Electronic Supplementary Material (ESI) for Chemical Communications

The Core-Inversible Micelles for Hydrophilic Drug Delivery

Wenzhe Huang [†], Changying Shi[†], Yu Shao[†], Kit S. Lam[‡] and Juntao Luo[†*]

[†]Department of Pharmacology, SUNY Upstate Cancer Research Institute, SUNY Upstate Medical University, Syracuse, NY 13210, USA

‡ Department of Biochemistry & Molecular Medicine, Division of Hematology and Oncology, Department of Internal Medicine, University of California Davis, Sacramento, CA 95817, USA

[*]**Corresponding Author**

luoj@upstate.edu

Supporting Information

1. Materials

Monomethylterminated poly(ethylene glycol) monoamine hydrochloride(MeO-PEG-NH2 ·HCl, Mw: 5000 Da) was purchased from Jenkem Technology (Dallas, TX). (Fmoc)Lys(Fmoc)-OH was obtained from AnaSpec Inc. (San Jose, CA). Toyopearl HW-55S resin was purchased from Tosoh corporation (Japan), Congo red, HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt), Cholic acid and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2. Methods

2.1 Preparation of CIMs

Telodendrimer $PEG^{5k}CA_8$ was prepared via solution peptide chemistry following the procedure in our previous publication. [1,2] 10 mg of $PEG^{5k}CA_8$ telodendrimer was dissolved in 1 mL of certain apolar solvents, e.g. ethyl acetate or toluene, to form CIMs. These two kinds of CIMs solution were used for further characterizations. Folic acid was coupled on an amino group on the peripheral end of telodendrimer NH2-$PEG^{5k}CA_8$ using DIC/HOBt as coupling reagents.

2.2 Loading HPTS, CR and PTX-CR in CIMs

HPTS (25 μg) and $PEG^{5k}CA_8$ (20 mg) were first dissolved in sodium hydroxide solution (pH=10, 1.0 mL) in a 1.5 mL tube and then lyophilized to yield a light yellow powder. Ethyl acetate (1.0 mL) was added into the powder, followed by the mild heat to disperse the HPTS- $PEG^{5k}CA_8$ conjugates into ethyl acetate to form HPTS loaded CIMs. Followed the same procedure above, HPTS-$PEG^{5k}CA_8$ conjugates in toluene, ethanol, acetonitrile and water were prepared.

Preparation of CR-CIMs: 10 mg $PEG^{5k}CA_8$ was dissolved in 1.0 mL water in a 1.5 mL tube, added 100 μL CR solution (1.25 mg/mL in water) into the tube, lyophilized, then added 1.0 mL of ethyl acetate into the tube to form CIMs.

Alternatively, 1.0 mg of CR and 10 mg of telodendrimer $PEG^{5k}CA_8$ was dissolved in 1.0 mL of water and lyophilized. The dry powder was dissolved with 2.0 mL of ethanol with 5% of water in volume. The solvent was removed on a rotaevaporator to form a thin film coated on the flask wall, which was further dried under high vacuum for 30 min. The thin film was dispersed into PBS by the aid of vortex and sonication.

2.3 Characterizations of CIMs

2.3.1 Dynamic Light Scattering (DLS) measurements of CIMs

The size and size distribution of the CIMs were measured using a Zetatrac (Microtrac Inc.) instrument. For the DLS particle size measurements in different organic solvents, the fluid parameters, such as refractive index and the viscosities of the solvent at two different temperatures (20 ^{o}C and 30 ^{o}C) have been input according to the Fluid Reference Manual along with the Zetatrac instrument. The mean sizes based on the area distribution (MA) were reported for size comparison in different solvents. The poldispersity index (PDI) was calculated based on the formula of $PDI = (SD/MA)^2$ The CIM concentrations were kept at 10 mg/mL for DLS measurements. All measurements were performed at room temperature.

2.3.2 Ultraviolet-visible (UV-Vis) spectra of CIMs

UV-Vis spectra were recorded on a Thermo Scientific Nanodrop 2000c spectrophotometer. The spectra of HPTS-CIMs in different solvent were recorded.

2.3.3 The morphology of CIMs study

The Transmission Electron Microscopy (TEM) images of CIMs were carried out on a JEOL JEM-2100 HR instrument operating at a voltage of 110 kV. The samples were prepared by dropping the solutions (100 μg/mL) onto carbon-coated grids, and the sample were stained by sodium urinate and dried by heating to 50 °C℃ for 30 min before measurement.

2.3.4 ^{1}H NMR spectroscopy of CIMs

^{1}H NMR spectra of the polymers were recorded using Bruker AVANCE 600 MHz spectrometer with different solvents (CCl4, toluene-d_8, CDCl3, DMSO-d_6, MeCN-d3 and D2O).

2.3.5 Study of the transfer of CR-CIMs from ethyl acetate to water

The CR-CIMs formed in ethyl acetate can be transported into water completely via extraction between two phases. Take 0.5 mL CR CIMs solution to the 1.5 mL tube, then add 0.5 mL water, shake gently for 5 min, spin down, upper layer is ethyl acetate phase, lower layer is water phase. The absorbance at 500 nm of two phases was measured to determine the transfer efficiency.

2.4 Loading efficiency of micelles

2.4.1 CR loading efficiency

The CR loading efficiency was determined in triplicate by UV-Vis. The detection wavelength was 500 nm. The UV absorbance vs CR concentration was calibrated with standard CR solutions in water from 2.5 to 160 μg/mL (correlation coefficients of R^2=0.9998). It has been determined that the solubility of CR in ethyl acetate is close to zero via UV-Vis measurement. Therefore, the loading efficiency (LE) of CR in CIMs in ethyl acetate was calculated based on the following equation (1).

$$\text{LE}\ (\%) = \frac{\text{Amount of CR in CIMs in EtOAC}}{\text{Initial amount of CR}} \times 100 \quad (1)$$

The CR-CIMs were transferred from ethyl acetate to water via simple extraction. To determine the percentage of CR loaded in CIMs vs the free CR dissolved into aqueous solution, the gravity size exclusive chromatography (SEC) was performed. The aqueous CR loaded CIMs solution with the determined amount of CR was loaded on a column (0.6×2 cm) of HW 55s equilibrated in water. The column was eluted with 300 μL of water and then with 500 μL of the Acetonitrile/water (3:1 v/v). The elution was performed at a flow rate of 20 μL/min. Each fraction was collected at a volume of 20 μL, and was measured via UV-Vis absorbance at 500 nm. The encapsulation efficiency (EE) of CR in CIMs after being extracted into aqueous solution was defined in equation (2) by the ratio of the earlier fraction of CR loaded in CIMs nanoparticle to the total CR amount in CIMs aqueous solution before loaded onto SEC column.

$$\text{EE}\ (\%) = \frac{\text{Amount of CR in CIMs}}{\text{Initial amount of CR loaded on SEC column}} \times 100 \quad (2)$$

2.4.2 Co-loading of hydrophilic CR with hydrophobic PTX into CIMs in aqueous solution

1.0 mg of CR and 10 mg of telodendrimer $PEG^{5k}CA_8$ was dissolved in 1.0 mL of water and lyophilized. The dry powder was dissolved with 2.0 mL of ethanol with 5% of water in volume, at the same time, 1.0 mg of PTX was dissolved in the above mixture solution. The solvent was removed on a rotaevaporator to form a thin film coated on the flask wall, which was further dried under high vacuum for 30 min. The thin film was dispersed into PBS by the aid of vortex and sonication. The separation of CR-PTX co-loaded CIMs, free CR and PTX was performed by Gel filtration Chromatography. The elution of CR was monitored via absorption at 500 nm and the determination of PTX was performed by HPLC analysis. The fractions of the CIMs indicated by CR absorbance were combined and the late eluted fractions for small molecules were combined in another collection. The aqueous solutions were lyophilized. Then the lyophilized powders were dissolved in acetonitrile and vortexed vigorously to get a clear solution for HPLC analysis. The mobile phase was composed of a gradient from 0-100% of acetonitrile in water. The elute time was 25 min, the reverse phase column was a C_{18} (5 µm, 4.6 x 150 mm). The column temperature was maintained at 30 °C. The flow rate was set at 1.0 mL/min and the detection wavelength was 227 nm. Sample solution was injected at a volume of 10 µL.

2.5 In vitro release profiles of CR from CR-CIMs

The release profile of free CR and CR loaded in $PEG^{5k}CA_8$ CIMs were evaluated by a dialysis method. CR-CIMs were prepared and characterized as mentioned in section 2.2 and 2.3. After CR-CIMs preparation, the CIMs was diluted with double distilled water, to yield about 100 µg/mL of CR, the concentration of free CR was also made at 100 µg/mL. The prepared samples (0.5 mL) were added into a D-Tube TM Dialyzer Midi with a MWCO of 3.5 kDa respectively. Three triplicates dialysis were used in each experiment. The dialysis bags were placed in a reservoir of 5 L dd-water, which was changed every 4 hrs for free CR sample and every 4 hrs in the first day, then changed every day for the CR loaded CIMs. A sample of 2.0 µL was drawn from each sample at various sampling time intervals. The sampling time intervals were 0, 1, 2, 4, 6, 7, 8 and 21 h for the free CR sample, and the sampling time intervals were 0, 1, 2, 3, 6, 8, 10, 12, 15, 17, 22 and 41 day for the CR loaded in CIMs. The Absorbance of CR in each sample was measured by Thermo Scientific Nanodrop 2000c UV-Vis spectrophotometer at maximum wavelenth of 500 nm for the free CR sample and 515 nm for the CR loaded in CIMs, the cumulative release percentage was calculated.

2.6 Cell staining

2.6.1 Cell culture

The HT-29 colon adenocarcinoma cell line was cultured in McCOY'S 5A medium (Hyclone Laboratories. Inc, Utah, USA) containing 1.5 mM glutamine, supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (MP Biomedicals LLC, Ohio, USA). The cultures were incubated at 37 ^{0}C in a humidified atmosphere of 5% CO2 and passaged when they reached > 90% confluence. The cells were supplied with fresh medium every second day and 20 hrs before each experiment.

2.6.2 Live cell staining via CRs

Cells were trypsinized to generate a single cell suspension and seeded into a Lab-Tek chamber slide (NUNC, NY, USA) to get a final concentration of ~50000 cells/mL and incubated for overnight (37 ^{o}C and 5% CO2) in fresh medium. Confluent cells were

stained with CR, CR-PEG^{5K}CA$_8$ and CR-(PEG^{5K}CA$_8$+FA-PEG^{5K}CA$_8$) to keep the CR final concentration at 10 μM.

After 3 hrs incubated at 37 ^{o}C and 5% CO2 in the dark, the treated cells were washed with PBS buffer 3 times and then fixed by 4% formaldehyde for 5 mins. The fixed cells then were washed with PBS buffer 3 times again. After being washed with PBS, cells were mounted by VECTASHIELD HardSet mounting medium (Vector Laboratories, Inc. CA, USA).

Images were obtained using a Nikon ECLIPSE TE2000-U inverted research microscope (Nikon corporation instruments company, Japan) and were acquired with a Nikon DS-Ri1 digital camera using NIS-Elements F3.0 software. The 20× objective was used.

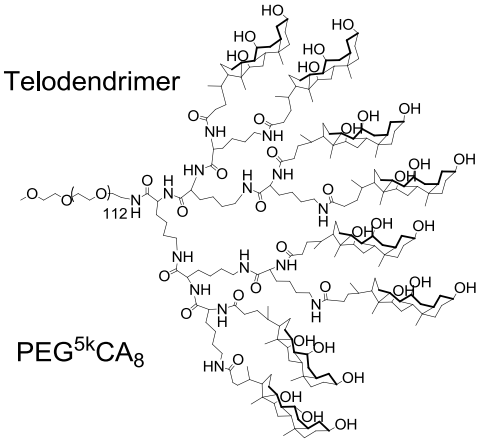

Figure S-1. Chemical structure of typical telodendrimer PEG^{5k}CA$_{8.}$

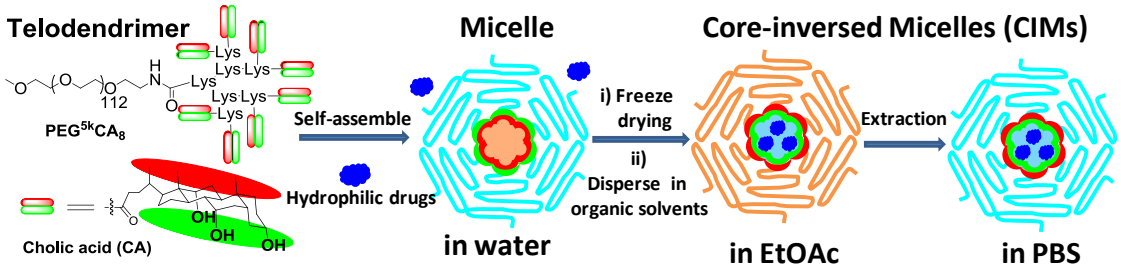

Figure S-2. The illustration of the CIM formation and the loading of the hydrophilic molecules (blue/green: hydrophilic; red/pink: hydrophobic).

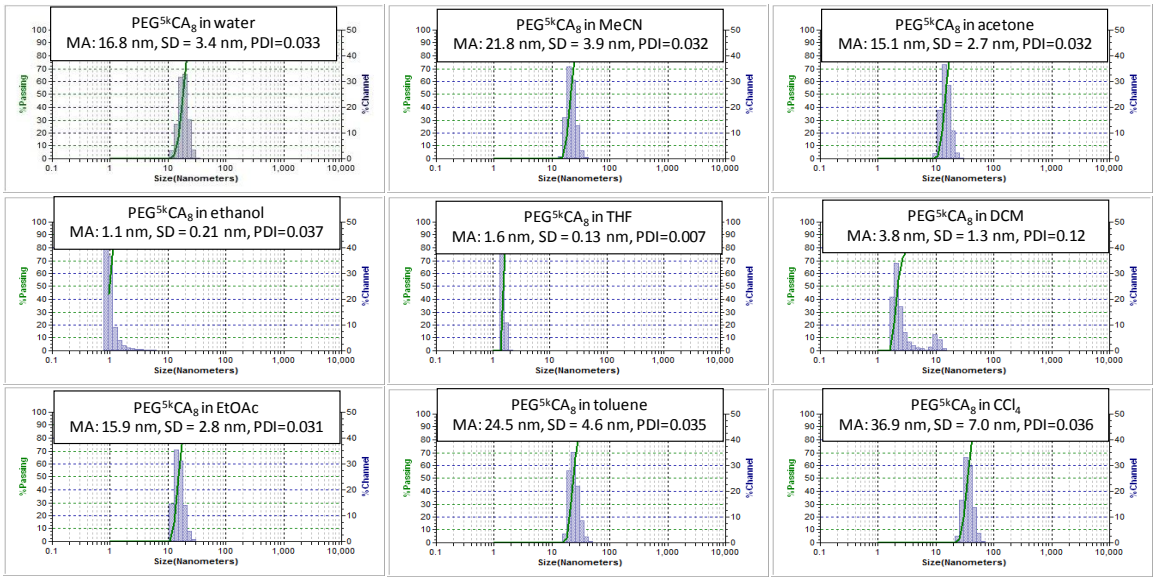

Figure S-3. Particle Sizes (MA, area distribution), standard deviation (SD) and the polydispersity (PDI = $(SD/MA)^2$) of the nanoparticles formed by $PEG^{5k}CA_8$ in different solvents via DLS: namely water, acetonitrile, acetone, ethanol, THF, DCM. (G) Ethyl acetate (EtOAc), toluene and carbon tetrachloride (CCl4).

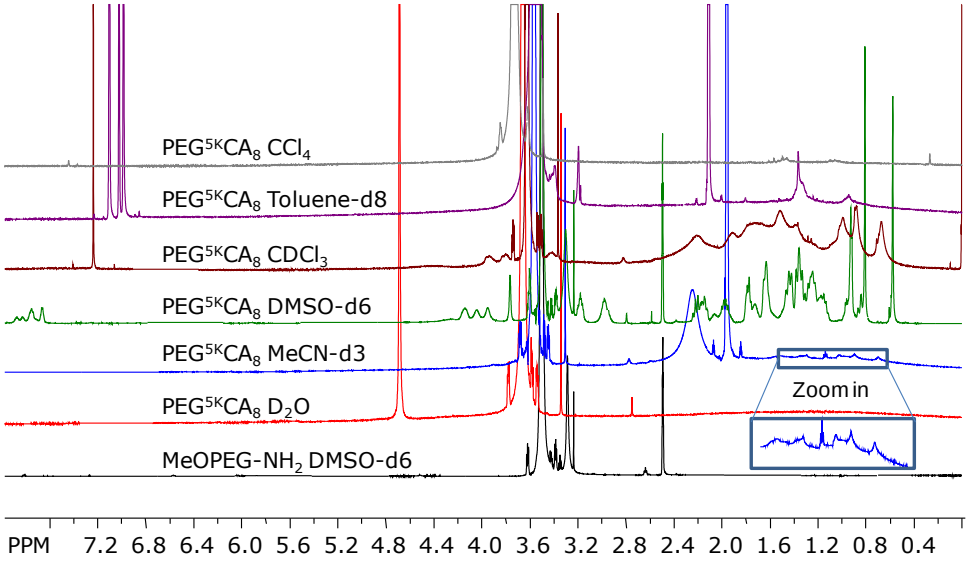

Figure S-4. ^1H NMR of $PEG^{5k}CA_8$ in different solvents

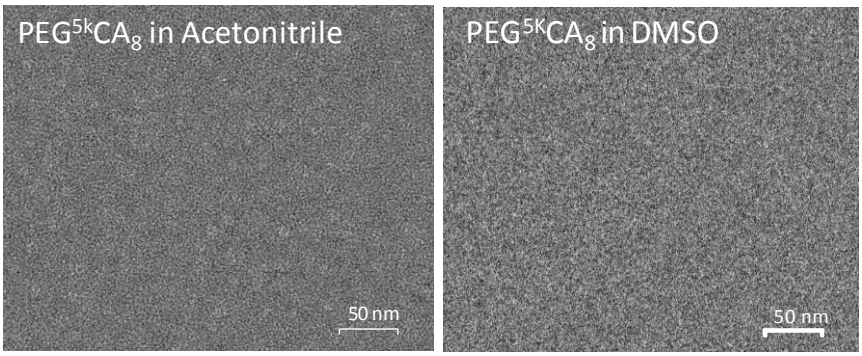

Figure S-5. TEM images of $PEG^{5k}CA_8$ in different solvents.

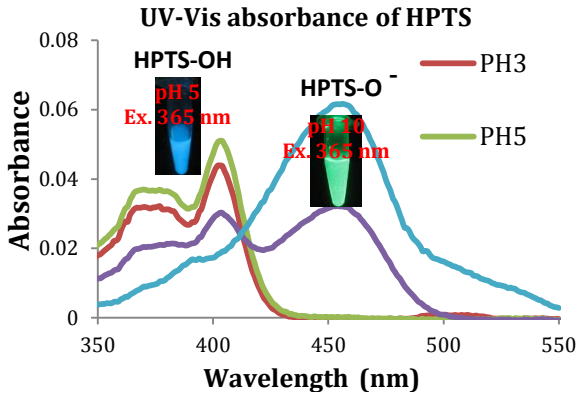

Figure S-6. UV-Vis spectra of HPTS in aqueous solutions with different pH values.

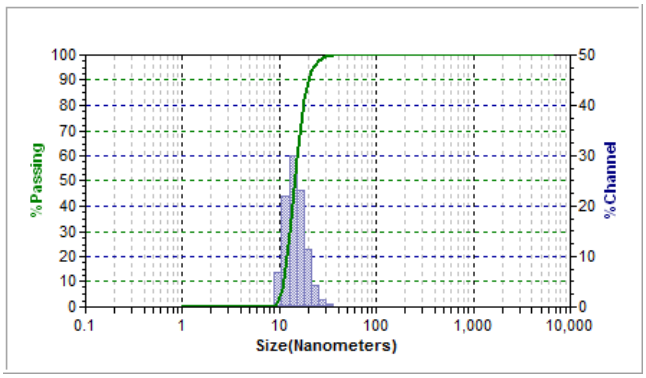

Figure S-7. Size of CR-CIMs in ethyl acetate measured via DLS particle sizer (14.4±3.4 nm).

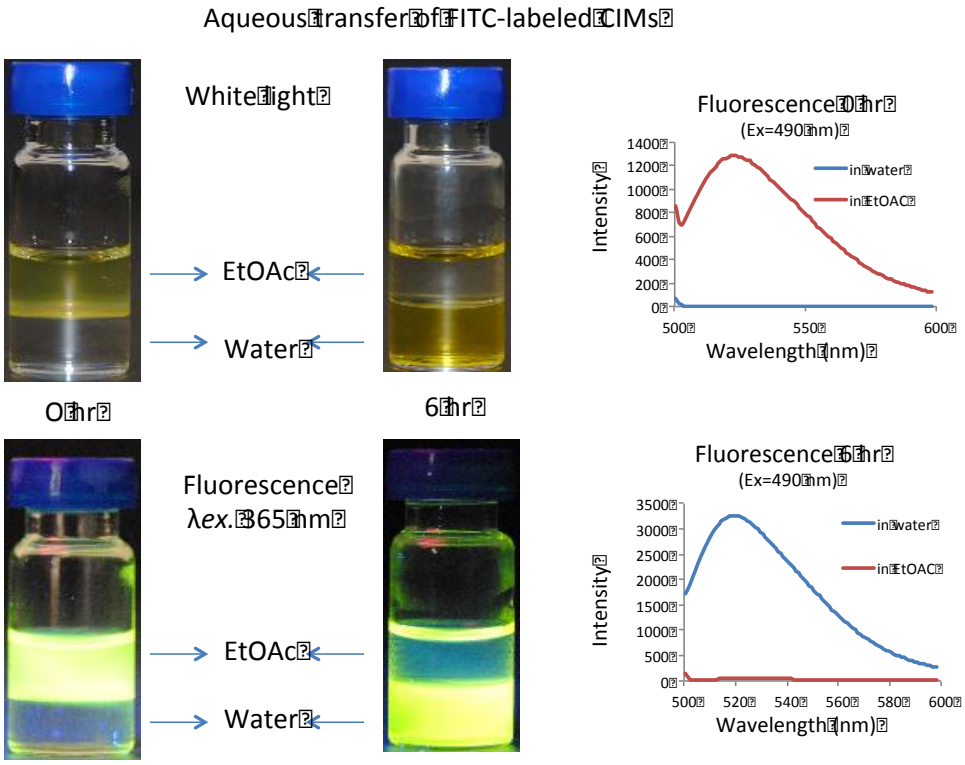

Figure S-8. The FITC labeled polymer micelles can be transferred completely from ethyl acetate phase into water phase. The UV absorbance ad fluorescence spectrum showed the complete extraction.

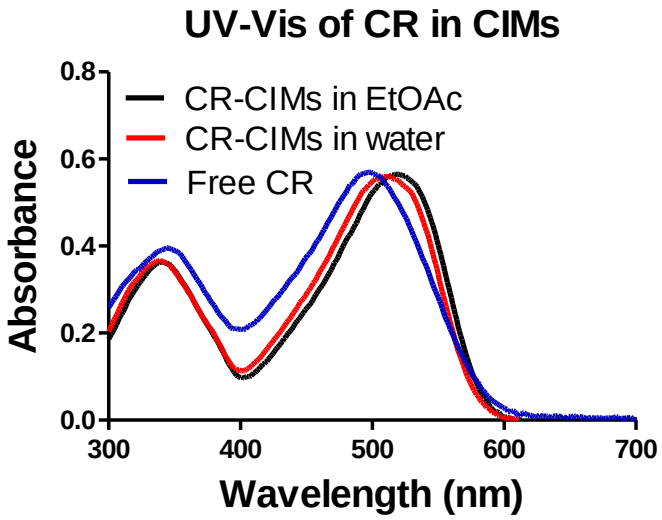

Figure S-9. Red shift of the UV-Vis absorbance of CR after being loaded in CIMs in both ethyl acetate and water.

(1) Luo, J.; Xiao, K.; Li, Y.; Lee, J. S.; Shi, L.; Tan, Y. H.; Xing, L.; Holland Cheng, R.; Liu, G. Y.; Lam, K. S. *Bioconjug. Chem.* **2010**, *21*, 1216.

(2) Xiao, K.; Luo, J.; Fowler, W. L.; Li, Y.; Lee, J. S.; Xing, L.; Cheng, R. H.; Wang, L.; Lam, K. S. *Biomaterials* **2009**, *30*, 6006.