Electronic Supplementary Information

A modular approach for assembling turn-on fluorescence sensors using molecularly imprinted nanoparticles

Qianjin Li, Tripta Kamra and Lei Ye*

Division of Pure and Applied Biochemistry, Lund University, Box 124, 221 00 Lund, Sweden
Tel: +46 2229560; Fax: +46 2224611; Email: lei.ye@tbiokem.lth.se

1. Materials

Trimethylolpropane trimethacrylate (TRIM, ≥90%), propargyl acrylate (PPA, 98%), atenolol (≥98%), potassium carbonate, sodium ascorbate, $CuSO_4 \cdot 5H_2O$ (≥98%), dansyl choride (DSC, ≥99%) and 11-azido-3,6,9-trioxaundecan-1-amine (ATAA, ≥90%) were purchased from Sigma-Aldrich. Methacrylic acid (MAA, 98.5%) was purchased from ACROS and was used as received. Azobisisobutyronitrile (AIBN) from ACROS was re-crystallized from methanol before use. (R,S)-Propranolol hydrochloride (99%) supplied by Sigma-Aldrich was converted into free base form before being used as template to prepare molecularly imprinted polymers. (R,S)-Atenolol (98%) was from Leiras (Helsiki, Finland). 1-Amino-3-(naphthalene-1-yloxy)propan-2-ol (ANOP) was from Aurora Feinchemie GmbH (Graz, Austria). Acetonitrile (ACN, 99.7%) of HPLC grade was from Honeywell (Seelze, Germany). Other solvents and inorganic salts were of analytical reagent grade and were used without further purification.

2. Equipments

UV−vis absorption spectra were recorded with a Beckman Coulter DU 800 UV/vis spectrophotometer. Fluorescence emission was measured using a QuantaMaster C-60/2000 spectrofluorometer (Photon Technology International, Lawrenceville, NJ). Scanning electron microscopy (SEM) imaging was carried out on a JEOL JSM-6700F Field Emission Scanning Electron Microscope (Tokyo, Japan). Particle size distribution was measured on a Zetasizer Nano ZS instrument equipped with a software package DTS Ver. 4.10 (Malvern Instruments Ltd., Worcestershire, UK).

3. Synthesis of molecularly imprinted nanoparticles MIP-CCH

MIP-CCH nanoparticles were synthesized by precipitation polymerization following a literature method with some modifications.[1] Propranolol (free base, 137 mg, 0.53 mmol) was dissolved in 40 mL ACN. After addition of MAA (111 μL, 1.31 mmol), PPA (58 μL, 0.53 mmol), TRIM (611 μL, 2.02 mmol) and AIBN (50 mg), the mixture was sonicated for 1 minute to give a clear solution. The solution was purged with a gentle flow of N_2 for 10 min and sealed. The reaction mixture was transferred into an oven and heated to 60 °C for 24 h while the glass reactor was rotated at a speed of 20 rpm.

After the polymerization, the nanoparticles formed (MIP-CCH) were collected by centrifugation, and washed with ACN once. For comparison, non-imprinted polymer nanoparticles (NIP-CCH) were synthesized in the same way except for the omission of the template propranolol.

4. Synthesis of MIP-NH_2

MIP-NH_2 nanoparticles were synthesized by introducing ATAA into MIP-CCH nanoparticles through Cu(I)-catalyzed click reaction. [2] Half of the MIP-CCH particles was dispersed in 100 mL of ACN/H_2O (1/1, v/v). Then, 400 μL $CuSO_4$ solution (100 mM), 2 mL sodium ascorbate (100 mM) and 80 μL (0.41 mmol) ATAA were added. The reaction mixture was stirred at room temperature in dark for 48 h. After the reaction, the MIP-NH_2 particles were collected by centrifugation and were washed with ACN for two times. The corresponding non-imprinted polymer NIP-NH_2 were prepared from NIP-CCH using the same procedure.

5. Synthesis of MIP-DSC

Half of the MIP-NH_2 particles were dispersed in 50 mL ACN. Then, 36 mg (0.13 mmol) DSC and 40 mg K_2CO_3 were added. The reaction mixture was stirred at room temperature in dark for 24 h. After the reaction, the MIP-DSC particles were collected by centrifugation. To remove the template propranolol, MIP-DSC particles were washed with MeOH/HAc (10/1, v/v) repeatedly until no template could be detected from the washing solution by UV measurement. The nanoparticles were finally washed with MeOH and dried in a vacuum chamber at room temperature. The corresponding non-imprinted polymer NIP-DSC were prepared from NIP-NH_2 using the same procedure.

6. Quantification of dansyl groups in MIP-DSC

The amount of dansyl groups in MIP-DSC were determined using a method described as the following: First, 60 mg MIP-NH_2, 11 mg DSC and 12 mg K_2CO_3 were mixed in 20 mL ACN. The mixture was stirred at room temperature in dark for 24 h. After the reaction, the mixture was centrifuged and the supernatant was collected. The amount of unreacted DSC was quantified using UV/vis spectrophotometer. A standard curve for quantifying DSC was obtained by diluting a stock solution of 1 mg DSC and 11 mg K_2CO_3 prepared in 2 mL ACN. The amount of dansyl groups in NIP-DSC were determined in the same way.

7. Kinetic fluorescence measurement

Kinetic FRET response measurement was performed as following: a suspension of nanoparticles (25 mg L^{-1}) was prepared in 2 mL ACN. The fluorescence intensity (F_0) of the suspension was measured at 500 nm using an excitation wavelength of 292 nm. After addition of propranolol that gave a final concentration of 1.0 μM, the mixture was shaken for 1 min before the fluorescence intensity (F) was measured at different time intervals.

8. Kinetic binding analysis

Kinetic binding analysis was carried out as following: (1) a suspension of nanoparticles (25 mg L^{-1}) was prepared in 2 mL ACN; (2) after addition of a concentrate propranolol solution (0.1 mM, 20 μL) to the suspension, the mixture was shaken on a rocking table for different periods; (3) the sample was centrifuged at 12500 rpm for 10 min, and the supernatant was collected; (4) the amount of propranolol in the supernatant was measured using a fluorescence spectrometer, and the result was used to calculate the amount of propranolol bound to the nanoparticles.

9. Dose-dependent fluorescence emission

A suspension of nanoparticles (25 mg L^{-1}) was prepared in 2 mL ACN. The fluorescent emission spectrum of the suspension was measured using an excitation wavelength of 292 nm. After addition of a concentrate propranolol solution, the mixture was shaken for 5 min before the new fluorescent emission spectrum was measured.

10. Equilibrium binding

A suspension of nanoparticles (25 mg L^{-1}) was mixed with propranolol solution at different concentrations in 2 mL ACN. After incubation overnight, the sample was centrifuged and the free propranolol in the supernatant was measured using a fluorescence spectrometer. The result was used to calculate the amount of propranolol bound to the nanoparticles.

11. Detection of propranolol in tap water

Tap water spiked with 20 μM propranolol (0.5 mL) was added to a mixture composed of 50 μg of MIP-DSC nanoparticles suspended in 1 mL ACN and 0.5 mL of 10 mM phosphate buffer (pH 7.0). The sample was shaken for 5 min before its fluorescence intensity at 500 nm was measured using an excitation wavelength of 292 nm. The experiment was repeated independently for three times. For propranolol quantification, a standard curve was first established using pure propranolol solution in purified water (0.5 mL).

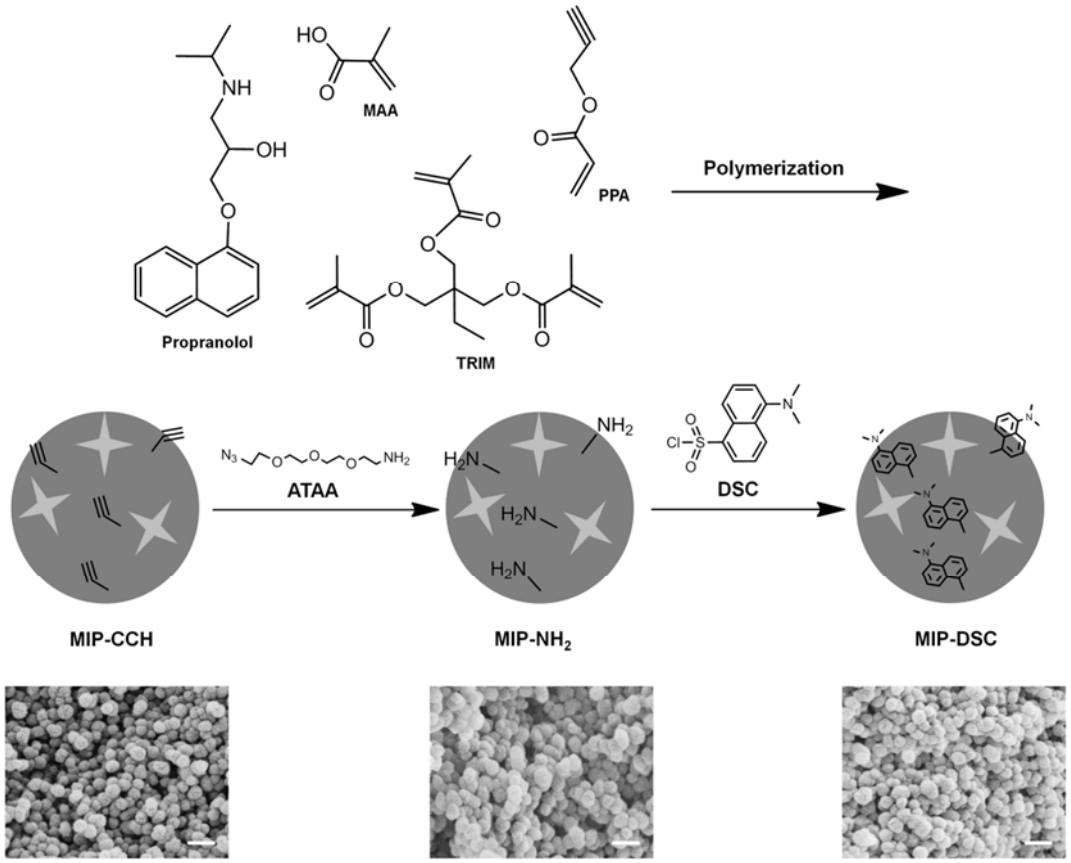

Figure S1. Synthesis of propranolol-imprinted, dansyl-labelled fluorescent nanoparticles, and their morphologies characterized by SEM. The length of the scale bar in SEM images is 200 nm.

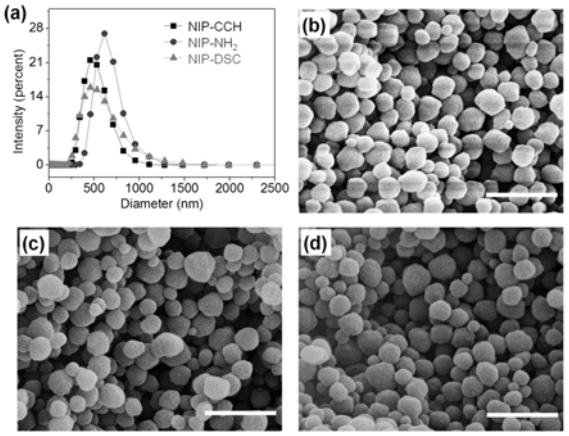

Figure S2. Characterization of NIP particles by dynamic light scattering (a), and by SEM imaging for NIP-CCH (b), NIP-NH_2 (c) and NIP-DSC (d). The length of the scale bar is 1 μm.

Table S1. Selectivity and DSC amount

Nanoparticle	Bound, %	Bound, μmol/g	IF [a]	Amount of DSC, mmol/g
MIP-CCH	14.1±0.4	56.2±1.6	3.7±0.1	/
NIP-CCH	3.8±0.0	15.2±0.1		/
MIP-NH2	15.3±0.1	61.3±0.3	3.7±0.0	/
NIP-NH2	4.1±0.0	16.6±0.1		/
MIP-DSC	11.3±0.1	45.2±0.2	3.7±0.0	0.19±0.01
NIP-DSC	3.1±0.0	12.3±0.1		0.40±0.03

Note: (a) IF (imprinting factor) = Propranolol bound to MIP / Propranolol bound to NIP

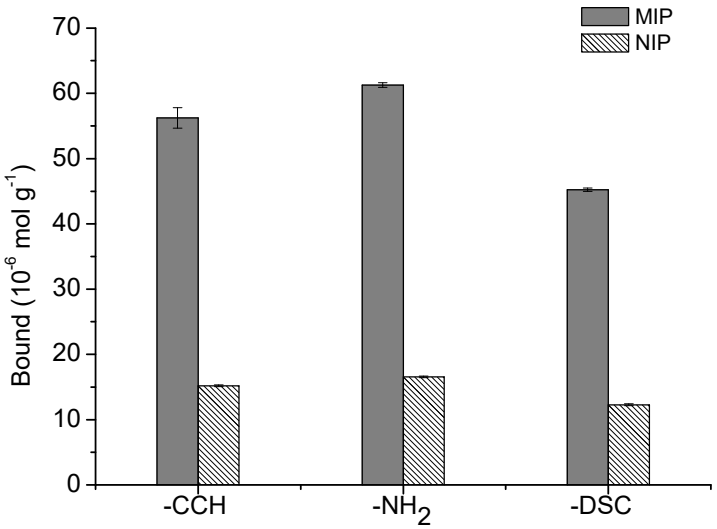

Figure S3. Uptake of propranolol by the MIP nanoparticles (MIP-CCH, MIP-NH_2 and MIP-DSC) and the NIP nanoparticles (NIP-CCH, NIP-NH_2 and NIP-DSC) in ACN. Initial concentration of propranolol: 10 μM; nanoparticle concentration: 25 mg L^{-1}.

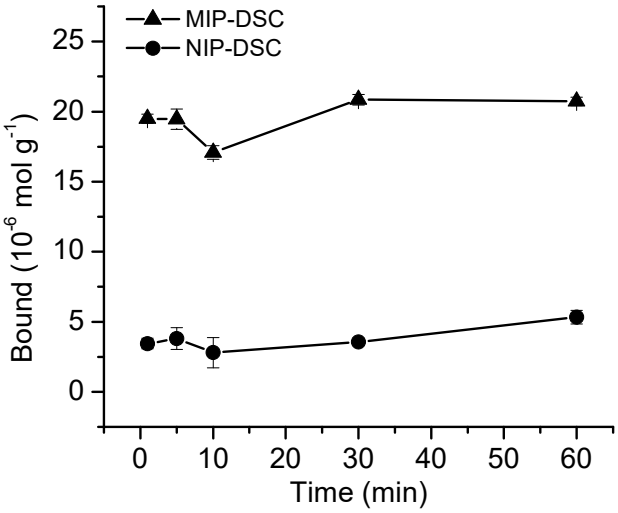

Figure S4. Kinetic binding of propranolol to MIP-DSC and NIP-DSC. Nanoparticle concentration: 25 mg L^{-1}; propranolol concentration: 1 μM.

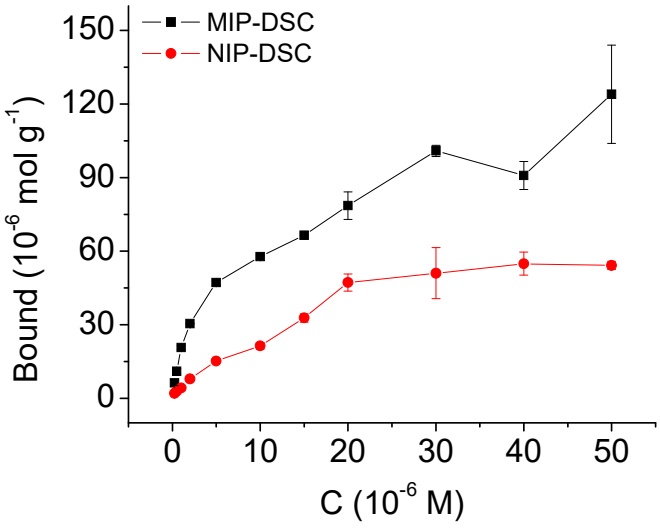

Figure S5. Uptakes of propranolol at different concentrations by MIP-DSC and NIP-DSC. Nanoparticle concentration used is 25 mg L^{-1}.

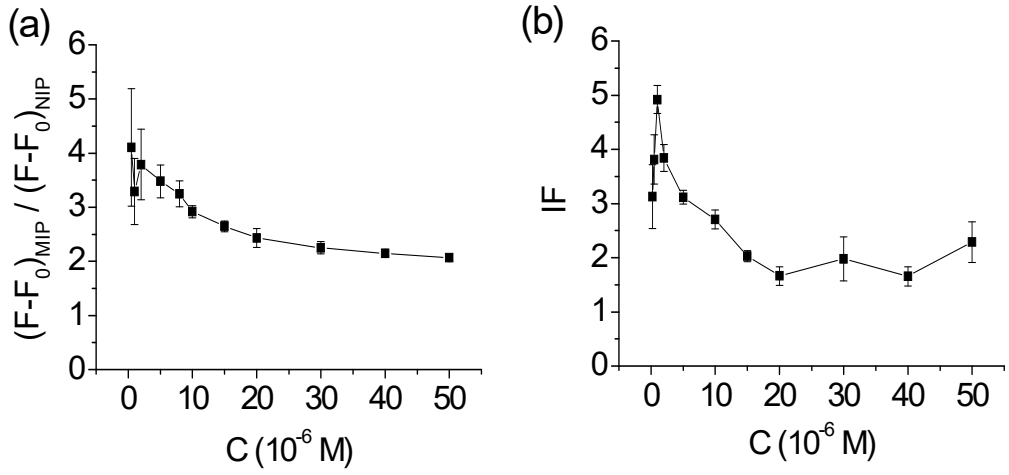

Figure S6. (a) Specificity of MIP-DSC system to propranolol evaluated by $(F\text{-}F_0)_{MIP}/(F\text{-}F_0)_{NIP}$, calculated from the data in Figure 3a; (b) Specificity of MIP-DSC nanoparticles evaluated by IF, calculated from the data in Figure S6.

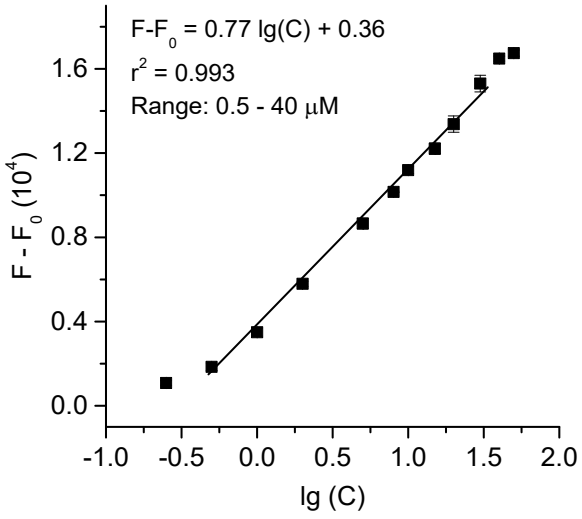

Figure S7. Linear curve of the turn-on fluorescence assay using MIP-DSC by plotting *F-F0* vs lg (*C*). The fluorescence intensity was measured at 500 nm using excitation wavelength of 292 nm, in the absence (F_0) and presence (F) of propranolol, respectively. Nanoparticle concentration: 25 mg L^{-1}.

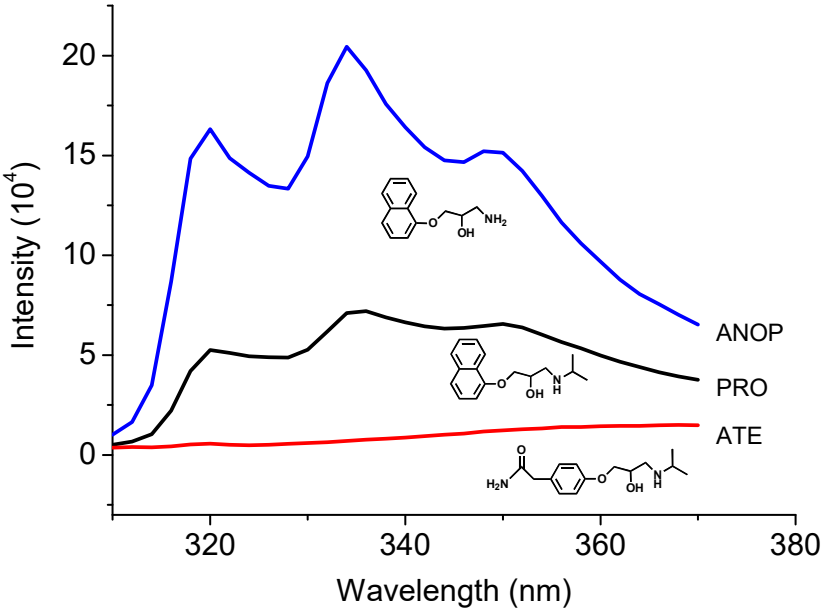

Figure S8. Fluorescent spectra of propranolol (PRO) and its analogues atenolol (ATE) and ANOP, measured using excitation wavelength of 292 nm in ACN. The concentration of each compound was 10 μM.

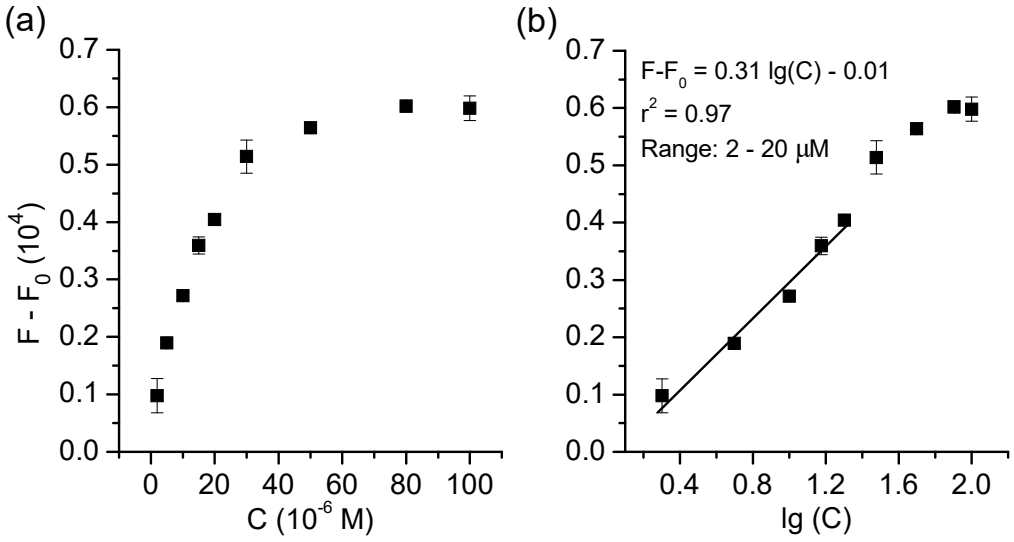

Figure S9. FRET response of the turn-on fluorescence assay system using MIP-DSC to propranolol concentrations (a) and its linear relationship (b). Fluorescence intensity (F) was measured at 500 nm using excitation wavelength of 292 nm. F_0 means that fluorescence intensity was measured in the absence of propranolol. Solvent: phosphate buffer (5 mM, pH 7.0)/ACN = 1/1 (v/v).

References

1. K. Yoshimatsu, K. Reimhult, A. Krozer, K. Mosbach, K. Sode, L. Ye, *Anal. Chim. Acta* 2007, **584**, 112.
2. C. G. Xu, L. Ye, *Chem. Commun.* 2011, **47**, 6096.