Rational Design of Biotinylated Probes: Fluorescent Turn-On Detection of (Strept)avidin and Bioimaging in Cancer Cells

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Supporting Information

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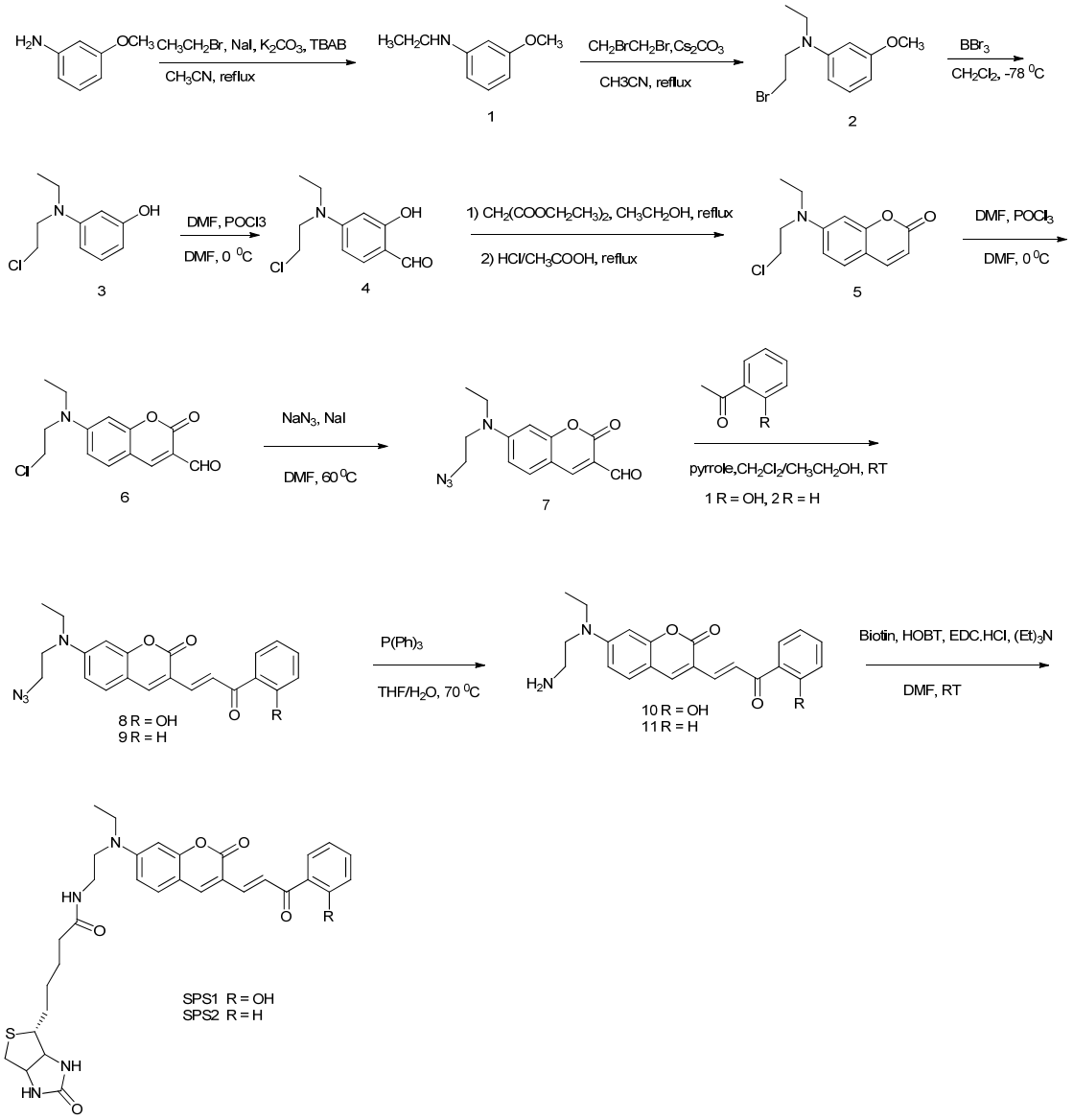
Experiments

1. Synthesis of probes SPS1-2



Scheme 1 synthesis procedures of SPS1 and SPS2.

Synthesis of compound 1: Commercially available 3-methoxyaniline (1.67 g, 13.6 mmol), K_2CO_3 (2.82 g, 20.4 mmol), NaI (203.9 mg, 1.36 mmol) and TBAB (438 mg, 1.36 mmol) were added to acetonitrile (80 mL) in a 250 mL flask. 1.48 g (13.6 mmol) of bromoethane were added slowly to the above solution, and the mixed solution was refluxed for 8 h. After filtration, the solvent was removed under reduced pressure, and the brown residue was purified by column chromatography to give colourless oil (1.14 g, yield: 55.4%). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 7.08 (t, J = 8.1 Hz, 1H), 6.26 (dd, J_1 = 1.9 Hz, J_2 = 8.1 Hz, 1H), 6.22 (dd, J_1 = 1.5 Hz, J_2 = 8.10 Hz, 1H), 6.16 (s, 1H), 3.77 (s, 3H), 3.14 (q, J = 7.2 Hz, 2H), 1.25 (t, J = 7.2 Hz, 3H).

Synthesis of compound 2: 12.42 g (66.1 mmol) of 1,2-dibromoethane were added slowly to acetonitrile (80 mL) with compound **1** (1.00 g, 6.61 mmol) and Cs_2CO_3 (3.23 g, 9.92 mmol). The above solution was refluxed for 18 h. After filtration, the solvent was removed under reduced pressure, and the brown residue was purified by column chromatography to give a brown oil (1.43

g, yield: 88.7%). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 7.08 (t, J = 8.1 Hz, 1H), 6.26 (dd, J_1 = 1.9 Hz, J_2 = 8.1 Hz, 1H), 6.22 (dd, J_1 = 1.5 Hz, J_2 = 8.1 Hz, 1H), 6.16 (s, 1H), 3.77 (s, 3H), 3.66 (t, J = 8.3 Hz, 2H), 3.46 (t, J = 8.3 Hz, 2H), 3.40 (q, J = 7.0 Hz, 2H), 1.18 (t, J = 7.0 Hz, 3H).

Synthesis of compound 3: Under nitrogen atmosphere and at -78 °C, 1 mL (10.6 mmol) BBr_3 was added slowly to 40 mL of compound **2** (1.05 g, 4.30 mmol) in dichloromethane. The solution was stirred at -78 °C for 30 min and at room temperature for another 3 h. Then the above solution was poured into 100 mL iced water and stirred for 10 minutes. The crude product was extracted with dichloromethane. The organic layer was washed with saturated $NaHCO_3$ (100 mL × 2) and brine (100 mL × 2), followed by concentrated under vacuo and purified by column chromatography to give a colourless oil (610 mg, yield: 61.6%). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 9.17 (s, 1H), 6.96 (m, 1H), 6.15 - 6.05 (m, 3H), 3.71 (t, J = 7.3 Hz, 2H), 3.59 - 3.53 (m, 4H), 1.07 (t, J = 7.0 Hz, 3H).

Synthesis of compound 4: Under nitrogen atmosphere and at 0 °C, 1.5 mL $POCl_3$ (16.51 mmol) were injected slowly into 2 mL freshly distilled DMF and the mixed solution was stirred for 30 min. Then compound **3** (1.90 g, 8.26 mmol) in 3 mL freshly distilled DMF were injected slowly to the above mixture. After stirred at 0 °C for 30 minand at 60 °C for 4 h, the reaction mixture was poured into 100 mL ice-water and extracted with dichloromethane. The organic layer was washed with saturated $NaHCO_3$ (100 mL × 2) and brine (100 mL × 2), followed by concentrated under vacuo and purified by column chromatography to give a white solid (1.25 g, yield: 70.9%). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 11.15 (s, 1H), 9.69 (s, 1H), 7.47 (d, J = 8.9 Hz, 1H), 6.41 (dd, J_1 = 8.9 Hz, J_2 = 2.3 Hz, 1H), 6.13 (d, $_1$ = 2.3 Hz, 1H), 3.75 (t, J = 5.3 Hz, 2H), 3.72 (t, J = 5.6 Hz, 2H), 3.49 (q, J = 7.1 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H).

Synthesis of compound 5: compound **4** (1.70 g, 7.96 mmol), diethyl malonate (1.53 g, 9.55mmol) and 1 mL piperidine were dissolved in 100 mL absolute ethanol. After refluxed for 8 h, the solvent was removed under the reduced pressure. Then 40 ml of mixed solution [concentrated HCl : glacial acetic acid = 1:1 (v/v)] were added to the above crude product and refluxed for another 7 h. After cooled to room temperature the reaction solution was poured into 300 mL of ice water. The solution's pH was adjusted to ~5 with NaOH solution. The precipitate was purified with column chromatography to give a yellow solid (1.23 g, yield: 61.4%). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 7.60 (d, J = 9.4 Hz, 1H), 7.29 (d, J = 8.7 Hz, 1H), 6.60 (dd, J_1 = 2.3 Hz, J_2 = 8.8 Hz,1H), 6.53 (d, J = 2.3 Hz, 1H), 6.10 (d, J = 9.4 Hz, 1H), 3.70 (t, J = 6.6 Hz, 2H), 3.64 (t, J = 6.6 Hz, 2H), 3.50 (q, J = 7.1 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H).

Synthesis of compound 6: Under nitrogen atmosphere and at 0 °C, 1.5 mL $POCl_3$ (16.51 mmol) were injected slowly into freshly distilled DMF and stirred for 30 min. Then compound **5** (1.23 g, 4.89 mmol) in 5 mL freshly distilled DMF were injected slowly to the $POCl_3$-DMF solution. After stirred at 0 °C for 30 minand at 60 °C for 4 h, the reaction solution was poured into 100 mL ice water. The solution's pH was adjusted to ~5 with NaOH solution. The precipitate was purified with column chromatography to give a orange-brown solid (800 mg, yield: 58.5%). ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 10.15 (s, 1H), 8.29 (s, 1H), 7.47 (d, J = 8.9 Hz, 1H), 6.68 (dd, J_1 = 1.8 Hz, J_2 = 8.9 Hz, 1H), 6.55 (d, J = 1.8 Hz, 1H), 3.77 (t, J = 6.6 Hz, 2H), 3.67 (t, J = 6.7 Hz, 2H), 3.50 (q, J = 7.1 Hz, 2H), 1.23 (t, J = 7.1 Hz, 3H).

Synthesis of compound 7: Compound **6** (1.2 g, 4.29 mmol), NaI (64.3 mg, 0.43mmol) and NaN_3 (1.39 g, 21.45mmol) were added to 5 mL freshly distilled DMF. After stirred under nitrogen at 80 °C for 6 h, the above solution was poured into 100 mL ice water and stirred for 10 minutes. The

precipitate was filtered to give an orange-brown solid (0.95 g, yield: 77.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 10.14 (s, 1H), 8.28 (s, 1H), 7.47 (d, J = 9.0 Hz, 1H), 6.68 (dd, J_1 = 2.1 Hz, J_2 = 9.0 Hz,1H), 6.54 (d, J = 2.1 Hz, 1H), 3.61-3.54 (m, 6H), 1.28 (t, J = 7.1 Hz, 3H).

Synthesis of compounds 8 and 9: Compound **7** (300 mg, 1.05 mmol) and 2-hydroxyacetophenone (256.8 mg, 1.89 mmol) were added to 20 mL of the mixed solvent [CH_2Cl_2/anhydrous CH_3CH_2OH = 1:1 (v/v)], then 10 drops of pyrrolidine were droped into the above solution. The mixture was stirred at room temperature for 1 d, and the solvent was removed under reduced pressure. The residue was purified by column chromatography to give a bright red solid (280 mg, 66.1%). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 12.58 (s, 1H), 8.55 (s, 1H), 8.15 (d, J = 15.4 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 15.3 Hz, 1H), 7.57-7.53 (m, 2H), 7.05-6.99 (m, 2H), 6.90 (d, J = 9.0 Hz, 1H), 6.74 (s, 1H), 3.67 (t, J = 5.4 Hz, 2H), 3.59 (t, J = 5.4 Hz, 2H), 3.55 (q, J = 7.0 Hz, 2H), 1.15 (t, J = 6.9 Hz, 3H). Compound **9** was obtained as a bright red solid (90 mg, 22.1%) by the same procedures with acetophenone (226 mg) instead of 2-hydroxyacetophenone. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.53 (s, 1H), 8.08-8.04 (m, 3H), 7.69-7.65 (m, 2H), 7.61-7.57 (m, 2H), 7.53 (d, J = 8.9 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 6.74 (s, 1H), 3.67 (t, J = 5.5 Hz, 2H), 3.59 (t, J = 5.4 H z, 2H), 3.55 (q, J = 6.8 Hz, 2H), 1.15 (t, J = 6.8 Hz, 3H).

Synthesis of compounds 10 and 11: Compound **8** (200 mg, 0.49 mmol) and PPh_3 (195 mg, 0.74 mmol) were added to 20 mL of the mixed solvent [THF/H_2O = 4:1 (v/v)] under nitrogen atmosphere. After stirred for 24 h at 60 °C, the solvent was removed under vacuum. The residue was purified by column chromatography to give a bright red solid (125 mg, 66.8%). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.52 (s, 1H), 8.14 (d, J = 15.3 Hz, 1H), 8.02 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 15.3 Hz, 1H), 7.56 (t, J = 7.2 Hz, 1H), 7.50 (d, J = 8.9 Hz, 1H), 7.05-6.99 (m, 2H), 6.90 (d, J = 9.0 Hz, 1H), 6.70 (s, 1H), 3.52 (q, J = 6.9 Hz, 2H), 3.43 (t, J = 7.3 Hz, 2H), 2.76 (t, J = 7.2 Hz, 2H), 1.14 (t, J = 6.9 Hz, 3H). Compound **11** was obtained as a bright red solid (60 mg, 64.3%) by the same procedures with compound **9** instead of compound **8**. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (s, 1H), 8.09 – 8.03 (m, 3H), 7.69 (d, J = 5.5 Hz, 2H), 7.66 (d, J = 2.6 Hz, 2H), 7.60 (d, J = 7.7 Hz, 2H), 7.56 (d, J = 9.1 Hz, 1H), 6.89 (dd, J_1 = 2.3 Hz, J_2 = 9.0 Hz, 1H), 6.79 (d, J = 2.2 Hz, 1H), 3.64 (t, J = 7.0 Hz, 2H), 3.52 (q, J = 6.8 Hz, 2H), 3.01 (t, J = 7.1 Hz, 2H), 1.14 (t, J = 7.0 Hz, 3H).

Synthesis of SPS1 and SPS2: Biotin (65 mg, 0.26 mmol), EDC·HCl (62 mg, 0.31 mmol), HOBt (53 mg, 0.31 mmol) and Et_3N (65 μL, 0.47 mmol) were dissolved in freshly distilled DMF (5 mL) and stirred at room temperature for 50 min. Then 100 mg of compounds **10** (0.26 mmol) were added to the above solution. After stirred for another 5 h, the mixture was poured into 100 mL of ice water. The precipitate was washed in turn with saturated NH_4Cl, saturated $NaHCO_3$, brine and then purified with column chromatography to give a bright red solid (95 mg, 59.5%). ^{1}H NMR (400MHz, DMSO-d_6) δ (ppm): 12.64 (s, 1H), 8.50 (s, 1H), 8.12 (d, J = 15.3 Hz, 1H), 8.04 – 8.00 (m, 1H), 7.75 (d, J = 15.3 Hz, 1H), 7.55 (t, J = 8.3 Hz, 1H), 7.49 (d, J = 9.0 Hz, 1H), 7.03 – 6.98 (m, 2H), 6.86 (dd, J_1 = 1.7 Hz, J_2 = 8.9 Hz, 1H), 6.71 (d, J = 1.4 Hz, 1H), 6.45 (s, 1H), 6.39 (s, 1H), 4.32 - 4.28 (m, 1H), 4.13- 4.10 (m, 1H), 3.51 - 3.43 (m, 4H), 3.25 (q, J = 6.1 Hz, 2H), 3.09 - 3.04 (m, 1H), 2.81 (dd, J_1 = 5.0 Hz, J_2 = 12.5 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.06 (t, J = 7.3 Hz, 2H), 1.64 – 1.23 (m, 6H), 1.13 (t, J = 6.8 Hz, 3H). ^{13}C NMR (400MHz, DMSO-d_6) δ (ppm): 193.7, 173.1, 163.2, 162.3, 160.4, 157.0, 153.1, 146.6, 140.7, 136.6, 131.2, 130.5, 121.2, 120.6, 119.7, 118.3, 113.7, 110.6, 109.2, 97.1, 61.5, 59.7, 55.9, 49.4, 45.9, 45.4, 36.6, 35.7, 28.7, 28.5, 25.6,

12.5. HR-MS *m/z*: 605.2430 $(M+H)^+$; calculated molecular weight of $C_{32}H_{36}N_4O_6S$: 605.2389 for $(M+H)^+$. SPS2 was obtained a bright red solid (95 mg, 59.5%) by the same procedures with compound **11** instead of compound **10**. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.51 (s, 1H), 8.07-8.03 (m, 3H), 7.69-7.65 (m, 2H), 7.61-7.57 (m, 2H), 7.53 (d, J = 8.9 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 6.74 (s, 1H), 6.43 (s, 1H), 6.37 (s, 1H), 4.31-4.28 (m, 1H), 4.12-4.10 (m, 1H), 3.52-3.44 (m, 4H), 3.26 (q, J = 6.0 Hz, 2H), 3.17 (d, J = 5.2 Hz, 1H), 2.81 (dd, J_1 = 5.9 Hz, J_2 = 12.4 Hz, 1H), 2.57 (d, J = 12.4 Hz, 1H), 2.07 (t, J = 7.2 Hz, 1H), 1.63 – 1.40 (m, 3H), 1.21 – 1.12 (m, 6H). ^{13}C NMR (400MHz, DMSO-d_6) δ (ppm): 189.4, 173.1, 163.2, 160.5, 156.8, 152.9, 146.1, 139.8, 138.4, 133.4, 131.1, 129.3, 128.6, 121.5, 114.0, 110.5, 109.1, 97.1, 61.5, 59.6, 55.9, 49.4, 45.9, 45.4, 36.6, 35.7, 28.7, 28.5, 25.6, 12.5. HR-MS *m/z*: 589.2487 $(M+H)^+$, 314.1015 $(M+H+K)^{2+}/2$; calculated molecular weight of $C_{32}H_{36}N_4O_5S$: 589.2440 for $(M+H)^+$, 314.1061 for $(M+H+K)^{2+}/2$.

2. Meterials and Reagents

All the chemicals were purchased from Aladdin Corporation and used without further purification. Ultra-pure water was prepared by Sartorius Arium 611DI system.

3. Spectral measurements

Stock solutions of SPS1-2 (3×10^{-4} M) in DMF, biotin (14.4×10^{-3} M) in phosphate buffer solution (PBS, 10 mM, pH 7.0) and 1 mg/mL of strepavidin (SA) /avidin (AV) in PBS were prepared in advance. The stock solutions of the dyes were diluted with corresponding solvents to acquired 3 μM dye solutions. Absorption spectra were measured with an Evolution 220 UV-vis spectrophotometer (Thermo Scientific). Fluorescent spectra were carried out in a Lumina Fluorescence Spectrometer (Thermo Scientific). NMR spectra were performed with a Bruker AV-400 spectrometer (400M Hz). Mass spectra were recorded on a MA 1212 Instrument using standard condition (ESI, 70 ev). Time-resolved fluorescent data were acquired with an Edinburgh FL900 Fluorescence spectrometer equipped with a laser (λ_{ex} = 441 nm). A di-exponential function was used to fit the fluorescence decay.

4. SA/AV Titrations

20 μL of the dye stock solution were added to 2 mL of phosphate buffer solution (10 mM, pH 7.0) to keep [SPS1] = [SPS2] = 3 μM / 0.3 μM. 0 - 100 μL of 1 mg/mL SA/AV-PBS were added into the above solution to obtain appreciate concentrations of SA/AV.

5. Binding ratio of SA/AV to SPS2

200 μL of 1 mg/mL SA (AV) stock solution were added to 1.80 mL of phosphate buffer solution to make [SA] = [AV] = 100 μg/mL (1.5 μM). 0-72 μL of the dye stock solution was added into the above solution to acquire different ratios of dye/protein.

6. Living cell culture and fluorescence imaging

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ^oC and under 5% CO_2 in a CO_2 incubator. The cells were washed with phosphate buffered solution (PBS) and incubated with SPS2 (10 μM) in DMEM for 1 h at 37 °C and washed 3 times with PBS. For the control experiment, the cells were pre-incubated with 10 μM biotin for 1 h, then incubated with 10 μM of SPS2 for another 1 h. Cell imaging was carried out after washing cells with PBS. Emission was collected at 550-560 nm for green channel and 590-600 nm for red channel. The excited wavelength was set at 488 nm.

7. Determination of the detection limit

The limit of detection (LOD) was obtained by $3S_b/k$, where S_b is the standard deviation of the blank measurements of 10 times, and k is the slope of the fitted line.

8. Cell viability assay

The cell viability of SPS2 was the same as that in the reference (S. Bhuniya, S. Maiti, E.-J. Kim, H. Lee, J. L. Sessler, K. S. Hong and J. S. Kim, *Angew. Chem. Int. Ed*., 2014, **53**, 4469).

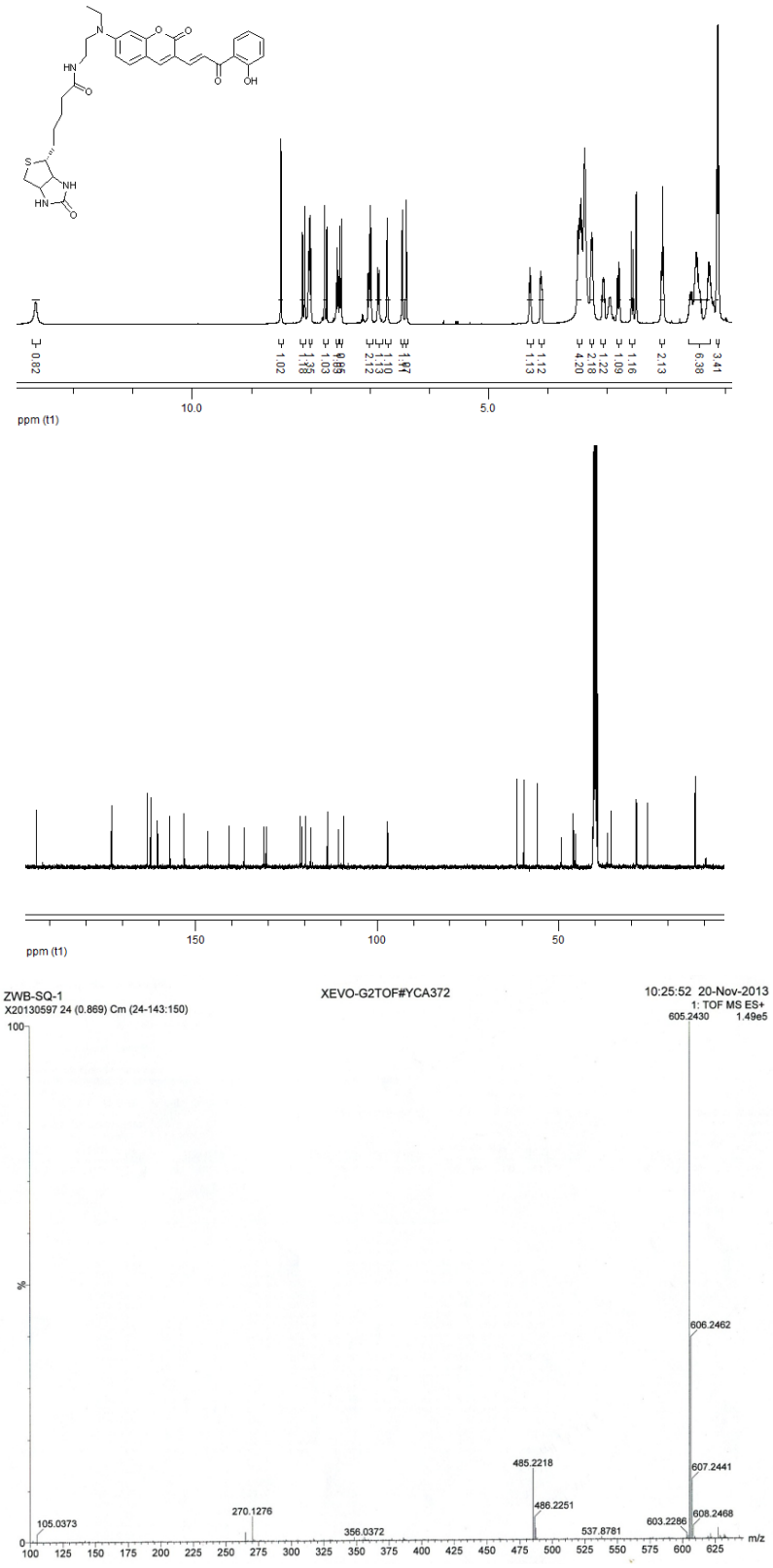


Fig. S1 ^{1}H-NMR, ^{13}C-NMR and HR-MS spectra of SPS1.

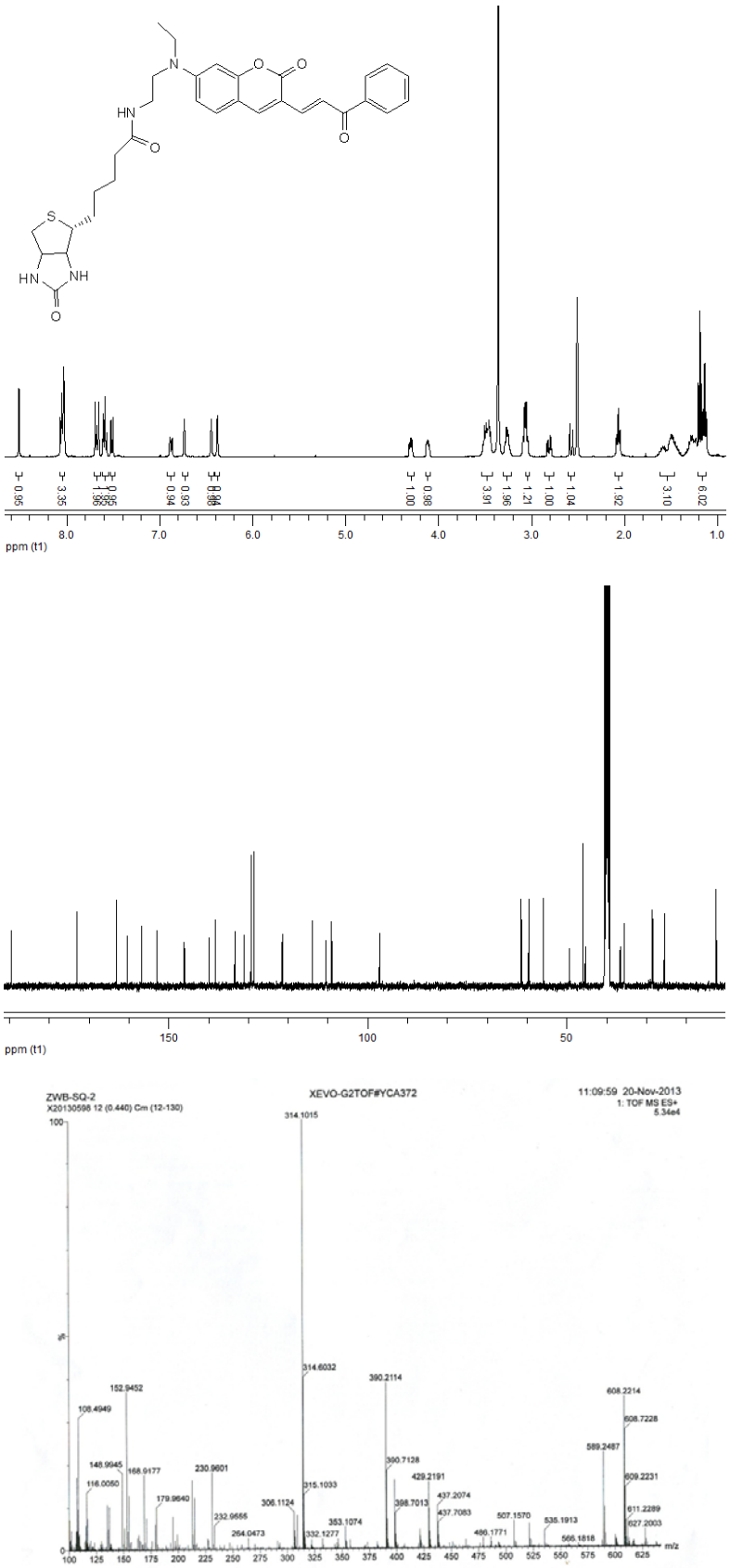


Fig. S2 ^{1}H-NMR, ^{13}C-NMR and HR-MS spectra of SPS2.

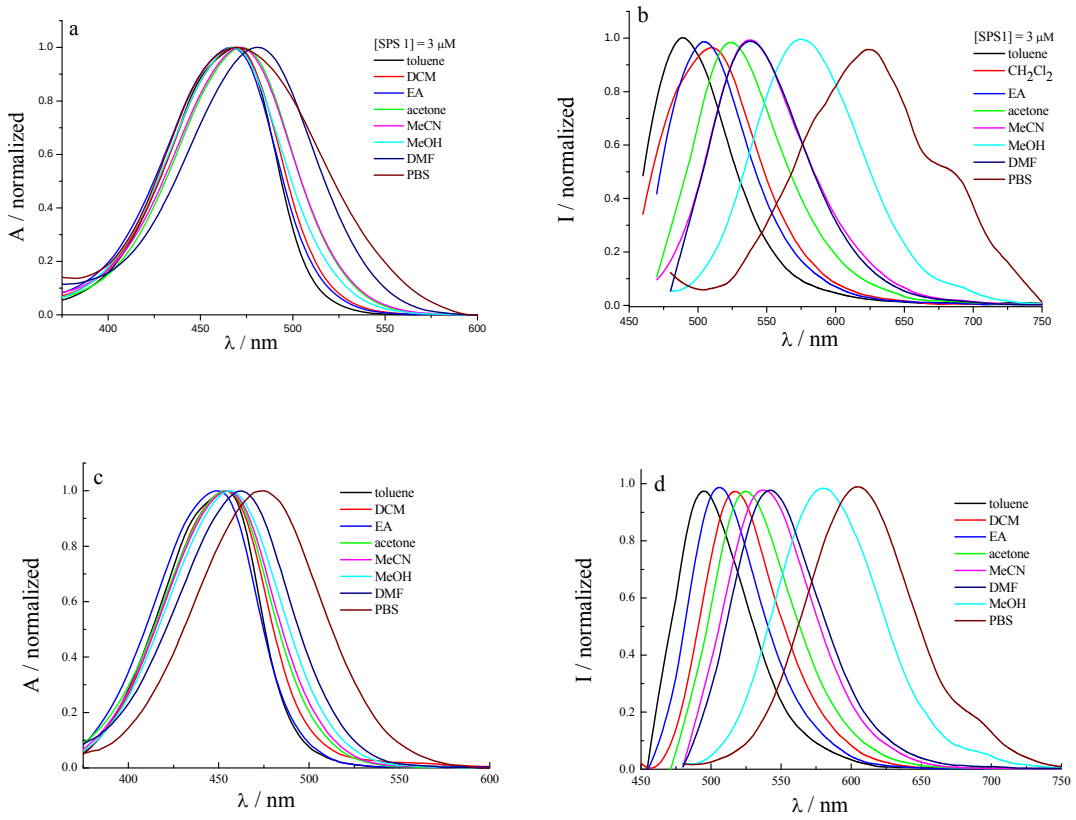


Fig. S3 The normalized absorption and emission spectra of SPS1(a, b) and SPS2 (c, d) in various solvents, [SPS1] = [SPS2] = 3 μM.

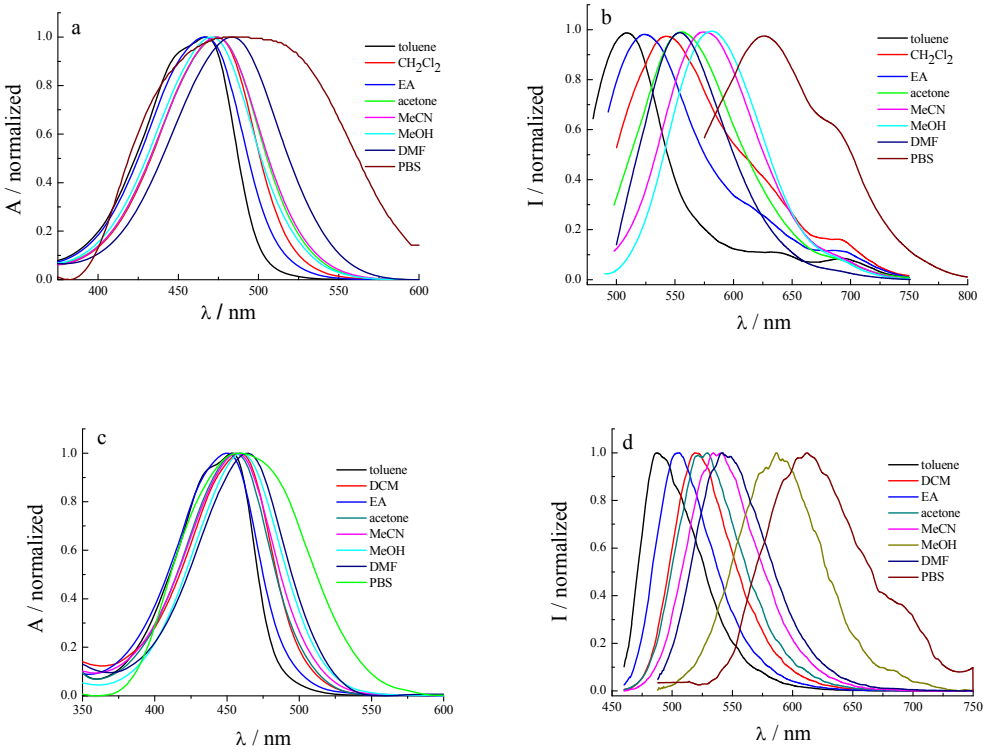


Fig. S4 The normalized absorption and emission spectra of RC1(a, b) and RC2 (c, d) in various solvents, [RC1] = [RC2] = 3 μM.

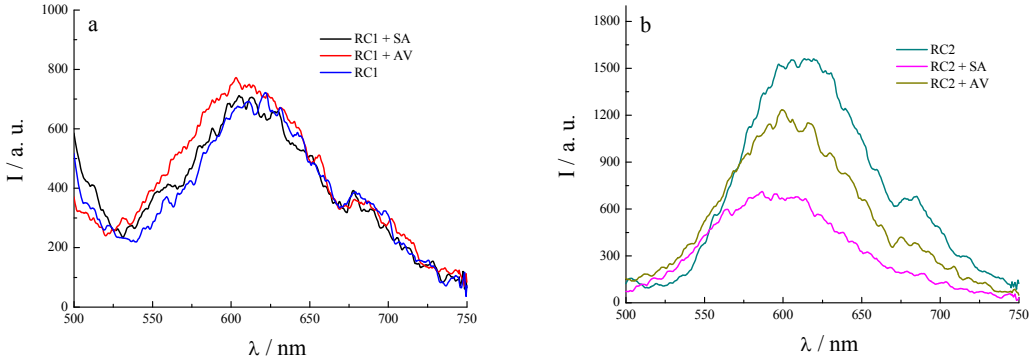


Fig. S5 The emission spectra of RC1 (a) and RC2 (b) with and without SA/AV. 10 mM PBS, pH 7.0; [RC1] = [RC2] = 3.0 μM, [SA] = [AV] = 1.5 μM; λ_{ex} = 465 nm.

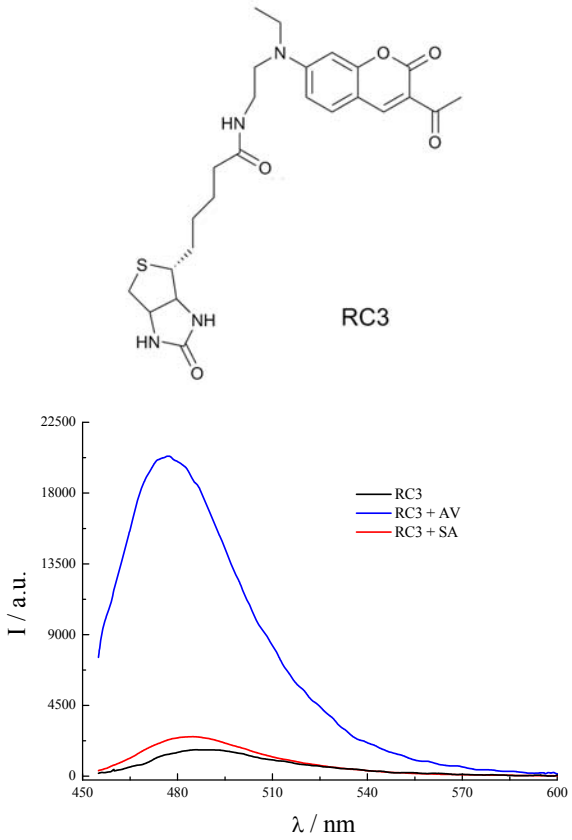


Fig. S6 The emission spectra of the refernece compound RC3 with or without SA/AV. 10 mM PBS, pH 7.0; [RC3] = 3.0 μM, [SA] = [AV] = 1.5 μM; λ_{ex} = 435 nm.

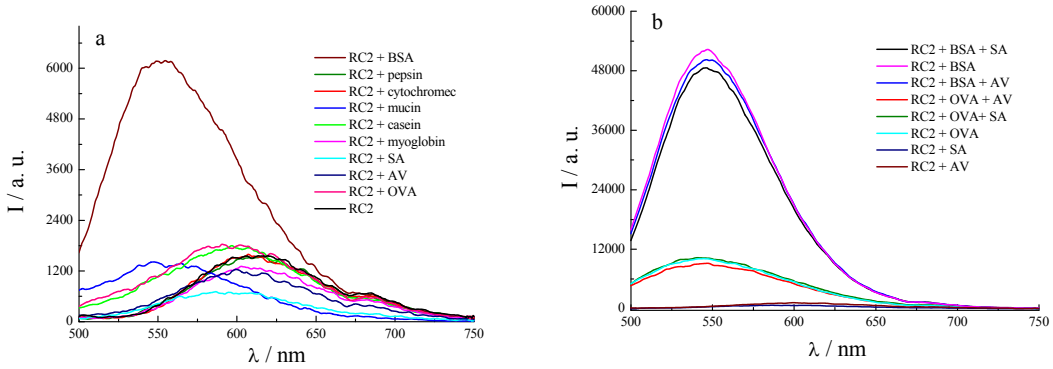


Fig. S7 The fluorescence spectra of RC2 in the presence of 0.1 mg/mL of various proteins (a) and the competitation of RC2 toward BSA and OVA over SA/AV (b). [RC2] = 3.0 μM, [SA] = [AV] = 0.1 mg/mL, [BSA] = [OVA] = 1.0 mg/mL; 10 mM PBS, pH 7.0; λ_{ex} = 465 nm.

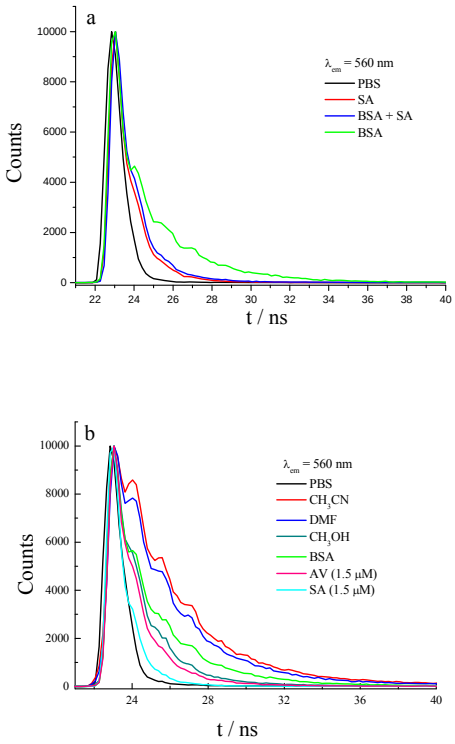


Fig. S8 The the fluorescence decay curves of SPS2 in different systems (λ_{ex} = 441 nm, λ_{em} = 560 nm).

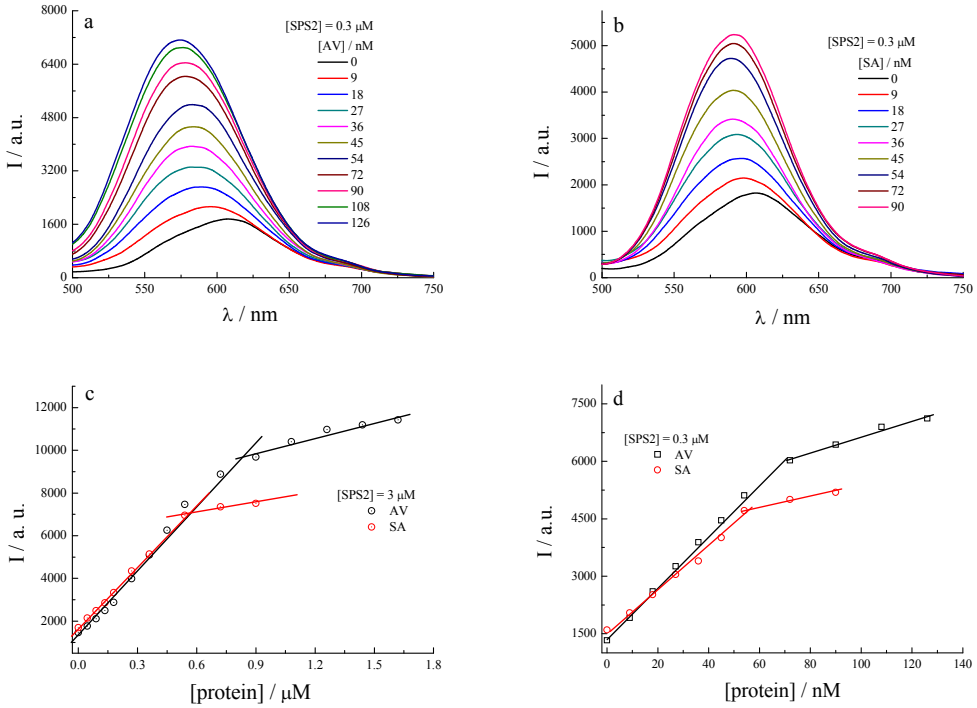


Fig. S9 The emission spectra of SPS2 (0.3 μM, both slits were 10 nm) with different concentrations of AV/SA (a-b), the fluorescence intensity of SPS2 at 576 nm as a function of AV/SA concentration (c, d) (10 mM PBS, pH 7.0, λ_{ex} = 465 nm).

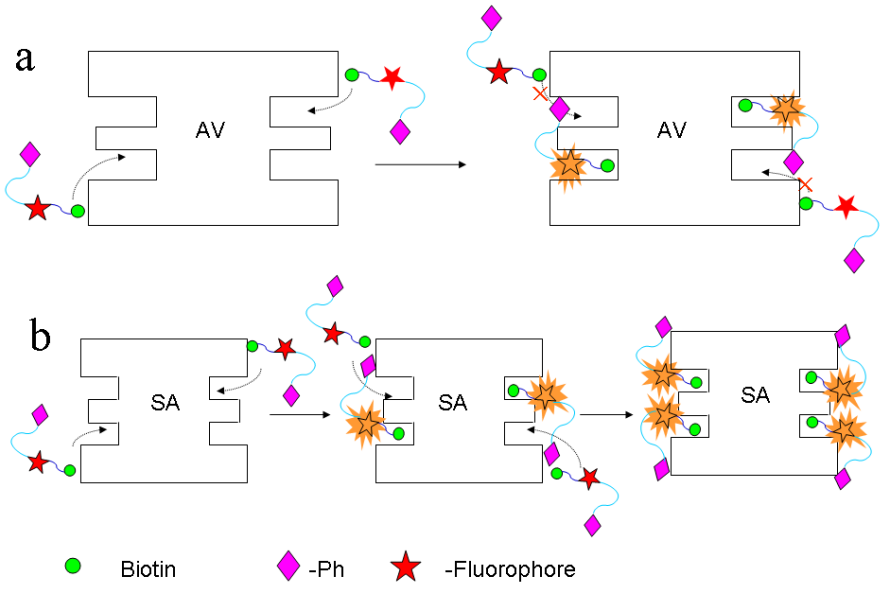


Fig. S10 The proposed binding models of SPS2 to AV/SA.

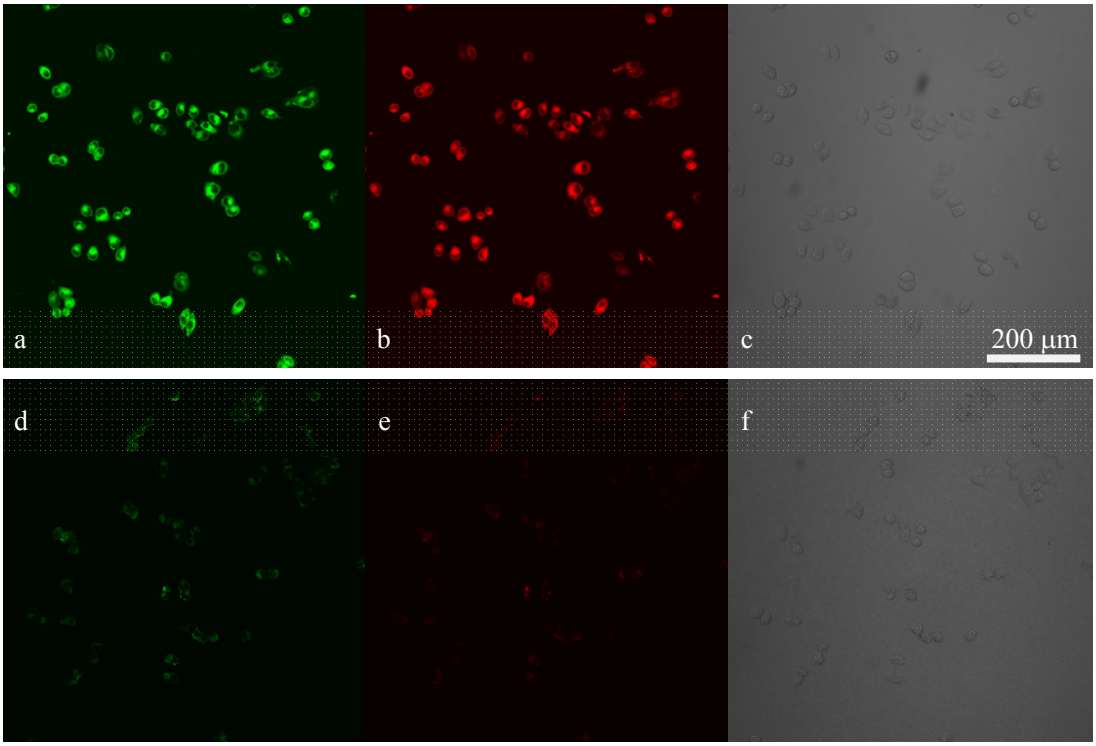


Fig. S11 Confocal laser fluorescence (a-b, d-e), bright-field (c, f) images of living HeLa cells incubated with SPS2 (10 μM) for 60 min (a-c) and pretreated with 10 μM biotin for 1h (d-f) followed by incubated with SPS2 for another 1h. Excited at 488 nm; (a, d) green channel; (b, e) red channel.

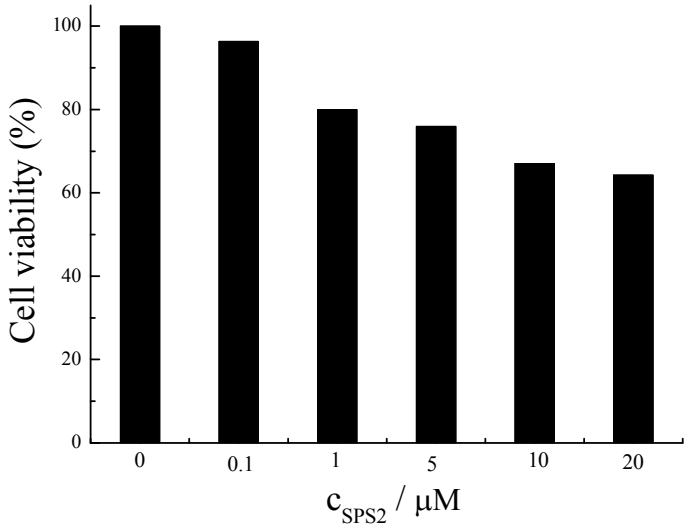


Fig. S12 Cell viability of HeLa cells pretreated with various concentrations of SPS2 in PBS. After treatment, the cells were incubated for 24 h. Cell viability was assessed using a standard MTT assay.

Table 1. The absorption and emission maxima, Stokes shifts and fluorescence quantum yields of SPS1-2 and RC1-2 in various solvents.

Solvents	dyes	λ_{ab} / nm	λ_{em} / nm	Stokes shift/ nm	Φ_{fl}[a]
toluene	SPS1	469	488	19	0.0047
	RC1	467	508	31	0.0001
	SPS2	454	495	41	0.58
	RC2	453	491	38	1.00
dichloromethane	SPS1	469	510	41	0.0036
	RC1	474	541	67	0.0002
	SPS2	454	517	63	0.41
	RC2	459	499	53	1.00
ethylacetate	SPS1	467	495	28	0.0039
	RC1	466	524	58	0.0002
	SPS2	449	506	57	0.48
	RC2	450	506	56	1.00
acetone	SPS1	472	514	42	0.0040
	RC1	474	556	82	0.0007
	SPS2	454	525	71	0.46
	RC2	456	526	70	1.00
acetonitrile	SPS1	471	528	57	0.0032
	RC1	474	573	99	0.0009
	SPS2	455	537	82	0.49
	RC2	457	537	80	0.88
Methanol	SPS1	468	555	87	0.0058
	RC1	471	581	100	0.0034
	SPS2	457	578	121	0.19
	RC2	459	587	128	0.18
DMF	SPS1	482	518	36	0.024
	RC1	482	553	71	0.0077
	SPS2	462	524	80	0.48
	RC2	464	544	80	0.71
PBS[b]	SPS1	470	625	155	0.0031
	RC1	488	626	138	0.0044
	SPS2	475	605	130	0.0090
	RC2	458	611	153	0.0016

Coumarin 153 (Φ_f = 0.38 in ethanol) was used as the reference; [b] containing 1% DMF

Table S2 the life time and relative amplitudes α of the two time components in the fluorescence decays of SPS2 (3 μM) in different systems at emission maxima (λ_{ex} = 465 nm)

Solvent	τ_1/ns (α_1 /%)	τ_2/ns (α_2 /%)	τ_f / ns
PBS	0.46 (74.1)	2.19 (25.9)	0.90
CH_3OH	1.10 (59.8)	3.69 (40.2)	2.08
CH_3CN	2.82 (72.7)	21.64 (27.3)	7.96
DMF	2.85 (65.9)	15.88 (43.1)	7.29
Toluene	2.83 (47.5)	12.24 (52.5)	7.77
SA	0.29 (13.4)	1.19 (86.6)	1.07
AV	1.52 (79.1)	2.82 (20.9)	1.79
BSA	3.06 (100)		3.06
BSA+SA	1.71 (100)		1.71