Enzyme-Responsive Snap-Top Covered Silica Nanocontainers

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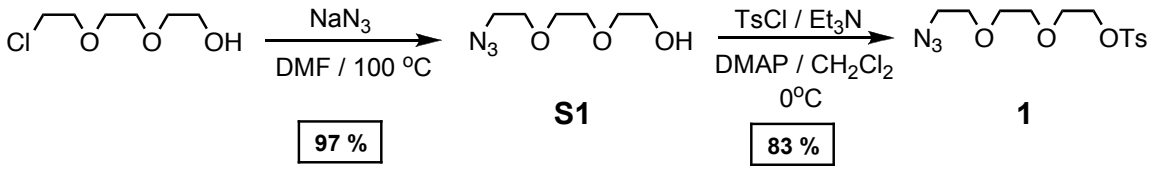
General Methods. All reagents were purchased from commercial suppliers (Aldrich or Fisher) and used without purification. Column chromatography was performed on silica gel 60F (Merck 9385, 0.040–0.063 nm). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 (^{1}H: 500 MHz; ^{13}C: 126 MHz) spectrometer. Chemical shifts are reported as parts per million (ppm) using $CDCl_3$ as the reference solvent, for both ^{1}H and ^{13}C NMR spectroscopies, unless otherwise indicated. SEM images were collected on a JEOL SM-71010 (fine powder profile). Au coating of the particles used for imaging was carried out by sputtering for 1 min (Hummer 6.2, Anatech LTD, plasma discharge current = 15 mA at 70 mTorr). IR spectra were collected on a Perkin-Elmer FT-IR Paragon 500 spectrometer using KBr pellets. UV-vis spectra were collected on a Cary 5000 UV-vis-NIR spectrophotometer. The controlled release profiles were obtained via luminescence spectroscopy using an Acton SpectraPro 2300i CCD, and a coherent Argon Innova 90C-5 excitation laser.

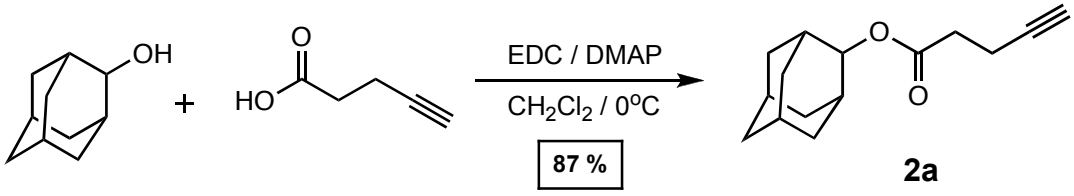
Scheme S1. Synthesis of Azido-Toluenesulfonate Derivative **1**.



2-(2-(2-Azidoethoxy)ethoxy)ethanol (S1):[S1] A mixture of 2-(2-(2-chloroethoxy)ethoxy)ethanol (5.0 g, 30 mmol) and NaN_3 (3.0 g, 45 mmol) in DMF (100ml) was stirred at 100°C for 48 h. The mixture was then cooled to RT, filtered and the solvent was removed under vacuum to afford the product **S1** (5.1 g, 97 %) as a colorless oil. Characterization was in agreement with the literature.[1]

2-(2-(2-Azidoethoxy)ethoxy)ethyltoluenesulfonate (1):[S2] A mixture of 2-(2-(2-azidoethoxy) ethoxy)ethanol **(S1)** (1.0 g, 5.7 mmol), DMAP (80 mg, 0.66 mmol) and Et_3N (4.0 g, 40 mmol) was dissolved in CH_2Cl_2 (150 ml) and cooled down to 0°C. A solution of *p*-toluenesulfonyl chloride (4.77 g, 25 mmol) in CH_2Cl_2 (50 ml) was added dropwise to this mixture. The solution was allowed to warm up to RT before being stirred for 18 h. The progress of the reaction was monitored by thin layer chromatography and the solvent removed under vacuum following disappearance of the starting material. The mixture was then passed through a silica plug with EtOAc as the eluent. The solution was then washed with H_2O (2 x 50 mL) and brine (1 x 50 mL) and the solvent evaporated to provide **1** (1.2 g, 83 %) as a pale orange oil. Characterization was in agreement with the literature.[2] HRMS (FAB): Calcd for $C_{13}H_{20}SO_5N_3$ *m/z* = 330.1124. Found *m/z* = 330.1134.

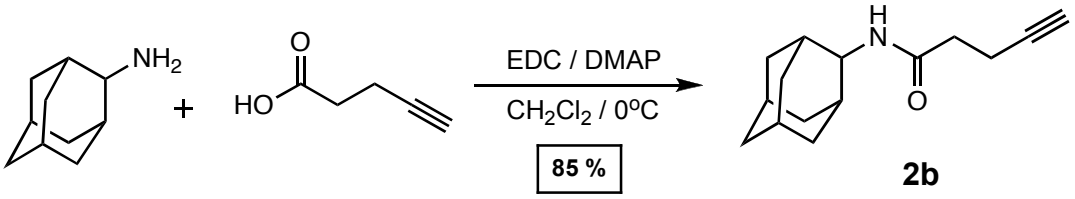
Scheme S2. Synthesis of Adamantyl-Ester Derivative **2a**.



(2-Adamantanyl)-4-pentynoic Acid Ester (2a): A solution of pentynoic acid (2.5 g, 25.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (6.54 g, 34 mmol) in CH_2Cl_2 (76 mL) was cooled down to 0°C before adding a solution of 2-adamantanol (2.59 g, 17 mmol) in CH_2Cl_2 (100 mL). The reaction was then warmed up to RT and stirred for 2 h before the addition of 4-methyldiaminopyridine (DMAP) (200 mg, 1.65 mmol). After stirring for 24 h, the reaction

was poured into H_2O (100 mL) and washed with CH_2Cl_2 (3 x 50 mL). The organic layer was washed with H_2O (3 x 50 mL), $NaHCO_3$ (2M, 30 mL) and brine (30 mL). Chromatography (SiO_2, 1:2 EtOAc : Hexanes eluent) provided **2a** as a white crystalline solid (3.45 g, 87 %), mp 60.5–62.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): d = 4.94 (t, J = 3 Hz, 1H), 2.59–2.53 (m, 2H), 2.53–2.48 (m, 2H), 2.05–1.90 (m, 5H), 1.86–1.78 (m, 4H), 1.78–1.67 (m, 4H), 1.57–1.50 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): 171.0, 82.6, 77.5, 69.0, 37.4, 36.3, 33.9, 31.9, 31.8, 27.2, 27.0, 14.6. HRMS (FAB): Calcd for $C_{15}H_{20}O_2$ *m/z* = 232.1463. Found *m/z* = 232.1468.

Scheme S3. Synthesis of Adamantyl-Amide Derivative **2b**.



(2-Adamantanyl)-4-pentynoic Acid Amide (2b): A solution of pentynoic acid (0.981 g, 10 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (2.5 g, 13 mmol) in CH_2Cl_2 (30 mL) was cooled down to 0°C before adding a solution of 2-adamantylamine (1 g, 6.6 mmol) in CH_2Cl_2 (30 mL). The reaction was then warmed up to RT and stirred for 2 h before the addition of 4-methyldiaminopyridine (DMAP) (100 mg, 0.825 mmol). After stirring for 24 h, the reaction was poured into H_2O (50 mL) and washed with CH_2Cl_2 (3 x 30 mL). The organic layer was washed with H_2O (3 x 20 mL), $NaHCO_3$ (2M, 20 mL) and brine (20 mL). Chromatography (SiO_2: 1:2 EtOAc/Hex eluent) provided **2b** as a white crystalline solid (1.3 g, 85 %), mp 126–128.5 °C. ^{1}H NMR (500MHz, $CDCl_3$, 25 °C): d = 6.02 (s, 1H), 4.06 (dt, 8 Hz, 4 Hz, 1H), 2.55–2.49 (m, 2H), 2.43–2.37 (m, 2H), 2.01 (t, 4 Hz, 1H), 1.94–1.59 (m, 14H); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): 170.0, 83.5, 69.7, 53.6, 37.7, 37.3, 35.9, 32.2, 32.1, 27.4, 27.3, 15.4. HRMS (FAB): Calcd for $C_{15}H_{21}NO$ *m/z* = 231.1623. Found *m/z* = 231.1629.

Preparation of Amine Modified Porous MCM-41 Silica Particles: Synthesis of mesostructured silica nanoparticles was performed using a one-pot sol consisting of a tetraethylorthosilicate (TEOS) silica precursor and cetyltrimethyl-ammonium bromide (CTAB) surfactant in aqueous solution[S3] at 80 °C. The synthesized nanoparticles were collected by filtration and washed extensively with MeOH and distilled H_2O. The CTAB surfactant was removed by calcination in air at 550˚C for 5 h. The surfactant-free particles were then suspended in a solution of 3-aminopropyltriethoxysilane (APTES) in dry PhMe (10 mM) and heated under reflux for 12 h under N_2. The amine-modified nanoparticles were collected by filtration, washed with dry PhMe, and dried under vacuum.

General Preparation of the MCM-41 Snap-Top System: A mixture of azide-functionalized nanoparticles (100 mg), Rhodamine B (10 mg, 0.023 mmol) and α-CD (480 mg, 0.493 mmol) were suspended in a 50:50 mixture of DMF and H_2O (20 mL) and stirred for 24 h at 5°C. A solution of **2a** or **2b** in DMF (5 mL, 39 mM) was added, followed by the addition of $CuSO_4 \cdot 5H_2O$ (1.5 mg, 0.0098 mmol) and ascorbic acid (4 mg, 0.019 mmol). The mixture was stirred at 5°C for 3 d. The nanoparticles were then filtered and washed thoroughly with MeOH and H_2O to remove unreacted and adsorbed molecules. The resulting red nanoparticles were finally dried under high vacuum.

General Procedure for Enzymatic Activation of the Snap-Top System. A sample of Rhodamine B loaded red nanoparticles (10 mg) was placed in the corner of a 15 mL quartz

cuvette and then filled with a HEPES buffer solution (12 mL, 50 mM, pH = 7.5). A sample of a PLE solution [0.12 mL, 10 mg/mL solution in 3.2M $(NH_4)_2SO_4$] was then added to the cuvette and the emission spectra of the solution (excitation at 580 nm) was monitored over a period of 40 min.

Dynamic Light Scattering

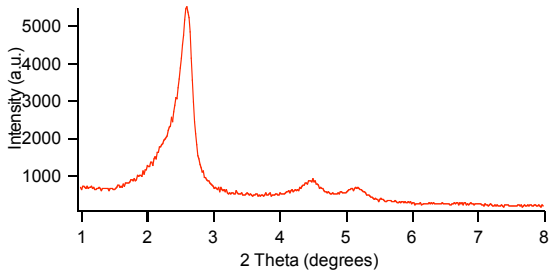
Dynamic light scattering was performed on a Beckman Coulter N4 Plus particle sizer, with a 633 nm HeNe excitation source. The measurements were carried out in MeOH on calcined nanoparticles prior to any surface modifications.

Table. Average Diameters of Calcined Mesoporous Silica Nanoparticles from DLS Measurements.

	Particle radius (nm)	Standard Deviation (nm)
Run 1	415	175
Run 2	407	65
Run 3	411	150
Average	**411**	**130**

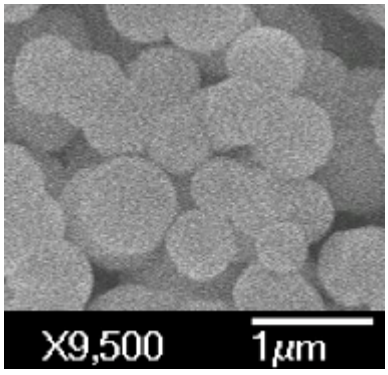
X-Ray Diffraction

Powder X-ray diffraction measurements were carried out using a Panalytical X'Pert Pro powder diffractometer. The radiation source is copper ($K_{\alpha 1}$ and $K_{\alpha 2}$ = 1.5418 Å)



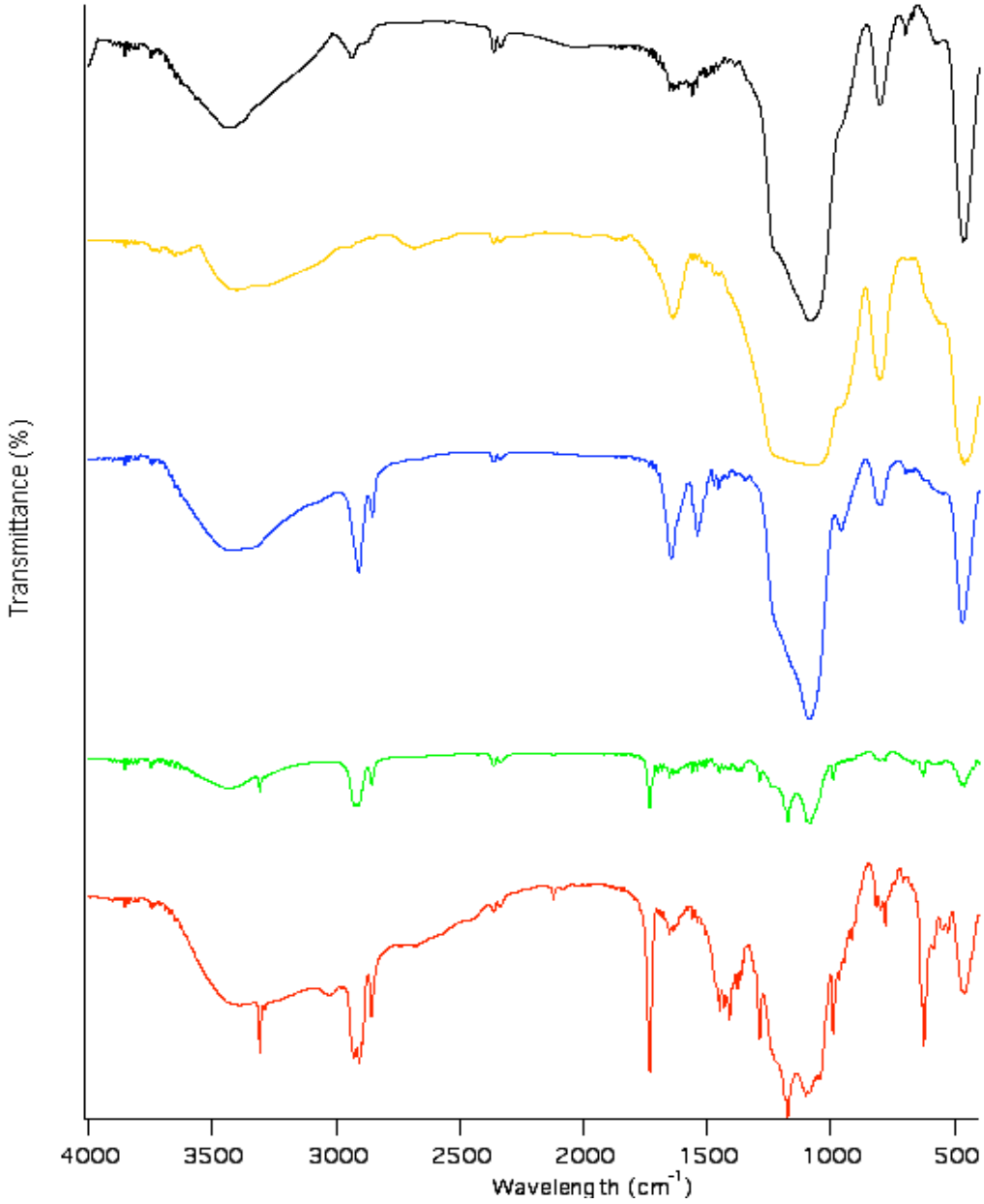
Supporting Figure 1. XRD pattern of calcined mesostructured silica nanoparticles. The 100 peak at $2\theta = 2.6^{o}$ corresponds to a 2D-hexagonal mesostructure with lattice spacing of 3.4 nm.

Scanning Electron Microscopy



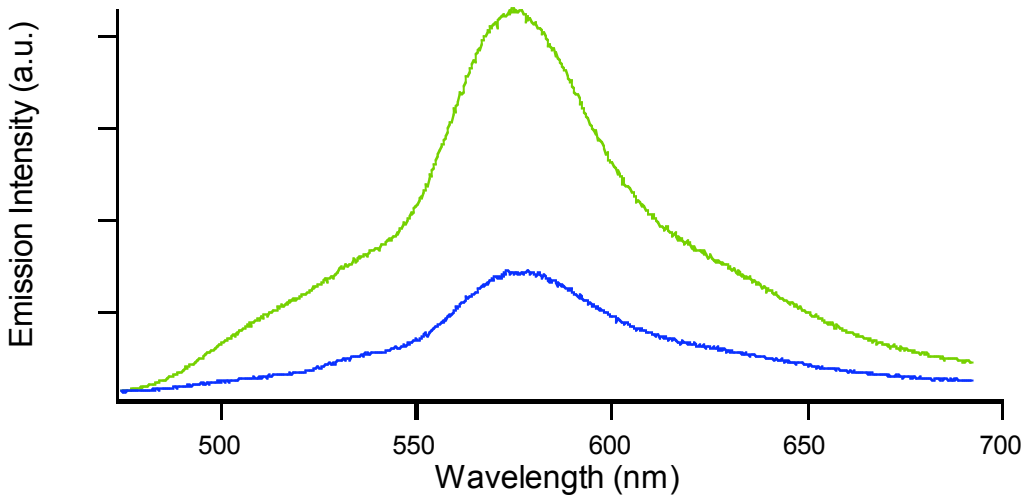
Supporting Figure 2. SEM image of calcined mesostructured amine-modified silica nanoparticles.

FT Infrared Spectroscopy



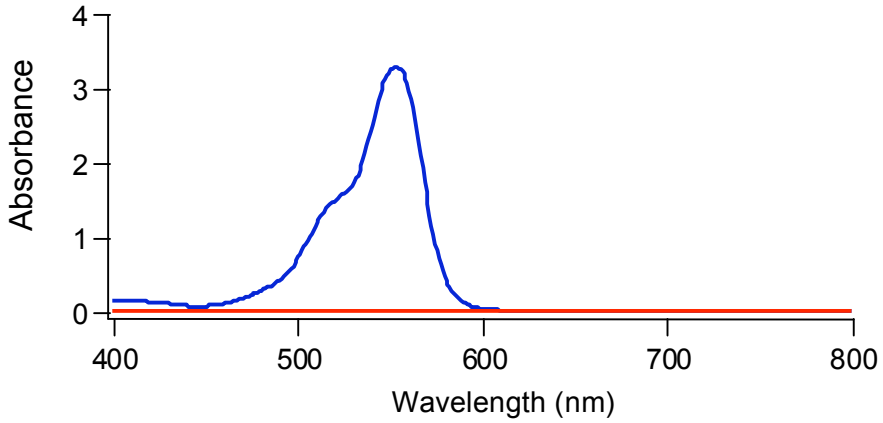
Supporting Figure 3. FTIR spectra of amine particles (**black**), azide particles (**orange**), amide-capped particles (**blue**), ester-capped particles (**green**) and released ester-capped particles (**red**)

Time-Dependent Release Profile



Supporting Figure 4. The emission intensity of Rhodamine B released into solution 100 seconds (blue) and 1000 seconds after addition of esterase.

Absorption Spectra



Supporting Figure 5. The absorption spectrum of Rhodamine B in the solution above the particles before (red) and after (blue) esterase-triggered release.

References

(S1) Fernandez-Megia, E.; Correa, J.; Rodriguez-Meizoso, I.; Riguera, R. *Macromolecules* **2006**, *39*, 2113.
(S2) Meunier, S. J.; Wu, Q.; Wang, S.-N.; Roy, R. *Can. J. Chem.* **1997**, *75*, 1472.
(S3) Huh, S.; Wiench, J. W.; Yoo, J. C.; Pruski, M.; Lin, V. S. Y. *Chem. Mater.* **2003,** *15*, 4247.