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Reversible Deformation-formation of a Multistimuli Responsive Vesicle by a Supramolecular Peptide Amphiphile

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A systematic study of the ternary complex formation process for aromatic amino acids with Cucurbit[8]uril (CB[8]) and a viologen amphiphile shows that the affinity of the amino acid needs to be higher or in comparable range to that of CB[8] for the amphiphile in order to form the ternary complex. Based on these observations, a supramolecular peptide amphiphile and its corresponding vesicle is prepared using a peptide containing an Azobenzene moiety. The Azobenzene group at the N-terminal of the peptide served as the second guest for CB[8]. The vesicles obtained from this peptide amphiphile shows response to a number of external triggers. The *trans-cis* isomerization of the Azobenzene group upon irradiation with UV-light of 365 nm, leads to the breakdown of the ternary complex and eventually to the disruption of the vesicle. The deformation-reformation of the vesicle can be controlled by illuminating the disrupted solution with light of 420 nm as it facilitates the *cis-trans* isomerization. Thus, the vesicle showed controlled and reversible response to UV-light with the ability of manipulation of the formation/deformation of the vesicle by choice of appropriate wavelength. The vesicle showed response to a stronger guest (1-adamantylamine) for CB[8] which displaces both the guests from the CB[8] cavity and consequently ruptures the vesicle structure. 2,6-dihydroxynaphthalene acts as a competitive guest and thereby behaves as another external trigger for replacing the peptide from the CB[8] cavity by self-inclusion to form ternary complex. Henceforth, it allows retaining the vesicle structure and results in release of the peptide from the vesicle.

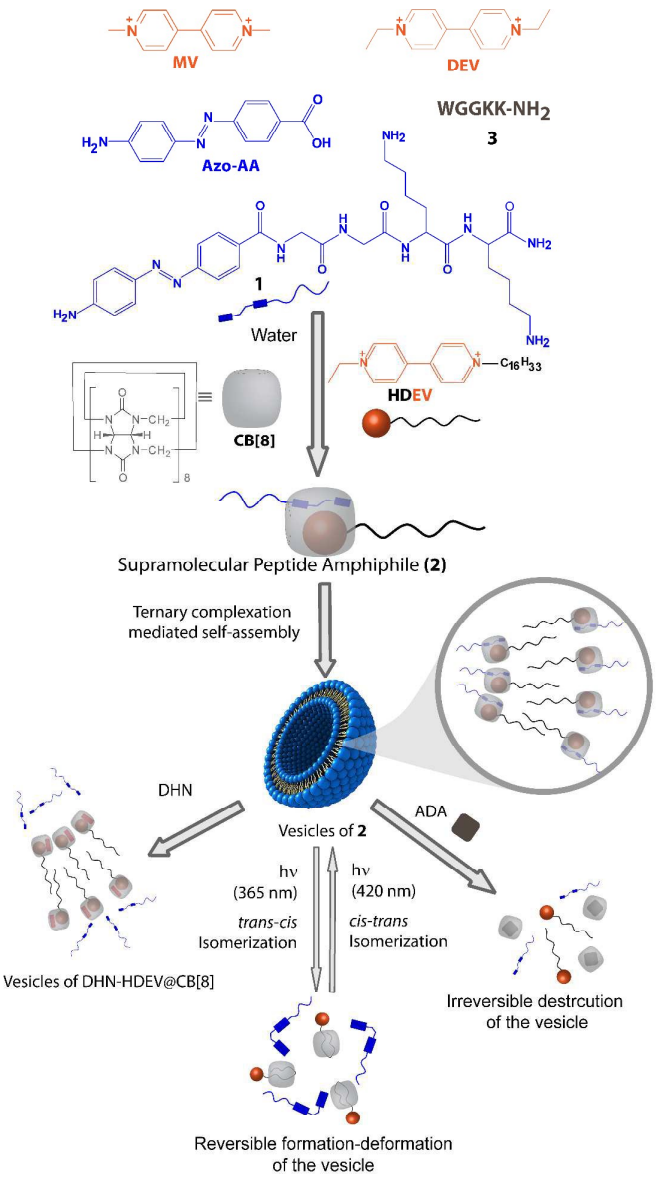
1. Introduction

Vesicles are integral building blocks of living systems. Vesicles have been attracting attention owing to its various applications across different scientific domains.[1-7] Construction of vesicles with defined size, shape and external stimuli responsiveness has become a fascinating challenge for the chemists. These stimuli responsive vesicles find applications in biomimetic systems, light-harvesting systems as well as in drug delivery vehicles.[8-16] Manipulating the formation/deformation process using an external switch is most desirable for these systems to be utilized for these applications.[8-16] Stimuli responsive vesicles have been reported in many occasions and some of them are having multiple switches in the same system.[17-19] The reversible formation/deformation by a single trigger can be of immense importance and to the best of our knowledge no such system has been reported so far. However, a system with multiple switches would be highly beneficial as it can be used under different environment and conditions.

In this regard, lipidated peptide amphiphiles (PA) have gained considerable attention as a new class of biomaterials having wide ranging applications from biomineralization, drug delivery, template for nano-material synthesis and templating to tissue engineering and therapeutics.[20-26] These PAs self-assemble into various structures such as vesicles, micelles or nanotubes and have been efficaciously applied for biomaterial conjugation and as bioactive scaffolds for tissue engineering.[17-23] Embedding multiple switches on these PAs via covalent attachment is always a challenging task. In this context, supramolecular host-guest chemistry paved the way to an alternative approach toward incorporating multiple responsiveness in the same system. Since the molecules are adhered together through multiple weak interactions, appropriate stimulus can be used to dis-assemble them easily.

The unique ternary complexation by Cucurbit[8]uril (CB[8], Scheme 1) has opened up the possibility of constructing such stimuli responsive soft-materials. CB[8] is a macrocyclic synthetic host capable of accommodating two guests inside its hydrophobic cavity simultaneously and hence acts as a "supramolecular handcuff".[27-30] CB[8] can stabilize a 1:1:1 ternary complex with methylviologen (MV, Scheme 1) and an

electron rich second guest like naphthalene, pyrene, tryptophan etc.[31-34] The charge transfer (CT) interaction between electron deficient MV and the electron rich second guests as well as the hydrophobic interaction facilitate the formation of the ternary complex.[30-33] Stimuli responsive materials derived from ternary complexes of CB[8] have recently gained significant attention.[34-36] Interestingly, Kim et al. reported that when an asymmetric viologen amphiphile is used, such ternary complexation eventually leads to the formation of uniform vesicles.[37] Recently, we have shown that this vesicle formation is a micelle to vesicle transformation and can be achieved with extremely low content of the ternary complex in the system (1% of the total surfactant concentration).[38,39]



Scheme 1. Chemical structures of Azo-AA, DEV, HDEV, and CB[8], pictorial presentation of the formation of SPA and its vesicle as well as responses to various stimuli.

This unique ternary complexation has been utilized to construct supramolecular glycolipid, peptide amphiphile and their corresponding vesicles.[40,41] Scherman et al. have shown the formation of a supramolecular peptide amphiphile (SPA) which can be taken up by HeLa cells and are responsive to external triggers.[41] Nevertheless, this system requires external triggers in the form of foreign chemicals and only the disruption of these vesicles can be achieved with these triggers as it is an irreversible process.

To this end, ternary binding of azobenzene-containing moieties with MV@CB[8] is already getting attention primarily owing to its ability to show photo response.[36,42,43] A *trans*-isomer can fit within the available cavity space of MV@CB[8] while the space is not adequate for the cis-isomer.[36,42,43] The Azobenzene-MV@CB[8] complex breaks down upon UV irradiation as the *trans-cis* isomerization occurs. We envisioned that the CB[8] assisted ternary complexation of a viologen surfactant and combining an azobenzene containing peptide may lead to a photo labile SPA and it's corresponding vesicle. In this case the formation/deformation of the vesicle can be controlled through irradiation of the system with appropriate light.

We report here, a systematic study of the ability of different aromatic amino acids to form ternary complexes with viologen amphiphiles in presence of CB[8]. Based on the results obtained from this study, we have successfully constructed a supramolecular peptide amphiphile (SPA) (**2**, Scheme 1) and its vesicle through ternary complexation inside the CB[8] cavity. The SPA-vesicle displayed response to multi-stimuli and controlled release of a model dye upon irradiation with UV light (Scheme 1). Notably, the system showed reversible formation/deformation based on the wavelength of light it is irradiated with.

2. Materials and Methods

2.1 General. 1-bromohexadecane, 4,4′-bipyridyl, HBTU, DIPEA, HOBT, Rink-amide HMBA resin (0.75 mmol/g loading) Fmoc-Lys(Boc)OH, Fmoc-Gly(OH), 5(6)-carboxyfluorescein (CF), D_2O, CF_3CO_2D, and $D2SO_4$ were obtained from Sigma-aldrich (USA) and used as received. All other chemicals, reagents and solvents were procured from Merck, India and Spectrochem, India. For preparing samples, Milli-Q water with conductivity of less than 2 μS.cm-1 was used. UV-visible spectra were recorded on Lambda 35 (Perkin-Elmer) spectrometers. ESI-MS was performed by using a Q-Tof Premier Quadrupole mass spectrometer. The dynamic light scattering were measured on a Zetasizer Nano ZS90 from Malvern using a 632.8 nm He–Ne laser. The syntheses of CB[8], HDEV (*N*-ethyl-*N*′-hexadecylviologen dibromide), DEV (*N*,*N*′-diethylviologen dibromide) and Peptide **1** and their characterization data are provided in the ESI.

2.2 Vesicle preparation. To a volumetric flask (10 mL) containing a measured quantity of HDEV (4.3 mg), CB[8] (12.8 mg, the overall molecular weight observed for the used CB[8] was 1730 from the elemental analysis data) and 1 equivalent of

the second guest (Tryptophan or Azo-AA ((E)-4-((4-aminophenyl)diazenyl)benzoic acid) or peptide **1**) were taken. To these mixtures, water was added to make up the mark. The resulting mixtures after sonication for 1 h at 358 K were kept undisturbed at 298 K for one day to give clear light violet coloured solutions before utilizing for further experiments.

2.3 Vesicle preparation for Dye Release experiment. The vesicles were prepared according to the method mentioned above but in these cases, a measured amount of CF (0.1 mg) was added to the 10 mL flask prior to the preparation of the samples. The resulting vesicle solutions were passed through a sephadex G-25 column (1 × 30 cm) and the fractions containing the vesicles were collected. The vesicle solution was then diluted and used for fluorescence measurements. The dye release was monitored on a Cary-Eclipse luminescence spectrometer (Agilent) by exciting the solutions at 492 nm and measuring the fluorescence intensity at 512 nm.

2.4 NMR spectroscopy. ^{1}H NMR, ^{13}C and diffusion-ordered spectroscopy (DOSY) were recorded on a NMR-Bruker AscendTM 600 MHz (Bruker, Coventry, UK). Spectra were recorded in heavy water (D_2O) at 298 K. In case of experiments containing DHN, DHN was added to the systems as a THF-d8 solution. The concentration of all the components was fixed at 0.75 mM for all the samples. The experiments were processed with standard Bruker 1D and 2D DOSY softwares. For ADA and DHN related experiments, 1.2 equivalents (with respect to **2**) of the stimuli (ADA or DHN) were added to the vesicle solutions of **2** and incubated for 24h prior to measurements. For UV irradiation related experiments, the NMR tube containing the vesicle solutions were exposed to the UV-lamp irradiating light of 365 nm wavelength for 8h before measuring the ^{1}H and DOSY NMR.

2.5 Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). FESEM images were recorded on zeiss (Sigma) microscope while TEM images were taken on a JEM-2100 microscope. FESEM samples were prepared by casting a drop of the vesicle solution (0.075 mM) on a glass slide and dried under ambient condition. For TEM, a drop of the vesicle solution (0.075 mM) was placed on a 300 mesh Cu grid with thick carbon film (Pacific Grid Tech, USA) and held in air for 2 to 3 min. Excess solution was then blotted with tissue paper. With a drop of 2% uranyl acetate solution the sample was negatively stained before removing the excess liquid with filter paper.

2.6 Isothermal Titration Calorimetry (ITC). The formation constants and thermodynamic parameters for the inclusion complexes were determined by isothermal titration calorimetry using a Nano-ITC instrument from MicroCal. CB[8] solutions (0.1 mM) in buffer (10 mM phosphate, pH 7) was placed in the reaction cell (volume = 200 mL). Amino acid/peptide solutions (each injection, 0.5 μL, 2.0 mM) were injected from a 40-μL micro-syringe at an interval of **2** min into the DEV@CB[8] solutions with stirring at 298 K. The first data point was omitted from the data set for curve fitting. All solutions were degassed prior to titration. Heats of dilution were checked by titration of the guest into a buffer solution and subtracted from the normalized enthalpies, but the heat of dilution data are relatively small in all cases. The data were fitted to a theoretical titration curve using software supplied by Microcal using the one set of sites binding model with K_a (M^{-1}), ΔH (cal/mol) and ΔS (cal/mol/deg) as adjustable parameters.[44]

2.7 Dye release Experiment. The vesicle solution containing entrapped CF was placed in a 3 mL fluorescence cuvette and the emission spectra were recorded at certain time interval by exciting the solution at 492 nm and measuring the emission intensity at 512 nm. The % CF release was calculated following the equation,

CF release (%) = $((I_t-I_0)/(I_\alpha-I_0))\times100$

Where, I_0 and I_t are the fluorescence intensities initially and at time t, and I_α is the fluorescence intensity when all the CF molecules were released from vesicles, which was measured by the addition Triton x-100 and heating for an additional 30 min. To monitor ADA and DHN (THF solution) induced release, the stimuli was added in portions to reach 2 equivalent concentration of the stimuli compared to **2**.

2.8 Photoresponsive dye release experiment. A vesicle solution (0.075 mM) loaded with entrapped CF, stored in a screw-capped 3 mL fluorescence cuvette with 10 mm path length, was exposed to UV light of 365 nm (8 W long wavelength lamp from SIAC, India with band path filter of 365 nm) for different times (Figure S1). The fluorescence intensity change at 512 nm was followed on a Cary-Eclipse luminescence spectrometer (Agilent) as a function of UV irradiation time. The time taken to reach intensity maxima was observed to be 6h but to ensure complete destruction of vesicles, for other studies, the samples were irradiated for 8h.

2.9 Tyndall Effect experiments. The solutions were taken in 3 mL fluorescence cuvettes and in dark, laser was irradiated through these solutions and the images were captured using a hand held digital camera.

3. Results and Discussions

3.1 Aromatic Natural Amino Acids as Second Guests for HDEV@CB[8]

In order to create supramolecular peptide amphiphiles, initially we have evaluated the possibility to use natural aromatic amino acids as a potential replacement of 2,6-dihydroxynaphthalene (DHN). Earlier, Urbach et al. reported that phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) can form ternary complexes with methylviologen (MV, Scheme 1) and CB[8] and the binding affinity is in the order of Trp>Phe>Tyr.[32] Moreover, the ternary complexation ability of these amino acids were observed to be sequence specific in case of peptides and highest when they occupy the N-terminal position.[42] Interestingly, among these three amino acids, only Trp showed the ability to form ternary complex when an asymmetric viologen amphiphile, HDEV (Scheme 1) is used in place of MV. While there is a significant up-field shift observed for the aromatic protons of Trp in the ^{1}H NMR of the ternary mixture of Trp, HDEV and CB[8], no noticeable movement was observed for Phe and Tyr in similar experiments (Figure 1).

Additional information on the ternary complexation was obtained from the ESI-MS spectra of the 1:1:1 Trp-HDEV-CB[8] mixture. A new signal corresponding to Trp-HDEV@CB[8] (*m/z* = 971.78, M^{2+}) appeared while no such signals were obtained in case of Phe and Tyr under similar conditions. Further conclusive evidences were observed for the encapsulation of Trp in the HDEV@CB[8] binary complexes from the appearance of a new CT band in the UV-visible spectra as well as the quenching of the fluorescence of Trp (Figures S1 and S2 of the ESI).[32]

Importantly, the ternary complex, Trp-HDEV@CB[8] transformed the solutions into spherical nano-aggregates as seen from the FESEM and TEM images (Figure 2). Based on the previous reports of formation of vesicles by similar systems, we assumed that these nano-aggregates are vesicles.[37-41] In order to confirm the formation of vesicles, we have entrapped a water soluble dye, CF, inside these nano-aggregates and followed the release of the encapsulated dye through enhancement of fluorescence intensity. The entrapment and slow release of the dye is a confirmatory experiment for the formation of vesicles. A slow release of the dye was observed. Upon addition of Triton X-100 (a well-known vesicle destroyer) a sudden enhancement of the fluorescence intensity confirms the formation of vesicles by Trp-HDEV@CB[8] (Figure 2).

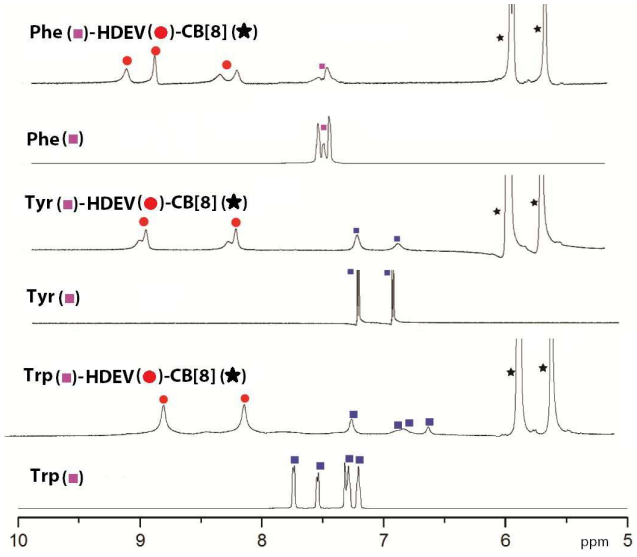


Fig. 1. 1H NMR of Phe, Tyr, and Trp in absence and presence of HDEV@CB[8].

In order to investigate the failure of Phe and Tyr to form ternary complexes with HDEV@CB[8], we have measured the association constants for all these amino acids with DEV@CB[8] binary complexes through isothermal titration calorimetry (ITC). It is worth mentioning that ITC experiments with such binary complexes of CB[8] and amphiphilic molecules like HDEV could not provide any conclusive results as was also observed earlier.[39] Under such circumstances, it is a common practice to assume that the binding constants for the amphiphiles (eg. HDEV) are similar to its shorter analogues (eg. DEV).[39,41] Earlier studies show that in absence of any second guest, CB[8] forms a binary complex at the tail of HDEV.[39,45] Upon addition of the second guest, CB[8] moves towards the head group of HDEV and forms the ternary complex.[39] The formation of this ternary complex thus depends on the comparative binding constants of "Tail of HDEV"@CB[8] versus the second guest@HDEV-CB[8]. The observed lower binding constants of Phe and Tyr with DEV@CB[8] (in the order of 10^3 M^{-1}) compared to that of CB[8] with hydrophobic tail of HDEV ($(2.3 \pm 0.1) \times 10^5$ M^{-1})[39] does not allow CB[8] to move from the tail group to the viologen head to form the ternary complex with these amino acids. On the other hand, similar binding constants for Trp-DEV@CB[8] ($(0.94 \pm 0.1) \times 10^5$ M^{-1}) and HDEV@CB[8] presumably allows a competitive binding at the viologen head of HDEV.

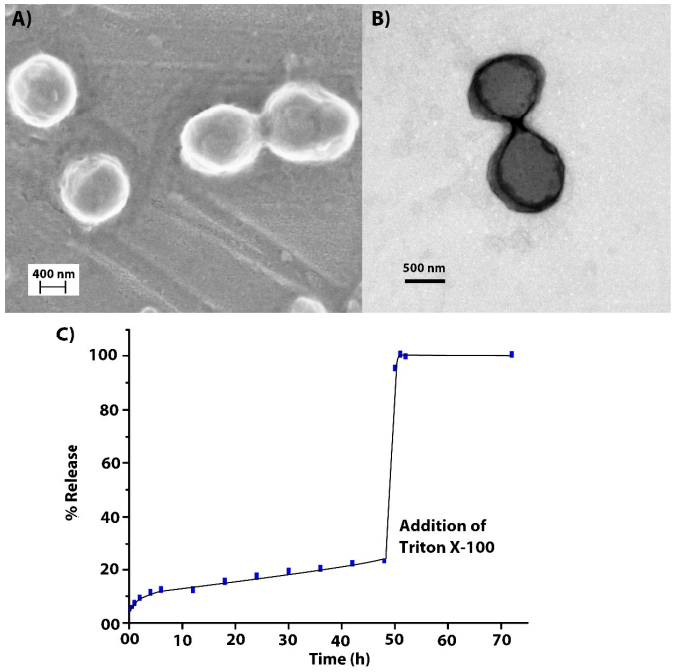


Fig. 2. A) FESEM and B)TEM images and C) Dye release profile for 1:1:1 Trp-AA-HDEV@CB[8] vesicles in deionized at RT. [Trp] = 0.075 mM.

3.2 Azo-AA as the Second Guest for HDEV@CB[8]

With this experience in hand, in order to create a stimuli responsive supramolecular peptide amphiphile, Azo-AA (Scheme 1) was tested for its ability to bind to the viologen head group of HDEV in presence of CB[8]. The binding constant for Azo-AA to DEV@CB[8] ($(0.84 \pm 0.05) \times 10^5$ M^{-1}) as obtained from the ITC experiment, was found to be similar to that of HDEV to CB[8].[39] In accordance with the results obtained for Trp, Azo-AA also formed ternary complex with HDEV@CB[8]. The up-field shifts in the proton peaks of the corresponding aromatic protons of Azo-AA in presence of CB[8] indicates the formation of the ternary complex (Figure 3A). Further confirmation on the formation of ternary complex was obtained from the appearance of Azo-AA-HDEV@CB[8] peak (*m/z* = 990.63, M^{2+}) in the ESI-MS spectrum. Similar to the case of Trp, the ternary complex undergoes self-assembly to

form vesicles as can be confirmed from the entrapment and slow release of CF from this system (Figure 3). Further supporting evidences were obtained from the FESEM, TEM and DLS experiments.

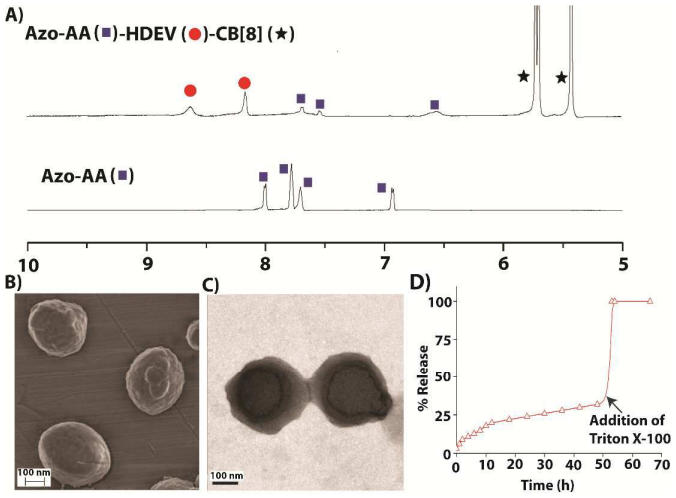


Fig. 3. A) ^{1}H NMR of Azo-AA in absence and presence of HDEV@CB[8] complex; B) FESEM image, C) TEM image, and D) release profile of the entrapped CF from the vesicles formed by 1:1:1 Azo-AA-HDEV@CB[8] (0.075 mM) at RT.

3.3 Formation of the Supramolecular Peptide Amphiphile and its Vesicles

The formation of the vesicles using Azo-AA encouraged us to construct a photo-responsive supramolecular peptide amphiphile. Peptide (**1**) was designed where the second guest (Azo-AA) is situated at the N-terminal and is separated by two Glycines from the hydrophilic Lysine residues at the C-terminal. The binding constant of the peptide **1** to DEV@CB[8] was determined through ITC and found to be $(0.91 \pm 0.1) \times 10^5$ M^{-1} (Figure 4A). The observed K_a was in the similar range to that of Azo-AA and Trp. The formation of the peptide amphiphile **2** (Scheme 1) through ternary complexation with HDEV and CB[8] was confirmed by ^{1}H NMR spectroscopy (Figure 5). The individual proton signals were found to be broadened in presence of CB[8] as normally observed in case of ternary complexes.[39,41] The up-field shifts of the aromatic protons for both **1** and HDEV in presence of CB[8] is an indication of encapsulation of viologen head group as well as the Azobenzene group of the peptide. Previously, we have shown that the aliphatic tail of HDEV gets buried inside the CB[8] cavity in absence of any second guest and the proton signals show an up-field shift.[39] Upon addition of an appropriate second guest, CB[8] moves toward the head group of the amphiphile leaving the hydrophobic tail free which is clearly observed as the up-field shifted aliphatic protons come back to their initial position.[39] When peptide **1** was added to HDEV@CB[8], similar down-field movement of these hydrophobic protons is also observed which further confirms the formation of the supramolecular peptide amphiphile (S6 of the ESI). In DOSY spectra (Figure 6), all three components of **2** showed similar diffusion coefficients (~-9.69 m^2S^{-1}) while different coefficients were observed when measured separately. Further confirmation on the formation of **2** was obtained from the appearance of the $\mathbf{2}^{2+}$ (m/z =1174.57) peak in the ESI-MS.

The SPA **2** also formed nano-aggregates as observed from the FESEM and TEM images (Figure 4). To confirm the type of these aggregation, we have performed the CF entrapment and release study. A slow release of the dye over a period of two days and sudden enhancement in the fluorescence intensity triggered by the addition of Ttriton X-100 confirms the formation of vesicular aggregates. The average diameter of these vesicles were found to be ~ 500 nm. A dilution experiment followed by DLS measurements showed a sharp decrease in the particle size after 0.0025 mM. The inflection point (0.0025 mM) can be considered as the critical aggregation concentration (CAC) for the SPA (Figure S8 of the ESI).[40,41]

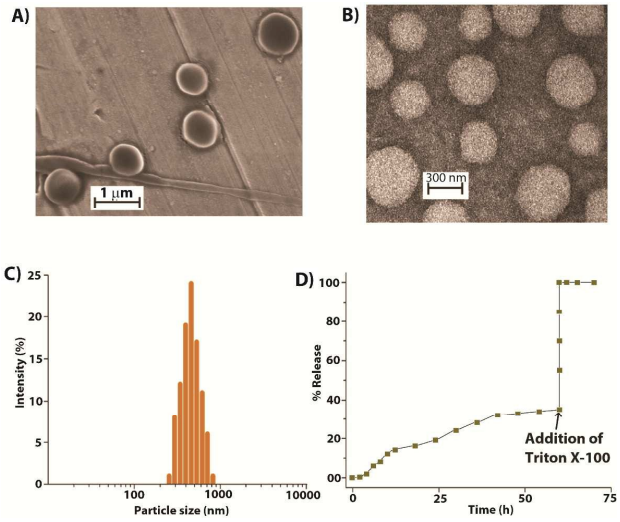


Fig. 4. A) FESEM image; B) TEM image, and C) intensity-weighted distributions obtained from DLS measurements of the vesicles formed by 1:1:1 **1**-HDEV@CB[8] (0.075 mM) at RT; D) release profile of the entrapped CF in the same vesicles under ambient condition.

3.4 Photo-Sensitivity of the Vesicles of SPA 2

To assess photo-lability of the SPA vesicles, the solution containing entrapped CF was subjected to UV irradiation (365 nm, 8 W lamp) and simultaneously the fluorescence intensity of CF was monitored at 512 nm. A continuous increase in the intensity was observed which is much faster than the ambient release (Figure 7). In order to confirm the light-induced leakage of the vesicles, we have constructed another vesicle with peptide **3**, where Trp is incorporated as the second guest in place of Azo-AA. As expected, peptide **3** also formed vesicles through ternary complexation with HDEV and CB[8]. A control experiment with the vesicles made of the SPA of **3** showed no enhancement in the emission intensity of the released dye when irradiated with UV light of 365 nm. The faster release in case of **2** is attributed to the quicker discharge

of the dye molecules from the vesicle. The UV irradiation leads to the conversion of *trans*-isomer of the Azobenzene group to *cis*-isomer which eventually comes out of the CB[8] cavity leading to break down of the ternary complex (Scheme 1). Consequently, the vesicle structure breaks down releasing the entrapped dye.

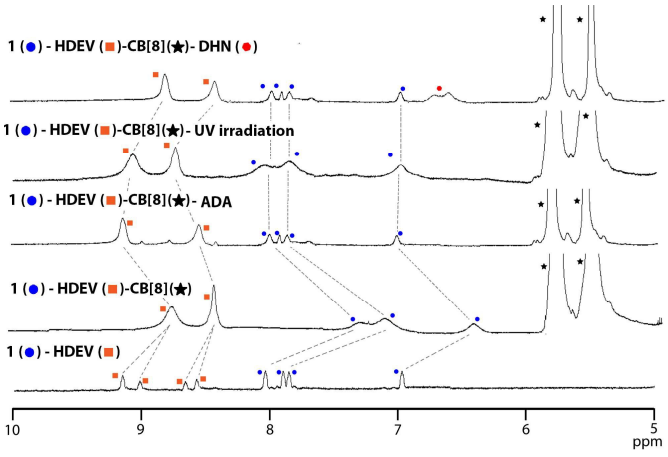


Fig. 5. ^{1}H NMR (aromatic region only) spectra of various compositions of **1** and under different conditions showing the chemical shifts of protons upon complexation and de-complexation.

Though the *trans-cis* isomerization could not be monitored using UV-visible spectroscopy owing to overlapping of absorbance regions of the peptide and HDEV, NMR techniques provided conclusive evidences for the dis-assembly of the vesicle. The disassembly of the ternary complex is evident from the DOSY spectrum of the UV-irradiated (for 8h) vesicle solution (without CF) where the free peptide showed a different diffusion coefficient (-9.64 m^2S^{-1}) than the other two components (-9.89 m^2S^{-1}). Similar diffusion coefficients for HDEV and CB[8] indicate that once the ternary complex breaks down, CB[8] moves back to the tail region to form the binary complex with the hydrophobic part of HDEV.[39] Moreover, a significant down-field shift was observed in the aromatic region in the 1H NMR spectrum for the UV-irradiated solution of **2**. The protons corresponding to the Azobenzene group as well as Viologen, showed down-field shift to their respective native position indicating decomplexation (Figure 5). The decomplexation of the ternary complex and formation of the U-shaped binary complex of HDEV tail inside the CB[8] cavity was further confirmed from the up-field shifts of the aliphatic peaks and especially the terminal methyl group in the UV-irradiated sample (Figure S6 of the ESI).

Though the UV-irradiated sample showed no Tyndall effect (Figure 8), in DLS analysis, particles with size distribution centered at around 130 nm were observed but with significantly low counts (Figure S7 of the ESI). Though the count is extremely low, the appearance of such particles could be attributed to a possible non-spherical micelle formed by the binary complexes of HDEV@CB[8] as observed earlier in similar cases.[39] At this point, the self-assembly process of such aggregation need to be addressed with more experimental evidences but beyond the scope of this communication.

3.5 Reversible Deformation-Formation of the Vesicle with Light

Interestingly, the vesicle showed reversible deformation-formation based on the wavelength of the light it was exposed to. After complete breakdown (as monitored by DOSY spectra), the solution was irradiated back with a lamp illuminating light of 420 nm (150 W) for 10 mins, sonicated for 1h and finally incubated in dark for two days. The DLS measurement showed appearance of larger particles of around 500 nm. The solution also showed positive Tyndall effect (Figure 8). TEM images of the sample showed similar vesicular structure as obtained from the freshly prepared vesicles. The irradiation of the solution 420 nm light facilitated the *cis-trans* conversion and sonication followed by incubation at dark allowed the self-assembly and consequently re-construction of the vesicles. DOSY spectra also showed similar pattern to the freshly prepared vesicles. Repetition of the cycle for several times leads to similar results showing the reversibility of the vesicle as can be seen from the particle size analysis of the samples during every cycle (Figure 9).

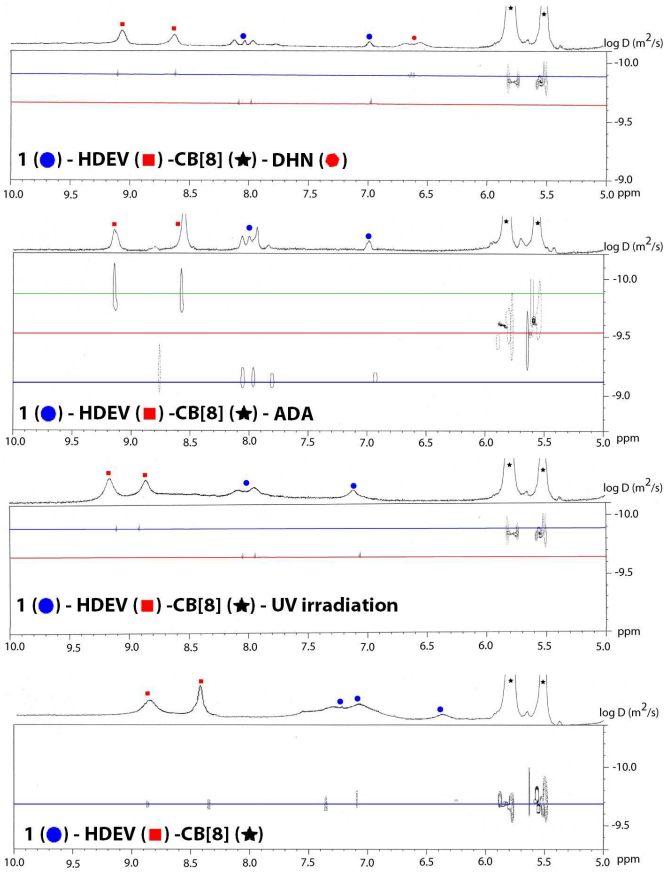


Figure 6. DOSY NMR spectra of 1-HDEV@CB[8] vesicles with and without various stimuli.

3.6 Photo-controlled Release of an Entrapped Dye from Vesicles of 2

Irradiating the vesicle with UV light (365 nm) in an alternate fashion leads to controlled release of the entrapped dye. The CF release was followed by alternately switching "on" and "off" the irradiation (Figure 7). The release ceased as the irradiation is off. After irradiating the solution for a certain period (on), the fluorescence intensity enhanced but did not change upon keeping the solution in dark for some time (off). The CF release was triggered again by switching on the irradiation when a steady increase in the fluorescence intensity was observed again. Reiteration of this "on-off" process resulted in the release of the dye in a controlled manner. This stepwise release evidently established a sequential controlled release mode and thereby facilitating the potential for this SPA based systems to be used as nano-carriers for a time dependent release of cargo.

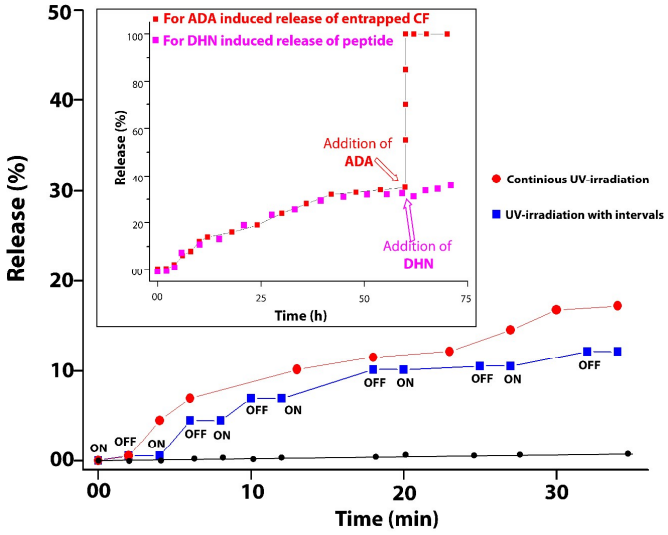


Fig. 7. UV-irradiation triggered controlled dye release profile of CF trapped inside the vesicles formed by **1**-HDEV–CB[8] in water at 298 K. (ON and OFF indicate the time when the irradiation was switched on and off respectively). Black line corresponds to the release profile of continuous UV-irradiated sample of **3**-HDEV–CB[8] in water containing entrapped CF at 298 K. Inset: The normal CF release profile from vesicles of **2**, red: for ADA induced release of entrapped CF and, magenta: for DHN induced release of **1** from the vesicles.

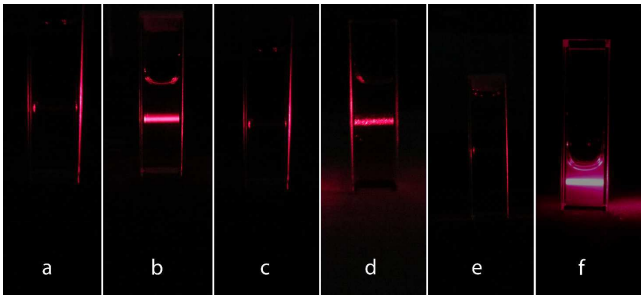


Fig. 8. Photographs from the Tyndall Effect experiments. a) water used for all the sample preparation; b) vesicle solution of **2**; c) solution of **2** after 8h of irradiation with UV light (365 nm); d) c after 10 mins of irradiation with 420 nm light and incubation for 48 h; e) solution **2** after addition of 2 equivalents of ADA; f) solution **2** after addition of 2 equivalents of DHN. [**2**] = 0.075 mM for all experiments.

3.7 A Stronger Guest Disrupts the Vesicle

The vesicle formed by SPA **2** was then analysed for its stimuli responsiveness to a number of other stimulus. To start with, we have checked its response to a stronger (than MV) guest of CB[8], 1-adamantylamine (ADA). ADA, having a significantly higher binding constant (K_a = (8.19 ± 1.75)×10^8 M^{-1}) and larger size, is known to replace other guests from the ternary complex.[46] When added to the vesicles of **2**, ADA replaced both the guests and occupied the space inside the CB[8] cavity (Scheme 1).[41] De-complexation of the ternary complex results in the breakdown of the SPA and consequently of the vesicles. When excess ADA was added to the vesicle of **2** containing entrapped CF, the enhancement of the fluorescence intensity was found to be similar to that observed when Triton X-100 was added (Fig. 4). Further confirmation was obtained from the DOSY NMR of the vesicle solution mixed with ADA where three different diffusion coefficients were observed corresponding to that of ADA@CB[8] (-9.52 m^2S^{-1}), HDEV (-9.89 m^2S^{-1}), and 1 (-9.12 m^2S^{-1}). ^{1}H spectrum of the ADA treated solution of **2** showed down-field shift of the aromatic protons resulting from the de-complexation of the ternary complex (Figure 5). Interestingly, the aliphatic region of the spectrum remained unaltered except the appearance of the peaks from ADA (Figure S6 of the ESI). The ^{1}H NMR of the aliphatic region clearly points to no involvement of the hydrophobic tail in any kind of complexation in this solution. Negative Tyndall effect as well as absence of any proper distribution in DLS also supports complete destruction of the vesicles (Figure 8).

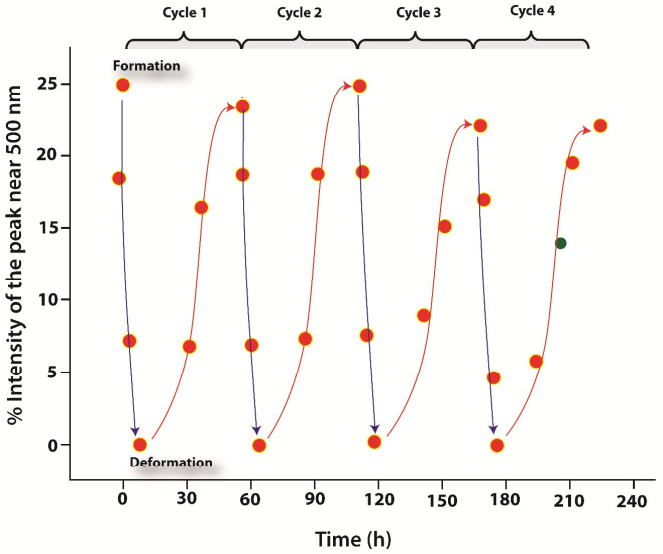


Figure 9. % Intensities of the peak near 500 nm in the DLS measurements of a solution of **2** at different times while illuminating it with UV-light of 365 nm (de-formation) followed by irradiating the same sample with light of 420 nm and incubation for 48h (deformation) for four cycles. Every Cycle: 8h irradiation with Light of 365 nm + 48 h incubation (10 mins irradiation with light of 420 nm + 1 h sonication + incubation in dark for the rest of the time).

3.8 A Competitive Second Guest Releases the Peptide from the Surface but Retains the Vesicle Structure

The vesicles of **2** showed interesting response to a competitive guest, DHN. Upon addition of one equivalent DHN to the vesicle of **2**, the DOSY spectra showed two different diffusion coefficients, one for the free peptide **1** (-9.63 m^2S^{-1}) and the other corresponding to DHN, HDEV and CB[8] (-9.89 m^2S^{-1}) (Figure 6). The proton NMR also showed downfield shift of the Azobenzene-protons to its unbound state indicating de-complexation of the azobenzene group (Figure 5). An observed up-field shift of the DHN protons compared to its native spectrum, and unchanged peak positions of the viologen protons indicate the inclusion of the DHN molecule in the CB[8] cavity to form DHN-HDEV@CB[8] ternary complex. The aliphatic region of the spectra showed no observable shift of the hydrophobic protons signifying that the CB[8] moiety remains at the head group of HDEV (Figure S6 of the ESI). Interestingly, in the dye release, no enhancement in fluorescence intensity was observed upon addition of DHN. The DLS distribution of the DHN treated solution showed a slight decrease in the particle size (Figure S7 of the ESI). When checked for Tyndall effect, the DHN treated solution also showed positive results (Figure 8). All these information lead to the conclusion that the vesicle structure remained intact even after the addition of DHN. DHN is well known for its charge CT mediated ternary complexation with HDEV and CB[8] and consequently transforming the amphiphile solution to vesicle.[37-40] The solution binding constant for DHN to MV@CB[8] complex, as reported by Scherman et al., was found to be higher than that of Azo-AA (K_a = (5.9 ± 0.5) × 10^5 M^{-1}).[47] Moreover, the second guest exchange is known to be dynamic and rapid whereas the vesicle formation by these supramolecular amphiphile is a relatively slow process.[48,49] In the present case, DHN replaces the peptide from the ternary complex keeping the vesicular structure intact. This particular response of the vesicle can be utilized for unloading peptide therapeutics in a controlled fashion.

4. Conclusions

A detailed investigation on the ability of the aromatic amino acids to form vesicles through ternary complexation with a viologen amphiphile and CB[8] has been done in this study. It shows that Trp and Azo-AA form stable vesicles through ternary complexes but Tyr and Phe failed to do so. The lower binding affinity for Tyr and Phe toward HDEV@CB[8] compared to that of CB[8] toward the tail of HDEV did not allow the self-sorting of CB[8] and formation of the ternary complex. Based on these results, a supramolecular peptide amphiphile is designed and prepared. In accordance with other such amphiphilic ternary complexes of CB[8], this SPA also transformed the solution into vesicles. The presence of the Azobenzene group as the second guest allowed us to utilize this vesicle as a photo-responsive system. This could be achieved through UV irradiation mediated *trans-cis* isomerization and consequent disassembly of the complex and thereby facilitating the controlled release of a dye molecule in a time dependent fashion. Importantly, the system showed reversible light responsivity as the formation-deformation of the vesicle can be controlled by choice of appropriate wavelength. The vesicle also responded to a stronger first guest in the form of ADA where the presence of ADA destroyed the vesicle structure and releases the entrapped dye. In response to a competitive guest like DHN, the vesicle structure remained unaltered while the peptide was released from the vesicle surface. Overall, this new multi-stimuli responsive SPA vesicle has the potential to be utilized in various applications pertaining to the delivery of the cargo at a time or in a controlled time dependent fashion depending on the need. Although the formation of vesicles and stimuli responsiveness of this type of SPAs are known, the incorporation of light sensitivity and most importantly controlling the reversible formation-deformation process using light will certainly pave the way to utilize these systems for future applications.

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