

Electronic supplementary information

Self-assembly of chiral foldamers with alternating hydrophilic and hydrophobic side chains into acid-sensitive and solvent-exchangeable vesicular particles

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1. Materials and Methods

All reagents were obtained from commercial suppliers, and, with the exception of CH_2Cl_2 , were used without further purification. CH_2Cl_2 was purified by distillation according to standard methods. All air-sensitive reactions were carried out under N_2 atmosphere. Thin-layer chromatography was carried out using Model ENF-240C/FE (100-200 mesh) fluorescent treated silica, visualized under UV light (254 nm). Some intermediates were synthesized according to the reference.^[1]

The ^1H NMR spectra were recorded at 400 MHz (Bruker AV) with TMS as the internal standard. All shifts were given in ppm. All coupling constants (J values) were reported in Hertz (Hz). Fourier-transform infrared spectra were recorded on a Bruker Vertex70 Win-IR instrument. High-resolution mass spectra were obtained by using IonSpec7.0 TMALDI-FTICRMs. Optical rotation was measured by the PerkinElmer341 LC polarimeter, using the 10 cm optical rotation pool. The number average molecular weights (M_n) and molecular weight distributions (MWDs) of foldamers were measured at 30 °C by using a gel permeation chromatograph (GPC) equipped with a Waters515 HPLC pump, four columns (HMW7THF, HMW6ETHF x 2, and HMW2THF) and a Waters 2414 refractive index detector. THF was used as the eluent at a flow rate of 1.0 ml/min. Sample solutions were filtered through a 0.45 μm microfilter before injection. TEM images were obtained by using a JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. A carbon-coated copper grid was quickly put into the dispersions for less than 1 s and dried in air at room temperature for 5 h. The TEM samples were stained with uranyl acetate. SEM was performed on an XL 30 scanning electron microscope (MicrionFEI PHILIPS). A drop sample solution was put on the silicon slice and dried in air at room temperature. The sample was then coated with Au in the ion coater for 40 s. CD and LD spectra were obtained by using a BioLogic Science MOS-450 scanning spectrometer. LD is the difference between absorption of light polarized parallel and polarised perpendicular to an orientation axis. Uv-vis spectra were observed by using a SHIMAD ZU UV-2550 ultraviolet spectrophotometer. Fluorescence signals of vesicular samples encapsulating fluorescein were captured by using Olympus Fluoview FV1000 confocal microscope and analyzed by FV10-ASW 1.6 Viewer program (Olympus, Japan). Melting points were determined for compounds where a preparative recrystallization was carried out. These were acquired on a Anke TGL-16C melting point apparatus. Values were given in °C and were uncorrected. HPLC analysis for PTX content was carried out on a Hypersil DOLD™ C18 analytical column (5 μm , 250 × 4.6 mm). Eluent: $\text{CH}_3\text{OH}/\text{H}_2\text{O} = 7/3$, v/v, 1 ml/min. The detection wavelength was set at 227 nm. The retention time of PTX was approximately 8.3 minutes.

2. Characterization of monomers (*R*)-**1a-b** and foldamers **A1-A4** [2]

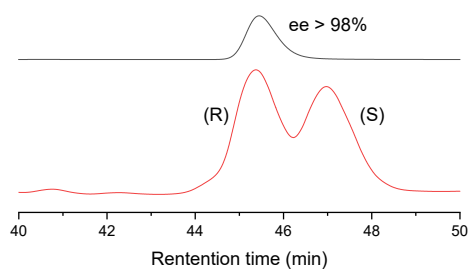


Fig. S1 HPLC spectra of racemic standard substance (red) and compound (*R*)-**1a** (black) using CHIRALPAK ID. Eluent: *n*-hexane/CH₂Cl₂/*i*-propanol, 37.5/37.5/25 (v/v/v), 0.15 ml/min

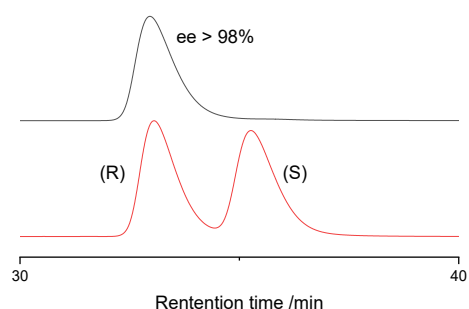


Fig. S2 HPLC spectra of racemic standard substance (red) and compound (*R*)-**1b** (black) using CHIRALPAK ID. Eluent: *n*-hexane/CH₂Cl₂/*i*-propanol, 35/35/30 (v/v/v), 0.17 ml/min.

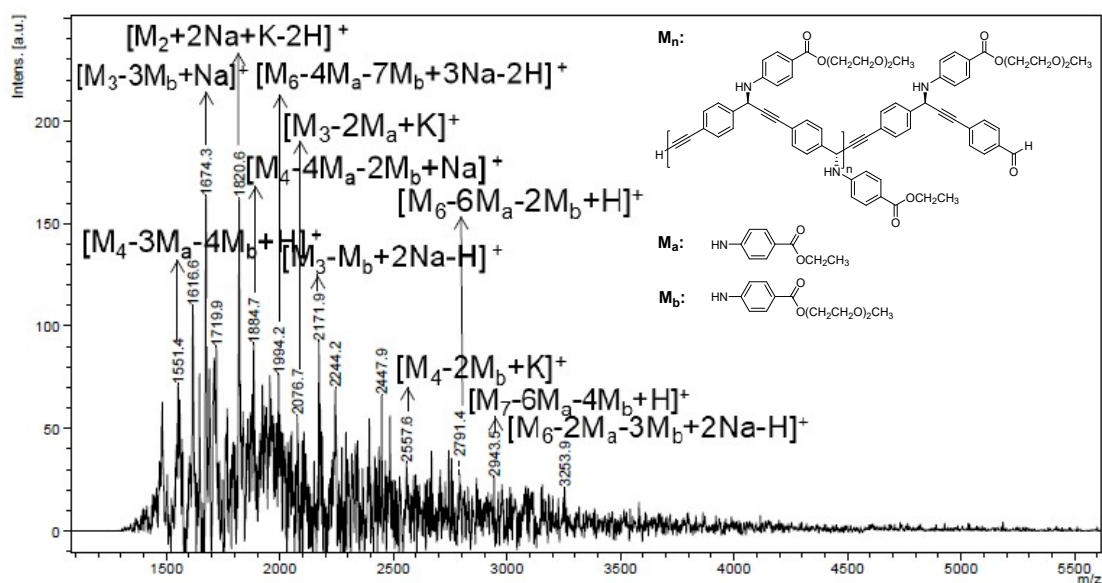


Fig. S3 Mass spectra of foldamer **A1**

M_n : molecular formula of **A1**; M_a : 4-ethyloxycarbonylanilino segment; M_b : 4-(2,5,8-trioxanonanoyl)anilino segment; n , number of repeat unit.

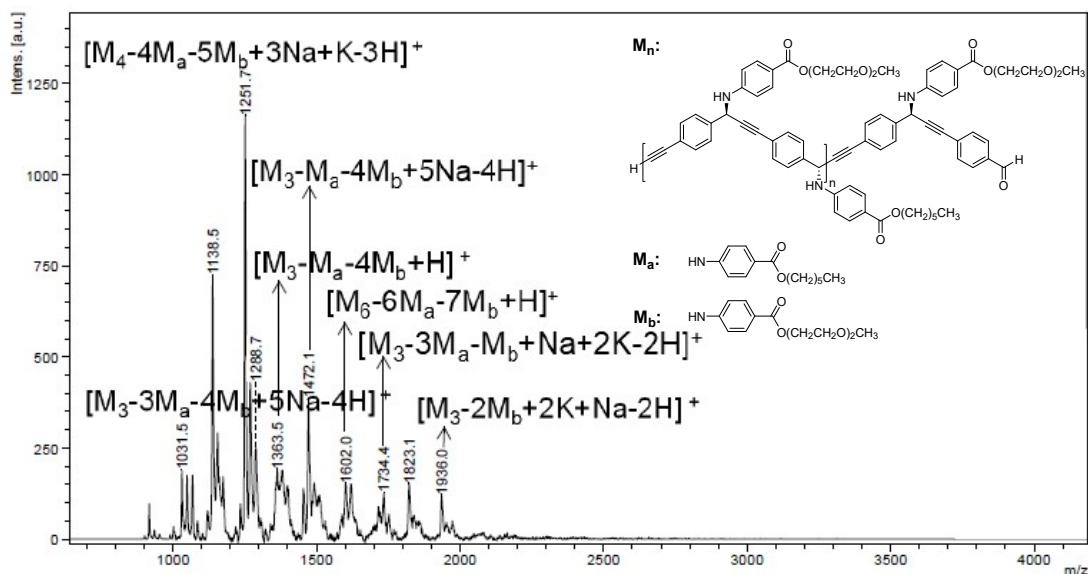


Fig. S4 Mass spectra of foldamer **A2**

M_n : molecular formula of **A2**; M_a : 4-*n*-hexyloxycarbonylanilino segment; M_b : 4-(2,5,8-trioxanonanoyl)anilino segment; *n*, number of repeat unit.

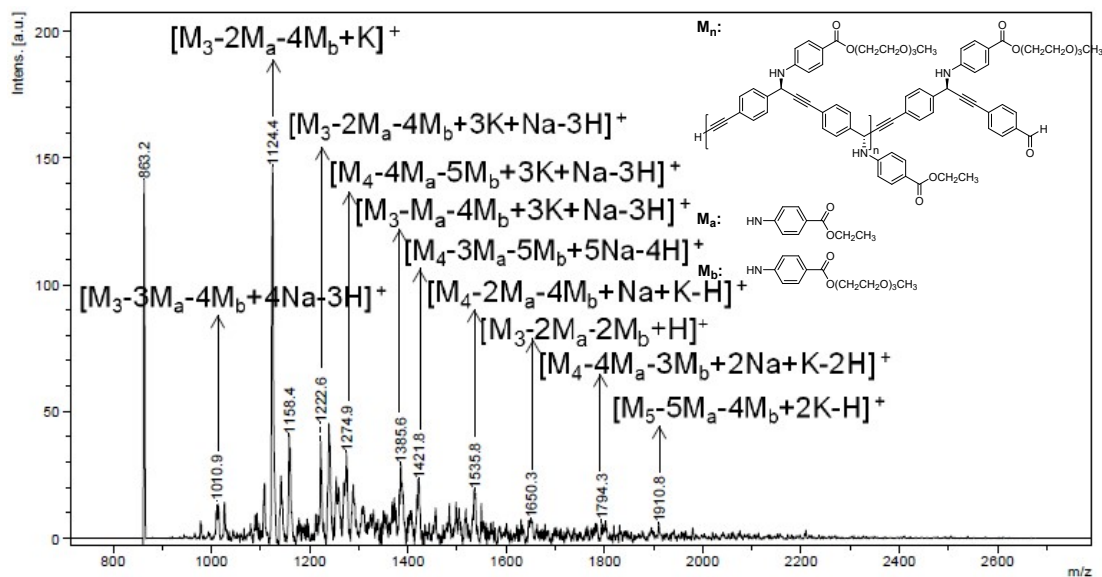


Fig. S5 Mass spectra of foldamer **A3**

M_n : molecular formula of **A3**; M_a : 4-ethyloxycarbonylanilino segment; M_b : 4-(2,5,8,11-tetraoxadodecanoyl)anilino segment; *n*, number of repeat unit.

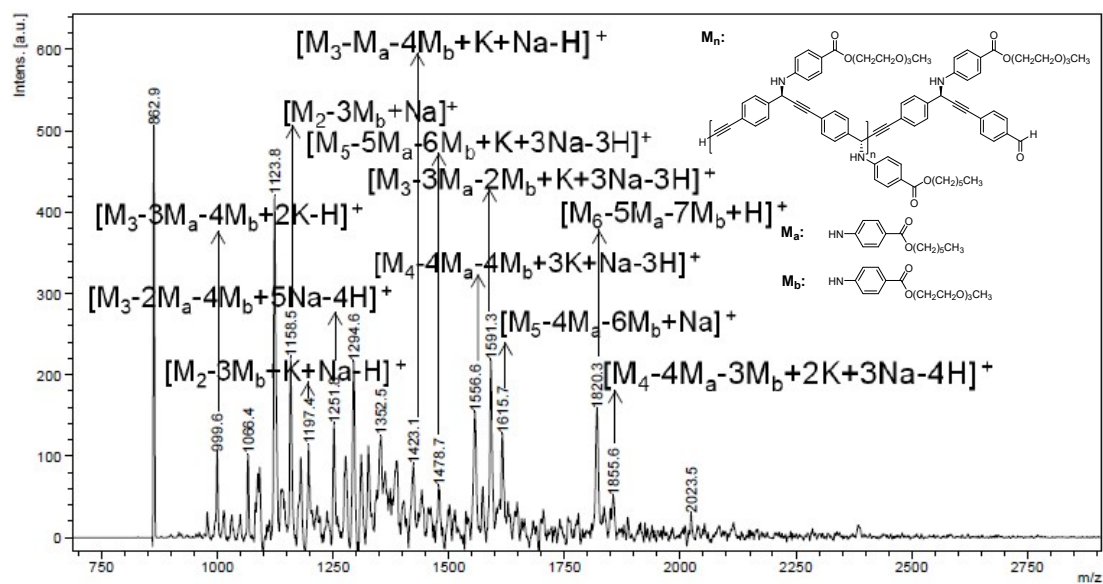


Fig. S6 Mass spectra of foldamer **A4**

M_n : molecular formula of **A4**; M_a : 4-*n*-hexyloxycarbonylanilino segment; M_b : 4-(2,5,8,11-tetraoxadodecanoyl)anilino segment; *n*, number of repeat unit.

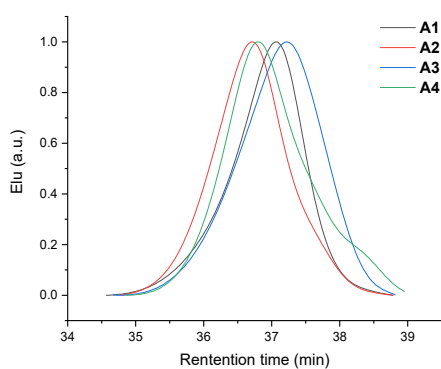


Fig. S7 GPC curves of foldamers **A1-A4**

THF was used as the eluent at a flow rate of 1.0 ml/min.

Table S1 GPC data of foldamers **A1-A4**

Foldamer	M_n ^{a)}	M_w ^{a)}	PDI	Average number of repeat unit, <i>n</i>
A1	3178	4330	1.36	4.3
A2	3315	3998	1.21	4.1
A3	3266	4225	1.29	4.1
A4	3357	4459	1.32	3.9

a) THF as solvent.

Table S2 The signal peaks of UV-vis and CD spectra of foldamers **A1-A4**^a

Foldamer	UV-vis ^b	CD ^b
A1	297 nm (2.171)	294 nm (+21.19), 267 nm (-5.60)
A2	294 nm (2.046)	290 nm (+12.61), 265 nm (-6.13)
A3	294 nm (1.739)	302 nm (+8.16), 261 nm (-5.39)
A4	295 nm (1.711)	298 nm (+7.06), 262 nm (-5.08)

^a In CH₂Cl₂, room temperature.

^b The data in the brackets are the intensity values of signal peaks.

In contrast to **A2-A4**, **A1** has close UV signal, but significant Cotton effect around 294 nm. This is possibly due to the short side chains of **A1**, resulting in weak intramolecular interaction between adjacent side chains. Notably, **A2** has relative strong CD signal than **A3-A4**, but **A3** and **A4** have similar CD spectra. This means that the interactions between long flexible hydrophilic side chains could be relatively strong, and the interactions between long hydrophobic side chains relatively weak. Another explanation is that hydrophilic ether chain is enough flexible so as to favor the interaction between them and the flexibility of hydrophobic alkyl chains is relatively poor.

3. CLSM images of samples encapsulating dye molecules

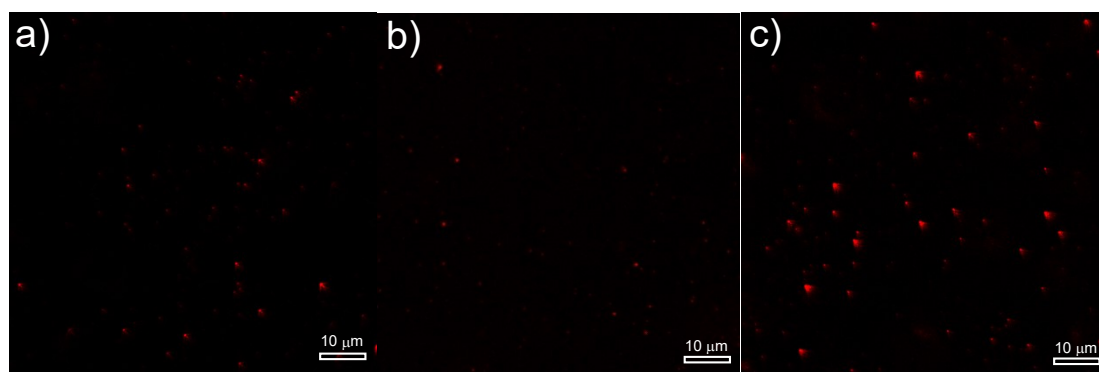


Fig. S8 CLSM images of samples **A1** encapsulating Nile Red ($\lambda_{\text{ex}} = 554$ nm) in CH₃OH (a) and H₂O (b), and Rhodamine B ($\lambda_{\text{ex}} = 554$ nm) in H₂O (c), respectively.

4. The dynamic light scattering diagrams of vesicular particles

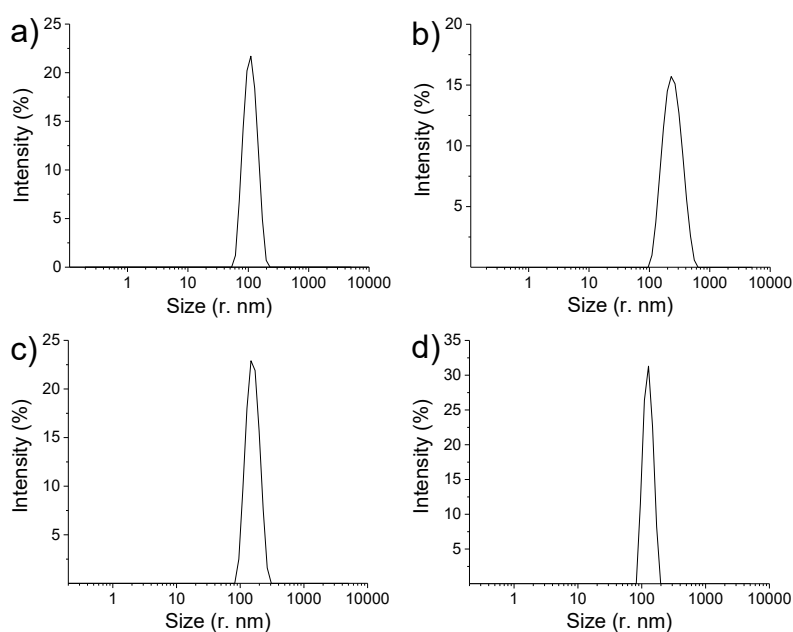


Fig. S9 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in $\text{CH}_2\text{Cl}_2/n\text{-C}_6\text{H}_{14}$ (1/5, v/v) at room temperature. Concentration: 0.02 mg/mL.

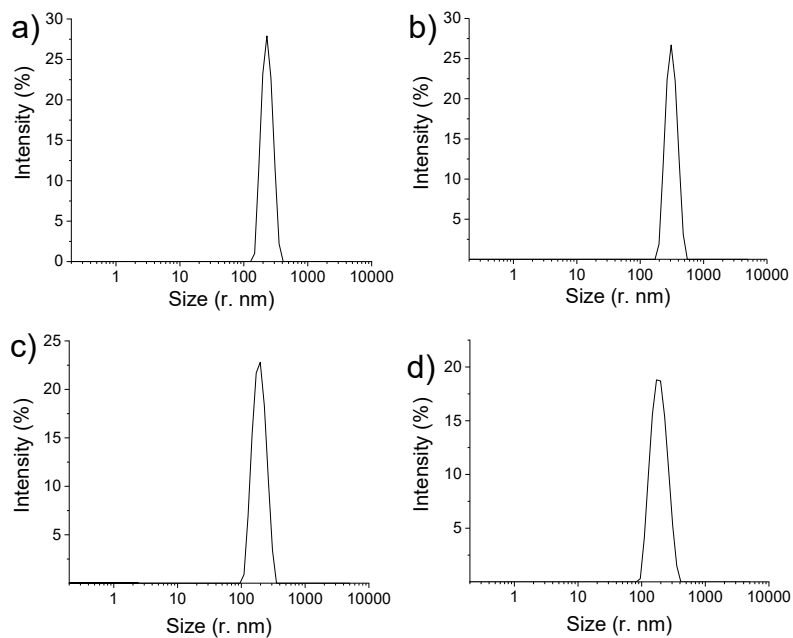


Fig. S10 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (1/5, v/v) at room temperature. Concentration: 0.02 mg/mL.

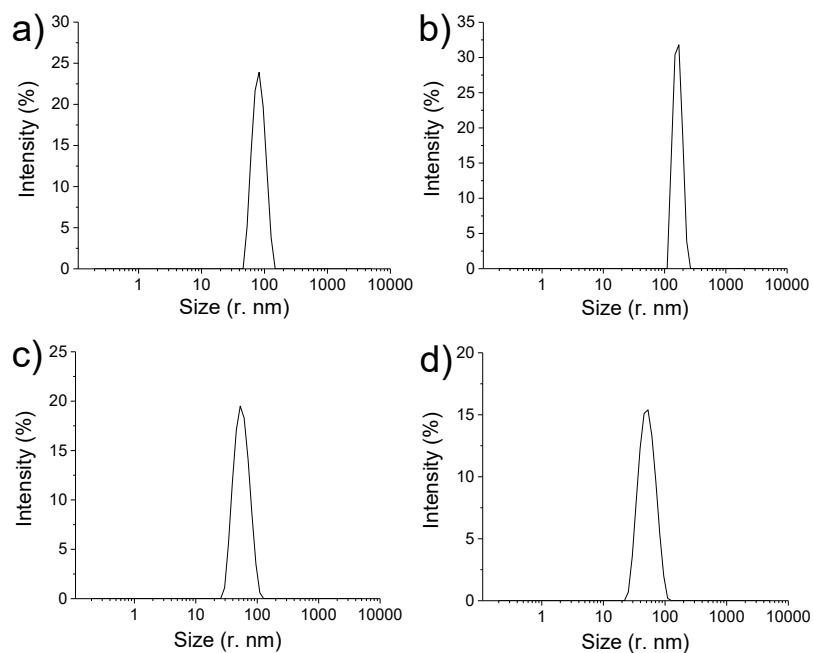


Fig. S11 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in THF/H₂O (1/5, v/v) at room temperature. Concentration: 0.02 mg/mL.

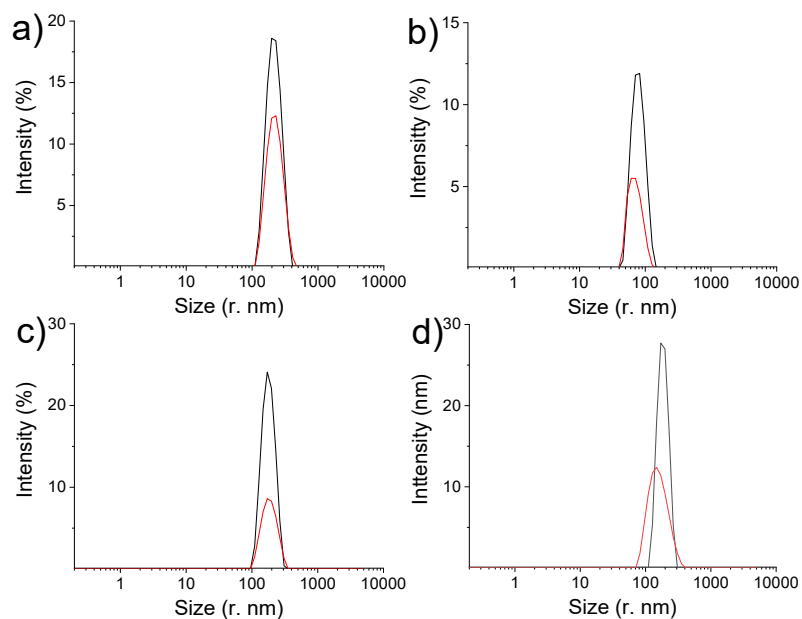


Fig. S12 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in *n*-C₆H₁₄ before (black) and after (red) kept for one month at room temperature. Concentration: 0.02 mg/mL.

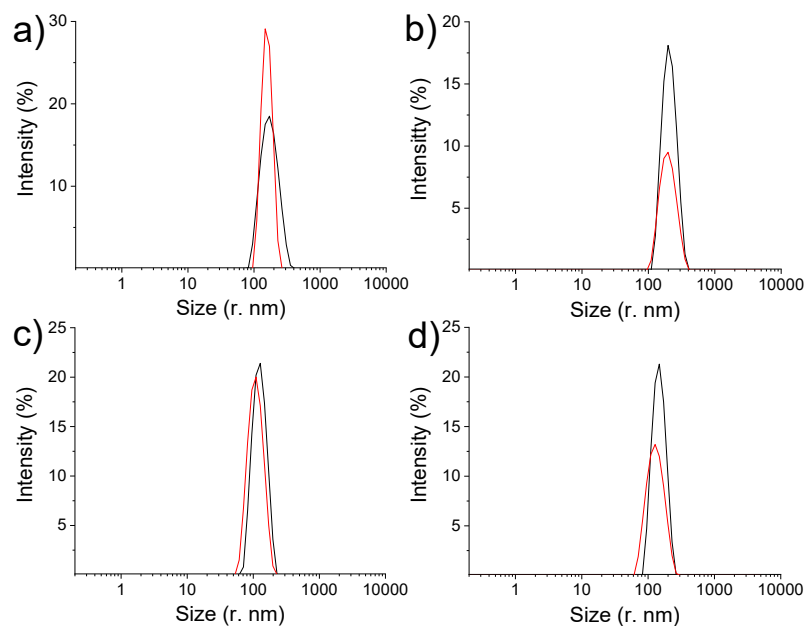


Fig. S13 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in CH_3OH before (black) and after (red) kept for one month at room temperature. Concentration: 0.02 mg/mL.

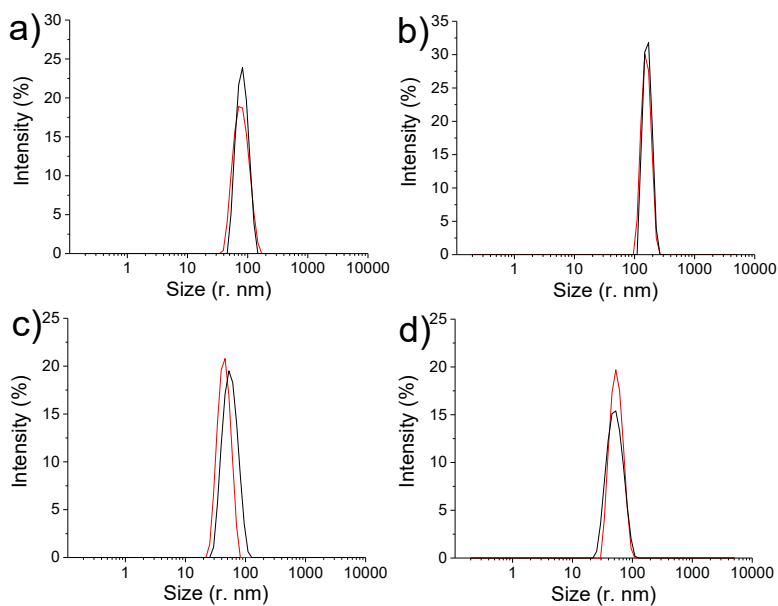


Fig. S14 The dynamic light scattering diagrams of vesicular samples **A1-A4** (a-d) in H_2O before (black) and after (red) kept for one month at room temperature. Concentration: 0.02 mg/mL.

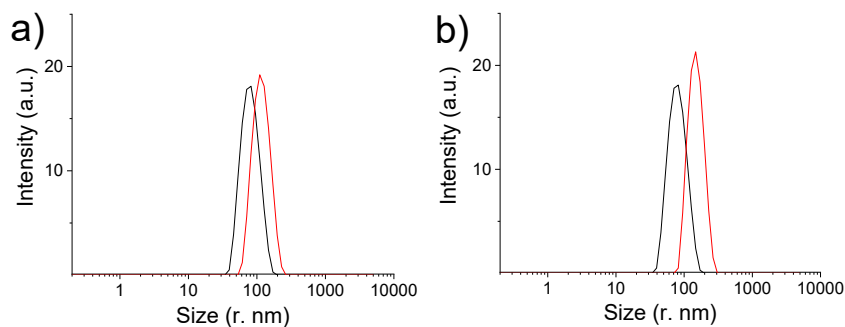


Fig. S15 The dynamic light scattering diagrams of RhB (a) and DOX-HCl-loaded (b) vesicular samples **A1** (red), respectively, and their unloaded ones (black). **A1** concentration: 0.02 mg/mL. Loading content: RhB, 20 wt%; DOX-HCl, 22 wt%.

5. Encapsulation and releasing experiment

General preparation method of vesicular samples encapsulating cargo: A certain amount of dye or drug solution (10mg/mL, 50 μ L) was added to a foldamer solution. Then, to the resultant mixed system, the non-solvent (water or *n*-hexane, or methanol) of five times of volume of solution was added under shaking, and the liquid system became cloudy. The cargo-loaded vesicular particles were purified by dialysis (molecular weight cutoff 8000) in water (or methanol, then water) for 1-2 days until the solution outside the dialysis bag exhibited no characteristic UV absorption of cargo. The ultimate loaded dye or drug loading content was determined by UV-vis or HPLC method.

0.1 M PBS (pH 5.6, 6.5, 7.4) and 0.1 M sodium acetate (pH 4.0) buffer solutions were used to cargo release experiment. A standard curve of dye concentration-absorbance was made. The dye loaded in the vesicle was completely released by adding dilute aqueous HCl of pH 2.0, and the absorbance value of this resulting sample in a determined volume was measured to afford its concentration by standard curve. Then the cargo load content (CLC) was calculated via the following Equation (1).

$$\text{CLC (wt\%)} = \frac{\text{total amount of cargo} - \text{amount of free cargo}}{\text{foldamer weight}} \times 100\% \quad (1)$$

6. Cell experiments

6.1 Cell lines and cell culture

HeLa (human cervical carcinoma) and L929 (mouse fibroblast cells) cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO). Cells were cultured in a humidified incubator at 37 $^{\circ}$ C with 5% CO₂, and the culture medium was replaced once every day.

6.2 Cellular uptake measured by CLSM

The cellular uptake behaviors of RhB-loaded vesicular samples **A1**, **A4** were examined by CLSM towards HeLa cells. The cells were seeded in 6-well plates (a clean cover slip was put in each well) at about a density of 200,000 cells per well in 1.5 mL of DMEM medium and allowed to adhere for 24 h. And then the medium was replaced with RhB-

loaded vesicular samples diluted with fresh culture medium to a final RhB concentration of $0.6 \mu\text{g mL}^{-1}$. Thereafter cells were incubated at $37 \text{ }^\circ\text{C}$ for additional different times. Besides, cells were incubated at $37 \text{ }^\circ\text{C}$. Subsequently, the supernatant was removed and the cells were washed gently three times with PBS (pH 7.4), fixed with 4% paraformaldehyde (1 mL/each well) for 15 min at ambient temperature and washed thrice with cold PBS. DAPI (4',6-diamidino-2-phenyl-indole) was employed to counterstain the cell nuclei. For lysosome colocalization visualization, before the cells were subjected to DAPI, pretreated with Lyso-Tracker Green for 30 min at $37 \text{ }^\circ\text{C}$ to stain lysosomes. The cellular uptake was obtained by CLSM, while RhB was excited at 555 nm. The testing experiment of cellular uptake behaviour of RhB-loaded vesicular samples was nearly the same.

6.3 Determination of cytotoxicity in vitro

The cytotoxicity of vector/pDNA complexes were characterized by CCK-8 assay. L929 and HeLa cells were seeded in 96-well plates (1×10^4 cells per well) and incubated for 24 h. Then the culture medium was replaced with fresh culture medium, and T(PD) complexes at various ratios were added ($0.2 \mu\text{g pDNA}$ per well) and incubated for 24 h. Afterwards, the culture medium was replaced with the culture medium containing 10% CCK-8 and incubated for 1 h. The cells were detected with Bio-Rad 680 microplate reader based on absorption value at 450 nm. The cell viability (%) was calculated by this equation: cell viability (%) = $(A_{\text{sample}}/A_{\text{control}}) \times 100$, where A_{sample} was the absorbance value of sample well and A_{control} was the absorbance value of control well. The relevant data were represented as mean \pm SD based on triplicate independent experiments.

6.4 Antitumor efficacy

HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at an initial density of 2×10^3 cells/well and incubated in $100 \mu\text{L}$ DMEM at $37 \text{ }^\circ\text{C}$ in 5 % CO_2 atmosphere for overnight. After removing incubation medium, PTX-loaded vesicles ($100 \mu\text{L}$) diluted with cell culture media to the desired concentration were added to cell wells at various PTX concentrations from $0.063 \mu\text{g/mL}$ to $1 \mu\text{g/mL}$. Cells free of PTX-loaded vesicular particles and PTX treatment were used as control. At the specific time intervals (48 h), $20 \mu\text{L}$ of MTT solution in PBS with the concentration of 5 mg mL^{-1} was added and the plates were incubated at $37 \text{ }^\circ\text{C}$ for another 4 h, straight after careful removal of the culture medium supernatant and addition of $150 \mu\text{L}$ of dimethyl sulfoxide to each well to dissolve the formed violet formazan crystals. Finally, the plates were shaken for 3 min, and the absorbance of violet product was quantified at 490 nm by a microplate reader. Cytotoxicity of M(PTX2) was conducted according to the similar procedure of 6.3. The compatibility of blank micelles was carried out in the same way, just change the treatment concentration.

7. References

- (1) Y. Chen, Z. Zhao, Z. Bian, R. Jin, C. Kang, X. Qiu, H. Guo, Z. Du, L. Gao, *ChemistryOpen*, **2016**, 5, 386-394.
- (2) Z. Zhao, Z. Bian, Y. Chen, C. Kang, L. Gao, G. Zhu, *Soft Matter*, **2021**, 17, 637-644.

- (3) X. Li, L. Guo, M. Casiano-Maldonado, D. Zhang, C. Wesdeminotis, *Macromolecules*, **2011**, *44*, 4555-4564