Supporting Information for

Programmed supramolecular nanoassemblies: enhanced serum stability and cell specific triggered release of anti-cancer drug

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Materials and Methods: All the reagents were purchased from commercial source and used as such without further purification. \(^1\)H NMR spectra were recorded on a Bruker DPX-300 MHz NMR spectrometer and all the spectra were calibrated against TMS. Dynamic Light Scattering (DLS) measurements were carried out on a Malvern Nanozetasizer. UV-Vis spectra were recorded in a Carry 100 Scan spectrometer. MASS spectra were recorded on using Qtof Micro YA263 mass spectrometer. TEM and AFM images were collected using a 200 KV Transmission Electron Microscope (MODEL: JEOL JEM 2100 HR with EELS) and Pico plus 5500 AFM instrument respectively. Fluorescence spectra were recorded on LS-55 PerkinElmer fluorescence spectrophotometer. The confocal images were taken using an Andor spinning Disc laser scanning confocal microscope. The cellular uptake was examined using BD LSRfortessa flow cytometry instrument (Beckton, Dickinson, San Jose, CA, USA).

Synthesis of the Bolaamphiphile (BA): The synthetic protocol of compound 3 (BA) is outlined below:

**Compound 1:** At first, 800 mg (3.84 mmol) of tetraethylene glycol monomethyl ether and 10 mL dry CH\(_2\)Cl\(_2\) were taken in a round bottom flask. To this solution, 582 mg (5.76 mmol) of triethylamine was added. The reaction mixture was stirred and cooled in an ice bath and a solution of acryloyl chloride (382 mg, 4.225 mmol) in 5 mL dry CH\(_2\)Cl\(_2\) was added drop-wise with constant stirring under N\(_2\) atmosphere. After the addition was over
the reaction mixture was allowed to come to room temperature and it was stirred for 12 h. Then the reaction was stopped and the solution was washed with water (3 x 40 mL) to remove the unreacted reactants and salts. The organic phase was collected to get the product. The combined organic part was dried over anhydrous Na$_2$SO$_4$ and CH$_2$Cl$_2$ was removed under reduced pressure to get the pure product as colorless oil. The product was used in the next step without any further purification.

$^1$H-NMR (300 MHz, CDCl$_3$, TMS): $\delta$ (ppm) = 6.29 (t, 1H), 6.45 (dd, 1H), 5.70 (dd, 1H), 4.17 (q, 2H), 3.40-3.62 (m, 14H), 3.244 (s, 3H).

ESI-MS: m/z calculated for C$_{12}$H$_{22}$O$_6$Na (M+Na)$^+$ = 285.1216, Found: 285.1280.

\[ \text{Scheme 1: Synthetic scheme of Bolaamphiphile} \]
**Compound 2:** 600 mg (2.228 mmol) of compound 1 and 10 mL dry THF were taken in a round bottom flask and it was well capped with septum. Then N₂ gas was passed through it for 5 minutes. The dithiothreitol (DTT) (319 mg, 2.072 mmol) and catalytic amount of dimethyl-phenylphosphine (4.18 mg, 0.03 mmol) were added to the solution and the reaction mixture was stirred at room temperature for 24 h under N₂ atmosphere. Then the reaction was stopped and solvent was evaporated under reduced pressure to get the crude product as yellow oil which was purified by column chromatography using silica gel as stationary phase and DCM/MeOH as eluent to get the pure product as light yellow oil. The pure product was isolated as colorless oil in 70 % yield.

¹H-NMR (300MHz, CDCl₃ TMS): δ (ppm) = 4.2 (d, 4H), 3.488-3.665 (m, 30H), 3.31 (s, 6H), 2.61-2.79 (s, 12H).

ESI-MS: m/z calculated for C₂₈H₅₄O₁₄S₂Na (M+Na)+ = 701.2853, Found: 701.2964.

**Compound 3:** At first, compound 2 (870 mg, 1.28 mmol) was dissolved in 10 mL of dry CH₂Cl₂ in a round bottom flask. To this solution, 272 mg (2.69 mmol) of triethylamine was added. Then the reaction mixture was stirred and cooled in an ice bath and a solution of acryloyl chloride (244 mg, 2.69 mmol) in 5 mL of dry CH₂Cl₂ was added drop-wise with constant stirring under N₂ atmosphere. After the addition was over the temperature was allowed to come to room temperature and the reaction mixture was stirred for 12 h. Then the reaction was stopped and the solution was washed with water (3 x 40 mL) to remove the unreacted reactants and salts. The organic phase was collected to get the product. The combined organic part was dried over anhydrous Na₂SO₄ and CH₂Cl₂ was
removed under reduced pressure to get the product as light yellow oil. The product was purified by column chromatography using silica gel as stationary phase and DCM/MeOH as eluent to get the pure product as very light yellow oil.

$^1$H-NMR (CDCl$_3$, 300MHz TMS): $\delta$ (ppm) =: 6.28 (t, 1H), 6.05 (dd, 1H), 5.7(dd, 1H), 4.17(t, 4H), 3.48-3.63(m, 28H), 3.26 (s, 6H), 2.56-2.78 (m, 12H).

ESI-MS: m/z calculated for C$_{34}$H$_{58}$O$_{16}$S$_2$Na (M+Na)$^+$ = 809.3064, Found: 809.3152.

FTIR (KRB, cm$^{-1}$): 3449.13, 2924.11, 2874.1, 2855.78, 1963.68, 1619.30, 1636.08, 1729.15, 1456.17, 1407.90, 1349.52, and 1295.22.

**NMR Characterization:** $^1$H-NMR spectra were recorded at room temperature on the Bruker DRX-300 (300 MHz). Unless stated otherwise, all spectra were recorded in deuterated chloroform purchased from Sigma-Aldrich. All chemical shifts are given in ppm ($\delta$) units relative to tetramethylsilane (singlet $\delta$H = 0.00). Calibration was achieved using the residual solvent signal of chloroform at $\delta$H = 7.27. Analysis followed first order and the following abbreviations were used throughout the text: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet.

**FTIR Characterization:** The sample was prepared by mixing it with KBr and then a pellet was prepared and placed in the chamber inside the instruments and spectrum was recorded at 25°C.

**ESI-MS Characterization:** The samples were dissolved in appropriate solvents and then injected in the column for recording mass spectra at room temperature.
Additional Figures:

**Fig. S1:** Encapsulation of Nile red in BA micelles of different concentration. (Concentration of Nile red = $1 \times 10^{-5}$M)

**Fig. S2:** AFM image of BA (uncross-linked nanoassemblies)
Fig. S3: NMR of BA after cross-linking (cross-linked nanoassemblies)

Fig. S4: Segmental representation of self-assembled structure of BA and cross-linking reaction in presence of dithiol and catalytic amount of butyl amine
Fig. S5: pH responsive release of pyrene molecules. a) Pyrene loaded UCNs at pH = 5.3, b) Pyrene loaded UCNs at pH = 7.4, c) Pyrene loaded CNs at pH = 5.3 and d) Pyrene loaded CNs at pH = 7.4. CNs = Cross-linked and UCNs = Uncross-linked nanoassemblies
Fig. S6: IC50 calculation of DOX loaded nanoassemblies and Free DOX against various cell lines a) HCT-116 cells, b) HEK-293T cells and c) PBMC

Table S1: IC50 values of DOX loaded nanoassemblies and free DOX against different cell lines are listed here.

| Cells    | IC50 of UCNs (µg/mL) | IC50 of CNs (µg/mL) | IC50 of Free DOX (µg/mL) |
|----------|----------------------|---------------------|--------------------------|
| HCT-116  | 6.62                 | 8.25                | 6.42                     |
| HEK-293T | 5.98                 | -                   | 6.09                     |
| PBMC     | 6.65                 | -                   | 6.55                     |

Fig. S7: FACS analysis for cellular uptake of empty nanoassemblies and drug loaded nanoassemblies. a) HCT-116 cells and b) HEK-293T cells
Fig. S8: $^1$H-NMR spectrum of compound 1. Solvent = CDCl$_3$.

Fig. S9: ESI-MS spectrum of compound 1.
Fig. S10: $^1$H-NMR spectrum of compound 2. Solvent = CDCl$_3$.

Fig. S11: ESI-MS spectrum of compound 2.
Fig. S12: $^1$H-NMR spectrum of compound 3. Solvent = CDCl$_3$

Fig. S13: $^{13}$C-NMR spectrum of compound 3. Solvent = CDCl$_3$
Fig. S14: ESI-MS spectrum of compound 3.