Support information for:

Supramolecular Fluorescent Vesicle Based on Coordinating Aggregation Induced Emission Amphiphile: Inspiration for Cancer Cell Division

Jie Li[1], Kangjie Shi[1], Markus Drechsler[2], Ben Zhong Tang[3,*]，Jianbin Huang[1,*], and Yun Yan[1,*]

[1]Beijing National Laboratory for Molecular Sciences (BNLMS), State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

[2]Bayreuth Institute of Macromolecular Research (BIMF)-TEM, university of Bayreuth, D-95440, Bayreuth, Germnay

[3]Department of Chemistry, Institute of Molecular Functional Materials, State Key Laboratory of Molecular Neuroscience, Institute for Advanced Study, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China.

This document includes: Experimental section; The synthetic path of TPE-BPA, Figures S1-S3:

1. Experiment section

Materials. TPE-BPA was synthesized according to path in Supporting Information. Cetyltrimethyl Ammonium Bromide (CTAB)，$Zn(NO_3)_2$ (AR) and $Ca(NO_3)_2$ (AR) was purchased from Beijing Chemical Works. Aqueous solutions were prepared using Milli-Q water of 18 MΩ.

Spectra measurements. UV/Vis absorbance measurements were carried out on the Pgeneral TU-1810 UV/Vis spectrophotometer at room temperature (RT). Fluorescence spectra were recorded on a Hitachi F7000 spectrometer equipped with a constant temperature bath to control the temperature at 298K (λex = 365 nm, bandwidth(ex) 2.5 nm, bandwidth(em) 2.5 nm).

Cryogenic transmission electronic microscope (Cryo-TEM). A few microliters of samples were placed on a bare copper TEM grid (Plano, 600 mesh), and the excess liquid was removed with filter paper. This sample was cryo-fixed by rapidly immersing into liquid ethane cooled to -170 to -180 °C in a cryo-box (Carl Zeiss NTS GmbH). The specimen was inserted into a cryo-transfer holder (CT3500, Gatan, Munich, Germany) and transferred to a Zeiss EM922 EFTEM (Zeiss NTS GmbH, Oberkochen, Germany). Examinations were carried out at temperatures around -180 °C. The TEM was operated at an acceleration voltage of 200 kV. Zero-loss filtered images were taken under reduced dose conditions (500-2000 e/nm^2). All images were recorded digitally by a bottom-mounted CCD camera system (UltraScan 1000, Gatan) and processed with a digital imaging processing system (Digital Micrograph 3.9 for GMS 1.4, Gatan).

Transmission Electron Microscopy (TEM). TEM images were recorded on a JEM-100 CX II transmission electron microscope (JEOL, Japan, 80kV). The samples were prepared by dropping solutions onto copper grids coated with Formvar film. Excess water was removed by filter paper, and the samples were stained with uranyl acetate and dried in ambient environment at room temperature for TEM observation.

Atomic force microscopy (AFM). AFM measurements were conducted on a VEECO D3100 AFM with a tapping mode under ambient conditions. One drop of the solution was spin-coated on silicon surface, and then dried at room temperature for AFM observation.

Dynamic light scattering (DLS). DLS data were obtained on a laser light scattering spectrometer ALV/DLS/SLS5022F with a 22 mW He−Ne laser (632.8 nm wavelength). The scattering angle was 90°and the samples were filtered with 450 nm filters.

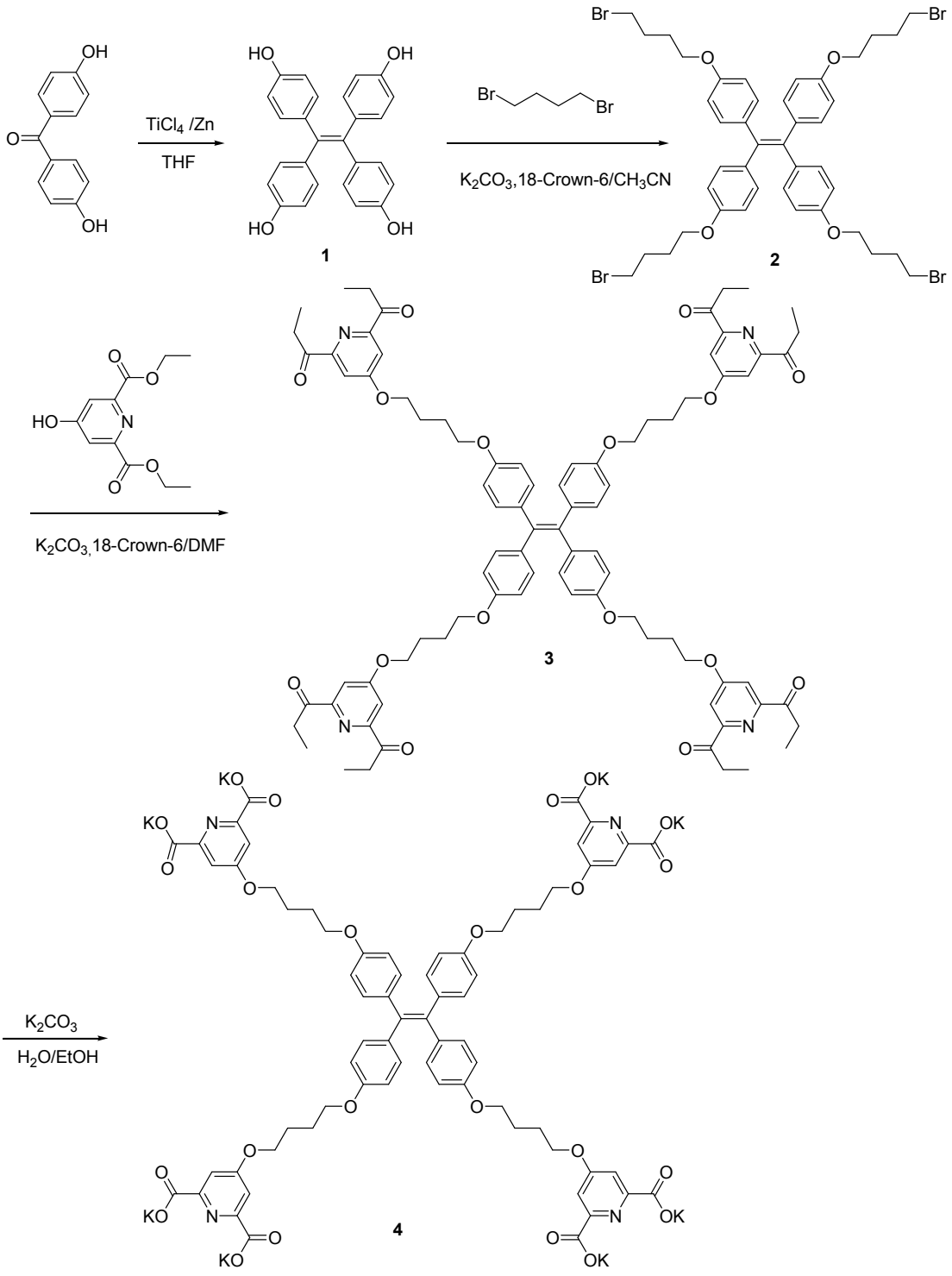
Confocal Laser Scanning Microscopy (CLSM). The samples were observed directly without any staining. A drop of the TPE-BPA@8CTAB solution was sealed between two slides at room temperature and ready for CLSM observation. The CLSM experiments were conducted in florescence modes on N-SIM CLSM.

Drug loading and release of DOX. Doxorubicin hydrochloride (DOX) was loaded

using an equilibrium dialysis method as described previously.[46] DOX hydrochloride (1 mg) was dissolved in 1 mL water, then 1 mL 1 mM TPE-BPA solution was added to prepare TPE-BPA/DOX mixed solution containing 0.5 mM TPE-BPA. Finally, CTAB solids were added to the TPE-BPA/DOX mixture to fabricate vesicles where the molar ratio between TPE-BPA and CTAB is 1:8. The solution was heated at 50 °C for 1 h, which was then placed in dark for 24 h at 25 °C. The solution was further transferred to a dialysis bag (MWCO = 3500) against water in the dark for 24 h to remove free DOX. The encapsulation rate of DOX determined by UV-vis spectrophotometry at 480 nm is about 12.6%.

For the releasing experiment, the above vesicle suspension loaded with DOX was divided into 2 fractions with equal volume and transferred into two dialysis bags, respectively. Addition of Zn^{2+} ion to generate 2Zn@vesicle in one bag, and both bags were incubated in 80 mL water to start new dialysis. At predetermined frequencies, 3 mL of incubated solution was taken out, and 3 mL of fresh water was added to refill the incubation solution to 80 mL. DOX-releasing profiles were determined by measuring the UV-vis absorbance of the solutions at 480 nm. The released DOX was analyzed with UV-vis spectrophotometer. The calibration curve was acquired with different DOX concentrations.

2. The synthetic path of TPE-BPA



Synthesis of compound 1. Into a 500mL flask was dissolved 4,4'-dihydroxybenzophenone (16.05g, 75mmol) and Zn dust (11.78g, 180mmol) in 350mL of THF under a N_2 atmosphere. The flask was cooled in an acetone-dry ice bath at -78℃ and 17.1g (90mmol) of $TiCl_4$ was added carefully. After stirring for 1h, the reaction mixture was warmed to room temperature and then

refluxed overnight. After filtration and solvent evaporation, the product was purified by silica gel column using petroleum ether/ethyl acetate (3:1 v/v) as eluent. Compound 1 was obtained as light brown red solid in 89% yield (13.2g).

Synthesis of compound 2. Compound 1(11g, 27.7mmol), 18-crown-6(1.45g, 5.5mmol), and 38.4g of K_2CO_3 (0.277mol) were dissolved in 500mL acetonitrile under an Ar atmosphere. Then 0.554mol of 1,4-dibromobutane was added, and the mixture was stirred overnight under 70℃. The reaction mixture was cooled to room temperature and filtered. The solvent was evaporated under vacuum and the crude product was purified by a silica gel column using dichloromethane/petroleum ether (5:1 v/v) as eluent. Compound 2 was obtained as yellow solid in 45% yield (11.6g). ^{1}H NMR (300MHz, $CDCl_3$), δ (TMS, ppm): 6.926-6.896 (d, 8H), 6.630-6.600 (d, 8H), 3.941-3.901(m, 8H), 3.499-3.455 (m, 8H), 2.073-2.024 (m, 8H), 1.924-1.877 (m, 8H).

Synthesis of compound 3. Compound 2 (1.87g, 2mmol), 18-crown-6 (0.26g, 1mmol), diethyl 4-hydroxypyridine-2,6-dicarboxylate (2.3g, 9.6mmol) and 5.54g (40mmol) of K_2CO_3 were refluxed in 100mL DMF under an Ar atmosphere, and the mixture was stirred overnight under 80℃. After filtration and solvent evaporation, the product was purified by silica gel column several times using petroleum ether/ethyl acetate (1:1 v/v) as eluent. Compound 3 was obtained as yellow solid in 43.6% yield (1.37g). ^{1}H NMR (300MHz, $CDCl_3$), δ(ppm): 7.778 (s, 8H), 6.941-6.911(d, 8H), 6.644-6.614 (d, 8H), 4.482-4.435 (m, 16H), 4.208-4.188 (m, 8H), 3.966 (m, 8H), 2.012-1.972 (m, 16H), 1.477-1.430 (t, 24H). MS-TOF, $C_{86}H_{96}N_4O_{24}$ $(M+Na)^+$:1591.6.

Synthesis of compound 4. Compound 3 (1.57g, 1mmol) and 1.1g of K_2CO_3 was dissolved in a 1:1 mixture of ethanol-water. This mixture was stirred overnight under 70℃. The solution was cooled to room temperature and HCl solution was then added to precipitate the product. The mixture was filtered, and washed with water several times. The product (1.24g, 0.888mmol) was dissolved in KOH (0.398g, 7.10mmol) water solution. The solvent was evaporated and compound 4 was obtained as yellow powder. ^{1}H NMR (300MHz, D_2O), δ(ppm):7.407 (s, 8H), 6.734 (s, 8H), 6.326 (s, 8H), 3.959 (s, 8H), 3.603 (s, 8H), 1.647 (s, 16H). Elemental analysis: C (45.32%), H (4.44%), N (2.88%), $C_{70}H_{56}N_4O_{24}K_8 \cdot 12H_2O$.

3. Figure S1-S4.

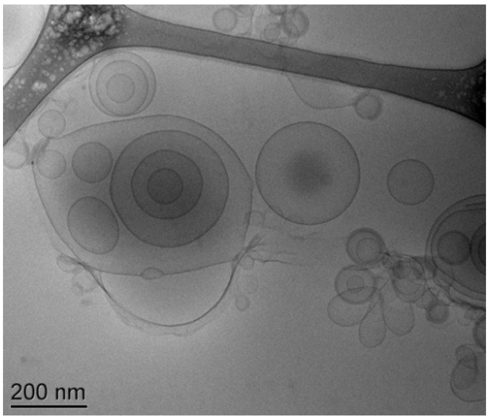


Figure S1. Cryo-TEM image of CTAB/TPE-BPA=8 vesicle. [TPE-BPA]=50 μM, [CTAB]=400 μM.

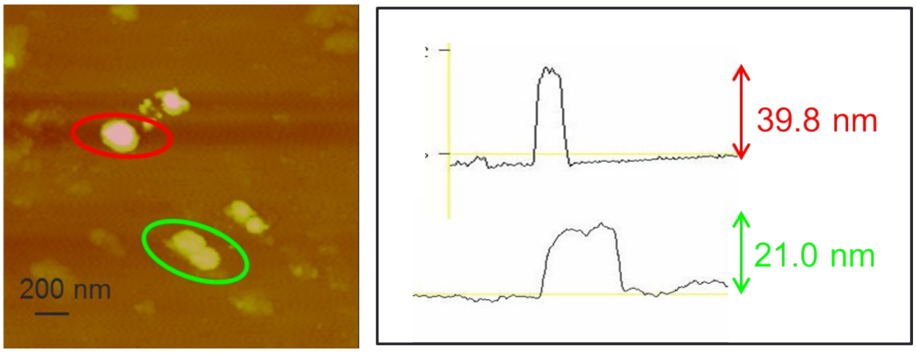


Figure S2. AFM image and the height profile of the CTAB/TPE-BPA=8 vesicle. [TPE-BPA]=50 μM, [CTAB]=400 μM.

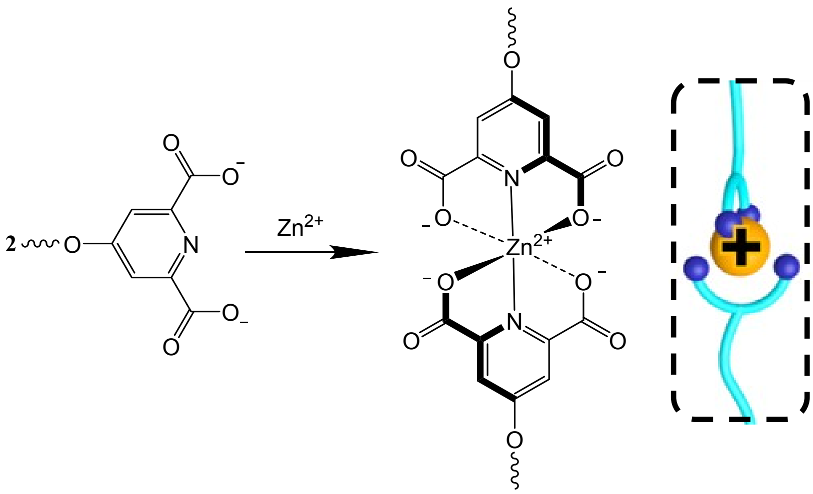


Figure S3. The vertical coordinated conformation of pyridine dicarboxylate groups of TPE-BPA with Zn^{2+}.

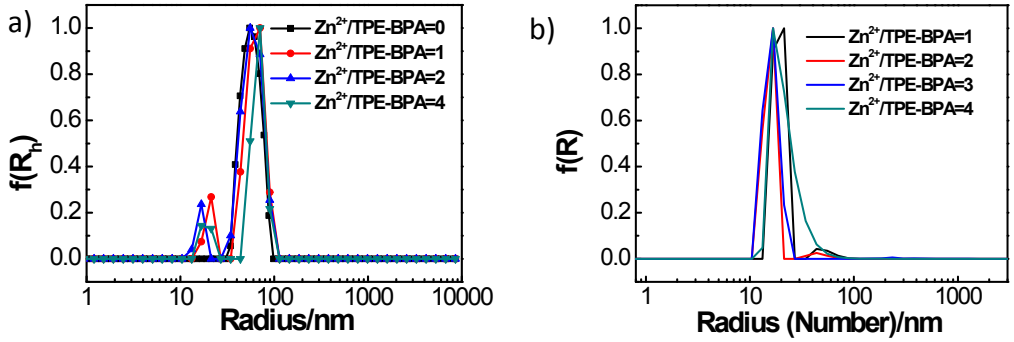


Figure S4. a) Intensity averaged DLS size distribution of the TPE-BPA@8CTAB vesicle upon addition of Zn^{2+}; b) Number averaged size distribution.

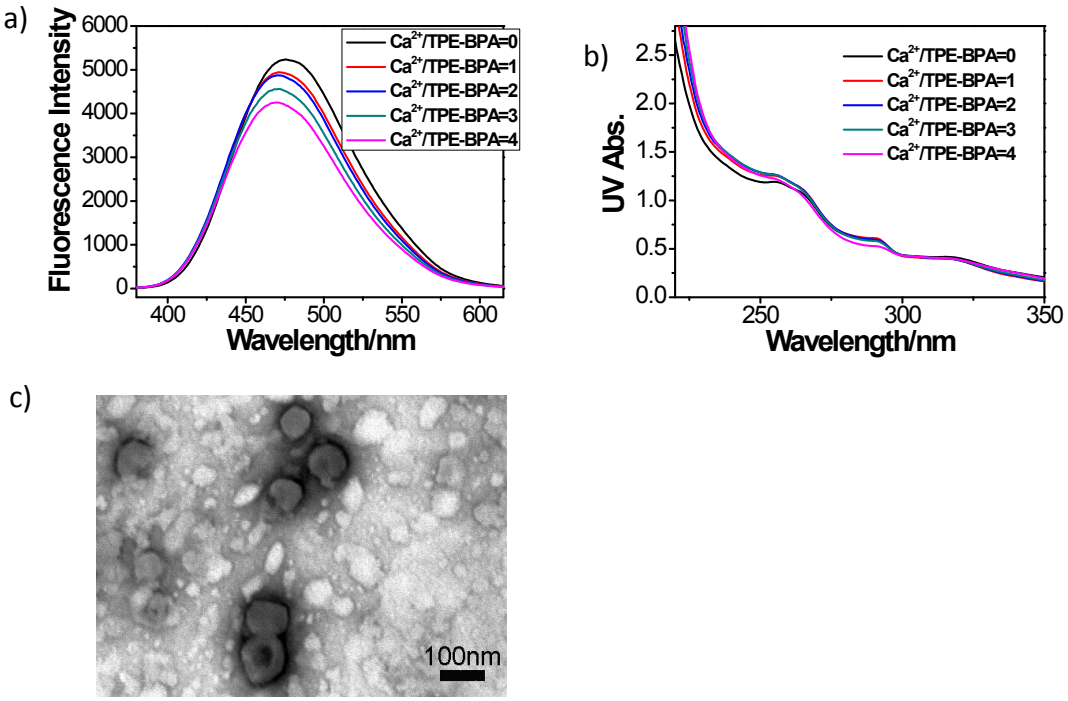


Figure S5. Influence of Ca^{2+} on the a) fluorescence and b) UV/Vis absorption of the TPE-BPA@8CTAB vesicle. c) TEM image of the $2Ca^{2+}$@TPE-BPA@8CTAB vesicle. [TPE]=50 μM, [CTAB]=400 μM, T=298 K.