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Supplementary information

An Amphiphatic Lytic Peptide For Enhanced and Selective Delivery of Ellipticine

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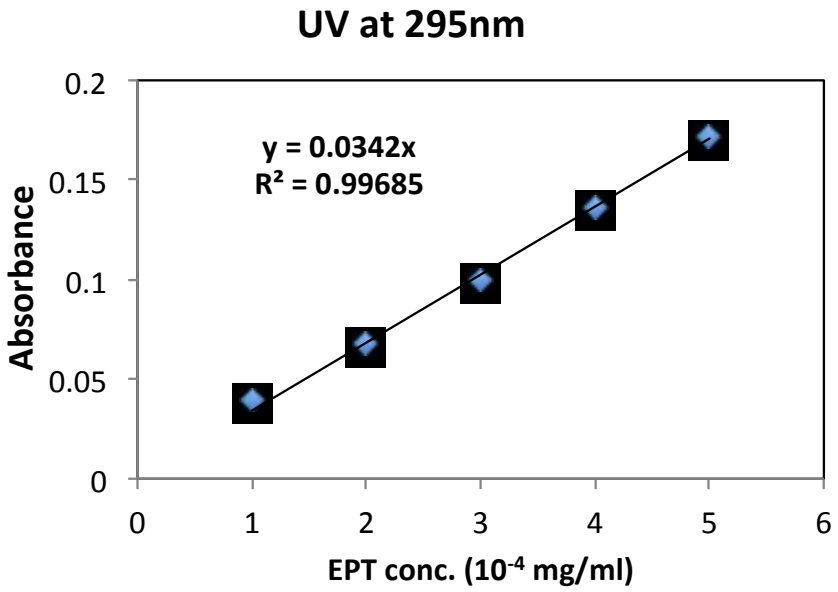

Figure S1. Calibration curve of EPT UV-adsorption at concentrations ranging from $1\times10^{-4}\sim 5\times10^{-4}$ mg/ml. The fitting equation of the calibration curve is shown in the plot.

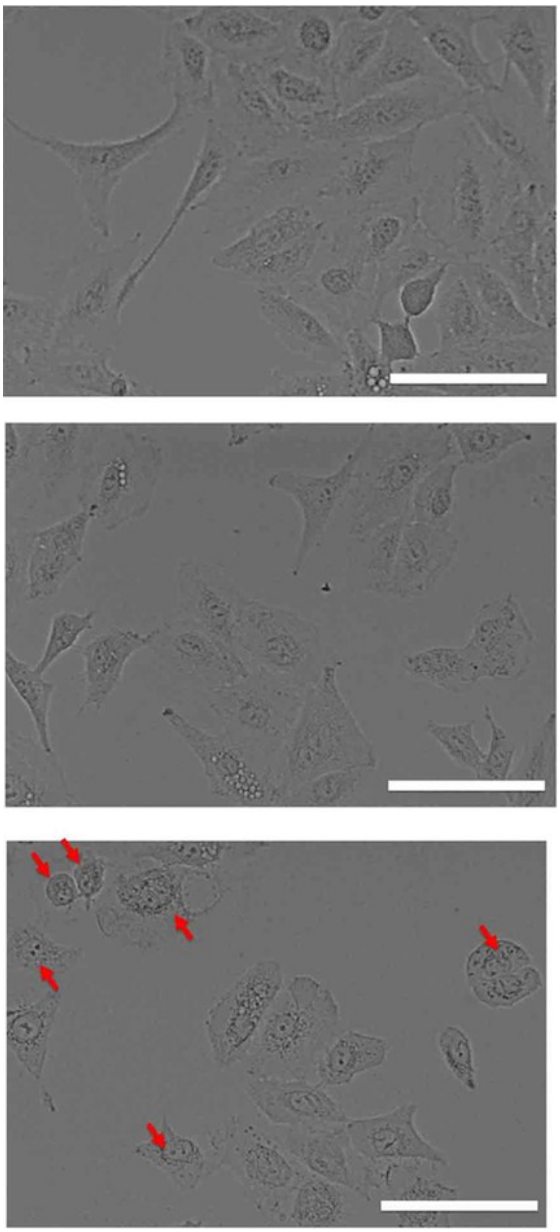

Figure S2. The morphologies of untreated A549 cells (top) and A549 cells treated with 25 μg/ml (10.1 μM) C6 (middle) or 50 μg/ml (20.2 μM) C6 (bottom) for 5 min. Arrows indicate the necrotic cells. Scale bars are 20 μm. The images were produced using an EVOS ® FL Cell imaging system (AMG).

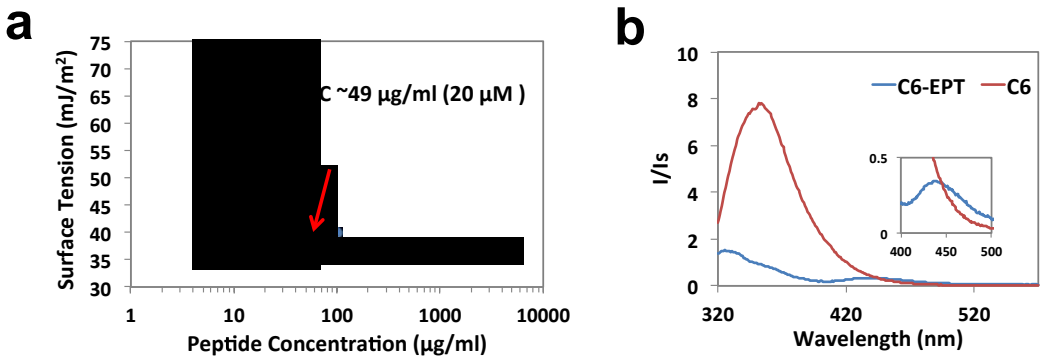

Figure S3. (a) Critical aggregation concentration of C6 determined by equilibrium surface tension of C6. (b) Molecular state of EPT in C6-EPT complex determined by fluorescence spectroscopy. The concentration of C6 is 500 µg/ml and the C6-EPT complex is consisted of 500 µg/ml with 14 µg/ml EPT. The inset shows the emission peak of EPT. Emission peak ~430 nm indicates neutral EPT.

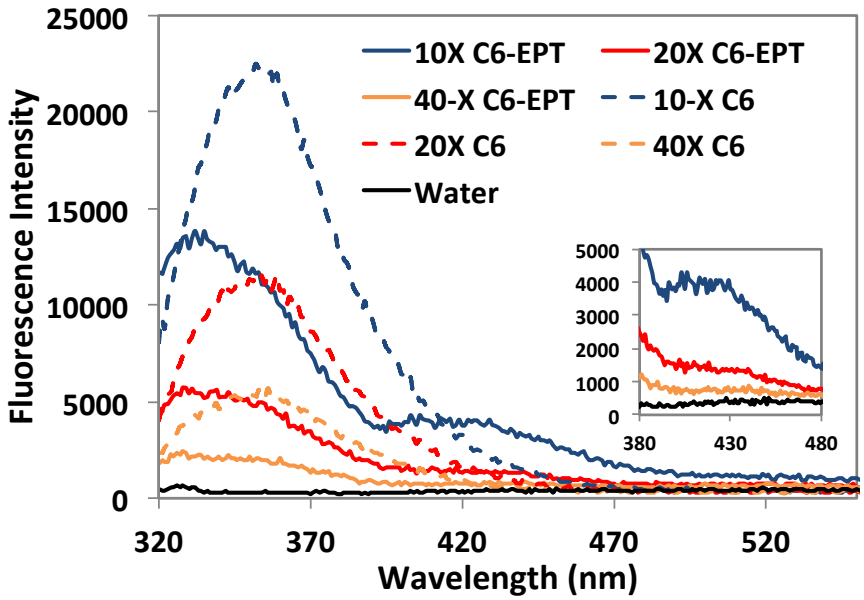

Figure S4. Fluorescence spectrum of the C6-EPT complex and C6 solution after serial dilutions. 10X, 20X and 40X represent 10-fold, 20-fold and 40-fold dilutions from the original C6-EPT complex (500 µg/ml of C6 with 14 µg/ml of EPT) or C6 (500 µg/ml) samples. The inset shows the emission peak of EPT.

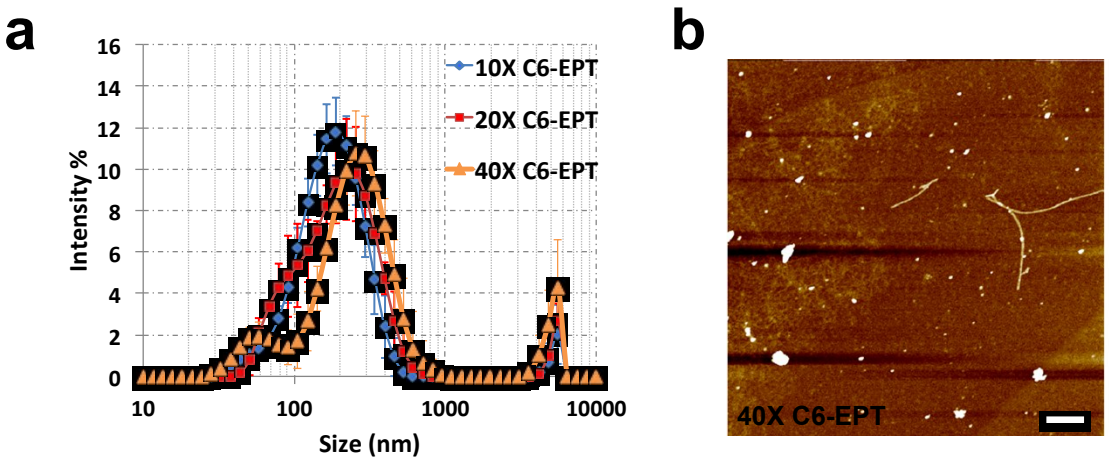

Figure S5. (a) The hydrodynamic size distributions of the C6-EPT complex after serial dilutions measured by DLS. 10X, 20X and 40X represent 10-fold, 20-fold and 40-fold dilutions from the original C6-EPT complex sample (500 μg/ml of C6 with 14 μg/ml of EPT). (b) AFM images of the C6-EPT complex after 40-fold dilution. Scale bars are 500 nm. Inset: amplified images. Scale bar is 500 nm.

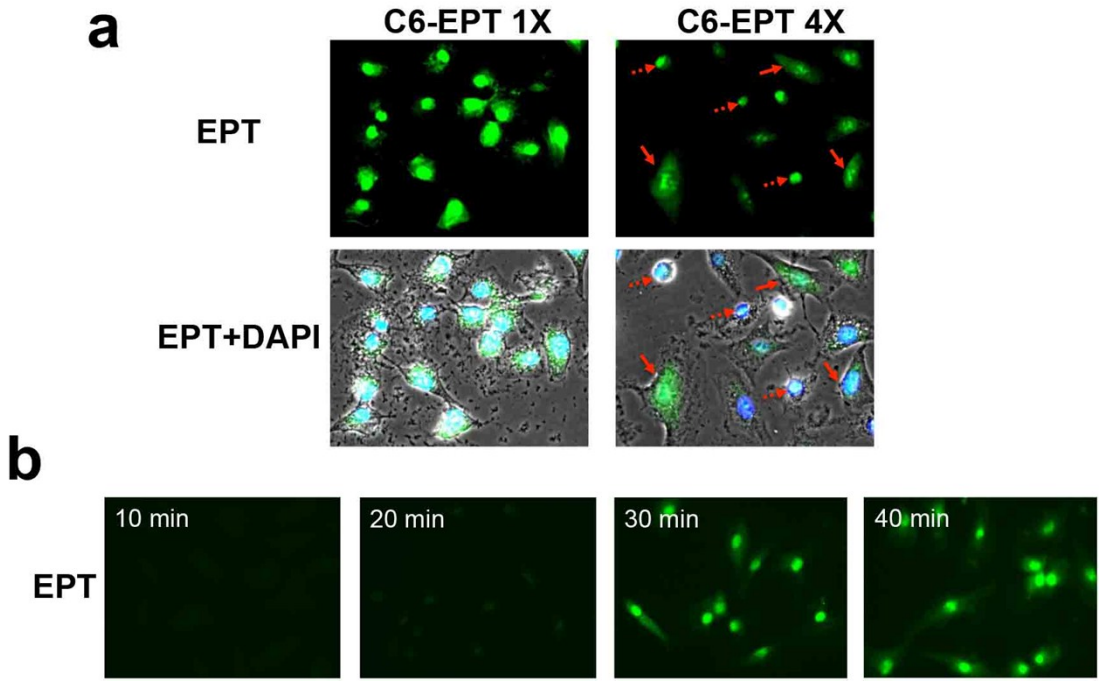

Figure S6. (a) Cellular uptake of EPT delivered by C6 monitored using fluorescence microscope. EPT is shown in green, A549 cell nuclei were stained with DAPI shown in blue. 1X sample refers to complex with with 125 μg/ml (53.6 μM) C6 and 3.5 μg/ml (14.2 μM) EPT in the final culture media. The concentration of C6 sample was 125 μg/ml (53.6 μM). Solid arrows indicate cells with intact membranes and dashed arrows indicate broken cells. (b) Time study of cellular uptake of EPT from 1X C6-EPT complex at 10 min, 20 min, 30 min and 40 min, respectively.

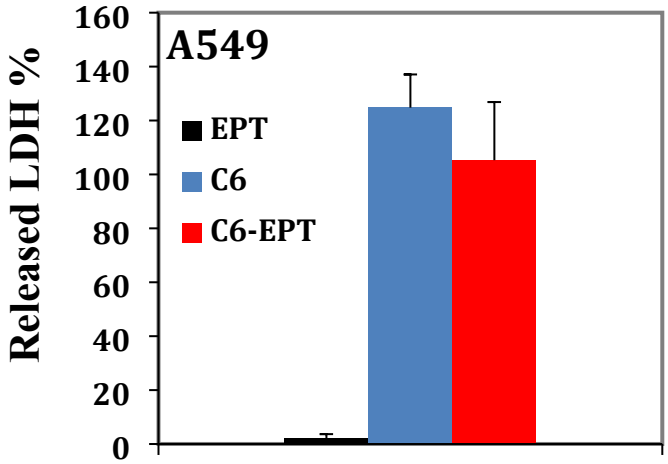

Figure S7. LDH release induced by EPT (10 μg/ml), C6 (125 μg/ml) or C6-EPT complex (125 μg/ml, 3.5 μg/ml) on A549 cells. Error bars indicate standard deviation (n=3).

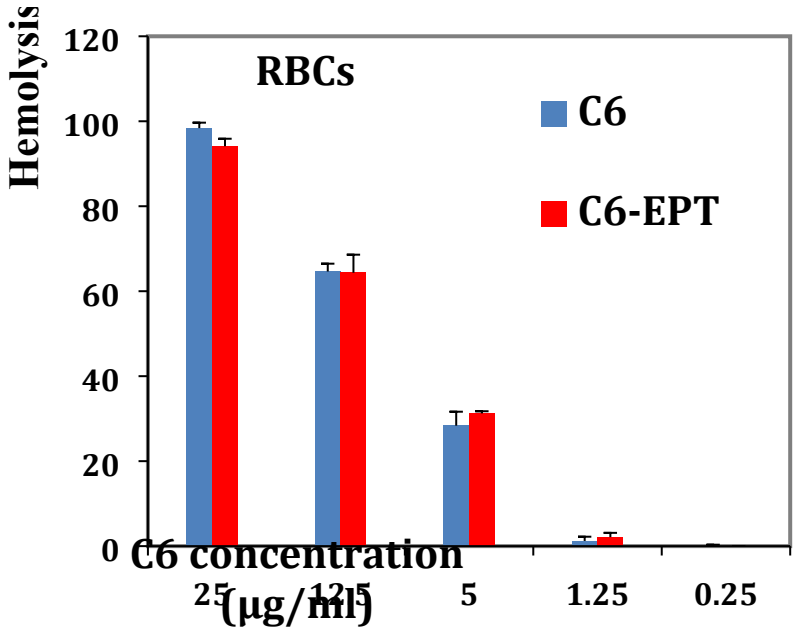

Figure S8. Hemolytic activity of C6 and C6-EPT complex evaluated using rabbit red blood cells after 2 h of incubation. The results reported here were averaged from at least three independent experiments. Error bars represent standard deviation.

Methods

Surface tension measurement

The dynamic surface tensions of C6 aqueous solutions at various concentrations were measured using ADSA-P technique. The experimental set-up was described in an earlier publication [1]. Briefly, a pendent drop of C6 solution at various concentrations was form at the tip of a motor-driven syringe (inner diameter of 0.92 mm) in a chamber saturated with pure water vapor to keep a consistent humid environment. For each sample, the images of the pendent drop were acquired by a CCD camera at 30s intervals for 1 h. The surface tension was obtained as a fitting parameter by fitting the drop profile to the theoretical curve governed by the Laplace equation of capillarity [2]. The equilibrium surface tensions were estimated by averaging 10 data points at the end of each dynamic surface tension profile. The equilibrium surface tensions were plotted as a function of C6 concentration to determine the critical aggregation centration (CAC) of C6.

Fluorescence spectroscopy

The molecular states of EPT in C6-EPT complex were identified using fluorescence spectroscopy. The samples were transferred into a square quartz cell and excited at 295 nm. The emission scans were performed on a QM4-SE spectra fluoremeter (PTI, London, Canada). The fluorescence emission of C6 aqueous solution was used as background. Detailed experimental settings have been described previously [3]. A standard (2 mM EPT in ethanol, sealed and degassed) sample was used in each run to correct the lamp intensity variations. The standard fluorescence intensity was obtained by taking an average of the fluorescence (I_s) from 424 to 432 nm (for the peak at 428 nm). The spectra of samples were normalized with I_s.

Cellular uptake of C6-EPT complexes

A549 cells were seeded into 24-well plates at a density of 5×10^4 cells per well and cultured overnight. The cells were then treated with C6 or C6-EPT complexes and incubated for 10 min, 20 min, 30 min, 40 min and 1 h, respectively. The cells were washed with phosphate-buffered saline three times, and then incubated with Fluoroshield™ mounting solution with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, Oakville, Canada) for 30 min. Afterwards, the cellular uptake of C6-EPT complexes was immediately imaged using an Axio Observer microscope (Carl Zeiss, Jena, Germany).

Lactate dehydrogenase (LDH) assay

An LDH assay kit (promega, Madison, USA) was applied to monitor cell membrane leakage induced by C6 or C6-EPT complex on A549 cells. Briefly, A549 cells were seeded into a 96-well plate and treated with sampels as described in MTT assay section and incubated for 24 h. The LDH assay was conducted according to the manufacture's

instructions. F-12K culture media with 10% FBS and 1% antibiotics was used as a control for background LDH release, and 3% triton X-100 in the same media was used as a positive control for LDH release. The absorbance of assay solution was measured at 485 nm using a FLUOstar plate reader (BMG Labtech, Ortenberg, Germany). The percentage of released LDH was calculated using the following equation: $((\text{Absorbance}_{\text{treated cells}} - \text{Absorbance}_{\text{background}}) / (\text{Absorbance}_{\text{positive control}} - \text{Absorbance}_{\text{background}}) \times 100$.

Hemolysis assay

The fresh rabbit ear venous blood (female New Zealand White rabbit) was collected in K2EDTA anticoagulant tubes, centrifuged at 1500 rpm for 15 min and washed three times with PBS buffer. After washing, the red blood cells (RBCs) were isolated and suspended in PBS buffer for a 2% hematocrit solution. Varying concentrations of C6 or C6-EPT complex were prepared by diluting samples in PBS buffer. The dilutions were mixed with RBC suspensions to make solutions with 1% RBCs with a final volume of 1 ml, and incubated at 37°C for 2 hours. RBCs mixed with 1% Triton X-100 and PBS buffer were used as positive and negative controls to obtain 100% and 0% hemolysis, respectively. The samples were centrifuged at 3000 rpm for 5 min and the supernatants were collected in a 96-well plate. The released hemoglobin was monitored at 540 nm. The percentages of hemolysis were then determined with the following equation:

$$Hemolysis\ \% = \frac{Abs(sample) - Abs(-)}{Abs(+) - Abs(-)} \times 100\%$$

where Abs(sample), Abs(+) and Abs(-) denote the absorbances of sample-treated, Triton-treated and buffer-treated samples, respectively. The animal experiments were approved by the Ethical Committee (approval ID: 2013-0019) of North Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine.

Reference

[1] Fung SY, Keyes C, Duhamel J, Chen P. Concentration effect on the aggregation of a self-assembling oligopeptide. Biophys J. 2003;85:537-48.
[2] Rotenberg Y, Boruvka L, Neumann AW. Determination of Surface-Tension and Contact-Angle from the Shapes of Axisymmetric Fluid Interfaces. J Colloid Interf Sci. 1983;93:169-83.
[3] Wu Y, Sadatmousavi P, Wang R, Lu S, Yuan YF, Chen P. Self-assembling peptide-based nanoparticles enhance anticancer effect of ellipticine in vitro and in vivo. International journal of nanomedicine. 2012;7:3221-33.