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

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The pH-controlled release of substrates from mesoporous SiO_2 nanoparticles, MP-SiO_2 NPs, is demonstrated by capping the pores with the Mg^{2+}- or UO_2^{2+}-dependent DNAzyme sequences and unlocking of the pores with Mg^{2+} ions or UO_2^{2+} ions at appropriate pH values. While the Mg^{2+}-dependent DNAzyme reveals high activity at pH=7.2, moderate activity at pH=6.0, and it lacks activity at pH=5.2, the UO_2^{2+}-dependent DNAzyme reveals high activity at pH=5.2, moderate activity at pH=6.0 and it is catalytically inactive at pH=7.2. Accordingly, the MP-SiO_2 NPs were loaded with methylene blue, MB^+, or thionine, Th^+, and locked in the pores by the Mg^{2+}- and UO_2^{2+}-dependent DNAzyme sequences, respectively. The pH programmed release of MB^+ or Th^+ from the loaded NPs proceeds, in the presence of Mg^{2+} ions or UO_2^{2+} ions, at pH=7.2 and pH=5.2, using the Mg^{2+}- and UO_2^{2+}-dependent DNAzyme as catalysts that cleave the protecting caps and unlock the pores. At pH=6.0 the MB^+ and Th^+-loaded NPs are concomitantly unlocked by the two DNAzymes. The unlocking processes are selective and other metal ions do not stimulate the release processes.

Introduction

The entrapment of substrates in the pores of mesoporous SiO_2 nanoparticles, MP-SiO_2 NPs, by stimuli-responsive caps, attracts growing interest since such NPs may be used for drug delivery,[1,2] controlled release,[3,4] sensing[5,6] and switchable catalysis.[7,8] Different triggers have been implemented to unlock the pore-gates of the NPs and to release the pore-entrapped materials. These included the application of photonic signals,[9,10] redox reagents,[11,12] pH,[13,14] and enzymes.[15,16] The information encoded in the base sequence of nucleic acids provides versatile means to program stimuli-responsive caps for trapping substrates in MP-SiO_2 NPs. For example, the formation of duplex nucleic acids, DNA hairpin structures,[17] metal-ion-assisted duplex nucleic acid structures,[18] and the formation of aptamer-substrate complexes[19,20] were used to block substrates in the pores of MP-SiO_2 NPs. Furthermore, the stimuli-triggered transitions between DNA structures, e.g., pH-stimulated i-motif/random-coil transitions were used to control interfacial adhesion processes[21] and to block and unlock the pores.[22,23] Also, catalytic nucleic acids (DNAzymes)[24] or enzymes[17] manipulating DNA were applied as catalytic labels for unlocking the pores. Here we wish to report on the pH-programmed unlocking of MP-SiO_2 NPs pores by means of different metal ion-dependent DNAzymes. We demonstrate the controlled opening of the pores at three different pH values.

Metal-ion-dependent catalytic nucleic acids (DNAzymes) reveal pH-controlled functions. For example, the Mg^{2+}-dependent DNAzyme exhibits optimal catalytic activity at pH=7.2 and it lacks catalytic functions at pH=5.2.[25] In contrast, the UO_2^{2+}-dependent DNAzyme reveals maximum catalytic activity at pH=5.2, while at pH=7.2 the DNAzyme is inactive.[26] At pH=6.0 the Mg^{2+}- as well as the UO_2^{2+}-dependent DNAzymes show ca. 50% residual activity as compared to their optimal activities at the respective pH values. These properties of the Mg^{2+}- and UO_2^{2+}-dependent DNAzymes were previously applied to construct field programmable logic gates and logic gate cascades.[27]

Results and discussion

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† Electronic Supplementary Information (ESI) available: SEM image of solid MP-SiO_2 NPs, N_2 adsorption-desorption isotherms, 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. See DOI: 10.1039/b000000x/

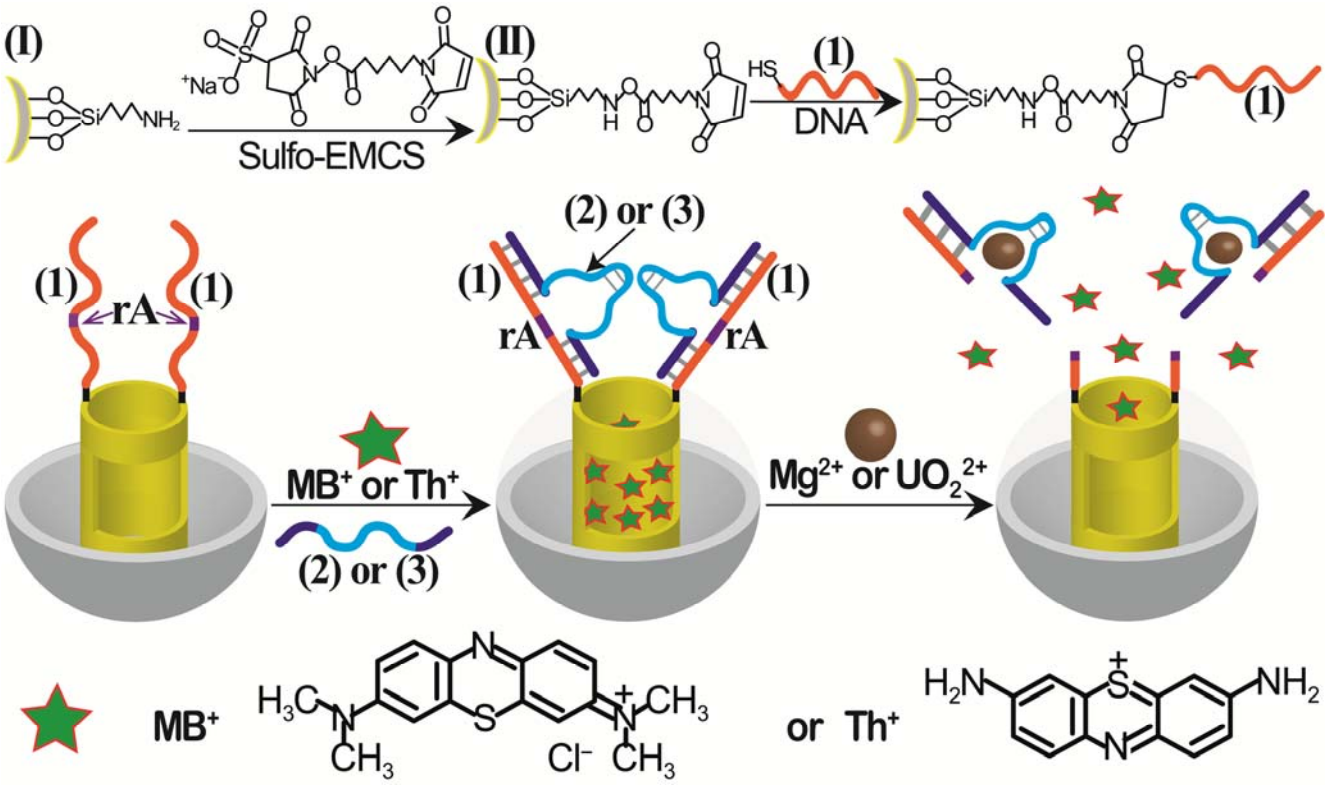

Figure 1. Synthesis of the methylene blue (MB^+)- or thionine (Th^+)-loaded mesoporous SiO_2 nanoparticles, MP-SiO_2 NPs, using the Mg^{2+}-DNAzyme or the UO_2^{2+}-DNAzyme sequences hybridized with **(1)** as pore gates. The Mg^{2+} ions or UO_2^{2+} ions trigger the cleavage of the gates, thus stimulating the release of MB^+ or Th^+.

Figure 1 outlines the preparation of the pH-controlled Mg^{2+}- or UO_2^{2+}-dependent DNAzyme capped MP-SiO_2 NPs. The NPs (390±10 nm diameter) were prepared according to the literature.[21] The pore size was estimated to be 2.8 nm, the surface area of the MP-SiO_2 NPs corresponded to 1057 m^2/g (for the characterization of the MP-SiO_2 NPs see **Figures S1** and **S2**, supporting information). The NPs were functionalized with aminopropyl siloxane **(I)**, and the surface amino-functionalities were reacted with Sulfo-EMCS to yield surface-active maleimide functionality groups **(II)**. The thiolated ribonucleobase-containing nucleic acids (**1**), being the substrates of the Mg^{2+}- and UO_2^{2+}-dependent DNAzymes, were then linked to the maleimide functions. The **(1)**-modified MP-SiO_2 NPs were loaded with methylene blue, MB^+, as fluorescence label, and the pores were capped by hybridization with the Mg^{2+}-dependent DNAzyme sequence (**2**). The resulting (**1**)/(**2**)-capped MP-SiO_2 NPs loaded with MB^+ were extensively washed to remove any MB^+ bound to the surface domains outside the pores. The loading of the pores by MB^+ was evaluated spectroscopically to be ca. 17.6 μmole/g of SiO_2 NPs (for details see experimental section). The free amino functionalities associated with the surface were then acetylated to eliminate any electrostatic or non-specific interaction of the fluorophore released, in the next steps, from the pores (**Figure S3**, supporting information). Using a similar procedure, the (**1**)-modified MP-SiO_2 NPs were loaded with thionine, Th^+, as fluorescence label. The pores were subsequently capped by the hybridization of the UO_2^{2+}-dependent DNAzyme sequence (**3**) with **(1)**, to yield the **(1)**/(**3**)-capped pores loaded with Th^+. The loading of the pores with Th^+ was determined spectroscopically to be ca. 21.5 μmole/g of SiO_2 NPs.

In the presence of Mg^{2+}- or UO_2^{2+} ions, and at the appropriate pH values, the respective active DNAzyme structures are anticipated to be formed, leading to the cleavage of the respective (**1**)/(**2**) or (**1**)/(**3**) caps, and the release of MB^+ or Th^+ from the pores. **Figure 2(A)** depicts the fluorescence spectra of MB^+ released from the (**1**)/(**2**)-capped MP-SiO_2 NPs upon subjecting the NPs to different concentrations of Mg^{2+}, at pH=7.2, for a fixed time-interval of 60 minutes. As the concentration of Mg^{2+} ions increases the release of MB^+ is improved, consistent with the higher degree of cleavage of the (**1**)/(**2**) caps. **Figure 2(B)** depicts the time-dependent release of MB^+ from the (**1**)/(**2**)-capped MP-SiO_2 NPs in the Mg^{2+} ions, 20 mM, pH=7.2, **curve (a)**, and in the absence of Mg^{2+}, at pH=7.2, **curve (b)**. The release of MB^+ proceeds for ca. 60 minutes, and then the released MB^+ levels off to a saturation value. From the saturated fluorescence intensity of MB^+, and using an appropriate calibration curve we estimate that ca. 3.1 μmole/g of MB^+ are released from the SiO_2 NPs. This value implies that ca. 18 % of the loaded MB^+ was released from the pores. The content of MB^+ released by unlocking the pores with the Mg^{2+}-dependent DNAzyme is ca. four-fold higher than the MB^+ released from the pores in the absence of Mg^{2+}, **Figure 2(B)**, **curve (b)**. It should be noted that the release of MB^+ from the pores in the absence of Mg^{2+} ions reaches a saturation value after ca. 60 minutes. The release of MB^+ from the MP-SiO_2 NPs

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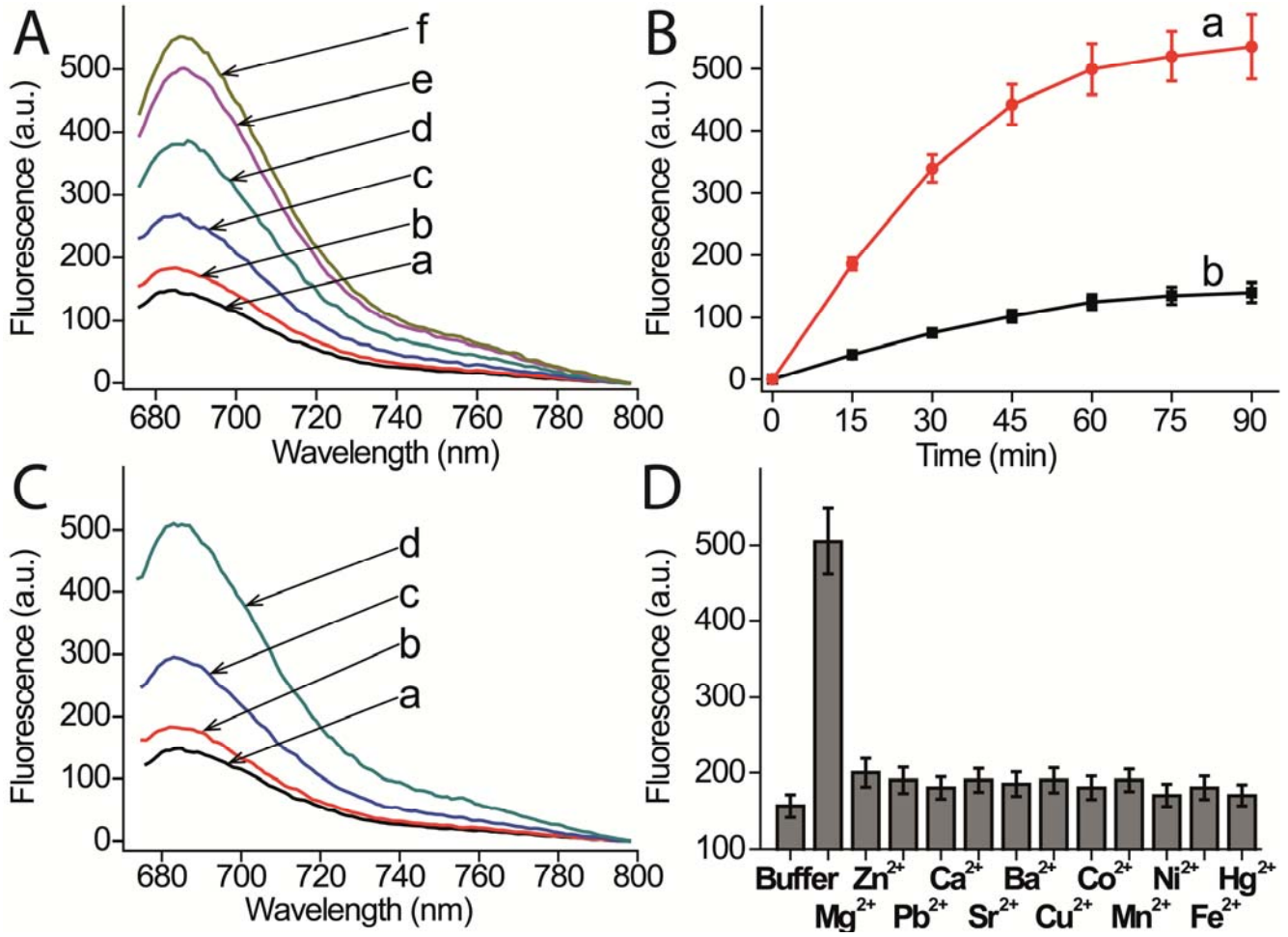

Figure 2. (A) Fluorescence spectra corresponding to the release of MB^+ upon subjecting the **(1)**/**(2)**-capped MP-SiO_2 NPs (10 mg) to different concentrations of Mg^{2+} ions: (a) 0, (b) 1 mM, (c) 5 mM, (d) 10 mM, (e) 20 mM, (f) 50 mM, for a fixed time interval of 60 min. (B) Time-dependent fluorescence changes upon releasing MB^+ from the MB^+-loaded MP-SiO_2 NPs, 10 mg: (a) Upon treatment of the MP-SiO_2 NPs with Mg^{2+} ions, 20 mM. (b) In the absence of added Mg^{2+} ions. (C) Fluorescence spectra corresponding to the release of MB^+ upon subjecting the MP-SiO_2 NPs (10 mg) to 20 mM of Mg^{2+} ions for a fixed time interval of 60 min at different pH values: (a) background, fluorescence spectra corresponding to the release of MB^+ in the absence of added Mg^{2+}, at pH=7.2. (b) At pH=5.2. (c) At pH=6.0. (d) At pH=7.2. (D) Fluorescence spectra corresponding to the release of MB^+ from 10 mg of MP-SiO_2 NPs, treated with a non-metal ions containing buffer or 20 mM of different metal ions, for a fixed time interval of 60 min.

in the absence of Mg^{2+} ions is attributed to the leakage of MB^+ from imperfectly-capped pores (either large pores or insufficiently blocked pores by the **(1)**/(**2**) caps). **Figure 2(C)** depicts the fluorescence spectra corresponding to the released MB^+ upon subjecting the **(1)/(2)**-capped MB^+-loaded NPs to Mg^{2+} ions, 20 mM, for a fixed time-interval of 60 minutes at different pH values. At pH=7.2 a high fluorescence spectrum is observed, indicating the effective Mg^{2+}-ion-stimulated biocatalytic unlocking of the pores. At pH=6.0, the fluorescence of MB^+ is lower, indicating less-efficient release from the pores. This is consistent with the lower activity of the Mg^{2+}-dependent DNAzyme at pH=6.0, that turns the cleavage of the (**1**)/(**2**) caps, and the subsequent release of MB^+, to be less efficient. At pH=5.2, the Mg^{2+}-dependent DNAzyme-stimulated release of MB^+ is almost fully blocked and the release of MB^+ is similar to the background leakage of MB^+ in the absence of Mg^{2+} ions (Figure 2(C) curves (a) vs. (b)). It should be noted that the fluorescence of MB^+ is unaffected within the pH range 5.2–7.2. **Figure 2(D)** depicts the selectivity of the DNAzyme-mediated release of MB^+ by Mg^{2+} ions at pH=7.2. While in the presence of Mg^{2+} effective release of MB^+ occurs, only the background leakage of MB^+ is observed with all other metal ions. All of these

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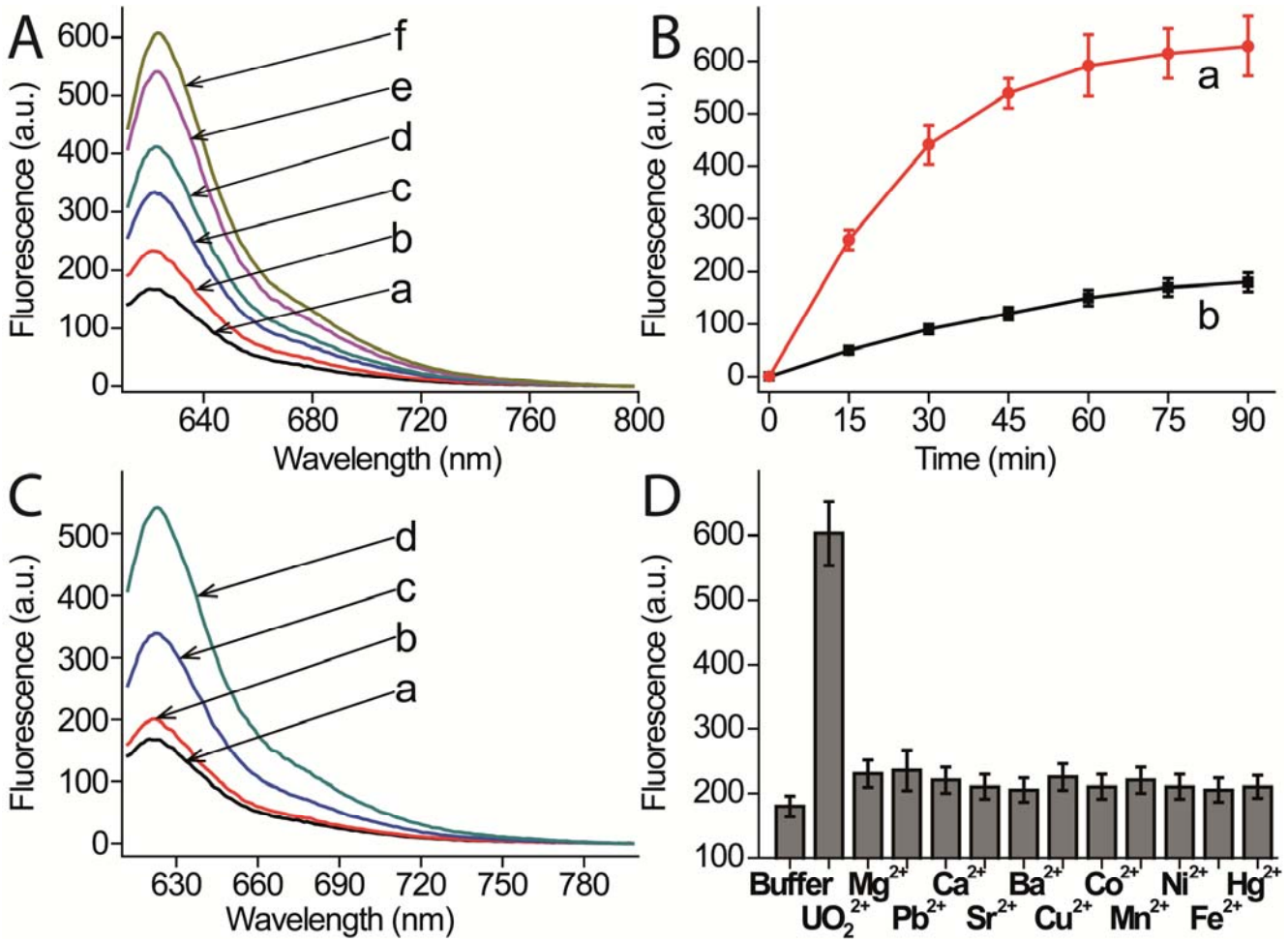

Figure 3. (A) Fluorescence spectra corresponding to the release of Th^+ upon subjecting the **(1)**/**(3)**-capped MP-SiO_2 NPs (10 mg) to different concentrations of UO_2^{2+} ions: (a) 0, (b) 0.1 μM, (c) 0.5 μM, (d) 1 μM, (e) 5 μM, (f) 10 μM, for a fixed time interval of 60 min. (B) Time-dependent fluorescence changes upon releasing Th^+ from the Th^+-loaded MP-SiO_2 NPs, 10 mg: (a) Upon treatment of the MP-SiO_2 NPs with UO_2^{2+} ions, 5 μM. (b) In the absence of added UO_2^{2+} ions. (C) Fluorescence spectra corresponding to the release of Th^+ upon subjecting the MP-SiO_2 NPs (10 mg) to 5 μM of UO_2^{2+} ions for a fixed time interval of 60 min at different pH values: (a) background, fluorescence spectrum in the absence of added UO_2^{2+} ions, pH=5.2. (b) At pH=7.2. (c) At pH=6.0. (d) At pH=5.2. (D) Fluorescence spectra corresponding to the release of Th^+ from 10 mg of MP-SiO_2 NPs, treated with the non-metal ions containing buffer solution (pH=5.2) or 5 μM of different metal ions, for a fixed time interval of 60 min.

results demonstrate that the Mg^{2+}-dependent DNAzyme selectively unlocks the pores through the cleavage of the (**1**)/(**2**) caps. The cleavage reaction is efficient at pH=7.2, moderately efficienct at pH=6.0, and fully blocked at pH=5.2.

The reverse pH-stimulated release of thionine, Th^+, substrate is observed with the (**1**)/(**3**)-capped MP-SiO_2 NPs, in the presence of the UO_2^{2+}-dependent DNAzyme. **Figure 3(A)** shows the fluorescence spectra of Th^+ released from the (**1**)/(**3**)-capped MP-SiO_2 NPs after a fixed time-interval of 60 minutes at pH=5.2, in the presence of different concentrations of UO_2^{2+} ions. As the concentration of UO_2^{2+} ions is elevated, the release of Th^+ is more efficient, consistent with the enhanced hydrolytic cleavage of the substrate units **(1)** that leads to unlocking of the pores by the DNAzyme. **Figure 3(B)**, **curve (a)** presents the time-dependent release of Th^+, at pH=5.2, using a constant concentration of UO_2^{2+}, 5 μM. For comparison, the time-dependent leakage of Th^+ from the NPs in the absence of added UO_2^{2+} is depicted in **Figure 3(B)**, **curve (b)**. From the saturated fluorescence intensity of the released Th^+, in the presence of UO_2^{2+}, and using an appropriate calibration curve we estimate that ca. 4.7 μmole/g of Th^+ are released from the SiO_2 NPs. This value implies that ca. 22 % of the loaded Th^+ was released from

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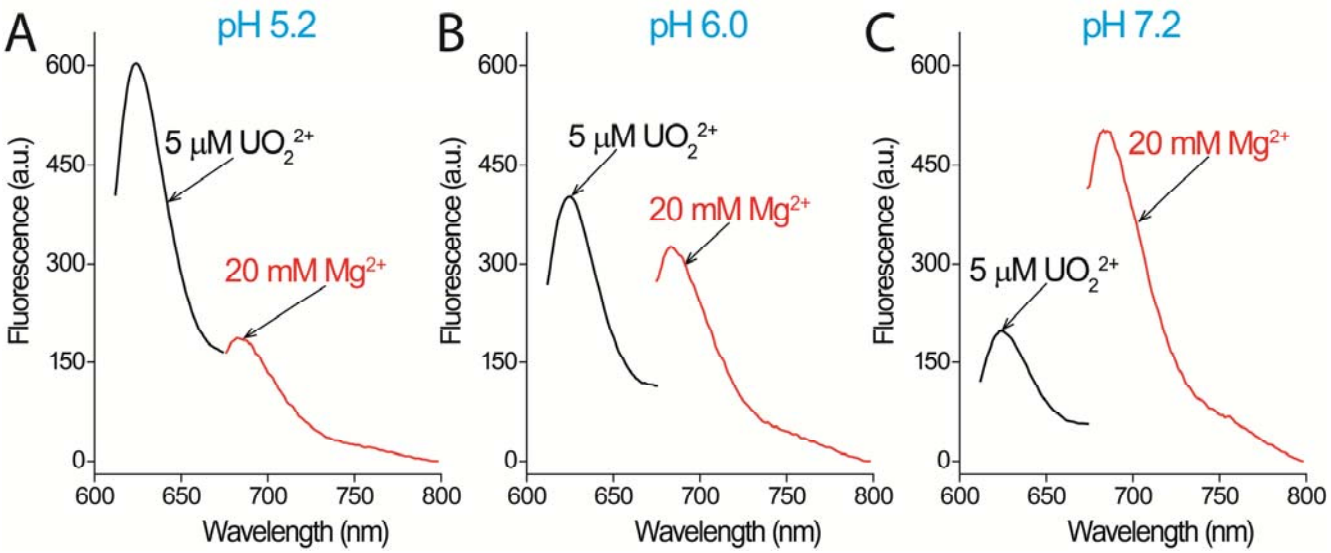

Figure 4. Dual fluorescence outputs of MB^+ and Th^+ in the presence of 20 mM of Mg^{2+} and 5 μM of UO_2^{2+}, and applying different pH values as inputs: (A) at pH=5.2; (B) at pH=6.0; and (C) at pH=7.2. The ratio of the MB^+- and Th^+-loaded mesoporous SiO_2 NPs is 1:1.

the pores. The release of Th^+ is ca. four-fold enhanced in the presence of UO_2^{2+} as compared to the non-catalytic leakage. **Figure 3(C)** depicts the effect of pH on the UO_2^{2+}-stimulated release of Th^+ from the (**1**)/(**3**)-locked SiO_2 NPs. The release of Th^+ is effective at pH=5.2, shows moderate efficienct at pH=6.0, and is totally blocked at pH=7.2 (similar to the background leakage). **Figure 3(D)** demonstrates the selective unlocking of the pores in the presence of UO_2^{2+} at pH=5.2. While UO_2^{2+} ions unlock effectively the pores, all other metal ions do not affect the release of Th^+. All of these results indicate that the UO_2^{2+}-dependent DNAzyme units selectively unlock the pores of the NPs. In contrast to the Mg^{2+}-dependent DNAzyme that reveals optimal activity in unlocking the pores at pH=7.2 (where the UO_2^{2+}-dependent DNAzyme is inactive), the UO_2^{2+}-dependent DNAzyme shows optimal unlocking of the pores at pH=5.2 (where the Mg^{2+}-dependent DNAzyme is inactive). At pH=6.0 both metal ions-dependent DNAzymes reveal partial activity, thus, allowing the concomitant unlocking of the two types of MP-SiO_2 NPs. **Figure 4** depicts the programmed pH-stimulated release of the substrates, MB^+ and Th^+, from a mixture of MP-SiO_2 NPs gated by the Mg^{2+}-(**1**)/(**2**) and UO_2^{2+}-(**1**)/(**3**) DNAzymes. At pH=5.2, the UO_2^{2+}-DNAzyme modified NPs are predominately activated, leading to the release of Th^+ and to its characteristic fluorescence at λ_{em}=623 nm, **Figure 4A**. At pH=6.0, the two DNAzymes are activated, resulting in the concomitant release of MB^+ and Th^+ from the two types of MP-SiO_2 NPs, **Figure 4B**. At pH=7.2 the activity of the UO_2^{2+}-dependent DNAzyme is blocked, and the Mg^{2+}-dependent DNAzyme is triggered-on with optimal activity, resulting in the release of MB^+, λ_{em}=690 nm, **Figure 4C**.

Conclusions

In conclusion, the present study has introduced the pH-programmed unlocking of substrate-loaded MP-SiO_2 NPs capped with metal-ion-dependent DNAzyme sequences. By loading the pores with different fluorophores, the controlled pH-regulated and switchable opening of the pores was demonstrated. The systems revealed high selectivities and the loaded pores were unlocked only in the presence of the ion-specific DNAzymes, operating at the appropriate pH conditions. As many different metal-ion-dependent DNAzymes are available, different applications of pH-programmed metal-ion-dependent DNAzymes may be envisaged. These include the multiplexed sensing of metal ions (using the released substrate as fluorescence labels), the programmed synthesis of pre-designed products by metal-ion-dictated release of reactants from the appropriate NPs containers, and the pH-controlled release of substrates. In the specific study we demonstrate that the mesoporous silica nanoparticles may be unlocked by the concentrations of Mg^{2+} ions that are available in cellular environments. Furthermore, the activity of the UO_2^{2+}-ion-dependent DNAzyme at acidic conditions may be implemented to specifically unlock the pores of MP-SiO_2 NPs in acid media present in cancer cells.

Experimental section

Reagents and Materials: Tetraethyl orthosilicate (TEOS), (3-aminopropyl)-triethoxysilane (APTES) and N-Hydroxysulfosuccinimide sodium salt (NHS) were purchased from Aldrich. Hexadecyltrimethylammonium bromide (CTAB), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES), methylene blue (MB^+), thionine (Th^+) and N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma. Uranyl acetate (UO_2^{2+} ions) was purchased from May & Baker Ltd. (Dagenham, England) and was dissolved in a 10 mM sodium citrate to yield a 10 mM stock solution. N-(ε-Maleimidocaproyloxy)sulfosuccinimide ester (Sulfo-EMCS) was purchased from Pierce Biotechnologies. All chemicals were of analytical grade and were used as received, without any further purification. Ultrapure water from a NANOpure Diamond (Barnstead Int., Dubuque, IA) source was used throughout the experiments. All DNA sequences were purchased from Integrated DNA Technologies. (Coralville, IA).

The sequences of the nucleic acids are as follows:

(1) 5'-SH$(CH_2)_6$CAGTGAATTrAGGACATAGAAGAAGAA G-3'

(2) 5'-CTTCTTCTTCTATGTCAGCGATTCCGGAACGGAC ACCCATGTATTCACTG-3'

(3) 5'-CTTCTTCTTCTATGTCAGCCGGAACGGCCTTGCA ATTCACTG-3'

Instruments: Fluorescence emission measurements were recorded using a Cary Eclipse instrument (Varian, Inc.). Methylene blue (MB^+) and thionine (Th^+) were excited at 664 nm and 599 nm, respectively. UV-vis absorption spectra were recorded with a Shimadzu UV-2401 spectrophotometer. Scanning electron microscopy (SEM) images were recorded on a Tecnai F20 G2 (FEI Co.) using an accelerating voltage of 200 kV. Surface areas and pore size of the MP-SiO_2 NPs were determined using a Nova 1200e Brunauer-Emmett-Teller (BET) meter (Quantachrome Instruments). The surface area was calculated using multi-point BET method. And the pore size was calculated using BJH pore size distribution method.

Synthesis of Mesoporous Silica Nanoparticles (MP-SiO_2 NPs): Amino-functionalized MP-SiO_2 NPs were synthesized according to a previously reported procedure.[22] The collected SiO_2 NPs were washed with large volume of distilled water and ethanol by repeated precipitation using a centrifuge at 6000 rpm for 3 minutes and re-suspenstion of the NPs. To remove the template CTAB, the SiO_2 NPs were refluxed for 16 hours in a solution composed of HCl (37%, 1 ml) and ethanol (80 ml). The resulting NPs were extensively washed with distilled water and ethanol, respectively. Finally, to remove the remaining solvent from the pores, the amino-functionalized, CTAB-free MP-SiO_2 NPs were dried under vacuum at 75℃ for 12 hours.

Loading the Dye and Capping the Pores: 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™ G-25 Column). 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. 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.

Subsequently, 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). The dye content in the different solutions was determined. Subsequently, the particles were washed at least six times with a HEPES buffer solution until low background fluorescence was observed. 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. Using this procedure, the loading of MB^+ for the Mg^{2+}-DNAzyme and Th^+ for the UO_2^{2+}-DNAzyme in the MP-SiO_2 NPs were determined to be ca. 17.6 µmole/g of SiO_2 NPs and ca. 21.5 µmole/g of SiO_2 NPs, respectively.

Release of the dye: Release of the dye from the different MP-SiO_2 NPs. The release of the dye from the MP-SiO_2 NPs capped with the Mg^{2+}-**(1)**/**(2)** DNAzyme, or capped with the UO_2^{2+}-**(1)**/**(3)** DNAzyme were analyzed as follows: The respective MB^+-loaded or Th^+-loaded MP-SiO_2 NPs, 10 mg, were introduced into 900 µl of HEPES buffer solution (10 mM, pH=7.0), and the mixture was

divided into five samples, 180 µl each sample. Subsequently, 20 µl of aqueous solution of different concentrations of Mg^{2+} ions or UO_2^{2+} ions were added to the samples that were allowed to react for a fixed time-interval of 60 minutes. The resulting mixtures were centrifuged and the NPs were separated. The fluorescence spectra of the supernatant solutions were then recorded. For the time-dependent release of the dye from the respective systems, a similar procedure was applied while subjecting the NPs, for different time-intervals, in the presence of a fixed concentration of Mg^{2+} ions or UO_2^{2+} ions. For the release of the dye from the Mg^{2+}-DNAzyme or UO_2^{2+}-DNAzyme capped MP-SiO_2 NPs triggered by different pH values, the dye-loaded NPs were introduced into 450 µl of water, and the mixture was divided into five samples, 90 µl each. Then 90 µl of 50 mM $NaNO_3$ in a 50 mM MES buffer with different pH values (pH=5.2, pH=6.0, pH=7.2), and 20 µl of Mg^{2+} ions or UO_2^{2+} ions aqueous solution were added to the mixtures, and the total volume of each sample was completed to 200 µl. The samples were allowed to react for a fixed time-interval of 60 minutes. Subsequently, the NPs were precipitated (centrifugation at 6000 rpm for 3 minutes) and the fluorescence spectra were recorded.

Acknowledgements

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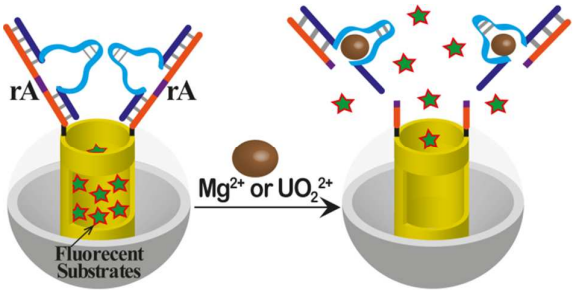

pH-programmed release of fluorescent substrates from mesoporous silica nanoparticles is stimulated by Mg^{2+}- or UO_2^{2+}-dependent DNAzymes unlocking the pores.