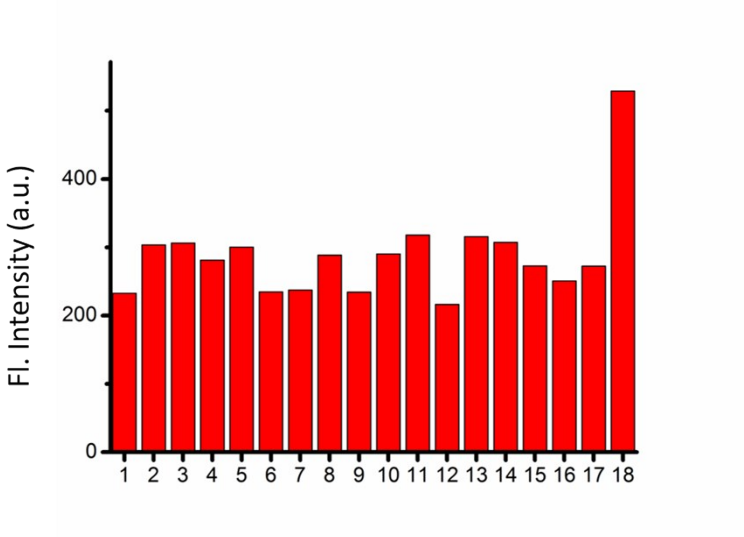


**Figure S3.** Fluorescence properties of probe in different analyses. From left to right: (1) **BT-PHIT**, (2) $Br^-$, (3) $Ca^{2+}$, (4) $Cl^-$, (5) $Cu^{2+}$, (6) Cys, (7) $Fe^{3+}$, (8) $I^-$, (9)$K^+$, (10) $Mg^{2+}$, (11) $Na^+$, (12) $Ni^+$, (13) $NO_2^-$, (14) $NO_3^-$, (15)$Zn^+$, (16) $SO_3^{2-}$, (17) $SO_4^{2-}$, (18)RNA.[ **BT-PHIT**]: 1 μM. [Cys]: 100 μM. [Other]: 1 mM.

**7.**

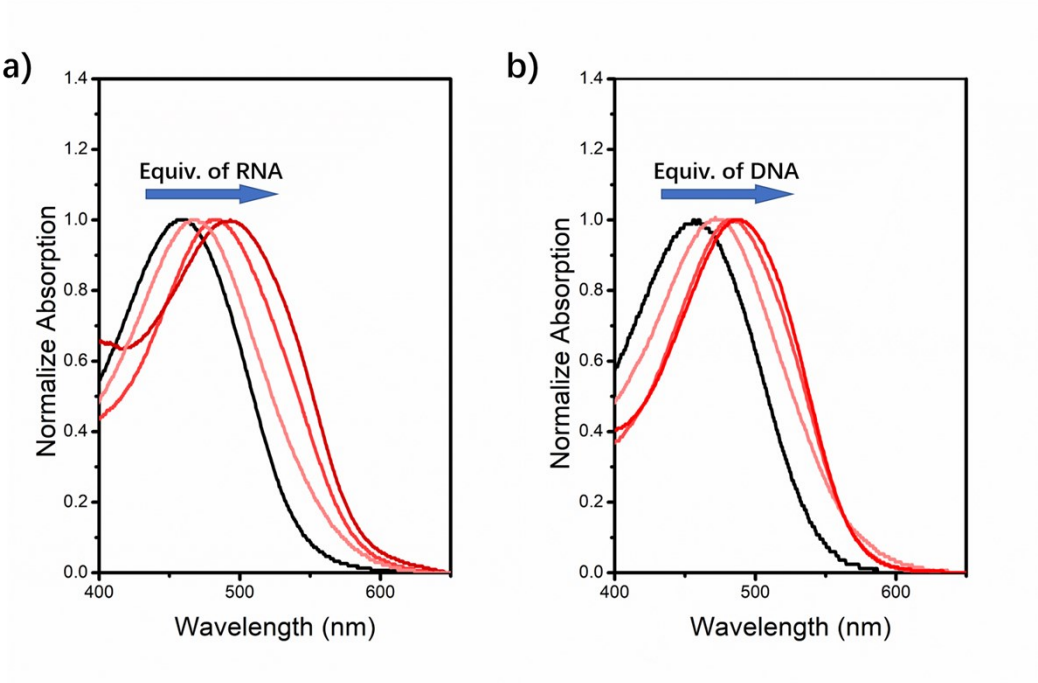


**Figure S4.** Normalize absorption spectra of **BT-PHIT** upon addition of RNA a) and DNA b) in buffer solution.

**8.**

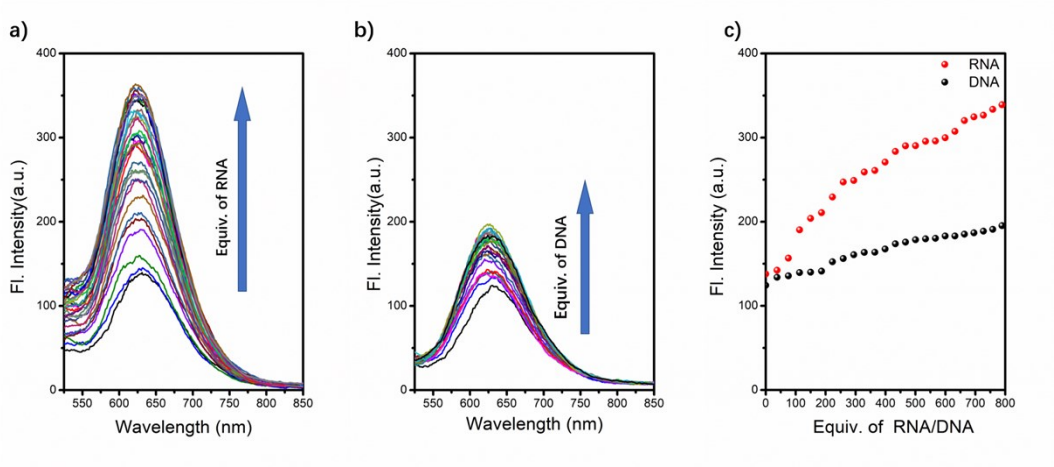


**Figure S5.** Fluorescence spectra of **BT-PHIT** upon addition of RNA a) and DNA b) in buffer solution. c): Fluorescence intensity changes at 630 nm of **BT-PHIT** with the amount of RNA and DNA. [**BT-PHIT**]: 1 μM. Buffer (10 mM Tris–HCl, 100 mM KCl, pH = 7.2). $\lambda_{ex}$ = 488 nm

**9.**

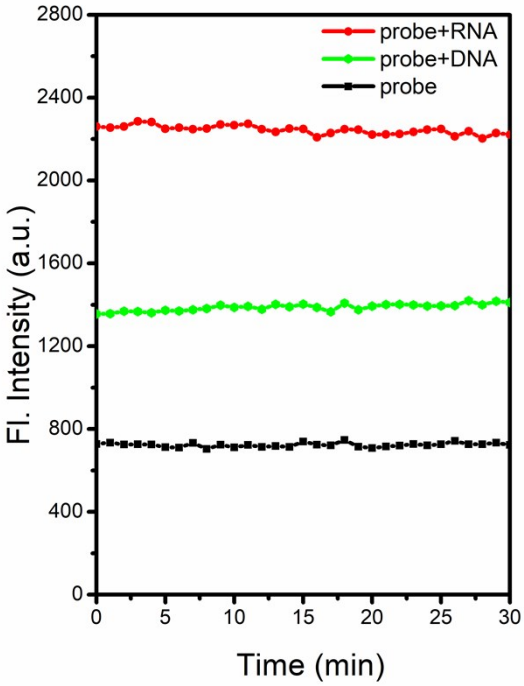


**Figure S6.** Time courses of fluorescence intensity at 630 nm of **BT-PHIT** (10 μM) containing different species in buffer solution. $\lambda_{ex}$ = 488 nm.

## 10. MTT assays

First, HeLa cells were inoculated into cell culture medium for 24 hours. The cells were then transferred to the medium containing different concentrations of probes **BT-PHIT** (0, 1, 5, 10, 20, and 30 mM). After 24 hours incubation in a constant temperature incubator, the medium containing the probe was aspirated and added with 10 μL MTT (5 mg $mL^{-1}$ in PBS). Subsequently, the culture medium was removed, and 100 μL of DMSO was added into the dishes to dissolve the formazan crystal product. Shake the plate for 10 min, and then measure the absorbance at 490 nm with a microplate reader. The cell viability (%) = ($OD_{490\ sample} - OD_{490\ blank}$)/ ($OD_{490\ control} - OD_{490\ blank}$) × 100%. $OD_{490\ sample}$ is the cells incubated with the probe for different incubation times, $OD4_{90\ control}$ denotes the cells without the probe, $OD_{490\ blank}$ stands for the wells containing only the culture medium.

**11.**

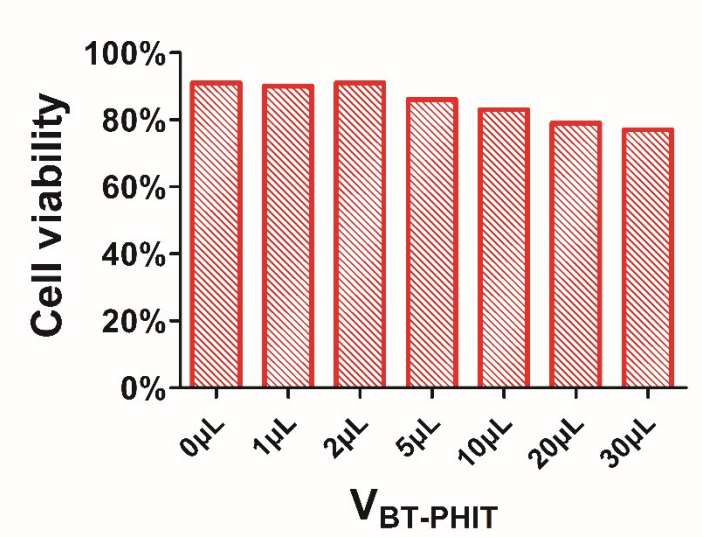


**Figure S7.** Viability of the HeLa cells treated with **BT-PHIT** stock solution at varying volume.

## 12. Characterization

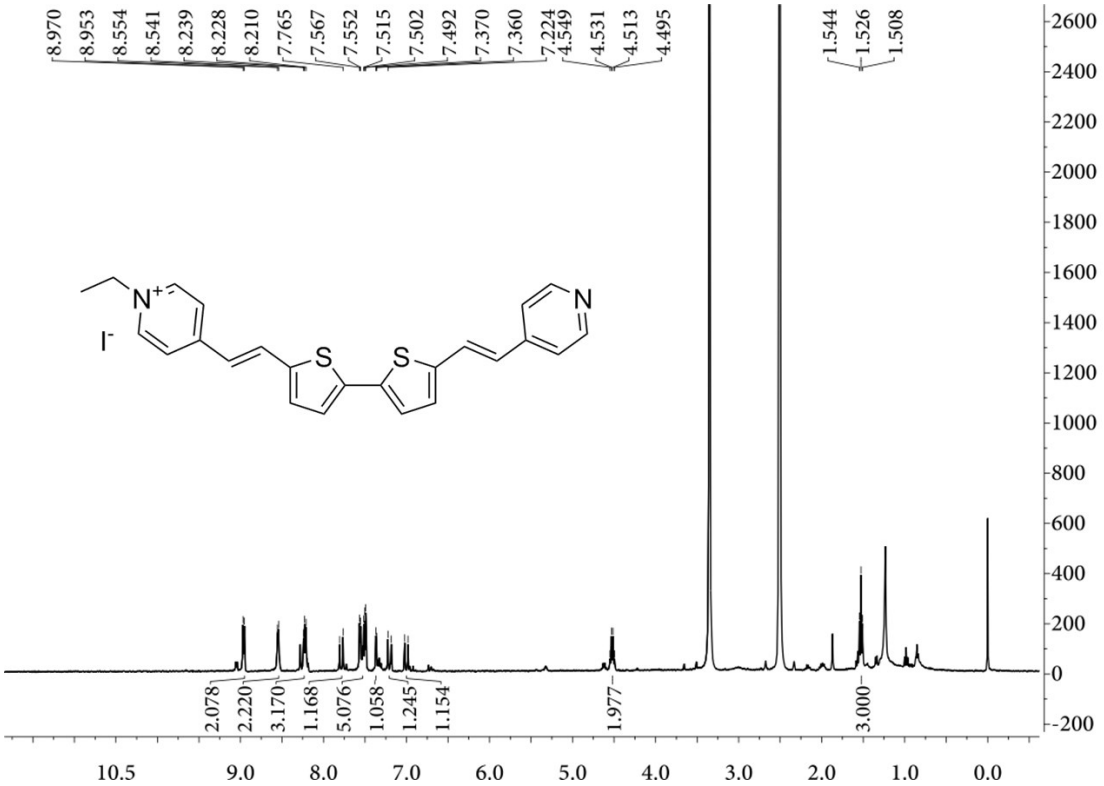


**Figure S8.** $^{1}$H NMR spectrum of the compound **BT-PHIT**

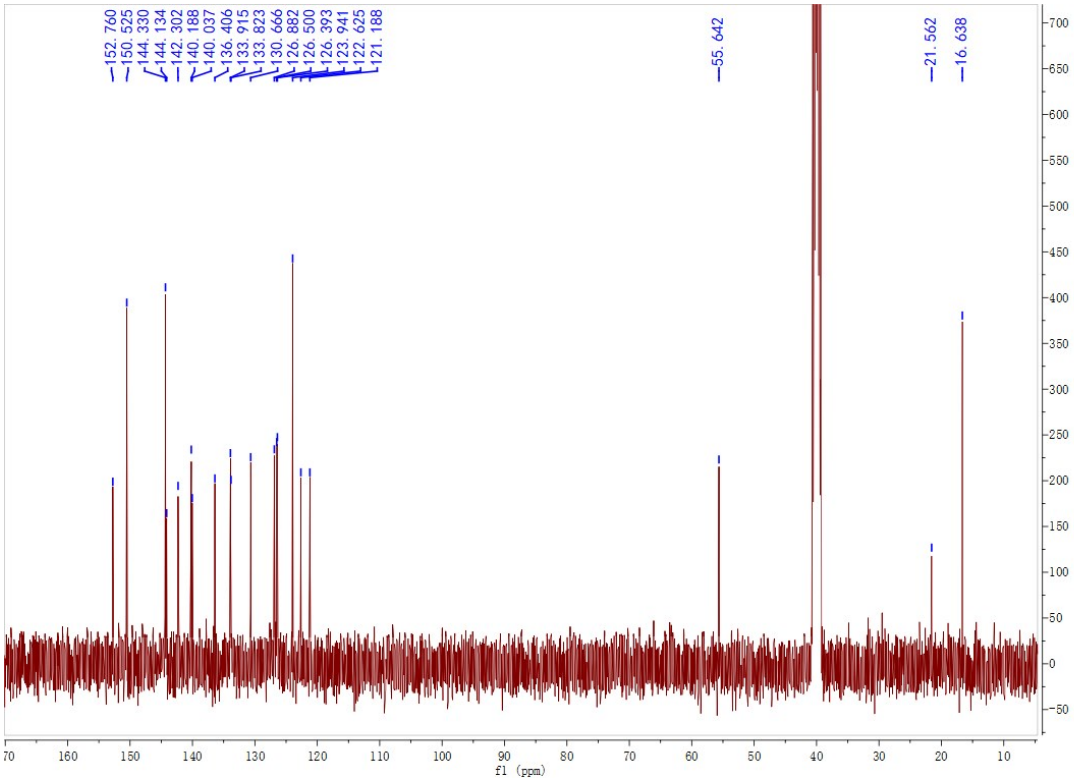


**Figure S9.** $^{13}$C NMR spectrum of the compound **BT-PHIT**

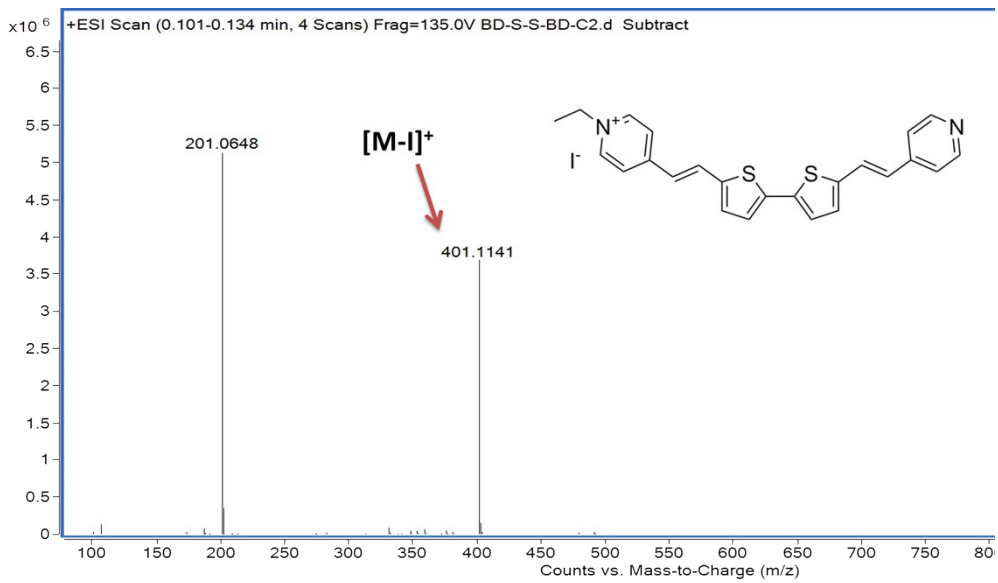


**Figure S10.** HRMS spectrum of the compound **BT-PHIT**