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Supporting Information

pH-Controlled Release of Substrates from Mesoporous SiO_2 Nanoparticles Gated By Metal Ion-Dependent DNAzymes

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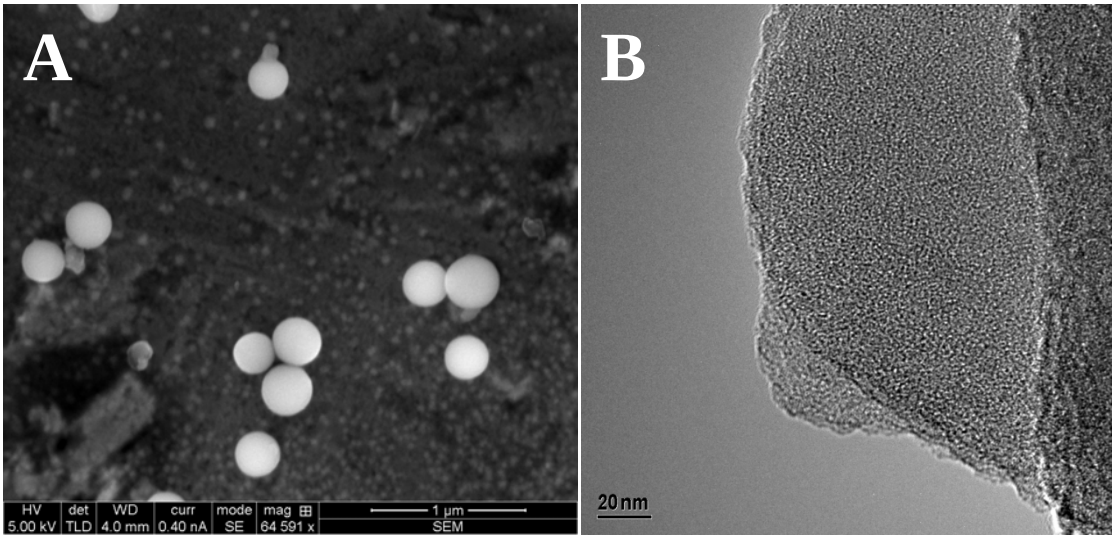

Figure S1. (A) SEM image of solid mesoporous SiO_2 nanoparticles (MP-SiO_2 NPs). (B) High resolution TEM image of the MP-SiO_2 NPs.

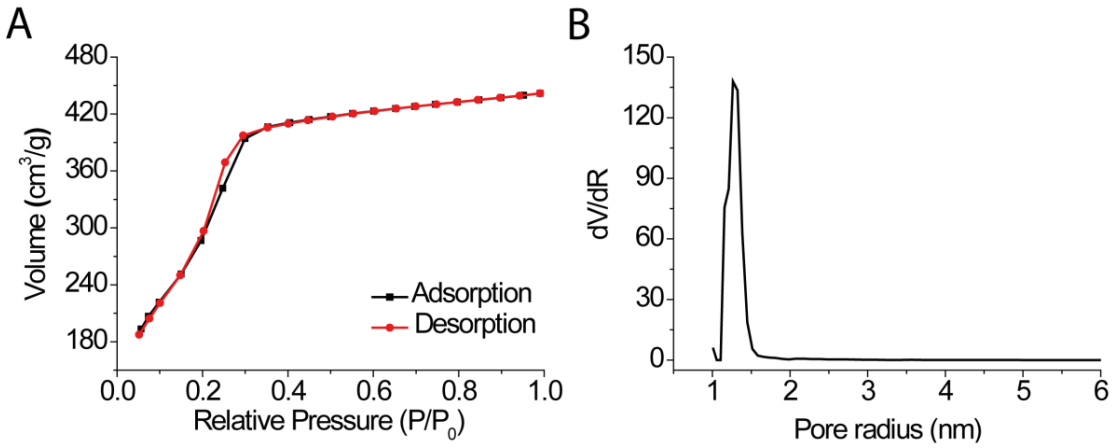

Figure S2. (A) N_2 adsorption-desorption isotherms and (B) Pore size distribution, of the MP-SiO_2 NPs. The surface area is 1057 m^2/g, and the pore diameter corresponds to ~2.8 nm.

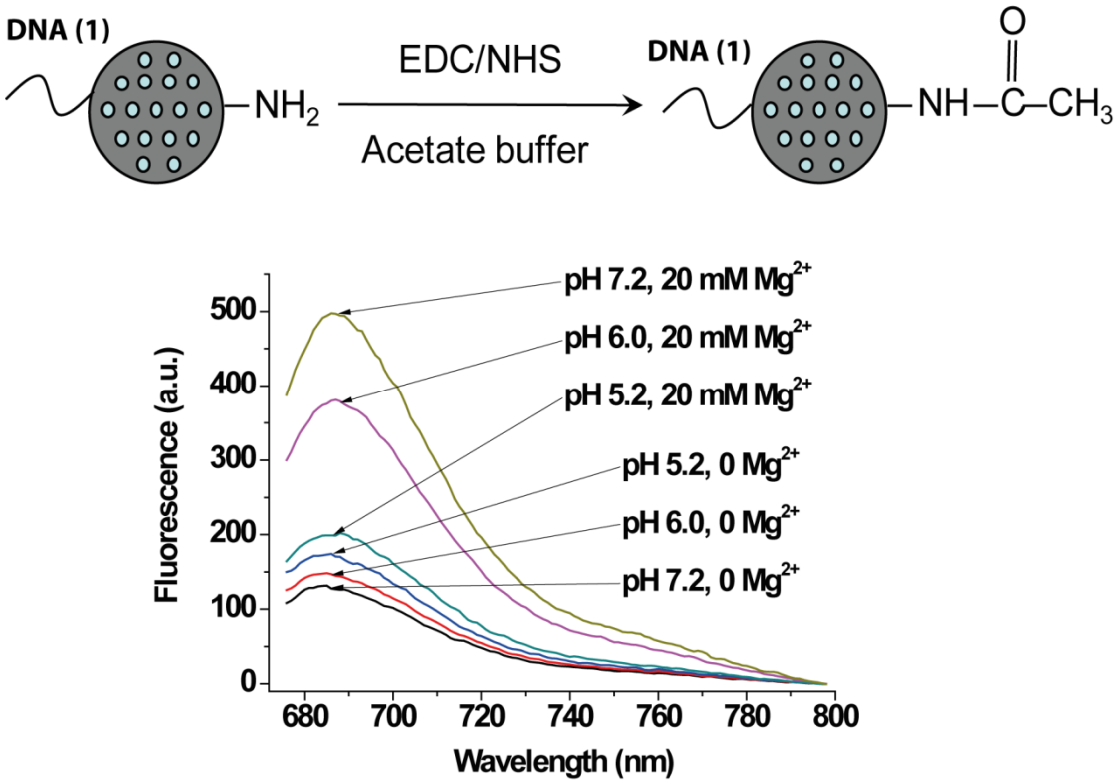

Figure S3. The background fluorescence spectra corresponding to the release of MB^+ from the MP-SiO_2 NPs without added Mg^{2+} ions at different pH values. For comparison the fluorescence spectra of the released MB^+ in the presence of add 20 mM Mg^{2+} ions, at different pH values are shown. All spectra were recorded after fixed a time-interval of 60 minutes of release.

Conjugation of the DNAzyme to the dye-loaded MP-SiO_2 NPs.

The preparation of the DNAzyme-capped and dye-loaded MP-SiO_2 NPs followed the following steps: (i) Covalent functionalization of the amine-functionalized MP-SiO_2 NPs with the DNAzymes substrate (**1**). (ii) Protection of the vacant amino functionalities by acetylation. (iii) Loading of the dyes (methylene blue or thionine) in the pores and capping the pores with the DNAzyme sequences (**2**) or (**3**). (iv) Rinsing off the dye associated with non-pore surface domains of the nanoparticles. (v) Characterization of the loading of the dyes in the pores of the respective MP-SiO_2 NPs.

The following procedures were implemented to follow each of these steps:

(i) Covalent functionalization of the amine-functionalized MP-SiO_2 NPs with the DNAzymes substrate

A mixture consisting of monodispersed amino-functionalized MP-SiO_2 NPs solution was prepared by placing 10 mg of silica NPs in 950 μl HEPES buffer (10 mM, pH=7.0) followed by the sonication of the mixture for 1 hour. The resulting solution was mixed with 50 μl of sulfo-EMCS (10 mg/ml) and allowed to react for 30 minutes. To remove excess of EMCS, the MP-SiO_2 NPs were collected using a centrifuge (at 6000 rpm for 3 minutes), and the NPs were re-dissolved in 950 μl of HEPES buffer (10 mM, pH=7.0). The purified SiO_2 NPs were reacted with freshly reduced and purified thiolated oligonucleotides (**1**) (100 μl, 1 mM), and incubated for 2 hours (the as-provided thiolated nucleic acids protected in the form of disulfide, were reduced with dithiothreitol, DTT, 0.1 M. The resulting thiolated nucleic acids were separated from excess of DTT using a MicroSpin^{TM} G-25 Column).

(ii) Protection of the vacant amino functionalities by acetylation.

For the blocking of the remaining amino groups on the surface of MP-SiO_2 NPs, 10 mg of MP-SiO_2 NPs were dissolved in 800 μl of acetate buffer (0.1 M, pH=5.5), 100 μl of EDC (100 mM) and 100 μl of NHS (50 mM) were added to the mixture and reacted for 2 hours. Then, the MP-SiO_2 NPs were precipitated by centrifugation at 6000 rpm for 3 minutes and washed using ultrapure water.

(iii) Loading of the dyes (methylene blue or thionine) in the pores and capping the pores with the DNAzyme sequences (2) or (3).

The dyes were loaded on the different MP-SiO_2 NPs: the **(1)**-modified MP-SiO_2 NPs, 10 mg, were introduced into 800 μl of HEPES buffer (10 mM, pH=7.0), 100 μl of MB^+ or Th^+ aqueous solutions (10 mM) were added to the NPs, and the mixtures were incubated for 12 hours. Afterwards, 100 μl of (**2**) or (**3**) (1 mM) were added to the resulting mixtures, and the systems were allowed to react for 2 hours. Finally, the loading of the dyes in the pores of the two different systems was evaluated by precipitating the loaded NPs of the different systems (centrifugation at 6000 rpm for 3 minutes).

(iv) Rinsing off the dye associated with non-pore surface domains of the nanoparticles.

The particles were washed at least six times with a HEPES buffer solution until low background fluorescence was observed.

(v) Characterization of the loading of the dyes in the pores of the respective MP-SiO_2 NPs.

*The loading of nucleic acids (**1**) on the MP-SiO_2 NPs was determined as follows:* the nucleic acid at a known concentration was reacted with the functionalized NPs, and the resulting particles

were precipitated by centrifugation at 6000 rpm for 3 minutes. The concentration of the unreacted nucleic acid in the solution was evaluated by absorbance spectroscopy. By the subtraction of the content of unreacted nucleic acid from the content of nucleic acid added to the reaction media, the loading of the nucleic acids (**1**) on the SiO_2 NPs was estimated to be 1.8 μmol/g silica NPs.

The dye content in the different solutions was determined as follows: the contents of the dye in the washing solution were determined, and these correspond to the dye that is physically adsorbed non-pore domains on the NPs. Knowing the content of the dye present in the solution, after the primary NPs precipitation process, and knowing the amounts of the dye eliminated by the washing procedure from the different systems the total content of residual non-bound dye in the different systems was evaluated. As the initial content of the dye added for the loading solutions of the NPs is known, the difference between the two values corresponds to the loading of the MP-SiO_2 NPs.