

Cooperative Metal Ion Binding to a Cucurbit[7]uril–Thioflavin T Complex: Demonstration of a Stimulus-Responsive Fluorescent Supramolecular Capsule

Sharmistha Dutta Choudhury, Jyotirmayee Mohanty,* Haridas Pal, and Achikanath C. Bhasikuttan*

Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

Received October 15, 2009; E-mail: jyotim@barc.gov.in; bkac@barc.gov.in

Abstract: We report an intriguing noncovalent interaction of thioflavin T (ThT), a fibril diagnostic dye, with the versatile macrocyclic host molecule cucurbit[7]uril (CB7) in the presence of metal cations. ThT forms both 1:1 (CB7·ThT) and 2:1 [(CB7)₂·ThT] complexes with CB7 host, leading to specific structural arrangements. Addition of competitive guests like metal cations to the 1:1 stoichiometric complex displays expected competitive binding interactions with CB7, leading to decreased fluorescence intensity from ThT. However, addition of metal ions to the 2:1 complex leads to unusual enhancement in the fluorescence emission (~270-fold in the presence of Ca²⁺ and ~160-fold in the presence of Na⁺). These contrasting observations on the fluorescence enhancement with change in the stoichiometric equilibrium have been investigated explicitly for a feasible binding model. Detailed photophysical characterization with supporting data from NMR and anisotropy measurements has led to the revelation of a novel stimulus-responsive cooperative metal ion binding to the stoichiometrically selected (CB7)₂·ThT complex, demonstrating a highly fluorescent supramolecular nanocapsule. The first example of a noncovalently packed fluorescent complex became feasible due to the structural arrangement of the host–guest complex in the 2:1 stoichiometry with two CB7 portals providing strong negative charge density for the metal ions to group and seal the complex, thus protecting the incorporated dye. To further strengthen the usefulness of the supramolecular capsule established here, rupture of the capsular complex has been demonstrated with a strong competitive guest, 1-amantadine hydrochloride, which helped in disrupting the capsule to release the dye. It is proposed here that by judicious design of the chromophore (guest) structure, such capsular assemblies can be explored for the binding and release of drug molecules, for fluorescence on–off systems, and as building blocks for molecular architectures displaying unique properties.

Introduction

Design and demonstration of structurally well-defined functional materials with dynamic and stimulus-responsive properties have attracted a great deal of attention in recent years because of their direct relevance in molecular architectures,^{1–3} targeted drug delivery,^{4–7} nanocapsules,^{4,8} on–off sensors,^{9,10} and cooperative and responsive phenomena, particularly in biological systems.¹¹ One of the many different strategies for making

synthetic photofunctional materials takes advantage of self-assembly, the method by which well-ordered architectures are formed spontaneously from individual components. Host–guest chemistry has been in the forefront of this area as it can direct and thread molecular components according to designs under proper selection criteria.^{1–3} Many attempts have been made over the last few decades to form such noncovalently linked host–guest complexes by use of classical synthetic macrocyclic host molecules like cyclodextrins, calixarenes, etc. but limitations often arose due to the solubility and stability of the complexes. A new entrant to this class of macrocycles is the cucurbit[*n*]urils (CB*n*), having excellent complexation properties toward cationic guest molecules.^{12,13} Cucurbiturils (CBs) are macrocyclic container molecules composed of *n* (5–10) gly-

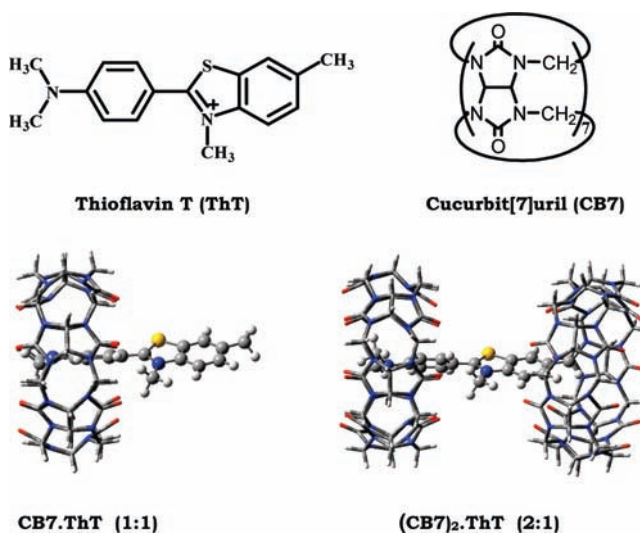
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coluril monomers, joined by pairs of methylene bridges.^{12,13} Structurally, CBs constitute highly symmetrical pumpkin-shaped supramolecular hydrophobic cages of low polarity and polarizability^{14,15} with two identical dipolar portal ends composed of carbonyl functional groups.^{12,13} CBs have gained immense research interest because of their ability to host in a highly selective manner certain types of guest molecules such as, small organic dyes through hydrophobic interaction,^{12,13} metal cations,^{12,13,16–18} protonated alkyl and aryl amines,^{12,13,19,20} and cationic dyes like rhodamines,^{21,22} triphenylmethane dyes,¹¹ and porphyrins,²³ mainly through ion–dipole interaction.

In general, the stability constants of the cucurbituril complexes are larger than those of the corresponding cyclodextrins with the same guest, and they can be several orders of magnitude larger when the guest is cationic.^{19,24} Since the supramolecular assemblies are held together by relatively weak noncovalent interactions, the preferential affinity of these forces and hence the structure and stoichiometry of the complex brings out significant modulation in the molecular properties of the guest.^{9,10,23} Simultaneous association of multiple hosts or guests by either cooperative binding¹¹ or competitive displacement^{5,25} is another idea to construct novel assembled systems with diverse characteristics. On the other hand, the fact that the binding interactions are mainly noncovalent in nature makes it convenient to tune them by wide range of stimuli including cooperative/competitive binding guests/hosts,^{5,11,25} pH,²⁶ temperature, light,⁹ redox control,²⁷ etc. In this context, recently we have shown that cucurbit[7]uril, CB7, macrocycle acts as an enhancer by cooperatively binding with a dye–protein assembly, projecting its usefulness in many biological applications.¹¹ This thought has attracted immense research interest due to its tremendous potential in many applied areas, especially in constructing sensors,^{9–11} substrate selective tandem assays,^{28,29} binding and release actions for drug delivery,^{4–7} on–off switches,^{9,10} etc. Advantage of this stimulus response has been exploited for the relocation of a biologically important dye,

Scheme 1. Chemical Structures of Thioflavin T, Cucurbit[7]uril, and the Geometry-Optimized Structures for the 1:1 and 2:1 (CB7:ThT) Complexes



neutral red, from CB7 cavity to a protein moiety⁵ by making use of the supramolecular pK_a shift^{19,20} and the action of a competing metal cation.⁵ The competing metal ion strategy has been further explored in affecting the highly fluorescent excimer emission from the complexation of cucurbit[8]uril (CB8) with a fibril diagnostic dye, thioflavin T (ThT) (Scheme 1), proposing a Ca^{2+} induced on–off control.⁹ ThT is well-known for its dramatic fluorescence enhancement upon binding to the structural cavities of amyloid fibrils³⁰ and is extensively used in the early detection of several neurodegenerative diseases like Alzheimer's and Parkinson's diseases.^{30,31} It is widely accepted that imposing restriction on the torsional motion among the benzthiazole and dimethylanilinium groups of ThT leads to significant reduction in the nonradiative processes, thus favoring its radiative transition.^{32,33} The intriguing specificity of ThT toward the nanopores of amyloid fibrils, resulting in amazing fluorescence enhancement (about 1000-fold),^{34,35} has prompted many curious studies in restricted host environments like cyclodextrins,³³ cucurbiturils,^{9,36} micelles,³⁷ Nafion,³⁸ cellulose,³⁸ etc. Structurally, the positioning of the cationic charge on the benzthiazole ring of ThT would allow the encapsulating host from either end, setting chances for multiple stoichiometric equilibrium. Our recent studies on ThT with CB7 and CB8 macrocycles have shown that the nature

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and stoichiometry of the CB7³⁶ and CB8⁹ complexes are strikingly different with contrasting spectroscopic features. ThT forms both 1:1 and 2:1 [(CB7)₂·ThT] complexes with CB7,³⁶ whereas 1:1, 1:2, and 2:2 (CB8·ThT) stoichiometries are preferred in the case of CB8 as the host.⁹ When the cases of multiple host/guest association are considered, as in the 2:1 complex of CB7 and the 2:2 complex of CB8, an increase in the ionic strength of the solution would increase the ability of the cations to compete with the organic guest for binding at the CB portals, thus releasing free dye.^{5,17,25,39} This is exactly what we have established in the case of the 2:2 CB8·ThT complex by using metal ions.⁹ However, attempting a similar competitive displacement with metal ions on the (CB7)₂·ThT complex, we were surprised to observe a remarkable fluorescence enhancement when salts were added to the solution, pointing to an unusual and intriguing interaction of the metal ions on the (CB7)₂·ThT complex resulting in a significant effect on the characteristics of ThT. In this article, we report for the first time a novel and interesting cooperative metal ion binding to a stoichiometrically selected cucurbit[7]uril–thioflavin T complex, demonstrating a fluorescent supramolecular capsule and its controlled release, projecting its possible implications in the host–guest molecular assembly.

Experimental Section

Thioflavin T [3,6-dimethyl-2-(4-dimethylaminophenyl)benzothiazolium cation; ThT] obtained from Sigma–Aldrich was purified by column chromatography on a silica gel column with mildly acidic methanol (1 mL of 1 N HCl in 500 mL of methanol) as eluent. Cucurbit[7]uril and 1-amantadine hydrochloride were obtained from Aldrich and Sigma, respectively, and were used as received. We mention here that commercial CB7 sample usually contains a considerable amount of salts, which may compete with the guest for binding at the CB7 portals. However, considering the very low binding constant values for the CB7–salt interaction ($K_{\text{CB7-Na}} \sim 100 \text{ M}^{-1}$, $K_{\text{CB7-Ca}} \sim 700 \text{ M}^{-1}$)⁵ and the molar concentrations of the salts used in the present experiments, the contribution from the salts present in the CB7 sample on the observed results can be neglected. Nanopure water (conductivity less than $0.06 \mu\text{S cm}^{-1}$), obtained from a Millipore Gradient A10 system, was used to prepare the sample solutions. Absorption spectra were recorded with a Jasco V530 UV–vis spectrophotometer (Tokyo, Japan). Steady-state fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). The samples were excited at 390 nm or at the isosbestic point, where the changes in the optical density were minimal. Time-resolved fluorescence measurements were carried out on a time-correlated single-photon-counting (TCSPC) spectrometer (IBH). In the present work, a 408 nm diode laser (100 ps, 1 MHz repetition rate) was used for excitation and a microchannel plate photomultiplier tube (MCP PMT) was used for fluorescence detection. With the present setup, the instrument time resolution is judged to be better than 50 ps. The ¹H NMR experiments were performed at ambient temperature in D₂O (99.8%). ¹H NMR spectra (500 MHz) were recorded on a Bruker Avance WB spectrometer at Tata Institute of Fundamental Research (TIFR), Mumbai, India. Throughout the text the stoichiometric composition has been represented as host:guest ratio.

Results and Discussion

i. Steady-State Absorption and Fluorescence Measurements. An aqueous solution of thioflavin T displayed very poor fluorescence properties with emission quantum yield ~ 0.0003 .^{34,35} Figure 1 presents the absorption and emission spectra of ThT ($\sim 3 \mu\text{M}$) in water, showing an absorption maximum at 412 nm

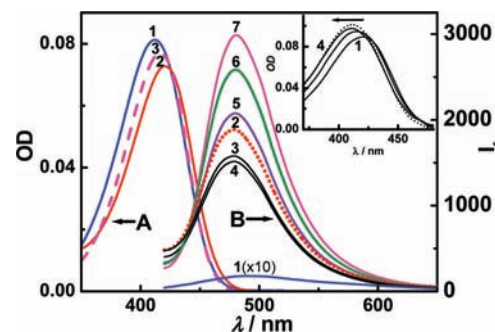


Figure 1. (A) Absorption spectra of ThT ($3 \mu\text{M}$) in water (spectrum 1), with 1 mM CB7 (spectrum 2), and with 1 mM CB7 and 1 M NaCl (spectrum 3). (B) Fluorescence spectra of ThT ($3 \mu\text{M}$) in water (enlarged by 10 times) (spectrum 1) and with 1 mM CB7 in the presence of [NaCl]/M: 0 (spectrum 2), 0.001 (spectrum 3), 0.05 (spectrum 4), 0.3 (spectrum 5), 0.5 (spectrum 6), and 1.0 (spectrum 7). (Inset) Absorption spectra for ThT ($3 \mu\text{M}$) in water in the presence of CB7 ($5 \mu\text{M}$) and [NaCl]/M: 0 (spectrum 1), 0.05 (spectrum 2), and 1.0 (spectrum 3). The dotted line (spectrum 4) represents the absorption spectrum of ThT alone.

and a weak emission band centered at $\sim 490 \text{ nm}$. Addition of increasing amounts of CB7 host to this ThT solution provided noticeable changes in the absorption and emission spectral profiles, and those recorded in the presence of 1 mM CB7 are shown in Figure 1. As reported earlier,³⁶ the details of the complexation interaction evaluated from the absorption and emission measurements have established a 1:1 stoichiometry for the CB7·ThT complex at lower concentration of CB7 ($\sim 5 \mu\text{M}$), whereas a 2:1 (CB7)₂·ThT complex is preferred when the CB7 concentration is much higher ($\sim 1 \text{ mM}$). These stoichiometric assignments were aptly supported from the changes in the proton NMR signals and also through the optimized geometrical structures, which are shown in Scheme 1.³⁶ Following the competing guest strategy to selectively achieve the dissociation of the complex, we further titrated the complex solution with metal ions. To our surprise, addition of NaCl to the CB7–ThT solution led to remarkable enhancement in the fluorescence intensity after an initial dip (Figure 1B). This striking observation, just opposite to that anticipated, was intriguing and an immediate qualitative trial indicated that the enhancement indeed depends on the amount of macrocycles present, that is, the stoichiometric ratio of the system, and also on the nature of added cation. In a control experiment it has been verified that the absorption and emission spectra of ThT do not show any significant change in the presence of salt (NaCl $\sim 1 \text{ M}$). Since CB7–ThT complexes exist in different stoichiometric combinations, we have undertaken a systematic study and carried out titrations with different metal ions under two specific solution conditions where the concentrations of CB7 and ThT were preset to have predominantly 1:1 or 2:1 complexes.

Figure 2 shows the binding curve measured at 490 nm for ThT upon titration with CB7 (curve a). The region where 1:1 stoichiometry is predominant (i.e., at lower concentrations of CB7) is separately shown in the inset of Figure 2. As indicated in the Figure 2 inset, addition of NaCl to the 1:1 complex ([CB7] $\sim 5 \mu\text{M}$) drastically decreased the fluorescence intensity, with a concomitant retraction of the absorption maximum of the complex from 421 to 412 nm (Figure 1 inset). These changes, indicating the release of ThT, are commensurate with the changes expected on the basis of a competitive binding interaction at the carbonyl portal by the metal ion. Interestingly, on the other hand, at higher CB7 concentration ($\sim 2 \text{ mM}$), where

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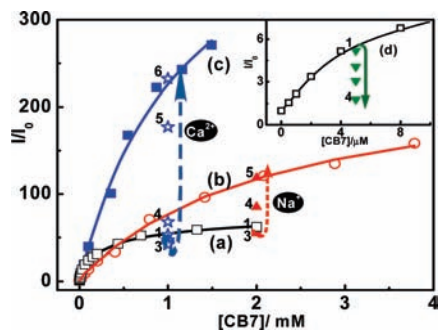


Figure 2. Fluorescence titration curves of ThT (3 μM) with CB7: (a) in the absence of any metal ion, (b) in the presence of 1 M NaCl, and (c) in the presence of 1 M CaCl_2 . Red triangles represent the intensity changes of spectrum a with NaCl/M : 0.001 (1), 0.004 (2), 0.02 (3), 0.1 (4), and 1.0 (5). Blue stars represent the intensity changes of spectrum a with CaCl_2/M : 0 (1), 0.001 (2), 0.005 (3), 0.01 (4), 0.3 (5), and 1.0 (6) at the respective host concentrations. (Inset, d) Titration curve corresponding to 1:1 complex. Green inverted triangles show the intensity changes with NaCl/M : 0.001 (1), 0.01 (2), 0.3 (3), and 1.0 (4).

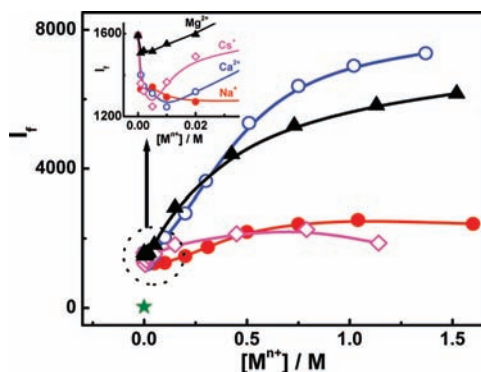


Figure 3. Changes in the fluorescence intensity of $(\text{CB7})_2 \cdot \text{ThT}$ complex in the presence of different concentrations of metal ions $[\text{M}^{n+}]$: Cs^+ (pink diamonds), Na^+ (red solid circles), Mg^{2+} (black triangles), and Ca^{2+} (blue open circles) ions. The green star indicates the fluorescence intensity of the free dye in water. (Inset) Initial region showing a decrease in the fluorescence intensity at lower salt concentration.

the equilibrium shifts to a 2:1 $(\text{CB7})_2 \cdot \text{ThT}$ complex (Figure 2), gradual addition of NaCl up to ~ 50 mM led to a decrease in the fluorescence intensity by $\sim 20\%$, followed by a remarkable increase by ~ 120 -fold upon further increasing the salt concentration to about 1 M (cf. Figures 2 and 3). It may be further noted here that the absorption maximum did not revert completely to that of the free dye, as observed in the case of 1:1 complex even at 1 M concentration of the salt, but remained at ~ 416 nm as shown in Figure 1A. Both these changes directly point to an unusual stoichiometric arrangement of the metal ion with the 2:1 complex. Alternatively, titration of the ThT solution containing NaCl (~ 1 M) displayed a steady increase in the fluorescence intensity to ~ 160 -fold and nearly attained saturation at ~ 4 mM CB7 (Figure 2, trace b). It should be noted here that because the solubility of CB7 in water increases appreciably in the presence of salt,¹³ higher concentration of CB7 could be used for the titration and hence larger enhancement was observed as compared to the results obtained when salt was added later. Though we could estimate the binding constants for the 1:1 [$K_1 = (1.2 \pm 0.7) \times 10^5 \text{ M}^{-1}$] and the 2:1 [$K_2 = (1.8 \pm 0.6) \times 10^3 \text{ M}^{-1}$] CB7-ThT complexes in the absence of added salt,³⁶ similar attempts to evaluate these parameters in the presence of salts did not provide any meaningful values,

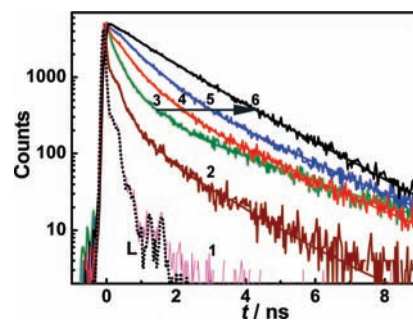


Figure 4. Fluorescence decay traces of ThT (3 μM) at 490 nm (λ_{ex} at 408 nm) under different conditions: ThT alone (trace 1), ThT with 2 μM CB7 (trace 2), ThT with 1 mM CB7 (trace 3), ThT with 1 mM CB7 and 1.5 M NaCl (trace 4), ThT with 1 mM CB7 and 1.5 M CaCl_2 (trace 5), and ThT with insulin fibril (1.5 mg of insulin/mL, pH 1.8 at 60 $^\circ\text{C}$ after 2 h; trace 6). The dotted line L represents the excitation lamp profile. The solid lines represent the fitted exponential decay traces whose values are tabulated in Table 1.

obviously due to various possible interactions and formation of different types of complexes.

In the next step, we examined these intriguing observations for the dependence on the ionic strength of the system. When a bivalent Ca^{2+} ion was used, the emission intensity of the 2:1 complex displayed a sharp decrease during the initial additions of CaCl_2 to ~ 10 mM (Figure 3 inset) but again showed remarkable enhancement to about 250-fold upon increasing the CaCl_2 concentration to about 1 M (Figure 2). Titration with preadded Ca^{2+} also displayed a steady increase in the fluorescence intensity, which was followed up to ~ 270 -fold (Figure 2, trace c), verifying the larger effect of Ca^{2+} on the complex compared to Na^+ . Similar measurements were carried out with other group metal ions like Cs^+ and Mg^{2+} and are compared with that of Na^+ and Ca^{2+} in Figure 3. For both Cs^+ and Mg^{2+} , the emission intensity during the initial additions showed a steeper increase as compared to Na^+ and Ca^{2+} ; however, the overall fluorescence enhancement remained slightly lower. Hence it is judged that the charge of the metal ion, rather than the size, contributes essentially to the observed phenomena. Precisely, the above contrasting observations with 1:1 and 2:1 stoichiometric complexes in the presence of metal ions emphasize that the unusual fluorescence enhancement observed is specific to the $(\text{CB7})_2 \cdot \text{ThT}$ complex and highlights its structural distinctiveness, having two CB7 moieties sandwiching the dye.

ii. Fluorescence Lifetime Measurements. It is well-known that ThT belongs to the class of molecular rotors, which in the excited state undergo very fast nonradiative decay and hence exhibit unusually low fluorescence quantum yield.^{34,35} This has been attributed to the highly efficient torsional movements of the benzothiazole and the dimethylaminobenzene rings of ThT in the excited state.³⁵ Hence, the excited-state lifetime of ThT has been reported to be very short, on the order of a few picoseconds in aqueous solution (beyond the time resolution of the present TCSPC instrument; Figure 4). In the presence of CB7, ThT displayed different decay patterns corresponding to 1:1 and 2:1 stoichiometries, which are presented in Figure 4. Both traces, however, followed triexponential decay kinetics, arguably due to different conformational structures possible.³⁶ The decay profile of the complex, especially for the 1:1 complex, conforms to that of free dye in presence of NaCl, due to the release of free dye. However, in agreement with the steady-state findings, the 2:1 complex registered a significant increase in the decay time constant in the presence of NaCl, especially in the faster decay component. The decay traces were further

Table 1. Excited-State Decay Time Constants Evaluated for CB7·ThT Complexes with Metal Ions and for ThT Bound to Insulin Fibrils

system	τ_1	τ_2	τ_3
CB7·ThT (1:1)	23 ps ^a (62%)	290 ps (21%)	1.6 ns (17%)
(CB7) ₂ ·ThT (2:1)	190 ps (50%)	1.12 ns (24%)	3.13 ns (26%)
(CB7) ₂ ·ThT + NaCl	32 ps ^a (13%)	544 ps (52%)	2.1 ns (35%)
(CB7) ₂ ·ThT + CaCl ₂		637 ps (55%)	2.1 ns (45%)
insulin fibril + ThT		140 ps (4%)	2.2 ns (96%)

^a These values are within the time resolution of the TCSPC instrument used in the present study.

slowed down with Ca²⁺ and the decay kinetics became nearly biexponential (cf. Figure 4). Details of the kinetic analysis for the above-mentioned systems are provided in Table 1. Essentially, all these changes well corroborate and support the contention that both the macrocycle and the metal ions impose severe restrictions on the intramolecular torsional motions of ThT, which otherwise independently are not that effective.

iii. Binding Mechanism. A number of host–guest complexation studies with cucurbiturils have demonstrated that the stability constants of the complexes are dependent on the nature and concentration of the metal ions present. This arises from binding of cations to the cucurbituril portals, modulating the complexation equilibrium and overall stability constant for the complex. Since the carbonyl group in the uril moiety is an excellent donor for cations, stronger interactions with metal ions are expected to occur with cucurbiturils. Kim and co-workers¹⁷ have reported the encapsulation of small and uncharged molecules like tetrahydrofuran or diethyl ether within the CB6 cavity, stabilized by the metal ion binding at the portals. The strong negative charge density at the CB portals encourages clustering of the metal ions along with their coordinated water molecules, which block the cavity entrance like a lid. Recently, Wyman and Macartney⁴⁰ have shown a decrease in the stability constant of small polar organic guests like acetone and acetophenone with CB7 in the presence of metal ion as a result of cation capping of the CB7 portals. When the present results are analyzed in light of the above possibilities, it is believed that the positioning of two carbonyl portals from each of the CB7 moieties increases the negative charge density around the guest ThT and influences the binding of the metal ions. With this concept, and the experimental evidence presented here, the probable structural arrangements and complexation mechanism for the ternary complex are envisaged as in Scheme 2. With the 1:1 complex (I), the metal ions effectively compete for the binding at the carbonyl portals and form ion lids at both the CB7 portals as represented in II, thus displacing the ThT, thereby decreasing the fluorescence intensity. In the case of the 2:1 complex (III), while the terminal portals are sealed by ion

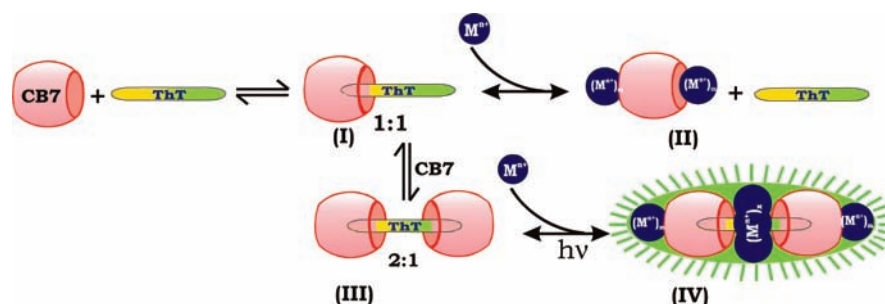
lids, the enhanced negative charge density from the CB7 portals in the central region extends strong attraction for the metal ions and allows assembly of the cationic charges, which cover the region effectively and seal the space in between the two CB7 units. The increased positive charge in the central region also reduces the repulsion between the two carbonyl portals, further stabilizing the complex. Therefore, it is judged that the binding of metal ions with (CB7)₂·ThT cooperatively stabilizes the included ThT in a more rigid and planar structure within a metal ion sealed supramolecular capsule, as envisaged in structure IV. Such an arrangement would indeed restrict the otherwise highly efficient nonradiative torsional motion among the benzothiazole and dimethylaminobenzene rings of ThT, thus reviving its radiative potential. The initial decrease in the fluorescence intensity with salt can be explained due to the dissociation of any available 1:1 complex. It should be noted here that although clustering of metal ions and formation of lids at the CB portals have been reported,^{17,40} the cooperative metal ion binding to a stoichiometrically selected host–guest complex, resulting in a fluorescent capsule, has never been reported before.

iv. Fluorescence Anisotropy Measurements. Since the anisotropy decay rate depends on the effective size of the fluorophore, additional information on the change in the hydrodynamic molecular volume due to the formation of structures I, III, and IV could be extracted from the measurement of anisotropy decay of the dye, $r(t)$, as given in eq 1, where I_{\parallel} and I_{\perp} represent the parallel and perpendicular polarized emission intensities, respectively, and G represents the correction factor for the polarization bias of the detection setup. From the rotational correlation time (τ_r) obtained from the fluorescence anisotropy decay, the hydrodynamic molecular volume can be estimated, which is directly related to rotational diffusion coefficients (D_r) and the viscosity (η) of the medium (water in the present case) according to the Stokes–Einstein relationship (eq 2).¹⁹

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad (1)$$

$$\tau_r = \frac{1}{6D_r} \quad \text{where } D_r = \frac{RT}{6V\eta} \quad (2)$$

where V is the hydrodynamic molecular volume of the complex and T is the absolute temperature. The anisotropy decay traces measured for the 1:1, 2:1 [(CB7)₂·ThT], and 2:1 complex in the presence of 1 M NaCl are shown in Figure 5. An exponential decay analysis of these traces provided $\tau_r \sim 450$ ps for the 1:1 complex (corresponding to an effective hydrodynamic diameter of ~ 15.5 Å),³⁶ ~ 900 ps for the 2:1 complex (diameter ~ 20

Scheme 2. Proposed Binding Interactions in the ThT, CB7, and Metal Ion System Leading to the Highly Fluorescent Supramolecular Capsule

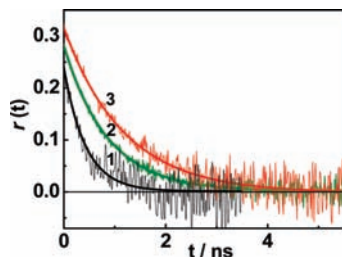


Figure 5. Anisotropy decay traces measured for ThT (3 μM) with 5 μM CB7 (trace 1), with 1 M CB7 (trace 2), and with 1 M CB7 and 1 M NaCl (trace 3). The solid line represents the fitted trace according to the single-exponential decay function.

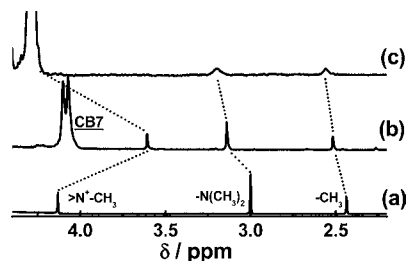


Figure 6. ^1H NMR spectra of ThT in D_2O at different environments: (a) ThT alone ($\sim 100 \mu\text{M}$), (b) ThT and 1 mM CB7, and (c) ThT with CB7 (1 mM) and NaCl (1 M). CB7 labels the methylene resonances of the CB7 macrocycle.

\AA),³⁶ and ~ 1200 ps for the 2:1 complex in the presence of NaCl (diameter ~ 22 \AA). The obvious increase observed in the hydrodynamic diameter of the complexes is in clear support of the increase in molecular volume expected due to 1:1, 2:1, and 2:1 with metal ion complexation, and hence the proposed mechanism.

v. ^1H NMR Measurements. Alternatively, monitoring the changes in the proton resonance signals of CH_3 groups on ThT would also be informative in analyzing the structure and interactions in the host–guest complex. It was considered that since the N^+CH_3 protons on the thiazole ring are positioned in between the two CB7 moieties, any change in the charge distribution in that region would be reflected in its chemical shift. Figure 6 shows the ^1H NMR spectrum for ThT and its CB7 complex in the absence and presence of metal ions providing distinct chemical shifts. In the absence of any metal ion, the N^+CH_3 protons on the thiazole ring experience a large upfield shift, due to strong negatively charged carbonyl groups from both CB7 portals.³⁶ Clustering of metal ions would largely affect these charge distributions and proton shielding characteristics of the carbonyl groups, thus shifting the proton resonance downfield, which gets buried under the large CB7 peak.⁴¹ It is also possible that, like the broadening observed with the other two methyl groups, the N^+CH_3 proton signals also become too broad to observe in the NMR spectrum. In other words, the significant deshielding effect on the methyl

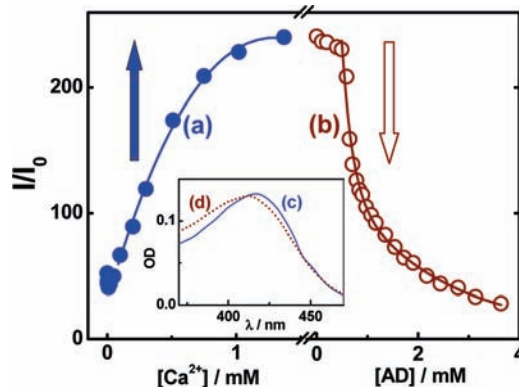


Figure 7. Changes in the fluorescence intensity of $(\text{CB7})_2\cdot\text{ThT}$ complex in the presence of Ca^{2+} (trace a) and followed by the addition of AD (trace b). (Inset) Absorption spectrum of Ca^{2+} bound $(\text{CB7})_2\cdot\text{ThT}$ complex (trace c) and after the addition of AD (trace d).

group on the thiazole ring verifies the clustering of metal ions in its vicinity, in strong support of the proposed capsular structure.

vi. Release Mechanism. In general, the ability to control and release the guest dyes/drugs from the container host(s) operates under a wide range of chemical, physical, or electrochemical stimuli responses. In the present case also, in order to validate the applicability of the capsular complex for its projected potential applications (vide infra), we set one such destabilizing condition to achieve a controlled rupture of the capsule so as to release the bound dye. For this we introduced a strong CB7 binder, 1-amanatidine hydrochloride (AD), into the solution to disturb the binding preferences of CB7 toward the other constituent guests. AD forms a very stable complex with CB7 and has a binding constant of $(4.23 \pm 1) \times 10^{12} \text{ M}^{-1}$ (in 50 mM sodium acetate buffer)⁴² as compared to the lower affinities of Na^+ ($K_{\text{CB7-Na}} \sim 100 \text{ M}^{-1}$),⁵ Ca^{2+} ($K_{\text{CB7-Ca}} \sim 700 \text{ M}^{-1}$),⁵ and ThT ($K_{\text{CB7-ThT}} 10^5 \text{ M}^{-1}$).³⁶ Fluorescence titration of Ca^{2+} ion-bound $(\text{CB7})_2\cdot\text{ThT}$ with AD provided the expected changes as noted in Figure 7. When the AD concentration was increased, the fluorescence intensity dropped drastically from 250-fold to nearly 10–15-fold with about 3.5 mM AD. Such a sudden drop in the fluorescence intensity is a clear validation of the disengagement of the metal ion and, most importantly, the dissociation of the 2:1 complex. Concomitantly, the absorption spectrum also shifted toward that of free dye as shown in the inset of Figure 7. Thus, the above experiment clearly manifests the collapse of the capsular structure, releasing the bound dye, and reaffirms its suitability for the projected applications. Schematically the interaction of AD and the release mechanism for the included dye is shown in Scheme 3.

Certainly, the results presented here demonstrate the construction of a novel fluorescent supramolecular capsule through noncovalent host–guest assembly and its rupture through competitive guest binding interactions. The mechanism is well-suited for application in protected drug carrier and release, fluorescence on–off systems, enhancing molecular characteristics (particularly that of guest), etc. It is apparent that the structure of the dye played an important role in bringing the specific orientations of the host moieties on it. This

(40) Wyman, I. W.; Macartney, D. H. *Org. Biomol. Chem.* **2008**, *6*, 1796–1801.

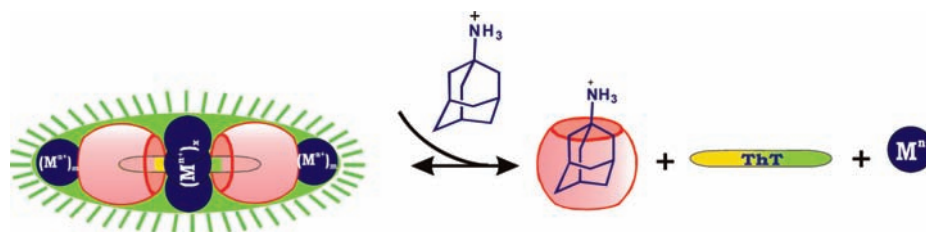
(41) Though different studies reveal that the methyl protons of the guest at the CB portal experience a downfield shift, in the CB7-ThT system, the methyl proton of the thiazole ring, residing between the two CB7 portals, displayed a significant upfield shift. Due to the lack of dedicated experimental data on this cause, we refrain from attributing this to any specific interaction; however, the stoichiometric arrangement of the complex with two carbonyl portals sandwiching the cationic thiazole ring methyl protons could be the major factor.

(42) (a) Gadde, S.; Batchelor, E. K.; Weiss, J. P.; Ling, Y.; Kaifer, A. E. *J. Am. Chem. Soc.* **2008**, *130*, 17114–17119. (b) Liu, S.; Ruspic, C. *J. Am. Chem. Soc.* **2005**, *127*, 15959–15967.

(43) Foderà, V.; Cataldo, S.; Librizzi, F.; Pignataro, B.; Spiccia, P.; Leone, M. *J. Phys. Chem. B* **2009**, *113*, 10830–10837.

(44) Harel, M.; Sonoda, L. K.; Silman, I.; Sussman, J. L.; Rosenberry, T. L. *J. Am. Chem. Soc.* **2008**, *130*, 7856–7861.

Scheme 3. Proposed Mechanism for the Release of ThT from the Metal Ion Bound Supramolecular Capsule by 1-Amantadine Hydrochloride (AD) as the Stimulant



provides vital information for the design and synthesis of tailor-made dyes/drugs in tandem fashion to exploit the utility of such assemblies for an extended chain. Moreover, the relative binding strengths of different noncovalent forces and the noteworthy modulation in the photophysical properties of the guest chromophore throw light on the understanding of the intramolecular motions and their remarkable contributions toward the radiative yield of the dye. This has been a point of discussion in relation to its application in characterizing protein fibrils, especially for ThT. Attempting a qualitative comparison of ThT binding to a fibril cavity, we prepared insulin fibrils as per standard protocols in the presence of ThT.⁴³ Our experimental data on ThT bound to insulin fibrils displayed huge fluorescence enhancements and the decay profile exhibited excited-state lifetimes almost similar to that recorded for the metal ion bound complex (cf. Figure 4, Table 1). It is intuitive that just as the CB7 and metal ions provide cooperative binding forces to ThT, affecting its planarity, rigidity, and protection from bulk solvent, in the fibril cavities as well, specific noncovalent interactions must be actively involved in arresting the intramolecular motions of ThT (as in acetylcholinesterase binding),⁴⁴ apart from the local hydrophobic environment. Efforts are being made to look into the details of these aspects.

Conclusion

In summary, this article reports a novel stimulus-responsive cooperative metal ion binding to the stoichiometrically selected

cucurbit[7]uril–thioflavin T complex demonstrating a highly fluorescent supramolecular nanocapsule. The first example of such an unusual assembly presented here became feasible due to the stoichiometry and the structural arrangement of the host–guest complex with two CB7 portals providing strong negative charge density for the metal ions to group and seal the complex, thus rigidizing and protecting the incorporated dye. To further strengthen the usefulness of the methodology established here, rupture of the capsular complex was demonstrated with a strong competitive guest, which helped in disrupting the capsule to release the dye. It is expected that, by proper design criteria of the guest chromophores, the methodology illustrated here can be explored for the binding and release of drug molecules and for application in on–off systems and will have immense potential as building blocks for molecular architectures displaying unique properties. These noteworthy results are being taken forward and further studies are in progress to acknowledge similar systems and explore its implications in a wide range of utilities.

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