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Synergistic Co-Entrapment and Triggered Release in Hollow Nanocapsules with Uniform Nanopores

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Supporting Information

ABSTRACT: We describe a new co-entrapment and release motif based on the combination of noncovalent and steric interactions in materials with well-defined nanopores. Individual components enter hollow nanocapsules through nanopores in the capsule shell. Their complex, larger than the pore size, remains entrapped. The dissociation of the complex upon external stimulus releases entrapped components. Reversible formation of complexes between diaza-18-crown-6 and metal ions was used to demonstrate the feasibility of new approach to co-entrapment and triggered release.

Reversible entrapment of molecules and ions in nanoporous materials is critical for creating supramolecular machines, molecular flasks, and functional nanodevices for the delivery of therapeutic and imaging agents.[1] Innovative methods for regulating the entrapment and release of molecules are essential for progress in this field. Triggered release of materials from capsules by using external stimuli has been demonstrated for micelles,[2a] soft materials,[2b] polymeric[2c] and rigid ceramic[2d] capsules. Kim et al. and Müller et.al. showed controlled release of the encapsulated content from supramolecular nanocapsules with changing pore size.[1a,b] Permanent entrapment can be achieved by creating a stable nanocapsule in the presence of the target compounds[3,4] or by ship-in-a-bottle assembly.[5] Usually, in order to release entrapped materials, stimuli-responsive hollow capsules undergo a change of shape or structural transition in response to external stimuli such as pH, temperature, or ionic strength.[1,2c,2d,6]

Here, we report a new motif for the reversible co-entrapment enabled by the differences between sizes of individual components and their complex. In contrast with previously reported methods,[3−5] this entrapment is reversible and is driven by noncovalent interactions. Previously, we reported a method for creating nanocapsules with uniform nanopores based on directed assembly of hydrophobic building blocks in the interior of the bilayers of lipid vesicles in the presence of hydrophobic pore-forming templates.[4] By using glucose pentaacetate as a pore-forming template, we created nanocapsules with imprinted nanopores having the diameter of 0.8 ± 0.2 nm.[4b,c] Nanocapsules used in this work have no functional groups on the pore orifice.

In our approach, individual components, which are smaller than the pore size, can enter and exit the capsule through the nanopores in the capsule shell. Once a complex is formed, it cannot escape from the capsule because its smallest cross section is bigger than the pore size (Figure 1a). When the complex is dissociated, for example, upon external stimulus, individual components can be released from the nanocapsules.

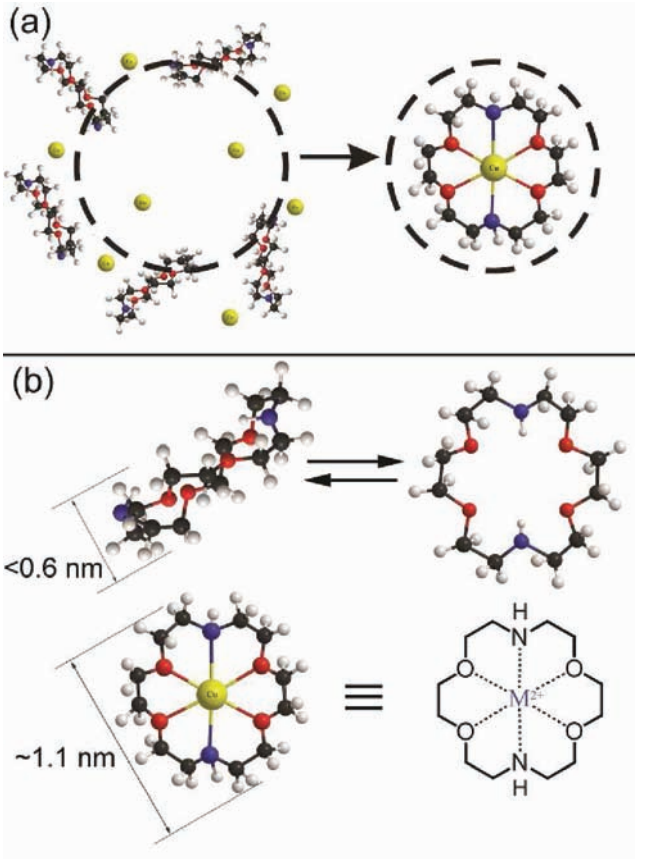

Figure 1. (a) Synergistic co-entrapment in porous hollow nanocapsules: individual components are smaller than the pore size and can diffuse into the capsule. Their complex is bigger than the pores and cannot escape from the capsule. (b) Free diaza-18-crown-6 (DA18C6) is conformationally flexible and can adopt a conformation with the smallest cross section of approximately 0.6 nm. The complex of DA18C6 with metals, such as Co^{2+} and Cu^{2+}, is conformationally rigid with approximately 1.1 nm cross section.

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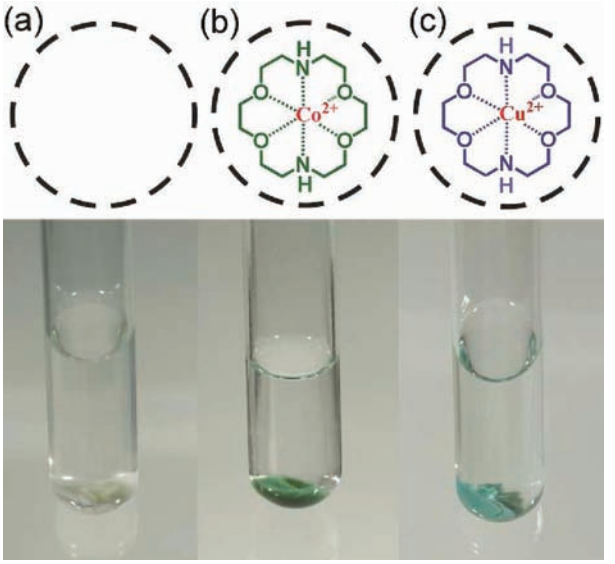

Figure 2. Schematic representation and photographs of (a) blank nanocapsules, (b) nanocapsules with entrapped complex of DA18C6 with Co^{2+}, and (c) nanocapsules with entrapped complex of DA18C6 with Cu^{2+} in methanol.

In this work, we used 1,10-diaza-18-crown-6 (DA18C6) to test this idea. DA18C6 is a conformationally flexible molecule that forms strong complexes with many metal ions.[7] Examination of three-dimensional models of different conformations of free DA18C6 shows that in the most flattened conformations, DA18C6 can pass through pores with the cross section of approximately 0.6 nm (Figure 1b). The complex, on the other hand, is conformationally rigid, and it would not be able to pass through pores smaller than 1.1 nm in size (Figure 1b). Although we use nanocapsules to demonstrate the feasibility of this co-entrapment motif, this approach should be applicable to a broad range of materials with well-defined nanopores.

Figure 2 shows successful entrapment of complexes with cobalt and copper cations in hollow porous nanocapsules. We chose these cations for this work because they are easy to observe and because they form strong complexes with DA18C6. In methanol, log K_a is 8.48 for Cu^{2+} and 3.56 for Co^{2+}.[7] In these experiments, a suspension of nanocapsules in methanol was centrifuged at approximately 2000*g* for 5 min. Methanol was decanted, and the culture tube with the compact precipitate was covered with Parafilm and kept upside down for several hours to drain excess methanol without drying the capsules. This method was used to allow rapid dispersion of nanocapsules in chloroform. In our experience, nanocapsules freeze-dried from benzene or *tert*-butanol can be readily dispersed in a broad range of solvents.

DA18C6 (20 mg, 7.62×10^{-2} mol) was dissolved in 1 mL of chloroform. Nanocapsules (30 mg) were then dispersed in this solution. One milliliter of aqueous $Co(NO_3)_2$ or $Cu(NO_3)_2$ (0.5 mmol each) was added dropwise while vortexing the solution. Nanocapsules with the entrapped complex were precipitated with methanol and washed several time with water, methanol, and methanol–water mixture until the supernatant appeared colorless. Aliquots of the supernatant were analyzed with UV–vis spectroscopy. The salts, DA18C6, and the complexes were well soluble in methanol, and all nonentrapped components were removed during this step. In control experiments, we found that free metal nitrates diffuse in and out of nanocapsules and preformed complexes of

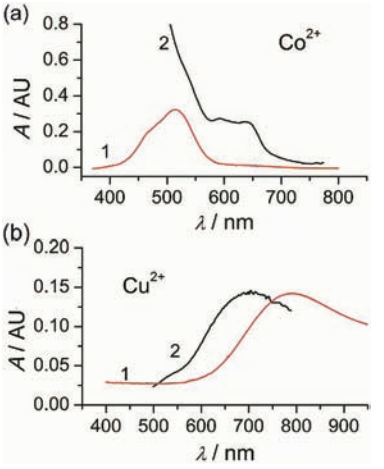

Figure 3. UV–vis spectra of free metal salts in methanol (red) and complexes of metal ions with DA18C6 entrapped in nanocapsules in methanol (black) for (a) $Co(NO_3)_2$ and (b) $Cu(NO_3)_2$.

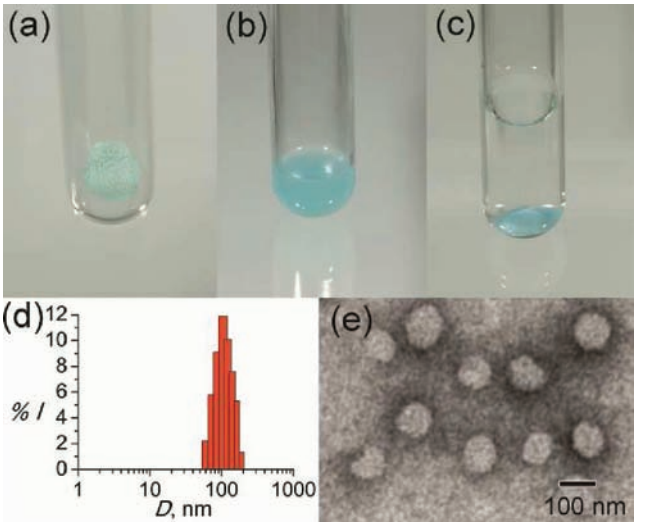

Figure 4. Retention of the complex of DA18C6 with Cu^{2+} in nanocapsules: (a) freeze-dried nanocapsules; (b) dried capsules resuspended in chloroform; (c) capsules precipitated in methanol. (d) Dynamic light scattering data of polymer nanocapsules with complex of DA18C6 resuspended in chloroform. (e) Transmission electron microscopy image of polymer nanocapsules after complex formation.

DA18C6 with Cu^{2+} and Co^{2+} do not enter the nanocapsules (see Supporting Information).

Nanocapsules containing the complexes can be kept in methanol with no noticeable release of entrapped content. In contrast with colorless empty nanocapsules (Figure 2a), nanocapsules containing metal complexes are colored (Figure 2b,c). Cobalt cation is colored green when forming complexes with nitrogen-containing ligands,[8] as opposed to being red in aqueous solutions in free form.

UV–vis spectra further confirmed the formation of the complexes (Figure 3). Both copper and cobalt complexes show characteristic shifts in absorbance maxima upon complexation.[7b,9] In the case of cobalt, the UV–vis spectrum illustrates the change from red color of free cobalt nitrate in water to green corresponding to the complex between cobalt cation and DA18C6.

Nanocapsules containing these complexes can be dried, resuspended, and precipitated without loss of content (Figure 4).

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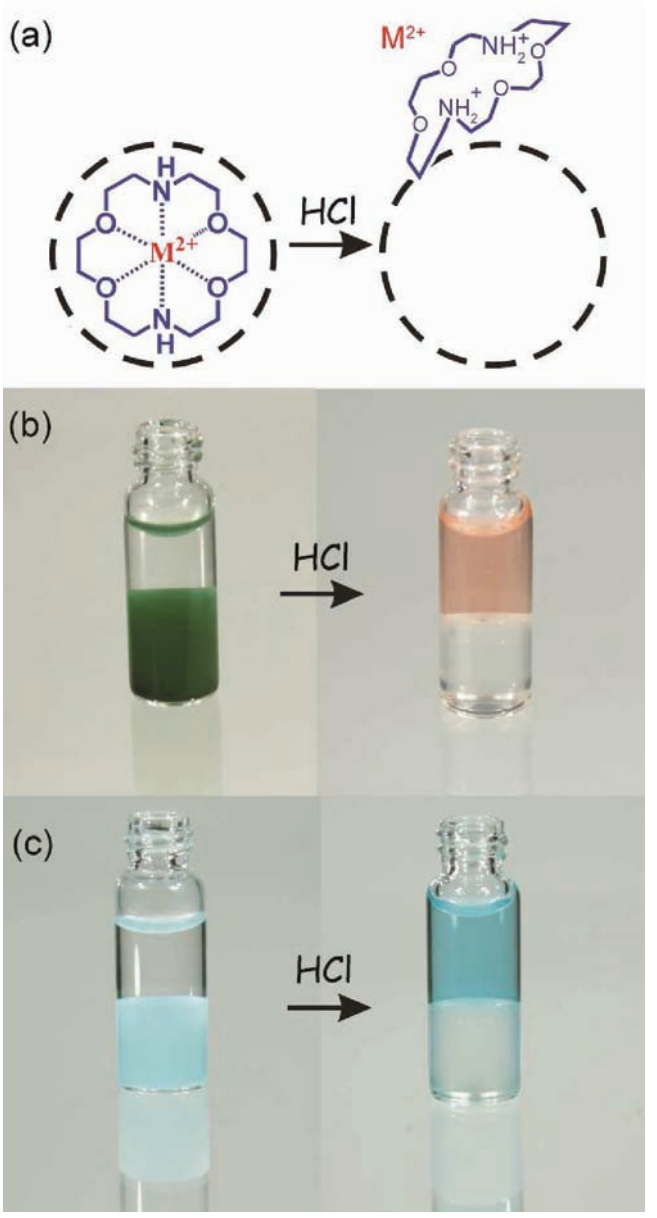

Figure 5. Triggered release of entrapped molecules from nanocapsules. (a) The complex between DA18C6 and metal cations is dissociated upon addition of HCl. The cation and DA18C6 are now free to leave the nanocapsule. (b and c) Acid-induced dissociation of complexes of DA18C6 with Co^{2+} and Cu^{2+}, respectively. Bottom layer, suspension of nanocapsules containing entrapped complexes in chloroform; top layer, water.

In these experiments, we freeze-dried the capsules from *tert*-butanol (Figure 4a), resuspended them in chloroform (Figure 4b), and precipitated them by the addition of methanol (Figure 4c). Colorless supernatant above the colored precipitated capsules suggests that complexes remained encapsulated during these manipulations. UV−vis spectra of the supernatants showed no metal ions. In this work, we used nanocapsules with the diameter of approximately 100 nm (Figure 4d,e), which is 2 orders of magnitude greater than the size of the entrapped complexes.

Dissociation of the complex causes the release of the encapsulated content. Complexes between DA18C6 and metal ions can be easily destroyed in acidic conditions via the protonation of DA18C6. Once the complex is broken, individual components are free to escape from the capsule (Figure 5a). To demonstrate this induced release of the encapsulated content, we placed water on top of the suspension of capsules containing complexes in chloroform and then added HCl to the aqueous layer. Before the HCl addition, the aqueous solution was colorless because the complex was retained within the capsules in the organic layer (Figure 5b,c). Shortly after the addition of HCl to the aqueous layer and brief agitation, aqueous solutions became colored (Figure 5b,c). As expected, the dissociation of the complex and extraction of the metal ions into the aqueous layer was accompanied with the color change, in agreement with UV−vis spectra of the free and complexed forms of Co^{2+} and Cu^{2+} (Figures 3 and 5). The color change is especially striking for cobalt (Figure 5b), where the transition from green to red unambiguously confirmed the dissociation of the complex.

In summary, we showed successful reversible co-entrapment of components that form a noncovalent complex. In this approach, components can enter the capsule individually through the nanopores. The complex is too big to pass through the pores, and it remains entrapped. The dissociation of the complex upon external stimulus triggers the release of individual components. In this work, we used a conformationally flexible crown compound. It is likely that this concept will be applicable to other systems, where the minimal cross section of the complex is bigger than the effective minimal cross sections of individual components. One can envision the evolution of this concept into metal-mediated release of crown compounds, for example, those with antibacterial activity[10] or into noncovalent assembly of functional devices, such as nanoreactors or nanosensors. This approach may be further extended to other porous materials with uniform nanopores.[11]

■ ASSOCIATED CONTENT

Supporting Information. Sample preparation procedures and control experiments including the synthesis of nanocapsules, entrapment of complexes, and triggered release from nanocapsules; complete ref 1a. This material is available free of charge via the Internet at http://pubs.acs.org.

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