Supplementary Information

Nanosensor platform for small molecules based on supraparticles of molecularly imprinted nanoparticles and gold nanoparticles

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Materials

Potassium carbonate, magnesium sulfate, styrene, citric acid anhydrous, sodium hydrogen carbonate, and bisphenol A (BPA) were purchased from Nacalai Tesque (Kyoto, Japan). Acetic anhydride, 55% divinylbenzene (DVB) 2,2'-azobis(2-methylpropionamidine) dihydrochloride (V-50), *N*,*N*'-methylenebisacrylamide (MBAAm), sodium hydroxide, potassium hydroxide, 4-(4,6- dimethoxy-1,3,5-triazine-2-yl)-4-methylmorpholinium chloride (DMT-MM), atrazine, Acetone, methanol, acetonitrile and ethyl acetate were obtained from Wako Pure Chemical Industries (Osaka, Japan). *N*-Hydroxysuccinimide (NHS) and BPA dimethacrylate were purchased from Sigma-Aldrich (USA). Diphenolic acid, bisphenol B (BPB), 4-nonylphenol and 4-α-cumylphenol were purchased from Tokyo Chemical Industries (Tokyo, Japan). 1-Ethyl-3-(3-dimethulaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Peptide Institute, Inc. (Osaka, Japan). 11-Amino-1-undecanethiol was purchased from Dojindo Laboratories (Kumamoto, Japan). Phenol was purchased from Katayama Chemical (Osaka, Japan). BPA-Au NPs were supplied from Tanaka Kikinzoku Kogyo K.K. (Tokyo, Japan). The average diameter and PDI of BPA-Au NPs were 12.07nm and 0.034 respectively. NPs with uniform diameter were confirmed from a TEM image.

Instruments

Polymer particle sizes, size distributions and polydispersity indexes (PDI) were determined by a dynamic light scattering system (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.). Images of nanoparticles ware measured by a transmission electron microscope (JEM-1230, JEOL Ltd., Japan) and a scanning electron microscope (VE-9800, Keyence Ltd. Japan). Turbidity of fractions collected in affinity chromatography were measured by an UV/Vis spectrophotometer equipped with an integrating sphere unit (V-550, JASCO Ltd., Japan). Other UV/Vis spectral measurements were performed using V-560 spectrophotometer (JASCO Ltd., Japan).

Preparation of 4,4-Bis(4-hydroxy-phenyl)-pentanoic acid (11-mercapto-undecyl)-amide

4,4-Bis(4-acetoxyphenyl)pentanoic acid (1)

Diphenolic acid (2.87 g, 9.95 mmol), acetic anhydride (3.8 mL, 40.4 mmol) and K_2CO_3 (5.57 g, 38.1 mmol) were dissolved in acetonitrile (80 mL), which was stirred for 8 h at 60 ^{o}C. A saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$, and concentrated in *vacuo* to obtain **1** as a white solid. Yield: 61.0%

^{1}H-NMR (300MHz, $CDCl_3$), σ_H=1.62 (3H, s, CH_3), 2.29 (6H, s, CH_3), 2.42 (2H, t, CH_2, J=4.5), 2.54 (2H, t, CH_2, J=4.5), 2.83 (3H, s, CH_3), 7.01-7.04 (4H, d, benzene), 7.19-7.19 (4H, d, benzene)

4.4-Bis (4-acetoxyphenyl)-pentanoic acid N-hydroxysuccinimide ester (2)

Compound **1** (483 mg, 1.30 mmol), EDC (713.39 mg, 3.71 mmol) and NHS (319.50 mg, 2.78 mmol) were dissolved in anhydrous dichloromethane (30 mL), which was stirred 1.5 h at 0 ^{o}C and for an additional 20h at room temperature. Brine was added, and the mixture was extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, and concentrated in *vacuo*. The crude product was purified by silica gel column chromatography (dichloromethane/ethyl acetate, 10:1, v/v) to afford **2** as a white solid. Yield: 64.8%

^{1}H-NMR(300MHz,TMS,$CDCl_3$),σ_H= 1.61 (3H, s, CH_3), 2.29 (6H, s, CH_3), 2.38 (2H, t, CH_2, J=4.5), 2.49 (2H, t, CH_2, J=4.5), 7.00 -7.01 (4H, d, benzene), 7.17-7.20 (4H, d, benzene)

4-[1-(4-Acetoxy-phenyl)-3-(11-mercapto-undecylcarbomoyl)-1-methyl-propyl-phenyl acetate (3)

Compound **2** (107.0mg, 0.229mmol), 11-amino-1-undecanethiol (47.0mg, 0.196mmol) was dissolved in anhydrous dichloromethane (10mL), which was stirred overnight under nitrogen atmosphere in dark,. Then, brine was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with distilled water and brine, and then dried over Na_2SO_4 and concentrated in *vacuo*. The crude product was purified by silica gel column chromatography (dichloromethane/methanol, 10:1, v/v) to afford **3** as a clear liquid. Yield: 85.2%

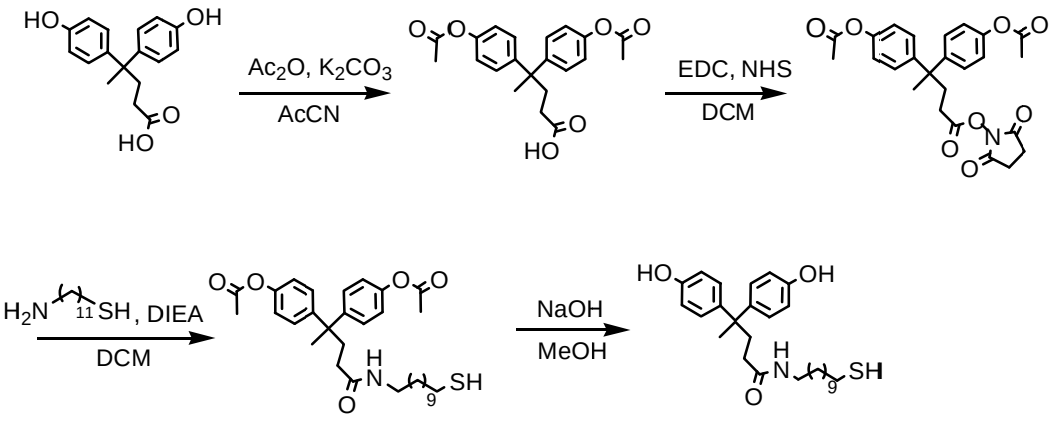
^{1}H-NMR (300MHz, TMS, $CDCl_3$), σ_H= 1.26 (18H, m, CH_2), 1.61 (3H, s, CH_3), 1.91 (2H, t, CH_2, J=4.02), 2.29 (6H, s, CH_3), 2.18-2.56 (4H, t, CH_2, J=4.4), 3.17 (2H, t, CH_2, J=4.5), 6.98-6.99 (4H, d, benzene), 7.18-7.20 (4H, d, benzene)

4,4-Bis(4-hydroxy-phenyl)-pentanoic acid (11-mercapto-undecyl)-amide

A freshly prepared, oxgen free (freeze-thaw) 0.1N NaOH-methanol solution (1.0 mL, 0.1 mmol) was added to a cooled (0 ^{o}C) solution of **3** in oxygen-free methanol. The reaction mixture was stirred at 0 ^{o}C under a nitrogen atmosphere for 1h. The reaction was quenched with cold 1N HCl solution, extracted with ethyl acetate and washed with water and brine. The organic layer was dried over $MgSO_4$ and concentrated in *vacuo*. The product **4** (yellowish brown solid) was used without further purification. This products was processed into BPA-Au NPs by Tanaka Kikinzoku Kogyo K.K. Yield: 94.5%

^{1}H-NMR (300MHz, TMS, $CDCl_3$), σ_H= 1.25 (18H, m, CH_2), 1.55 (3H, s, CH_3), 1.90 (2H, t, CH_2, J=8.4), 2.29 (4H, s, CH_2), 2.32 (1H, s, NH), 2.53 (2H, t, CH_2, J=7.3), 3.20 (2H, t, CH_2, J=6.4), 6.73-6.76 (2H, d, benzene), 6.94-6.69 (4H, d, benzene), 7.16-7.18 (2H, d, benzene)

(a)



(b)

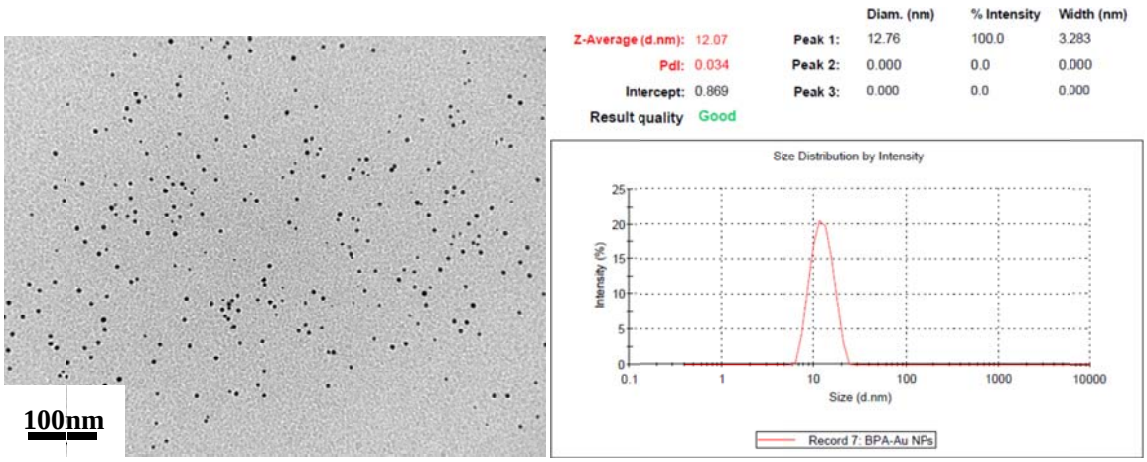


Figure S1. Synthesis scheme of BPA-thiol derivative (a) and TEM image of BPA-Au NPs (b) and DLS data of BPA-Au NPs (c)

Preparation of P(S-co-DVB) seed NPs

Styrene (760 mg, 7.30 mmol), DVB (40 mg, 0.31 mmol), 45% acetone/water (v/v) mixed solvent and V-50 (41.26 mg, 0.15 mmol) were mixed in a 100 mL Schlenk flask. After the mixture was degassed by vacuum/nitrogen, it was stirred at 1000 rpm and stood at 80 ˚C for 24 h. After cooling the emulsion, the seed particles were filtered using DISMIC-13CP 0.20 μm (ADVANTEC Ltd., Japan) to remove the small amount of coagulation. The final conversion of the polymerization was 77 %, which was measured by gravimetry.

Preparation of MIP-NPs and NIP-NPs

The seed emulsion (20 g, solid content: 0.76 %), MBAAm (95.0 mg, 0.62 mmol) and BPA dimethacrylate (5.0 mg, 13.7 μmol) were stirred, and after adding V-50 (2.62 mg, 9.66 μmol), the mixture was degassed, and stirred at 80 ˚C for 24 h. The obtained polymer nanoparticles were filtered, and the BPA moieties in the obtained NPs were removed by alkaline hydrolysis in 1M KOH for 24 h to yield MIP-NPs. NIP-NPs were prepared in a same manner as MIP-NPs with phenyl methacrylate (4.45 mg, 27.4 μmol), instead of BPA dimethacrylate. It was difficult that the measurement of the conversions by gravimetry due to non-volatility of monomer species (MBAA and BPA dimethacrylate).

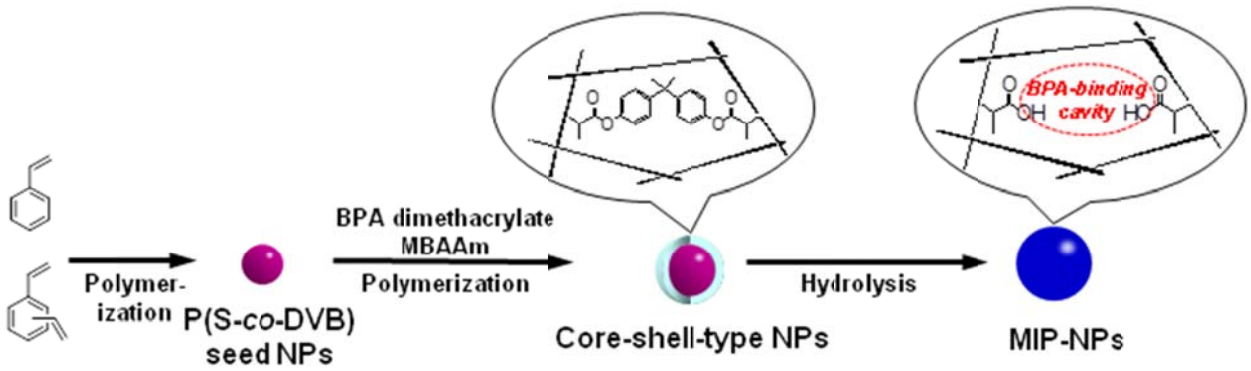


Figure S2. Schematic diagrams of the preparation of MIP-NPs for BPA by two-step emulsifier-free emulsion copolymerization

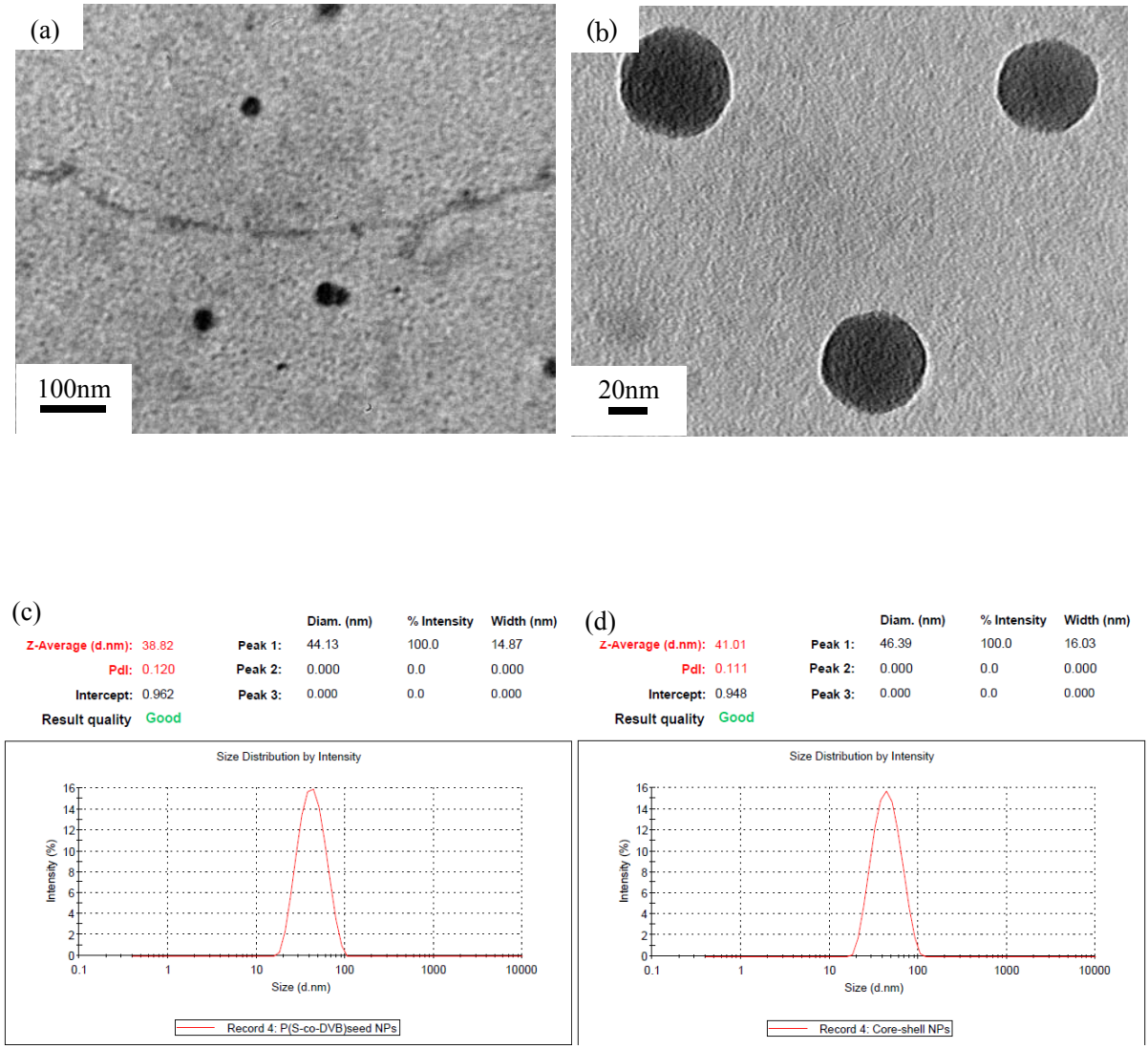


Figure S3. TEM images of P(S-*co*-DVB) seed NPs (a) and core-shell type NPs (b), and DLS data of P(S-*co*-DVB) seed NPs (c) and core-shell type NPs (d)

Purification by affinity chromatography

BPA- immobilized silica gel was prepared by the following procedure. Diphenolic acid (5.00 g, 17.5 mmol), NH_2 modified silica gel (20.0 g), DMT-MM (3.00 g) and methanol (200 mL) in a flask were shaked for 3h. This silica gel was washed with citric acid aq., $NaHCO_3$ aq, water and methanol consecutively, and then dried in *vacuo*. The obtained BPA-immobilized silica gel (1.00 g) was filled into a Pasteur pipette to obtain BPA affinity column. The column was conditioned with acetonitrile, and the MIP-NPs emulsion (2.0 mL in water containing 45 %(v/v) acetone) were applied. Then the NPs were eluted with acetonitrile, ethyl acetate and methanol. The column eluate was fractionated every 1 mL and the NPs were detected by turbidity (700 nm). Figure S4 shows the stepwise elution profile of MIP-NPs, in which the turbidity at 700 nm was monitored to detect the elution of MIP-NPs. A dispersion of MIP-NPs in 45% (v/v) acetone/water was introduced to the BPA-immobilized silica gel column.

Acetonitrile was selected as the initial eluent due to its compatibility with 45% (v/v) acetone/water. Then the eluent was changed to ethyl acetate (the solvent for binding experiments) and the 5th fraction, a large number of MIP-NPs with high affinity to BPA, were collected. Finally, methanol was applied to wash the column.

The yield of MIP-NPs was estimated to be approximately 40 % from % peak height based on turbidity in the elution profile (Figure S4). % Peak height was obtained from the ratio of the peak height of the fraction 5 to the summation of each peak height.

A TEM image and DLS data of the eluted MIP-NPs show that the particle size was similar to that of the unpurified particles (Figure S5).

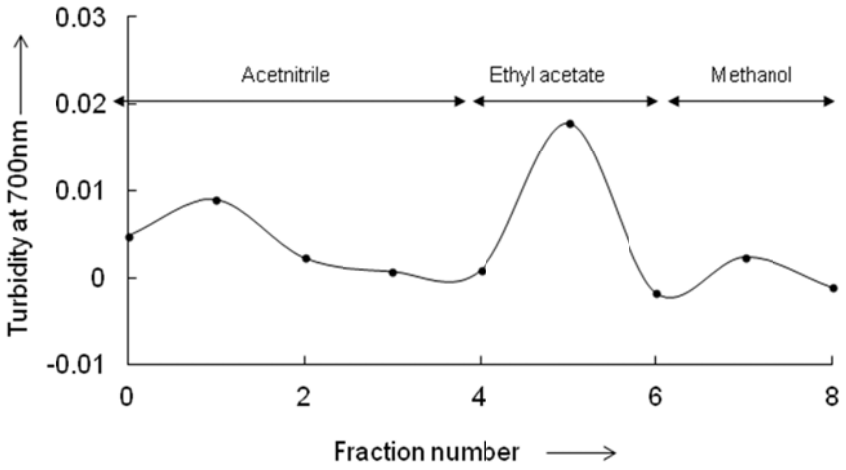


Figure S4. Elution profile of MIP-NPs in BPA-immobilized affinity chromatography with three different eluents (acetonitrile, ethylacetate and methanol). MIP-NPs elution was detected by turbidity at 700nm.

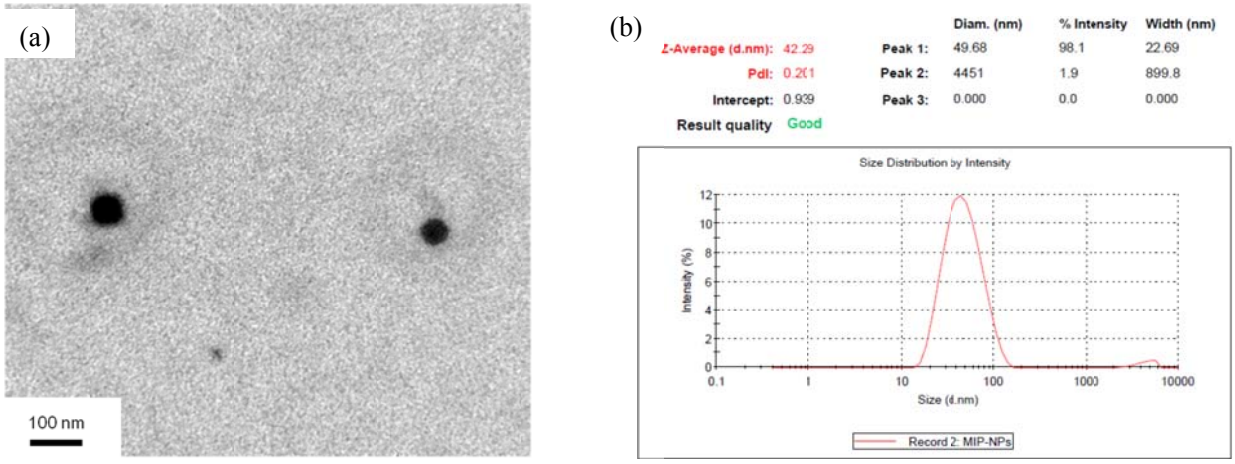


Figure S5. TEM image (a) and DLS data (b).of MIP-NPs in the fraction 5th from affinity chromatography utilizing BPA-immobilized silica gel

Concentration dependence on the BPA-AuNPs extinction

Various concentration of BPA-AuNPs suspended in ethyl acetate were prepared (0.05, 0.10, 0.25, 0.50, 0.88 mg/mL) and UV/Vis spectra (450-800nm) were measured respectively.

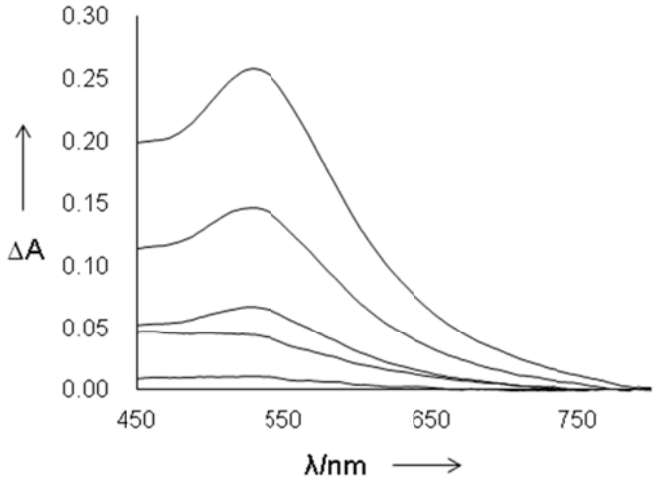


Figure S6. Effect of BPA-Au NPs concentrations on the change of BPA-Au NPs extinction. BPA-Au NPs concentration: 0.05, 0.10, 0.25, 0.50, 0.88 mg/mL, from bottom to top.

Calculation of A/D parameter

A/D parameter (A/D) was given by a ratio of the integral of the spectra of BPA-Au NPs in the range from 550 to 700 nm (A value) to that of the spectra in the range from 490 to 540 nm (D value). Δ(A/D) value was given by (A/D)-$(A/D)_0$, and a relative A/D change against an initial A/D parameter was calculated by $\Delta(A/D)/(A/D)_0$, where $(A/D)_0$ was an initial A/D parameter

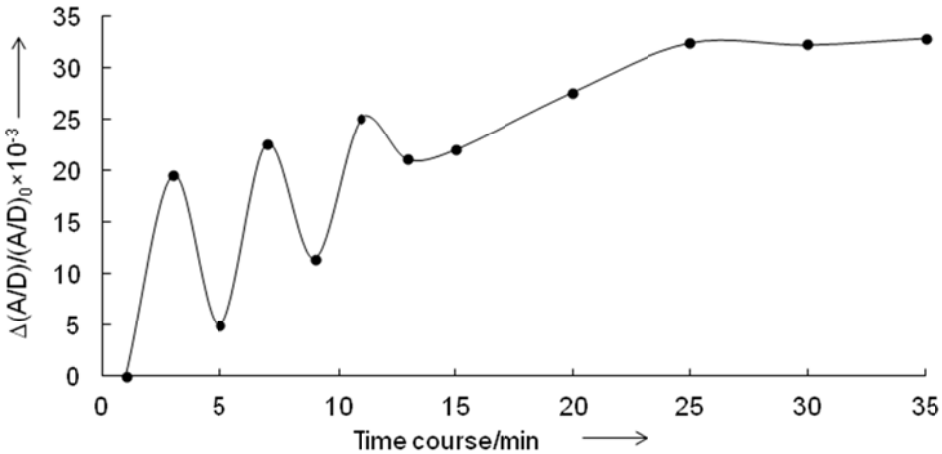


Figure S7. Time course of the spectral change of BPA-Au NPs (0.88 mg/mL) by the addition of MIP-NPs (0.22 mg/mL).

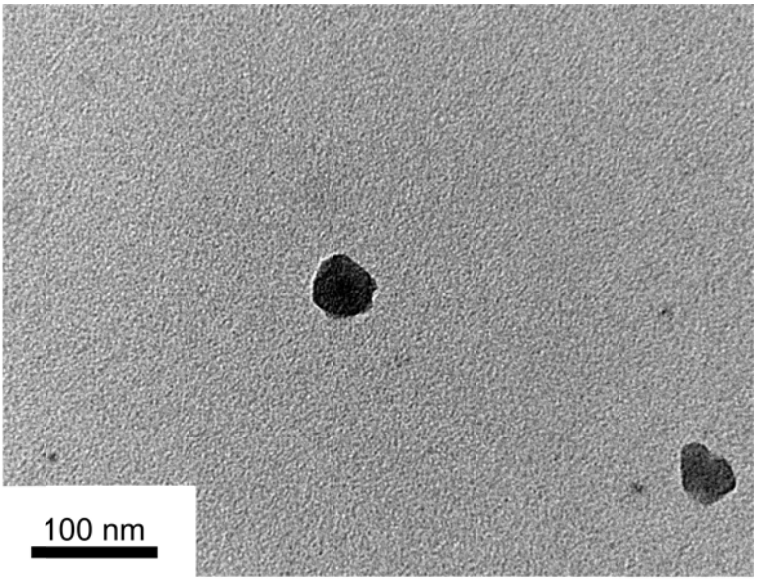


Figure S8. TEM photographs of NIP-NPs prepared by two-step emulsifier-free emulsion polymerization

BPA sensing

After 20 min from mixing MIP-NPs (0.22 mg/mL in ethyl acetate) with BPA-Au-NPs (0.88 mg/mL in ethyl acetate) to assemble the supraparticles, various concentrations (0-5.0 μM) of BPA dissolved in ethyl acetate were added. UV/Vis spectra were measured after 20 min-incubation at 25˚C. For selectivity tests, reference compounds (0.625 μM of BPB, phenol, 4-α-cumylphenol, atrazine and 4-nonylphenol) were incubated separately.

BPA sensing in human serum

The supraparticles assembled from MIP-NPs (56 μg/mL) and BPA-Au-NPs (220 μg/mL) were used for detecting lower concentrations (0-5.0 nM) of BPA and a diluted serum sample. BPA was dissolved in human serum, and BPA in the human serum was extracted with an equal volume of ethyl acetate, then the precipitation was removed by centrifugation. The extract (30 μL) was added into the supraparticles emulsion (2.97 mL) in ethyl acetate, where the final concentration of each BPA sample was 0, 0.63, 1.25, 2.5, or 5.0 nM. UV/Vis spectra were measured after 20 min-incubation at 25˚C.

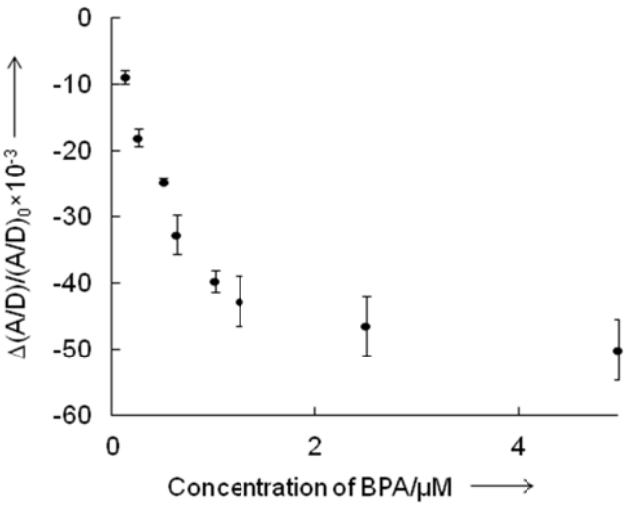


Figure S9. Binding isotherm of BPA in ethylacetate towards MIP-NPs in the presence of BPA-Au NPs. MIP-NPs: 0.22 mg/mL; BPA-Au NPs: 0.88 mg/mL Estimation of affinity constant of BPA to MIP-NPs in the presence of BPA-Au NPs by using the below equation.

$$Y = \left[(1 + KG + KH) - \sqrt{(1 + KG + KH) - 4K^2HG}\right] * \frac{D}{2KG}$$

Y: A/D parameter calculated; *K*: affinity constant; *H*: concentration of BPA-AuNPs / BPA-INPs complexes, *G*: BPA concentration; *D*: maximum A/D parameter value.