Electronic Supplementary Information

Clickable molecularly imprinted nanoparticles

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1. Materials

Trimethylolpropane trimethacrylate (TRIM, technical grade), propargyl acrylate (98%), ethyleneglycol dimethacrylate (EDGMA, 98%), sodium ascorbate, 4-vinylpyridine (95%), sodium azide (≥99.0%), propargyl amine (98%), atenolol (≥98%), 11-azido-3,6,9-trioxaundecan-1-amine, $CuSO_4 \cdot 5H_2O$ (≥98%), 2,4-dichlorophenoxyacetic acid carboxyl-^{14}C and 2,4-dichlorophenoxyacetic acid (≥98%) were purchased from Sigma-Aldrich (Dorset, UK). 4-vinylpyridine was purified by vacuum distillation before use. Acetic acid (glacial, 100%), acetonitrile (99.7%) and azobisisobutyronitrile (AIBN, 98%) used for polymer synthesis were purchased from Merck (Darmstadt, Germany). AIBN was re-crystallized from methanol before use. Methacrylic acid (MAA, 98.5%) was purchased from ACROS (Geel, Belgium) and used as received. Glycidyl methacrylate (≥97.0%), fluorescein isothiocyanate (FITC, ≥90%, HPLC) and (*R,S*)-Propranolol hydrochloride (99%) were supplied by Fluka (Dorset, UK). (*R,S*)-Propranolol hydrochloride was converted into free base form before use. (*S*)-[4- ^{3}H]-Propranolol (specific activity 555 GBq mmol^{-1}, 66.7 μM solution in ethanol) was purchased from NEN Life Science Products Inc. (Boston, MA), and 2,4-dichlorophenoxyacetic acid-*carboxy*-^{14}C ([^{14}C]-2,4-D, specific activity 15.7 mCi mmol^{-1}) was obtained from Sigma. Scintillation liquid, Ecoscint A was from National Diagnostics (Atlanta, GA).

2. Synthesis of propranolol imprinted core-shell nanoparticles

Propranolol imprinted core nanoparticles were synthesized following a procedure described by Yoshimatsu *et al.*[1] The template molecule, (*R,S*)-propranolol (137 mg, 0.53 mmol) was dissolved in 40 mL of acetonitrile in a 150 mm × 25 mm borosilicate glass tube equipped with a screw cap. MAA (113 mg, 1.31 mmol), TRIM (648 mg, 2.02 mmol) and

AIBN (28 mg) were then added. The solution was purged with a gentle flow of nitrogen for 5 min and then sealed. Polymerization was carried out by fixing the borosilicate glass tube horizontally in a Stovall HO-10 Hybridization Oven (Greensboro, NC, USA), and rotated at a speed of 20 rpm, at 60°C for 24 h (note: the glass reactor was introduced into the oven pre-heated at 60°C). This polymerization step led to propranolol imprinted core particles. To obtain imprinted core-shell particles, a mixture of propargyl acrylate (550.1 mg, 5 mmol), EDGMA (248.1 mg, 1.25mmol) and AIBN (24 mg) was added into the reaction tube. The mixture was purged with nitrogen for 5 min before the polymerization was continued for another 48 h at 60°C under the same gentle rotation in the Stovall HO-10 Hybridization Oven. After polymerization, polymer particles were collected by centrifugation. The template was removed by wash with methanol containing 10% acetic acid (v/v), until no template could be detected from the washing solvent using UV spectrometric measurement. The polymer particles were finally washed with acetone and dried in a vacuum chamber. For comparison, the non-imprinted nanoparticles were synthesized under the same condition except that no template was added.

3. Synthesis of 2,4-D imprinted core-shell nanoparticles

Core nanoparticles imprinted against 2,4-D were synthesized following a procedure described by Hunt *et al*. with minor modification.[2] The template molecule, 2,4-dichlorophenoxyacetic acid (150 mg, 0.679 mmol) was dissolved in 36 mL of acetonitrile in a glass tube equipped with a screw cap. The functional monomer, 4-vinylpyridine (285.3 mg, 2.71 mmol), EGDMA (1141.2 mg, 5.757 mmol) and AIBN (36 mg) were then added. The solution was purged with a gentle flow of N_2 for 5 min and sealed. The reaction tube was rotated at a speed of 20 rpm at 60°C in the Stovall HO-10 Hybridization Oven for 16 h. This polymerization step led to 2,4-D imprinted core particles. After the first polymerization step, a mixture of glycidyl methacrylate (156 mg, 1.1 mmol), EDGMA (54.3 mg, 0.274 mmol) and AIBN (5 mg) was added into the reaction tube. The reaction mixture was purged with N_2 for 5 min before it was rotated in the Stovall HO-10 Hybridization Oven for another 16 h. After the second polymerization step, a mixture of 10 mL of 0.66 M aqueous sodium azide and 10 mL of acetonitrile were added into the glass reaction tube. The mixture was purged with N_2 for 5 min, then rotated in the Stovall HO-10 Hybridization Oven at a speed of 20 rpm at 50°C for 20 h. Polymer particles were collected by ultracentrifugation, washed with acetonitrile, followed by methanol containing 10% acetic acid until no template could be detected from the washing solvent by UV spectrometric measurement. Polymer particles were finally washed in

acetone and dried in a vacuum chamber. For comparison, the non-imprinted nanoparticles were synthesized under the same condition except that no template was added.

4. Dynamic light scattering (DLS) analysis

The hydrodynamic size of the nanoparticles was determined using DLS measurement. Acetonitrile was used as the dispersant.

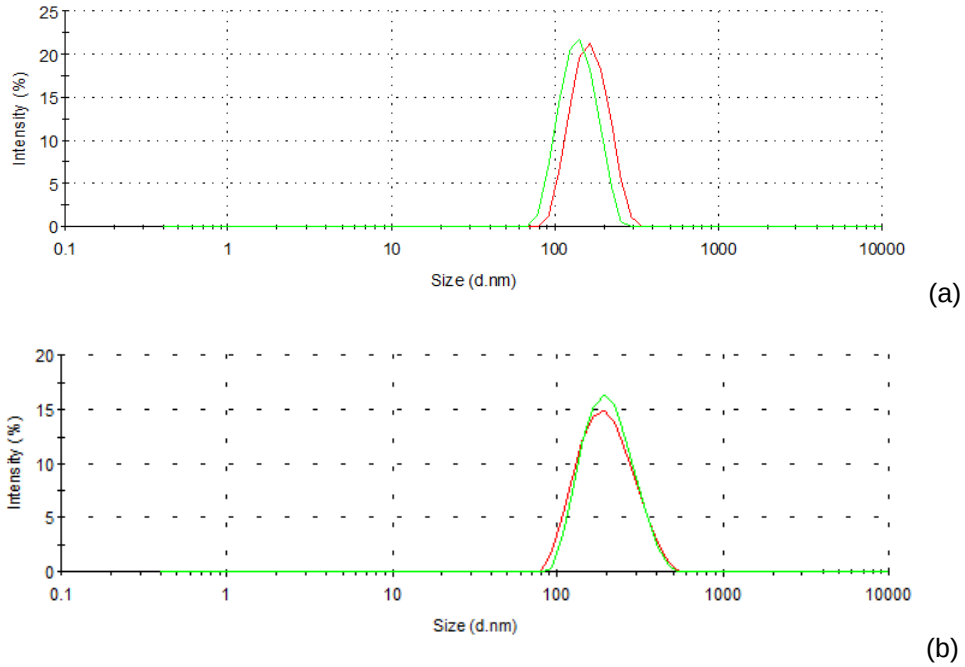
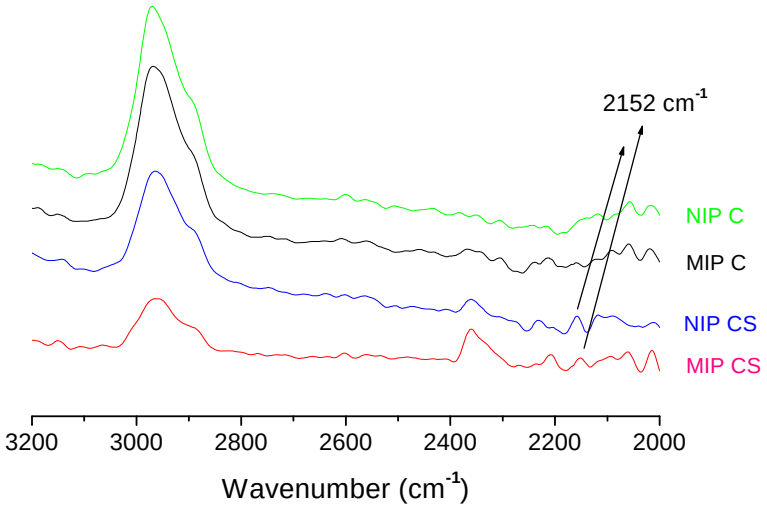


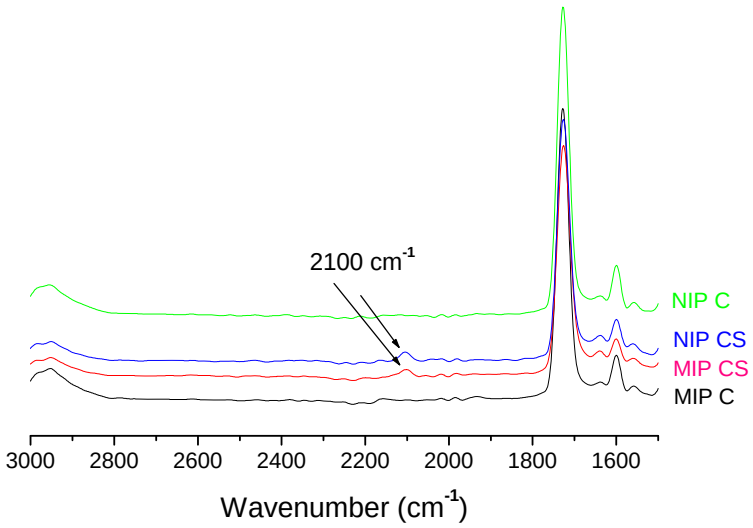
Fig. S1 Hydrodynamic size of propranolol-imprinted nanoparticles (a) and 2,4-D-imprinted nanoparticles (b) measured by DLS. The distribution curve shown in green is from the core particles, and the curve in red is from the core-shell particles.

5. Detection of alkynyl and azide groups by FT-IR analysis

The presence of alkynyl and azide groups in the core-shell nanoparticles was confirmed by FT-IR analysis. Attenuated total reflection (ATR) infrared spectra were recorded on a Perkin-Elmer FTIR instrument (Perkin-Elmer Instruments). All spectra were collected in the 4000-375 cm^{-1} region with a resolution of 4 cm^{-1}, with 32 scans, and at 25 °C.



(a)



(b)

Fig. S2 FT-IR analysis of molecularly imprinted polymer (MIP) and non-imprinted polymer (NIP) nanoparticles in the propranolol-imprinting system (a) and the 2,4-D-imprinting system (b). C: core particles; CS: core-shell particles. Characteristic IR absorption bands for terminal alkyne and azide are at 2152 cm^{-1} and 2100 cm^{-1}, respectively.

6. Fluorescent labelling of nanoparticles using Cu(I)-catalyzed click reactions

FITC-derivatives containing terminal azide or alkynyl groups were used to label nanoparticles containing terminal alkynyl or azide groups, respectively (Figure S3). After fluorescent labelling, the nanoparticles were washed thoroughly with solvent before their

fluorescence emission spectra were measured. For comparison, core nanoparticles without the clickable groups were treated under the same conditions.

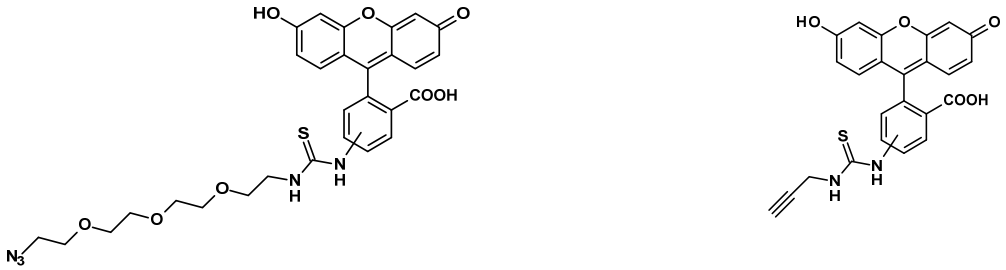


Fig. S3 FITC-N_3 and FITC-alkyne used for fluorescent labelling of nanoparticles.

6.1. Fluorescent labelling of alkynyl-nanoparticles with FITC-N_3

FITC-N_3 was prepared by reacting FITC with excess of 11-azido-3,6,9-trioxaundecan-1-amine. Briefly, FITC (10 mg, 0.0256 mmol) and 11-azido-3,6,9-trioxaundecan-1-amine (92 µL, 0.46 mmol) were dissolved in 108 µL of DMF in a microcentrifuge tube. The mixture was stirred at room temperature in dark for 24 h, before it was dissolved in 1.9 mL of acetonitrile:water (1:1) to give the stock solution of FITC-N_3.

Polymer nanoparticles (2 mg) were suspended in 500 µL of acetonitrile. The mixture was sonicated for 10 min to give well dispersed suspension. To the particle suspension, 250 µL of the FITC-N_3 stock solution, 440 µL water, 10 µL of 100 mM $CuSO_4 \cdot 5H_2O$ and 50 µL of 100 mM sodium ascorbate were added. The mixture was stirred in dark for 48 h at room temperature. After the reaction, the nanoparticles were collected by centrifugation, washed repeatedly with acetonitrile:water (1:1) until no fluorescin emission band can be detected from the washing solvent. The particles were dried in a vacuum chamber for 1 h, and then re-dispersed in 2 mL of acetonitrile:water (1:1).

6.2. Fluorescent labelling of azide-nanoparticles with FITC-alkyne

FITC-alkyne was prepared by reacting FITC with excess of propargyl amine. Briefly, FITC (5 mg, 0.0128 mmol) and propargyl amine (45 µL, 0.703 mmol) were dissolved in 55 µL of DMF in a microcentrifuge tube. The mixture was stirred at room temperature in dark for 24 h, before it was dissolved in 950 µL of acetonitrile: water (1:1) to give the stock solution of FITC-alkyne.

Polymer nanoparticles (2 mg) were suspended in 500 µL of acetonitrile. The mixture was sonicated for 10 min to give well dispersed suspension. To the particle suspension, 250 µL of the FITC-alkyne stock solution, 440 µL water, 10 µL of 100 mM $CuSO_4 \cdot 5H_2O$ and 50 µL of

100 mM sodium ascorbate were added. The mixture was stirred in dark for 48 h at room temperature. After the reaction, the nanoparticles were collected by centrifugation, washed repeatedly with acetonitrile:water (1:1) until no fluorescin emission band can be detected from the washing solvent. The particles were dried in a vacuum chamber for 1 h , and then re-dispersed in 2 mL of acetonitrile:water (1:1).

6.3. Fluorescence emission measurement

Fluorescence emission of the FITC-labeled nanoparticles was measured with a QuantaMaster C-60/2000 spectrofluorometer (Photon Technology International, Lawrenceville, NJ, USA). Before measurement, particle concentration was adjusted to 50 μg mL^{-1} by dilution with acetonitrile:water (1:1). The excitation wavelength was fixed at 494 nm for all the measurements.

7. Radioligand binding analysis

Various amounts of polymer particles were mixed with 1 mL of acetonitrile containing 0.5% acetic acid in a series of microcentrifuge tubes. (*S*)-[4-^{3}H]-Propranolol (246 fmol) or 2,4-dichlorophenoxyacetic acid carboxyl-^{14}C (260 pmol) was then added. The mixture was incubated on a rocking table at room temperature overnight. After the incubation, the polymers were sedimented by centrifugation at 13 000 rpm for 10 min. Supernatant (500 μL) was withdrawn and mixed with 10 mL of scintillation liquid (Ecoscint A). The radioactivity of the samples was measured with a Tri-Carb 2800TR liquid scintillation analyzer (PerkinElmer). The amount of radioligand bound to the polymer particles was calculated by subtracting the free radioligand from the total radioligand added. The data are mean values of measurements on three independent samples.

In competitive radioligand binding experiments, the propranolol-imprinted core-shell particles (0.5 mg) were mixed with (*S*)-[4-^{3}H]-propranolol (246 fmol) and various amounts of (*R*,*S*)-propranolol or atenolol in 1 mL of acetonitrile containing 0.5% acetic acid. The amount of radioligand bound to the polymer particles was calculated by subtracting the free radioligand from the total radioligand added.

Fig. S4 shows the uptake of radioligand [^{14}C]-2,4-D by 2,4-D imprinted and non-imprinted particles.

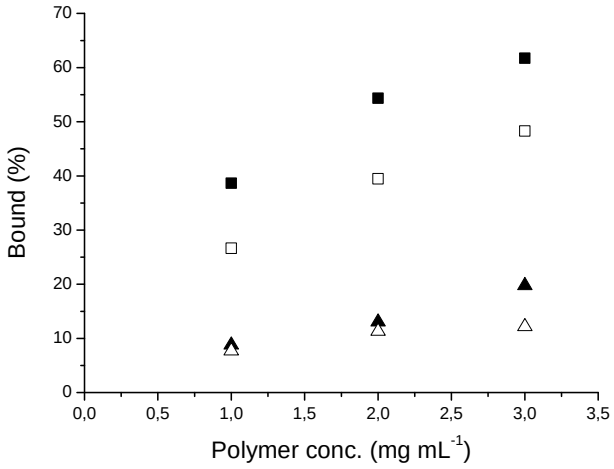


Fig. S4 Uptake of radioligand [^{14}C]-2,4-D by imprinted (solid symbol) and non-imprinted particles (open symbol). The core-shell particles and the core particles are represented by square and triangle symbols, respectively.

8. Click conjugation of two different MIP nanoparticles

Propranolol-imprinted core-shell nanoparticles (2 mg) were mixed with 2 mg of 2,4-D imprinted nanoparticles in 1 mL acetonitrile in a microcentrifuge tube. To this particle suspension, 880 µL water, 20 µL of 100 mM $CuSO_4 \cdot 5H_2O$ and 100 µL of 100 mM sodium ascorbate were then added. The reaction mixture was stirred on a rocking table for 48 h at room temperature. The particles were then collected by centrifugation, washed with acetonitrile:water (1:1) repeatedly, and dried in a vacuum chamber. As a control, the same particle mixture was treated under the same conditions except for omission of $CuSO_4 \cdot 5H_2O$. Fig. S5 shows the different tendency of sedimentation of particles between the clicked and the non-clicked particle mixtures.

Fig. S5 Sedimentation of the click-conjugated particles (left) and the non-conjugated particles (right). The image was taken after the particle suspensions have been left to stand for 1 min. Total particle concentration: 4 mg mL^{-1} in acetonitrile.

References

1. K. Yoshimatsu, K. Reimhult, A. Krozer, K. Mosbach, K. Sode and L. Ye, *Anal. Chim. Acta*, 2007, **584**, 112.
2. C. E. Hunt, P. Pasetto, R. J. Ansell and K. Haupt. *Chem. Commun.*, 2006, 1754.