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A pH-Responsive Molecular Capsule with an Acridine Shell: Catch and Release of Large Hydrophobic Compounds

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Unlike common polyaromatic hydrocarbons, acridine is a characteristic compound bearing both π-stackable large surfaces and a protonable nitrogen atom. Here we report the first synthesis of a supramolecular capsule with multiple acridine panels. In water, the assembly and disassembly of the capsule reversibly occur under neutral and acidic conditions, respectively (≥10 cycles). Notably, the pH-responsive capsule encapsulates a variety of large hydrophobic compounds (up to 1.6 nm in diameter) such as coumarins, phthalocyanines, and subphthalocyanine in neutral water and subsequently releases them by simple addition of acid.

π-Stacking interactions are superior tools to construct well-controlled supramolecular assemblies with characteristic photo- and electrochemical properties.[1,2] Various columnar nanostructures composed of polyaromatic rings (e.g., triphenylene, hexabenzocoronene, and phthalocyanine rings) have been prepared through infinite π-stacking interactions.[3] However, as compared with other non-covalent interactions such as hydrogen-bonding interactions and hydrophobic effects, π-stacking interactions themselves are less sensitive to changes in external environment (e.g., temperature, light, and pH). Anthracene is an exceptional polyaromatic compound capable of changing its stereostructure upon light irradiation, reversibly (Fig. 1a, left)[4] but insensitive to pH change like most of other polyaromatic rings. On the other hand, acridine is an anthracene-shaped polyaromatic compound but provides both π-stackable large surfaces and a reversibly protonable nitrogen atom (Fig. 1a, right).[5] We thus envisioned that the incorporation of multiple acridine switches into supramolecular systems, which has not been accomplished in contrast to that of anthracene and other polyaromatic panels,[6,7] could develop novel pH-responsive polyaromatic nanostructures (Fig. 1b). Although there are copious reports on stimuli-responsive supramolecular cages and capsules consisting of *aliphatic and small aromatic frameworks*, their host capabilies toward large hydrophobic compounds (>1 nm) have been so far less explored.[8]

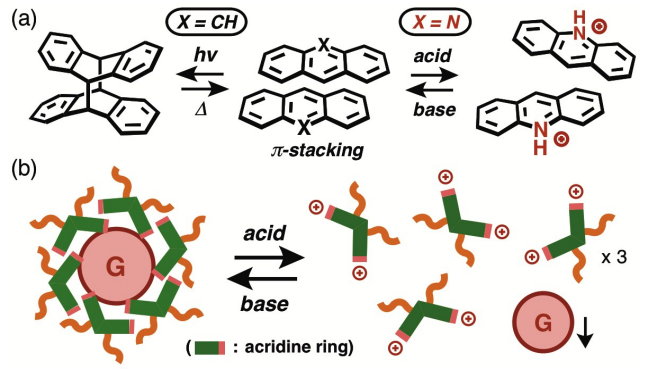

Fig 1. (a) Reversible dimerization of anthracenes (left side) and protonation of acridines (right side). (b) Cartoon representation of the catch and release ability of a pH-responsive capsule with multiple acridine rings toward large hydrophobic compounds (G).

pH-Responsive compound **1** designed here is composed of two acridine rings and two trimethylammonium groups connected by a *meta*-phenylene spacer (Fig. 2a). The simple and rigid V-shaped framework is characteristic of this new amphiphilic compound (Fig. 2b). Herein we report that bent bisacridine amphiphiles **1** assemble into spherical capsule **2** in water through π-stacking interactions and the hydrophobic effect *under neutral conditions*, in a manner similar to bisanthracene-based analogues.[9] In marked contrast, *under acidic conditions*, the neutral polyaromatic panels of **2** convert to cationic ones due to protonation of the terminal nitrogen atoms. Accordingly, the π-stacked polyaromatic capsule can disassemble into monomeric species (**1'**) by electrostatic repulsion (Fig. 1b). In addition, we demonstrate, by the combination of the dynamic behavior and host capability, the catch and release of various hydrophobic compounds with diameters of 0.6-1.6 nm (*e.g.*, coumarins, phthalocyanines, and subphthalocyanine) by pH-responsive capsule **2** in water at ambient temperature.

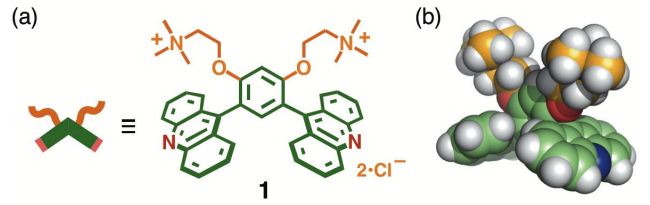

Fig. 2 (a) Bent bisacridine amphiphile **1** designed herein and (b) the structure of the cationic part optimized by DFT calculation (B3LYP/6-31G* level).

Six-step syntheses including Negishi cross-coupling reaction afforded amphiphilic compound **1** in ~30% overall yield and the structure of the obtained product was characterized by NMR and ESI-TOF MS analyses (Fig. S1-18).[10] In neutral water (2.0 mL), compounds **1** (1.4 mg, 2.0 μmol) were quantitatively assembled into spherical capsule **2** at room temperature within 1 min. The ^{1}H NMR spectrum of **2** showed the upfield shifts of the aromatic signals $H_{a\text{-}f}$ ($\Delta\delta_{max}$ = –1.65 ppm for H_b) as compared with that of **1** in CD_3OD (Fig. S13), which indicates efficient π-stacking interactions between the bisacridine moieties (Fig. 3a). Whereas micellar product **2** is not stable enough under ESI-TOF MS conditions, the dynamic light scattering (DLS) analysis and atomic force microscopy (AFM) of **2** confirmed the quantitative formation of spherical assemblies $(\mathbf{1})_n$ (n = ~5) with a core diameter of approximately 1 nm (Fig. S23,24).[10]

pH-Responsive assembly and disassembly behavior of supramolecular capsule **2** was observed in water at room temperature. When an aqueous HCl solution (8.0 equiv. based on **1**) was added to a D_2O solution of **2** (2.0 mM based on **1**), the colorless solution quickly turned yellow and the proton NMR signals were dramatically changed (Fig. 3a,b). All of the aromatic signals ($H_{a\text{-}f}$) were shifted downfield, indicating disassembly of **2** due to protonation of the acridine panels. The aromatic signal H_b was shifted downfield by +1.55 ppm. Notably, protonated compounds **1'** (= **1**•2HCl) were re-assembled into capsule **2** through deprotonation by addition of NaOH aq. (8.0 equiv.) within 1 min, as confirmed by the ^{1}H NMR spectrum (Fig. 3c). The UV-visible absorption and fluorescence studies of capsule **2** also supported the reversible assembly-disassembly process. The new absorption band derived from **1'** was observed at λ_{max} = 418 nm upon addition of HCl aq. (8.0 equiv.) to capsule **2** in water (Fig. 3d, step *i*). The original absorption bands of **2** (280-430 nm) were recovered by addition of an equivalent molar of NaOH aq. to **1'** (Fig. 3d, step *ii*). The emission band of **2** (λ_{max} = 473 nm) also reversibly converted to that of protonated species **1'** (λ_{max} = 650 nm) under the similar acidic conditions (Fig. 3e, steps *i-ii*). Importantly, the assembly-disassembly cycle could be repeated more than 10 times (Fig. 3f and S29), without decomposition of the component of capsule **2** and notable influence of the increase in ionic strength.

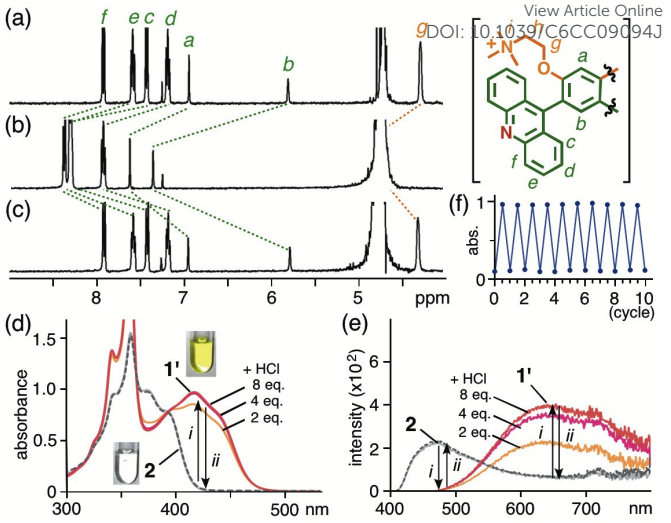

Fig. 3 ^{1}H NMR spectra (400 MHz, D_2O, r.t.) of (a) capsule **2**, and the aqueous solution after (b) addition of HCl aq. (8.0 equiv. based on **1**) and (c) subsequent addition of NaOH aq. (8.0 equiv.). (d) UV-visible spectra (r.t., H_2O) and (e) fluorescence spectra (r.t., H_2O, λ_{ex} = 357 nm) of **2** after (*i*) addition of HCl aq. (up to 8.0 equiv. based on **1**) and (*ii*) subsequent addition of NaOH aq. (up to 8.0 equiv. based on **1**, a dotted line). (f) Assembly-disassembly cycles of **2** under neutral-acidic conditions, monitored by the UV-visible spectra (plot of the intensity of the absorption band at 415 nm).

Next we revealed that acridine-shelled capsule **2** can encapsulate hydrophobic compounds, which are subsequently released by pH-stimuli in water. When hydrophobic coumarin **3a** (1.6 μmol) was stirred in a D_2O solution (2.0 mL) of capsule **2** (2.0 mM based on **1**) for 30 min at room temperature, 1:2 host-guest complex $\mathbf{2}\supset(\mathbf{3a})_2$ was quantitatively obtained (Fig. 4a, left side) after separation of excess **3a** suspended in the resultant solution by centrifugation and filtration.[10] In the ^{1}H NMR spectrum (Fig. 4b), the signals derived from the polycyclic framework ($H_{C\text{-}J}$) of **3a** were shifted significantly upfield ($\Delta\delta_{max}$ = –3.24 ppm) due to the encapsulation in the polyaromatic shell of **2**. On the other hand, the ethyl signals ($H_{A,B}$) of **3a** were almost unchanged, indicating their penetration through the capsule shell. The NMR integrals of the product indicated the **1**:**3a** molar ratio to be 3:1 and the DLS analysis elucidated the diameter of the product being 1.9 nm (Fig. 4e, right side), which agrees with the formation of a spherical $(\mathbf{1})_6\supset(\mathbf{3a})_2$ structure in water (Fig. 4f). Next, an aqueous HCl solution (8.0 eq. based on **1**) was added to the resultant solution to release the encapsulated **3a** from the cavity of capsule **2** (Fig. 4a, right side). The ^{1}H NMR signals of both **2** and **3a** were replaced by those of **1'** within 1 min at room temperature (Fig. 4c). Released molecules **3a** were suspended in the aqueous solution due to the hydrophobic feature. It should be noted that the released **3a** was re-encapsulated into capsule **2** after (i) addition of an aqueous NaOH solution (8.0 eq. based on **1**) and (ii) sonication (42 kHz, 70 W, 30 min), through the deprotonation of **1'** and the formation of **2**. The resultant ^{1}H NMR spectrum revealed the quantitative regeneration of the original host-guest complex, $\mathbf{2}\supset(\mathbf{3a})_2$ (Fig. 4d).

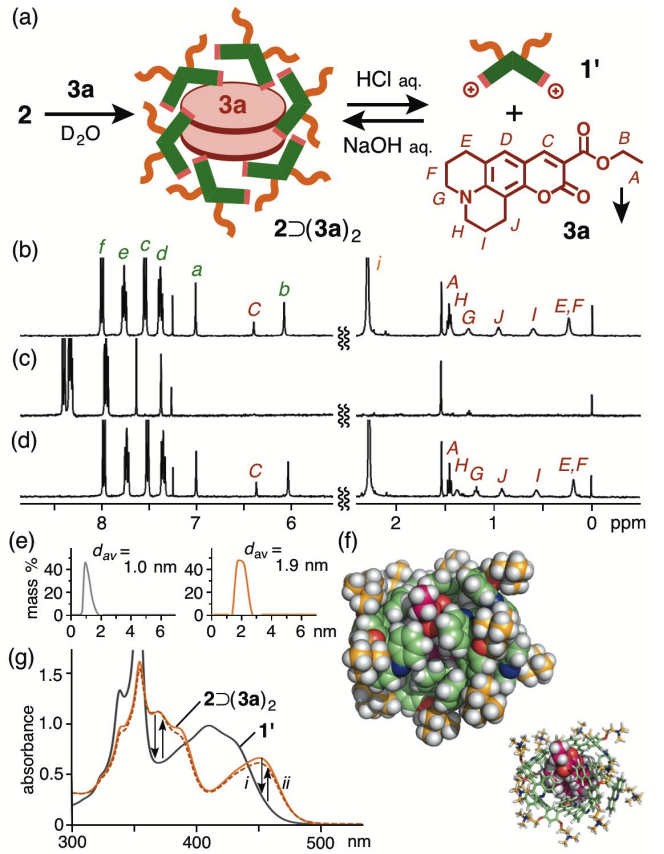

Fig. 4 (a) pH-Responsive encapsulation and release of coumarin **3a** by capsule **2** in water. ^{1}H NMR spectra (400 MHz, D_2O, r.t.) of **2**⊃(**3a**)$_2$ (b) before and (c) after the addition of HCl aq. (8.0 equiv. based on **1**) and then (d) after the addition of NaOH aq. (8.0 equiv. based on **1**). (e) DLS analysis (H_2O, r.t.) of **2** (left side) and **2**⊃(**3a**)$_2$ (right side). (f) Molecular modeling of **2**⊃(**3a**)$_2$ without the counterions. (g) UV-visible spectra (r.t., H_2O) of **2**⊃(**3a**)$_2$ after (*i*) addition of HCl aq. (8.0 equiv. based on **1**) and (*ii*) subsequent addition of NaOH aq. and sonication (8.0 equiv. based on **1**, a dotted line).

The encapsulation and release processes were also monitored by UV-visible spectroscopy. A new absorption band derived from encapsulated **3a** was observed around 415-500 nm by mixing an aqueous solution of **2** with **3a** (Fig. 4g). Relative intensity of the host-guest absorption bands also supports the formation of 1:2 host-guest complex **2**⊃(**3a**)$_2$. The absorption band of **3a** disappeared under acidic conditions (Fig. 4g, step *i*) and subsequently reappeared upon neutralization (Fig. 4g, step *ii*).[11] Fluorescent emission of **3a** (Φ_F = 76% in CH_2Cl_2) was suppressed within **2** (Φ_F = 7% in H_2O), indicating the relatively strong interactions between the acridine frameworks of **2** and **3a** in neutral water (Fig. S34). In a manner similar to **3a**, the catch and release of medium-sized hydrophobic compounds such as DCM (**3b**) and Nile Red (**3c**) were carried out using pH-responsive capsule **2** in water (Fig. S35). In sharp contrast, the analogous host-guest complexes composed of the anthracene-shelled capsule[9] and **3a-c** displayed no releasing ability under acidic conditions (Fig. S39).[10] Therefore, protonation of the polyaromatic panels of capsule **2** is essential for the guest release.

Flexibility and pH-responsiveness of the polyaromatic shell of capsule **2** make it possible to encapsulate and release large hydrophobic compounds. Planar blue pigment, Cu(II)-phthalocyanine (**3d**), with a diameter of 1.6 nm was solubilized in water upon encapsulation within capsule **2** by a sequence of grinding a 2:1 mixture of amphiphile **1** and **3d** for 1 min, addition of H_2O, and filtration of the resultant suspended solution.[10] Then, to the obtained blue solution of host-guest complex **2**⊃(**3d**)$_2$, an aqueous HCl solution (8.0 equiv. based on **1**) was added at ambient temperature to release the encapsulated **3d** from **2**. The UV-visible spectrum of **2**⊃(**3d**)$_2$ showed new broad absorption bands derived from encapsulated **3d** around 480-820 nm.[12] The absorption bands vanished upon addition of the acid to the aqueous solution owing to precipitation of the released **3d** (Fig. 5a). By the same way, two molecules of perfluorinated Cu(II)-phthalocyanine **3e** accommodated in the cavity of capsule **2** were also released by the acid stimulus in water.

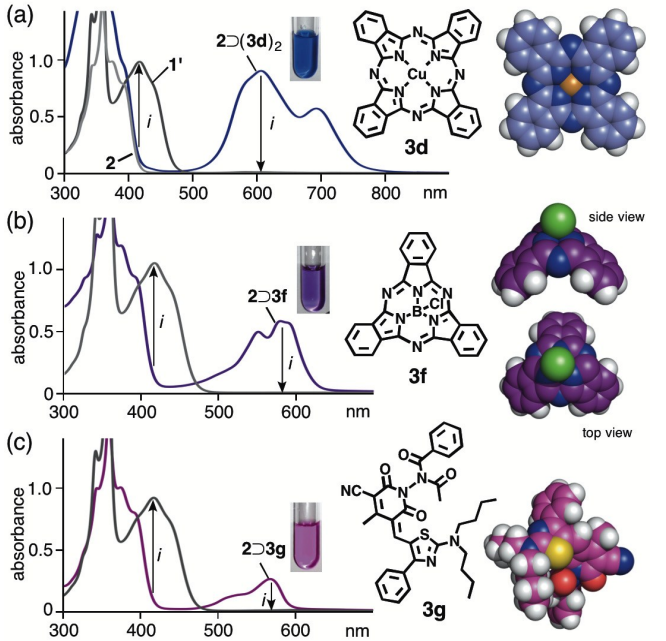

Fig. 5 UV-visible spectra (r.t., H_2O) of (a) **2**⊃(**3d**)$_2$, (b) **2**⊃**3f**, and (c) **2**⊃**3g** before and after (*i*) addition of HCl aq. (8.0 equiv. based on **1**). The structures of **3d**, **3f**, and **3g**, and their molecular modeling.

Non-planar and bulky large compounds, *i.e.*, subphthalocyanine (**3f**)[13] and pigment **3g**,[14] were also caught and released by capsule **2** in water. Whereas many water-soluble **3f** derivatives have been synthesized as building blocks in biological applications, reversible switching the water-solubility of **3f** by non-covalent functionalizations has been undemonstrated to date.[13,15] In a manner similar to the preparation of **2**⊃(**3d**)$_2$, a violet aqueous solution of **2**⊃**3f** was obtained through the grinding of a mixed solid of **1** and hydrophobic **3f** (1.1 nm in diameter). The UV-visible spectrum displayed new absorption bands corresponding to the encapsulated **3f** in the range of 430-660 nm (Fig. 5b). The broadening and red-shifts (+22 nm) of the bands, as compared with absorption bands of free **3f** in CH_2Cl_2 (Fig. S37), indicate the strong interactions between **3f** and the acridine panels of **2**. In contrast to planar compounds such as **3a** and **3d**, one molecule of bowl-shaped **3f** was encapsulated in the cavity of **2**, which was confirmed by UV-visible and DLS analyses (Fig. S36,38).[16] Similarly, a magenta aqueous solution of 1:1 host-

guest complex **2⊃3g** was formed from **1** and bulky hydrophobic pigment **3g** (1.5 nm in diameter) (Fig. 5c). The encapsulated compounds **3f** and **3g** were quantitatively released from the capsule cavities under acidic conditions at room temperature. The UV-visible absorption bands of **3f** and **3g** completely disappeared by addition of HCl solutions to the aqueous solutions of **2⊃3f** and **2⊃3g**, respectively (Fig. 5b,c).[17] It is worthy of note that the released water-insoluble dyes could be fully recovered by simple filtration.

In conclusion, we have designed and constructed a pH-responsive host-guest system using multiple acridine panels, which provide both π-stackable large surfaces and a protonable nitrogen atom. Simple bisacridine amphiphiles assemble into a spherical capsule with an acridine shell in neutral water. In contrast, under acidic conditions, the capsule reversibly disassembles into monomeric species due to the protonation of the acridine panels. The assembly-disassembly cycle can be repeated more than 10 times without decomposition of the capsule component. Moreover, the pH-responsive capsule catches a variety of large hydrophobic compounds (up to 1.6 nm) such as planar metallophthalocyanines and bowl-shaped subphthalocyanine in neutral water and subsequently releases them by simple acidification. The present strategy for the reversible control of π-stacking interactions by pH change would prompt further development of stimuli-responsive supramolecular compounds and materials.

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† Electronic Supplementary Information (ESI) available: Experimental procedures and physical properties of amphiphilic compound **1**, molecular capsule **2**, and its host-guest complexes. See DOI: 10.1039/x0xx00000x

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10 See the Supporting Information.

11 Fig. 4g (after step *i*) was obtained after (i) the addition of HCl aq. (8.0 eq. based on **1**) to the **2⊃(3a)**$_2$ solution and (ii) the filtration of the resultant solution to remove suspended **3a**. Fig. 4g (after step *ii*) was obtained after (i) the addition of NaOH aq. (8.0 eq. based on **1**) and **3a** (0.5 mg) to the acidic **1'** solution, (ii) the stirring and sonication of the resultant mixture for 30 min, and (iii) the removal of the excess **3a** by filtration.

12 The relative intensity of the host and guest absorption bands indicated the formation of 1:2 host-guest complexes **2⊃(3d)**$_2$ and **2⊃(3e)**$_2$.[10] In addition, the DLS analysis of **2⊃(3d** or **3e)**$_2$ elucidated the presence of small particles with an average diameter of 1.9-2.0 nm (Fig. S38).

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16 In a manner similar to previous systems[9] using anthracene panels, ^{1}H NMR signals derived from the encapsulated, highly hydrophobic guests (*e.g.*, **3d** and **3f**) were significantly broadened within **2** owing to restriction of the molecular motion by the limited cavity (Fig. S43)

17 As control experiments, bulky large compounds **3f** and **3g** were poorly dissolved in water even by using excess SDS (10 mM; Fig. S40).[10] In addition, the host-guest composites showed no pH-responsive properties at ambient temperature (Fig. S42).

