Towards Intelligent Bioreactor Systems: Triggering the Release and Mixing of Biomolecules Based on DNA-Functionalized Hydrogels

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Experimental Section

Materials and Measurements: Nanopure water (18.2 MΩ; Millpore Co., USA) was used in all experiments and to prepare all buffers. Tetraethylorthosilicate (TEOS), sodium hydroxide, tris(hydroxymethyl)aminomethane (Tris), (3-aminopropyl)trimethoxysilane (APTES), 4-morpholineethanesulfonic acid (MES), β-D-galactosidase (β-gal) and 5-Bromo-4-chloro-3-indolyl (X-gal) were purchased from Sigma-Aldrich. N-cetyltrimethylammonium bromide (CTAB), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), trisodium citrate dihydrate, fluorescein isothiocyanate (FITC) and succinic anhydride were obtained from Alfa Aesar. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was purchased Sangon Biotechnology Inc. (Shanghai, China). N-hydroxysulfosucnimide sodium salt (sulfo-NHS) was obtainedfrom Pierce Biotechnology. All the chemicals were used as received without further purification. The oligonucleotide used in this paper was synthesized by Sangon Biotechnology Inc. The sequence is as follows:

DNA1: 5'-NH_2-$(CH_2)_6$-AACCCCAAAACCCCAAATCTCTTGGACC-3'

DNA2: 5'-Acrydite-$(CH_2)_6$-<u>TGGAAGGAGGCGTTATGAGGGGGTCCA</u>AGAGAT-3' (aptamer with underline)

DNA3: 5'-NH_2-$(CH_2)_6$-ACACACACACCACCAAATCTCTTGGACC-3'

DNA4: 5'-NH_2-$(CH_2)_6$-AACCCCAAAACCCCAAACTCTACCTTGG-3'

FT-IR analyses were carried out on a Bruker Vertex 70 FT-IR Spectrometer. X-ray measurements were performed on a Bruker D8 FOCUS Powder X-ray Diffractometer using Cu Kα radiation. Thermogravimetric analyses were carried out on a PerkinElmer Pyris Diamond TG / DTA Analyzer, using an oxidant atmosphere (Air) with a heating program consisting of a dynamic segment (10 °C / min) from 373 to 1173 K. SEM images were obtained with a Hitachi S-4800 FE-SEM. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The samples were degassed at 150 °C for 5 h. The specific surface areas were calculated from the adsorption data in the low pressure range using the BET model and pore size was determined following the BJH method. Solid-state ^{13}C CP-MAS NMR spectra were obtained on Bruker AVANCE III 400 WB spectrometer equipped with a 4 mm standard bore CPMAS probe whose X channel was tuned to 100.62 MHz for 13C and the other channel was tuned to 400.18 MHz for broad band 1H decoupling, using a magnetic field of 9.39T at 297 K. UV-vis spectroscopy was carried out with a JASCO V-550 UV / vis spectrometer.

Synthesis and chemical modification of the MSP surface: N-cetyltrimethylammonium bromide (CTAB, 0.80 g) was first dissolved in 384 mL of pure water. Sodium hydroxide (2.8 mL, 2 M) was added to CTAB solution, followed by adjusting the solution temperature to 80 °C. The mixture was stirred for 15 min and TEOS (3.88 mL) was added rapidly while stirring was continued. TEOS (60 μL) and APTES (60

μL) was introduced 30 minutes later. The mixture was allowed to stir for 2 h to give rise to white precipitates. The solid product was filtered, washed with deionized water and methanol, and dried in air. To remove the surfactant template (CTAB), the white powder was refluxed for 16 h in a solution of 1.00 mL of HCl (37%) and 80.00 mL of methanol followed by extensively washing with deionized water and methanol. The resulting surfactant-removed MCM-41 was placed under high vacuum to remove the remaining solvent in the mesopores. The resulting surfactant-removed amine-functionalized MSP (MSP-NH_2) was placed under high vacuum to remove the remaining solvent in the mesopores. The MSP-NH_2 (50 mg) was reacted with succinic anhydride (1.00 g) in N,N-dimetylformamide solution (20 mL) under N_2 gas for 8 h with continuous stirring. By doing so, carboxyl groups were formed onto the MSP surface for conjugation of DNA. After a thorough water wash, the carboxylated nanoparticles (MSP-COOH) were activated using EDC (10 mg / mL, 15 mL) and sulfo-NHS (10 mg / mL, 15 mL) in a MES buffer (pH 6.0) for 15 min at room temperature with continuous stirring. 20 mL of PBS buffer (100 mM, pH 7.4) was then added in the mixture, followed by the addition of DNA1 or DNA3 (3 mL 132.1 μM) at room temperature with continuous stirring for 6 h and washing in PBS buffer (0.1 M, pH 7.4) to form the resultant DNA-conjugated nanoparticles (MSP-DNA). The unreacted DNA was purified with vivaspin ultrafiltration spin column (MW 2kDa).

As for fluorescein-labeled mesoporous silica nanoparticles, fluorescein isothiocyanate (FITC, 2 mg) was reacted with 44 μL of APTES in 1 mL ethanol

overnight in the dark. CTAB (0.40 g) was first dissolved in 192 mL of pure water. Sodium hydroxide (1.4 mL, 2 M) was added to CTAB solution, followed by adjusting the solution temperature to 80 °C. TEOS (2 mL) and APTES-modified dye solution (200 μL) added by dropwise while stirring was continued. The mixture was allowed to stir for 2 h to give rise to white precipitates. Finally, the surfactant template, CTAB, was removed by refluxing in acidic ethanol solution to give FITC-labeled MSP.

X-gal loading and proton-fueled MSP performance: The purified MSP-DNA1 was incubated in the phosphate-buffered saline (10 mM, 25 mL, pH 7.4) of X-gal (5 mg) for 24 h. The pH value of the suspension was adjusted to 5.5 by the addition of HCl. The solution was stirred for 16 h, followed by centrifuging and repeated washing with citrate buffer (25 mM pH 5.5) to remove physisorbed X-gal molecules from the exterior surface of the material. All the washing solutions were collected, and the loading of X-gal was calculated to be approximately 38.7 μmol/g SiO_2. MSP-DNA1 with X-gal (10 mg) material was dispersed in 25 mL of citrate buffer at a certain pH value (pH 5.5, 7.0 or 8.0) with the presence of β-gal. Aliquots were taken from the suspension and the delivery of X-gal from the pore to the buffer solution was monitored via the absorbance band of the enzymatic product centered at 401 nm.

Hydrogel preparation: MSP-DNA1 (1 mg/mL) was incubated with DNA2 (50 μM) in 50 mM MES buffer (100 mM NaCl, 50 mM $MgCl_2$, pH = 5.5) for 24 h. Then, the mixture was washed for several times with MES buffer to remove adsorbed molecules. The resulting MSP-DNA1/DNA2 was re-dispersed in MES buffer and stored at 4 ℃ until use.

In order to form the ATP responsive hydrogel, a mixture solution was first prepared. The solution contained: 50 mM MES buffer (pH 5.0), 50 mM $MgCl_2$, 100 mM NaCl, 5% acrylamide, 0.003% bis-acrylamide and 13 mg/mL MSP-DNA1/DNA2. Directly after mixing, nitrogen was bubbled through this solution for 10 min. To this stock solution was added 1.5% of a freshly prepared initiator-catalyst mixture consisting of 0.5 mL H_2O, 0.05 g ammonium persulfate, and 200 μL TEMED. After reaction at 25 °C for 30 minutes, the mixture became a hydrogel. To reduce the DNA concentration that need for the hydrogel, a small amount of bis-acrylamide (0.003%) was employed in the experiment. It was worth to note that the bis-acrylamide employed alone could not cross-link the gels due to its low concentration. This ATP responsive hydrogel was subsequently dissociated by immersion in a solution of 5 mM ATP. To investigate the distribution of MSP inside the sample, the hydogels were frozen at -20 °C for 1 h and lyophilized for SEM analysis.

For a bioreaction experiment, the hydrogel was prepared with addition of enzyme (β-D-galactosidase, final concentration, 4.0 units/mL) under identical conditions.

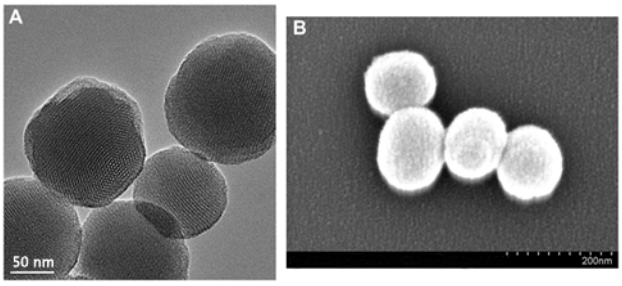

Fig. S1. TEM and SEM images of as-synthesized MCM-41.

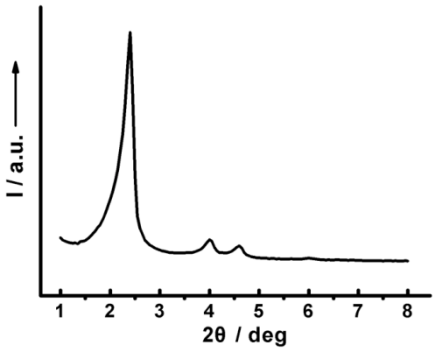

Fig. S2. X-ray diffraction pattern of as-synthesized MCM-41.

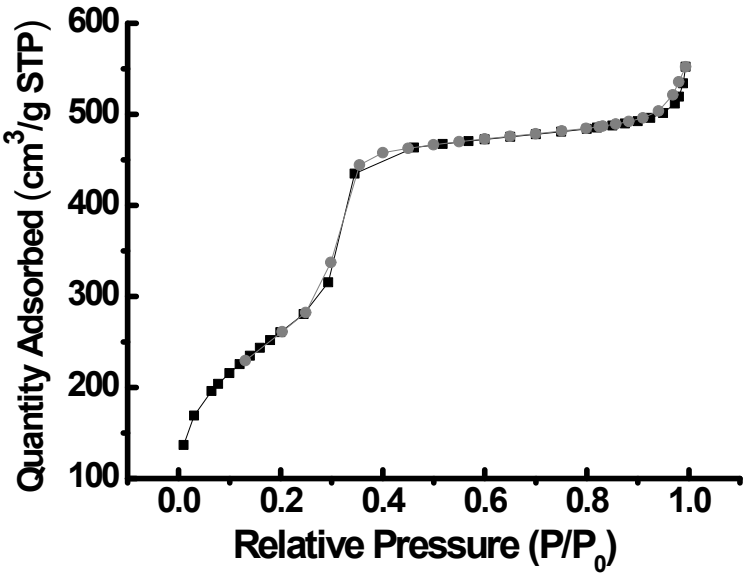

Fig. S3. Nitrogen sorption isotherms of as-synthesized MSP-NH_2.

Table S1. BET specific surface values, pore volumes, and pore sizes calculated from the N_2 adsorption-desporption isotherms of MSP-NH_2.

	S_{BET} [m^2g^{-1}]	Pore volume [cm^3g^{-1}]	Pore size [nm]
MSP-NH_2	995	0.79	3.2

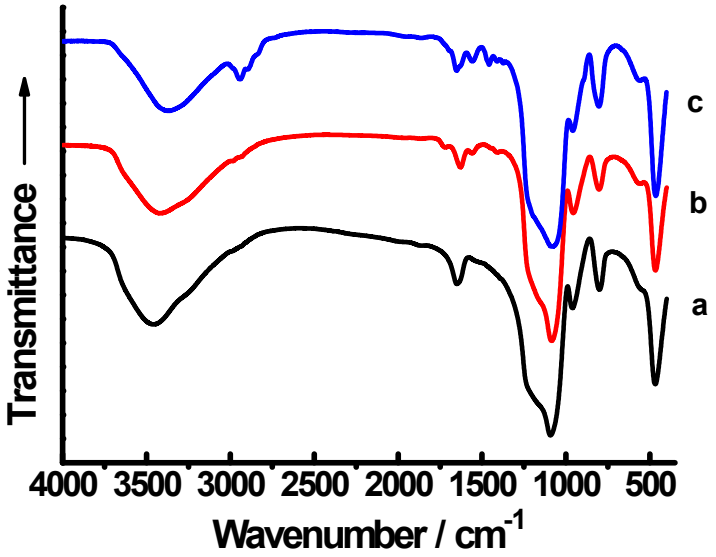

Fig. S4. FTIR spectra of the samples (a) MSP-NH_2, (b) MSP-COOH, and (c) MSP-DNA. The emerging absorption band at around 1700 cm^{-1} in the sample MSP-COOH can be ascribed to C=O stretching of the carboxyl groups contained within the attached succinic acid molecules. The efficient grafting of DNA onto MSPs was validated by the appearance of an enhanced band at 1562 cm^{-1}, which was characteristic of acylamide vibration.

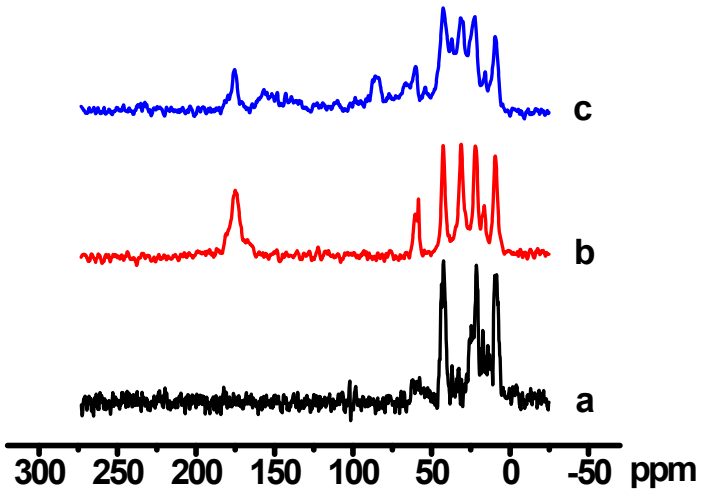

Fig. S5. ^{13}C NMR spectra of the samples (a) MSP-NH_2, (b) MSP-COOH, and (c) MSP-DNA. The MSP-NH_2 material exhibited three peaks at 8.7 ppm (C1), 23.9 ppm (C2) and 43.0 ppm (C3). The resonances at 173.0 ppm and 175.4 ppm were typical positions for C=O of the acylamide and carboxyl groups. The successfully anchoring of DNA was confirmed by the declined intensity of the resonance at 175.0 ppm and a band shift to 164.9 ppm for C=O of the acylamide.

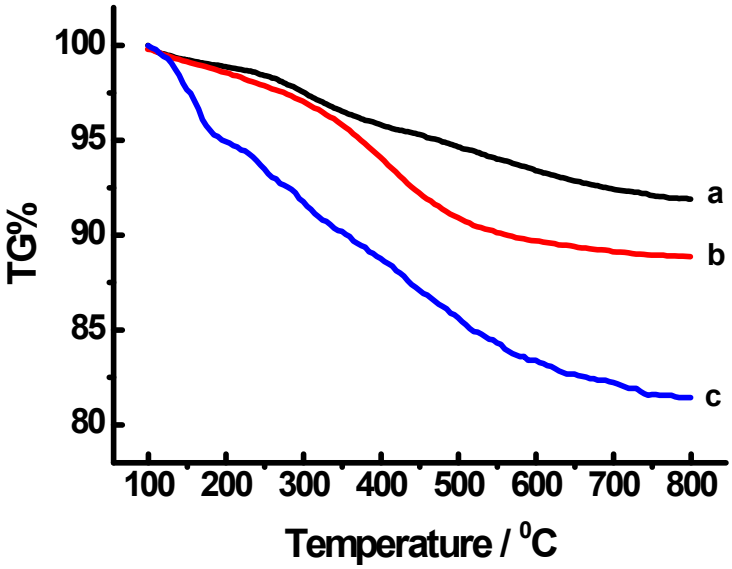

Fig. S6. Thermogravimetric analysis of the samples (a) MSP-NH_2, (b) MSP-COOH, and (c) MSP-DNA. Based on 7.43% more weight loss for MSP-DNA compared with MSP-COOH, the grafting density of DNA was calculated to be 8.72 μmol/g SiO_2.

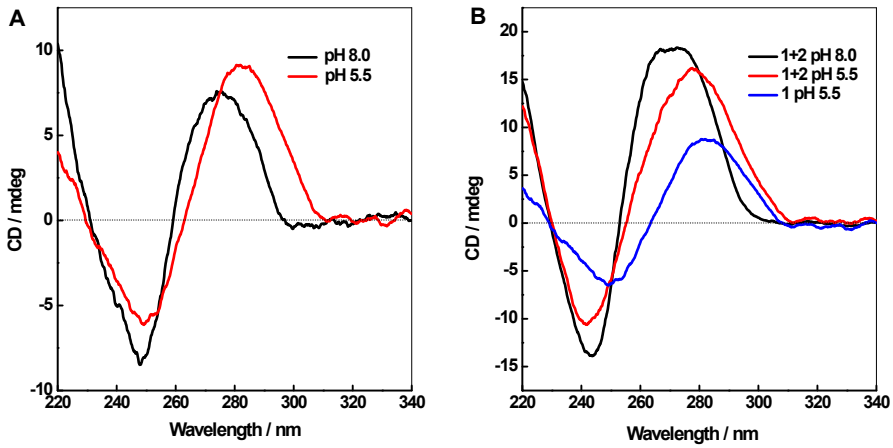

Fig. S7. (A) CD spectra of DNA1 (3 μM) in 50 mM Tris-HCl, 100 mM NaCl, pH 8.0 or 50 mM MES, 100 mM NaCl, pH 5.5. (B) DNA1/DNA2 duplex (3 μM) in 50 mM Tris-HCl, 100 mM NaCl, pH 8.0 or 50 mM MES, 100 mM NaCl, pH 5.5.

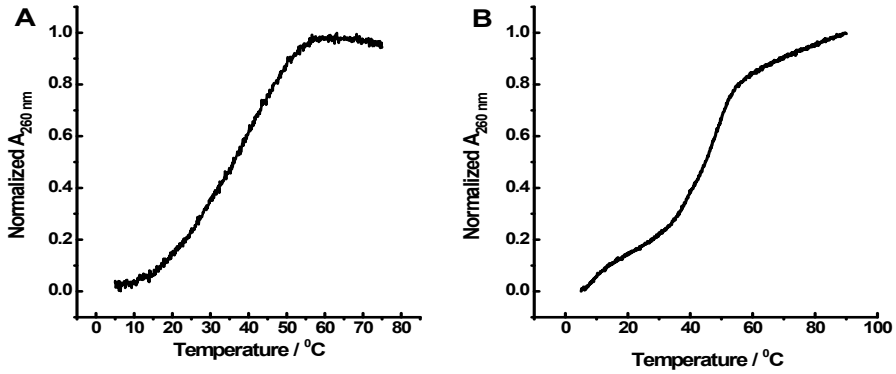

Fig. S8: The melting profile of DNA1 (A) and DNA1/DNA2 (B) with a self-complementary duplex structure after annealing in 50 mM MES-HCl, 100 mM NaCl,

pH 5.5. Absorbance changes at 260 nm versus temperature were collected at a heating rate of 1 °C·min^{-1}.

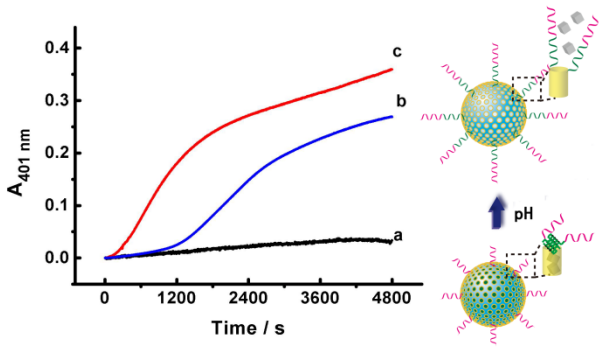

Fig. S9. Release profiles of X-gal from MSPs-DNA1 at pH 5.5 (a) pH 7.0 (b) and 8.0 (c).

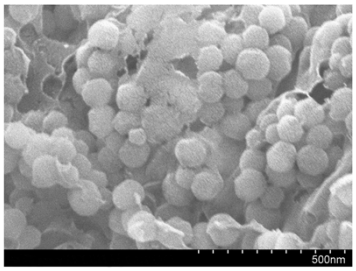

Fig. S10 SEM image of the prepared MSP-DNA hydrogel. The picture was taken from the broken edge of the lyophilized sample, which depicted the distribution of MSP inside the hydrogel.

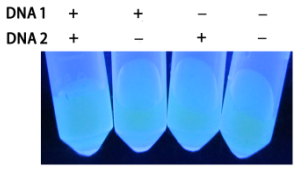

Fig. S11 Photograph of the sol transition switched by co-polymerization under UV illumination.

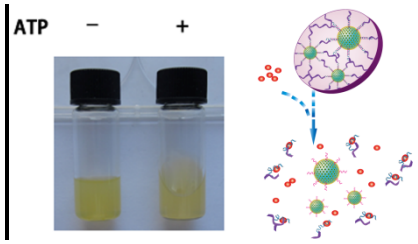

Fig. S12 Gel transition switched by adding of the target (ATP).

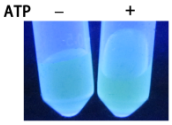

Fig. S13 The photograph of the gel transition switched by adding of the target (ATP) under UV illumination.

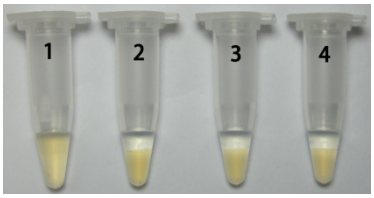

Fig. S14 Photograph of the gel transition by adding ATP (1), GTP (2), UTP (3) and CTP (4).

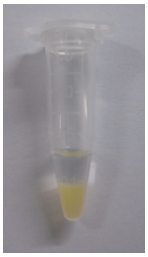

Fig. S15 Photograph of the on-demand enzymatic reactions carried out in the MSPs entrapped DNA-functionalized hydrogel at pH 8.0 but without ATP.

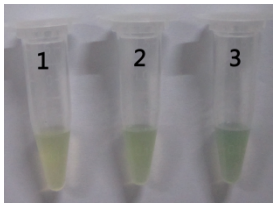

Fig. S16 Photograph of the enzymatic reactions carried out in the MSPs entrapped DNA-functionalized hydrogel at pH 6.0 (1), 7.0 (2) and 8.0 (3) with ATP.

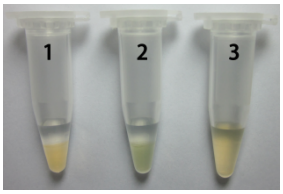

Fig. S17 Photograph of the gel with MSPs-DNA1/DNA2 (1), MSP-DNA3/DNA2 (2) and MSP-DNA4/DNA2 (3) through co-polymerization after loading the enzyme and the substrate.