Supplementary Information

Heat and light dual switching of a single-walled carbon nanotube/thermo-responsive helical polysaccharide complex: a new responsive system applicable to photodynamic therapy

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Materials

Curdlan, copper (I) chloride (CuCl, 99.9 %), 4-dimethylaminopyridine (DMAP), L(+)-ascorbic acid, copper (II) bromide ($CuBr_2$) and propylamine were purchased from Wako Pure Chemical Ltd. *N*-Isopropylacrylamide (NIPAM), 2-propyn-1-ol, 2-chloropropionic acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were obtained from Tokyo Chemical Industry Co. NIPAM was recrystallized twice from benzene/*n*-hexane (4:15 v/v). Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) and poly(N-isopropylacrylamide) (pNIPAM, Mw = 1.0-1.5 × 10^4) for use of reference experiments were obtained from Sigma-Aldrich Co. Alumina (activated, neutral) was bought from Merk KGaA. Single-walled carbon nanotube (SWNT) was purchased from Carbon Nanotechnologies, Inc. and was purified by removal of metallic catalyst according to a literature.[S1] Synthesis of 6-azido-6-deoxy Cur (Cur-N_3) was conducted according to the method that we developed.[S2]

Synthesis

Akyne-terminated poly(*N*-isopropylacrylamide) (pNIPAM-alkyne) was synthesized through an atom transfer radical polymerization (ATRP) method.[S3]

Propargyl 2-chloropropionate (PCP) [S3,S4]

In 25 mL of CH_2Cl_2, 2-chloropropionic acid (4.3 mL, 50 mmol) was dissolved. After cooling with an ice bath, DMAP (0.5 g, 4 mmol) and 2-propyn-1-ol (3.0 mL, 50 mmol) were added. A solution of EDC (10.6 g, 55 mmol) in 250 mL of CH_2Cl_2, which was cooled with an ice baht, was slowly added. The reaction mixture was stirred at 0 °C and allowed to room temperature. After stirring for 24 h, the solvent was evaporated to dryness. The obtained oil was dissolved in 200 mL of ethyl acetate and washed with 200 mL of 0.5 M HCl *aq.* five times and 5 wt% $NaHCO_3$ *aq.* five times. The separated organic layer was dried over anhydrous sodium sulfate. After filtration, the solvent was evaporated to dryness and PCP was obtained as pale yellow oil (5.49 g, 81 %). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 4.78 (t, 2H), 4.44 (q, 1H), 2.52 (t, 1H), 1.72 (d, 3H) ppm.

pNIPAM-alkyne [S3,S5]

The general procedure to synthesize pNIPAM-alkyne was as follows; NIPAM (3.6g, 32 mmol) and Me_6TREN (0.54 mL, 2.0 mmol) were dissolved in 10 mL of 2-propanol and the mixture was deoxygenated by bubbling with argon for 30 min. To the solution, CuCl (198 mg, 2.0 mmol) was added under protection of argon flow. The reaction mixture was stirred for 30 min and PCP (0.29 g, 2.0 mmol) in 2 mL of 2-propanol that was deoxygenated by 30 min bubbling with argon was mixed. The resultant solution was stirred at 40 °C for 5 h. The reaction was stopped by exposure to air. The mixture was precipitated into an excess of *n*-hexane. The solid product was recovered by filtration and redissolved in CH_2Cl_2. To remove copper catalysts, the solution was passed through a neutral alumina column using CH_2Cl_2 as eluent. The collected eluent was concentrated and precipitated into an excess of diethyl ether. This purification cycle was repeated three times. Drying of the product in vacuo afforded a colourless solid (0.54 g, 15 %). FT-IR (powder, ATR): ν_{max}/cm^{-1} 3434, 3287, 3080, 2972, 2932, 2876, 1636, 1540, 1458, 1387, 1366, 1172, 1130, 1040, 1025, 969, 929, 882, 836; SEC (Shodex GF-710, DMF, 40 °C, polystyrene standard) Mn = 2.9×10^3, degree of polymerization (DP) ~ 26, Mw/Mn = 1.15). [S6]

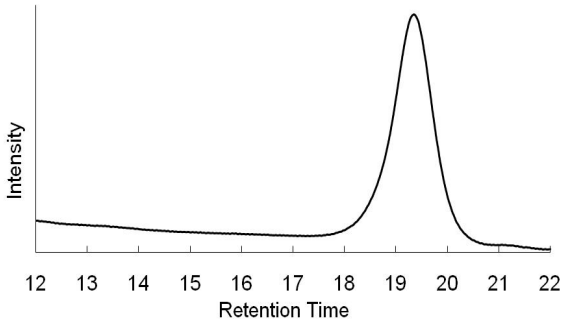


Chart S1 SEC chromatogram of pNIPAM-alkyne.

Cur-pNIPAM[S2]

Cur-N_3 (0.050 g, 0.54 mmol (monomer unit)) and pNIPAM-alkyne (0.90 g) was dissolved in 10 mL of DMSO. To the solution, $CuBr_2$ (4.8 mg), ascorbic acid (19 mg), propylamine (0.25 mL) and water (0.25 mL) were added. The reaction mixture was stirred at 40 °C and the reaction was monitored by FT-IR measurements until a peak at 2107 cm^{-1} ascribable to the N_3 group disappeared. After 24 days, the product was purified by dialysis against distilled water (MWCO 8000). Freeze-dry treatment afforded a colourless solid (0.76 g). FT-IR (powder, ATR): ν_{max}/cm^{-1} 3282, 3076, 2972, 2931, 2876, 1636, 1542, 1458, 1387, 1367, 1276, 1171, 1131, 1079, 1039, 1027, 980, 929, 882, 840; SEC (Shodex OHpak SE-806M HQ, DMSO containg 5 mM LiBr, 40 °C, pullulan standards) Mn = 1.4×10^5 (Mw/Mn = 1.58).[S7,S8]

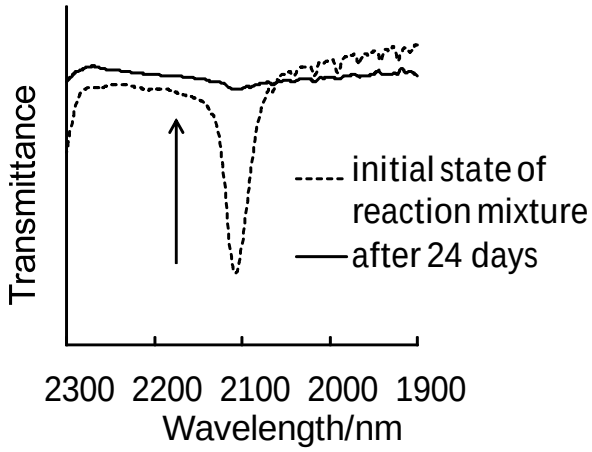


Chart S2 FT-IR spectra of the reaction mixture soon after mixing and after 24 days.

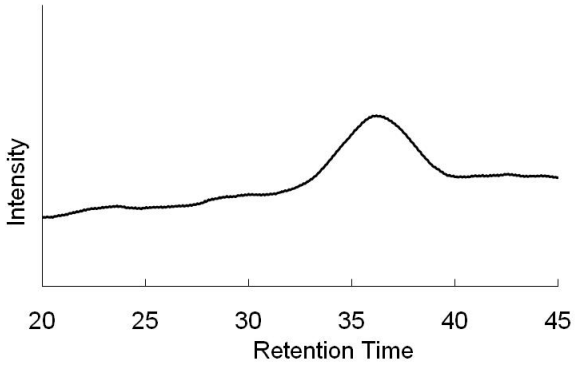


Chart S3 SEC chromatogram of Cur-pNIPAM.

Instruments

Ultrasonication was performed by using a SMT UH-50 with an ice bath. For centrifugation, TOMY MX-300 and Eppendorf Centrifuge 5415R were employed. ^{1}H NMR spectra were obtained on a JEOL JNM-ECS400. UV/Vis/NIR absorption was recorded on a JASCO V-670 spectrophotometer with a Peltier type thermostatic cell holder. Fluorescence spectroscopy was conducted on a Perkin Elmer LS55 luminescence spectrometer and temperature was controlled by a thermostatic bath. The path length of the quartz cells was 1 mm. FT-IR spectra were collected on a JASCO FT/IR-4200 with an ATR PRO450-S (Diamond). For transmission electron microscopy (TEM), a JEOL JEM-2010(acceleration voltage 120 kV) was used and hydrophilic TEM girds (carbon-coated copper mesh grids, Okenshoji Co.) were prepared by treatment with a Vacuum Device PIB-10 for 30 seconds. Surelite I (Nd:YAG laser, Continuum Inc.) was utilized for NIR laser irradiation (λ = 1064 nm, 10 Hz, 240 mW). The laser power was measured by OPHIR AN/2.

Sample Preparation

A stock solution of Cur-pNIPAM was prepared with a concentration of 50 mg/mL in pure water. SWNT wrapped with Cur-pNIPAM (SWNT/Cur-pNIPAM complex) was prepared by a following method; SWNT 1.0 mg was immersed in 1.8 mL pure water and sonicated in an ice bath for 10 min. Aqueous Cur-pNIPAM (0.2 mL) was added in the solution and the mixture was sonicated for 30 min in the ice bath. Undispersed portion was removed by centrifugation with 20,000 *g* at 20 °C for 15 min twice. As the insoluble carbon mass was removed after centrifugation (which was precisely weighed), one can estimated that the final solution used for the experiments consists of 0.39 mg/mL of SWNT and 5 mg/mL of Cur-pNIPAM. DHP was dissolved in DMSO and the solution was mixed with aqueous SWNT/Cur-pNIPAM complex in an ice bath to obtain the DHP and SWNT/Cur-pNIPAM complex mixture. The mixed solutions were left overnight at room temperature before measurements. A final concentration of DHP was 25×10^{-6} M and the mixing ratio of water and DMSO was 97.5:2.5 (v/v). TEM samples were prepared by the following methods. On a carbon-coated copper mesh grid, one drop of a sample solution was placed. After 10 minutes standing, the solution was absorbed with filter paper and the grid was dried in vacuo. The TEM samples in the heated condition were prepared as follows; the SWNT/Cur-pNIPAM complex solution was heated at 40 °C and was placed on a heated TEM grid after vigorous shaking to break the large aggregate into small pieces. After absorbing the residual liquid with filter paper, the sample was further dried at 40 °C.

Notes and References

S1 D. M. Guldi, H. Taieb, G. M. A. Rahman, N. Tagmatarchis and M. Prato, *Adv. Mater.*, **2005**, *17*, 871.

S2 (a) T. Hasegawa, M. Umeda, M. Numata, C. Li, A. H. Bae, T. Fujisawa, S. Haraguchi, K. Sakurai and S. Shinkai, *Carbohydr. Res.*, **2006**, *341*, 35; (b) T. Hasegawa, M. Umeda, M. Numata, T. Fujisawa, S. Haraguchi, K. Sakurai and S. Shinkai, *Chem. Lett.*, **2006**, *35*, 82.

S3 J. Xu, J. Ye and S. Liu, *Macromolecules*, **2007**, 4*0*, 9103.

S4 N. V. Tsarevsky, B. S. Sumerlin and K. Matyjaszewski, *Macromolecules*, **2005**, *38*, 3558.

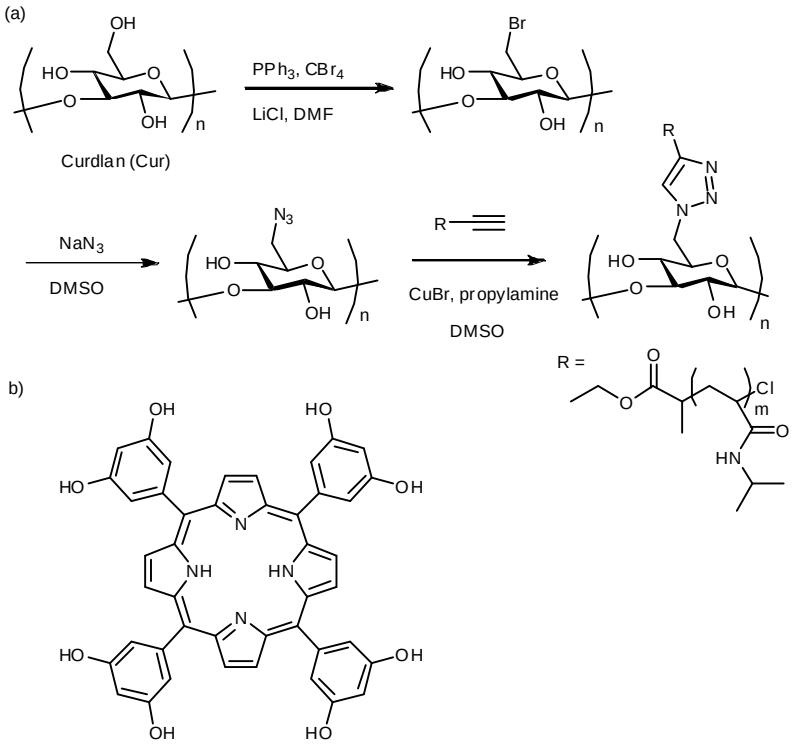
S5 Y. Xia, X. Yin, N. A. D. Burke and H. D. H. Stöver, *Macromolecules*, **2005**, *38*, 5937.

S6 The appearance of a proton signal from -CH_2- next to the alkyne group (δ = 4.66 ppm) was confirmed by ^{1}H NMR.

S7 In a ^{1}H NMR spectrum, Cur-pNIPAM showed peaks at δ = 4.74, 5.36 and 8.20 ppm ascribable to -CH_2- next to the triazole ring, -OH in the sugar moiety and H-triazole, respectively. In addition, an IR peak at 1079 cm^{-1} ascribable to the C-O stretching vibration characteristic in the sugar group appeared in the product. The SEC experiment indicates that the product was isolated as a single component. Moreover, the IR peak for the N_3 group in Cur-N_3 disappeared after the click reaction and the resultant product had the molecular weight much higher than those of Cur-N_3 and pNIPAM alkyne. These results are entirely coincident with the proposed structure.

S8 The molecular weight of Cur-N_3 was estimated by SEC in the same conditions to be 3.6×10^4.

Scheme S1 (a) Synthetic route of Cur-pNIPAM and (b) chemical structure of DHP.



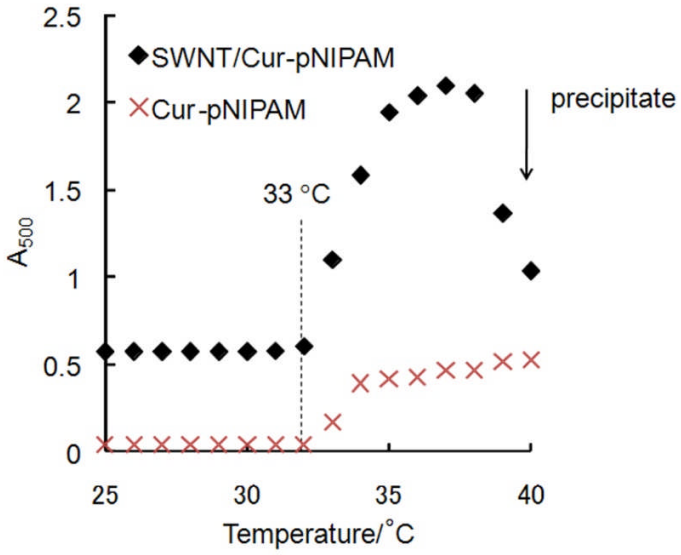


Fig. S1 Temperature dependence of absorbance at 500 nm (A_{500}) in SWNT/Cur-pNIPAM complex and Cur-pNIPAM itself in water; 0.2 °C/min, from 25 °C to 40 °C. Upon cooling, a drastic absorbance change was observed at 33 °C as well as that in the heating process.

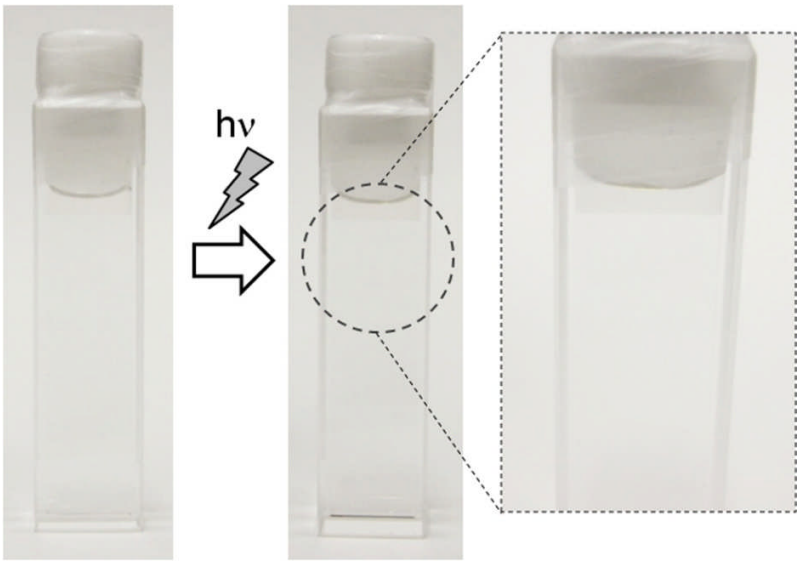


Fig. S2 Photos of Cur-pNIPAM itself in water before or after NIR laser irradiation: Nd:YAG laser 1064 nm, 240 mW, 60 minutes, room temperature.

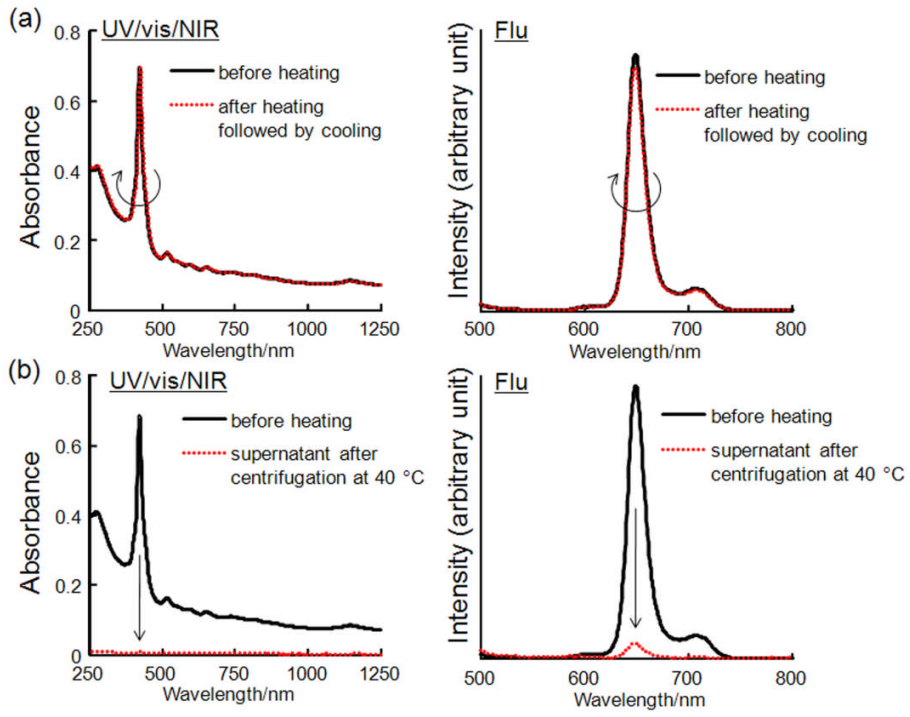


Fig. S3 UV/Vis/NIR absorption and fluorescence spectra (left side and right side, respectively) for the DHP and SWNT/Cur-pNIPAM complex mixture in water containing 2.5 vol.% DMSO; (a) solutions before heating and after heating followed by cooling (Fig. 3a) and (b) a solution before heating and a supernatant collected after centrifugation with 15,000 g for 15 minutes at 40 °C (Fig. 3a and 3d); λ_{ex} = 422 nm, [DHP] = 25×10^{-6} M.

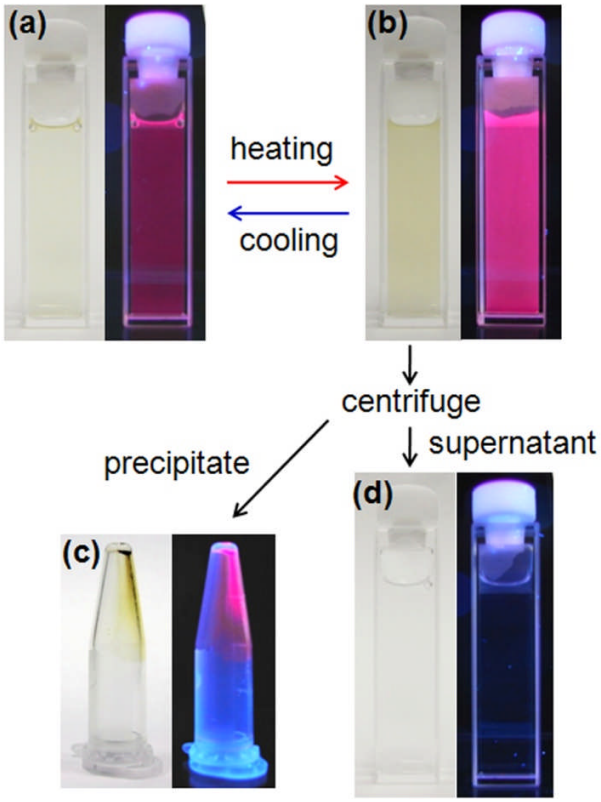


Fig. S4 Photos of the DHP and pNIPAM aqueous mixture (a) at 25 °C, (b) at 40 °C, (c) solid portion collected by centrifugation at 40 °C and (d) supernatant after centrifugation at 40 °C. Left: bright image, right: fluorescence image (λ_{ex} = 365 nm), [DHP] = 25×10^{-6} M.

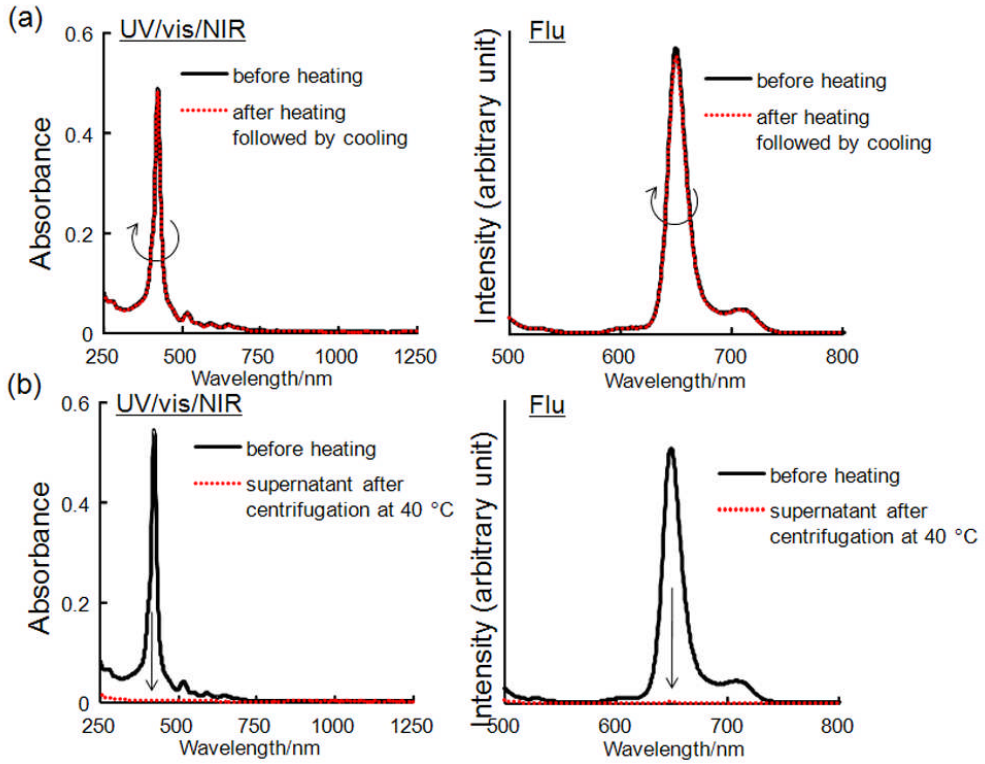


Fig. S5 UV/Vis/NIR absorption and fluorescence spectra (left side and right side, respectively) for the DHP and pNIPAM mixture in water containing 2.5 vol.% DMSO; (a) solutions before heating and after heating followed by cooling (Fig. S4a) and (b) a solution before heating and a supernatant collected after centrifugation with 15,000 g for 15 minutes at 40 °C (Fig. S4a and S4d); λ_{ex} = 422 nm, [DHP] = 25×10^{-6} M.

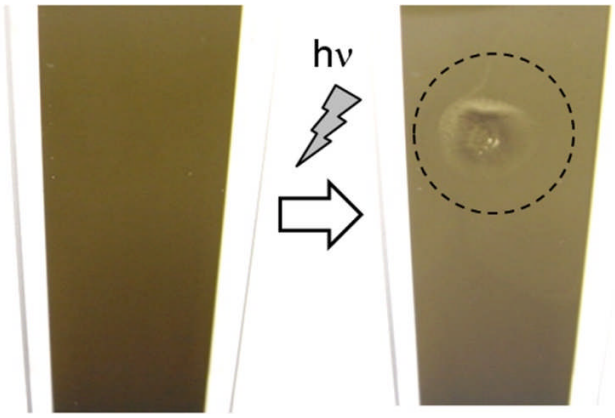


Fig. S6 Photos of the DHP and SWNT/Cur-pNIPAM complex mixture in water containing 2.5 vol.% DMSO before or after NIR laser irradiation: Nd:YAG laser 1064 nm, 240 mW, 15 minutes, room temperature.