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pH Clock-Operated Mechanized Nanoparticles

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Mesoporous silica nanoparticles, mechanized with nanovalves[1] and other artificial molecular machines,[2] are promising vehicles for drug delivery because they are biologically compatible and are capable of storing and releasing molecules on command. Previously, mechanized nanoparticles (MNPs) designed to release their contents in response to an externally controlled light source were used to deliver therapeutic molecules to human cancer cells.[3] However, for some applications, self-opening drug carrier vehicles that respond autonomously to a particular biostimulus are preferred. In the work reported here, we present tunable pH-operable MNPs in which biologically relevant pH changes are used to trigger the release of guest molecules.

One of the design challenges inherent in preparing MNPs is selecting suitable mechanical components such that the system operates under the desired conditions. Central to the design of these systems is the knowledge that mesoporous silica nanoparticles are noncytotoxic and undergo cellular uptake into acidic lysosomes through endocytosis.[4] We conceived that a MNP system designed to remain closed at neutral pH levels (i.e., the bloodstream) but open under mildly acidic conditions (i.e., in lysosomes) could release its contents autonomously upon cell uptake.

The supramolecular machines described here that fulfill these requirements are based[5] on the pumpkin-shaped macrocycle, cucurbit[6]uril (CB[6]), which forms[6] pH-dependent complexes with aminoalkanes when they are protonated. We recently reported[1a] a type of MNP based on the interaction of CB[6] with a bisammonium pseudorotaxane that operates under basic conditions, but it is unsuitable for biological applications because of the high pH (~10) that is required for activation. The unique biologically relevant supramolecular machines described here consist[7] of bistable CB[6]/trisammonium pseudorotaxanes that are attached to the surface of mesoporous silica nanoparticles and operate to encapsulate Propidium Iodide (PI) guest molecules at neutral pH and then release the contents under mildly acidic conditions. This design (Figure 1) incorporates the novel feature that the pH required for activation of this MNP system can be selectively "dialed-in."

The operation of the MNPs presented here relies on three prominent design features: (1) the difference in basicity of the anilinium nitrogen atom in comparison with the other two nitrogen atoms, (2) the difference in the length of the oligomethylene spacers, and (3) the identity of functional group R. The anilinium nitrogen atom is approximately 10^6-fold less basic than the alkyl nitrogen atoms, resulting in it being unprotonated at neutral pH. By this design, at neutral pH, the CB[6] ring resides on the tetramethylenediammonium recognition unit so that both portals of the macrocycle are able to engage in ion−dipole binding interactions,

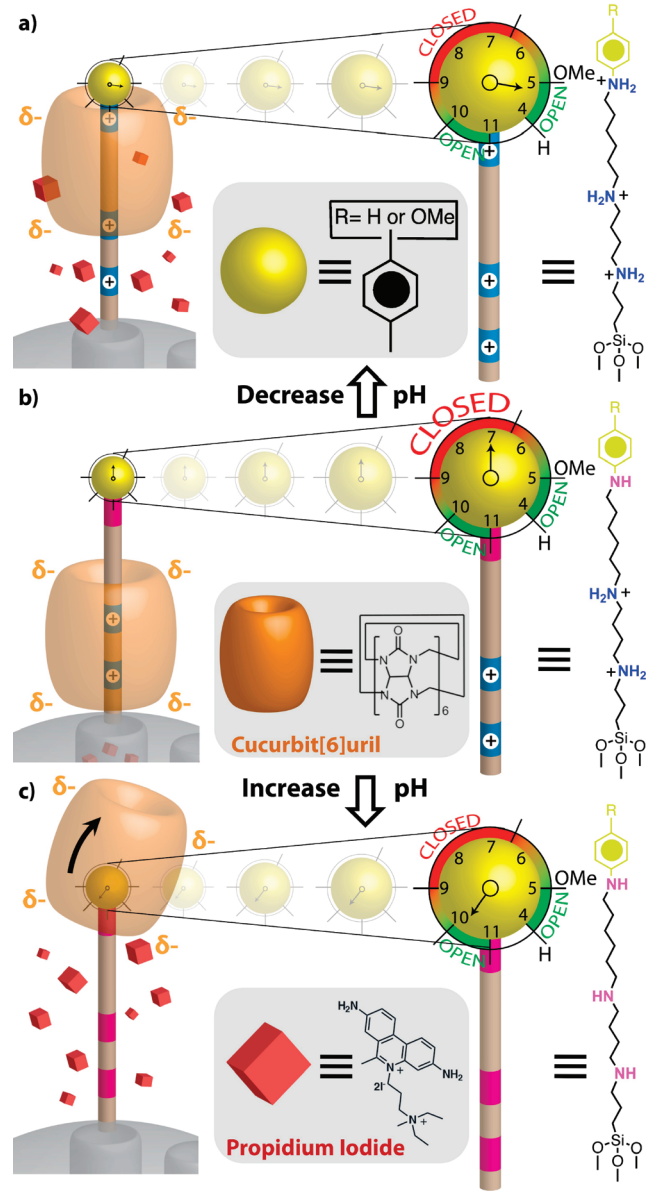

Figure 1. Design and pH-dependent operation of MNP-**1** (R = H) and MNP-**2** (R = OMe). When the pH is lowered such that the anilinium nitrogen atom is protonated, the CB[6] ring shuttles to the distal hexamethylenediammonium station, and PI is released (a). At neutral pH, the CB[6] ring sits on the tetramethylenediammonium recognition unit, blocking the nanopores (b). When the pH is raised, all of the nitrogen atoms on the stalk are deprotonated resulting in dethreading of the CB[6] ring (c). The system is designed such that the pH-response can be fine-tuned by changing R to modulate the pK_a of the anilinium nitrogen atom.

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thereby blocking the pore orifices and encapsulating the guest molecules. The difference in the oligomethylene spacer length is an important aspect of the design because the stability constant for the complexation of $NH_3^+(CH_2)_6NH_3^+$ with CB[6] is an order of magnitude greater than that for $NH_3^+(CH_2)_4NH_3^+$.[7] The six-carbon spacer is a better match with the inner dimensions of CB[6] and allows for optimal ion−dipole interactions between both occuli of the CB[6] ring and an ammonium center. Therefore, once the pH is lowered such that the anilinium nitrogen atom becomes protonated, the CB[6] ring shuttles to the distal hexamethylenediammonium recognition unit, such that the pore orifice is unblocked and the encapsulated contents are released (Figure 2). Finally, by tuning the pK_a of the anilinium nitrogen through chemical modification at the para postion R of the terminal phenyl group, the pH at which the MNP is opened can be adjusted. The pK_a of the nitrogen atom in aniline is approximately 1 pK_a unit less basic than that of the nitrogen atom in *p*-anisidine.[8] In following this trend, we have prepared two different MNP systems, MNP-**1** (R = H) and MNP-**2** (R = OMe), which are tuned to respond at different pH levels. Because the identity of the substituent R affects the pK_a of the anilinium nitrogen atom, it serves to modulate the acidic pH that is required for activation. Alternatively, but of less importance to biological applications, activation of these MNPs can also occur through addition of base: if the pH is raised such that each of the ammonium groups is deprotonated, all binding interactions are disrupted and the nanopore orifices are unblocked as a result of complete dethreading of the CB[6] rings.

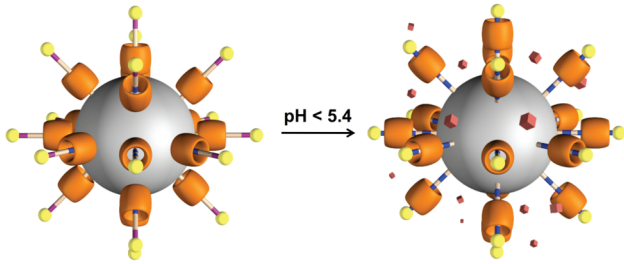

Figure 2. MNPs operate by keeping guest molecules contained at neutral pH, but release contents when the pH is lowered. Trisammonium stalks with two different recognition units are spread all over the surface of mesoporous silica nanoparticles. At neutral pH, the CB[6] ring encircles the tetramethylenediammonium station, and the MNP is in its closed configuration. When the pH is lowered, the CB[6] ring shuttles to the distal hexamethylenediammonium recognition unit such that the pore orifices are unblocked and the contents are released.

Mesoporous (pore diameter ~2 nm) silica nanoparticles[9] (~130 nm diameter) were prepared[10] through a base-catalyzed sol−gel process involving the use of cetyltrimethylammonium bromide (CTAB) surfactant and a tetraethylorthosilicate (TEOS) silica precursor. The assembly of the dye-loaded MNPs was then completed in a stepwise process from the nanoparticle surface outward (Supporting Information). Briefly, beginning with as-synthesized nanoparticles, acid extraction was performed to remove the CTAB template and generate empty, accessible nanopores. Next, amine modification of the silica nanoparticle surface was accomplished through treatment with aminopropyltriethoxysilane (APTES). To the amine-modified nanoparticles, the trisammonium stalks were attached to the nanoparticle surfaces. Dye loading was accomplished by soaking the trisammonium-terminated nanoparticles in a solution of propidium iodide (PI) to allow the dye molecules to diffuse into the empty nanopores. Finally, the pseudorotaxanes were formed—and the synthesis of the MNP system was completed—by adding CB[6] to the dye-loading mixture to allow for complexation between the CB[6] rings and the trisammonium stalks. The final mechanized nanoparticles are highly dispersible in water with an average diameter of 134 nm as measured by dynamic light scattering (Supporting Information).

Testing and characterization of the MNP operation were performed in solution using luminescence spectroscopy. A sample of dye-loaded MNPs (~10 mg) was loaded into the corner of a cuvette, and H_2O (~12 mL) was added carefully. A probe beam was directed into the H_2O, and the emission intensity of the dissolved dye was collected as a function of time to generate a release profile. At ~300 s, the pH was adjusted to a specific level through the addition of either 0.01 M HCl or 0.01 M NaOH. The percentage of release was calculated using absorbance spectroscopy to enable quantitative comparison of the release efficiency (Supporting Information).

The release profiles (Figure 3) demonstrate that MNPs are able to contain guest molecules at neutral pH but release them in response to pH changes. When different acidic pH values are used to activate MNP-**1** (Figure 3a), it is apparent that the rate of release for the MNPs depends directly on the pH that is used for activation, with the highest release rate occurring at the most acidic pH. When the pH is adjusted to 3.4, 100% release is obtained after 1800 s. However, when the pH is adjusted to 4.4, only 71% release occurs in the same amount of time. The response is even slower when pH 5.0 is used: only 35% of the maximum release is observed after 1800 s. Virtually no release is observed over the course of the experiment when the pH is adjusted to 6.5. The observed pH-

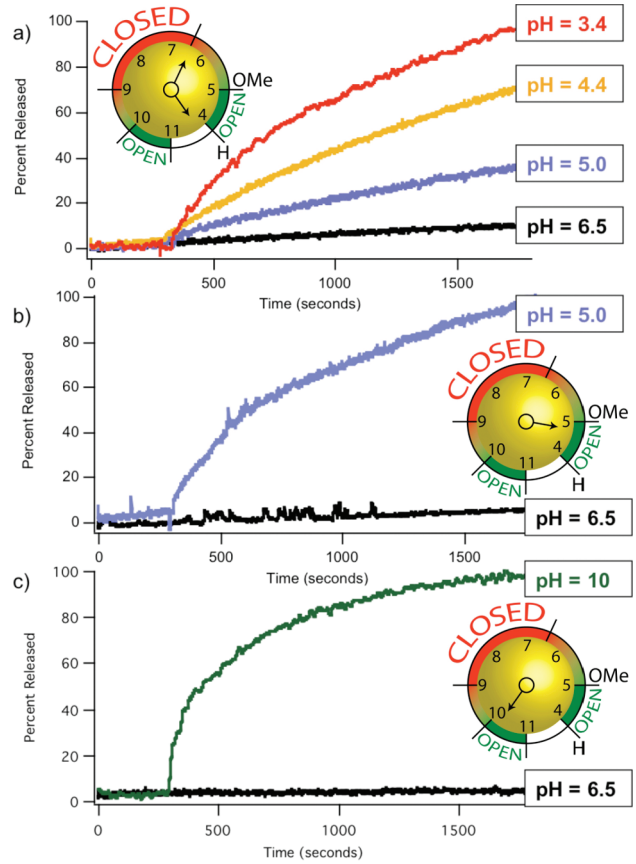

Figure 3. MNPs are able to contain PI molecules at neutral pH levels but release them under pH control. (a) The rate of release of PI from MNP-**1** depends on the amount of acid that is added. (b) The pH level required to fully activate MNP-**2** is less acidic than that for MNP-**1**. (c) Adjustment of the pH to 10 causes MNP-**1** to release PI molecules as a result of complete deprotonation of the trisammonium stalks and dethreading of the CB[6] rings.

dependent release rate is consistent with the mechanism of operation for the MNP system: release of guest molecules depends on the rate of protonation of the anilinium nitrogen atom, which occurs more rapidly at more acidic pH levels.

By comparing the release profiles for MNP-**1** and MNP-**2** (Figure 3a and 3b), a difference in the pH-sensitivity of the two systems is observed. When pH = 5.0 is used to activate the MNPs, the response exhibited by MNP-**2** is more pronounced than that by MNP-**1**. For MNP-**2**, 50% of the maximum release is obtained after 600 s (Figure 3b), while, for MNP-**1**, just 13% of the maximum release is reached after 600 s (Figure 3a). The difference in the operation of MNP-**1** and MNP-**2** corresponds to the difference in the pK_a of the nitrogen atom in aniline and *p*-anisidine. The difference in the release efficiency at pH 5 demonstrates that the pH sensitivity of the MNP system can be rationally tuned through chemical modification of the stalks at position R.

The response of MNP-**1** to high pH conditions (Figure 3c) illustrates that the MNP system can be operated through an alternative base-driven mechanism. When the pH is adjusted to 10 such that all of the ammonium groups on the stalk are deprotonated, the ion−dipole interactions between the CB[6] ring and the stalk are disrupted, resulting in dethreading of the ring and release of the encapsulated guests. As shown in the release profile, the rate of the base-driven release is rapid: 50% of the maximum release occurs at 400 s, just ∼100 s after the base is added.

In conclusion, we have designed and synthesized a novel MNP system. Luminescence spectroscopy has been used to demonstrate that the MNPs are able to keep guest molecules encapsulated at neutral pH but release them when the pH is lowered. Furthermore, the pH at which the MNP system responds can be tuned through rational chemical modification of the stalk. For biological applications, the ability to fine-tune the system for optimal response enhances the versatility of this MNP system for use in a wide range of different tissues with different pH values. Efforts are now underway to explore the applications of these MNPs by testing their delivery capabilities with various types of human cancer cells with varying lysosomal pH levels.

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Supporting Information Available: Synthetic procedures and spectral characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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