An Enzymatic Amplifier Based on Mechanized Nanoparticles

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SUPPORTING INFORMATION

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1. Synthetic Procedures

Mesoporous Silica Nanoparticles (MSNP): The synthesis of mesoporous silica nanoparticles using Pluronic P104 is based on published procedure. [1] In a typical synthesis, 0.35 g of P104 was dissolved in 13.2 mL of HCl (1.6 M). The solution was stirred at 56 ^{o}C for an hour before 0.53 g of tetramethyl ortholsilicate (Sigma Aldrich, 99%) was added. The solution was stirred at 56 ^{o}C for another 24 hours and the resulting white precipitates were collected via centrifugation. The nanoparticles were washed with H_2O and methanol dried under vacuum.

MSNP-Aldehyde: 1 g of the as-synthesized MSNP was resuspended in 50 mL of toluene and degassed with Argon. 300 uL of 1-butylaldehydetriethoxysilane (Gelest, 90%) was added into the suspension drop wise. The suspension was then stirred at 80 ^{o}C under Argon for 10 hours and then cooled to room temperature. The resulting MSNP-Aldehyde was collected through centrifugation and washed with methanol. The P104 surfactant was then removed using a soxhlet extraction with methanol and 5 % HCl under Argon. The extracted MSNP-Aldehyde was then dried under vacuum. The amount of the aldehyde modification is around 4% wt. determined by TGA analysis.

MSNP-Cyclodextrin: 500 mg of extracted **MSNP-Aldehyde** was suspended in 50 mL of anhydrous dimethylformamide (DMF) and mixed with 500 mg of 6-(2-aminoethyl)amino-6-deoxy-β-cyclodextrin [2] and 500 mg of anhydrous $MgSO_4$. The mixture was heated at 60^{o}C for 24 hr under Argon. After cooling to room temperature, the resultant precipitates were collected by filtration and washed with H_2O. The product was dried under vacuum. The amount of attached cyclodextrin is around 3% wt. determined by TGA analysis.

MSNP-Cyclodextrin-Amine: The reduction of the imine bond was carried out following published procedure. [3] 350 mg of **MSNP-Cyclodextrin** was suspended in 20 mL of anhydrous DMF under Argon and 200 mg of $NaBH_4$ (Sigma Aldrich, 96%) was added portion wise. The mixture was stirred at room temperature for 5 hours and the product was collected through centrifugation, washed with DMF and H_2O, and dried under vacuum.

Loading of Porcine Liver Esterase: 100 mg of **MSNP-Cyclodextrin** or **MSNP-Cyclodextrin-Amine** was suspended in a mixture of pH 7.4 PBS buffer (6 mL) and dimethyl sulfoxide (DMSO, 2 mL) at 4 ^{o}C. This suspension was mixed with 200 uL of Porcine Liver Esterase suspension (Sigma Aldrich, in 3.2 M ammonium sulfate, > 150 units/mg protein, 5 kU/ mL). This mixture was stirred at 4 ^{o}C for two days.

Capping the Nanogate: The hexamer cap molecules were synthesized through a Cu-catalyzed azide-alkyne coupling reaction following an established procedure.[2] 30 mg of this capping molecule was dissolved in 1 mL of DMSO and added into the previous loading mixture. The suspension was further stirred at 4 ^{o}C for 5 hours and the resulting nanoparticles were collected through centrifugation. The excess amount of adsorbed esterase was removed by extensively washing the nanoparticles with a 3:1 mixture of pH 7.4 PBS buffer and Polyethylene glycol

(Sigma Aldrich, MW. 2000). For the **No-Capping control** samples, the loaded nanoparticles were directly centrifuged without adding any capping molecules and then washed extensively with PBS-Polyethylene glycol mixture. All nanoparticles were dried under vacuum after washing.

2. Operation of the Enzymatic Amplifier

General Procedures: The operation of the enzymatic amplifier was monitored using a continuous-monitoring fluorescence spectroscopic method. Nanoparticle samples were first carefully placed at the bottom of a cuvette. A substrate solution (ACA or CFDA) was added and the solution was gently stirred, without any particles floating in the solution. An excitation laser beam was directed into the solution and the solution fluorescence was constantly recorded by a CCD camera. The fluorescence intensity was plotted over the time course to reveal the process of the enzymatic reaction.

Studies Using ACA: 6 mL of ACA solution (0.2 mg/mL in 25 mM pH 8.0 TAPS buffer) and 6 mg of nanoparticle samples were employed in each experiment. A 351 nm laser (15 mW) was used as the probe beam and the corresponding fluorescence intensity between 430 nm and 460 nm was integrated and normalized among different samples for comparison.

Studies Using CFDA: 6 mL of CFDA solution (0.1 mg/mL in 3 mL H_2O and 3 mL DMSO) and 6 mg of nanoparticle samples were employed in each experiment. 200 uL of ACA solution (0.2 mg/mL in H_2O) was added into the solution to initiate the amplification. A 448 nm laser (5 mW) was used as the probe beam for the hydrolyzed CFDA molecules and the corresponding fluorescence intensity between 530 nm and550 nm was integrated to monitor the enzymatic process.

3. TEM Images of Nanoparticles

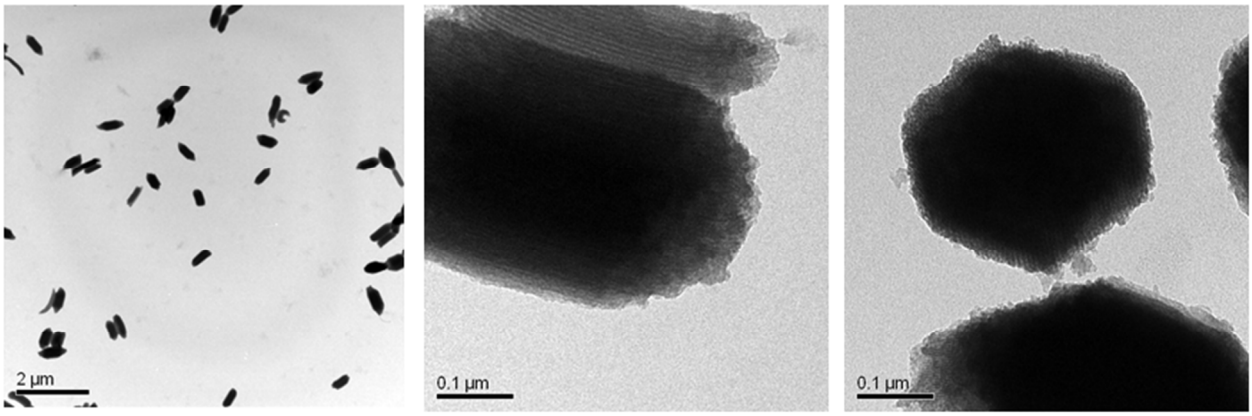

Figure S1. TEM images of the nanovalve-modified silica nanoparticle showing the overall size distribution around 300 nm by 500 nm (**Left**), the direction of the pore channels (**Middle**) and the pore openings (**Right**).

4. N_2 Absorption Isotherm and XRD of Nanoparticles

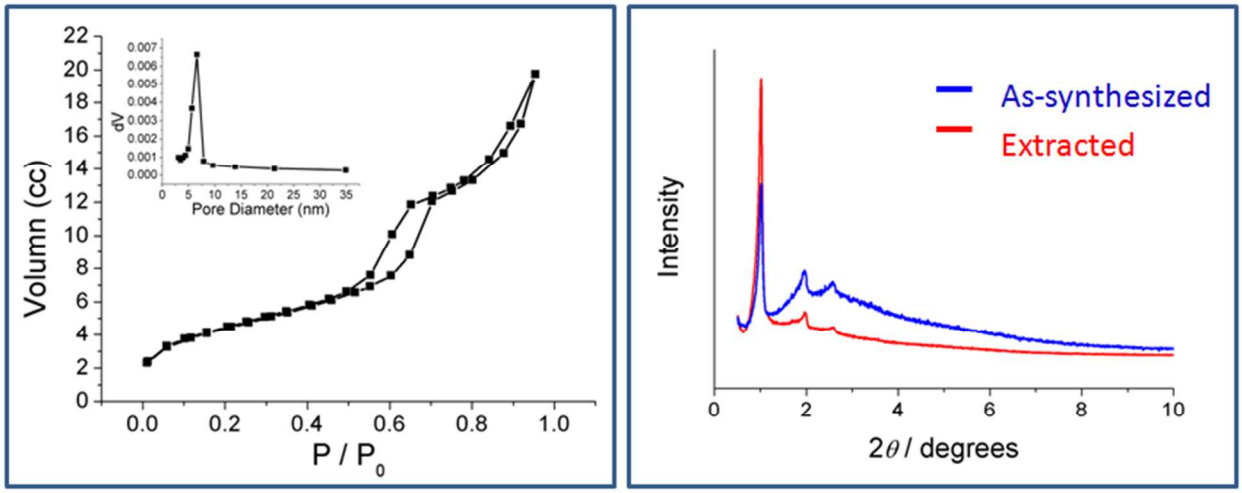

Figure S2. Left) N_2 Absorption isotherm and the pore size distribution of the extracted unmodified nanoparticles. **Right)** XRD patterns of the as-synthesized and extracted nanoparticles.

5. Fluorescence Spectrum of ACA and CFDA Before and After the Enzymatic Process

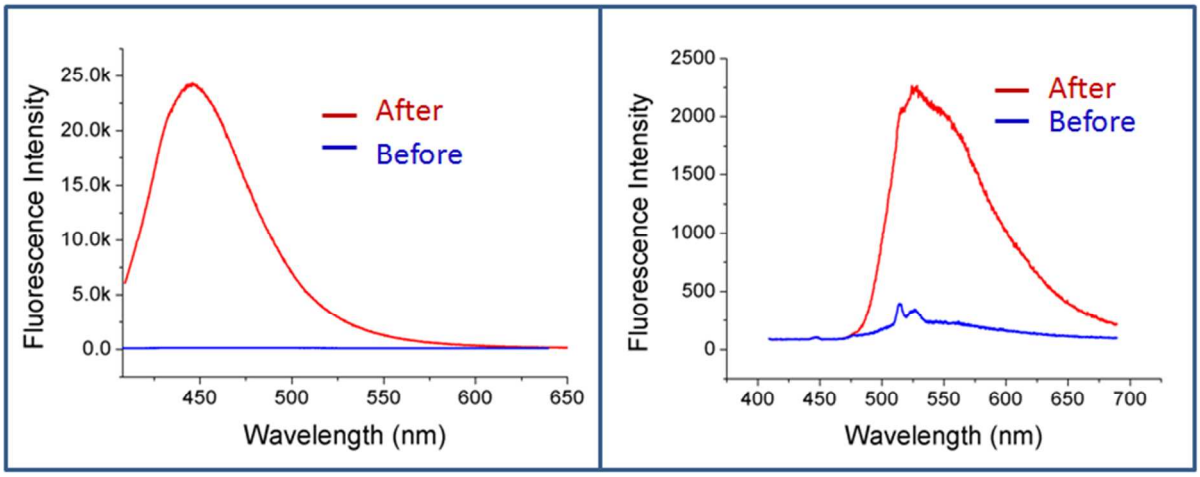

Figure S3. Fluorescence spectrum of the ACA (**Left**) and CFDA (**Right**) solutions before and after the enzymatic conversion.

6. Fluorescence Profile of Acetic Acid Induced Nanogate Opening

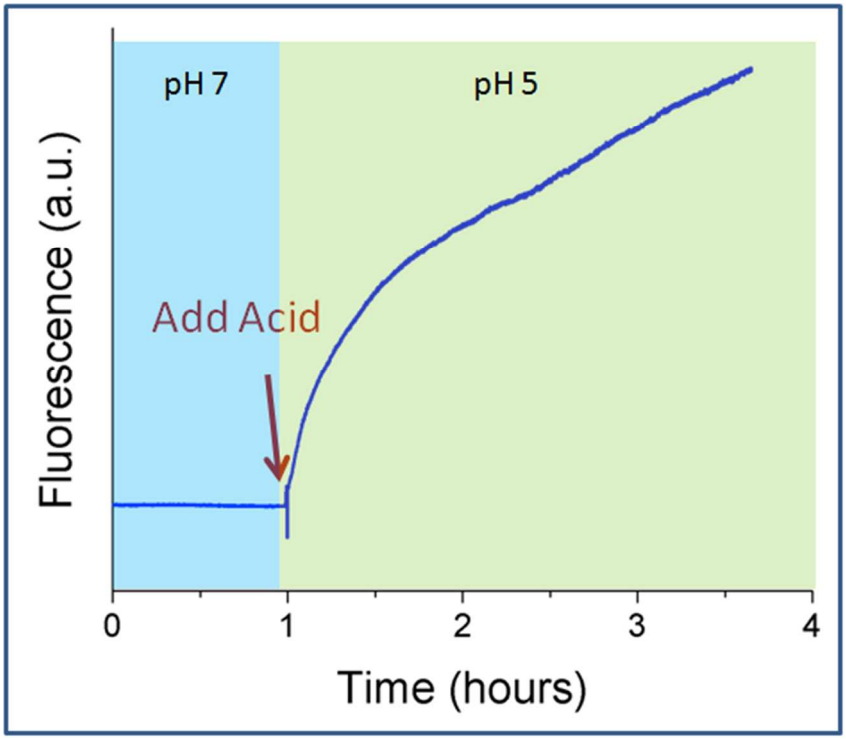

Figure S4. Fluorescence profile of acetic acid induced nanogate opening. The fully-assembled nanoparticles with enzymes loaded in the pores were placed in a CFDA solution and the corresponding hydrolyzed CFDA fluorescence in the solution was continuously monitored. The solution pH was adjusted through the addition of acetic acid after the collection of a flat baseline. Since the acetic acid causes all the caps being removed, there is no amplification mechanism and therefore the fluorescence profile shows a typical curve with decreasing slopes. This result is consistent with the previously report. [2]

7. Fluorescence Profile of the Nanoparticle Removed Control

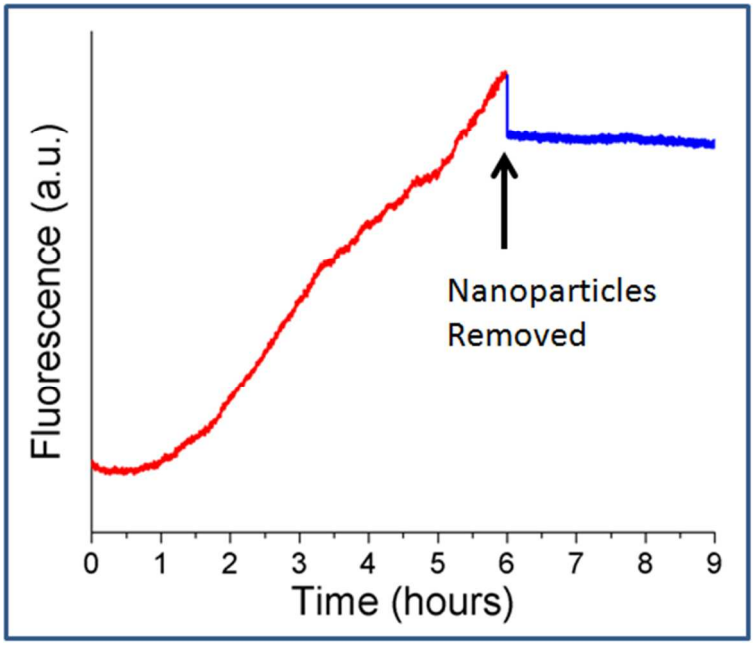

Figure S5. Fluorescence profile of the control experiment where the nanoparticles were removed from the solution after the amplification process started. The fully-assembled nanoparticles with enzymes loaded in the pores were placed in a CFDA solution and the corresponding hydrolyzed CFDA fluorescence in the solution was continuously monitored. The amplification process was initiated by the addition of ACA. After several hours, the nanoparticles were removed from the solution, which stopped the increase of the solution fluorescence. This result demonstrates that the amplification process is not a result of the enzymes leaking out from the pore.

8. References

[1]. Y. Zhao, X. Sun, G. Zhang, B. G. Trewyn, I. I. Slowing, V. S.-Y. Lin *ACS Nano*, **2011**, *5*, 1366-1375.

[2]. M. Xue, D. Cao, J. F. Stoddart, J. I. Zink *Nanoscale,* **2012**, *4*, 7569-7574.

[3]. Y.-L. Zhao, Z. Li, S. Kabehie, Y. Y. Botros, J. F. Stoddart, J. I. Zink *J. Am. Chem. Soc.*, **2010**, *132*, 13016-13025.