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Supporting information for

Dual-Template Docking Oriented Molecular Imprinting: A Facile Strategy for Highly Efficient Imprinting within Mesoporous Materials

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EXPERIMENTAL SECTION

Reagents and materials. 3-Aminopropyltriethoxysilane (APTES), tetraethoxysilane (TEOS), 3-glycidyloxypropyl triethoxysilane (GPTES), adenosine, deoxyadenosine (DA), guanosine, cytidine, uridine, adenosine monophosphate (AMP), guanosinemonophosphate (GMP), cytidine monophosphate (CMP), deoxyadenosine monophosphate (DAMP), and uridine monophosphate (UMP) were obtained from Sigma (St. Louis, MO, USA). The structures of AMP and its analogues are shown in Figure S1. 3-Aminophenylboronic acid (APBA) was purchase from J&K scientific (Shanghai, China). *N*-Cetyltrimethyl ammonium bromide (CTAB) and all other chemical reagents were of analytical grade and obtained from Sinopharm Chemical Reagent (Shanghai, China). Water used in all the experiments was purified by a Milli-Q Advantage A10 ultrapure water purification system (Millipore, Milford, MA, USA). Fused-silica capillaries with 75 μm i.d× 365 μm o.d were from the Reafine Chromatography (Hebei, China).

Instruments. Transmission electron microscopy (TEM) characterization was performed on a JEM-2100 system (JEOL, Tokyo, Japan). The X-ray diffraction (XRD) patterns of samples were acquired on an ARL XTRA diffractometer (Thermo Fisher Scientific, Waltham, MA, USA) with Cu Kα radiation in the 2θ range of 0.5-6°. The UV absorbance measurement was performed on a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), the wavelength was adopted at 260 nm for all the analytes. Fourier transform infrared (FT-IR) spectrometry was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher, MA, USA). Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromeritics, Norcross, GA, USA). The surface areas were calculated by the Brunauer-Emmett-Teller (BET) method and the pore size distributions were calculated by Barrett-Joyner-Halenda (BJH) method. Micellar electrokinetic chromatographic (MEKC) analyses were carried out on a P/ACE MDQ system

(Beckman Coulter, Fullerton, CA, USA). A fused-silica capillary (60 cm in total, 50 cm to the detector) was used as the separation column. The running buffer contained 25 mM borate, 25 mM phosphate buffer and 25 mM CTAB, pH 9.5. The separation voltage was −15 kV and the UV detection wavelength was set at 254 nm. Sample was injected by a pressure of 3.4 kPa for 15 s.

Synthesis of APBA-GPTES. 3-Aminophenylboronic acid appended 3-glycidyloxypropyl triethoxysilane (APBA-GPTES) was synthesized via a method reported by Yan and co-workers[1] with major modifications. Briefly, GPTES (2.36 g, 10 mmol) and APBA (1.37 g, 10 mmol) were added to a 25-mL round bottom flask. The flask was vacuumed and refilled with nitrogen three times. Then 15 mL tetrahydrofuran (THF) was injected through a rubber stop. The resulting light yellow solution was stirred at room temperature under nitrogen atmosphere for 12 h. After carefully vaporizing the THF, the obtained brown oil was redissolved in 10 mL methanol to get APBA-GPTES solution (denoted as S-1, 1 mmol/mL).

Dual-template docking oriented molecular imprinting. The DTD-OMI based molecularly imprinted MSNs were synthesized via a method reported by Lin and co-workers[2] with major modifications. Briefly, 0.6 g CTAB, 168 mg NaOH, 288 mL H_2O were added into a 500-mL round bottom flask. After the mixture was heated to 80 °C, 69.4 mg (0.2 mmol) AMP was added and then stirred for 15 min. Then a mixture of TEOS (9.8 mmol, 2.5 mL) and S-1 (0.2 mmol, 200 μL) was added dropwise to the solution under vigorous stirring and the resulting mixture was allowed to react for 2 h to produce white precipitate. The solid crude product was filtered, and dried under high vacuum to yield the as-synthesized material.

To prepare non-imprinted MSNs for comparison, the synthetic procedure was the same except that no template was added.

Bulk imprinting. For bulk imprinting, the synthetic procedure was the same as DTD-OMI except that AMP was replaced with adenosine of identical molar weight (0.2 mmol, 53.4 mg).

Template removal. To remove the surfactant template and imprinting template, Soxhlet extraction method was employed. Briefly, a methanolic acidic solution prepared by mixing 1.50 mL HCl (37.2%) with 150 mL methanol was used as the extraction solvent. After extracted for 24 h, the material was placed under high vacuum with heating at 60°C to remove residual solvent within the mesopores to get molecularly imprinted MSNs. The UV absorbance of the extraction solvent was measured and the amount of extracted imprinting template (recovered template) was determined through corresponding calibration curve established by the UV absorbance of standard solutions of known template concentration.

Binding capacity and imprinting factor. The binding capacity of molecularly imprinted MSNs was evaluated with AMP as the target molecules. For details, 900 μL of 1 mg/mL AMP dissolved in 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) was added with 5 mg molecularly imprinted MSNs. The tube was shaken on a rotator for 1 h at room temperature. The molecularly imprinted MSNs were then collected by centrifugation and washed with 500 μL of 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) for three times. After washing, the molecularly imprinted MSNs were resuspended and eluted in 50 μL of 100 mM acetic acid solution for 1 h on a rotator. Finally, the molecularly imprinted MSNs were centrifuged again and the eluate was collected by pipetting carefully. The eluate was applied for UV absorbance measurement, and the binding capacity was calculated through the corresponding calibration curve. The measurement was repeated for 5 parallel experiments and the averaged value was used as the final binding capacity. The same procedure was carried out for non-imprinted MSNs.

The imprinting factor was calculated according to the ratio of the binding capacity of AMP captured by imprinted MSNs over that by non-imprinted MSNs.

Imprinting efficiency. Imprinting efficiency, which is defined as the ratio of the number of imprinted cavities of the molecularly imprinted MSNs over the total number of template molecules used in the synthetic procedure, was calculated using related parameters.

Template usage efficiency. Template usage efficiency, which is defined as the percentage of the amount of recovered template over the amount of template used in the synthetic procedure, for both DTD-OMI and bulk imprinting was calculated using related parameters.

Optimization of imprinting conditions. In order to optimize the synthetic procedure of the imprinted material, molar ratio of APBA-GPTES to TEOS was changed and evaluated in terms of imprinted factor (IF). For details, totally 6 different molar ratios of APBA-GPTES: TEOS were investigated including 1:9; 0.8:9.2; 0.6:9.4; 0.4:9.6; 0.2:9.8; 0.1:9.9. In the optimization procedure, the molar ratio of template molecule to APBA-GPTES was fixed at 1:1 and was not optimized, because the binding between template and APBA-GPTES follows a 1:1 stoichiometry. Among the six ratios, the ratio 0.2: 9.8 provided the highest IF value. Because both AMP and adenosine are strong hydrogen-bonding molecules, APTES, which contains an amino group, was further added as a co-monomer for the imprinting, and the molar ratio of APTES to APBA-GPTES to TEOS was set 0.2:0.2:9.8. The APTES containing protocol was found to provide the best IF value among all the conditions (See Figure S3), the presence of amino group not only enhance the affinity (from 260 μM to 100 μM, see Figure S4 and Figure S9) but also improve the cross-reactivity (from 7% ~ 52% to 2% ~ 23%, see Figure S5 and Figure 3) of the obtained material.

Selectivity. To examine the selectivity of MIP prepared by DTD-OMI, a series of AMP analogues were used as interferences, including DA, guanosine, cytidine, uridine, DAMP, GMP, CMP and UMP (see Figure S1). For molecularly imprinted mesoporous silica, standard solutions of 1 mg/mL AMP or other analogues dissolved in 50 mM NH_4HCO_3 buffer containing 500mM NaCl (pH 8.5) were first prepared. Then 5 mg material was added to 900 μL of above mentioned solutions respectively. The tubes were shaken on a rotator for 1 h at room temperature. The molecularly imprinted MSNs were then collected by centrifugation and then rinsed with 500 μL of 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) for three times. After washing, the molecularly imprinted MSNs were resuspended and eluted in 50 μL 100 mM acetic acid solution for 1 h on a rotator. Finally, the molecularly imprinted MSNs were centrifuged again and the eluates were collected by pipetting carefully. UV absorbance of the eluates was measured at the wavelength 260 nm. The same procedure was carried out for bulk imprinting based and non-imprinted MSNs.

Cross-reactivity. Cross-reactivity experiment was carried out with AMP-imprinted and adenosine-imprinted MSNs and a series of template analogues, including DA, guanosine, cytidine, uridine, GMP, CMP, DAMP and UMP. Standard solutions of 1 mg/mL AMP or other analogues dissolved in 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) were first prepared. Then 5 mg material was added to 900 μL of above mentioned solutions respectively. The tubes were shaken on a rotator for 1 h at room temperature. The molecularly imprinted MSNs were then collected by centrifugation and rinsed with 500 μL of 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) for three times. After washing, the molecularly imprinted MSNs were resuspended and eluted in 50 μL 100 mM acetic acid solution for 1 h on a rotator. Finally, the molecularly imprinted MSNs were centrifuged again and the eluates were collected by pipetting carefully. Then the UV absorbance of the eluates was measured.

The cross-reactivity was roughly estimated in terms of the percentage of the absorbance of interfering analogues over the intensity of template under identical concentration.

Binding constant. Equivalent molecularly imprinted mesoporous silica (2 mg) was added to solutions (900 μL) of AMP or adenosine at different concentrations in 1.5-mL plastic microcentrifugal tubes. The tubes were shaken on a rotator for 2 h at room temperature. The washing and elution procedures were the same as those described above. The amounts of AMP or adenosine extracted by the molecularly imprinted mesoporous silica were determined by measuring the AMP or adenosine in the eluates by UV absorbance. Three parallel measurements were carried out for each experimental point. The amount of AMP or adenosine bound to molecularly imprinted mesoporous silica was plotted according to the Scatchard equation to estimate the binding properties of the materials. The Scatchard relationship can be established using the following equation:

$$Q_e / [S] = (Q_{max} - Q_e) / K_d$$

Where Q_e, $[S]$, Q_{max} and K_d are the amount of AMP or adenosine bound to molecularly imprinted MSNs at equilibrium, the free concentration of AMP or adenosine at adsorption equilibrium, the saturated adsorption capacity and the dissociation constant, respectively. By plotting $Q_e/[S]$ versus Q_e, K_d and Q_{max} can be calculated from the slope and the intercept, respectively.

Selective extraction of adenosine in human urine. Human urine sample (pH 6.5) was collected from a healthy male adult individual. The collected urine sample was frozen immediately and stored at -20 °C. Prior to use, the urine sample was thawed at room temperature. A 2-mL urine sample was centrifuged for 30 min at 12,000 r/min. The supernatant was used as a working sample for analysis. For extraction, 5 mg molecularly imprinted MSNs were first ultrasonically dispersed in 500 μL of 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) and then 500 μL of pretreated urine

sample was added. The tube was shaken on a rotator for 1 h at room temperature. The molecularly imprinted MSNs were collected by centrifugation and rinsed with 500 μL of 50 mM NH_4HCO_3 buffer containing 500 mM NaCl (pH 8.5) for three times. After washing, the molecularly imprinted MSNs were resuspended and eluted in 50 μL of 100 mM acetic acid solution for 1 h on a rotator. Finally, the molecularly imprinted MSNs were centrifuged again and the eluate was collected by pipetting carefully and used for MEKC separation.

References:

(1) Tan, J.; Wang, H. F.; Yan, X. P., *Anal. Chem.* **2009**, *81*, 5273-5277.

(2) Zhao, Y. N.; Brian, G. T.; Igor, I. S.; Lin, V. S.-Y., *J. Am. Chem. Soc.* **2009**, *131*, 8398-8400.

SUPPLEMENTARY DATA

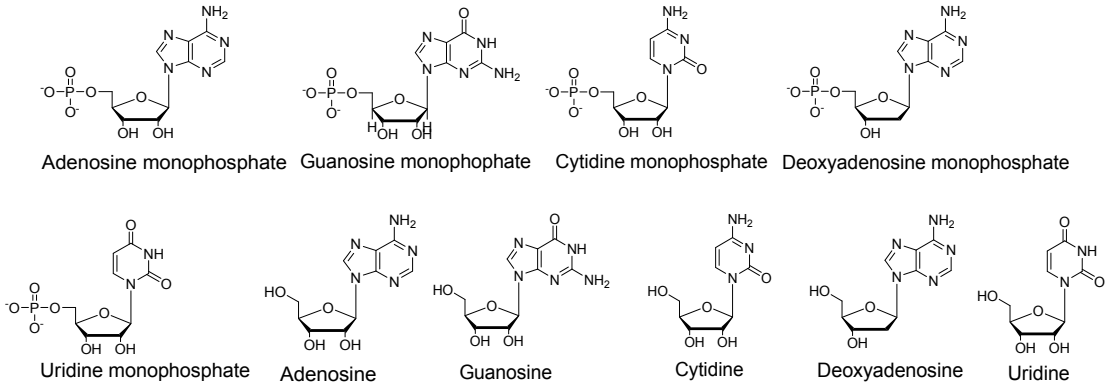

Figure S1. The structures of the AMP and its analogues.

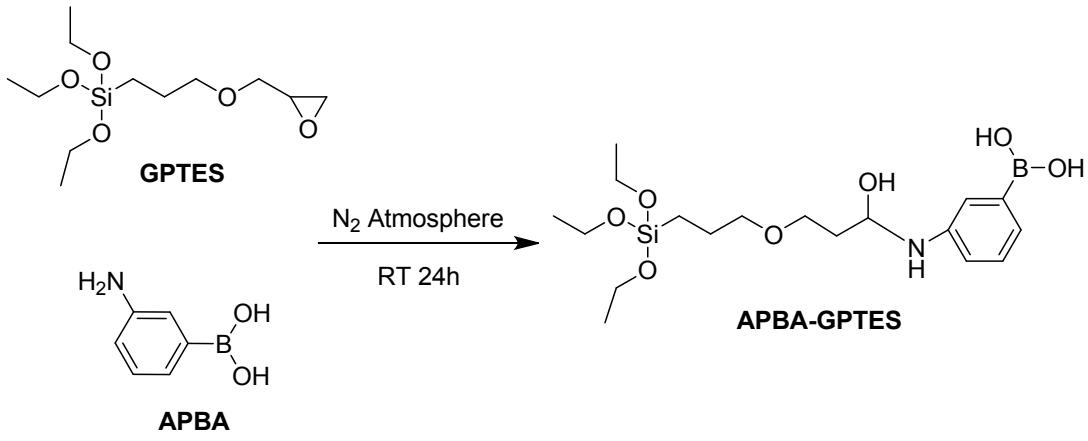

Figure S2. Synthetic procedure of APBA-GPTES.

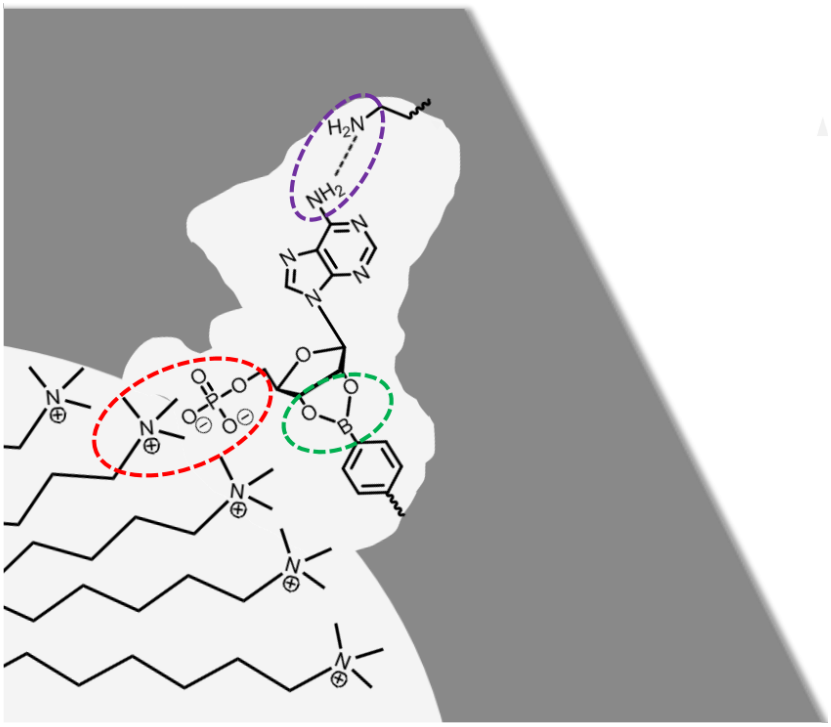

Figure S3. Schematic illustration of the possible chemical interactions during the imprinting process. Green circle: boronate affinity interaction (covalent), Red circle: electrostatic attraction (non-covalent); Purple circle: possible hydrogen bonding (non-covalent).

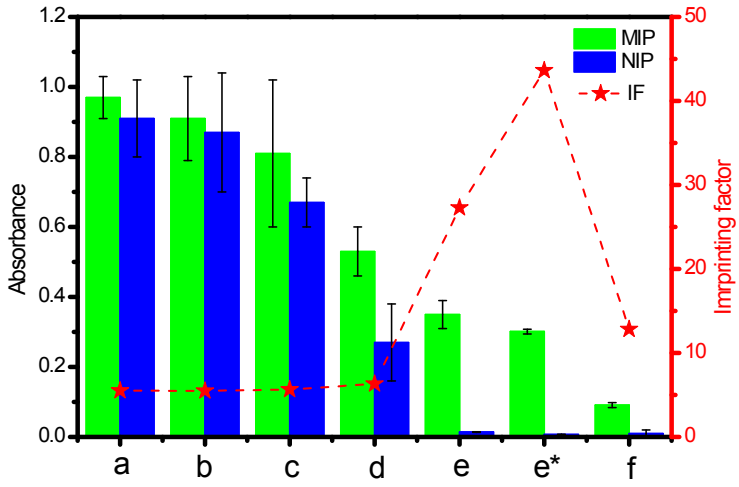

Figure S4. Effects of imprinting condition on the target amount captured by the AMP-imprinted MIP and NIP and on the imprinting factor. Imprinting condition: molar ratio of APBA-GPTES to TEOS, a) 1:9, b) 0.8:9.2, c) 0.6:9.4, d) 0.4:9.6, e) 0.2:9.8, f) 0.1:9.9. e*) Equivalent APTES and APBA-GPTES were added, the ratio of APTES to APBA-GPTES to TEOS: 0.2:0.2:9.8.

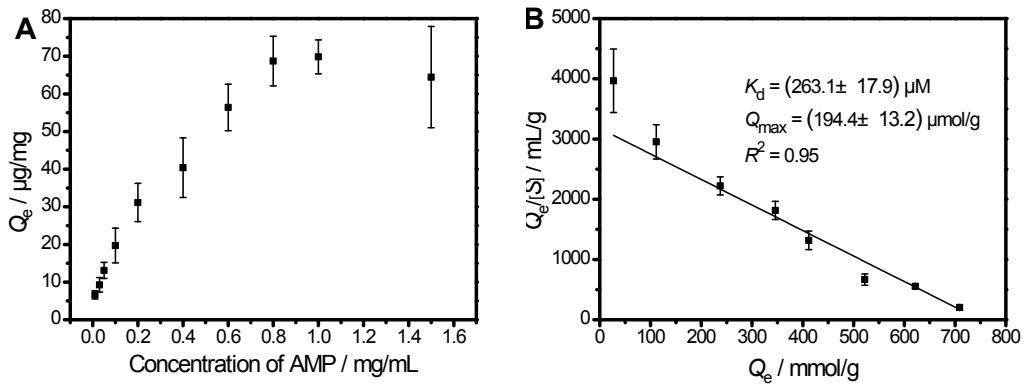

Figure S5. Binding isotherms and corresponding Scatchard plots for DTD-OMI based AMP-imprinted MSNs (test compound, AMP; APBA-GPTES : TEOS = 0.2 : 9.8).

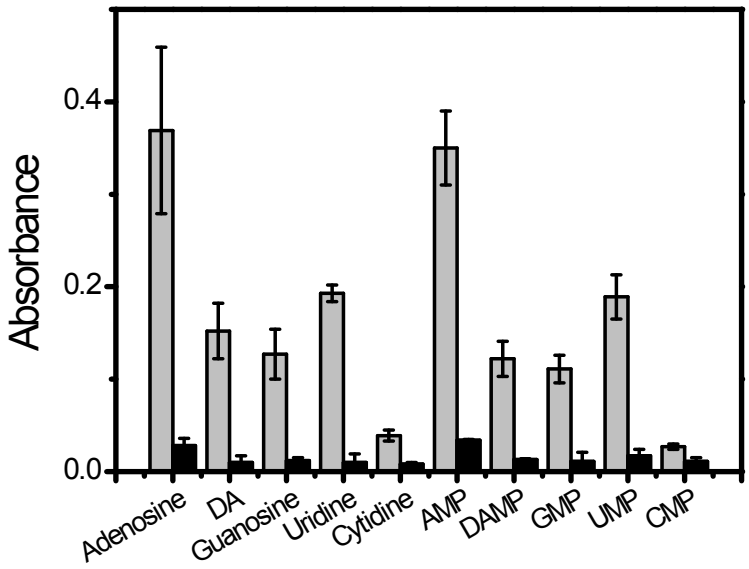

Figure S6. Selectivity of AMP-imprinted MSNs by DTD-OMI prepared at the molar ratio of APBA-GPTES: TEOS at 0.2: 0.98 towards the template and its analogues. Gray: Imprinted MSNs, black: non-imprinted MSNs.

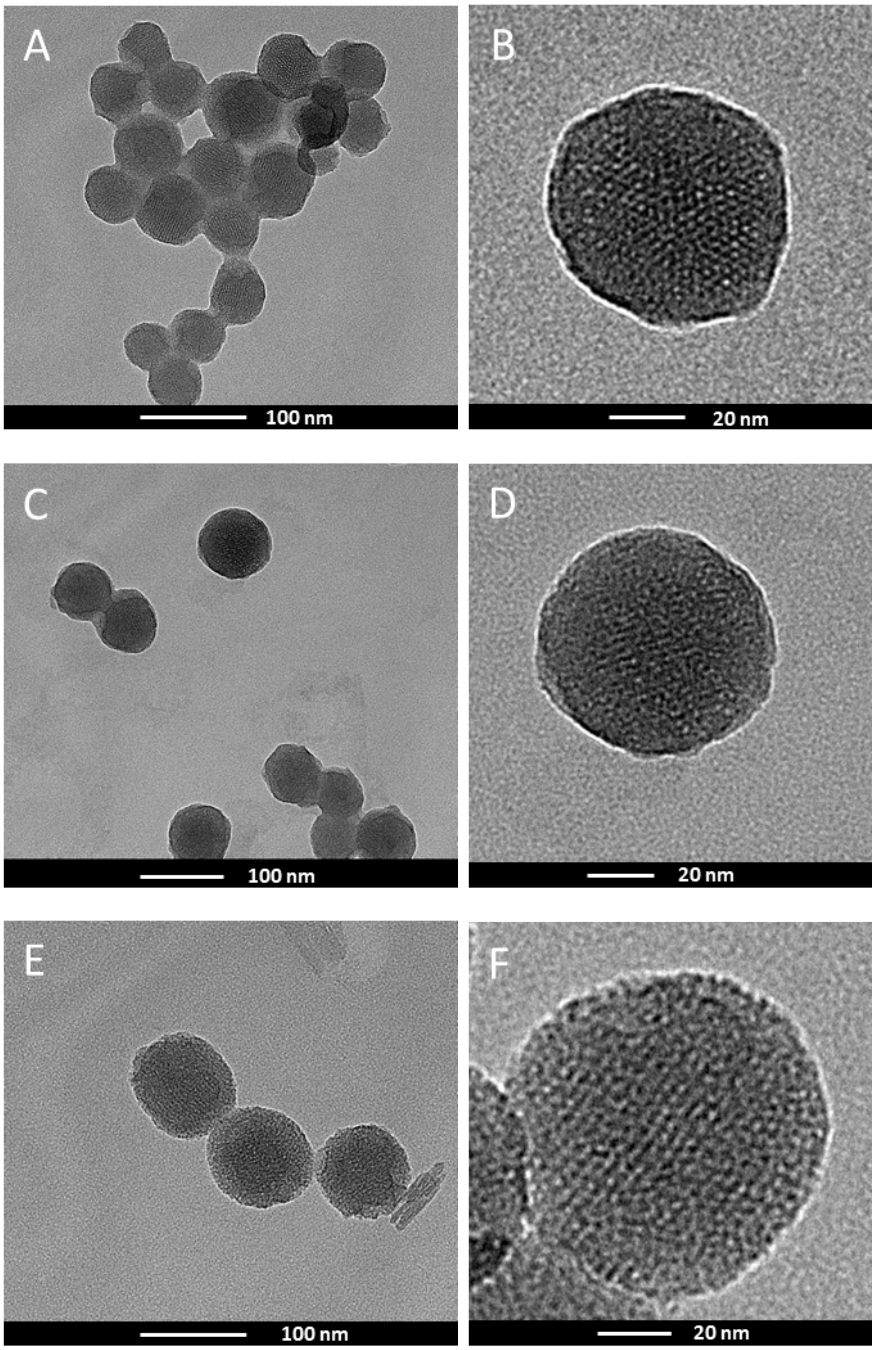

Figure S7. TEM images for (A,B) AMP-imprinted mesoporous silica by DTD imprinting, (C,D) adenosine-imprinted MSNs by bulk imprinting, and (E,F) non-imprinted MSNs.

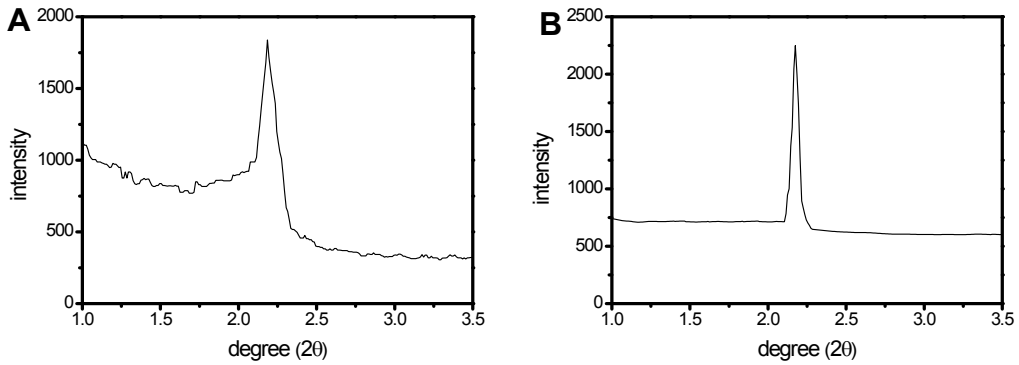

Figure S8. XRD patterns for (A) adenoisne-imprinted MSNs by bulk imprinting, (B) non-imprinted MSNs.

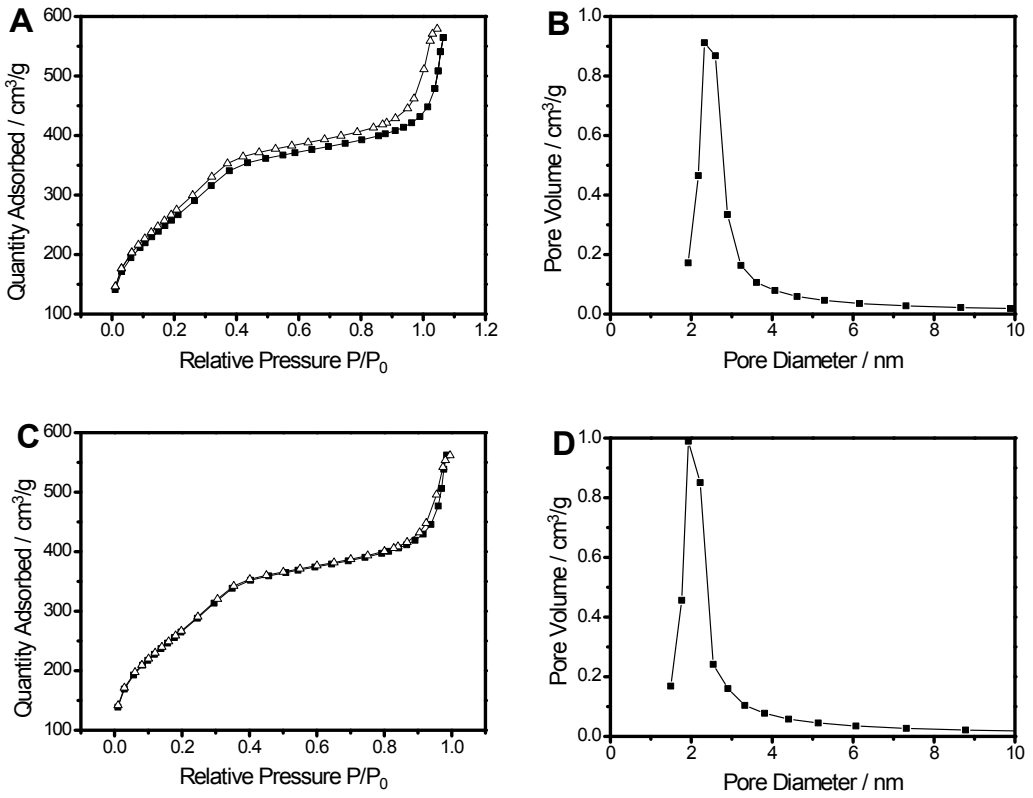

Figure S9. BET nitrogen adsorption/desorption isotherms and BJH pore size distributions for (A,B) adenosine-imprinted MSNs by bulk imprinting, (C,D) non-imprinted MSNs.

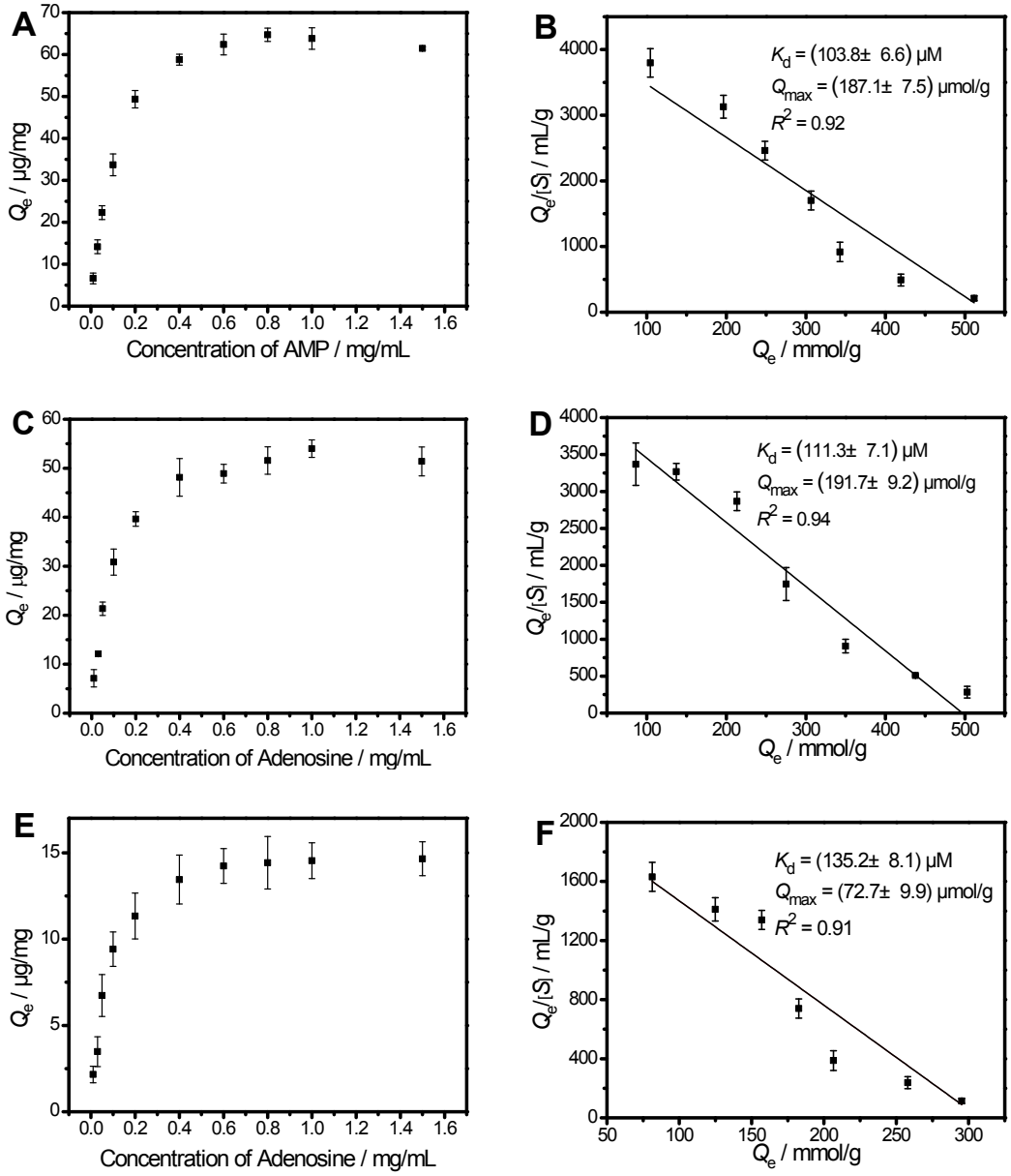

Figure S10. Binding isotherms and corresponding Scatchard plots for imprinted MSNs by different approaches.(A-D) AMP-imprinted MSNs by DTD-imprinting (A and B: test compound, AMP; C and D: test compound, adenosine), (E, F) adenosine-imprinted MSNs by bulk-imprinting.

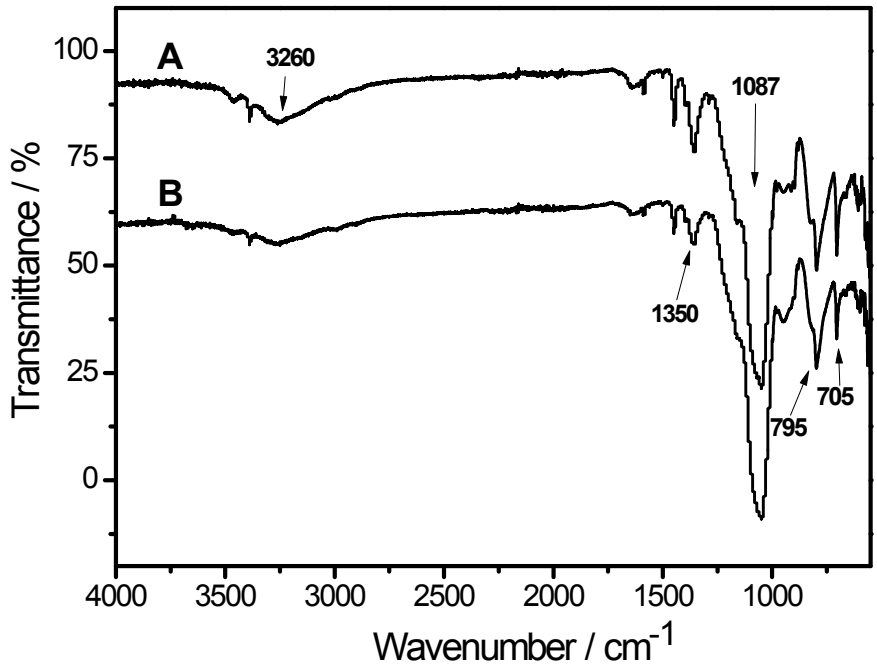

Figure S11. FI-IR spectra for A) AMP-imprinted mesoporous silica prepared by DTD-OMI and B) adenosine-imprinted mesoporous silica prepared by bulk imprinting.

Table S1. Cross-reactivity of DTD-OMI and bulk imprinting.[a]

	DTD-OMI	Bulk imprinting
A	103%	-
G	22%	72%
U	18%	70%
C	3%	14%
AMP	-	109%
GMP	23%	61%
UMP	22%	69%
CMP	2%	10%

[a] – not measured

Table S2. BET and BJH parameters of molecularly-imprinted MSNs prepared by DTD-OMI and bulk imprinting.

Material	BET surface area S_{BET} (m^2/g)	BET Pore Volume V_P (cm^3/g)	BJH Pore diameter W_{BJH} (Å)
AMP-imprinted MSNs by DTD-OMI	833.7	0.671	23
Adenosine-imprinted MSNs by bulk imprinting	778.2	0.602	23
AMP-rebounded AMP-imprinted MSNs	628.3	0.497	21