Electronic Supplementary Information for

Insights into the effect of nanoconfinement on molecular interactions

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EXPERIMENTAL SECTION

Reagents and materials. Horseradish peroxidase (HRP), RNase A, RNase B, 4-carboxyphenylboronic acid (CPBA), 3-aminopropyltrimethoxysilane (APTMS), tetraethoxy silane (TEOS), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) were obtained from Sigma (St. Louis, MO, USA). Adenosine and deoxyadenosine were purchased from Alfa Aesar China (Tianjin, China). 3,3,5',5'-Tetramethylbenzidine dihydrochloride substrate solutions (A, B two components) were obtained from Zhongkai Keyue Biotech (Beijing, China). 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).

Instruments. Transmission electron microscopy (TEM) characterization was carried out on a Tecnai G2 F30 S-Twin electron microscope (FEI Company, Hillsboro, OR, USA) operated at 300 kV. 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 adsorption isotherm measurement was performed with a NanoDrop 2000/2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The digitalization analysis of TEM images was implemented using an Image Processing Program kindly provided by Wayne Rasband (Research Services Branch, National Institute of Mental Health, USA). Nitrogen adsorption-desorption measurements were conducted at 77 K on an ASAP2020 instrument (Micromeritics, Norcross, GA, USA).

Synthesis of CPBA-mesoporous silica nanoparticles. The CPBA-mesoporous silica was synthesized via a method by Lin and co-workers[1] with slight modification. Briefly, 1.00 g of *N*-cetyltrimethylammonium bromide (CTAB) or 0.85 g *N*-

dodecantrimethylaminium bromide (DTAB) was dissolved in 480 mL water. 3.50 mL of sodium hydroxide aqueous solution (2.00 M) was introduced to the surfactant solution and the temperature of the mixture was adjusted to 80 °C. 5.00 mL of TEOS (22.4 mmol) was added dropwise to the surfactant solution under vigorous stirring. The mixture was allowed to react for 2 h to produce white precipitate. The solid crude product was filtered, washed with water and methanol, and dried under high vacuum to yield the as-synthesized material (M-1). To remove the surfactant template, 1.50 g of M-1 was refluxed for 6 h in a methanolic acidic solution containing 1.50 mL HCl (37.2%) and 150 mL methanol. The resulting material was filtered and extensively washed with water and methanol. The surfactant-free M-1 was placed under high vacuum with heating at 60 °C to remove the remaining solvent from the mesopores to get M-2. M-2 (1.00 g) was refluxed for 20 h in 80.0 mL of anhydrous toluene with 1.00 mL (5.67 mmol) of APTMS to yield the 3-aminopropyl-functionalized M-2 (M-3). The purified M-3 (400 mg) was dispersed in 20 mL dimethyl sulfoxide (DMSO). 0.15 g (0.90 mmol) CPBA was reacted with 0.10 g (0.87 mmol) NHS and 0.20 g (1.04 mmol) EDC in 5.0 mL DMSO, stirring at room temperature for 30 min before adding to the M-3 suspension. The mixture was stirred at room temperature for another 24 h, followed by filtration and washing with DMSO, water and methanol and dried to get M-4. Due to the different size of the surfactant templates, the obtained mesopore size was different.[2] The average pore size for CTAB-templated CPBA-functionalized mesoporous silica was measured to be 2.6 nm by TEM (Figure S1Aa), which was exactly the same as the literature value for CTAB-templated mesoporous silica and the average pore size for DTAB-templated CPBA-functionalized mesoporous silica was measured to be 2.1 nm by TEM (Figure S1Ba).

Synthesis of CPBA-nonporous silica sphere. Synthesis of CPBA-nonporous silica was carried out according to the reported seed-growth methods[3] with slight modification. 100 mL of ethanol, 5 mL of water, and 5 mL of $NH_3 \cdot H_2O$ (35.3%) were added consequently into a 250-mL flask and heated gradually to 40 °C under constant vigorous stirring. A mixed solution of 2 mL of TEOS and 8 mL of ethanol was added

to the solution quickly. After maintaining solution temperature at 40 °C for 5 h, the colloidal suspension was obtained, which was used as the seeds for subsequent particle growth. To obtain larger particles, 10 mL of the colloidal suspension from the first step was mixed with 50 mL of ethanol, 10 mL of water, and 10 mL of $NH_3 \cdot H_2O$ in a 250-mL flask. A mixture of 1 mL TEOS and 9 mL ethanol was added dropwise to the flask, followed by continuous stirring for 8 h. After centrifugation of the solution mixture, the precipitate, i.e., SiO_2 particles, was washed with ethanol four times and dried under vacuum. The enlarged SiO_2 spheres (S-1) were stored until needed. The obtained S-1 (1.00 g) was refluxed for 20 h in 80.0 mL of anhydrous toluene with 0.2 mL (1.1 mmol) of APTMS to yield the 3-aminopropyl-functionalized S-1 (S-2). S-2 (400 mg) was dispersed in 20 mL DMSO. 0.3 g (0.18 mmol) CPBA was reacted with 0.2g (0.17 mmol) NHS and 0.4 g (0.2 mmol) EDC in 5.0 mL DMSO, stirring at room temperature for 30 min before adding to the S-2 suspension. The mixture was stirred at room temperature for another 24 h, followed by filtration and washing with DMSO, water and methanol and dried to get CPBA-nonporous silica (S-3).

Characterization. For TEM analysis, 1 mg material was added to 1 mL solution of 1 mg/mL of different molecules in 30 mM phosphate buffer (pH 8.5). The tubes were shaken on a rotator for 6 h at room temperature. Then the mixture was diluted by 10 times with the same buffer. Drop-and-dry method was used to prepare the samples for TEM. For XRD analysis, 30 mg material was added to 30 mL solution of 3 mg/mL of different molecules. The tubes were shaken on a rotator for 6 h at room temperature. The solution was vacuum freeze dried and the obtained powder was used for XRD characterization.

The selectivity of CPBA-mesoporous and CPBA-nonporous silica spheres. For the extraction by CPBA-mesoporous silica, 2 mg material was added to 900 μL solution of 1 mg/mL adenosine or deoxyadenosine. The tubes were shaken on a rotator for 1 h at room temperature. The CPBA-mesoporous silica were then collected by centrifuge and rinsed with 500 μL of 30 mM sodium phosphate buffer (pH 8.5) for

3 times each. After washing, the CPBA-mesoporous silica was resuspended and eluted in 100 μL 100 mM acetic acid solution for 1 h on a rotator. Finally, the CPBA-mesoporous silica was centrifuged again and the eluates were collected by pipetting carefully. The eluates were used for UV absorbance measurement. The same procedure was carried out for CPBA-nonporous silica.

Digitalization analysis of TEM images. The digitalization analysis of TEM images was implemented using an Image Processing Program kindly provided by Wayne Rasband (Research Services Branch, National Institute of Mental Health, USA). More specifically, for each TEM image, the normalized pixel intensity was calculated through the following equation:

$$Normalized\ pixel\ intensity = \frac{Averaged\ intensity\ for\ selected\ pixels}{Average\ intensity\ for\ backgroud}$$

The intensity of 20 randomly selected pixels in the background area and within the images for the mesoporous silica nanoparticle and the nonporous silica nanoparticle were read out and averaged. For mesoporous silica nanoparticles, pixels in dark spots within the image were selected.

Nitrigen adsorption/desorption isotherms. The surface areas were calculated by the Brunauer–Emmett–Teller (BET) equation. The pore size distribution was calculated by the Barrett-Joyner-Halenda (BJH) equation.

Protein stability test by fluorescence spectrum and UV-vis absorbance. In the above adsorption isotherm measurement, to ensure an equilibrium between the free interacting molecule outside of mesoporous silica and the bound interacting molecule inside of mesoporous silica, a long equilibrium time (12 h at room temperature) under shaking condition was applied. To ensure such condition will not result in apparent denaturation or conformational change of proteins under test, fluorescence spectrum (excited at 295 nm) was used to evaluate the denaturation degree of proteins under different conditions. According to the fluorescence method,[4-5] if a protein is denatured,

the fluorescence peak for tryptophan will apparently red-shift. Different protein solutions were prepared for fluorescence spectrum measurement. For native protein solutions, protein lyophilized powders (1 mg/mL) were dissolved in freshly prepared with 30 mM phosphate buffer (pH 8.5) and the fluorescence spectra were instantly recorded. Then, the native protein solutions were shaken on a rotator for 12 h at room temperature, which was the same conditions for the extraction, and the fluorescence spectra were recorded. For denatured protein solutions, the native protein solutions were heated to 100 °C and kept for 10 min, and after cooling to room temperature fluorescence spectra were recorded. To further verify our results, TMB colorimetric method was used to test the catalytic activity of HRP that under long-time shaking, which is highly related to the conformation of HRP. Briefly, 5 μL HRP solutions (1 ng/mL) under test were mixed with 100 μL TMB stock solution (A:B, 1:1 v/v), and after shaken for 1 min the absorbance at 650 nm was recorded.

Adsorption isotherms and Scatchard analysis. Equivalent CPBA-mesoporous silica (2 mg) was added to solutions (900 μL) of glycoprotein or adenosine at different concentrations in 1.5-mL plastic microcentrifugal tubes. The tubes were shaken on a rotator for 12 h at room temperature. The washing and elution procedures were the same as those described above. The amounts of glycoprotein or adenosine extracted by the CPBA-mesoporous silica were determined by measuring the glycoprotein or adenosine in the eluates with the Nanodrop-2000C UV-Vis spectrophotometer. UV absorbance was adopted at 260 nm for adenosine, 403 nm for HRP, 280 nm for RNase B and RNase A. The same procedure was carried out for CPBA-nonporous silica. Three parallel measurements were carried out for each experimental point.The amount of glycoprotein or adenosine bound to CPBA-mesoporous silica or CPBA-nonporous 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 glycoprotein or adenosine bound to

CPBA-mesoporous silica or CPBA-nonporous silica at equilibrium, the free concentration 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.

References:

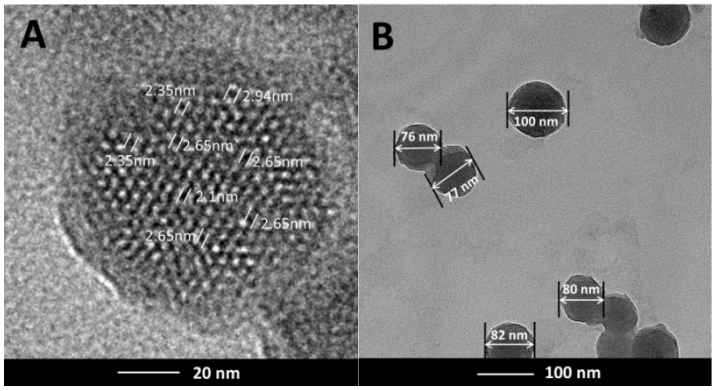
(1) Zhao, Y.N.; Trewyn, B.G.; Slowing, I.I.; Lin, V.S.-Y. *J. Am. Chem. Soc.* **2009**, *131*, 8398-8400.

(2) Itof, T.; Yano, K.; Inada, Y.; Fukushma, Y. *J. Mater. Chem.* **2002**, *12*, 3275-3277

(3) Chang, S. M.; Lee, M.; Kim, W.-S. *J. Col. Interf. Sci.* **2005**, *286*, 536-542.

(4) Abou-Zied O.K., Al-Shihi. O.I.K. *J. Am. Chem. Soc.* **2008**, *130*, 10793-10801.

(5) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.



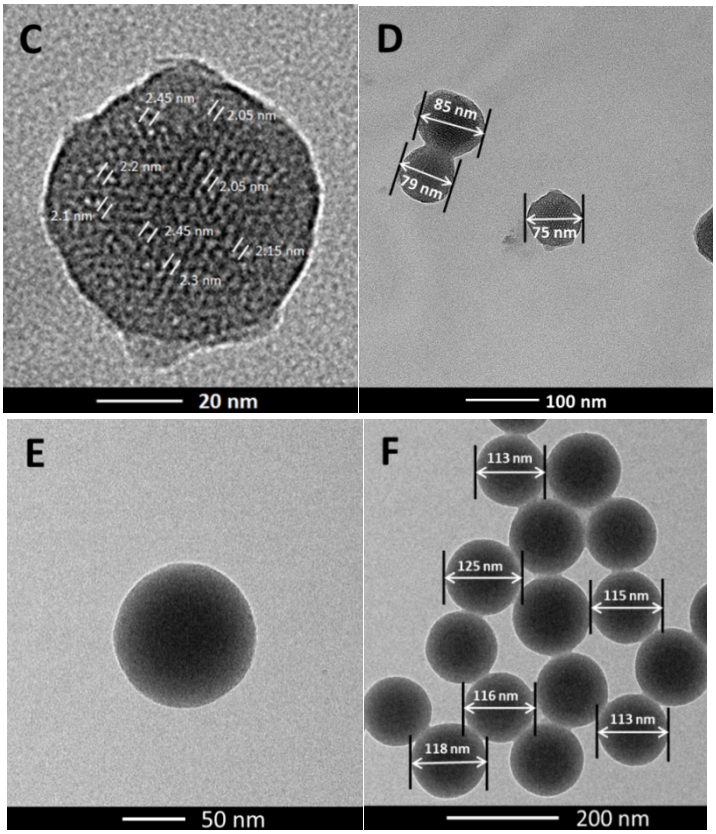


Fig. S1. TEM images for A, B) the CPBA-mesoporous silica nanoparticles with CTAB as template; C, D) the CPBA-mesoporous silica nanoparticles with DTAB as template; and E, F) the CPBA-nonporous silica sphere. Based on the TEM images, the pore size of the CPBA-mesoporous silica nanoparticles with CTAB and DTAB as templates were measured to be 2.6 ± 0.25 and 2.1 ± 0.2 nm, respectively. The diameters for these mesoporous silica nanoparticles and the nonporous silica sphere were measured to be 83 ± 10, 80 ± 5 and 117 ± 4 nm, respectively.

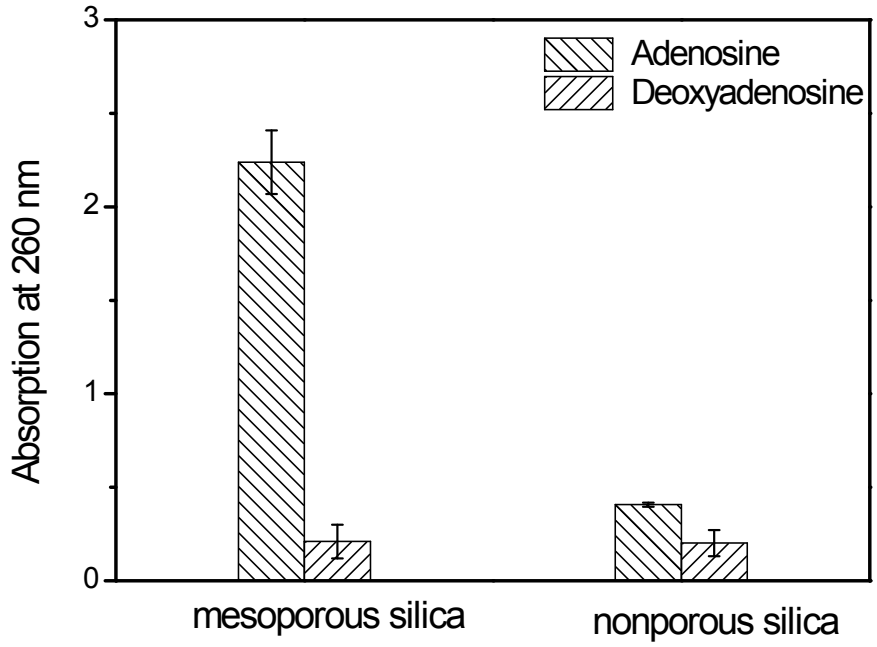


Fig. S2. Selectivity of the CPBA-functionalized mesoporous (pore size 2.6 nm) and nonporous silica toward adenosine against deoxyadenosine.

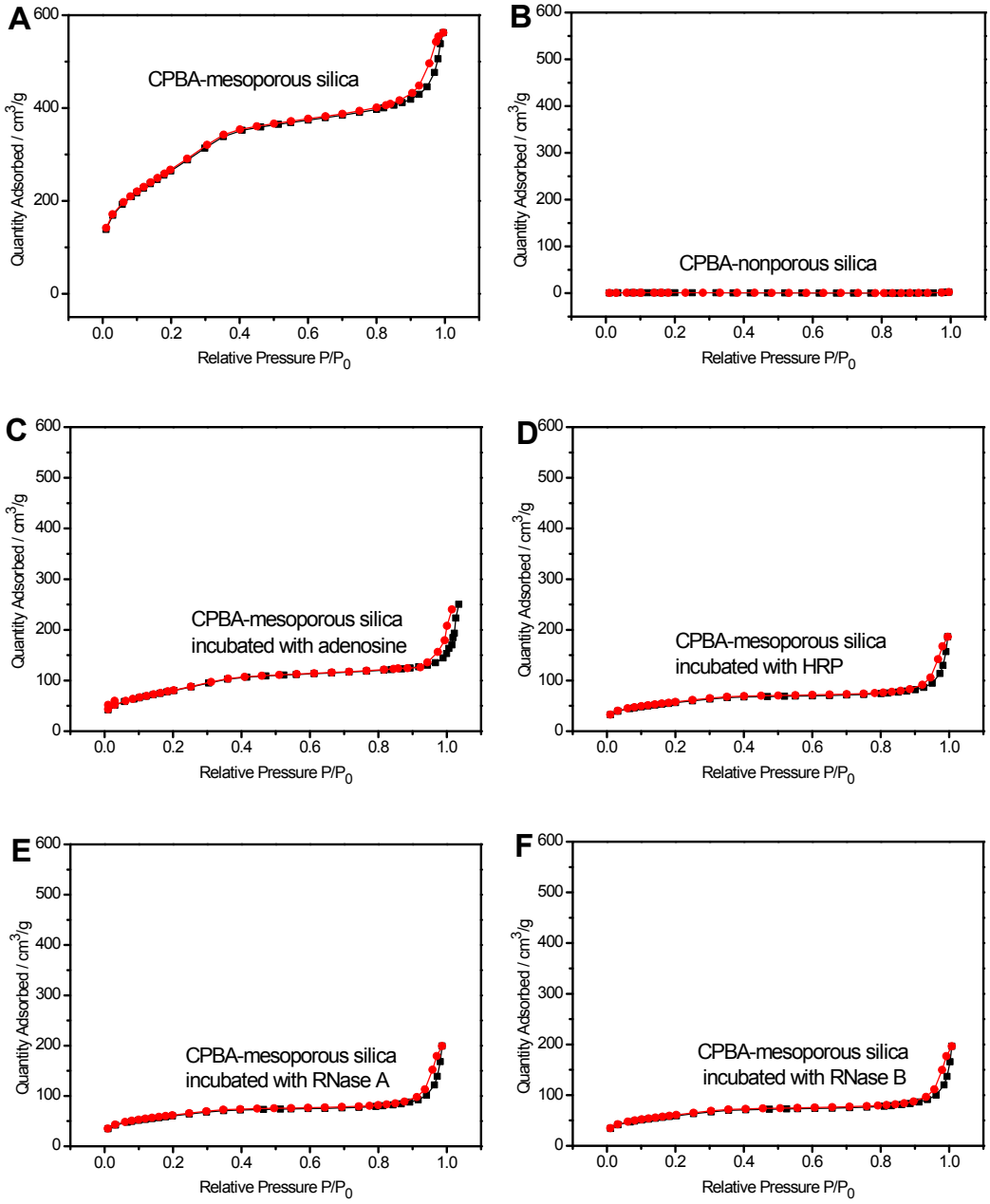


Fig. S3. BET nitrogen adsorption/desorption isotherms for A) CPBA-mesoporous silica, B) CPBA-nonporous silica, C-F) interacting species-bound CPBA-mesoporous silica. Interacting species: C) Adenosine, D) HRP, E) RNase A, and F) RNase B. The absorbed amount of N_2 by interacting species-incubated CPBA-mesoporous silica is apparently lower than that by bare CPBA-mesoporous silica. This can be attributed to that the mesopores were occupied by the interacting species with molecular size less than the mesopore (adenosine, RNase A and RNase B) or covered by the interacting species with molecular size larger than the mesopore (HRP).

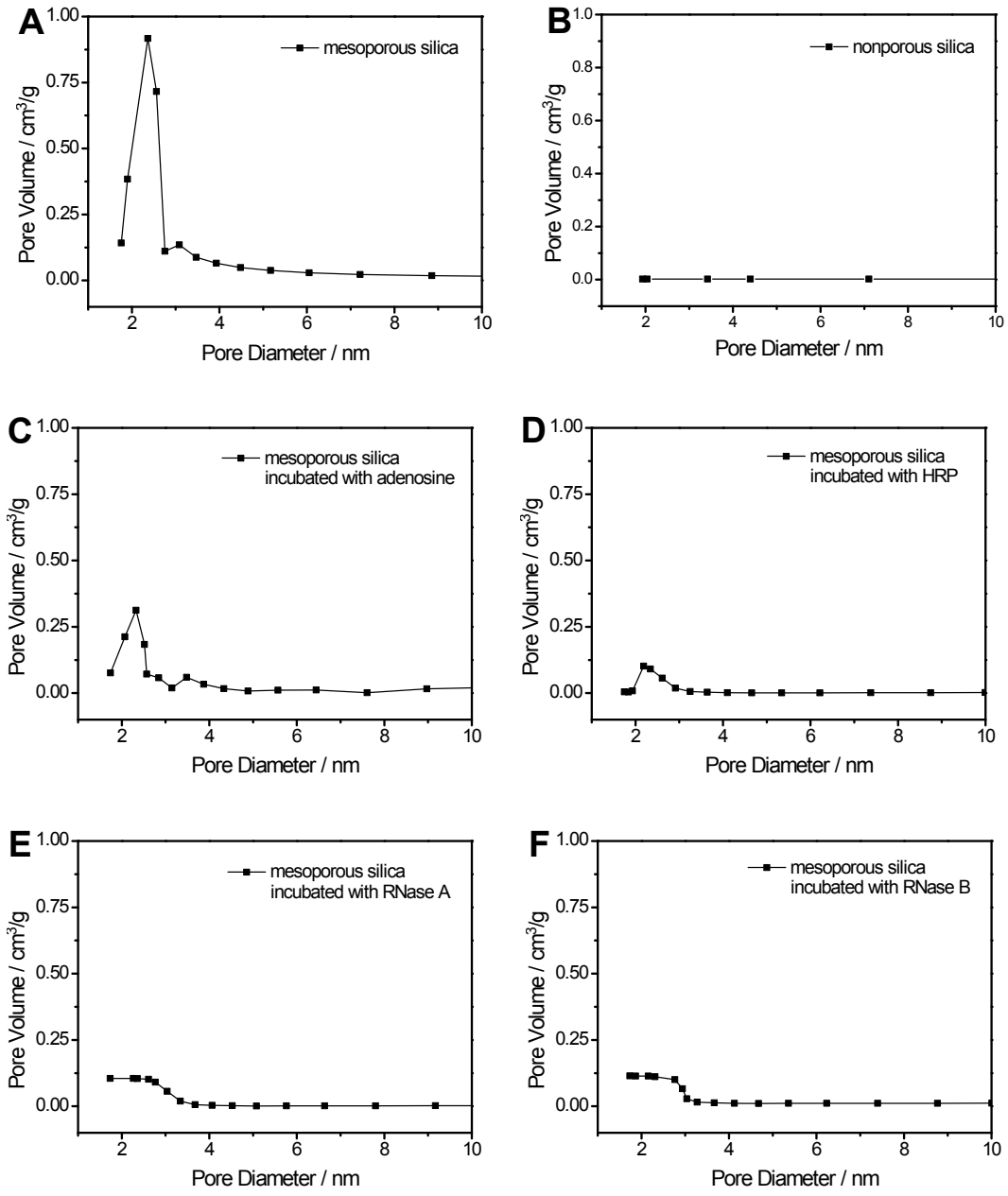


Fig. S4. BJH pore size distributions of A) CPBA-mesoporous silica, B) CPBA-nonporous silica, C-F) interacting species-bound CPBA-mesoporous silica. Interacting species: C) Adenosine, D) HRP, E) RNase A, and F) RNase B. The pore volume of the mesopore of CPBA-mesoporous silica is significantly decreased after been incubated with the interacting species. This can be attributed to that the mesopores were occupied by the interacting species with molecular size less than the mesopore (adenosine, RNase A and RNase B) or covered by the interacting species with molecular size larger than the mesopore (HRP).

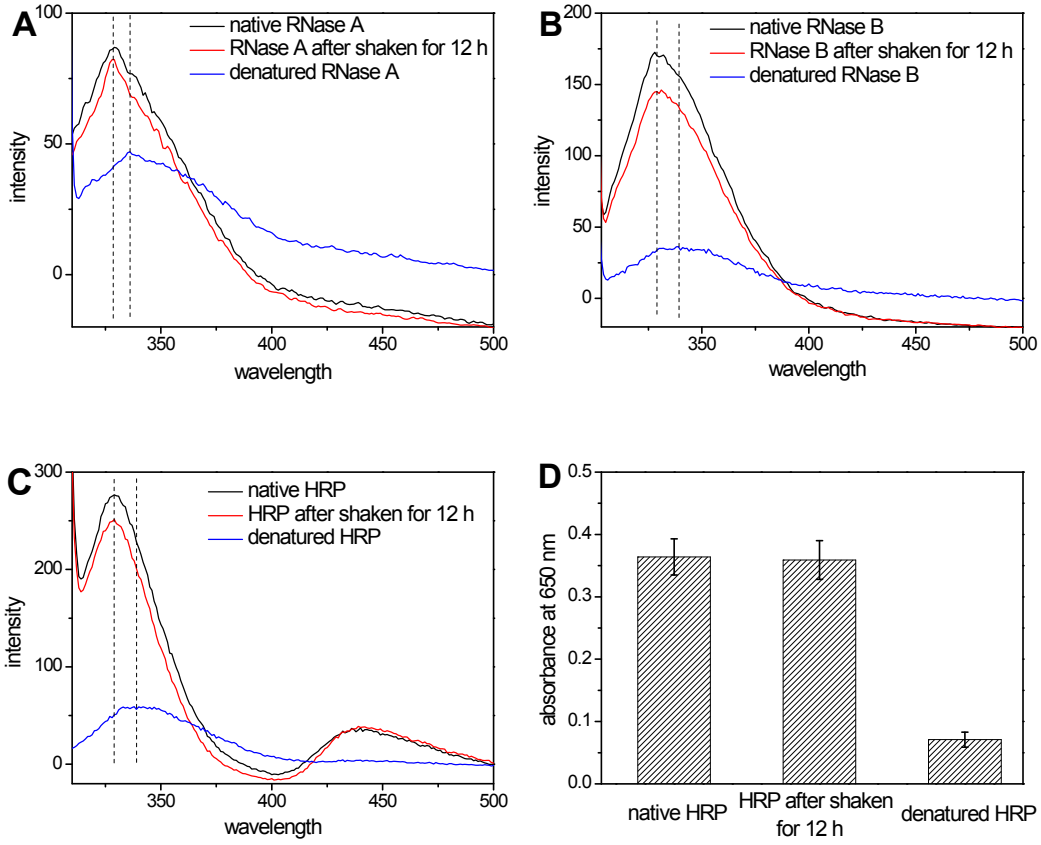


Fig. S5. A-C) Fluorescence spectra for the test proteins at different status (native, denatured, and after shaken for 12 h at room temperature) and D) UV-vis absorbance of HRP at different status after TMB staining. Since the peaks for tryptophan of the test proteins after shaken for 12 h at room temperature were all the same as those for native proteins, it can be concluded that all the test proteins did not experience apparent conformational change or denaturation during the shaking procedure. Besides, the TMB staining test also confirmed that HRP did not experience apparent conformational change or denaturation.

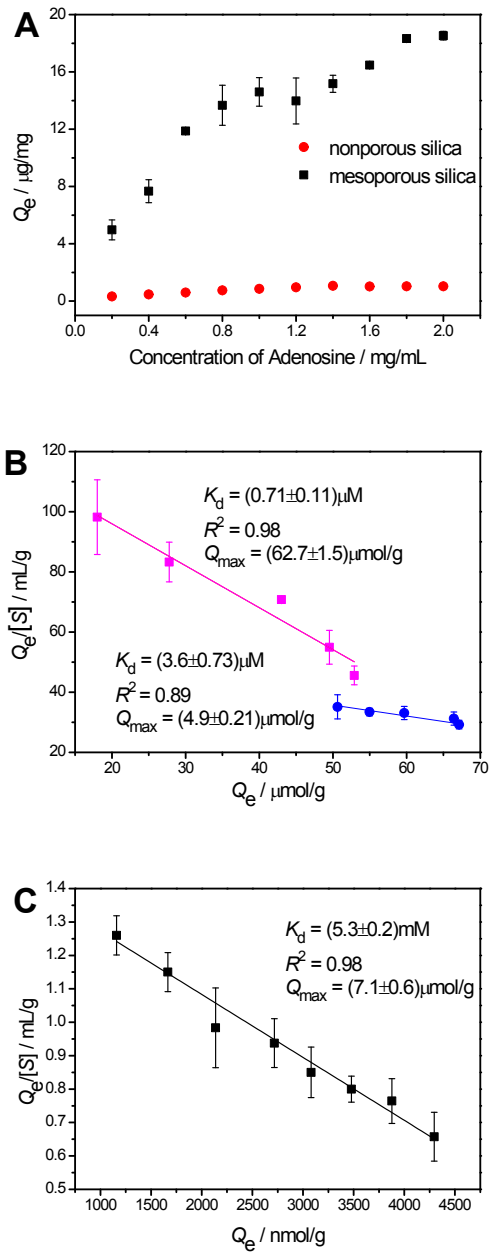


Fig. S6. A) Binding isotherms for the binding of adenosine with CPBA-mesoporous (pore size 2.6 nm) and CPBA-nonporous silica in 30 mM phosphate buffer pH 8.5; B) Scatchard plots for the bindings of adenosine with CPBA-mesoporous silica (pore size 2.6 nm); C) Scatchard plots for the binding of adenosine with CPBA-nonporous silica.

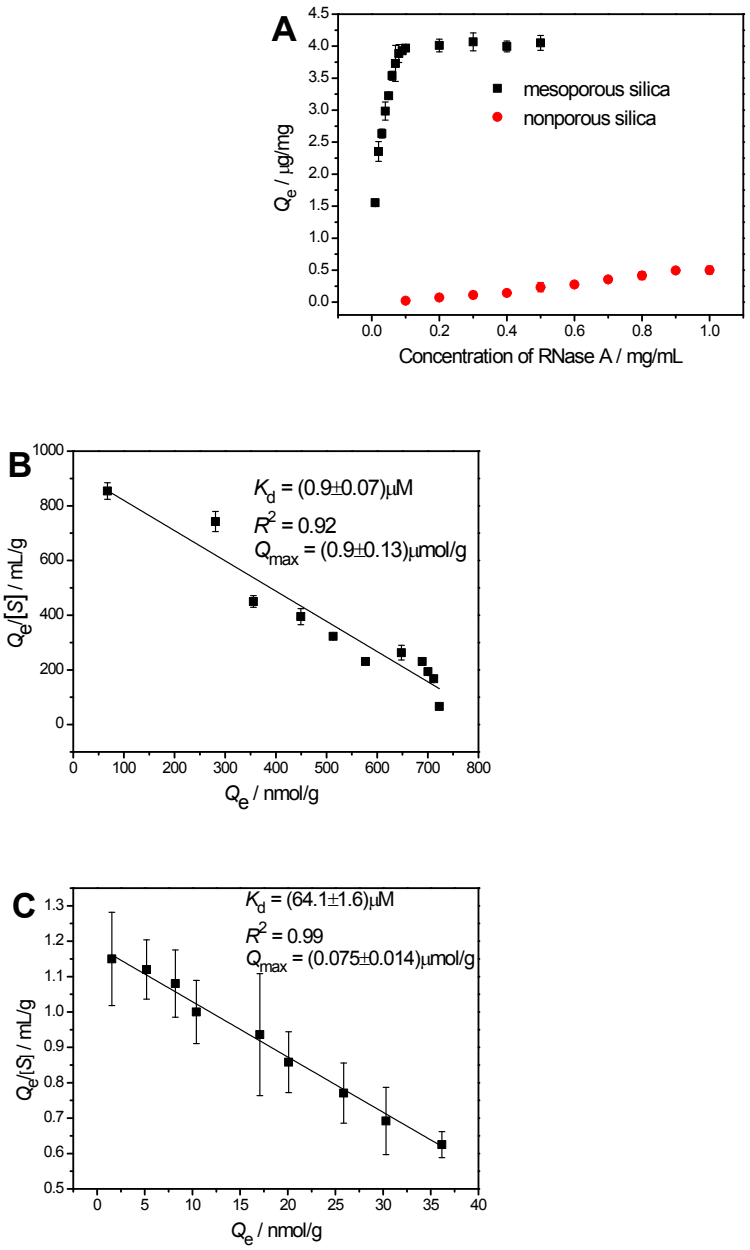
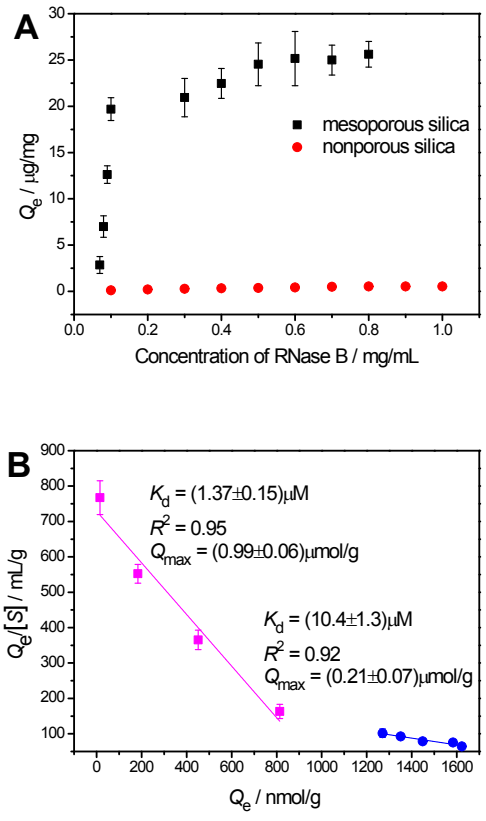


Fig. S7. A) Binding isotherms for the binding of RNase A with CPBA-mesoporous silica (pore size 2.6 nm) and CPBA-nonporous silica in 30 mM phosphate buffer pH 8.5; B) Scatchard plots for the binding of RNase A with CPBA-mesoporous silica

(pore size 2.6 nm); C) Scatchard plots for the binding of RNase A with CPBA-nonporous silica.



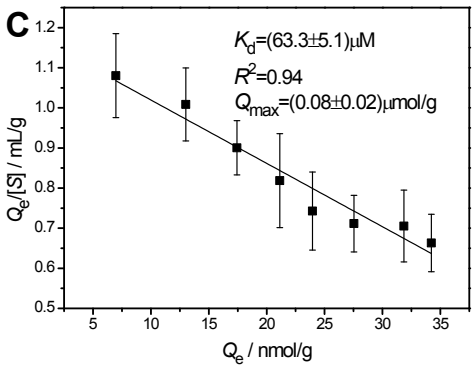
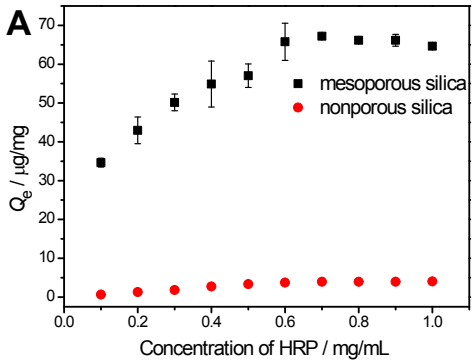


Fig. S8. A) Binding isotherms for the binding of RNase B with CPBA-mesoporous silica (pore size 2.6 nm) and CPBA-nonporous silica in 30 mM phosphate buffer containing 500mM NaCl pH 8.5; B) Scatchard plots for the bindings of RNase B with CPBA-mesoporous silica (pore size 2.6 nm); C) Scatchard plots for the binding of RNase B with CPBA-nonporous silica.



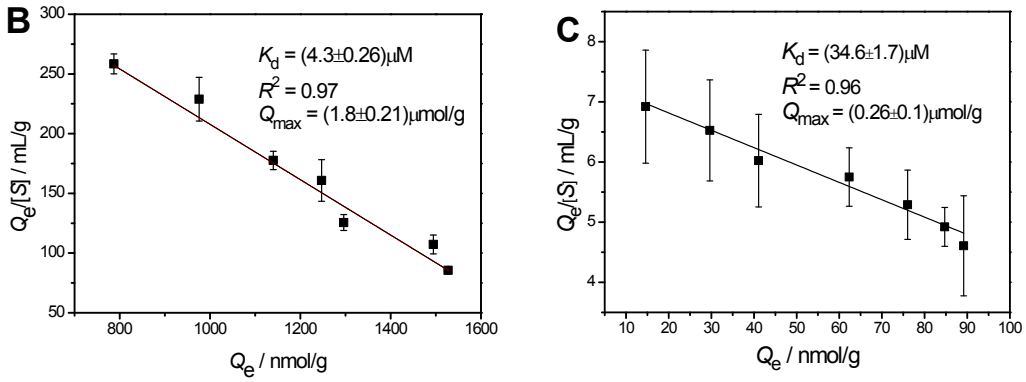
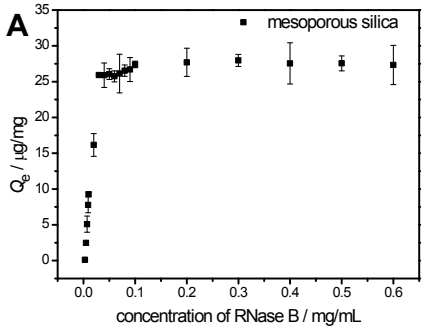


Fig. S9. A) Binding isotherms for the binding of HRP with CPBA-mesoporous silica (pore size 2.6 nm) and CPBA-nonporous silica in 30 mM phosphate buffer pH 8.5; B) Scatchard plots for the binding of HRP with CPBA-mesoporous silica (pore size 2.6 nm); C) Scatchard plots for the binding of HRP with CPBA-nonporous silica.



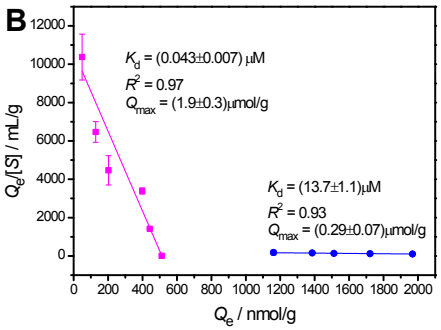


Fig. S10. A) Binding isotherms and B) Scatchard plots for the binding of RNase B with CPBA-mesoporous silica (pore size 2.1 nm) in 30 mM phosphate buffer pH 8.5.