Supporting Information

Photoreleasable Thiol Chemistry for Facile and Efficient Bioconjugation

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1 Experimental Section.

1.1 General materials. All chemical reagents were purchased from commercial available sources such as Aldrich or Fisher and used without further purification. dimethyl formamide and dichloromethane were distilled from CaH_2 before use, and NEt_3, DIEA was redistilled from and dried over KOH pellets.

1.2 Characterization instrumentation: Proton and carbon magnetic resonance spectra (^1H, ^{13}C NMR) were recorded on a Bruker Avance 500 (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from the Me_4Si resonance which was used as the internal standard when recording ^1H NMR spectra. Mass spectra were recorded on a Micromass GCTTM and a Micromass LCTTM. Absorption spectra were recorded on a Shimadzu UV-2550 UV-Vis spectrometer. MALDI-TOF mass spectrometry was performed on a AB SCIEX 4800Plus MALDI TOF/TOFTM Analyzer equipped with Nd:YAG 200 Hz laser at 355 nm. Sinapic acid (SA) was used as a matrix dissolved in a solution of

H_2O/CH_3CN (w/w=1/1) and 0.1% TFA. SDS-PAGE was carried out with a 10% separating gel and 5% polyacrylamide gel in Tris/Glycine/SDS buffer, pH=8.3, at 120 V. The steady-state fluorescence experiments were performed on a Varian Cary Eclipses fluorescence spectrometer.

1.3 Synthesis of photocaged thiol polymers (CTP, NTP and CNTP):

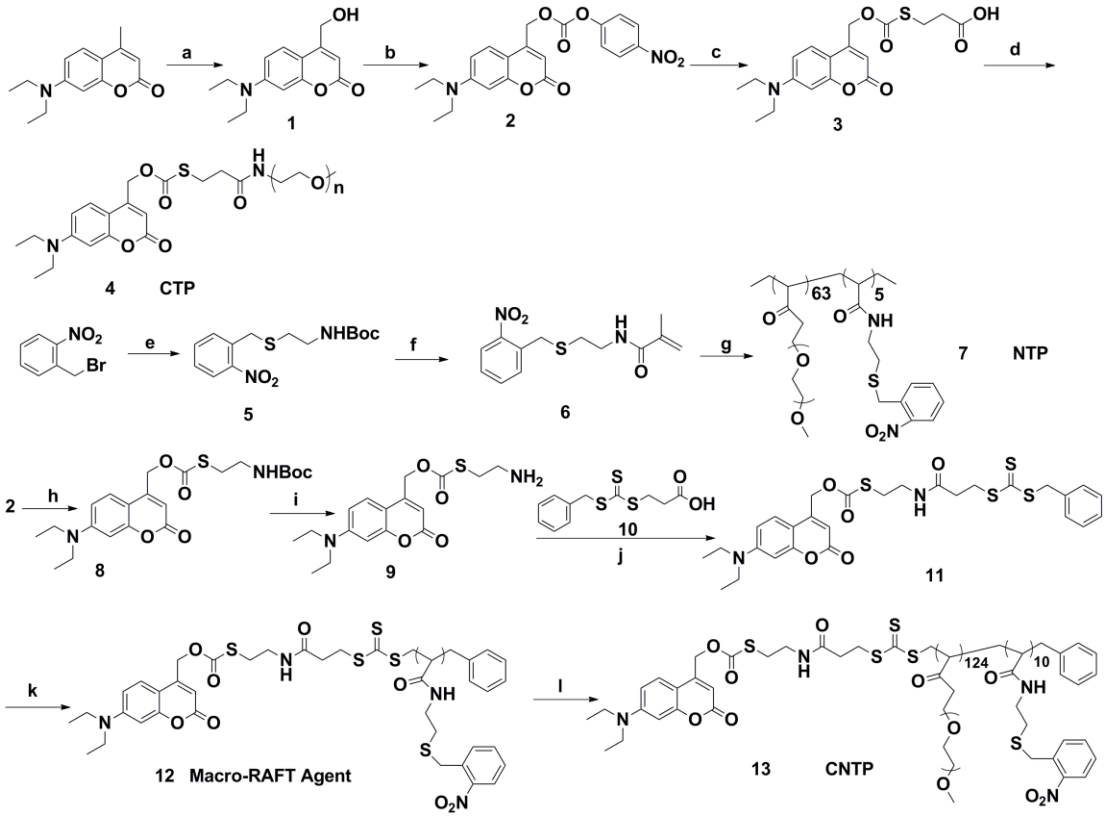

Fig. S1 (a), SeO_2, p-xylene, refluxed; then $NaBH_4$, CH_3OH; (b), 4-nitrophenyl chloroformate, 4-DMAP, CH_2Cl_2, 1 h, ice bath; (c), 3-mercaptopropionic acid, 4-DMAP, DMF, 1 h, rt; (d), PEG-NH_2, PyBOP, DIEA, Et_3N; (e), 2-(BOC-Amino) ethanethiol, K_2CO_3, acetone; (f), TFA/CH_2Cl_2; methacryloyl chloride, Et_3N; (g), PEG-MA, AIBN, 75 ℃, THF, 24 h; (h), 2-(BOC-Amino) ethanethiol, 4-DMAP, CH_2Cl_2, 1 h, rt; (i), TFA, CH_2Cl_2, 30 min, rt; (j), EDC, NHS, Et_3N, CH_2Cl_2, rt; (k), compound 6, AIBN, 75 ℃, THF, 24 h; and (l), PEG-MA, 75 ℃, THF, 24 h.

Compound 1: SeO_2 (1.10 g, 10 mmol) was added to a solution of 7-(diethylamino)-4-methyl-coumarin (2.06 g, 8.9 mmol) in p-xylene (25 mL, mixture of isomers). The reaction mixture was heated under reflux with vigorous stirring for 3 h. Then, the solvent was removed carefully under reduced pressure. The dark-brown residual oil was dissolved in acetone and the dark solid was filtered off. The obtained dark-orange solid was dissolved in methanol (50 mL) and then sodium borohydride (1.5 eq.) was added. The solution was stirred for 1 h at room temperature and the reaction was followed by thin layer chromatography (TLC). Once the oxydate can not be noticed ethyl acetate (100 mL) and water (150 mL) were added. The red solution was extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were washed with water and brine (saturated sodium chloride) and dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure gave a dark-brown oil. Column chromatography using hexane: acetone (3:1) as the eluent afforded desired alcohol **1** (320 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32 (d, *J* = 9.0 Hz, 1H), 6.58 (d, *J* = 8.7 Hz, 1H), 6.51 (s, 1H), 6.27 (s, 1H), 4.84 (s, 2H), 3.40 (q, *J* = 7.1 Hz, 4H), 1.20 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.09, 156.03, 155.47, 150.47, 124.41, 108.69, 106.37, 105.14, 97.63, 60.76, 44.72, 12.44. MS (ESI): 248.1 $[M+H]^+$

Compound 3: **1** (0.42 g, 1.7 mmol) was dissolved in dry dichloromethane (40 mL). 4-dimethylaminopyridine (0.53 g, 4.3 mmol) and 4-nitrophenylchloroformate (0.462 g, 2.3 mmol) were added and the yellow solution was stirred at room temperature. After 18 h stirring the formation of the carbamate intermediate (**compound 2**) was obtained

after a flash Column chromatography. The carbamate intermediate **2** (0.4 g, 0.97 mmol), 4-DMAP (0.258 g, 2.11 mmol), 3-mercaptopropionic acid (127 uL, 1.46 mmol) were then added to the stirring solution. After 24 h at room temperature, dichloromethane was evaporated. The obtained orange crude was purified by chromatography using EtOAc: Hexane = 1:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 (s, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 6.53 (s, 1H), 6.14 (s, 1H), 5.34 (s, 2H), 3.42 (q, *J* = 7.1 Hz, 4H), 3.15 (t, *J* = 6.8 Hz, 4H), 1.21 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, DMSO): δ = 172.64, 169.89, 160.45, 155.81, 150.48, 149.56, 125.43, 108.73, 105.56, 105.07, 96.81, 64.14, 43.98, 33.89, 33.52, 32.97, 26.07, 12.23. MS (ESI): 380.1 $[M+H]^+$.

Compound 4 (coumarin-caged thiocarbonate modified PEG_{5K}, CTP): A mixture of compound **3** (9.2 mg, 0.024 mmol), PyBOP (15.6 mg, 0.03 mmol) and DIEA (7 μL, 0.04 mmol) were dissolved in dry CH_2Cl_2 (10 mL), PEG-NH_2 (0.1 g, 0.02 mmol) was added into this solution and was stirred overnight at rt. Then the solvent was evaporated under reduced pressure, after purification by flash column chromatography using 5% CH_3OH/CH_2Cl_2, **4** (90 mg, 90%) was obtained as a pale yellow solid. The grafting percent was determined at 98% by calculating from the ^{1}H NMR spectrum as shown in Fig. S2.

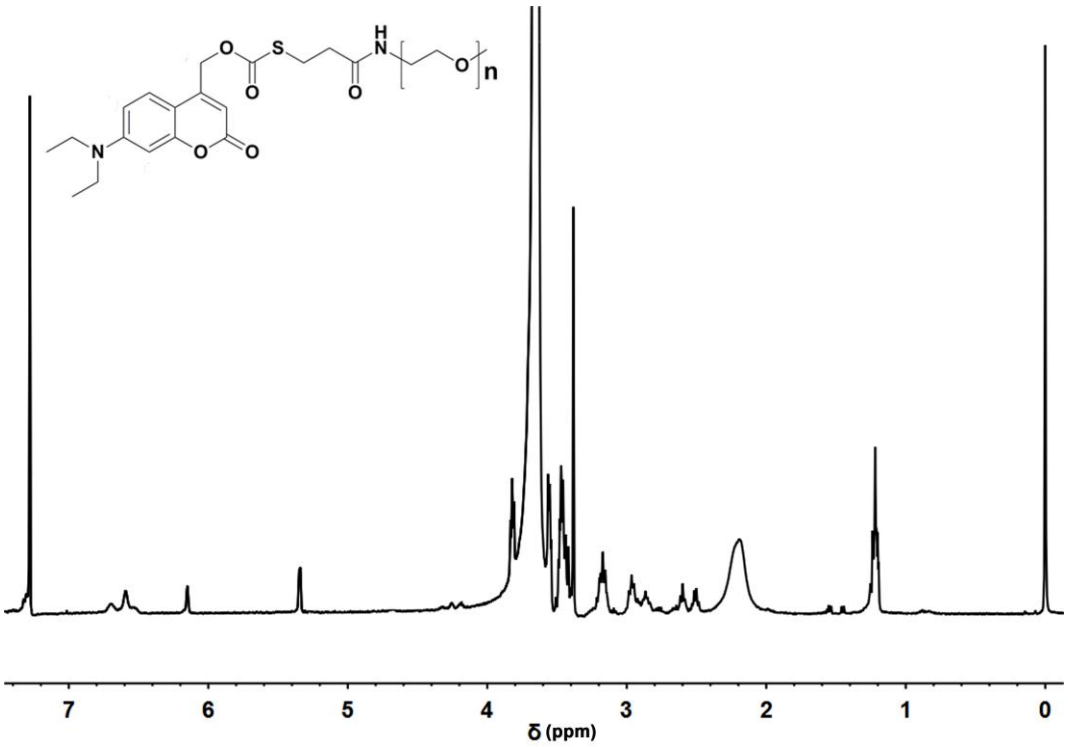

Fig. S2 ^{1}H NMR spectra ($CDCl_3$) of **CTP**.

Compound 5: 2-nitrobenzyl bromide (0.5 g, 2.33 mmol) was dissolved in dry acetone and 2-(BOC-Amino) ethanethiol (600 μL)，K_2CO_3 (0.64 g, 4.6 mmol) were added to this solution. Then the mixture was allowed at room temperature to stir for 30 min. The solvents were removed under reduced pressure. Purification by flash chromatography yielded 0.65 g of **5** as a white solid (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ= 8.00 (d, *J* = 7.4 Hz, 1H), 7.58 (t, *J* = 7.0 Hz, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 1H), 4.10 (s, 2H), 3.35 – 3.29 (m, 2H), 2.60 (t, *J* = 6.5 Hz, 2H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 155.85, 148.62, 133.10, 128.32, 125.45, 79.54, 38.43, 33.13, 28.39. MS (ESI): 335.1 $[M+Na]^+$.

Compound 6: The compound **5** was stirred in a mixture (10 mL) of TFA/CH_2Cl_2 (1:9) at room temperature for 30 min. The solvents were evaporated and the residue was coevaporated two times with Et_2O. The obtained product amino is no further

purification to the next reaction. In a 100 mL round-bottom flask, the amino (0.5 g, 2.4 mmol) and triethylamine (314 μL) were dissolved in dry dichloromethane. A dropping funnel was connected to the flask and filled with a solution of methacryloyl chloride (344 μL) in dry dichloromethane. The flask was cooled to 0 ℃, and the methacryolyl chloride solution was added dropwise under vigorous stirring over a period of 30 min. The mixture was then allowed to warm to ambient temperature and left to stir for another 3 h. After washing with 2 × 40mL of brine and 40 mL of a $NaHCO_3$-saturated solution, the organic residue was purified by flash column chromatography to give a yellow solid upon standing (0.4 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98 (dd, *J* = 8.1, 0.9 Hz, 1H), 7.57 (td, *J* = 7.6, 1.1 Hz, 1H), 7.45 (ddd, *J* = 15.5, 8.1, 4.0 Hz, 2H), 5.71 (s, 1H), 5.35 (s, 1H), 4.10 (s, 2H), 3.48 (dd, *J* = 12.5, 6.2 Hz, 2H), 2.66 (t, *J* = 6.4 Hz, 2H), 1.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 168.40, 148.61, 139.67, 133.82, 133.24, 132.10, 128.46, 125.48, 119.97, 38.24, 33.16, 31.78, 18.59. MS (ESI): 281.0953 $[M+H]^+$.

Copolymerizations of 6 and PEG-MA (7, o-nitrobenzyl-caged thioether polymer, NTP): In a typical experiment, **6** (56 mg, 0.2 mmol), PEG-MA (0.54 g, 1.8 mmol) and AIBN (7 mg) were dissolved in 10 mL THF, the polymerization was performed in a schlenk tube, following three freeze-vacuum-thaw cycles, the tube was immersed in an oil bath at 75 ℃ for 24 h. Afterward the polymer was precipitated 3 times into diethyl ether, isolated by centrifugation and dried for 12 h at 30 ℃ under vacuum. In the end, slightly yellow oil was obtained. (GPC/THF) M_n= 21636 g mol^{-1}, PDI= 1.67.

Compound 8: The carbamate intermediate **2** (0.4g, 0.97 mmol), 4-DMAP (0.258 g,

2.11 mmol), 2-(BOC-Amino) ethanethiol (246 uL, 1.46 mmol) were then added to the stirring solution. After 24 h at room temperature, dichloromethane was evaporated. The obtained orange crude was purified by chromatography using EtOAc: Hexane = 1:1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 (s, 1H), 6.61 (d, *J* = 8.9 Hz, 1H), 6.54 (s, 1H), 6.14 (s, 1H), 5.34 (s, 2H), 3.42 (dd, *J* = 14.3, 7.1 Hz, 6H), 3.04 (t, *J* = 6.3 Hz, 2H), 1.45 (s, 9H), 1.21 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.69, 161.89, 156.27, 150.71, 148.67, 126.13, 124.43, 115.75, 108.88, 106.84, 97.91, 79.77, 63.94, 44.84, 40.23, 31.41, 28.37, 12.41, MS (ESI): 451.2 $[M+H]^+$.

Compound 9: The compound **8** was stirred in a mixture (10 mL) of TFA/CH_2Cl_2 (1:9) at room temperature for 30 min. The solvents were evaporated and the residue was coevaporated two times with Et_2O. The obtained compound **9** is no further purification to the next reaction.

Compound 10: **10** was synthesized according to a previously reported procedure.[1]

Raft agent 11: In a 100 mL round-bottom flask, **10** (0.5 g, 1.83 mmol) was dissolved in dry dichloromethane and EDC (0.42 g, 2.2 mmol), NHS (0.25 g, 2.2 mmol) were added, the mixture was stirred at room temperature for 30 min. Then the solution of compound **9** was added to this mixture and left to stir for another 3 h. After purification by flash column chromatography, **11** was obtained as a yellow solid (0.66 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 - 7.30 (m, 5H), 7.28 (d, *J* = 2.4 Hz, 1H), 6.68 (s, 1H), 6.58 (s, 1H), 6.16 (s, 1H), 5.35 (s, 2H), 4.60 (s, 2H), 3.66 (t, *J* = 6.9 Hz, 2H), 3.54 (q, *J* = 6.1 Hz, 2H), 3.42 (q, *J* = 6.9 Hz, 4H), 3.07 (t, *J* = 6.2 Hz, 2H), 2.63 (t, *J* = 7.0 Hz, 2H), 1.21 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ =

170.86, 170.72, 161.81, 156.27, 150.75, 148.64, 134.83, 129.28, 128.72, 127.82, 124.45, 108.80, 106.66, 105.74, 97.80, 64.07, 44.80, 41.49, 39.46, 34.84, 32.11, 30.82, 12.45. MS (ESI): 627.1096 $[M+Na]^+$.

Macro-RAFT Agent 12: raft agent **11** (72 mg, 0.12 mmol), the monomer **6** (0.5 g, 1.8 mmol) and AIBN (6 mg) were dissolved in 10 mL dry dioxane, the polymerization was performed in a schlenk tube, following three freeze-vacuum-thaw cycles, the tube was immersed in an oil bath at 75 ℃ for 24 h. The polymerization was stopped by cooling the flask to ambient temperature. The macro-raft agent was obtained as a yellow oil by 3-fold precipitation in Et_2O at ambient temperature.

S**ynthesis of amphiphilic polymer (13, CNTP):** The Macro-RAFT Agent **12** (0.15 g), PEG-MA (1 g) and AIBN (10 mg) were dissolved in 10 mL dry dioxane, the polymerization was immersed in an oil bath at 75 ℃ for 24 h. Afterward the polymer was precipitated 3 times into diethyl ether, isolated by centrifugation and dried for 12 h at 30 ℃ under vacuum. In the end, a slightly yellow oil was obtained. (GPC/THF) M_n= 41832 g mol^{-1}, PDI= 1.15.

1.4 Bioconjugations between the photocaged thiol polymers and proteins and QDs (protein-polymer, QDs-polymer, and QDs-polymer-protein).

Preparation of maleimide-activated protein: Tf or avidin (10 mg) was dissolved in 1 mL PBS (pH= 7.2), and add the appropriate amount of crosslinker (Sulfo-SMCC, 100 μL, 4.8 mg mL^{-1}) to the protein solution. Then incubate reaction mixture for 30 min at room temperature or 2 h at 4 ℃. Then remove excess crosslinker using a desalted column equilibrated with conjugation buffer, we can get a white solid

through lyophilization (90% yield).

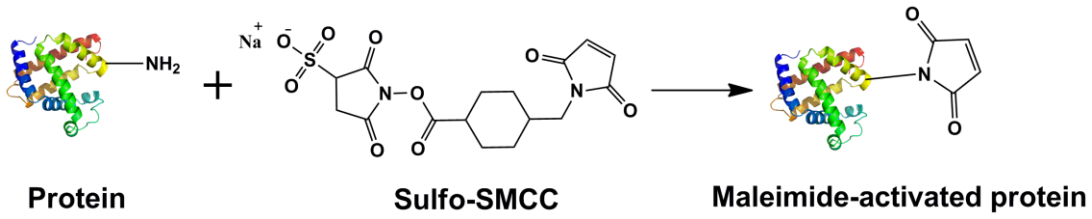

Fig. S3 Schematic representation of the synthesis of Maleimide-activated protein.

Bioconjugation between CTP and maleimide-activated proteins (Protein-polymer): Maleimide-avidin or Maleimide-Tf (5 mg) was dissolved in 5 mL PBS (pH= 7.2) to yield a final protein concentration of 1 g L^{-1}. The calculated amount of CTP was added to the protein solution and the reaction mixture was irradiated for 20 min at room temperature. Samples were taken from the reaction mixture and dialyzed against deionized water (molecular weight cutoff of 10 KDa) to remove salts and subsequently freeze-dried. Yield: 95% quantitative (compared to protein).

Bioconjugation between NTP and QDs (QDs-polymer): The TOPO-coated QDs (CdSe/ZnS QDs) were precipitated twice from the chloroform solution (2 mL) by addition of acetone (10 mL) and subsequent centrifugation for 10 min at 3000 rpm. The resulting precipitate was dissolved in n-hexane again. 200 µL (NTP) polymer solution (containing TMAH as base) was added to the solution of QDs. The mixture was shaken at room temperature under the irradiation of Xe lamp 365 nm for 5 min (10 mW cm^{-2}), all the particles were transferred to the water phase and the n-hexane phase became clear. The water phase with QDs solution was separated from the n-hexane, and the excess of the polymer ligand was removed by centrifugation for 20 min at 8000 rpm. The obtained QDs were re-dispersed in 2 mL water, and kept in

dark.

Bioconjugation among CNTP, protein and QDs (QDs-polymer-protein): Mal-avidin (5 mg) was dissolved in 5 mL PBS (pH= 7.2) to yield a final protein concentration of 1 g L^{-1}. The calculated amount of raft polymer (CNTP) was added to the protein solution and the reaction mixture was irradiated by Xe lamp (420 nm, 10 mW cm^{-2}) for 20 min at room temperature. Then the avidin modified polymer was followed to transfer oil-soluble QDs to water-soluble QDs-avidin bioconjugates under the excitation of Xe lamp (365 nm, 10 mW cm^{-2}, 5 min), and the fabricating process was similar to the preparation of QDs-polymer.

2. Photolysis experiments of CTP and SDS-PAGE and MALDI-TOF characterizations of protein-polymer.

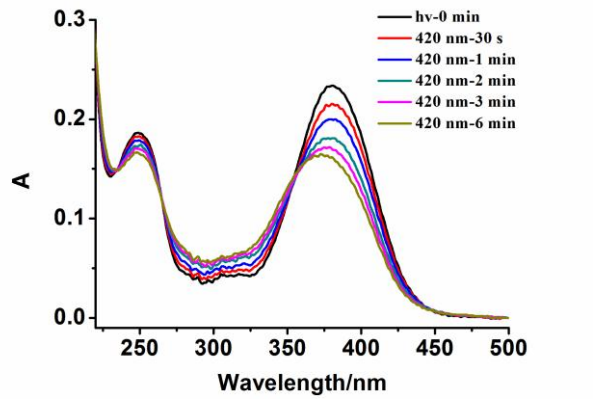

Fig. S4 UV-vis spectra of **CTP** (20 μmol L^{-1}, PBS, pH= 7.4) by 420 nm irradiation (10 mW cm^{-2}).

SDS-PAGE was carried out with a 10% separating gel and 5% polyacrylamide gel in Tris/Glycine/SDS buffer, pH= 8.3, at 120 V. The number of PEG bound to protein was determined by MALDI-TOF mass spectrometry which was performed on a AB SCIEX 4800Plus MALDI TOF/TOF^{TM} Analyzer equipped with Nd:YAG 200 Hz laser

at 355 nm. Sinapic acid (SA) was used as a matrix dissloved in a solution of H_2O/CH_3CN (w/w=1/1) and 0.1% TFA.

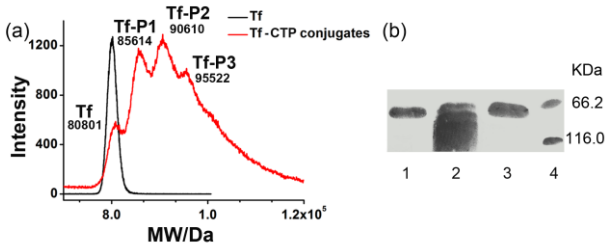

Fig. S5 (a) MALDI-TOF mass spectra of Tf and Tf-CTP conjugates. (b) SDS-PAGE picture of (1) Tf, (2) the mixture of CTP and Mal-Tf on light condition and (3) on dark condition, and (4) marker.

3. Photolysis experiments of NTP.

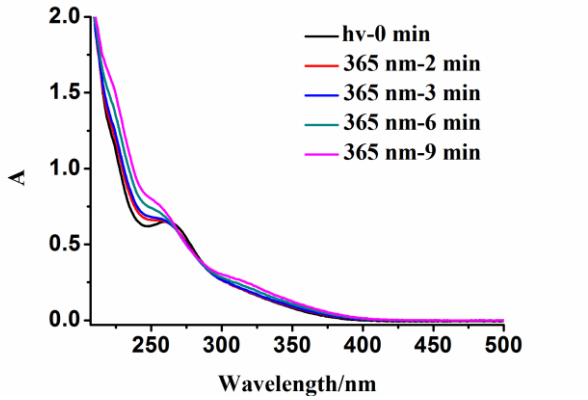

Fig. S6 UV-vis spectra of **NTP** (50 μmol L^{-1}, PBS, pH= 7.4) by 365 nm irradiation (10 mW cm^{-2}).

4. Quantum Yield Measurements of QDs.

The quantum yield (QY) for the QDs was measured relative to the dye Rhodamine 6G (QY≈ 95% in EtOH). Fluorescence spectra of QDs and dye were measured under identical conditions and the optical density of each sample did not exceed 0.1 at the excitation wavelength. The QY of QDs in n-hexane was approximately 0.4, whereas

for the samples in water after NTP and CNTP bioconjugation, both of their QY was measured to be about 0.2.

Reference

1. M. H. Stenzel, T. P. Davis and A. G. Fane, *J. Mater. Chem.*, 2003, **13**, 2090-2097.