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Supporting Information

Unusual self-assembly of a hydrophilic β-cyclodextrin inclusion complex into vesicles capable of drug encapsulation and release

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1. Methods and Materials:

A. General methods: β-CD and Doxorubicin were purchased from Aldrich used as received. The solvents and reagents were dried and purified by standard methods prior to use. The electronic absorption spectra were recorded on a Shimadzu UV-3101 or 2401PC UV-VIS-NIR scanning spectrophotometer. ITC data were obtained using microcal iTC 200. The raw data obtained were fitted and analysed using origin 7.0 software provided along with the instrument. All NMR data were

recorded in D_2O purchased from Aldrich, using a 500 MHz Bruker Avance DPX spectrometer. AFM measurements were carried out using NTEGRA (NT- MDT) instrument using micro fabricated TiN cantilever tips (NSG-10) with a resonating frequency of 299 KHz and a spring constant of 20-80 Nm^{-1} and multimode scanning probe microscope (Nanoscope IV controller, digital instruments), using tapping mode techniques. The samples for AFM were prepared by drop casting the solution on freshly cleaved mica surface and the excess solvent was evaporated. TEM analyses were performed using a FEI-TECNAI T30 G^2S-TWIN, 300 kV HRTEM microscope with an accelerating voltage of 100 kV and the samples were prepared by drop casting the solution on a formvar coated copper grid (400 mesh) and evaporating excess solvent. Confocal laser scanning microscopy (CLSM) images were obtained on a Lecica-DMIR2 optical microscope by collecting the emission in the 550 - 610 nm region at 20× magnification. Samples for CSLM were prepared by drop casting the above solution on a glass slide followed by slow evaporation. The SEM images were recorded by using ZEISS EVO MA and LS series scanning electron microscope. The operating range was between 100-230V at 50-60Hz single phase with a consumption of 2.5 kVA. The sample solution in water was drop casted directly on the top of the aluminium grid and the solvents were allowed to evaporate at ambient conditions.

B. Procedure for preparation of vesicles: The compounds AD-AD and β- CD were taken in the 1:2 ratios in water. The solution was heated to ensure dissolution of both the compounds and allowed to cool to room temperature. The formed supramolecular complex spontaneously self assembled into vesicles.

C. Procedure for loading and releasing of DOX into vesicles: **DOX** loaded vesicles were prepared by mixing a solution of **DOX** hydrochloride (1 mM) with **β-CD/AD-AD** vesicle (5×10^{-4} M **AD-AD** and 1×10^{-3} **β-CD**). The solution was kept aside overnight and then dialyzed in a dialysis membrane

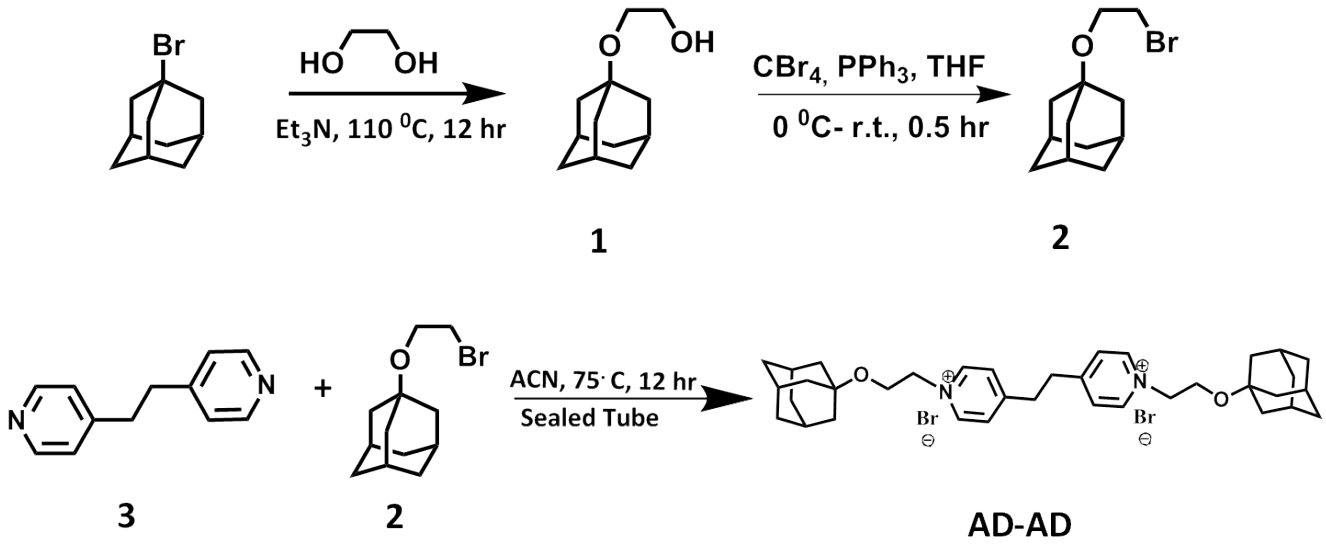
(MW cut off = 3500) to remove free DOX for 3 h using distilled water. For drug release study, **ADC** (2.25×10^{-3}) was added to the above solution and kept aside for 2 h.

D. Calculation of DOX encapsulation efficiency of vesicles: The DOX encapsulation efficiency was calculated using following equation.

Encapsulation efficiency (%) = (mass of DOX encapsulated x 100) ÷ mass of DOX loaded into vesicles

An aqueous solution of DOX was prepared and its concentration determined using the known extinction coefficient value of 11,400M^{-1} cm^{-1} at 480 nm.[1] The solution was then loaded to preformed vesicles and after 12 hrs it was dialyzed for 3 hrs. Concentration of DOX encapsulated in the vesicles was determined from the absorption of the vesicle solution at 480 nm. DOX masses were calculated from the concentration values.

2. Synthetic scheme for AD-AD:



Scheme 1 : Scheme for the synthesis of AD-AD

Synthesis of molecule AD-AD:

Synthesis and characterization of molecule 2 in the scheme is already reported by our group.[2] Compound 3 (0.050 g,0.27 mmole) and compound 2(0.140g,0.54 mmole) were heated in dry acetonitrile (5ml) for 12 hours in a sealed tube.The white precipitate formed was filtered and washed repeatedly with dry acetonitrile.Yield of the product:0.063g (42.8 %). ^{1}H NMR (500 MHz, D_2O) δ: 8.60 (d, 4H), 7.8(d, 4H), 4.5(t, 4H), 3.8(t, 4H), 3.3(t, 4H) 1.95(s, 6H), 1.5(m, 6H) and 1.3(m, 18H). ^{13}C NMR (125 MHz D_2O) δ:160.89, 144.29, 127.72, 74.89, 61.44, 58.80, 40.36, 35.36,34.06, 30.14.

ESI-HRMS: Mass calculated for $C_{36}H_{50}N_2O_{22}^{+}$ is 542.39 and mass obtained was 542.38.

3. ^{1}H NMR of molecule AD-AD:

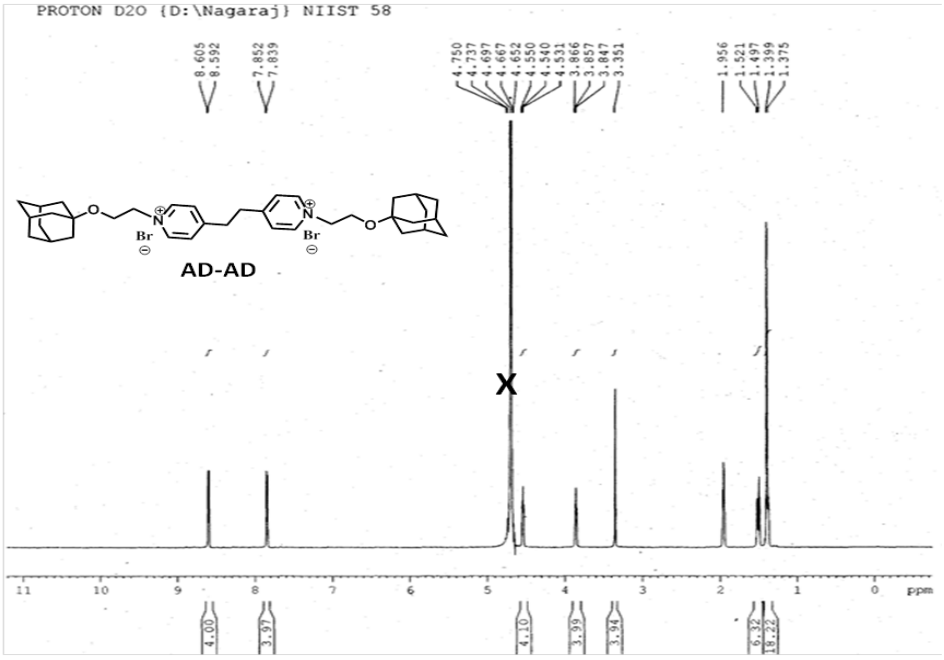


Figure S1 : ^{1}H NMR of AD-AD

4. ^{13}C NMR of Molecule AD-AD:

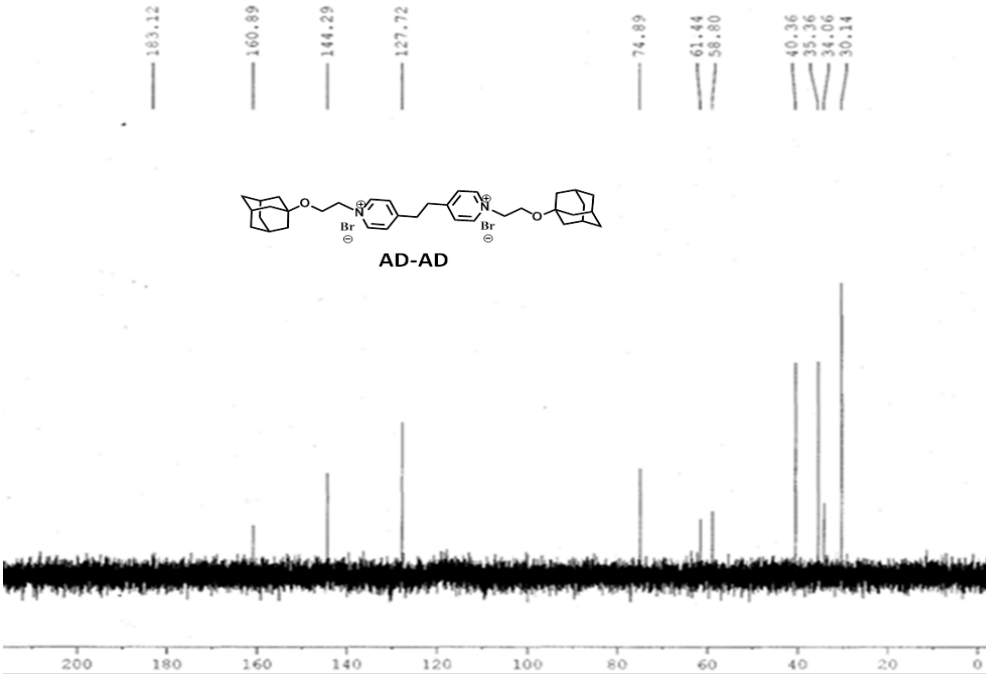


Figure S2 : ^{13}C NMR of AD-AD

5. ESI-HRMS of AD-AD:

Figure S3 : **The ESI-HRMS of AD-AD**

6. Chemical structures of DOX, β-CD and ADC:

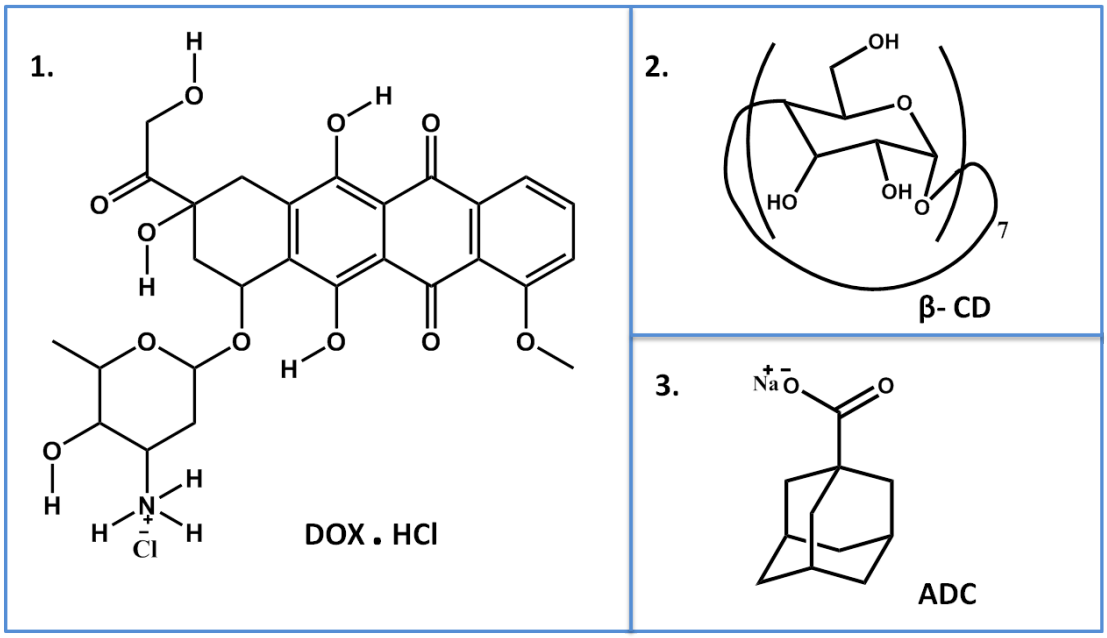


Figure S4: Chemical structures of Doxorubicin (DOX), β-Cyclodextrin (β-CD) and Adamantane Carboxylate (ADC).

7. ITC graph for the titration of AD-AD with β-CD:

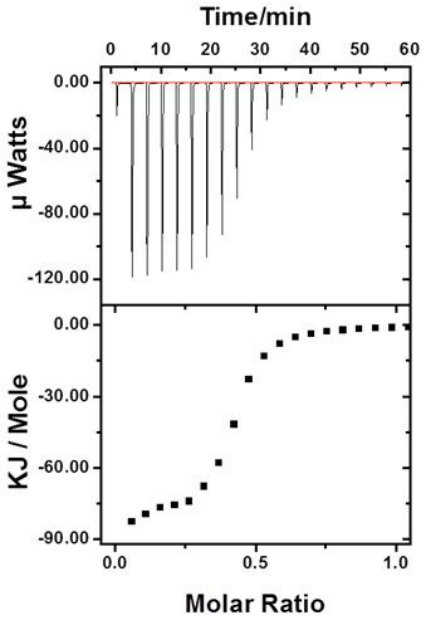


Figure S5 : The ITC Titration curve for titration of AD-AD with β-CD

8. 2D NMR (ROSEY) for inclusion complex of AD-AD with β-CD

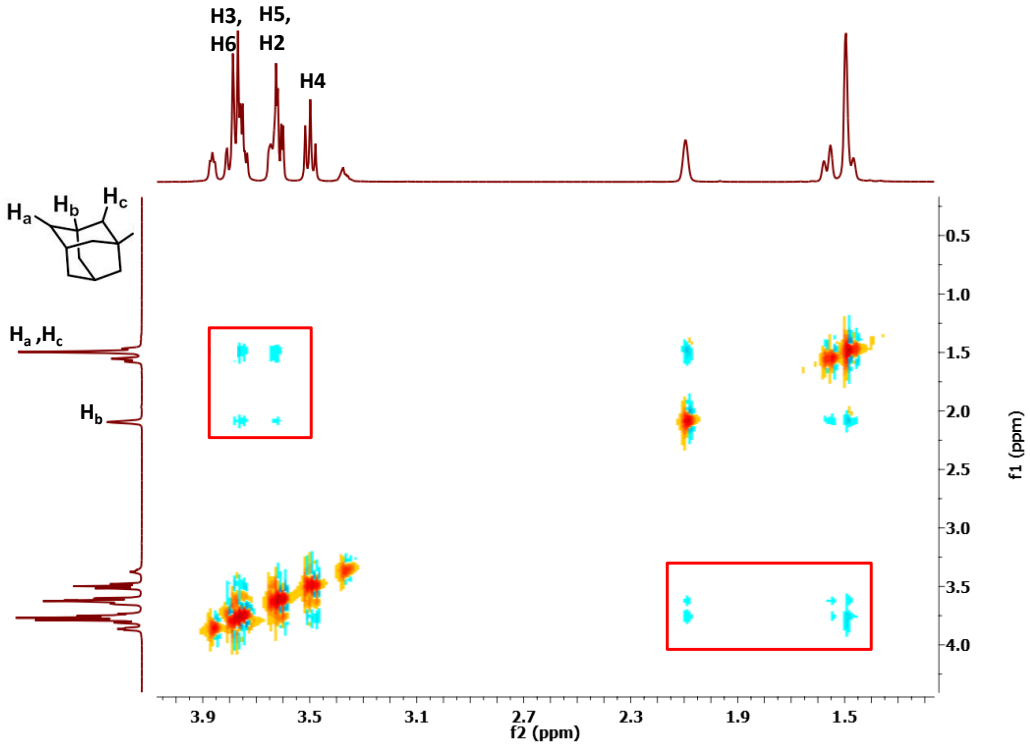


Figure S6 : **The selected ROSEY spectrum of AD-AD/ β-CD supramolecular complex.**

9. Additional AFM and TEM images of AD-AD/ β-CD vesicles

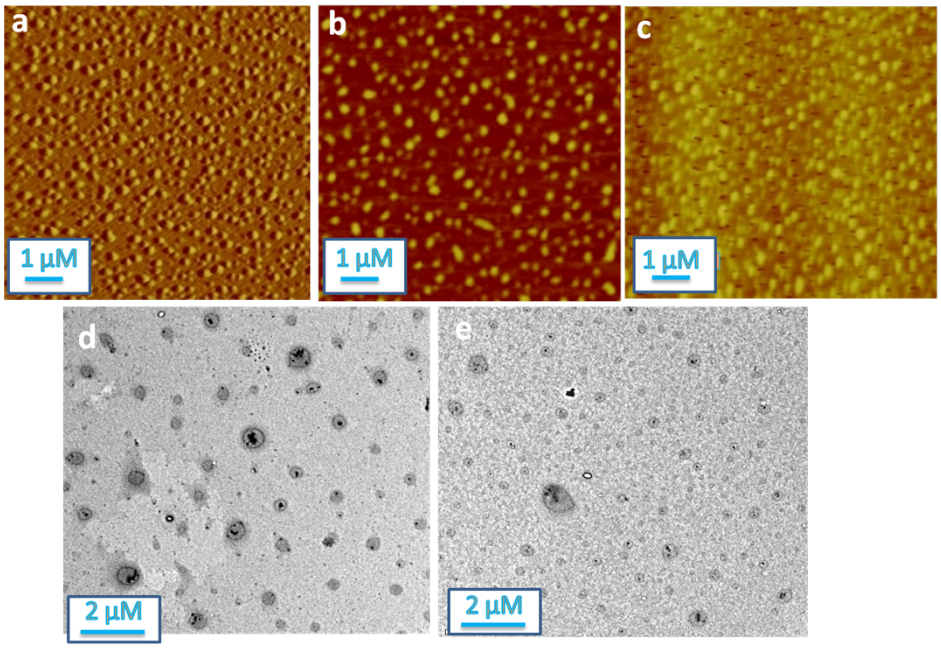


Figure S7 : AFM magnitude (a), AFM height (b, c) TEM (d, e) images of AD-AD/β-CD vesicles.

10. SEM images of vesicles

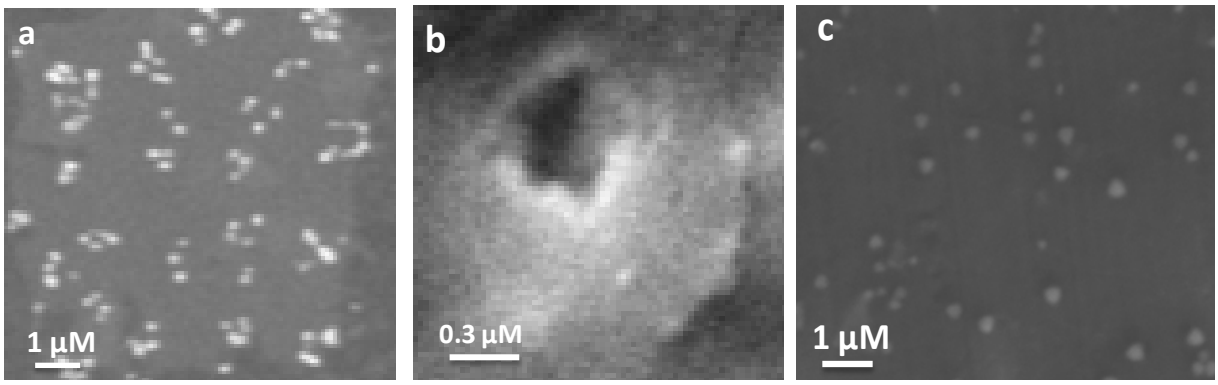


Figure S8 : (a) SEM image of AD-AD/β-CD vesicles, (b) SEM image of single broken vesicle and (c) SEM image of DOX loaded vesicles taken after 3 weeks

11. ^{1}H spectrum showing disassembly of vesicles in the presence of ADC

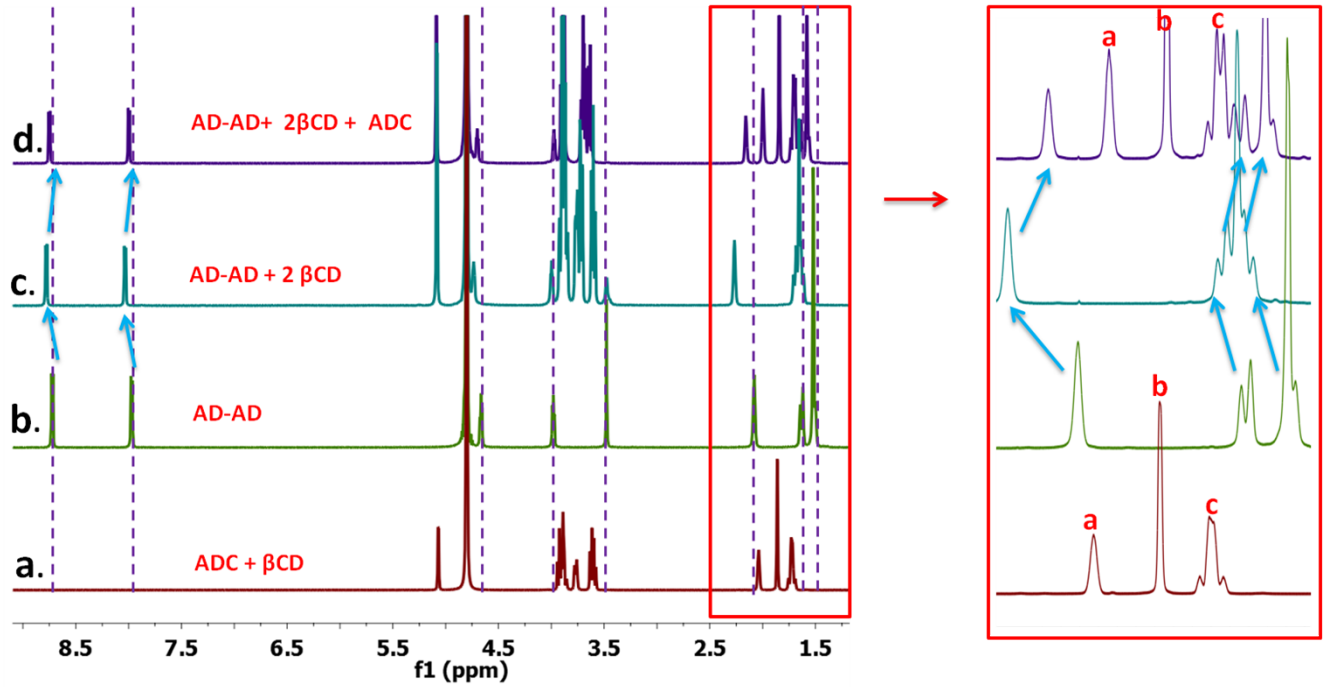


Figure S9 : ^{1}H spectra of (a) ADC + β -CD, (b) AD-AD, (c) AD-AD/ β -CD (2:1) vesicle and (d) spectrum after addition of ADC (2.25 eq.) to (c).

12. Additional CLSM images of DOX loaded vesicles

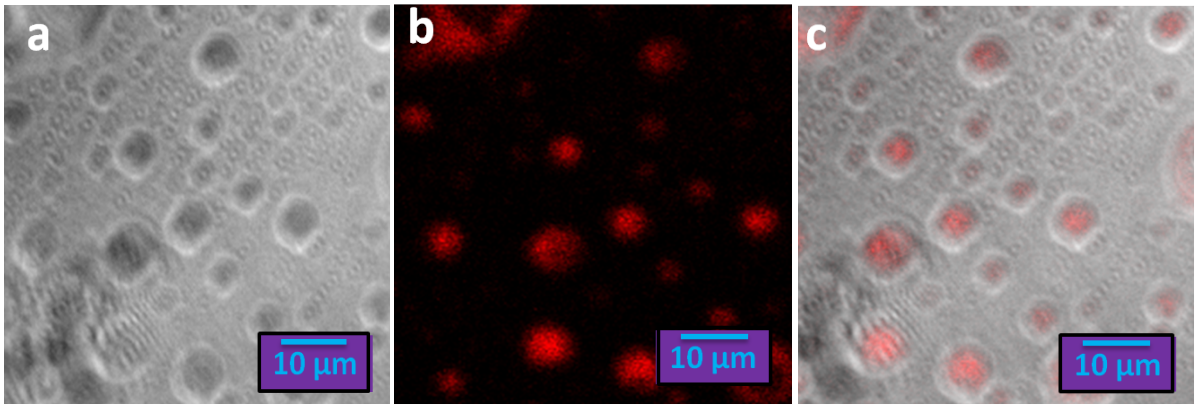


Figure S10 : Transmitted image (a), confocal laser scanning microscopy image (b) and merged image (c) of DOX loaded vesicles.

13. Additional CLSM images of DOX release

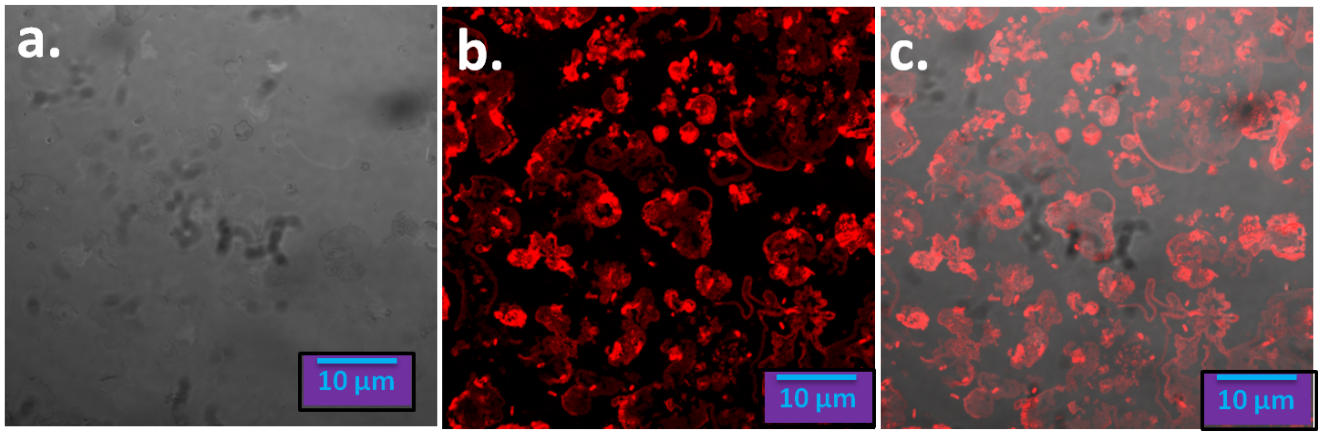


Figure S11 : Transmitted image (a), confocal laser scanning microscopy image (b) and merged image (c) of DOX release from vesicles upon addition of ADC.

Reference :

1 S. M. Stepankova J, Malina J, Kasparkova J, and N. O. Liskova B, Ulbrich K and Brabec V, *Biochem. Pharmacol. (In press).*

2 R. Krishnan and K. R. Gopidas, *J. Phys. Chem. Lett.*, 2011, 2, 2094-2098.