Supporting Information for

Reversible Fluorescence Switching of Spiropyran-Conjugated Biodegradable Nanoparticles for Super-Resolution Fluorescence Imaging

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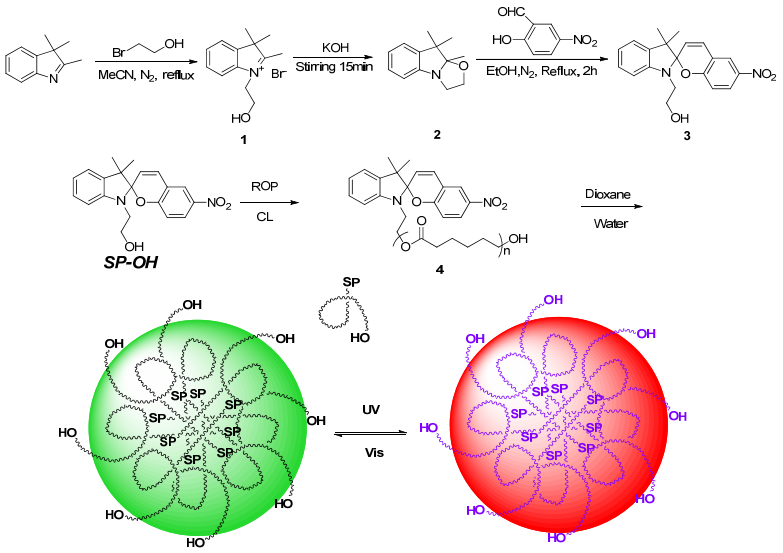
1. Synthetic procedures.



Scheme S1. Synthetic route employed for the preparation of SP-OH monomer and SP-PCL nanoparticles.

2-(3',3'-Dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)ethanol (SP-OH, 3). Compound 2-(3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)ethanol, ***3***, was prepared according to the previous literature[1] with slight modifications. The mixture of 2,3,3-trimethyl-3H-indole (16.06 g, 100.86 mmol), and 2-bromoethanol (13.23 g, 105.87 mmol) in acetonitrile (20 mL) was heated to reflux for 24 h under N_2. After cooling down to room temperature, the precipitate was filtered, washed with dichloromethane (DCM, 3 × 20 mL), collected and dried in the oven to obtain ***1*** (26.33 g, yield 91.87%), which was used in next step without purification. A solution of ***1*** (6.02 g, 21.82 mmol), KOH (2.78 g, 49.64 mmol) in distilled water (40 mL) was stirred at room temperature for 30 min, and then was extracted with Et_2O (3 × 30 mL). The organic layer was collected, dried with Na_2SO_4 and concentrated under the reduced pressure to afford ***2*** as yellow oil. To the oil, a solution of 5-nitrososalicylaldehyde (4.21 g, 25.2 mmol) in ethanol (40 mL) was added and the mixture was heated for 3 h under reflux and N_2. After cooling to room temperature, the precipitate was filtered, washed with ethanol and dried to afford ***3*** as a purple solid (6.87 g, yield 89.34%). ^{1}HNMR (400 MHz, $CDCl_3$): 1.21 (s, 3H), 1.31 (s, 3H),

3.32-3.38 (m, 1H), 3.44-3.51 (m, 1H), 3.71-3.85 (m, 2H), 5.88 (d, 1H, J = 10.4 Hz), 6.67 (d, 1H, J = 8.0 Hz), 6.76 (d, 1H, J = 8.8 Hz), 6.90 (m, 2H), 7.10 (d, 1H, J = 7.6 Hz), 7.19 (t, 1H), 8.00 (m, 2H). MS m/z (APCI): $[M+1]^+$: 353.0.

2. DSC spectra of SP-PCL nanoparticles

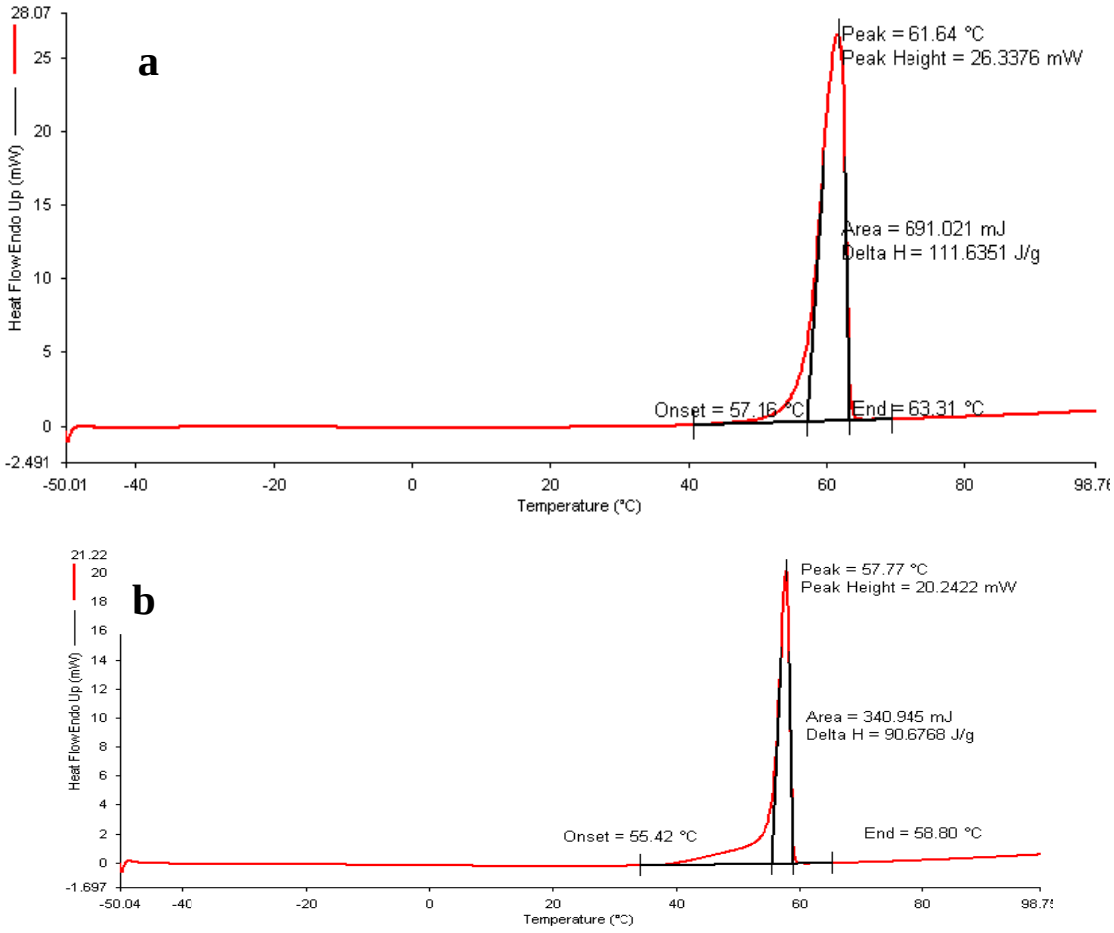


Fig. S1. DSC spectra of SP-PCL and SP-PCL nanoparticles. The DSC data indicate that the endothermic peak belongs to melting of SP-PCL crystal. **(a)** SP-PCL sample was first dissolved in chloroform and then precipitated in dry ether. Its melting point is about 61.6 ^{o}C, and the melting enthalpy is 111.63 $J{\cdot}g^{-1}$ that is calculated from the area of melting peak. Relative crystallinity is about 80%，based on the heat of melting of a 100% crystalline PCL (139.5 J/g)[2] by integration of the DSC curve. **(b)** SP-PCL sample was first dissolved in dioxane and then precipitated in water. Its melting point is about 57.7 ^{o}C, and the melting enthalpy is 90.67 $J{\cdot}g^{-1}$ that is calculated from the area of melting peak. Relative crystallinity is about 65%, based on the heat of melting of a 100% crystalline PCL (139.5 J/g) [2] by integration of the DSC curve.

3. On and off fluorescence switching of single SP-PCL nanoparticle.

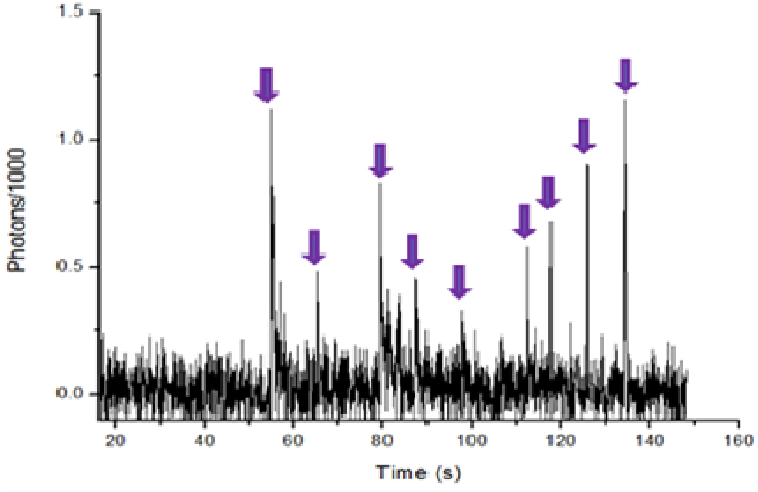
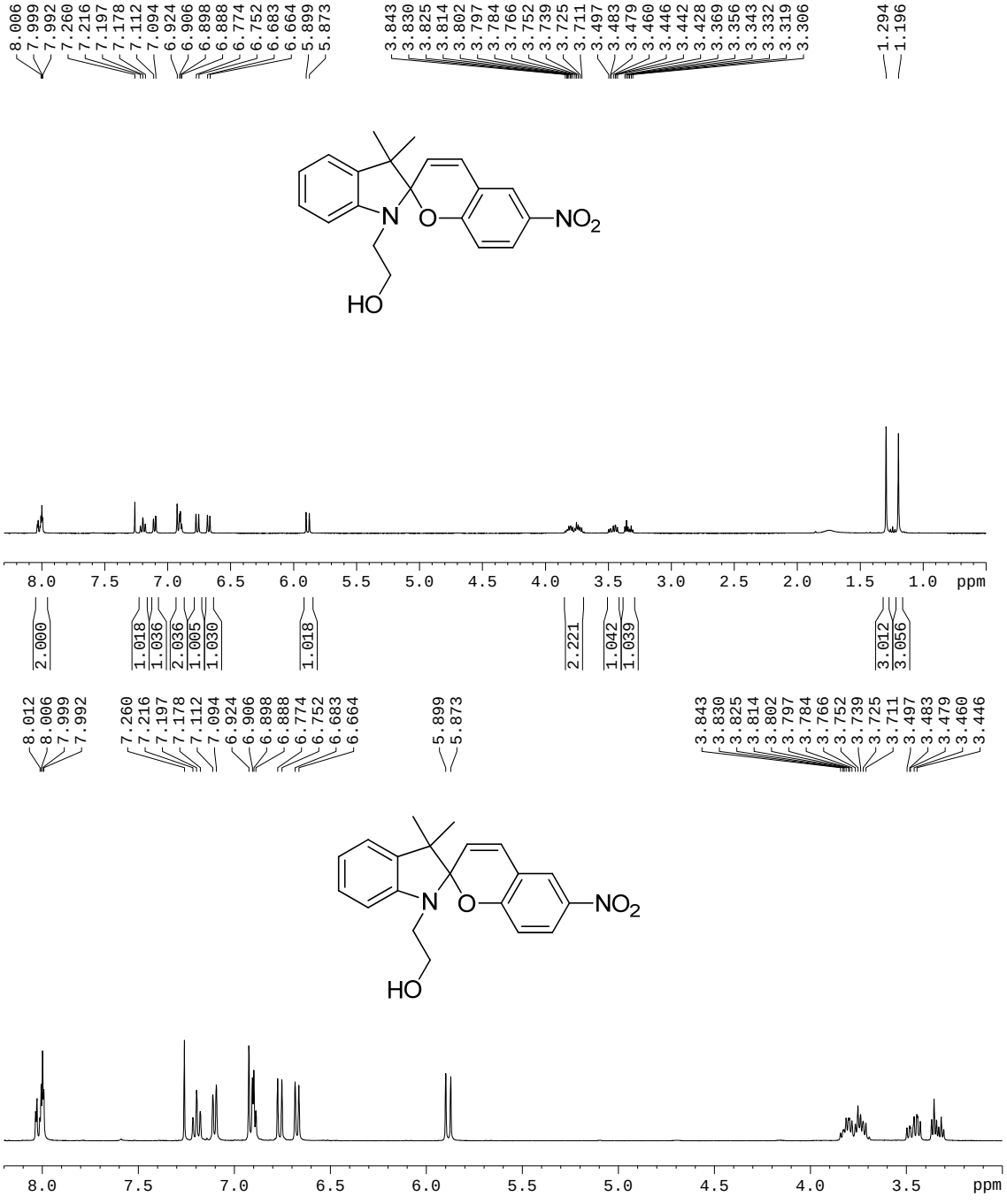
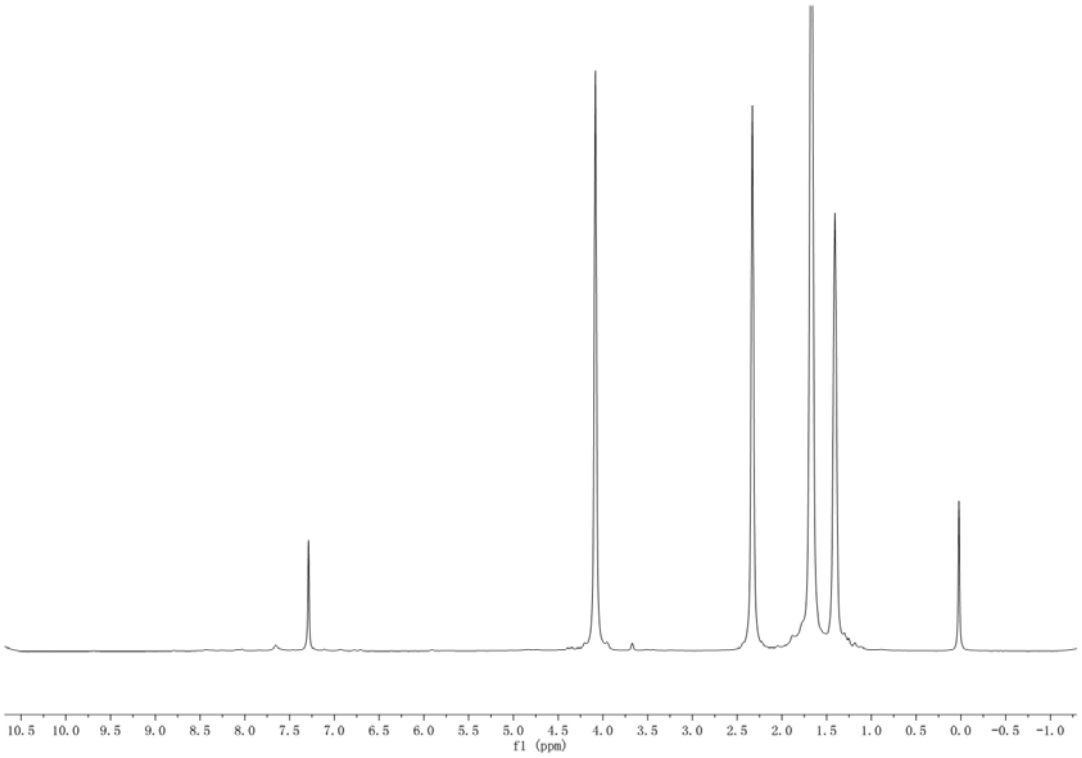


Fig. S2. Fluorescence of single SP-PCL nanoparticle can be turned on and off. A green laser (561 nm) is used to excite fluorescence from MC form inside nanoparticles, and switch fluorescent MC form to dark SP form inside nanoparticles. A 405 nm laser is used to return nonfluorescent SP form to fluorescent MC form inside nanoparticles. The purple arrows indicate the after 405 nm laser illumination, the fluorescence of single SP-PCL nanoparticle could be recovered immediately.

4. ^{1}HNMR spectra

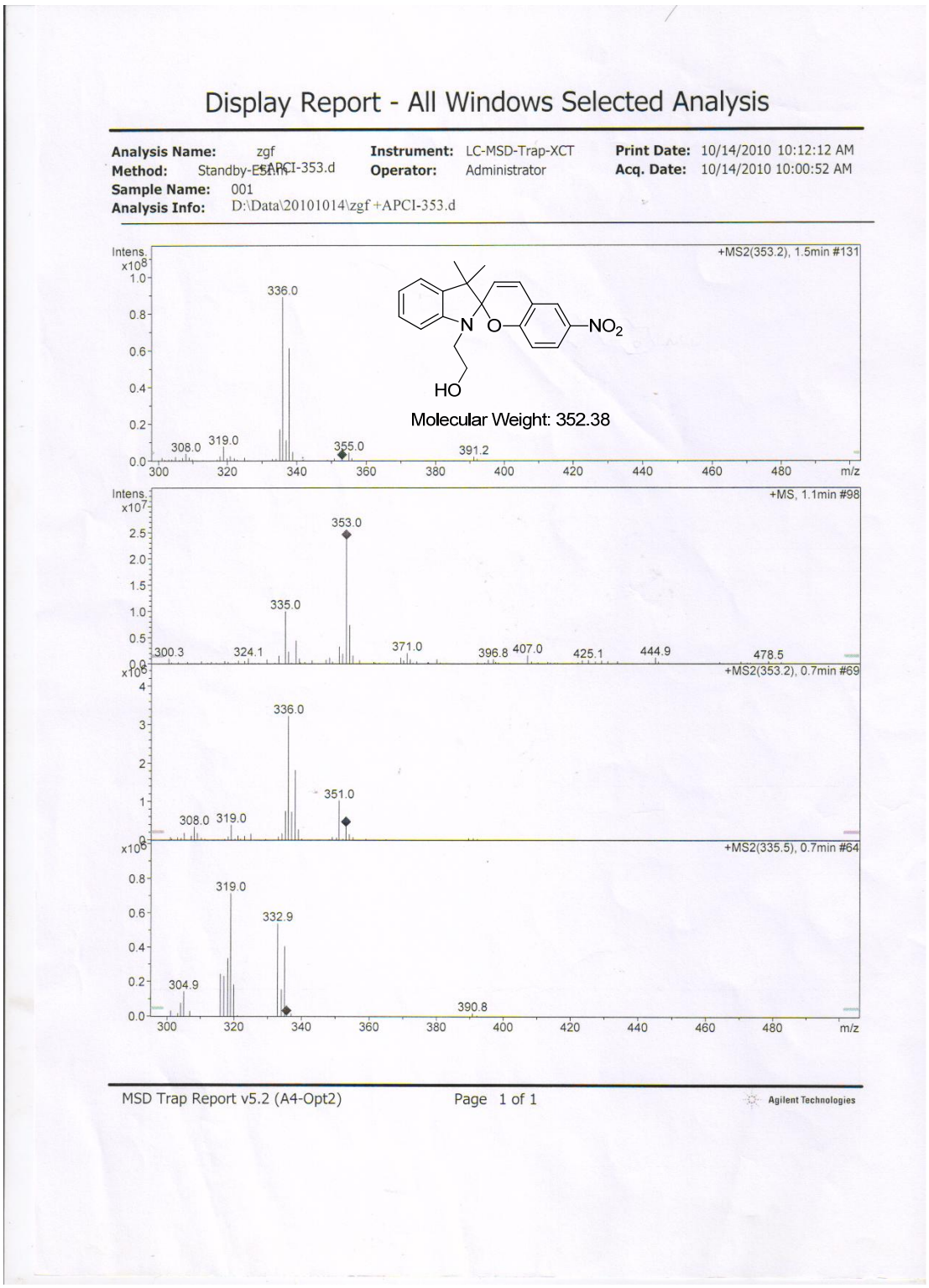
2-(3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indolin]-1'-yl)ethanol *SP-OH* (***3***).




10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

SP-PCL 1

5. Mass spectra



6. References

[1] Raymo FM, Giordani S. Signal Processing at the Molecular Level. *J. Am. Chem. Soc.* **2001**;*123*:4651-2.