

pH Clock-Operated Mechanized Nanoparticles

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Supporting Information

Revised Version

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Materials and Methods. All reagents are commercially available and were used as received unless otherwise noted. Cucurbit[6]uril (CB[6]) was synthesized^{S1} by the condensation of glycoluril and HCHO in concentrated HCl. High resolution electrospray ionization (HR ESI) mass spectra were measured on an Micromass Q-TOF Ultima mass spectrometer. The reported molecular mass (m/z) values were the most abundant monoisotopic mass peaks. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrometer at 25 °C. Chemical shifts were reported in parts per million (ppm) downfield from the Me₄Si resonance which was used as the internal standard when recording ¹H NMR spectra. Powder X-ray diffraction (XRD) measurements were carried out using a Panalytical X'Pert Pro powder diffractometer. The radiation source was copper ($K_{\alpha 1}$ and $K_{\alpha 2}$ = 1.5418 Å). Scanning electron microscopy images were collected on a JEOL SM-71010 microscope (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). Dynamic light scattering (DLS) was performed on a Beckman Coulter N4 Plus particle sizer, with a 633 nm HeNe excitation source. The controlled release profiles were obtained via luminescence spectroscopy using an Acton SpectraPro 2300i CCD, and a coherent Argon Innova 90C-5 excitation laser.

Preparation of MNP Stalks

4-(6-(Phenylamino)hexylamino)butyl 4-Methylbenzenesulfonate (4): Compound **2** (1 equiv.) was heated under reflux with 4-amino-1-butanol (10 equiv.) in PhMe overnight (Figure S1). The solvent was then evaporated and the reaction mixture dissolved in CH₂Cl₂ followed by the addition of TsCl and Et₃N. The tosylation was allowed to continue for 8 h after which time the solvent was evaporated and the product purified by column chromatography (SiO₂: EtOAc/Hexanes gradient) to afford the pure tosylate **4** as a yellow oil (23%). ¹H-NMR (500 MHz, CDCl₃): δ 7.61 (d, *J* = 8.1 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 7.12–7.08 (m, 2H), 6.61 (t, *J* = 7.5 Hz, 1H), 6.53 (d, *J* = 8.1 Hz, 2H), 3.57 (t, *J* = 7.1 Hz, 2H), 3.08–3.00 (m, 7H), 2.36 (s, 3H), 1.97 (s, 1H), 1.58–1.46 (m, 8H), 1.34–1.30 (m, 4H); ¹³C-NMR: 138.5, 129.9, 129.7, 127.4, 125.3, 114.5, 69.6, 69.6, 55.6, 41.9, 41.9, 31.9, 31.9, 31.0, 31.0, 21.8, 21.0; ESI (*m/z*): [*M*]⁺ calcd for C₂₃H₃₄N₂O₃S, 418.2; found [*M*+H]⁺, 419.2.

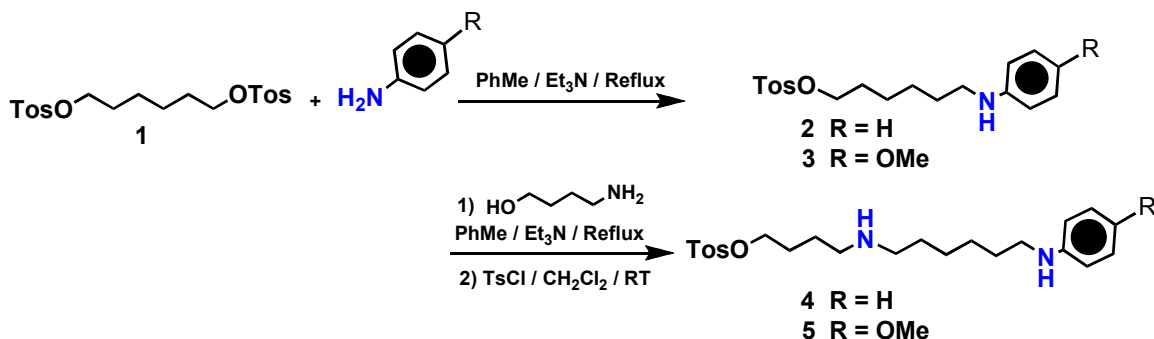


Figure S1: Synthesis of the tosylated trisammonium stalks.

4-(6-(Phenylamino)hexylamino)butyl 4-Methylbenzenesulfonate (5): Compound **3** (1 equiv.) was heated under reflux with 4-amino-1-butanol (10 equiv.) in PhMe overnight (Figure S1). The solvent was then evaporated and the reaction mixture dissolved in CH₂Cl₂ followed by addition of TsCl and Et₃N. The reaction was allowed to proceed for

8 h, before the solvent was evaporated and the product purified by column chromatography (SiO₂: EtOAc/Hexanes gradient) to afford pure **5** as a brown oil (15%).
¹H-NMR (500 MHz, CDCl₃): δ 7.48 (d, *J* = 8.1 Hz, 2H), 7.10 (d, *J* = 8.1 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 6.65 (d, *J* = 8.8 Hz, 2H), 3.65 (s, 3H), 3.52–3.49 (m, 4H), 3.40–3.35 (m, 4H), 3.05–3.02 (m, 2H), 2.68–2.65 (m, 4H), 2.28 (s, 3H), 1.63–1.53 (m, 4H), 1.51–1.42 (m, 4H); ¹³C-NMR: 129.6, 127.4, 125.4, 114.5, 63.5, 62.6, 55.9, 42.2, 42.2, 41.8, 41.8, 32.0, 32.0, 31.2, 31.1, 21.9; HRMS (*m/z*): [*M*]⁺ calcd for C₂₄H₃₆N₂O₄S, 448.2400; found [*M*+H]⁺, 449.2424.

Preparation of Mesoporous Silica Nanoparticles. Mesoporous silica nanoparticles were prepared by a base-catalyzed sol-gel process involving cetyltrimethyl-ammonium bromide (CTAB) surfactants and a tetraethylorthosilicate (TEOS) silica precursor. The CTAB templating agents were removed using an acid extraction process: The synthesized nanoparticles (1.5 g) were suspended in MeOH (160 mL) and concentrated HCl (12 M, 9 mL) was added. The suspension was heated under reflux and N₂ for 24 h, and the mesoporous materials were then collected via vacuum filtration, washed extensively with MeOH, and dried under vacuum.

Amine-Modification of Nanoparticles. Amine modification of the silica surface was performed by suspending the mesoporous nanoparticles (200 mg) in a solution of 3-aminopropyltriethoxy-silane (APTES) (1 mM) in dry PhMe (20 mL) and heating them under reflux for 24 h. The nanoparticles were collected by vacuum filtration, washed thoroughly with PhMe, and dried under vacuum.

Attachment of the Trisammonium Stalks 4 and 5. Amine-modified nanoparticles (150 mg) were suspended in a solution of **4** or **5** (0.1 mmol) in MeOH (10 mL) and 3 drops of Et₃N were added. The suspension was heated under reflux and N₂ for 2 d. The originally white nanoparticles will acquire a yellowish tint. The resulting trisammonium-modified nanoparticles were collected by vacuum filtration, washed thoroughly with MeOH, and dried under vacuum.

Propidium Iodide (PI) Loading. A 1 mM PI solution was prepared in deionized H₂O. Trisammonium-modified nanoparticles (150 mg) were suspended in the PI solution (50 mL) and the solution was sonicated to maximize the dispersion of the nanoparticles. The solution was then stirred at RT overnight to allow the PI to diffuse into the empty nanopores.

CB[6] Capping. The final step in the synthesis of the mechanized nanoparticles (MNPs) is capping with CB[6] to bring about the formation of the pseudorotaxanes on the nanoparticles' surfaces. CB[6] (80 mg) and NaCl (45 mg) were dissolved in a NaOH solution (10 mL, 5 mM) and the mixture was sonicated until it became clear. After complete dissolution of CB[6], the solution was added to the dye loading / particle suspension. The pH of the resulting mixture was measured and adjusted to pH 9. The suspension was stirred at RT for 3 d to maximize the association of CB[6] with the tethered stalks and complete the preparation of the entire MNP system. The MNPs were collected by vacuum filtration, washed extensively with aqueous NaOH (5 mM) and H₂O, before been dried under vacuum.

Spectroscopic Testing. The operation of the pH-responsive MNPs was tested using luminescence spectroscopy.

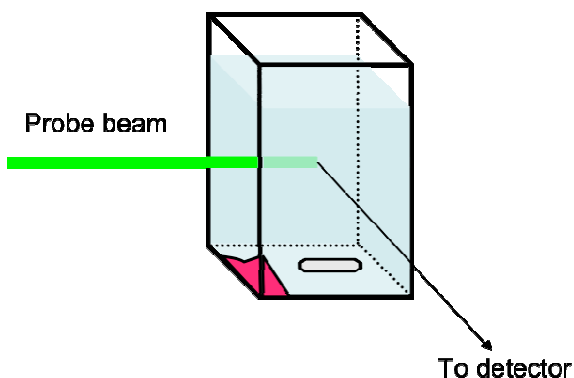


Figure S2. Spectroscopic setup for controlled release experiments. 15 mg of PI-loaded MNPs are placed in the corner of a cuvette, and 12 mL of DI water is added. The solution in the cuvette is stirred slowly so as not to perturb the nanoparticles. A 10 mW, 514 nm probe beam directed into the liquid is used to excite the dissolved PI molecules. The luminescence spectrum of the dissolved dye is collected in 1 second intervals over the course of the experiment. The luminescence intensity at the emission maximum of the dye is plotted as a function of time to generate a release profile.

Particle Size Characterization

Scanning Electron Microscopy and X-ray Diffraction

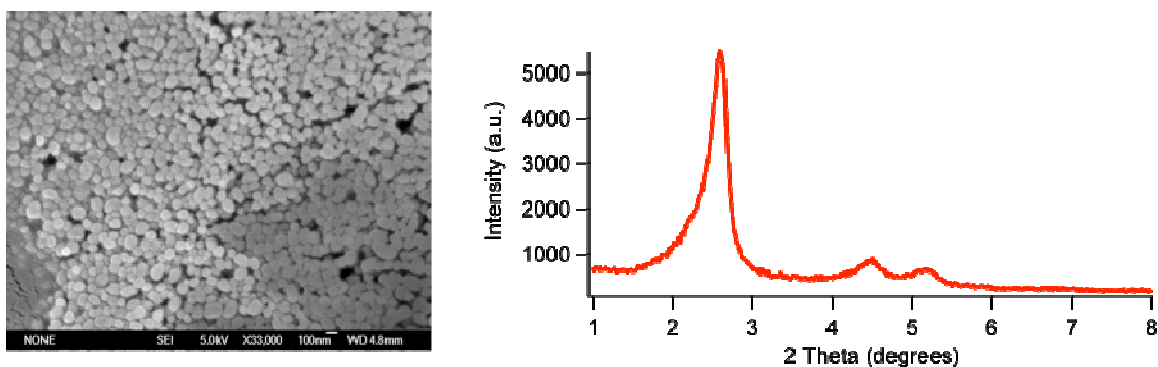


Figure S3. The scanning electron micrograph (left) of the MNPs shows relatively monodisperse particles (diameter ~ 130 nm). The X-ray diffraction pattern (right) of the MNPs confirms the presence of a 2D-hexagonal mesostructure (lattice spacing ~ 4 nm).

Dynamic Light Scattering

Table 1. Dynamic light scattering of nanovalve-modified mesoporous silica nanoparticles in water.

	Particle Size (nm)	Standard Deviation (nm)
Run 1	135.8	65.8
Run 2	135.3	65.6
Run 3	134.1	63.5
Average	134.1	65.0

Absorption Spectroscopy

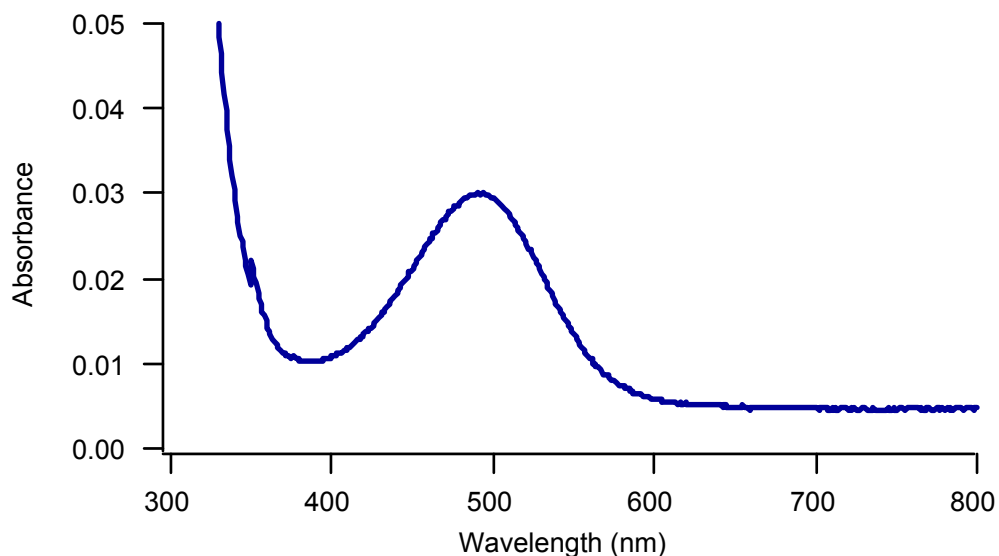


Figure S4. Absorption spectrum of supernatant after completion of the release profile for DCNP-1. Using the absorbance at 493 nm, it is calculated that ~ 0.1 μmol of dye is released after 1800 seconds. This release capacity corresponds to a maximum of ~ 1 wt%.

References.

- (S1) a) Behrend, R.; Meyer, E.; Rusche, F. *Justus Liebigs Ann. Chem.* **1905**, 339, 1–37;
b) Freeman, W.A.; Mock, W.L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1981**, 103, 7367–7368.