Electronic Supplementary Material (ESI) for Soft Matter.

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

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Synthesis

Cucurbit[8]uril (CB[8])[1], N-ethyl-N'-hexadecylviologendibromide (HDEV),[2] N,N'-diethylviologendibromide (DEV),[2] 4-[(4-Aminophenyl)azo]-benzoic acid (Azo-AA),[3] and 4-[(4-(N-Fmoc)-Aminophenyl)azo]-benzoic acid (Fmoc-Azo-AA-OH)[3] were synthesized following previously published protocols.

Cucurbit[8]uril (CB[8])

^{1}H NMR (600 MHz, $D_2O/CF_3CO_2D/D_2SO_4$ (1:1:0.15)): δ 4.25 (d, 16H), 5.55 (s, 16H), 5.86 (d, 16H); MS (ESI): m/z 1461.41 $(CB[8] + Cs)^+$.

***N*-ethyl-*N*'-hexadecylviologen dibromide (HDEV)**

1g (6.4 mmol) of 4,4'-dipyridyl was mixed with 5 mL (67 mmol) ethyl bromide in dichloromethane and refluxed for three days and excess (0.5 ml) ethylbromide was added after every 6h. A yellow precipitate formed. The volatiles were removed on a rotory evaporator before washing the residue several times with toluene and finally with diethyl ether to get a yellow solid as mono-ethylviologen (yield: 1.6 g, 95%). The purity of the product was confirmed by ^{1}H NMR spectroscopy. The obtained solid was then further alkylated on the other pyridyl group by taking it in 7:3 acetonitrile/methanol and refluxing the mixture in presence of 2.75 g (9.05 mmol, 1.5 equiv.) of 1-bromohexadecane for 24 h. The precipitate obtained was filtered and washed several times with toluene followed by diethyl ether. The surfactant was further purified by recrystalizing it three times from methanol/diethyl ether (Yield: 70%). ^{1}H NMR (600 MHz, D_2O) δ= 9.39 (d, *J* = 6.0 Hz, 2H), 9.24 (d, *J* = 6.6 Hz, 2H), 8.73 (d, *J* = 6.0 Hz, 2H), 8.68 (d, *J* = 6.6 Hz, 2H), 4.94 (t, *J* = 7.2 Hz, 2H), 4.84 (m, 2H), 2.26 (t, *J* = 6.6 Hz, 3H), 1.75 (m, 2H) 1.45 (br, 26H), 0.95 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz): δ 149.89, 149.56, 145.67, 145.34, 127.30, 127.21, 62.01, 57.73, 32.10, 31.28, 30.19, 30.01, 29.86, 26.38, 22.77, 15.85, 14.01; MS (ESI): m/z calcd for $[M-Br]^+$ $C_{28}H_{46}N_2Br$: 489.28, found: 489.35.

Diethylviologen dibromide (DEV)

Excess (7.5 ml, 99.8 mmol) Ethylbromide was mixed with 4,4'-dipyridyl (1.56 g, 9.98 mmol) in a glass tube and the mouth of the tube was sealed. The tube was heated to 80 °C for 24 h. After cooling down to room temperature the seal was broken and the material was concentrated on a rotory evaporator and the residue was crystallized three times from methanol-diethyl ether to get a yellow solid (Yiled: 35%). ^{1}H NMR (600 MHz, D_2O) δ 9.15 (d, *J* = 6.0 Hz, 4H), 8.56 (d, *J* = 6.0 Hz, 4H), 4.77(m, 4H), 1.73 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz)): δ/ppm = 150.1, 146.5, 126.5, 57.9, 17.1; MS (ESI): m/z calcd for $[M-Br]^+$ $C_{14}H_{18}N_2Br$: 293.06, found: 293.15.

4-[(4-Aminophenyl)azo]-benzoic acid (Azo-AA)

1.67 g (0.01 mol) 4-Nitrobenzoic acid and 2.0 g (0.018 mol) 1,4-diaminobenzene were suspended in 50 mL 3 % aqueous NaOH and heated up to 95 °C for several hours. After cooling, the red precipitate was collected and washed several times with dichloromethane before drying under reduced pressure(Yiled:66 %). ^{1}HNMR (DMSO-d_6, 400 MHz): δ/ppm = 7.98-7.96 (d, *J* = 8.8 Hz, 2H), 7.66-7.62 (t, *J* = 6.4 Hz, 4H), 6.68-6.66 (d, *J* = 8.8 Hz, 2H), 6.09 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz)): δ/ppm = 167.5, 156.2, 149.2, 145.3, 133.1, 130.5, 124.8, 123.0, 113.0. m/z calcd for $[M+H]^+$ $C_{13}H_{11}N_3O_2$: 242.25, found: 242.11.

4-[(4-(*N*-Fmoc)-Aminophenyl)azo]-benzoic acid (Fmoc-Azo-AA-OH)

0.48 g (2 mmol) 4-[(4-Aminophenyl)azo]-benzoic acid was dissolved in 10 mL of 10 % aqueous Na_2CO_3 solution and 5 mL THF. 0.54 g (2.08 mmol) Fmoc-Cl in 5 mL THF was added drop wise at 0 °C to the stirred solution. After 1 h the mixture was allowed to return to room temperature and was then stirred

overnight. The solution was poured into 150 mL ice water, washed with ether and acidified with conc. HCl to pH 1. The protected amino acid was extracted with ethyl acetate, the organic phase was dried over Na_2SO_4 and the solvent was removed in a rotory evaporatir, purified by column chromatography to get an orange solid (yield: 48 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ/ppm = 10.19 (s, 1H), 8.13-8.11 (d, *J* = 7.6 Hz, 2H), 7.94-7.90 (m, 6H), 7.78-7.76 (d, *J* = 7.2 Hz, 2H), 7.69 (d, *J* = 7.2 Hz, 2H), 7.46-7.42 (t, *J* = 7.2 Hz, 2H), 7.39-7.35 (t, *J* = 7.2 Hz, 2H), 4.57-4.56 (d, *J* = 5.2 Hz, 2H), 4.36-4.33 (t, *J* = 5.6 Hz, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz)): δ/ppm = 168.1, 157.8, 153.6, 150.8, 143.5, 142.5, 140.8, 133.0, 130.5, 126.8, 126.1, 125.1, 124.7, 122.8, 120.0, 66.1, 45.2; m/z calcd for $[M+H]^+$ $C_{28}H_{21}N_3O_4$: 464.15, found: 464.16.

Peptide 1 & 3

The peptides were prepared using solid phase peptide synthesis technique employing Rink-amide MBHA resin as the solid support. Sequence elongation at the N-terminus was performed by coupling the appropriate Fmoc protected amino acids under standard conditions employing HBTU, HOBT and DIPEA as coupling reagents. Fmoc deprotection was achieved by treating the resing bound peptide with 20% piperidine in DMF. The peptide was cleaved from the resin employing 95% TFA containing 1% triethylsilane in dichloromethane. Precipitation from dry ether followed by lyophilisation provided the peptides.

Peptide 1: Yield: 90%; ^{1}H NMR (D_2O, 600 MHz): δ/ppm = 8.03 (d, *J* = 8.8 Hz, 2H), 7.89 (d, *J* = 7.2 Hz, 2H), 7.85 (d, *J* = 6.4 Hz, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 6.90 (br, 1H), 4.85 (m, 4H), 4.37 (brm, 2H), 4.09 (s, 2H), 4.02 (s, 2H), 2.99 (m, 4H), 1.80 (m, 4H), 1.70 (m, 4H), 1.44 (m, 4H); ^{13}C NMR (D_2O, 100 MHz)): δ/ppm =173.1, 172.9, 172.4, 171.2, 167.5, 158.9, 150.2, 145.0, 135.5, 129.8, 129.7, 125.2, 123.4, 123.3, 113.0, 112.8, 56.2, 56.1, 43.7, 42.7, 42.1, 42.0, 31.5, 31.2, 28.2, 28.2, 22.3, 22.1; m/z calcd for $[M+H]^+$ $C_{29}H_{42}N_{10}O_5$: 611.33, found: 611.45.

Peptide 3: Yield: 94%; ^{1}H NMR (D_2O, 600 MHz): δ/ppm = 7.62 (d, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 7.2 Hz, 1H), 7.22 (s, 1H), 7.06 (t, *J* = 8.8 Hz, 1H); 6.96 (d, *J* = 8.8 Hz, 1H), 4.39 (m, 1H); 4.25 (m, 1H); 4.12(m, 2H); 4.08 (m, 2H); 3.85 (m, 1H); 3.36 (m, 1H); 3.10 (m, 1H); 2.92 (m, 4H), 1.80 (m, 4H), 1.74 (m, 4H), 1.41 (m, 4H); ^{13}C NMR (D_2O, 100 MHz)): δ/ppm =175.1, 173.9, 171.4, 170.2, 136.5, 127.8, 123.4, 121.2, 119.0, 118.8, 111.0, 107.5, 57.2, 56.8, 56.4, 42.7, 42.0, 31.7, 31.4, 28.9, 27.8, 22.3; m/z calcd for $[M+H]^+$ $C_{29}H_{44}N_9O_5$: 574.35, found: 574.37.

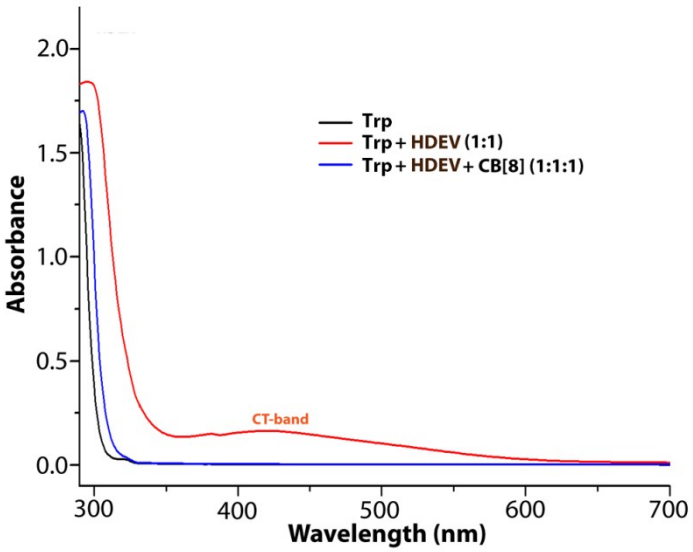

Figure S1. Absorption spectra of Tryptophan in absence and presence of HDEV and CB[8] showing the appearance of the charge transfer band upon ternary complexation at the head group of HDEV inside CB[8] cavity.[Trp] = 0.75 mM.

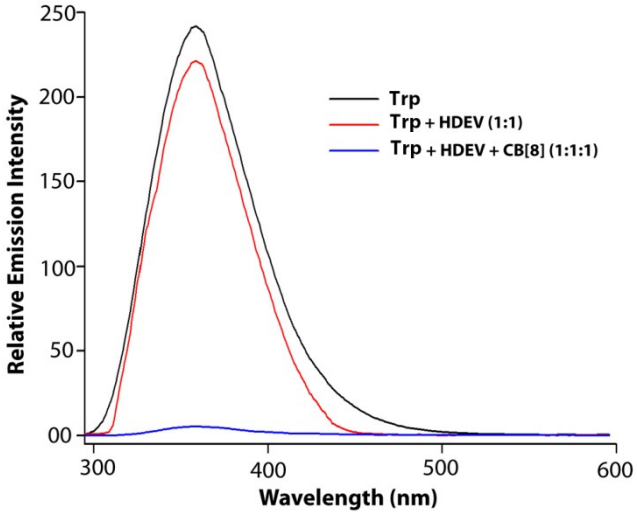

Figure S2. Fluorescence spectra of Tryptophan in absence and presence of HDEV and CB[8] in deionized water at room temperature showing the decrease in the fluorescence intensity upon ternary complexation at the head group of HDEV. [Trp] = 0.75 mM; λ_{ex} =280 nm.

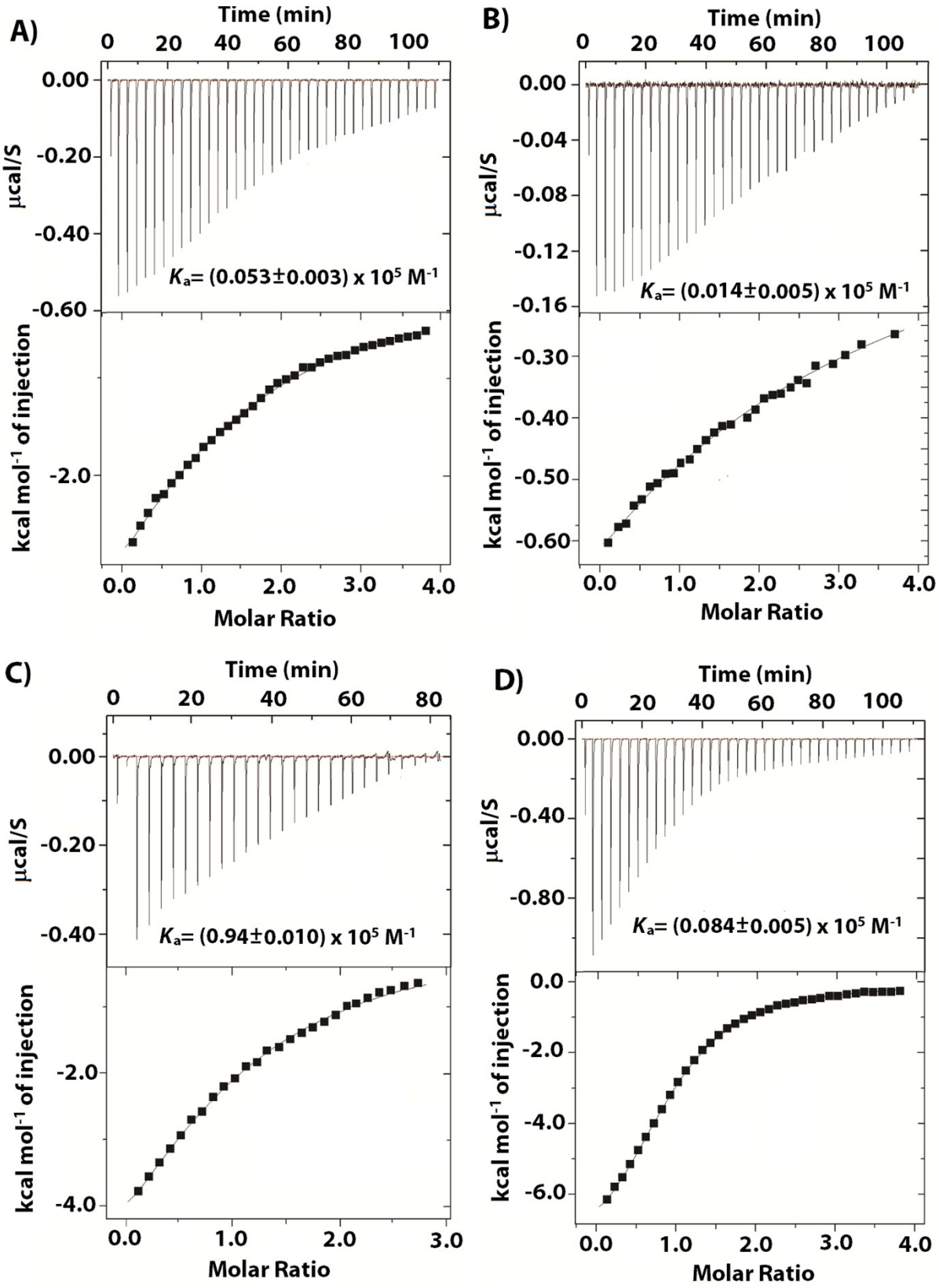

Figure S3. Fig. 2. ITC Thermogram (top) and binding isotherms (bottom) of DEV@CB[8] with A) Phe, B) Tyr, C) Trp, and D) Azo-AA at 298 K.

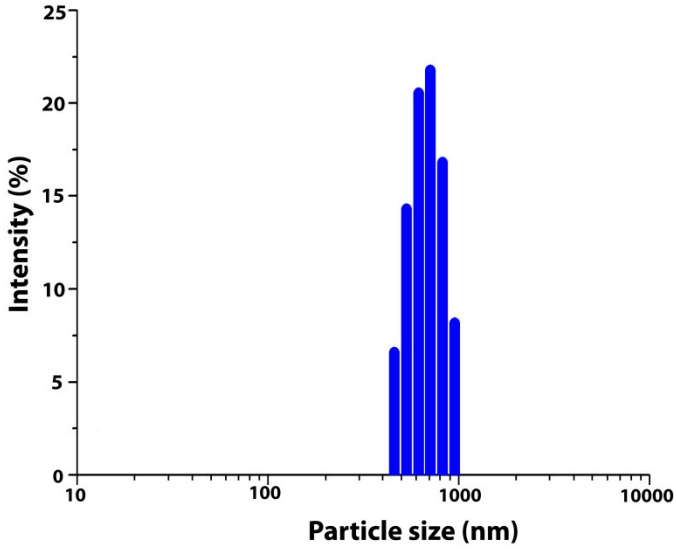

Figure S4. Intensity-weighted distributions obtained from DLS measurements of the vesicles formed by 1:1:1 Trp-HDEV@CB[8] at RT. [Trp] = 0.075 mM.

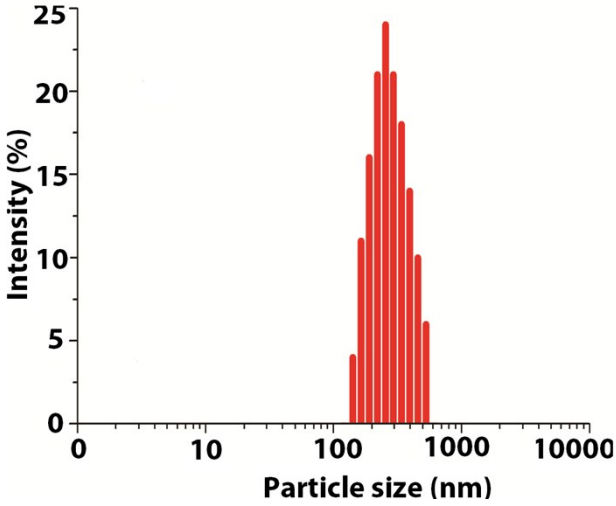

Figure S5. Intensity-weighted distributions obtained from DLS measurements of the vesicles formed by 1:1:1 Azo-AA-HDEV@CB[8] at RT. [Trp] = 0.075 mM.

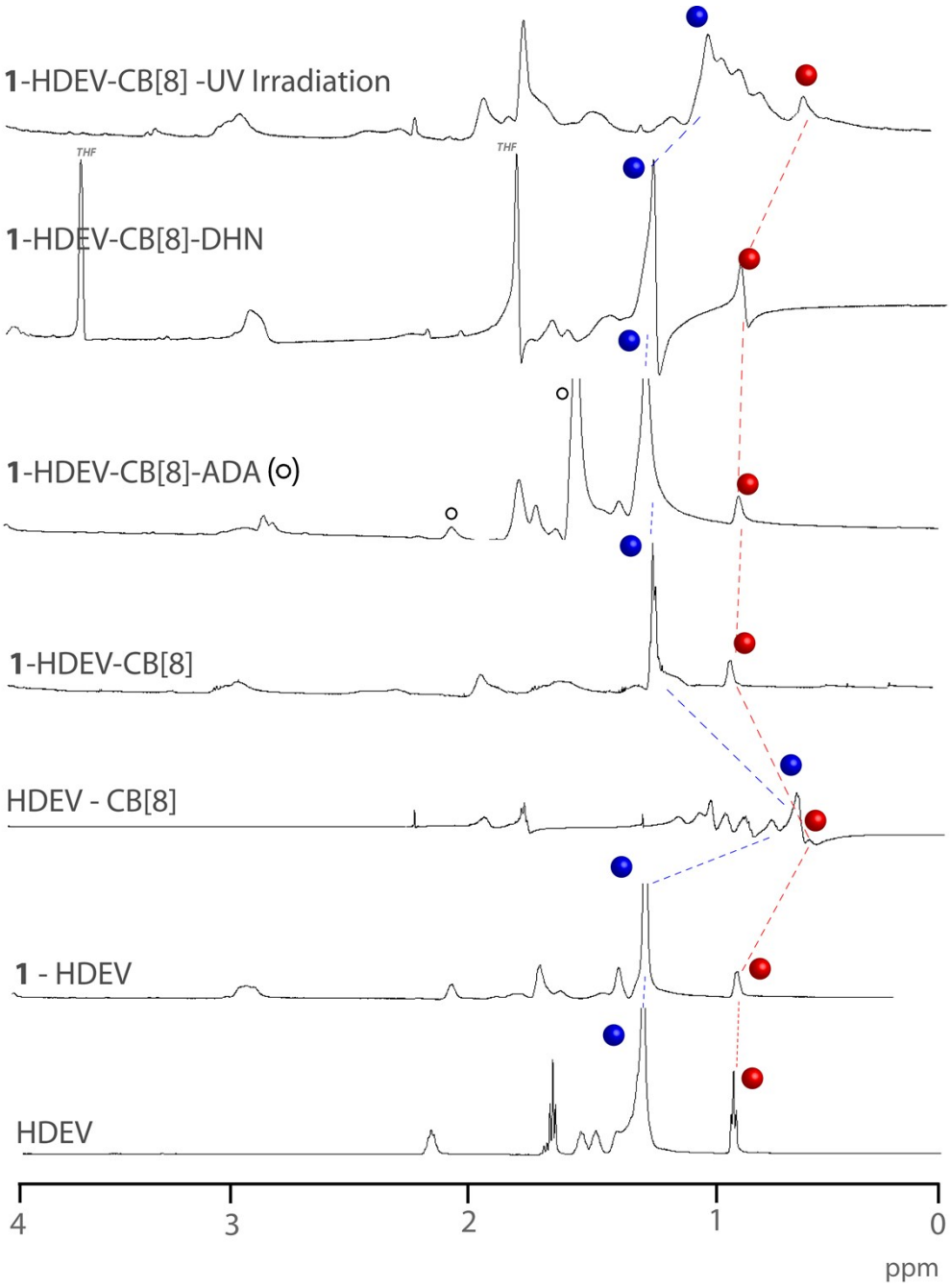

Figure S6. The aliphatic regions of the ^{1}H NMR spectra of HDEV with different compositions and under different conditions showing the shift in the ^{1}H signals originating from the hydrophobic tail of HDEV. The red-sphere and blue-sphere marked peaks correspond to the terminal methyl group of the tail and the other aliphatic protons of the tail respectively.

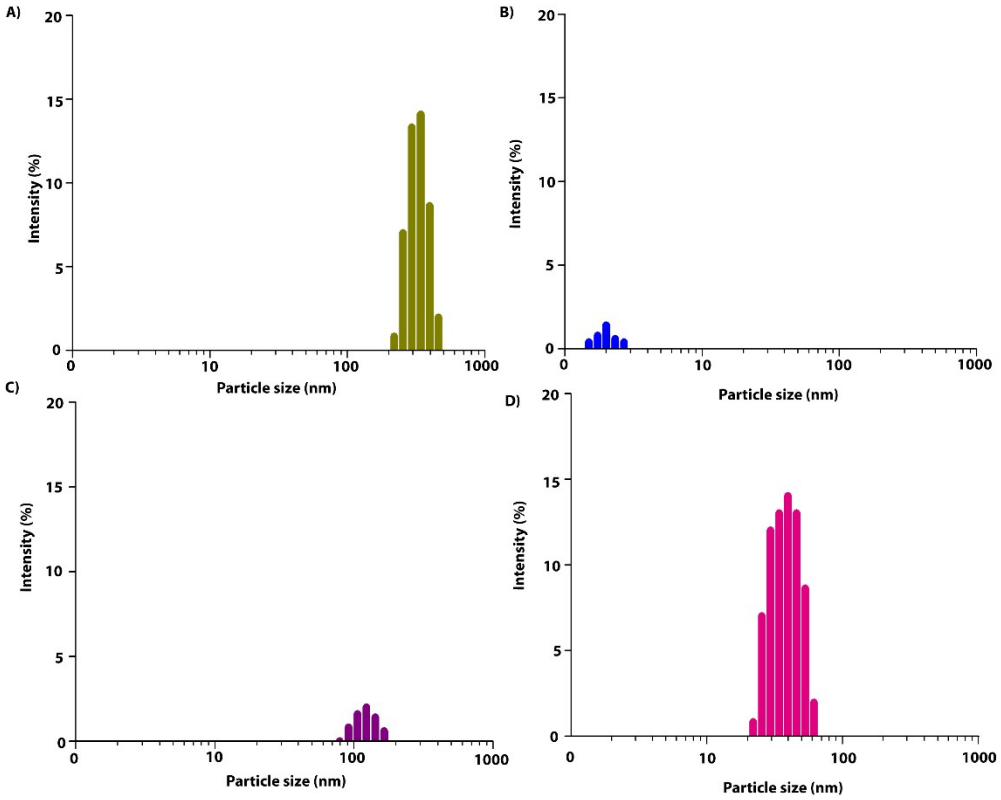

Figure S7. Intensity-weighted distributions obtained from DLS measurements 1:1:1 **1**-HDEV-CB[8] (0.075 mM) under various conditions. A) after treatment with 2 equivalent DHN; B) after treatment with 2 equivalent ADA; C) after UV irradiation with UV light (365 nm) for 8h; D) after irradiating the sample from E with a 420 nm lamp for 10 mins and incubation in dark for 48h at RT. All samples were prepared in deionized water.

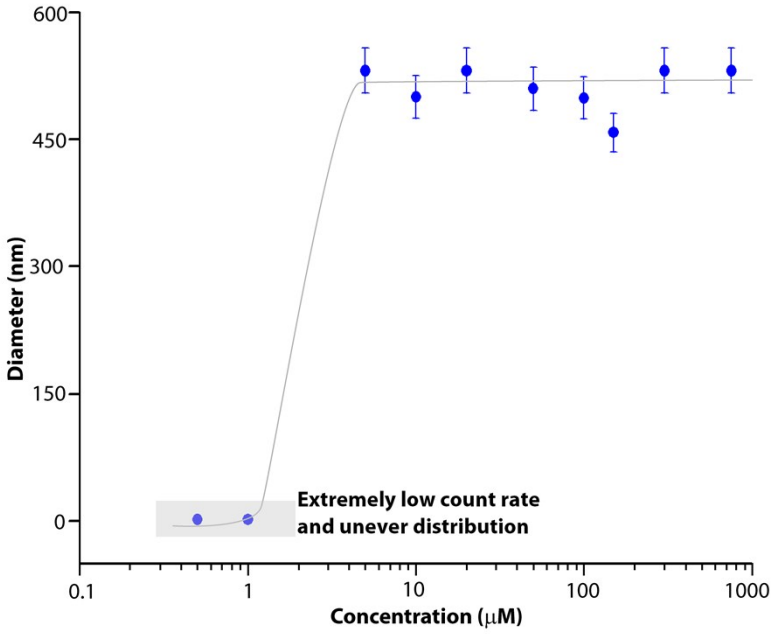

Figure S8. Concentration dependent average size of the vesicles formed by **2** in deionized water at 298 K.

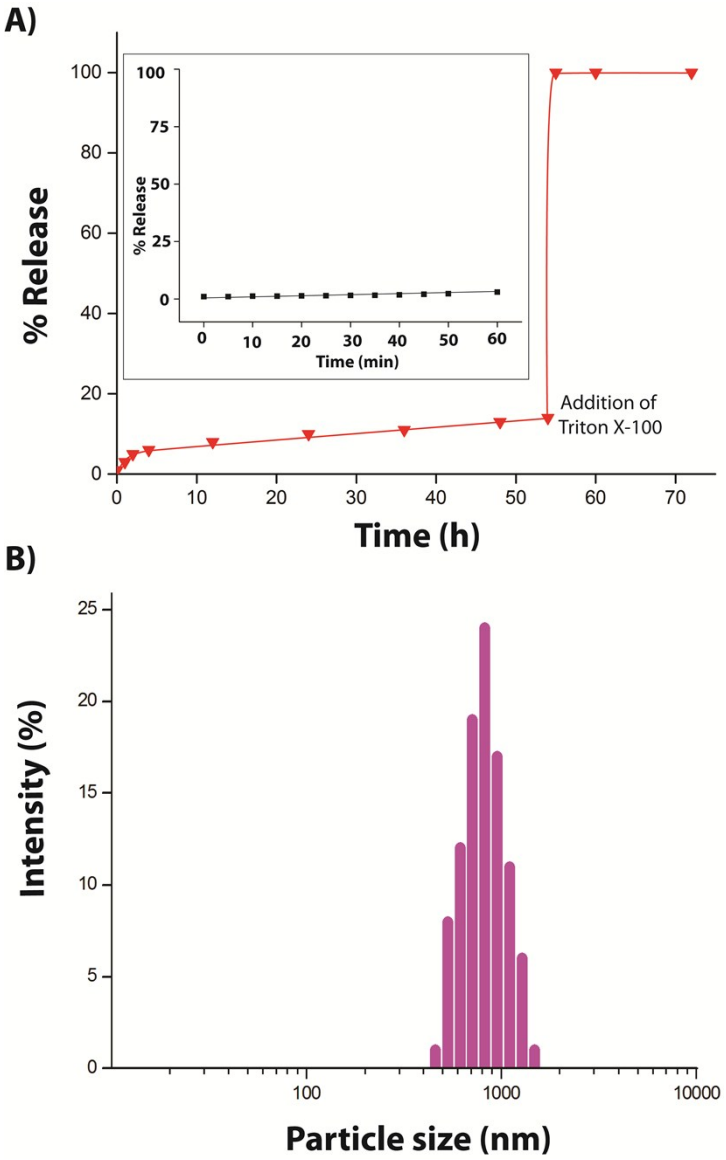

Figure S9. A) Dye release profile for 1:1:1 **3** -HDEV@CB[8] vesicles in deionized at RT. [**3**] = 0.075 mM.; Inset: dye release profile of the same system upon UV light irradiation; B) Intensity-weighted distributions obtained from DLS measurements of the vesicles formed by1:1:1 **3** -HDEV@CB[8] vesicles in deionized at RT.

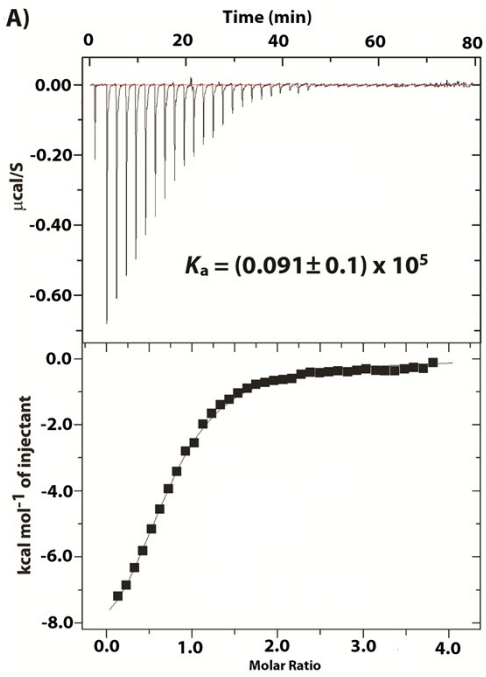

Figure S10. ITC Thermogram (top) and binding isotherms (bottom) of DEV@CB[8] with **1** at 298 K

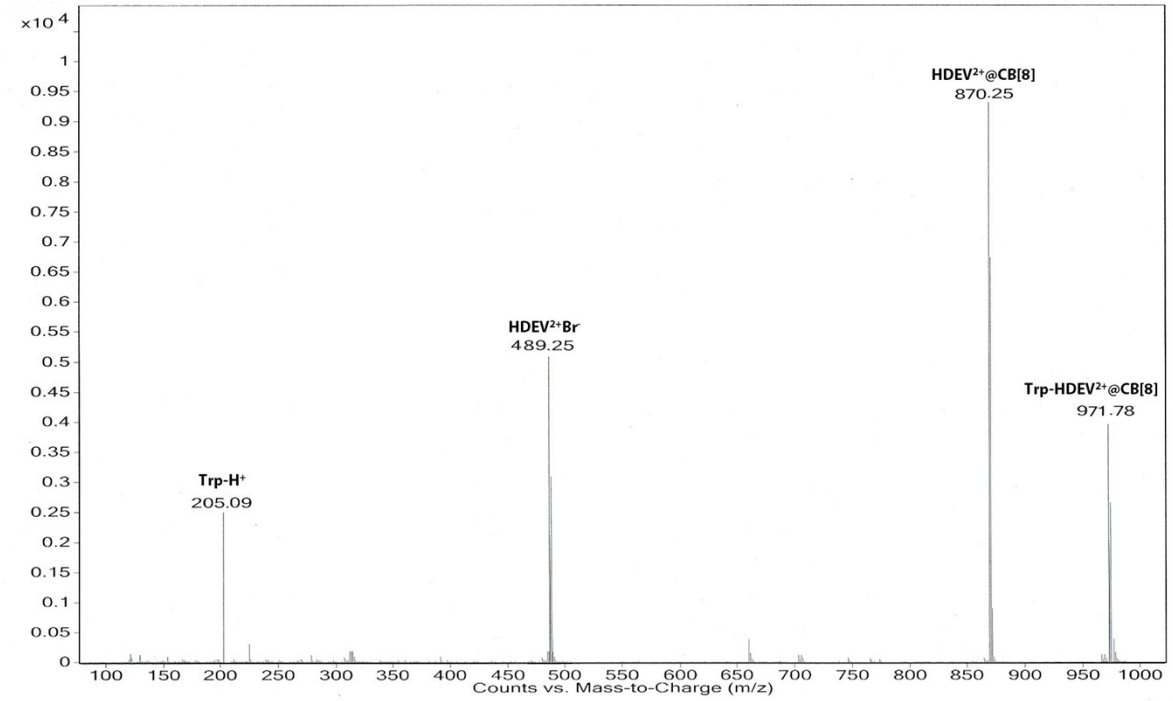

Figure S11. The ESI-MS spectrum of the 1:1:1 mixture of Trp, HDEV, and CB[8] in deionized water showing the appearance of the signal from the ternary complex (Trp-HDEV-CB[8]).

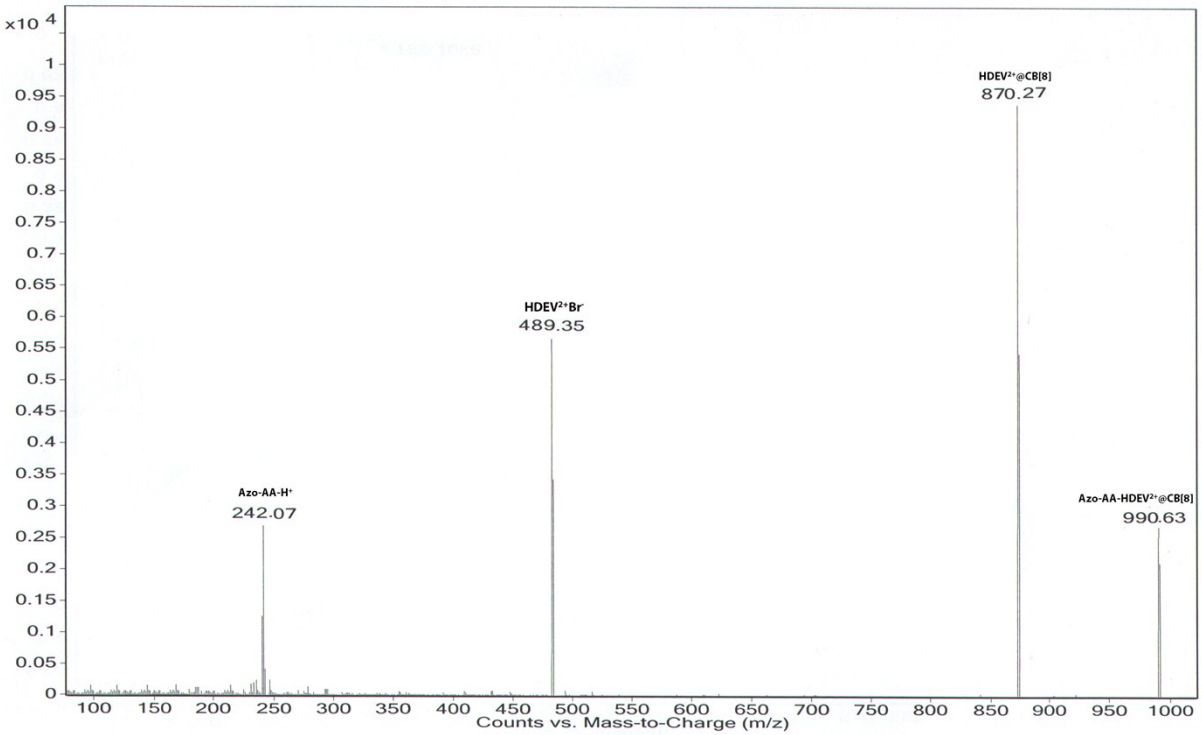

Figure S12. The ESI-MS spectrum of the 1:1:1 mixture of Azo-AA, HDEV, and CB[8] in deionized water showing the appearance of the signal from the ternary complex (Azo-AA-HDEV-CB[8]).

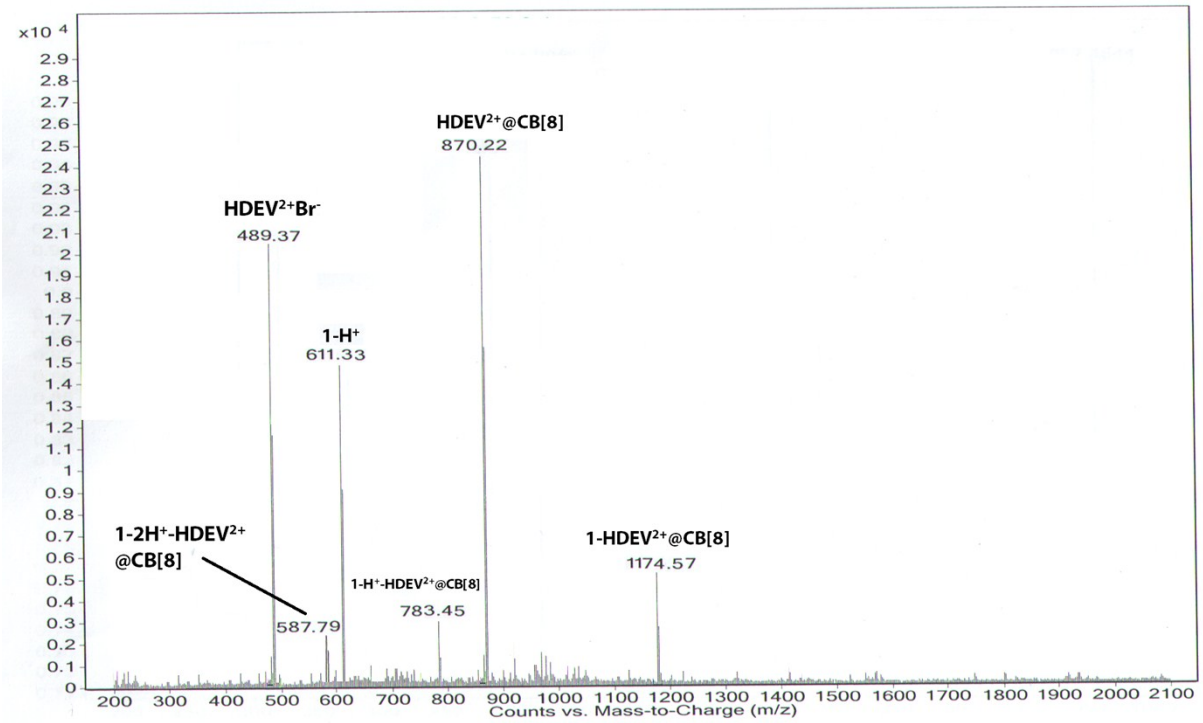

Figure S13. The ESI-MS spectrum of the 1:1:1 mixture of **1**, HDEV, and CB[8] in deionized water showing the appearance of the signal from the ternary complex (**1**-HDEV-CB[8]).

Table S1. Solution Binding constants (K_a) and related thermodynamic parameters for the complexation different amino acids with DEV@CB[8] at 298 K in 100 mM phosphate buffer pH 7.

Guest	N (sites)	K_a (10^5 M^{-1})	ΔH (cal/mol)	$T\Delta S$ (cal/mol/deg)
Phe	1.15	0.053 ± 0.003	-7276 ± 59.04	-7.35
Tyr	0.82	0.014 ± 0.005	-7723 ± 75.41	-12.10
Trp	0.95	0.94 ± 0.01	-8798± 25.01	-7.84
Azo-AA	0.94	0.84 ± 0.005	-8470 ± 65.01	-7.74
Peptide 1	1.18	0.91 ± 0.01	-5568± 86.60	2.19

References

1. J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.* **2000**, *122*, 540−541.
2. J. H. Mondal, T. Ghosh, S. Ahmed, D. Das, *Langmuir* **2014**, *30*, 11528-11534.
3. J. Auernheimer, C. Dahmen, U. Hersel, A. Bausch, H. Kessler, *J. Am. Chem. Soc.* **2005**, *127*, 16107−16110.