Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Controlling the release of hydrophobic compounds by supramolecular amphiphilic assembly

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Materials

Synthesis of 2,2'-bibenzimidazole was described elsewhere.[1] 1-Phenylazo-2-naphthol (Sudan I) and cetyltrimethylammonium bromide (CTAB) (both from Acros Organics), Triton X-100 (Sigma) were used as received. Deionized water used for the preparation of all solutions was provided by the Millipore Direct-Q 5 UV system. The pH of the solutions was measured with a glass electrode (HANNA HI-2210 pH meter) with a precision of ±0.01 at 25ºC.

Synthesis of calix[4]resorcinarene

A mixture 0.15 g (5 mmole) of paraformaldehyde and 0.4 ml (5 mmole) of 2-(methylamino)ethanol in 15 ml of ethanol was stirred at 80 °C under argon for 15 minutes. A suspension of 1 g (1 mmole) of sodium (2-sulfonatoethyl)resorcinarene in 30 ml of ethanol was added in portions within two hours. The reaction mixture was stirred at 80°C for 48 hours. The precipitation was filtrated, washed with ethanol and dialyzed against 1000 ml of water (3 times × 30 min). Yield, 1.14 g (85 %), ^{1}H NMR (600 MHz, D_2O, δ): 2.55 (s, 12H, CH_3N), 2.68 (m, 8H, $C\underline{H}_2CH_2SO_3$), 2.89 (m, 8H, $CH_2C\underline{H}_2SO_3$), 3.06 (m, 8H, $HOC\underline{H}_2CH_2N$), 3.70 (t, 8H, $HOC\underline{H}_2CH_2N$), 4.16 (s, 8H, $ArC\underline{H}_2N$), 4.56 (t, 4H, CH), 7.22 (s, 4H, ArH). MALDI-TOF, MS $[C_{52}H_{72}N_4O_{24}S_4^{4-} + 3H^+ + Na^+]$: calcd. 1291, found 1291; $[C_{52}H_{72}N_4O_{24}S_4^{4-} -2CH_2CH_2OH+ H^+ + K^+]$: calcd. 1216, found 1216. Elemental analysis ($C_{52}H_{72}N_4Na_4O_{24}S_4\times12H_2O$): calcd. C 39.69, H 6.15, N 3.56, S 8.15; found C 39.29, H 5.88, N 3.17, S 7.76.

Methods

Surface tension measurements were performed using the du Nouy ring detachment method. Each data point represented the average of ca. ten to fifteen measurements of surface tension. Each of concentration dependence was obtained three times, and the results were within 2 to 3%. Besides, solutions were monitored in time, e.g. after one, two and five days to test that they are equilibrated.

DLS studies were conducted at 25°C by using a Zetasizer Nano instrument (Malvern, UK) equipped with a 4 mW He-Ne laser operating at λ=633 nm. Correlation data were fitted by using the method of cumulants to the logarithm of the correlation function to yield the diffusion coefficient. Backscattered light was detected at 173°, and the number-average hydrodynamic diameter was calculated by using the Stokes–Einstein equation. The diffusion coefficient was measured at least 5 times in 10 runs, so that ≥50 scans were obtained for each sample. Zeta-potential measurements were conducted by using the same Malvern Zetasizer Nano instrument described above. Zeta potentials were calculated from electrophoretic mobilities by using the Smoluchowski relationship. All light scattering data (DLS and aqueous electrophoresis) were processed by using Malvern Zetasizer Software.

Fluorescence emission spectra and steady-state anisotropy measurements were performed on a Cary Eclipse fluorescence spectrophotometer (USA). The embedded software automatically determined the correction factor and anisotropy value. A fixed concentration of fluorescence probe DPH of 0.05 mM was used. Excitation and emission wavelengths used for DPH were set to 344 and 430 nm, respectively. A temperature of 25 °C was maintained.

Transmission electron microscopy (TEM) images were recorded on a Hitachi HT7700 TEM instrument (Japan) operated at 110 kV accelerating voltage. The samples were ultrasonicated in water for 10 min and then dispersed on 300 mesh carbon-coated copper grid.

The Sudan I solubilization experiments were performed by adding an excess of crystalline Sudan I dye to solutions. These solutions were allowed to equilibrate for about 48 h at room temperature. They were filtered with Millipore filters (0.45 μm), and their absorbancy was measured at λ=486 nm (molar extinction coefficient 17400M-1cm-1) on a Specord 250 Plus spectrophotometer (Analitic Jena, Germany) equipped with WinAspect software at (25±0.1)°C in a 0.1 cm path length cell. Absorbance of the dye for each sample was obtained by subtraction of the contribution of components to the summary spectrum. Reproducibility was checked for selected samples and no significant differences were observed.

Encapsulation of BBI and its CR-mediated release were detected by fluorescence techniques. An excess of BBI was added into 20 mM CTAB solution which then was kept for about 48 h at room temperature. Untrapped free BBI was removed by filtration with Millipore filter (0.45 μm) from solution. The 20 mM CTAB solution was aliquoted from a fraction collected after filtration. Immediately, the initial fluorescence intensity of one of aliquoted 20 mM CTAB solution was measured using a Cary Eclipse fluorescence spectrophotometer (USA). And then the appropriate amount of CR was added to the 20 mM CTAB solution to achieve CR concentration of 0.5 mM and CTAB concentration of 10 mM and then the fluorescence

intensity was measured. To confirm overall intensity, another aliquoted 20 mM CTAB solution was diluted by adding water (2 folds).

Reference

1. V. A. Mamedov, T. N. Beschastnova, N. A. Zhukova, S. F. Kadyrova, A. T. Gubaidullin and O. G Sinyashin, Method of Producing 2,2-Bisbenzidazole, *R.F. patent,* 2413722, 2011.

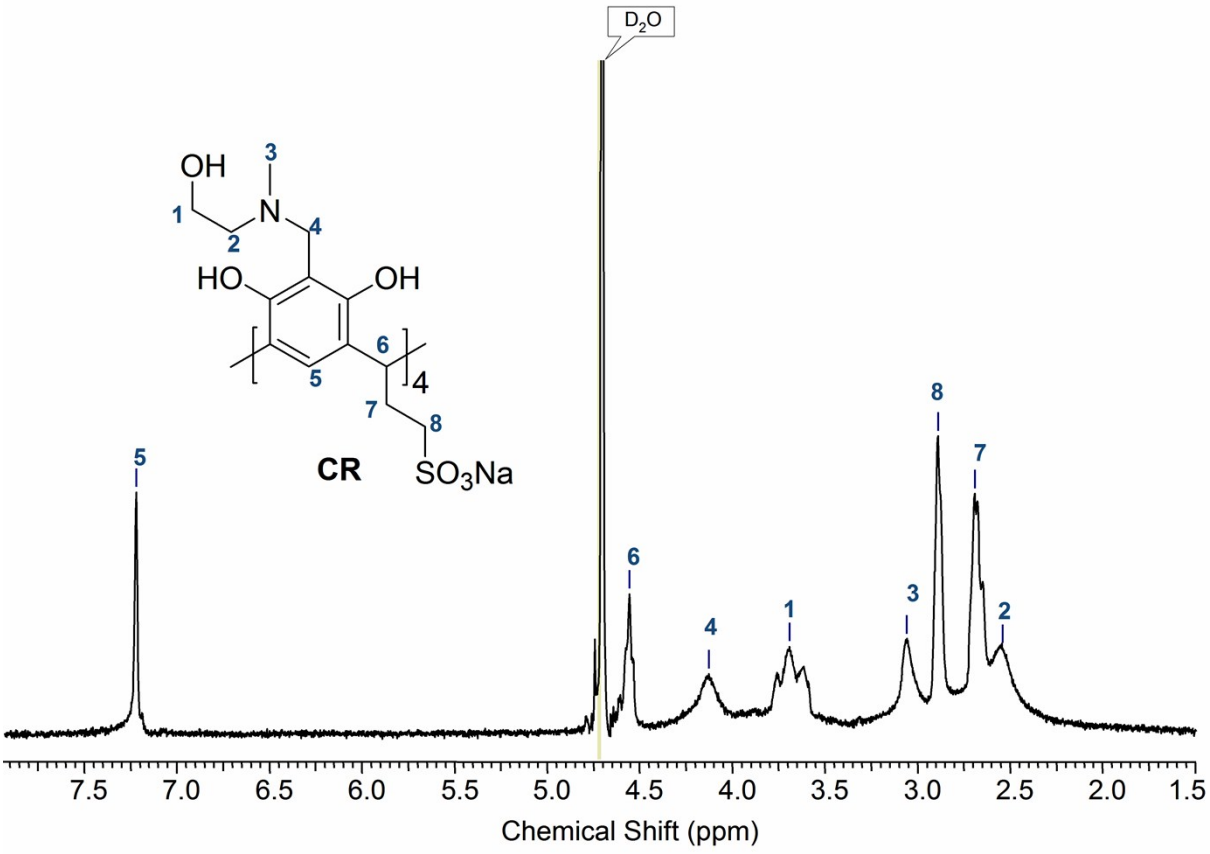


Fig. S1. ^{1}H NMR spectrum of CR in D_2O, 25 °C.

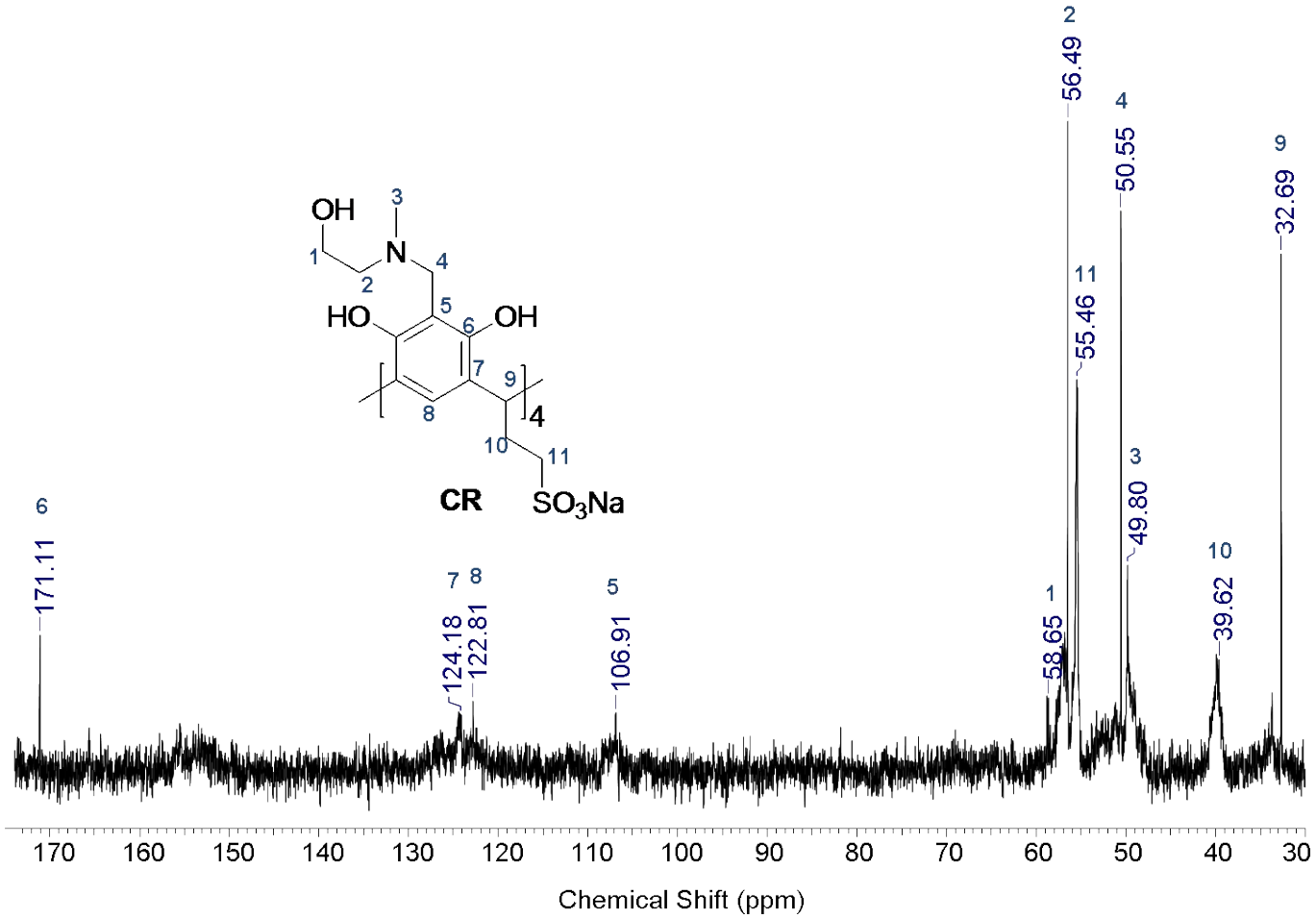


Fig. S2. ^{13}C NMR spectrum of CR in D_2O, 25 °C.

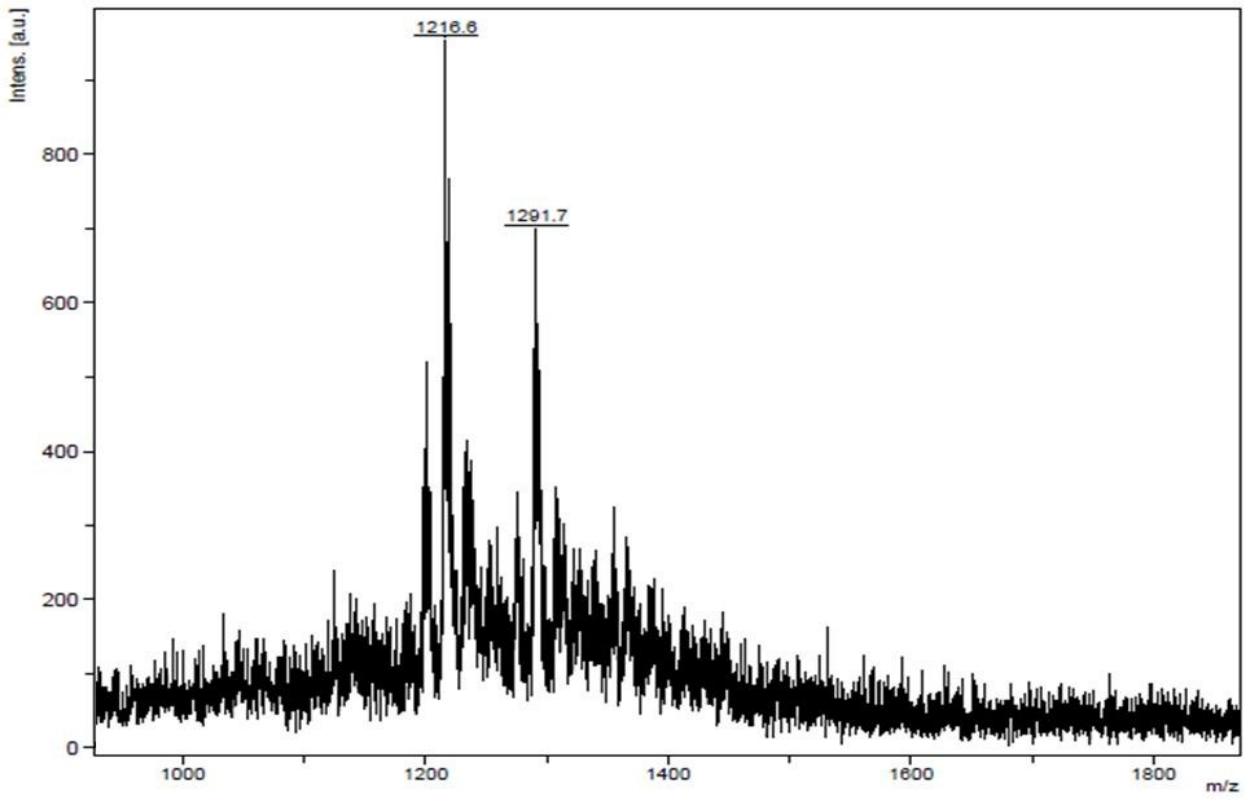


Fig. S3. MALDI-TOF spectrum of CR.

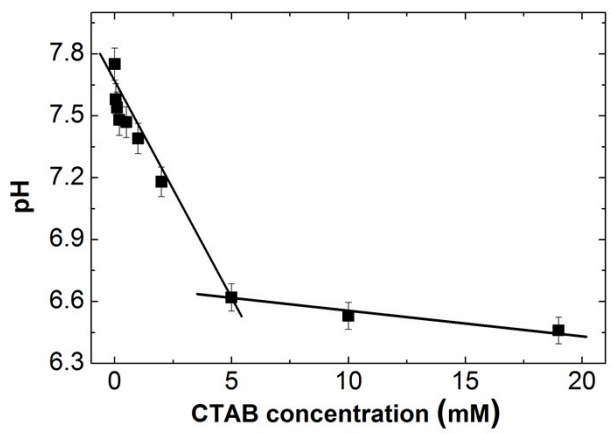


Fig. S4. CTAB concentration dependence of pH for CR–CTAB aqueous solutions with fixed CR concentration of 1 mM, 25°C.

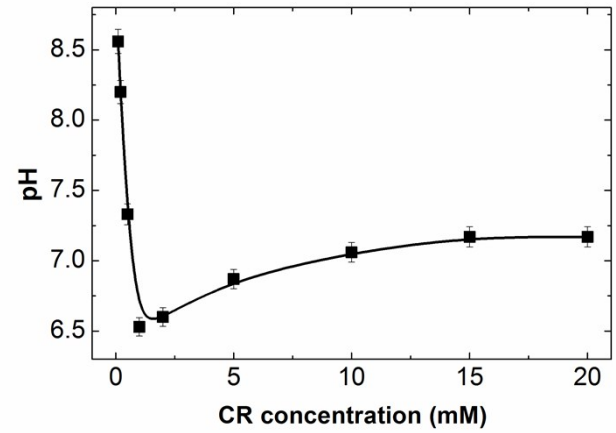
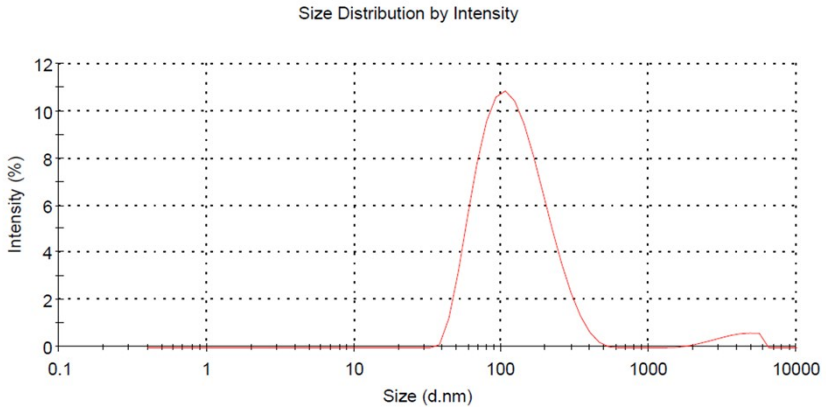


Fig. S5. CR concentration dependence of pH for CR–CTAB aqueous solutions with fixed CTAB concentration of 10 mM, 25°C.

1 mM CR – 1 mM CTAB

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 109.8	**Peak 1:**	131.2	96.5	69.64
PdI: 0.225	**Peak 2:**	3916	3.5	1114
Intercept: 0.928	**Peak 3:**	0.000	0.0	0.000

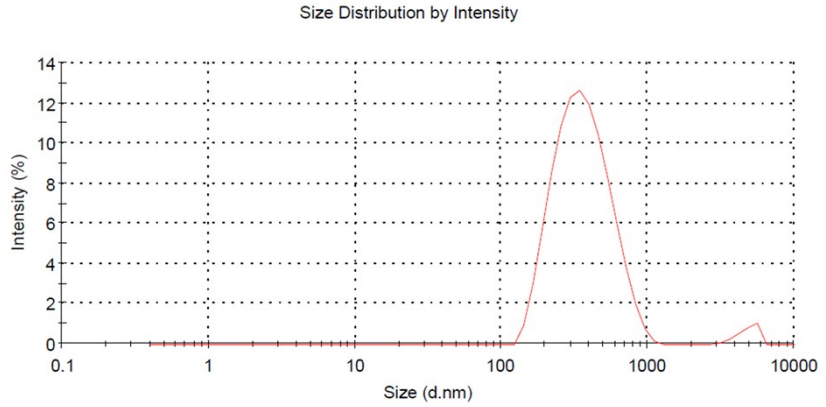
Result quality Good



1 mM CR – 5 mM CTAB

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 342.1	**Peak 1:**	381.8	97.2	164.8
PdI: 0.205	**Peak 2:**	4804	2.8	723.1
Intercept: 0.903	**Peak 3:**	0.000	0.0	0.000

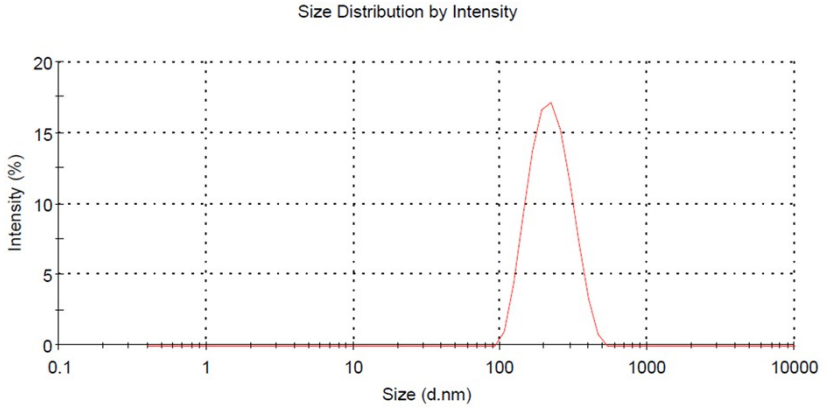
Result quality Good



1 mM CR – 10 mM CTAB

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 204.3	**Peak 1:**	225.5	100.0	71.18
PdI: 0.099	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.938	**Peak 3:**	0.000	0.0	0.000

Result quality Good



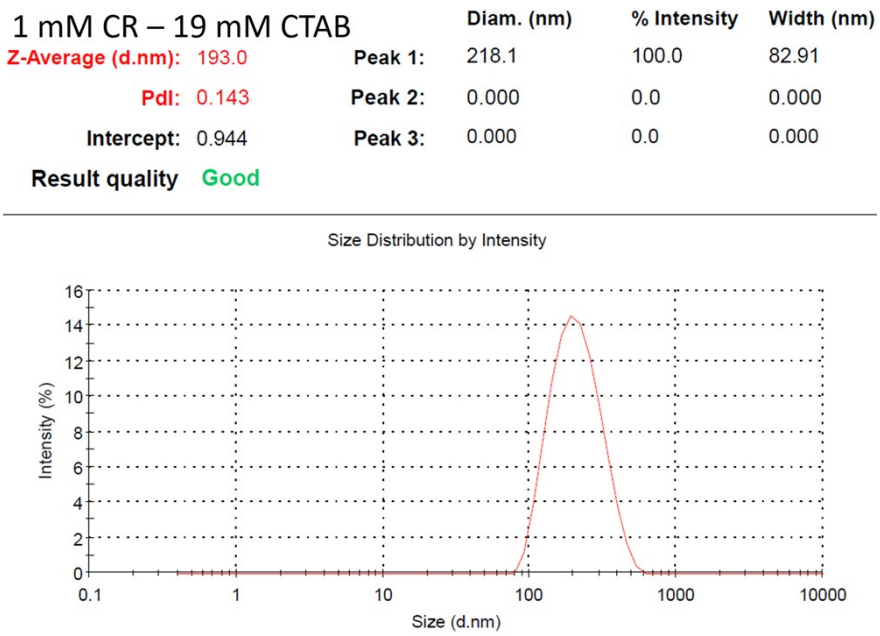


Fig. S6. DLS results obtained from the mixed aqueous 1 mM CR – CTAB solutions, 25°C.

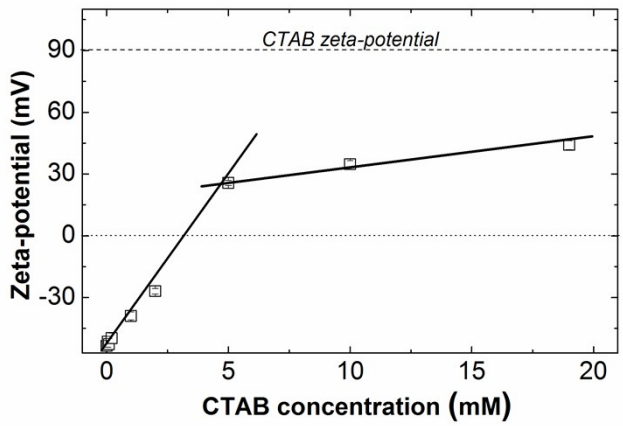
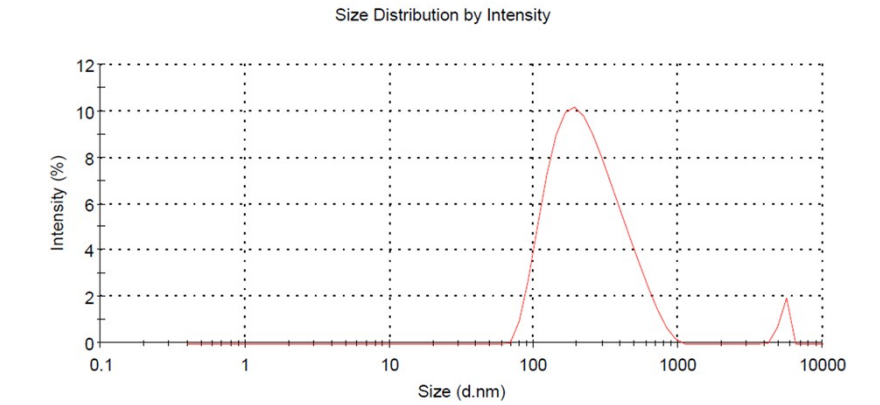


Fig. S7. Zeta-potential for individual CTAB solution and CR–CTAB mixture with fixed CR concentration of 1 mM, H_2O, 25°C.

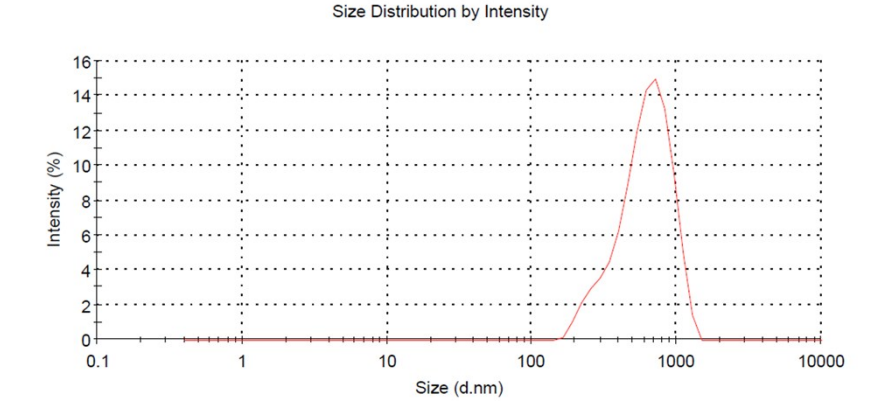
1 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 230.0	Peak 1:	259.4	97.3	149.7
PdI: 0.373	Peak 2:	5346	2.7	357.1
Intercept: 0.900	Peak 3:	0.000	0.0	0.000
Result quality Good				



5 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 548.4	Peak 1:	638.5	100.0	242.3
PdI: 0.265	Peak 2:	0.000	0.0	0.000
Intercept: 0.929	Peak 3:	0.000	0.0	0.000
Result quality Good				



10 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 322.9	Peak 1:	612.7	61.9	239.4
PdI: 0.270	Peak 2:	204.8	38.1	50.45
Intercept: 0.930	Peak 3:	0.000	0.0	0.000
Result quality Good				

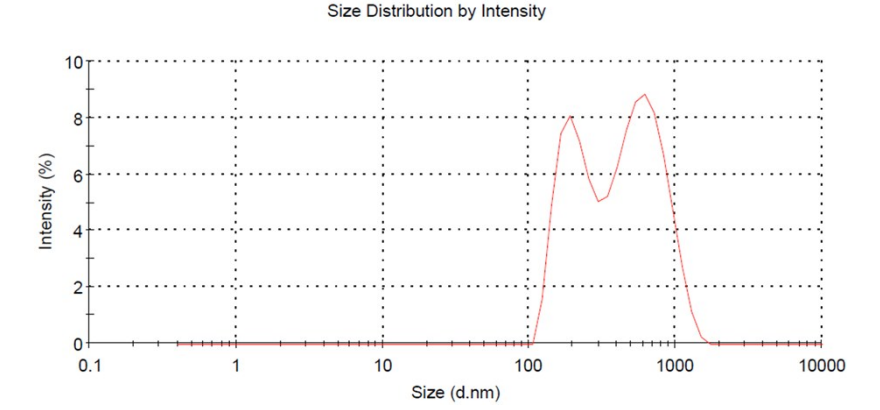
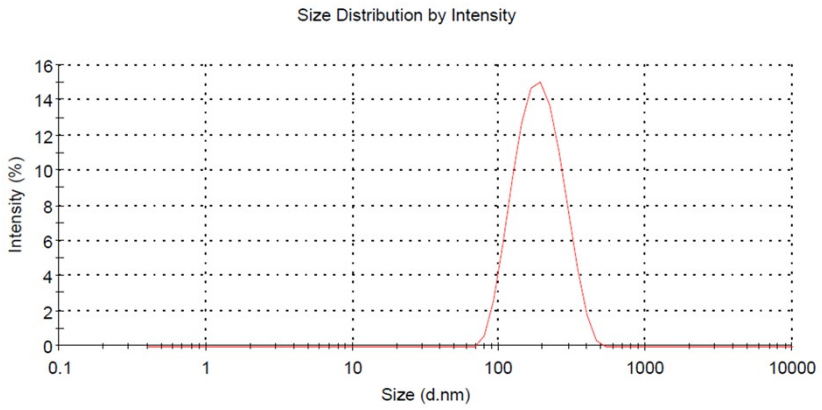


Fig. S8. DLS results obtained from the aqueous single CR solutions, 25°C.

10 mM CTAB – 0.5 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 171.1	**Peak 1:**	196.3	100.0	70.82
PdI: 0.127	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.946	**Peak 3:**	0.000	0.0	0.000

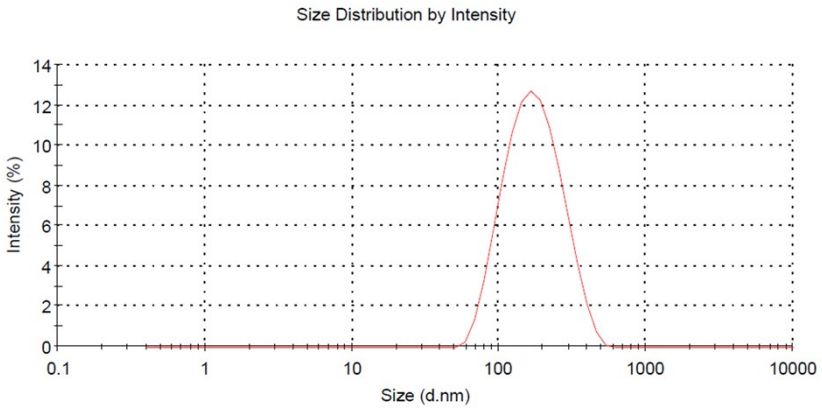
Result quality Good



10 mM CTAB – 0.5 mM CR – 20 mM Triton X-100

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 150.4	**Peak 1:**	184.1	100.0	79.68
PdI: 0.183	**Peak 2:**	0.000	0.0	0.000
Intercept: 0.948	**Peak 3:**	0.000	0.0	0.000

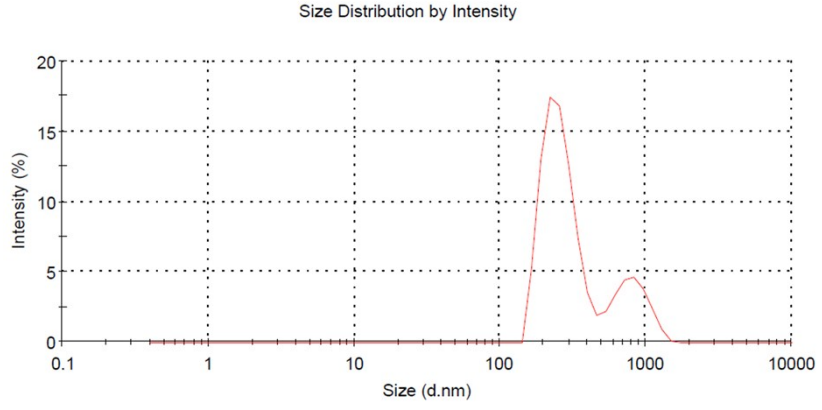
Result quality Good



10 mM CTAB – 5 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 298.1	**Peak 1:**	256.5	76.5	66.73
PdI: 0.221	**Peak 2:**	787.9	23.5	217.1
Intercept: 0.923	**Peak 3:**	0.000	0.0	0.000

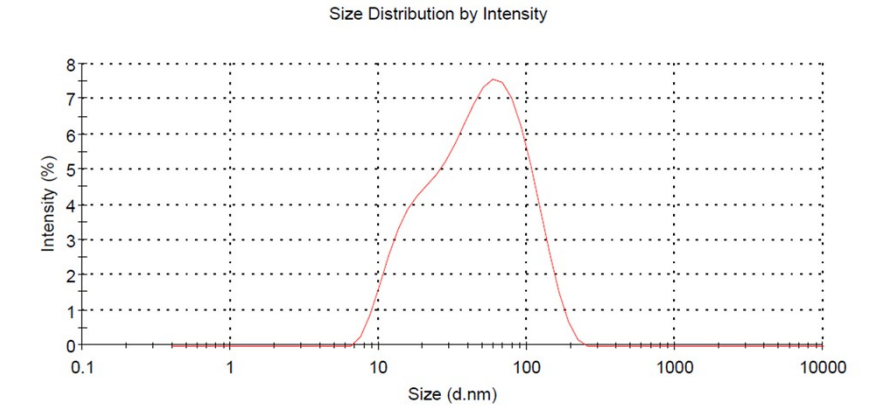
Result quality Good



10 mM CTAB – 5 mM CR – 20 mM Triton X-100

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 35.34	Peak 1:	55.87	100.0	38.39
PdI: 0.305	Peak 2:	0.000	0.0	0.000
Intercept: 0.932	Peak 3:	0.000	0.0	0.000

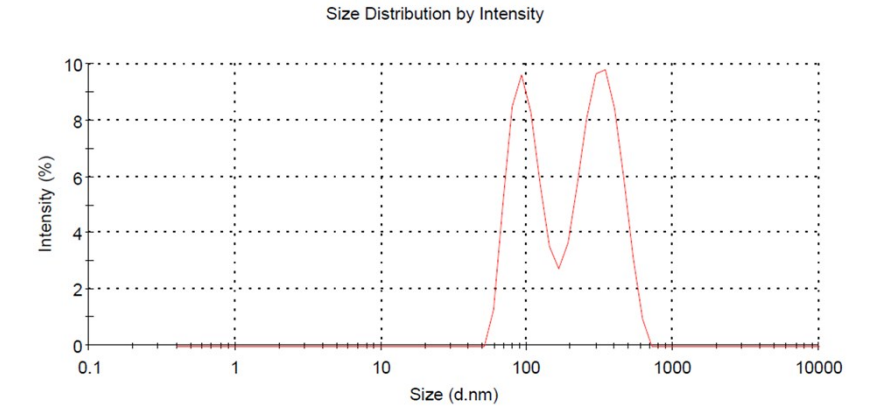
Result quality Good



10 mM CTAB – 10 mM CR

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 164.6	Peak 1:	326.6	56.5	102.8
PdI: 0.355	Peak 2:	100.5	43.5	26.96
Intercept: 0.929	Peak 3:	0.000	0.0	0.000

Result quality Good



10 mM CTAB – 10 mM CR – 20 mM Triton X-100

		Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 30.02	Peak 1:	58.10	100.0	48.11
PdI: 0.457	Peak 2:	0.000	0.0	0.000
Intercept: 0.938	Peak 3:	0.000	0.0	0.000

Result quality Good

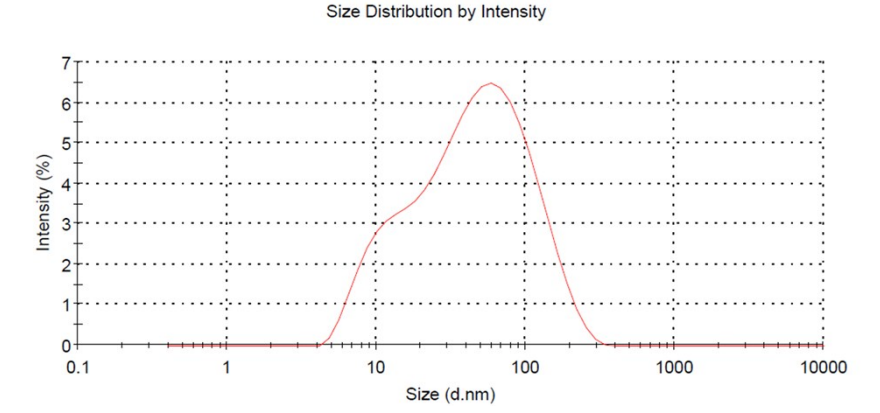


Fig. S9. DLS results obtained from mixed 10 mM CTAB – CR aqueous solutions in absence and presence of Triton X-100, 25°C.

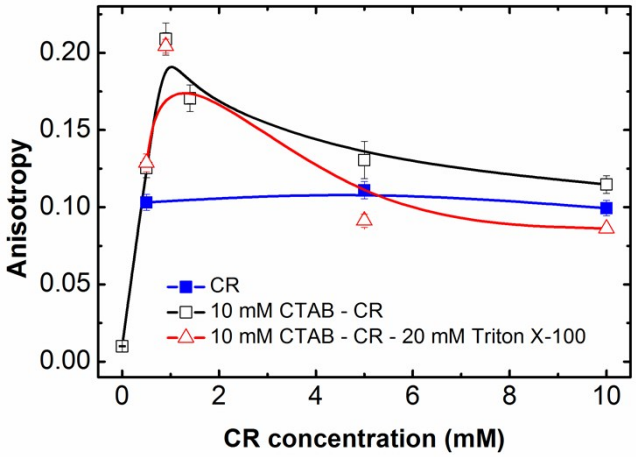


Fig. S10. Variation of fluorescence anisotropy of DPH (0.05 mM) in single CR solutions and mixed 10 mM CTAB – CR solutions in absence and presence of Triton X-100 as a function of concentration of CR, H_2O, 25°C.

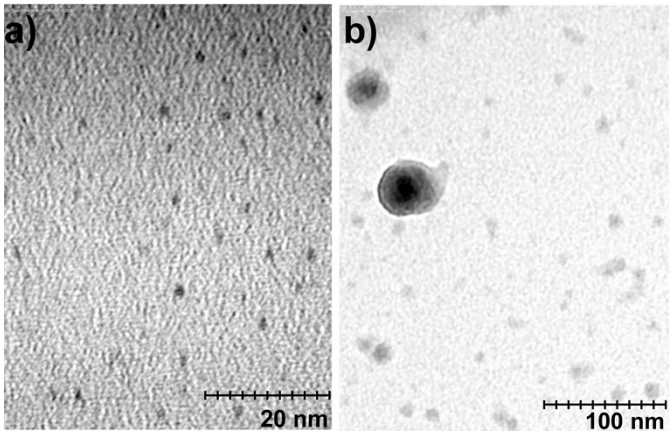


Fig. S11. TEM images of (a) 10 mM CTAB micelles and (b) 10 mM CTAB – 0.5 mM CR vesicles

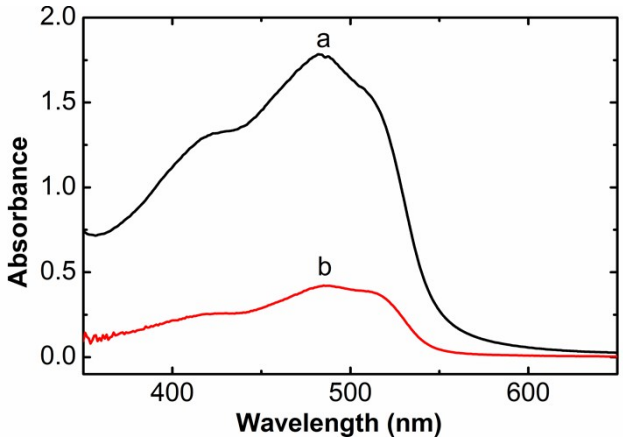


Fig. S12. UV spectra of Sudan I (a) in 19 mM CTAB solution and (b) in mixed 19 mM CTAB – 1 mM CR aqueous solution, 25ºC; optical length 0.1 cm.

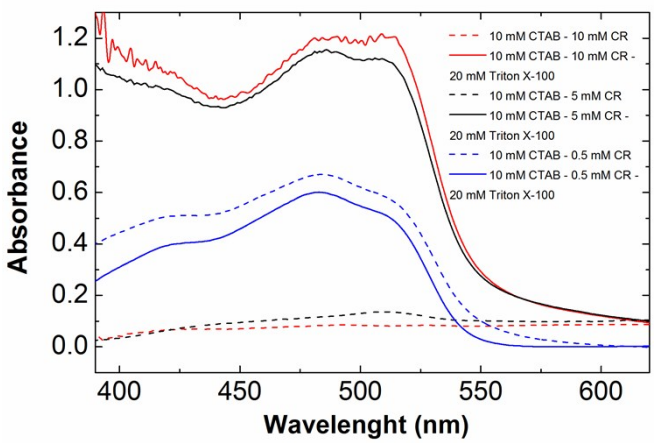


Fig. S13. UV spectra of mixed 10 mM CTAB – CR aqueous solutions in absence and presence of Triton X-100, 25ºC, optical length 0.1 cm.

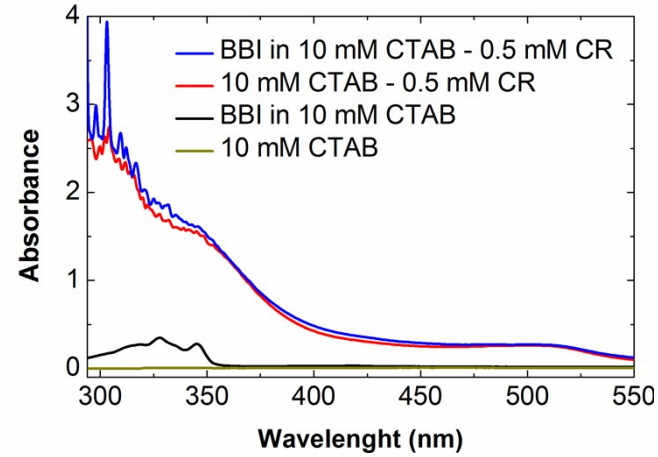


Fig. S14. UV spectra of single 10 mM CTAB solution and mixed 10 mM CTAB – 0.5 mM CR aqueous solutions in absence and presence of BBI, 25ºC; optical length 0.5 cm.