Electronic Supplementary Information (ESI)

Responsive mesoporous silica nanoparticles for sensing of hydrogen peroxide and simultaneous treatment toward heart failure

Si Yu Tan,[a,‡] Cathleen Teh,[b,‡] Chung Yen Ang,[a,‡] Menghuan Li,[a,c] Peizhou Li,[a] Vladimir Korzh,[d,*] Yanli Zhao[a,c*]

[a]Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, 21 Nanyang Link, Singapore 637371. Email: zhaoyanli@ntu.edu.sg

[b]Institute of Molecular and Cell Biology, 61 Biopolis Drive, Singapore 138673.

[c]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798.

[d]International Institute of Molecular and Cell Biology, 4 Ks. Trojena Street, 02-109 Warsaw, Poland. Email: vkorzh@iimcb.gov.pl

‡These authors contributed equally to this work.

General procedure

All solvents and reagents were of analytical grade and used directly upon received from commercial sources. All reactions were performed at room temperature (23°C) and atmospheric pressure (1.0 atm) unless otherwise stated. Ultrapure water (18.2 Ω·cm resistivity at 25 °C and < 10 ppb Total Organic Carbon) was used directly upon dispensed from Sartorius Ultrapure Water dispenser system.

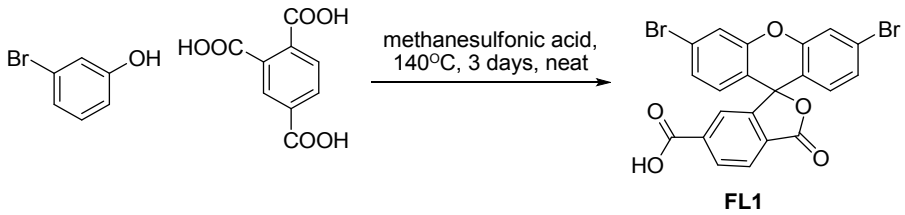
Compound purification by flash chromatography was performed with Merck Geduran® Si 60 silica gel with particles size of 40 to 63 μm. Column was stacked with the slurry mixture of the silica gel and the eluting solvent, and the pressure was applied on the column with an aquarium pump.

^{1}H and ^{13}C NMR measurements were carried out on a Bruker AMX 400 MHz NMR at ambient temperature. Chemical shifts δ for ^{1}H NMR spectra were reported in unit of parts per million (ppm)

downfield from TMS (δ 0.00 ppm) or relative to the deuterated DMSO (δ 2.50 ppm for DMSO) or deuterated water (δ 4.79 ppm for D_2O) used. All multiplicities were reported as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), dt (doublet of triplet) or m (multiplet). Coupling constants (*J* values) were reported in Hz and the number of equivalent protons for a given resonance was reported as *n*H. Chemical shifts δ for ^{13}C NMR spectra were reported in units of ppm relative to the residual signals from the deuterated DMSO (δ 39.5 ppm) used.

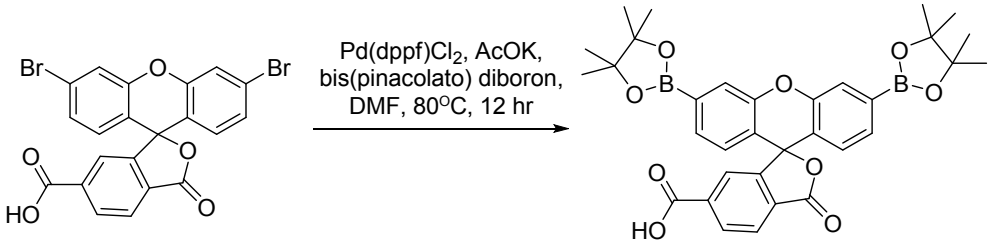
High resolution mass spectrometry (HRMS) was performed with Waters Q-tof Premier MS. Fourier transform infrared (FTIR) spectroscopy was carried out on a SHIMADZU IR Prestige-21 spectrophotometer. UV-Vis spectroscopy was carried out on a SHIMADZU UV-3600 UV-VIS-NIR spectrophotometer using a 10 mm by 10 mm 1.5 mL quartz cuvette. Fluorescence emission spectra were recorded using a Varian Cary Eclipse fluorescence spectrophotometer, equipped with water circulated temperature controller. Samples were contained in a 10 mm by 10 mm 1.5 mL quartz cuvette (four side transparent). Transmission electron microscope (TEM) images were recorded on JEOL : JEM 1400 thermionic emission electron microscope operating at an acceleration voltage at 100 kV. X-Ray photoelectric spectroscopy was carried out on a Phoibos 100 spectrometer, and a monochromatic Mg X-ray radiation source (SPECS, Germany) was used for the wide-range and high resolution S 2p scans at 12.53 kV. N_2 adsorption/desorption isotherms were measured on a Micromeritics ASAP 2020M automated sorption analyzer at a temperature of 192 K. Pre-treatment of the MSNPs was performed by degassing the powder samples at 100 °C for 12 h prior to the analysis. Thereafter, specific surface areas were calculated from the adsorption data in low pressure range using the Brunauer-Emmett-Teller (BET) model and pore size was determined using the Density Functional Theory (DFT) model. Powder X-ray diffraction (XRD) patterns were measured using the SHIMADZU XRD-6000 Labx diffractometer with Cu K α radiation (λ = 1.5405980 Å). Zeta potential measurements were performed using dynamic light scattering on a Malvern Zetasizer Nano ZS. Analytical liquid chromatography-mass spectrometry (Analytical LCMS) was conducted on ThermoFinnigan LCQ Fleet Mass Spectrometer equipped with Thermo Accela Liquid Chromatography.

Synthesis of organic compounds



FL1

Synthesis of FL1: This compound was prepared according to a previously reported procedure.[S1] Briefly, to a 3-neck flask equipped with a reflux condenser, 3-bromophenol (27.6 g, 159.8 mmol) and trimellitic acid acid (16.8 g, 79.9 mmol) were added. Gas exchange with nitrogen over 3 cycles was performed before the addition of methanesulfonic acid (60 mL). The mixture was heated to 140 ºC and reacted over 3 days. Thereafter, the solution was cooled down to room temperature and poured into ice water (400 mL). The precipitate was collected by vacuum flitration and dried in vauco. The resulting brown solid was then dissolved in 3:1 acetic anhydride : pyridine mixture (160 mL) to induce a white precipitate. The white precipitate was isolated and washed 6 times with 2:1 acetic anhydride : pyridine (60 mL) to give a light pink solid. The solid was then washed 3 times with HCl (100 mL, 1M) and 3 times with water to obtain a bone white solid (10.73 g, 27%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, J = 8.00 Hz, 1H), 8.18 (d, J = 8.00 Hz, 1H), 7.86 (s, 1H), 7.71 (s, 2H), 7.34 (dd, J = 8.60 Hz, 1.80 Hz, 2H), 6.88 (d, J = 8.40 Hz, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 168.0, 166.4, 152.7, 151.2, 138.3, 131.9, 130.5, 129.1, 128.2, 126.2, 125.2, 124.2, 120.4, 118.0. HRMS (H_2O/MeOH): m/z: calcd: 500.8973 $[M+H]^+$; found: 500.8986. IR (KBr) $\bar{\nu}$/cm^{-1}: 2980.0, 2881.7, 1766.80, 1722.4, 1614.1, 1593.2, 1562.3, 1479.4, 1408.0, 1267.2, 1230.58, 921.9, 756.1.

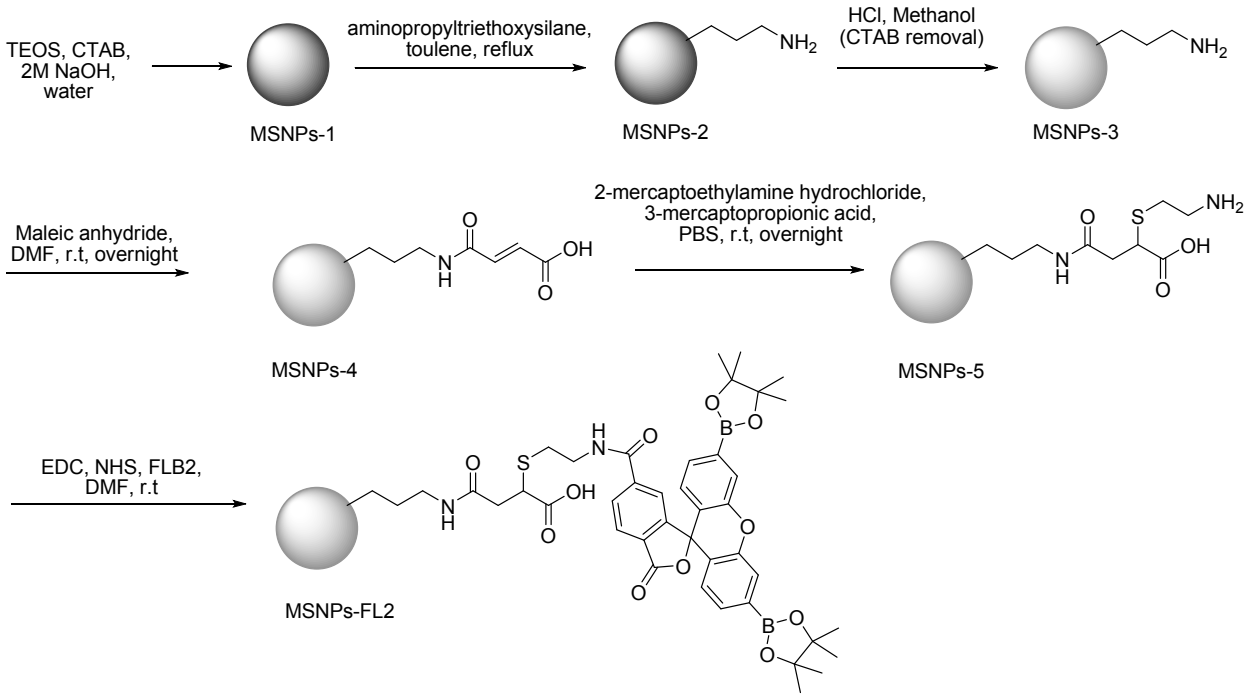


FL2

Synthesis of FL2: This compound was prepared according to a previously reported procedure.[S1] Briefly, Compound FL1 (2.0 g, 4.0 mmol), Pd(dppf)Cl_2 (980 mg, 1.2 mmol), potassium acetate (3.9 g, 40.1 mmol) and bis(pinacolato) diboron (4.0 g, 15.8 mmol) were charged into a 100 mL 3-neck flask under nitrogen. Anhydrous DMF (50 mL) was added and the solution was stirred at room temperature for 5 mins. The reaction was then heated to 80ºC overnight and cooled down to room temperature in the following day. DMF was distilled off under reduced pressure to leave a black mass, which was dissolved in ethyl acetate and washed 3 times with 0.5 N HCl, once with water, and once with brine separately. Thereafter, the organic layer was dried over Na_2SO_4, solvent was removed under reduced pressure, and the resulting mixture was purified by column chromatography. A reddish brown product was obtained by column chromatography, and it was washed with diethyl ether to give an light brown product (0.78 g, 33 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.26 (d, J = 8.00 Hz, 1H), 8.19 (d, J = 8.00 Hz, 1H), 7.68 (s, 1H), 7.63 (s,

2H), 7.41 (d, J = 8.00 Hz, 2H), 6.95 (d, J = 7.60 Hz, 2H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 168.3, 166.3, 153.6, 150.3, 138.2, 131.8, 130.0, 128.6, 128.0, 126.2, 124.8, 123.3, 121.2, 84.7, 25.1. HRMS (H_2O/MeOH): m/z: calcd: 596.2504 $[M+H]^+$; found: 596.2534. IR (KBr) $\bar{\nu}$/cm^{-1}: 3176.8, 2980.0, 1757.2, 1735.9, 1550.8, 1406.1, 1359.8, 1273.0, 1215.2, 1143.8, 974.0, 688.59.

Synthesis of functional mesoporous silica nanoparticles (MSNPs)



Synthesis of MSNPs-1: The MSNPs were synthesized according to previous reports. Briefly, to a 500 mL round bottom flask, cetyltrimethylammonium bromide (CTAB, 0.5g) was dissolved in distilled water (250 mL). Thereafter, 2M NaOH (1.75 mL) was added to the solution and the reaction mixture was heated to 80 ºC. Once the temperature has stabilized, tetraethyl orthosilica (TEOS, 2.5 mL) was added to the mixture and the resulting mixture was stirred for 2 h. Later, the precipate was isolated by centrifugation (8000 rpm, 10 min) and washed 2 times with distilled water and 2 times with methanol before it was dried under vacuum to afford MSNPs-1.

Synthesis of MSNPs-2: MSNP-1 (0.5 g) was dissolved in toulene (40 mL) and 3 aminopropyltriethoxysilane (0.125 mL) was added to solution. The resulting mixture was refluxed under inert conditions overnight, and the precipitate was collected in the next day by centrifugation (8000 rpm, 10 min). The precipitate was then washed 3 times with methanol and dried under vacuum to afford MSNPs-2.

Synthesis of MSNPs-3: MSNP-2 (0.5 g) was dissolved in a mixture of methanol (55 mL) and hydrochloric acid (3 mL). The resulting mixture was refluxed under overnight and the precipitate was collected in the following day by centrifugation (8000 rpm, 10 min). The precipitate was washed 3 times with methanol and dried under vacuum to afford MSNPs-3.

Synthesis of MSNPs-4: MSNP-3 (0.1 g) was dispersed in DMF (10 mL) before maleic anhydride (21 mg) was added. The mixture was stirred at room temperature overnight and the precipitate was collected in the next day by centrifugation (8000 rpm, 10 min). The precipitate then was washed 3 times with methanol and dried under vacuum to afford MSNPs-4.

Synthesis of MSNPs-5: MSNP-4 (0.1 g) was dispersed in PBS (5 mL) before 2-mercaptoethylamine hydrochloride (9.6 mg) and 3-mercaptopropionic acid (1.9 μL) were added. The mixture was stirred at room temperature overnight and the precipitate was collected in the next day by centrifugation (8000 rpm, 10 min). The precipitate then was washed twice with water and twice with methanol and dried under vacuum to afford MSNPs-5.

Synthesis of MSNPs-FL2: FL2 (51 mg) was dispersed in DMF (5 mL) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 81 mg) and N-hydroxysuccinimide (49 mg) were added. The solution was allowed to stir for 4 h before the addition of MSNPs-5 (0.1 g). The mixture was allowed to stir at room temperature overnight and the precipitate was collected by centrifugation (8000 rpm, 10 min) in the next day. The precipitate was washed 3 times with DMF and twice with methanol, and dried under vacuum to yield MSNPs-FL2.

Procedure for characterization of MSNPs-FL2

a. Kinetics of MSNPs-FL2

MSNPs-FL2 (2 mg) was added to PBS (1 mL) and the suspension was added to a 1.5 mL cuvette. At zero minute, H_2O_2 solution (10 μL, 0.5 M) was added to the cuvette to give a final concentration of 5 mM for H_2O_2. The readings were taken every 10 min at λ_{ex} = 494 nm / λ_{em} = 520 nm.

b. Detection limit of MSNPs-FL2

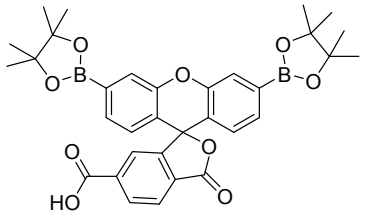
A series of solutions with concentrations of 1000 μM, 500 μM, 100 μM, 50 μM, 10 μM, 5 μM, 1 μM, 0.5 μM and 0.1 μM in volume 100 μL were prepared, which were added to MSNPs-FL2 solution (900 μL, 0.22 mg/mL), respectively. The solutions were stirred overnight at 37 ºC and the measurements at λ_{ex} = 494 nm were taken in the following day.

c. Determination of FL2 amount on the surface of MSNPs

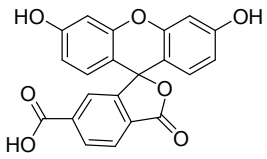
In order to determine the amount of FL2 on the surface of MSNPs, a 10 μL sample of FL2 was retained before the rest of the reagents were added for the synthesis of MSNPs-FL2. After the reaction, the MSNPs-FL2 was centrifuged down and the supernatant was retained. MSNPs-FL2 was washed 2 more times, and the supernatants obtained were combined with the previous one. Thereafter, both solutions containing FL2 (before the conjugation on MSNPs and supernatant after the conjugation on MSNPs) were diluted with 10 mM H_2O_2, and their concentrations were measured by high performance liquid chromatography (HPLC). In addition, a standard curve of FL2 was obtained in order to determine the concentration in the unknown solutions.

Procedure of obtaining job plot and Benesi–Hildebrand plot

a. Job Plot



FL2



6-FLCOOH

Stock solutions of FL2 (25 mM), α-CD and 6-carboxyfluorescein (6-FLCOOH) were prepared in D_2O, and the following solutions were prepared accordingly.

Solution no.	Volume of stock FL2 solution (μL)	Volume of stock α-CD solution (μL)	Volume of D_2O (μL)
1	80	0	920
2	70	10	920
3	60	20	920
4	50	30	920
5	40	40	920
6	30	50	920
7	20	60	920
8	10	70	920
9	0	80	920

Solution no.	Volume of stock 6-FLCOOH solution (μL)	Volume of stock α-CD solution (μL)	Volume of D_2O (μL)
1	40	0	960
2	35	5	960
3	30	10	960
4	25	15	960
5	20	20	960
6	15	25	960
7	10	30	960
8	5	35	960
9	0	40	960

Thereafter, each solution was analyzed on a Bruker AMX 400 MHz NMR.

b. Benesi-Hildebrand Plot

Stock solution of FL2 (25 mM), α-CD and 6-carboxyfluorescein (6-FLCOOH) were prepared in D_2O, and the following solutions were prepared accordingly. Both Benesi-Hildebrand plots of FL2 and 6-FLCOOH were plotted using the same table below.

Solution no.	[α-CD] (mM)	[Guest] [a] (mM)	Volume of stock α-CD solution (μL)	Volume of stock Host[a] solution (μL)	Volume of D_2O (μL)
1	12.5	0.625	500	25	475
2	10.9	0.625	438	25	538
3	9.375	0.625	375	25	600
4	7.81	0.625	313	25	663
5	6.25	0.625	250	25	725

[a] Guest refers to FL2 or 6-FLCOOH.

Thereafter, each solution was analyzed on a Bruker AMX 400 MHz NMR.

Characterization data of MSNPs

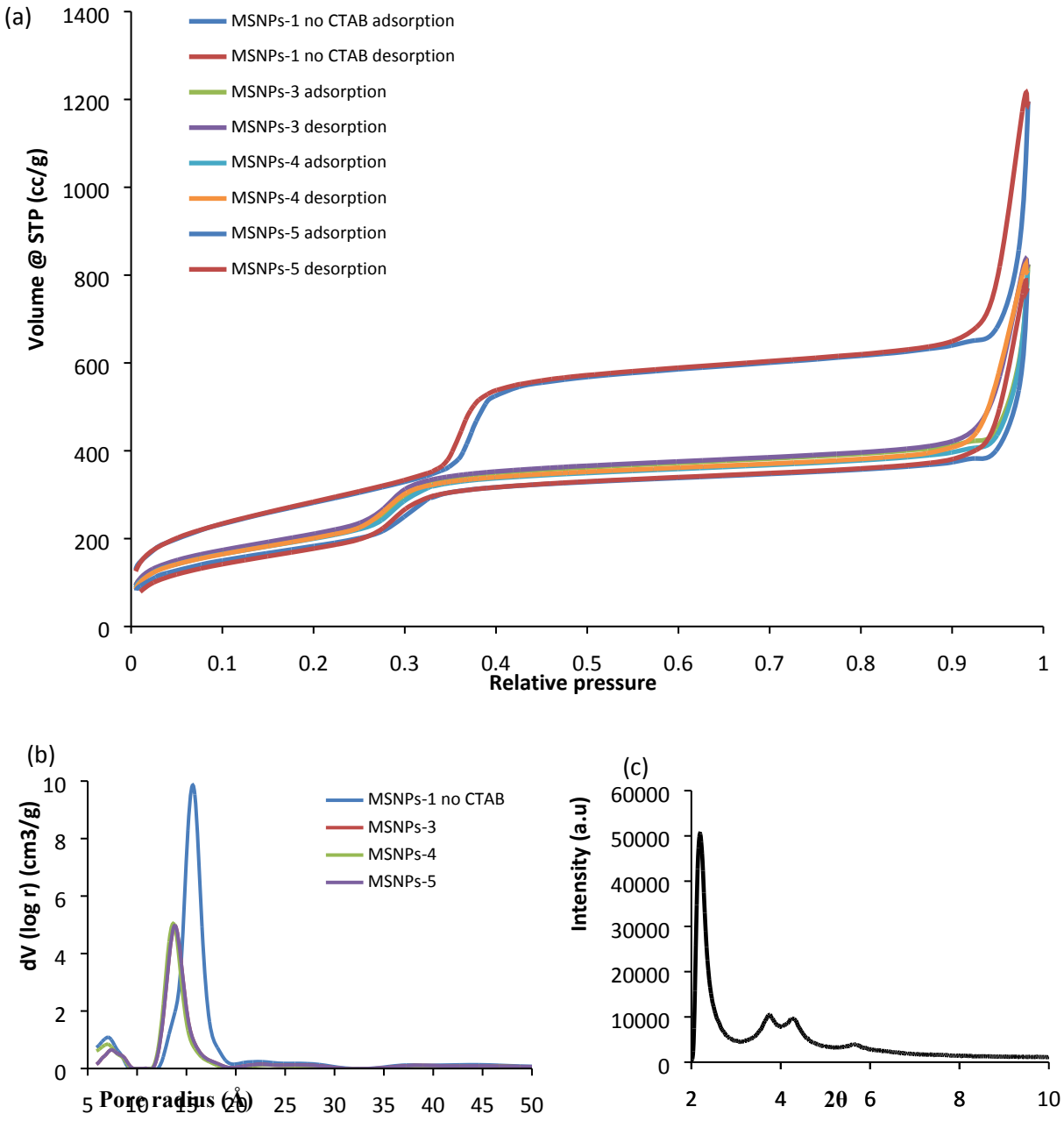


Figure S1. (a) BET isotherm plots, (b) DFT pore size distribution, and (c) powder XRD of MSNPs after stepwise functionalization.

MSNPs-FL2 Kinetics

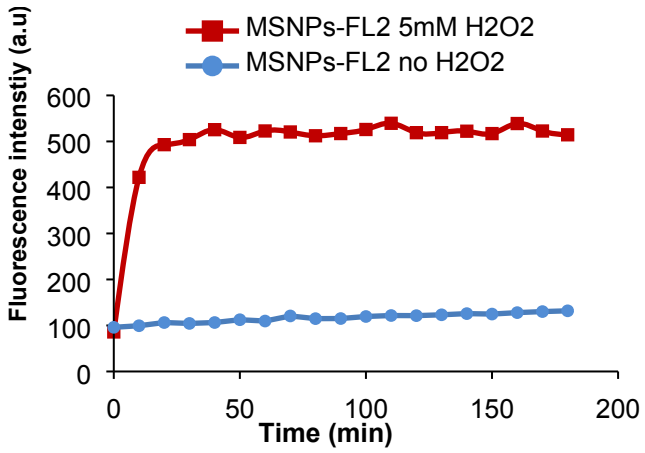


Figure S2. Kinetics of the "turn-on" fluorescence of the MSNPs-FL2 in the absence and presence of H_2O_2.

FL2 and 6-FLCOOH complexation with α-CD

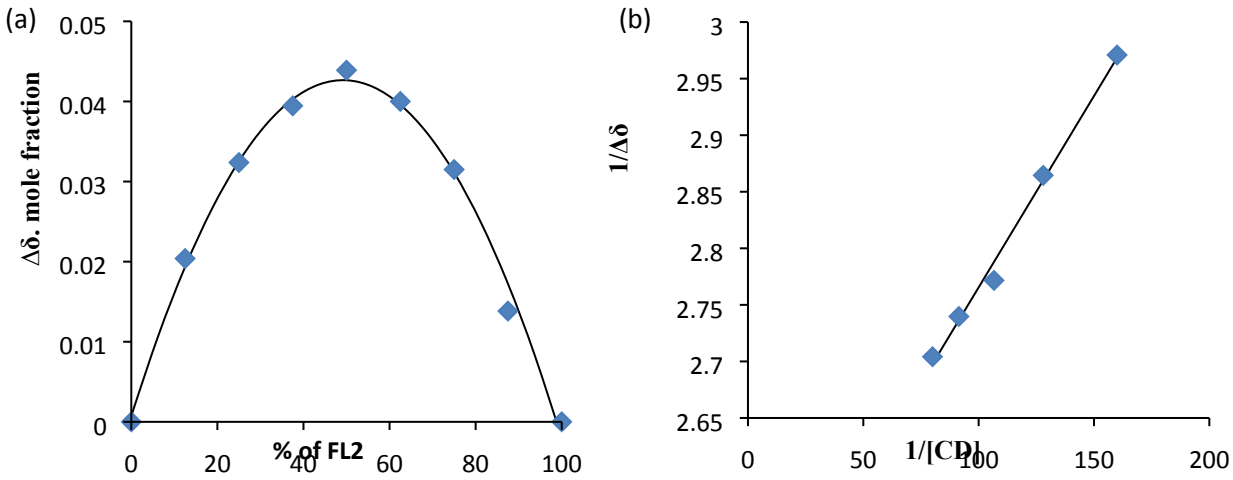


Figure S3. (a) Job plot of FL2 with α-CD at 7.80 ppm and (b) the Benesi-Hildebrand plot obtained at 7.80 ppm to afford the binding constant.

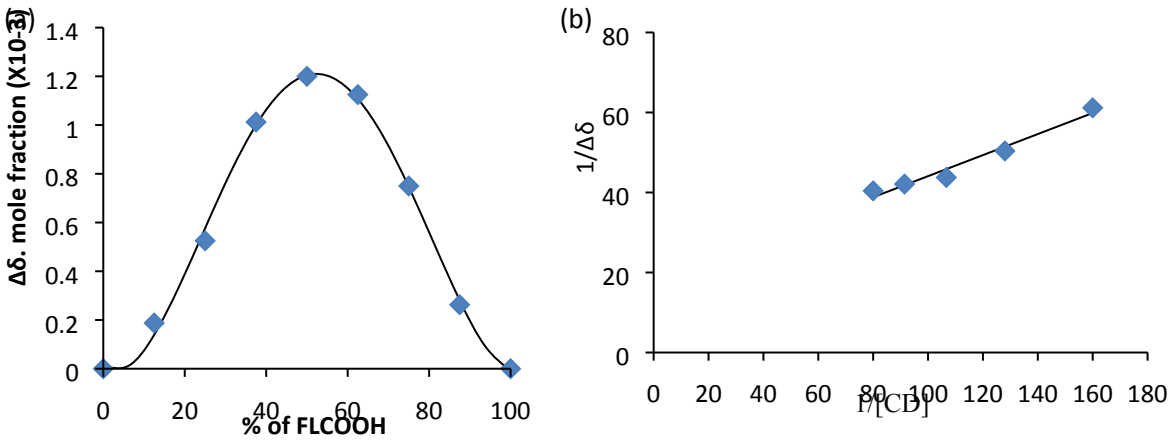


Figure S4. (a) Job plot of 6-FLCOOH with α-CD at 6.88 ppm and (b) the Benesi-Hildebrand plot obtained at 6.88 ppm to afford the binding constant.

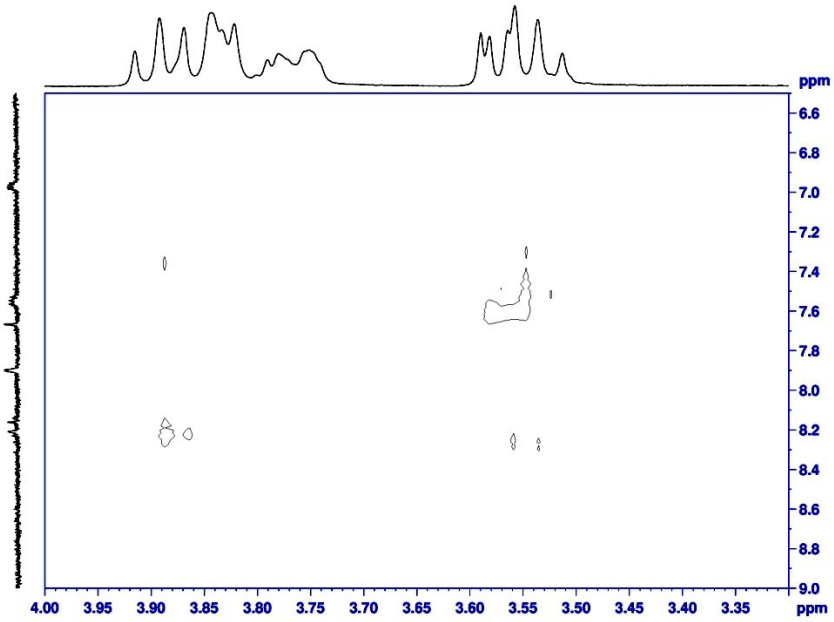


Figure S5. ^{1}H ROESY spectrum of FL2/α-CD complex.

In vivo experiments

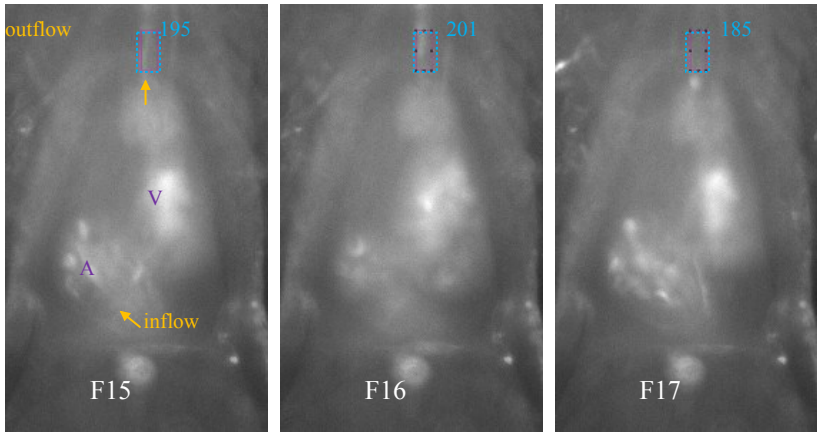


Figure S6. Cardiac output was estimated from sequential images of fluorescent red blood cell outflow (blue box) from the heart. Fluorescent intensity from red blood cells was computed in gray values. Time variation fluctuations of which are depicted as plot profile for all analyzed larvae.

Reference

S1. D. Srikun, A. E. Albers and C. J. Chang, *Chem. Sci.* 2011, **2**, 1156-1165.