

Supporting Information

Intracellular temperature sensing by a ratiometric fluorescent polymer thermometer

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Computational details

The experimental structure of NBDAA was used to evaluate the influence of hydrogen bonding for its fluorescence intensity in different solvent. All DFT and TDDFT calculations were performed using the Gaussian 09 suite of ab initio programs^[1] for a hybrid meta-GGA density functional M06^[2] in conjunction with all-electron 6-31++G(d,p) basis set. The M06 functional was selected for this study because it contains long-range and dispersion corrections, which are very important for the modeling of weak non-covalent interactions.

In order to simulate the solvent environment and the influence of hydrogen bonding more reliably, we have calculated the structures of NBDAA with five chloroform molecules (NBDAA-CHCl₃) and with eighteen water molecules (NBDAA-H₂O), respectively. Both structures were fully optimized with solvent effect corrections using the integral equation formalism polarizable continuum model (IEFPCM)^[3] with Truhlar and co-workers' SMD atomic radii^[4] for chloroform ($\epsilon = 4.7113$, for the calculation of NBDAA-CHCl₃) and water ($\epsilon = 78.3553$, for the calculation of NBDAA-H₂O). An ultrafine integration grid (99,590) was used for numerical integrations. The DFT optimized ground state structures of NBDAA-CHCl₃ and NBDAA-H₂O were used for the TDDFT calculations of the absorption spectra of NBDAA-CHCl₃ and NBDAA-H₂O, respectively. The emission spectra of NBDAA-CHCl₃ and NBDAA-H₂O were simulated at the optimized structures of their first singlet excited states by using the TDDFT method.

Simulated UV-Vis spectra

The simulated UV-Vis spectra of **NBDAA-CHCl₃** and **NBDAA-H₂O** based on TDDFT calculations are shown in Figure S1. The excitation energies and the oscillator strengths of the first excited singlet states at different structures are listed in Table S1. The wavelengths of the first absorption peaks of **NBDAA-CHCl₃** and **NBDAA-H₂O** are 466 nm and 467 nm, respectively, right in the middle of the observed absorption peaks of 459 nm and 470 nm. The oscillator strength of **NBDAA-H₂O** is 0.6935 slightly smaller than **NBDAA-CHCl₃**'s oscillator strength of 0.7632. At the optimized structures of the first singlet excited states of **NBDAA-CHCl₃** and **NBDAA-H₂O**, we can see the wavelengths of their first emission peaks are 523 nm and 526 nm, respectively, which also match well with the observed emission peaks of 521 nm and 535 nm (The UV-Vis absorption spectra and the fluorescent emission spectra of **NBDAA** in **CHCl₃** and **H₂O** have been investigated and shown in Figure S2). The corresponding oscillator strengths of **NBDAA-CHCl₃** and **NBDAA-H₂O** are 0.5861 and 0.5058, respectively.

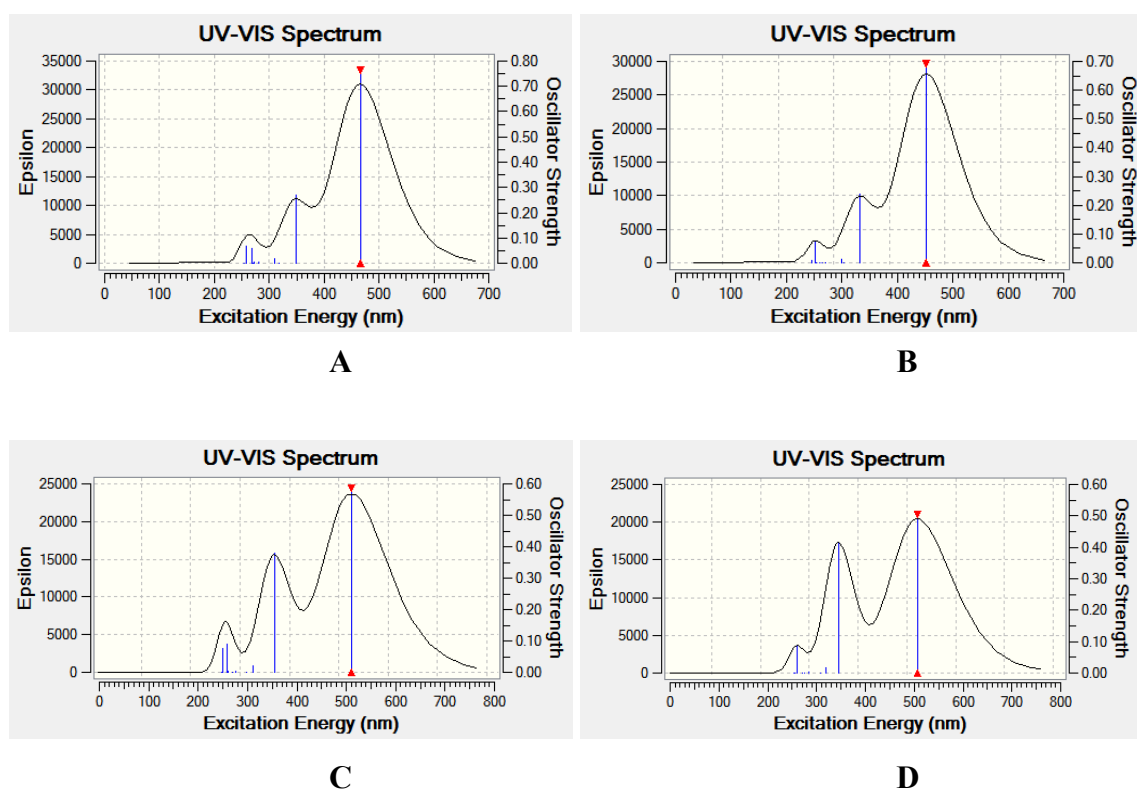


Figure S1. Simulated UV-Vis spectra of **NBDAA-CHCl₃** and **NBDAA-H₂O** based on TDDFT calculations: (A) the absorption spectrum at the optimized ground state structure of **NBDAA-CHCl₃**; (B) the absorption spectrum at the optimized ground state structure of **NBDAA-H₂O**; (C) the emission spectrum at the optimized structure of the first excited singlet state of **NBDAA-CHCl₃**; (D) the emission spectrum at the optimized structure of the first excited singlet state of **NBDAA-H₂O**.

Table S1. TDDFT calculated excitation energies and corresponding oscillator strengths of the first excited singlet states at the optimized structures.

Structure of the state		Excitation Energy (nm)	Oscillator Strength
Ground	NBDAA-CHCl₃	465.9	0.7632
	NBDAA-H₂O	466.8	0.6935
First excited singlet	NBDAA-CHCl₃	522.6	0.5861
	NBDAA-H₂O	526.4	0.5058

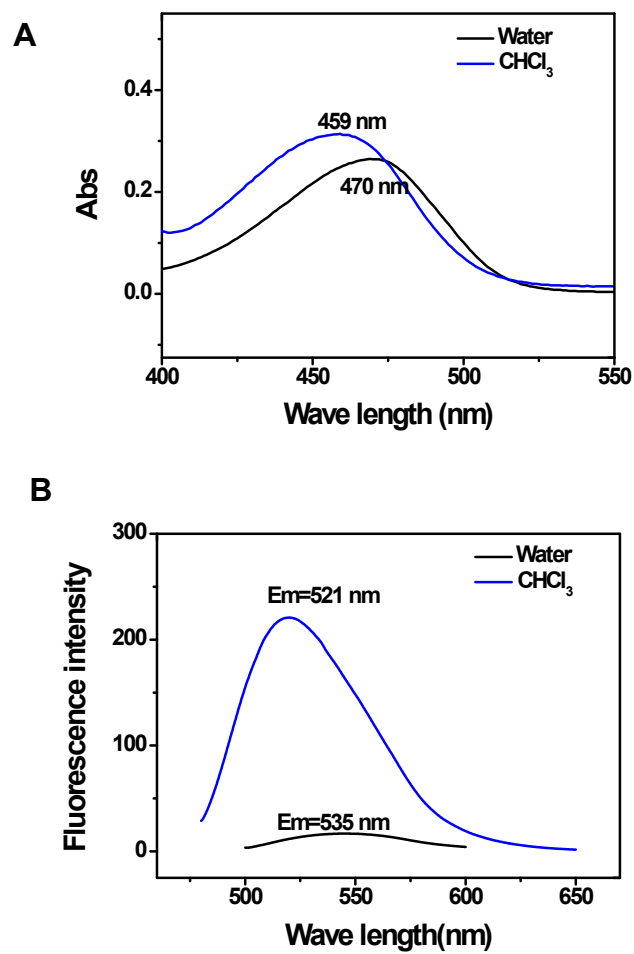


Figure S2. (A) The UV-Vis absorption spectra of NBDAA in CHCl_3 and H_2O ; (B) the fluorescent emission spectra of NBDAA in CHCl_3 and H_2O .

Frontier orbital analysis

In addition to the TDDFT simulated absorption and emission spectra, the components of the frontier orbitals of **NBDAA-CHCl₃** and **NBDAA-H₂O** also indicate the influence of hydrogen bonding. Figure S3 shows the diagrams of the highest occupied molecular orbitals (HOMOs) and the lowest unoccupied molecular orbitals (LUMOs) of **NBDAA-CHCl₃** and **NBDAA-H₂O**. We can see that the chloroform molecules have almost no contribution to the HOMO and LUMO of **NBDAA-CHCl₃**, but the water molecules have clear contributions to the HOMO and LUMO of **NBDAA-H₂O**.

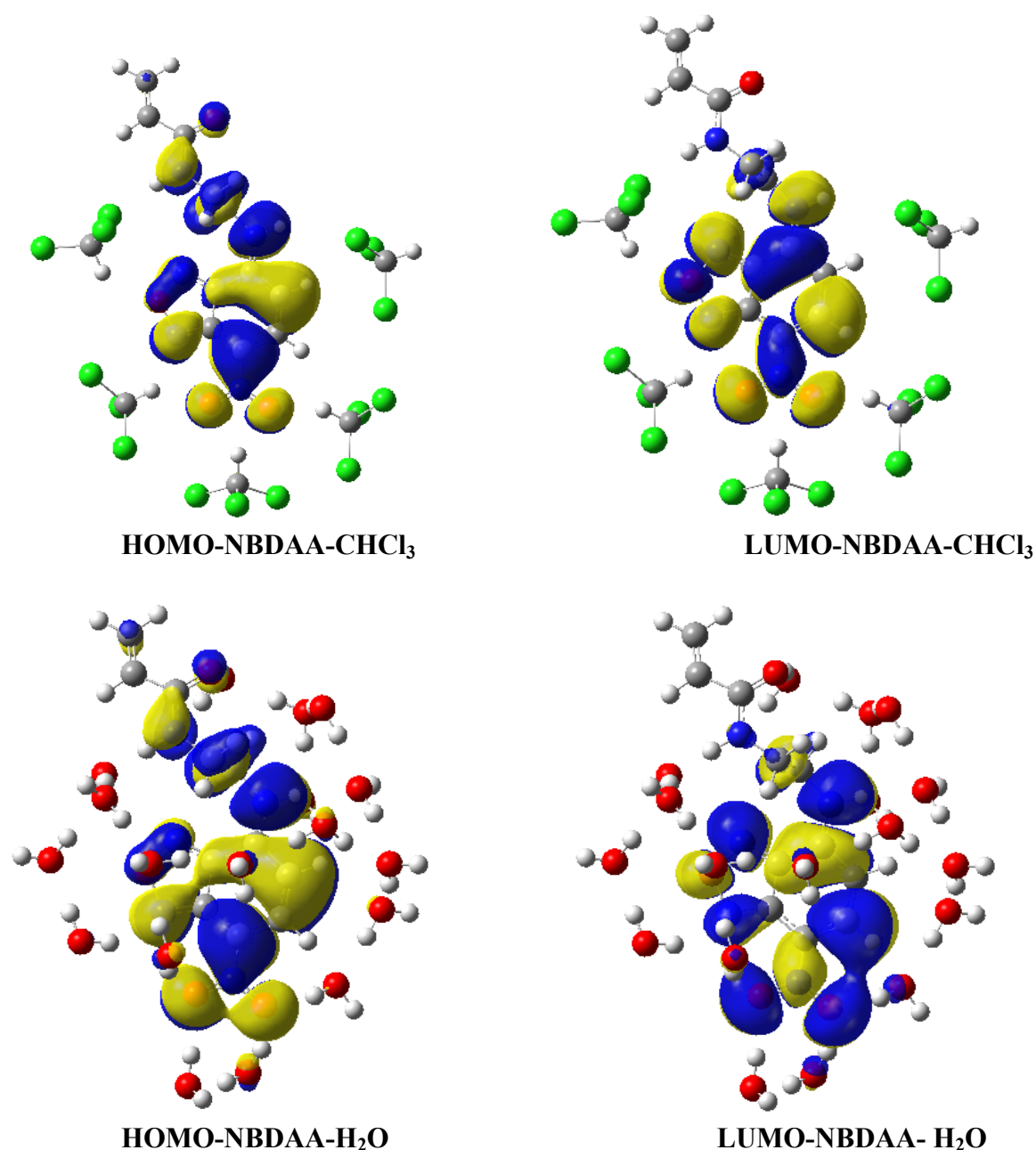


Figure S3. Molecular orbital diagrams of the HOMOs and LUMOs of **NBDAA-CHCl₃** and **NBDAA-H₂O** at their optimized ground state structures.

Synthesis of NH₂-NBD

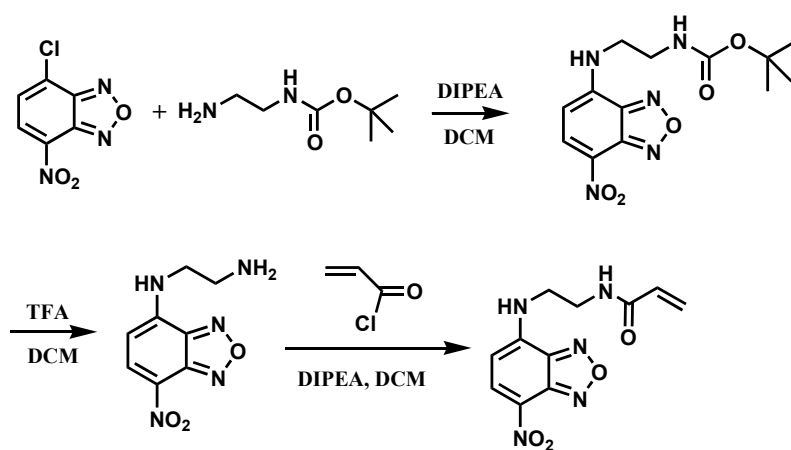


Figure S4. Synthesis of NBDAA. [5-11]

Synthesis of NBDAA

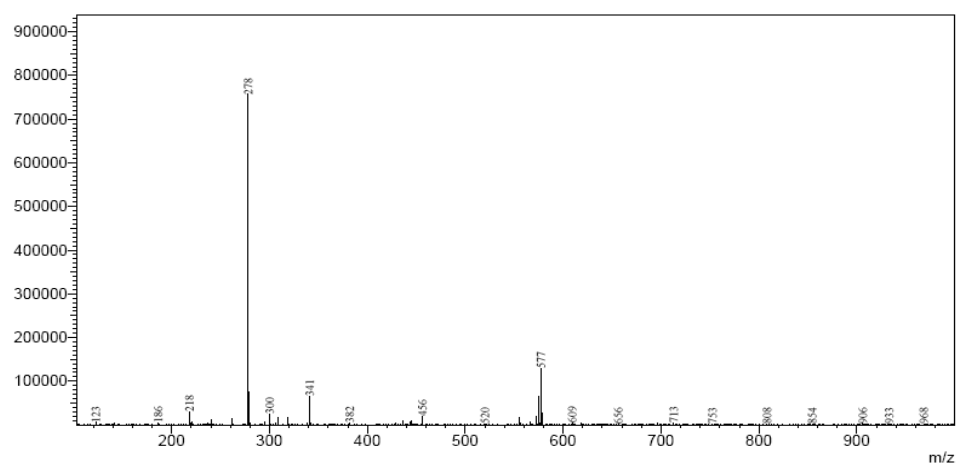


Figure S5. ESI-MS spectrum of NBDAA.

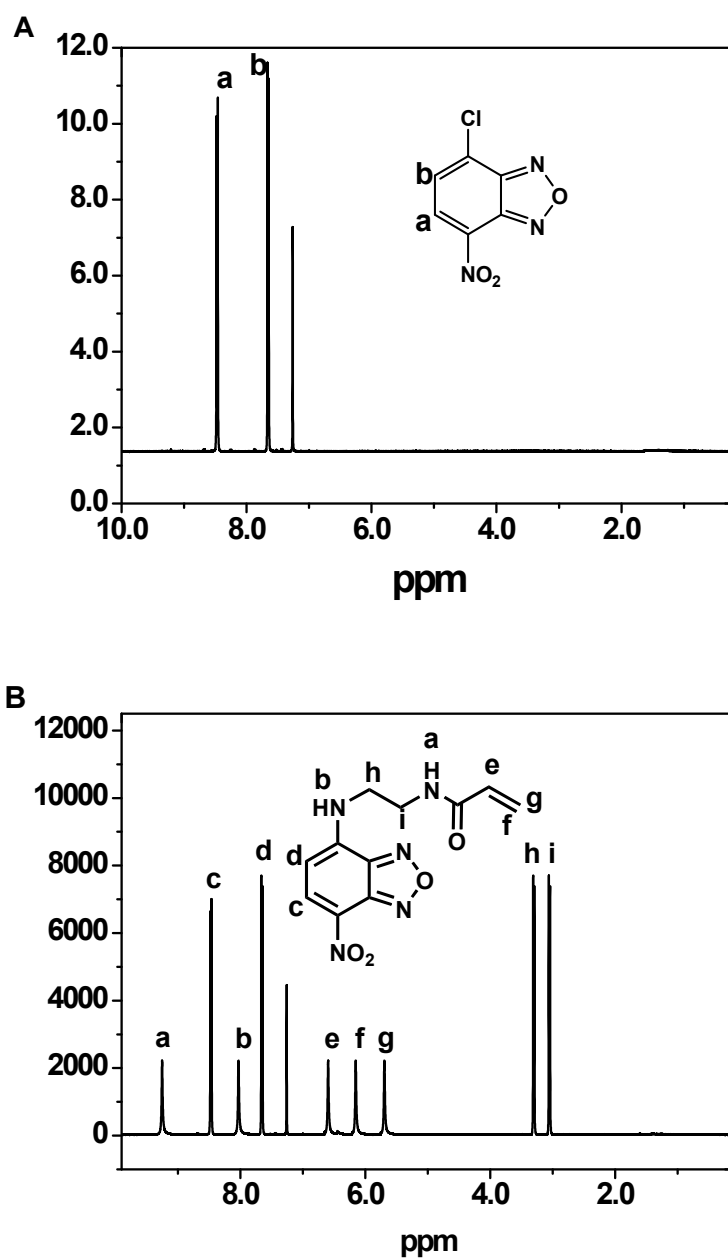


Figure S6. ^1H NMR spectrum of NBD-Cl (A) and NBDAA (B).

Synthesis of RhB hydrazide

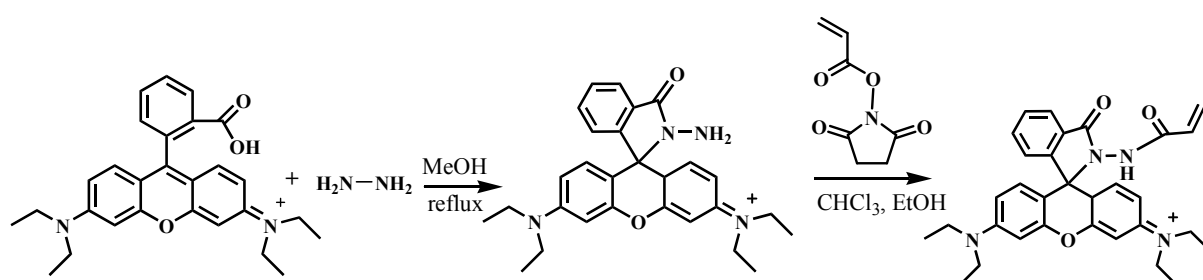


Figure S7. Synthesis of RhBAM. ^[12]

Synthesis of RhBAM

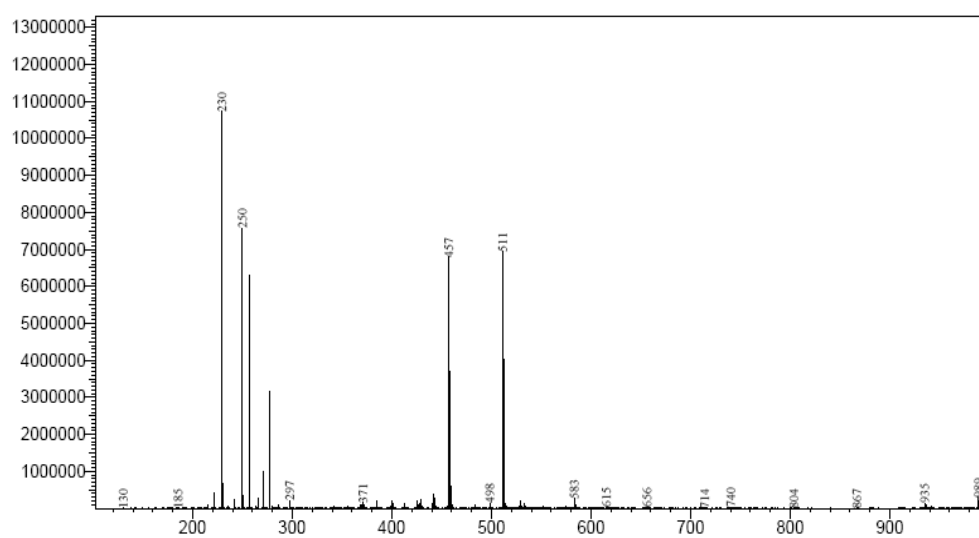


Figure S8. ESI-MS spectrum of RhBAM.

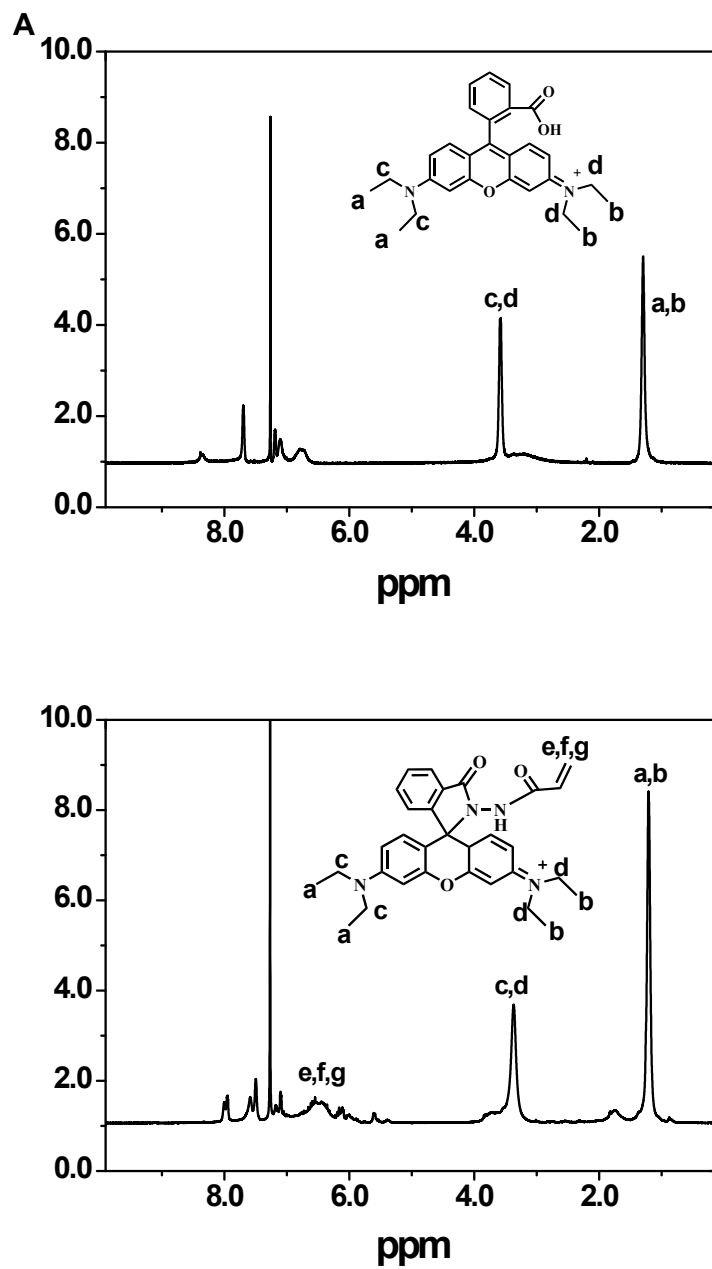


Figure S9. ^1H NMR spectrum of RhB (A) and RhBAM (B).

Polymerization of PNIPAm-*co*-NBDAA and PNIPAm-*co*-RhBAM

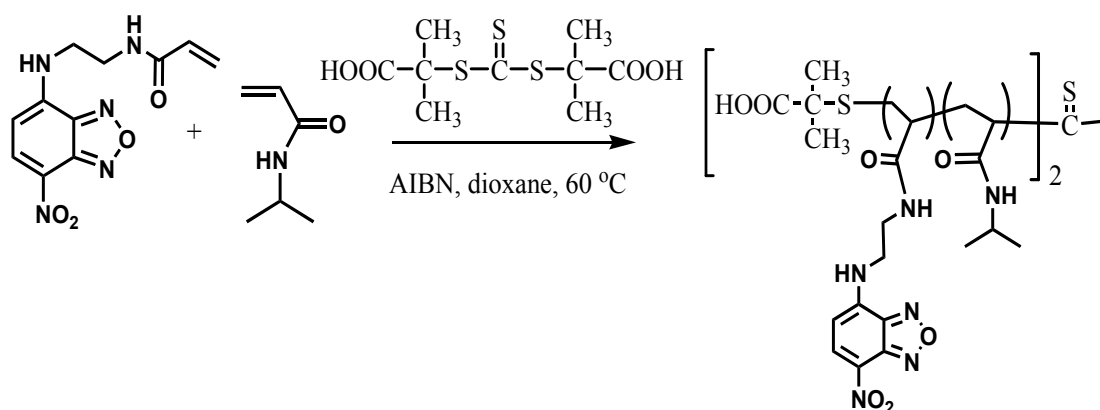


Figure S10. Polymerization of PNIPAm-*co*-NBDAA.^[13-17]

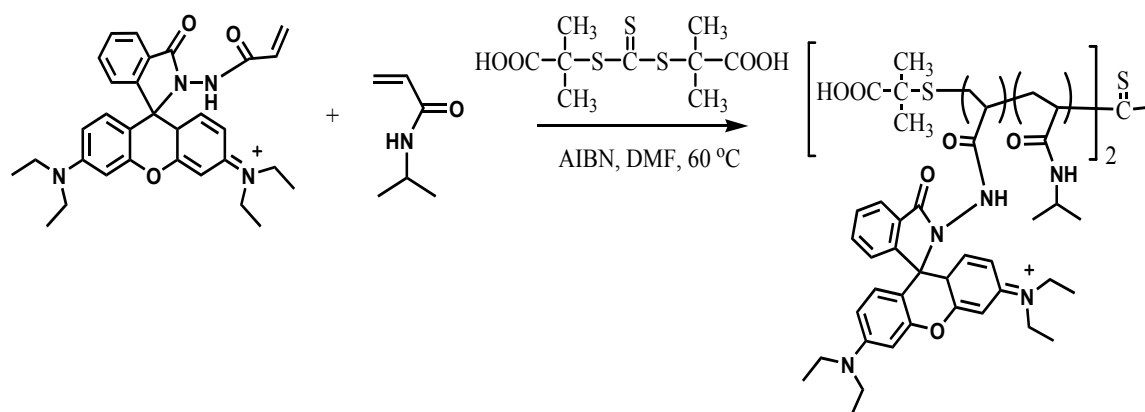


Figure S11. Polymerization of PNIPAm-*co*-RhBAM.^[18-28]

Table S2. Physical properties of the polymers.

Polymers	PNIPAM-<i>co</i>-NBDAA	PNIPAM-<i>co</i>-RhBAM
Solvent	Dioxane	DMF
Polymerization time (h)	24	24
Polymerization temperature (°C)	60	60
Mn	11062 ^[a]	9896 ^[a]
PDI	1.3	1.4

^[a] Mn was obtained by GPC (Gel Permeation Chromatography)

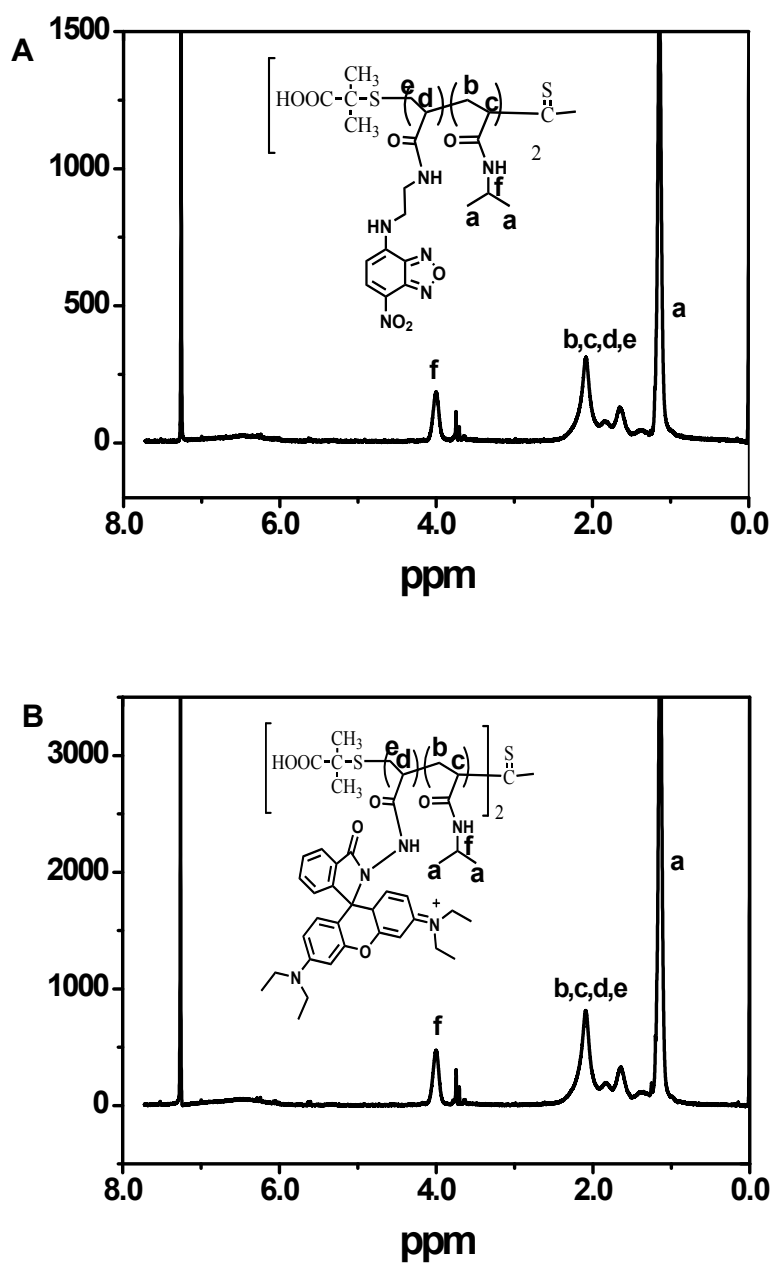


Figure S12. ^1H NMR spectrum of PNIPAm-*co*-NBDAA (A) and PNIPAm-*co*-RhBAM (B).

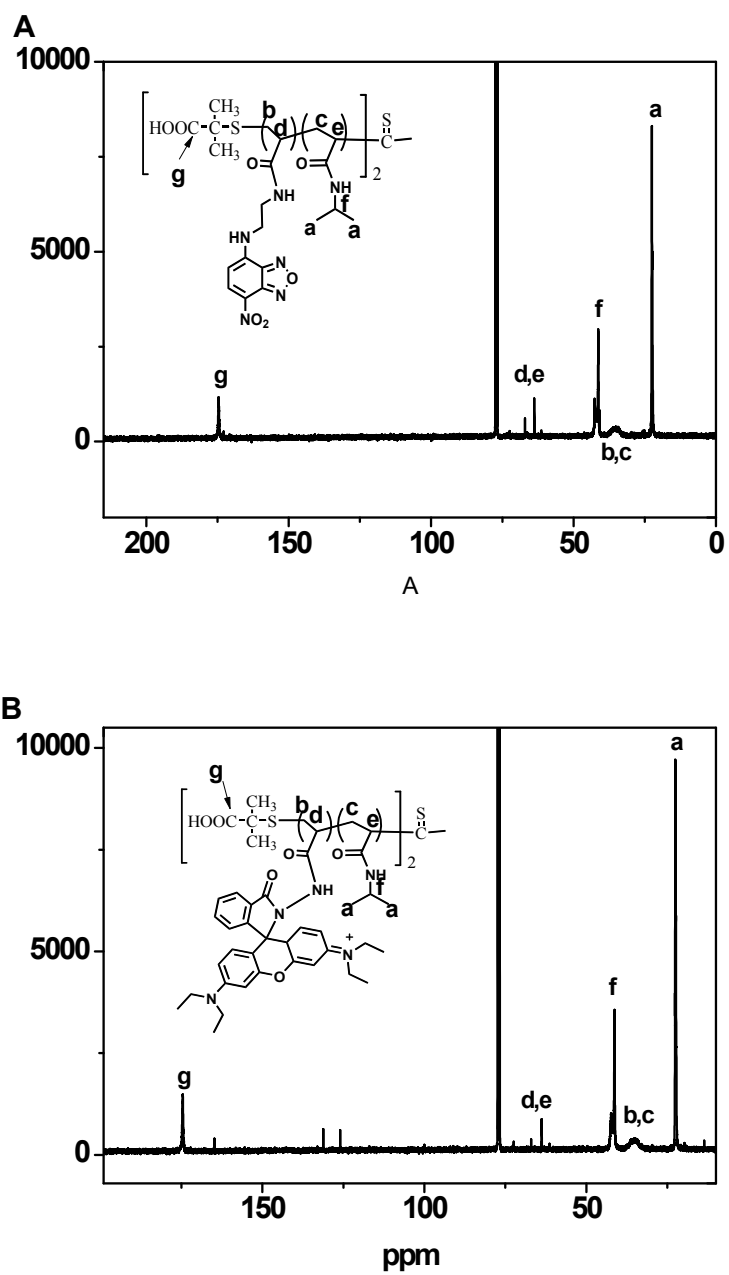


Figure S13. ^{13}C NMR spectrum of PNIPAm-*co*-NBDAA (A) and PNIPAm-*co*-RhBAM (B).

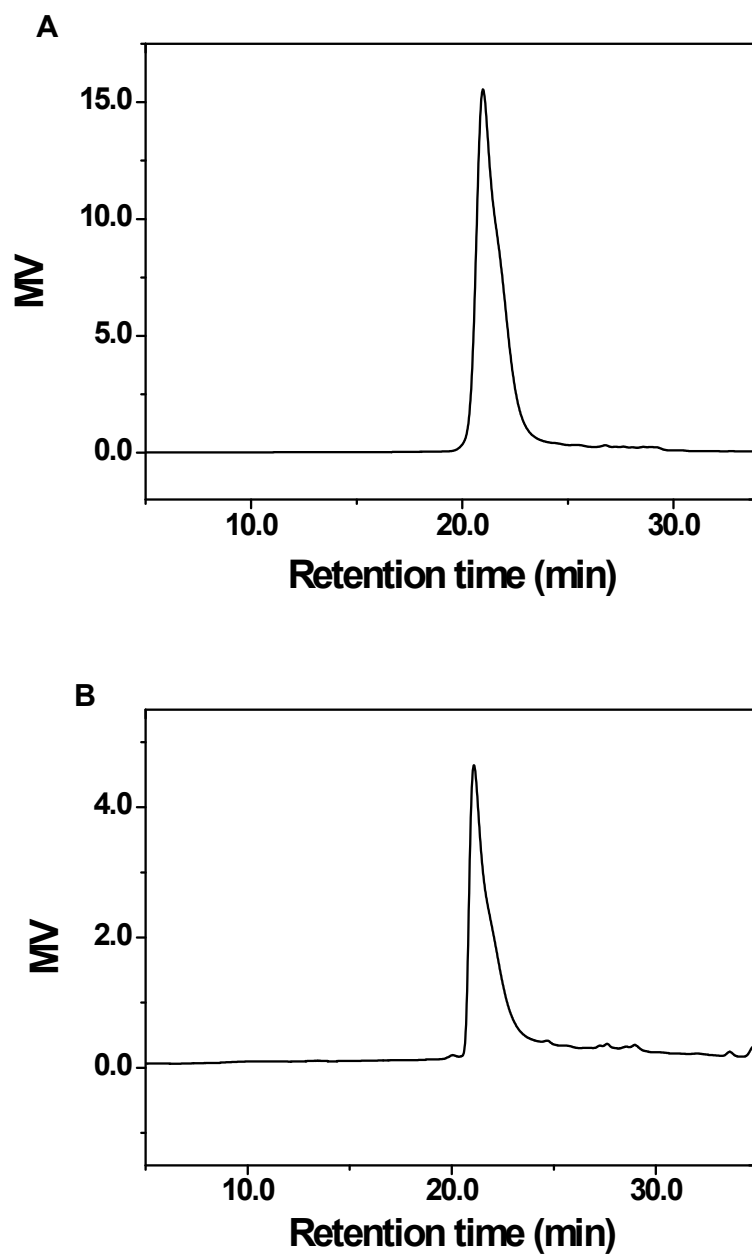


Figure S14. GPC spectrum of PNIPAm-*co*-RhBAM (A) and PNIPAm-*co*-NBDAA (B).

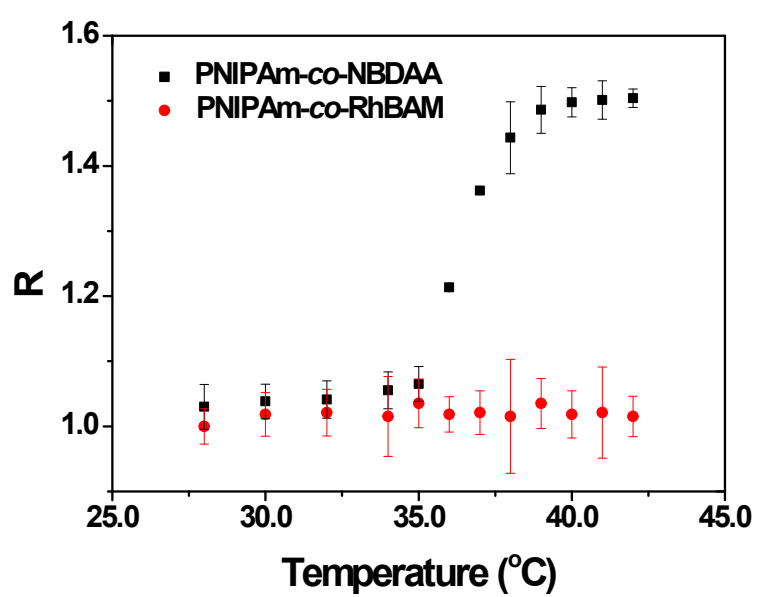


Figure S15. Temperature-responsive study of PNIPAm-co-RhBAM and PNIPAm-co-NBDAA between 28 °C and 42 °C.

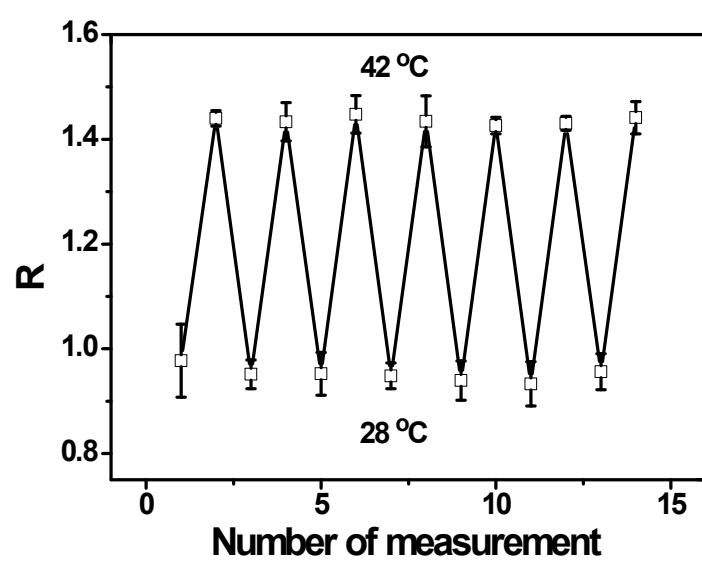


Figure S16. Temperature-responsive reversibility study of RFPs between 28 °C and 42 °C.

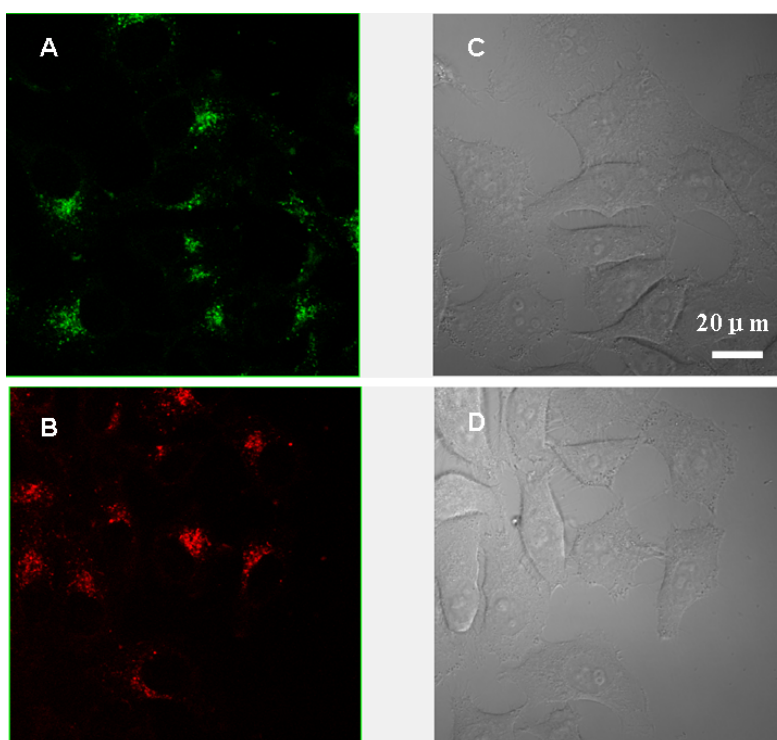


Figure S17. Fluorescent images of RFPs loaded HeLa cells obtained from different channel: NBDA channel (A) and RhBAM channel (B), respectively; (C) and (D) are the corresponding differential interference contrast images of (A) and (B).

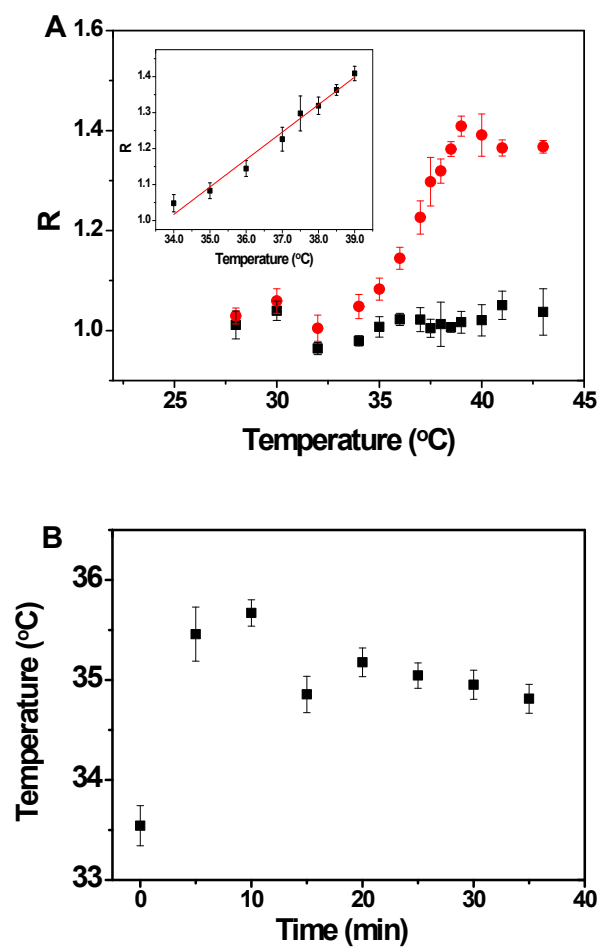


Figure S18. (A) The intracellular fluorescent intensity ratios changing with temperature increasing. Insert figure: intracellular temperature responsive calibration curve of RFPs obtained from the fluorescence spectra. (B) Changes in the temperature after the additions of FCCP in HeLa cells calculated from the fluorescence spectra.

Table S3. Comparison of temperature sensing range and resolution

Nano thermometer	Temperature controller	Temperature sensing range	Temperature sensing resolution	Ref.
Hydrophilic fluorescent nano-gel	a stage plate heater (Tokai Hit)	27~40 °C	0.29~0.50 °C	C. Gota, <i>et al</i> , <i>J. Am. Chem. Soc.</i> 2009, 131 , 2766-2767
CdSe-QDs nano-particles	an open oven	30~70 °C	1.0 °C	L. M. Maestro, <i>et al</i> , <i>Nano Lett.</i> 2010, 10 , 5109-5115.
NaYF₄:Er³⁺,Yb³⁺ nano-particles	an electric power dissipated with a resistor	26~63 °C	1.0 °C	F. Vetrone, <i>et al</i> , <i>ACS Nano</i> 2010, 4 , 3254-3258.
Fluorescent polymeric nano-thermometer	a stage plate heater (Tokai Hit)	29~39 °C	0.18~0.58 °C	K. Okabe, <i>et al</i> , <i>Nat. Commun.</i> 2012, 3 , 705.
Ratiometric fluorescent polymer nano-thermometer	incubation system for microscope (WSKM, Tokai hit)	25~50 °C	0.3~0.5 °C	This work

References:

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