Electronic supplementary information for:

A Supramolecular Approach for Versatile Biofunctionalization of Magnetic Nanoparticles

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1   Experimental section

1.1   Materials

Iron (III) chloride (FeCl$_3$·6H$_2$O), trisodium citrate dehydrate (C$_6$H$_5$O$_7$Na$_3$·2H$_2$O), urea and polyacrylamide (PAM, 300 kDa) were purchased from Sinopharm (Shanghai, China). Tetraethyl orthosilicate (TEOS), 3-Glycidyloxypropyl trimethoxysilane (GLYMO) and 1-adamantanamine hydrochloride were purchased from ALDRICH. All organic solvents were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Mannose-persubstituted β-cyclodextrin (CD-M), biotin-persubstituted β-cyclodextrin (CD-B) and QAS-persubstituted β-cyclodextrin (CD-Q) were synthesized as reported previously. [1] Fluorescein isothiocyanate (FITC)-labeled Concanavalin A (FITC-ConA) and fluorescein isothiocyanate (FITC)-labeled avidin (FITC-avidin) were obtained from Sigma. Fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (FITC-BSA) was obtained from Solarbio. Deionized water was purified with a Millipore water purification system, resulting in a resistivity of 18.2 MΩ·cm.

Escherichia coli (*E. coli*, ATCC-700926) and *Staphylococcus aureus* (*S. aureus*, ATCC-6538) were used in our experiments. Prior to the experiments, bacteria were incubated in Luria-Bertani broth medium (LB, Sigma-Aldrich) and nutrient bouillon medium (NB, Sigma-Aldrich), grown overnight under shaking at 37 °C, and harvested during the exponential growth phase via centrifugation. The supernatant was discarded, and the cell pellet was re-suspended in phosphate-buffered saline (PBS, pH=7.4) with a final concentration of approximately 1×10^5 cells·mL^{-1}.

1.2   Synthesis of Porous Magnetic Particles (MNPs)

Magnetic porous nanoparticles were prepared using the previously reported procedure. [2] In brief, 1.08 g FeCl$_3$·6H$_2$O (4 mmol), 2.35 g sodium citrate (8 mmol) and 0.72 g urea (12 mmol) were dissolved

in 80 mL ultrapure water with stirring until well combined. An amount of 0.6 g PAM was slowly added to the above solution with continuous stirring to form a homogeneous solution. After 1 h of mixing, the solution was transferred and sealed into a 100 mL Teflon-line stainless-steel autoclave, followed by heating to 200 °C for 12 h. The resulting solution containing the hollow nanoparticles was cooled to room temperature, and the black precipitate was obtained by centrifugation. The precipitate was washed several times with ultrapure water and ethanol and re-dispersed in water at certain concentrations.

1.3   Preparation of MNP@SiO$_2$

The core-shell structured MNP@SiO$_2$ was synthesized using the sol-gel approach. Specifically, 50 mg MNPs were fully dispersed in a solution containing 160 mL ethanol, 40 mL ultrapure water and 2 mL aqueous ammonia (28 wt%). After 3 min of ultrasonication vibration, 1 mL TEOS was added, and the resulting dispersion was continuously stirred at 30 °C for 6 h. The obtained product was separated using a magnetic field and washed with ethanol to remove excess unreacted agents. The final MNP@SiO$_2$ microsphere was dried under vacuum for further use.

1.4   Preparation of MNP@SiO$_2$-Ada

First, the adamantine-functionalized siloxane was synthesized from epoxy-containing siloxane via ring-open reaction with an anhydrous methanol solution of adamantanamine. Typically, 1.24 g 1-adamantanamine hydrochloride (6.61 mmol) was dissolved in 30 mL anhydrous methanol, and the pH was adjusted to 10 with sodium methylate. The reaction flask was cooled to 0 °C using an ethanol/ice bath before 1.41 g GLYMO (6.35 mmol) was added dropwise into the mixture with vigorous stirring under a nitrogen atmosphere. After another 30 min of stirring, the temperature was increased to 65 °C for 6 h. The resulting solution was referred to as the GLYMO-Ada solution.

An amount of 50 mg MNP@SiO$_2$ was added into the GLYMO-Ada solution with ultrasonication vibration for 10 min. The dispersion was held at 65 °C with mechanical stirring for 2 h. The product, known as MNP@SiO$_2$-Ada, was washed several times with methanol and dried under vacuum.

1.5 Preparation of MNP@SiO$_2$-X

An aqueous solution (25 mL) containing 50 mg CD-X (CD-B, CD-M or CD-Q) was stirred in a nitrogen atmosphere. The MNP@SiO$_2$-Ada (25 mg) in 25 mL aqueous solution was added into the above-functionalized CD solution. The resulting mixture was stirred continuously for 12 h. The magnetic nanoparticles with the CD-X inclusion complex (known as MNP@SiO$_2$-X) were washed with ultrapure water with the aid of a magnetic field and re-dispersed in 25 mL 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer containing 10 µL Triton X-100.

1.6 Characterization

The surface morphology and structure of the MNPs were investigated via transmission electron microscopy (TEM) (G-120, Hitachi, Tokyo, Japan) and scanning electron microscopy (SEM) (S-4700, Hitachi). The surface potential of the nanoparticles was measured via the Zeta potential using a Zetasizer Nano-ZS90 zeta potential analyzer (Malvern Instrument Ltd. UK) at 25 °C. Fourier transform infrared (FTIR) spectra were obtained using a Nicolet 6700 FTIR spectrometer (Thermo Inc. USA).

1.7 Protein Adsorption

Prior to the protein adsorption experiments, the magnetic particle suspension was separated from the solution using a magnetic plate and washed several times with equal HEPES buffer solution. The solution containing fluorescein-labeled protein (FITC-avidin, FITC-ConA and FITC-BSA) with a certain concentration was mixed with the magnetic particles and incubated at ambient temperature for 30 min in the dark. After incubation with dissociation by pipette, a magnetic field was applied to the

mixture through a magnet, thus inducing aggregation of nanoparticles on the side of the centrifuge tubes. The residual fluorescence intensity of the supernatant with an emission wavelength (em = 525 nm for FITC) was measured with a Varioskan Flash spectrophotometer with an excitation wavelength (ex = 488 nm for FITC).

Selected experimental data are presented as the adsorption capacity per unit mass (mg) of the nanoparticles, and the adsorption capacity constant (Q) is calculated using the difference in protein amount with the following equation:

$$Q = \frac{(C_0 - C_t)V}{m} \times 10^3$$

where C_0 (mg/mL) and C_t (mL), respectively, represent the original and equilibrium concentrations of the protein solution; V (mL) is the volume of protein solution; and m (mg) is the mass of the applied nanoparticles.

1.8   Bacteria Capture Experiment

The magnetic particle suspension was separated using a magnetic plate and washed several times with PBS. The solution containing bacteria (*E. coli*) with a concentration of approximately 1×10^5 CFU/mL was mixed with the magnetic particles (2 mg/mL) and shaken gently at ambient temperature for 30 min. After incubation, the bacteria-combined magnetic particle complexes were isolated using a magnetic plate on the side of the centrifuge tubes. The supernatant was sampled and analyzed for bacterial concentration via the plate counting method. The number of bacteria in each agar plate image was analyzed with Image-Pro Plus software.

The capture efficiency of bacteria was calculated using the following equation:

$$\text{Capture efficiency} = \frac{(N_0 - N_s)}{N_0} \times 100\%$$

where N_0 and N_s represent the number of bacteria in the initial sample and the supernatant after

magnetic separation, respectively.

1.9   Bacteria Killing Experiment

The magnetic particle suspension was separated using a magnetic plate and washed several times with PBS. The solution containing bacteria (*S. aureus*) with a concentration of approximately 1×10^5 CFU/mL was mixed with magnetic particles (2 mg/mL) and shaken gently at ambient temperature for 3 h. After incubation, the suspension solution containing bacteria-combined magnetic particle complexes was sampled and analyzed for bacterial concentration via the plate counting method.

2   Supporting Results

2.1   Magnetic property of MNP@SiO₂-Ada particles
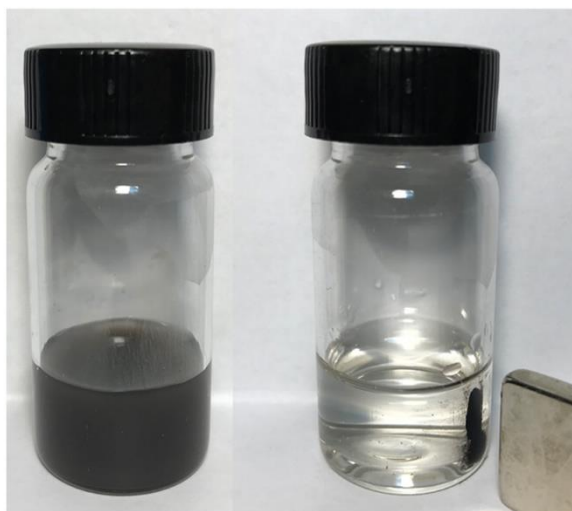
Fig. S1. Photographs of MNP@SiO₂-Ada dispersed in water (1 mg/mL) (left) and magnetically induced aggregation of MNP@SiO₂-Ada from water (right).
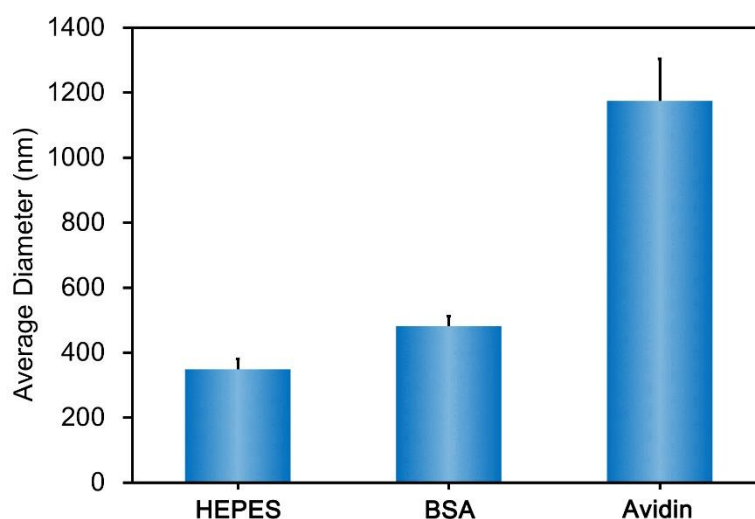
2.2   Protein adsorption

Fig. S2. Diameters of MNP@SiO₂-B particles in HEPES, 0.1 mg/mL BSA in HEPES, and 0.1 mg/mL avidin in HEPES. Error bars represent the standard deviation of the mean ($n = 3$).
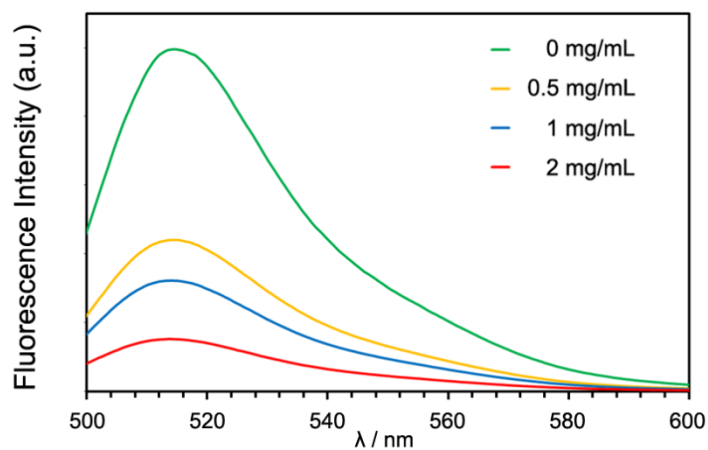
Fig. S3. Fluorescent emission spectra of the supernatants from FITC-ConA solution after incubation with different concentrations of MNP@SiO$_2$-M and separation by magnetic field.
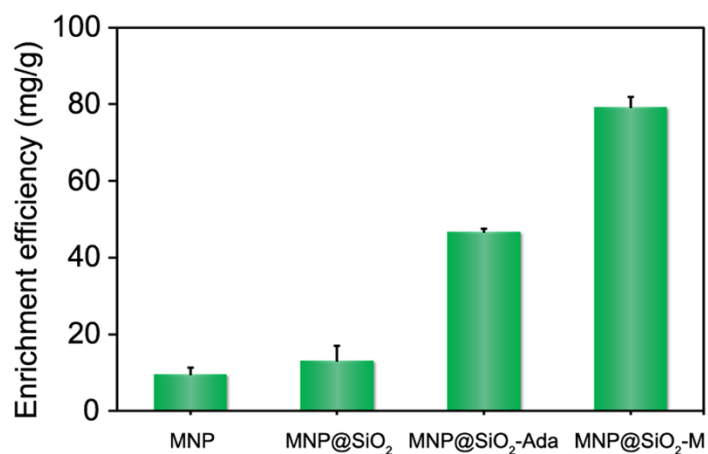

Fig. S4. Adsorption of 0.1 mg/mL FITC-ConA on MNP, MNP@SiO$_2$, MNP@SiO$_2$-Ada and MNP@SiO$_2$-M. Error bars represent the standard deviation of the mean (n = 3).
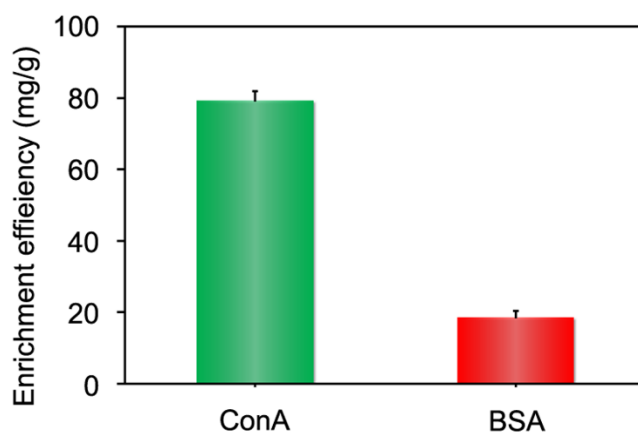
Fig. S5. Comparison of adsorption of 0.1 mg/mL FITC-ConA and 0.1 mg/mL FITC-BSA on MNP@SiO$_2$-M. Error bars represent the standard deviation of the mean (n = 3).

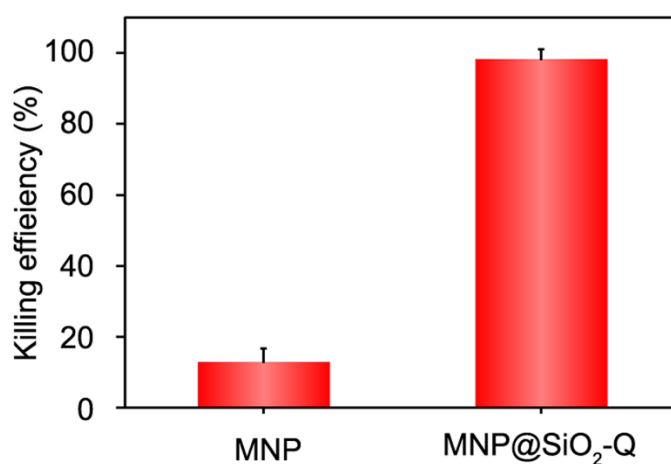## 2.3 Bactericidal activity evaluated via colony-counting assay

Fig. S6. Comparison of killing efficiency of MNP and MNP@SiO$_2$-Q against *S. aureus*. Error bars represent the standard deviation of the mean (n = 3).

References

1. L. Cao, Y. Qu, C. Hu, T. Wei, W. Zhan, Q. Yu and H. Chen, *Adv. Mater. Interfaces*, 2016, **3**, 1600600.

2. W. Cheng, K. Tang, Y. Qi, J. Sheng and Z. Liu, *J. Mater. Chem.*, 2010, **20**, 1799-1805.