Supporting Information

Angiopep2-Conjugated Star-Shaped Polyprodrug Amphiphiles for Simultaneous Glioma-Targeting Therapy and MR Imaging

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Characterizations

^{1}HNMR were recorded on a Bruker AV300 NMR 300 MHz spectrometer (Germany). FT-IR spectra were recorded with a Bruker VECTOR-22 IR spectrometer (Germany). Molecular weights and molecular weight distributions were determined with gel permeation chromatography (GPC, Agilent, 1260, USA). The hydrodynamic size distributions of micells (1.0, 0.1, 0.01 and 0.001 mg/mL) were measured using dynamic laser light scattering (DLS, Zetasizer Nano, Malvern, U.K.). The critical aggregation concentration (CAC) of the star amphiphiles was determined by surface tensiometry. The serum stability of CPP-2 was examined at predetermined time points by incubating CPP-2 in DMEM containing 10% FBS at 37 °C under gentle stirring. Inductively coupled plasma atomic emission spectrometry (ICP-AES, 7300 DV, Perkin-Elmer, USA) was used for Gd^{3+} content analysis. The morphology of CPP-2 was examined with transmission electron microscopy (TEM, H-800, Hitachi, Japan). All data were averaged over three measurements. Flow cytometric analysis was conducted using a BD FACS Calibur flow cytometer (USA). The fluorescence images were obtained using confocal laser scanning microscopy (CLSM, TCS SP5, Leica, Germany).

Cell Culture and Animals

Rat C6 glioma cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Penicillin-streptomycin, Dulbecco Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and 0.25% (w/v) trypsin solutions were bought from Corning Incorporated (Corning, NY, USA). The adult male Sprague Dawley (SD) rats (250 ± 15 g) were obtained from the animal center of Anhui Medical University laboratory Animal Center (AHMU, Hefei, China). We confirmed that all animal experiments were performed in accordance with protocols approved by the ethics committee of AHMU.

Dual-Targeting Effect of CPP-2

To analyze the dual-targeting effects of CPP-2 *in vitro*, the BCECs and astrocyte cells (ACs) co-culture BBB model was established as described.[1] Briefly, newborn rat astrocytes and brain endothelial cells (BCECs) were isolated. The cell material was characterized on the basis of specific cell-type properties and functional expression of specific BBB properties, e.g., expression of endothelial cell-specific Factor VIII-related antigen and Astrocytes cell-specific glial fibrillary acidic protein (GFAP). Subsequently, ACs (5×10^4 cells) and BCECs (3×10^4 cells) were successively grown on the opposite sides of a semi-permeable transwell insert（Corning Costar, Cambridge, MA, USA), with astrocytes on the bottom of the filter.

After the co-culture BBB model was established, we firstly evaluated the ability of different CPT preparations across the BBB. The trans-endothelial electrical resistance (TEER) of the BCECs layer was kept above 300 Ωcm2 throughout the experiment to ensure the integrity of the BBB model. CPT, CPP-1 and CPP-2 (equivalent CPT concentration of 10 μg/mL) were added into donor chambers, respectively. After treatment, a volume of 200 μL solution was removed from the acceptor compartments at 1, 2 and 4 h time intervals, and immediately replaced with 200 μL D-Hank's solutions. Drug content in the acceptor compartment was determined using HPLC as described above.

Then, the transwells inserts were transferred to acceptor chambers, which seeded with confluent C6 cells. The DMEM, CPT, CPP-1, CPP-2 (equivalent CPT concentration of 10 μg/mL) were separately added in the donor chamber of transwells for 6 h. After that, the inserts were moved away, and C6 cells were further incubated until for 24 h. The percentage of apoptotic cells was also detected by flow cytometry using an Annexin-VFITC Apoptosis Detection Kit (Bestbio, China) according to the manufacturers instruction exactly.

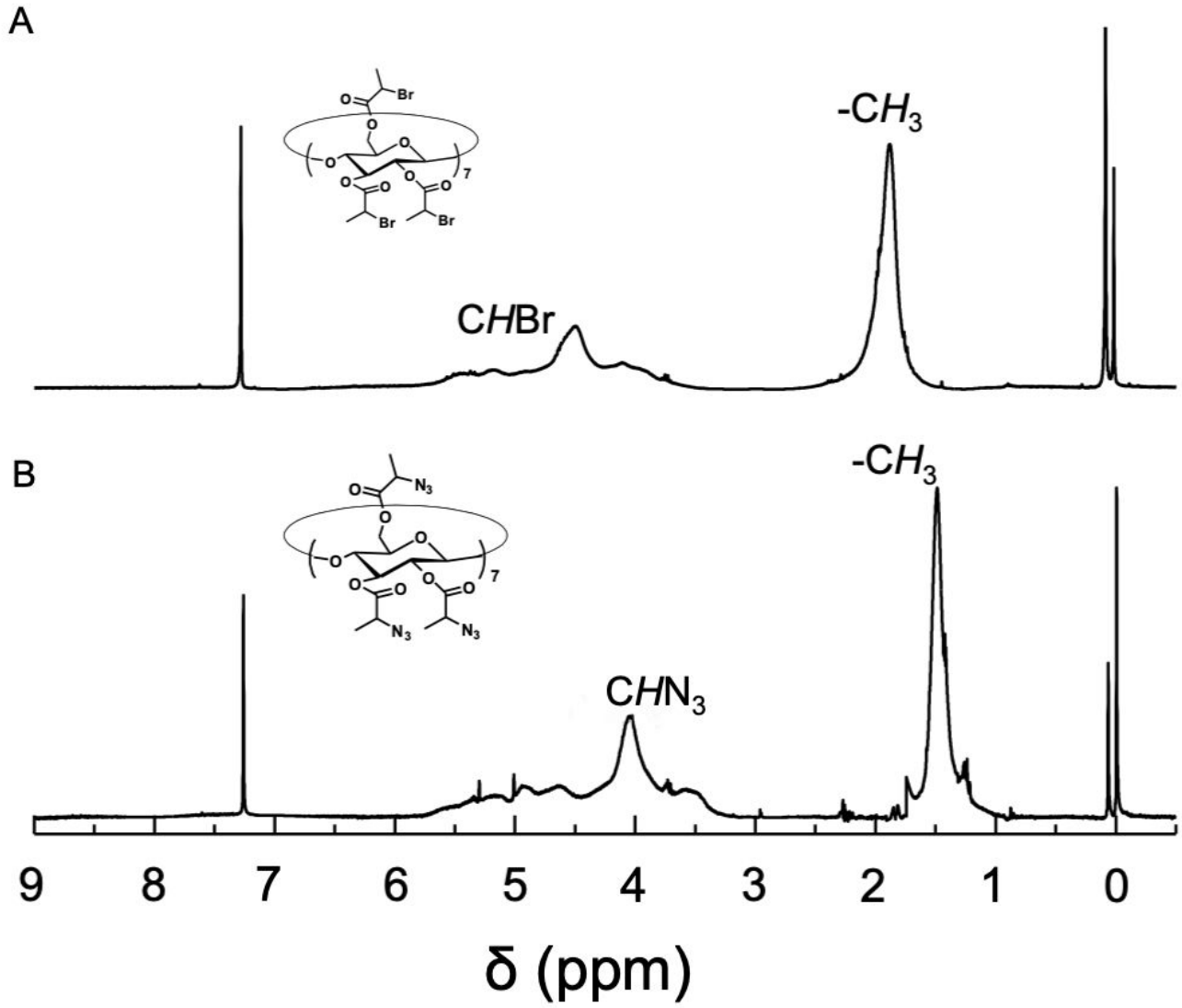


Figure S1. ^{1}HMRS spectra recorded in $CDCl_3$. (A) CD-Br_{21}. (B) CD-$(N_3)_{21}$.

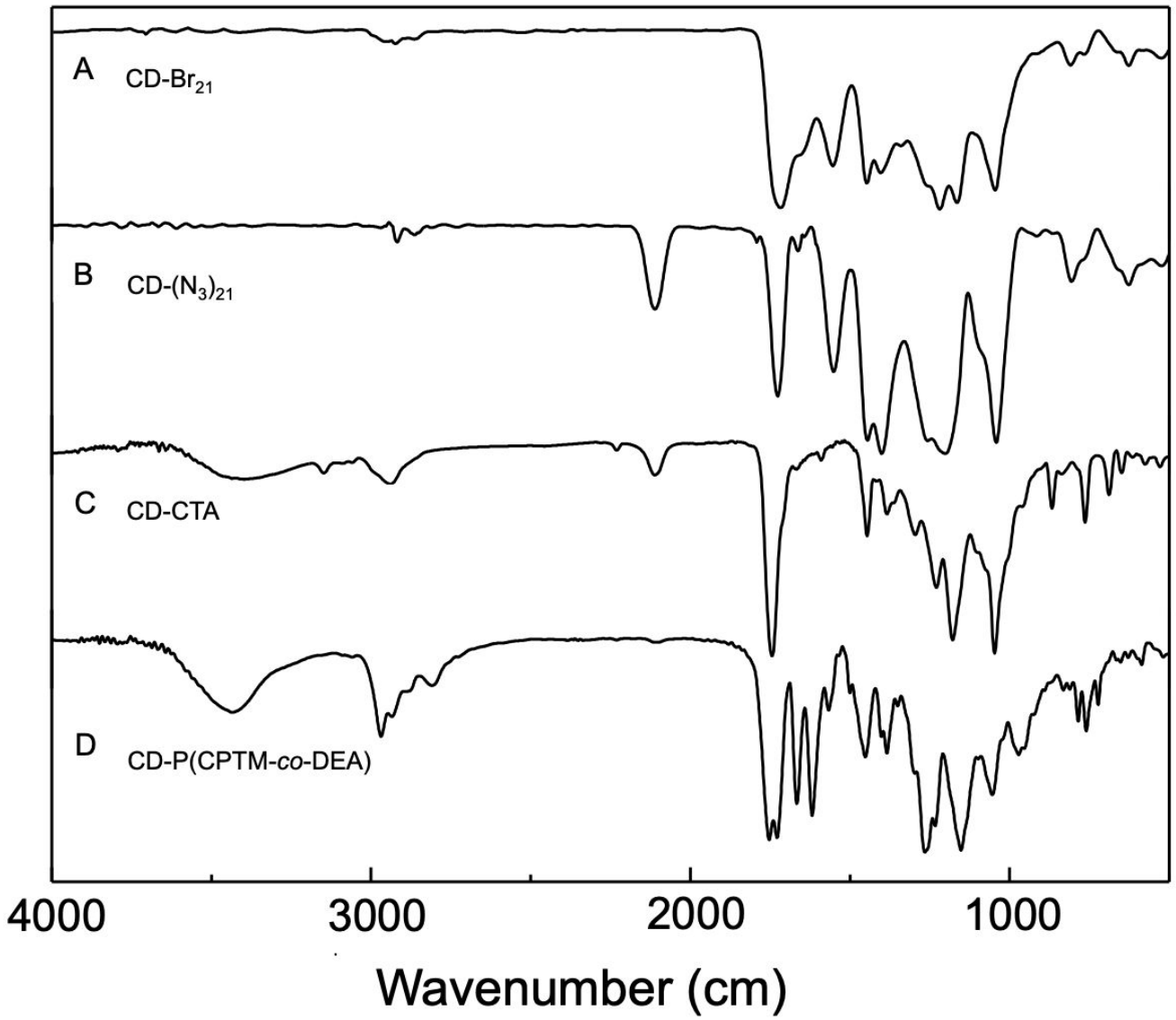


Figure S2. FT-IR spectra. (A) CD-Br_{21}. (B) CD-$(N_3)_{21}$. (C) CD-CTA. (D) CD-P(CPTM-*co*-DEA).

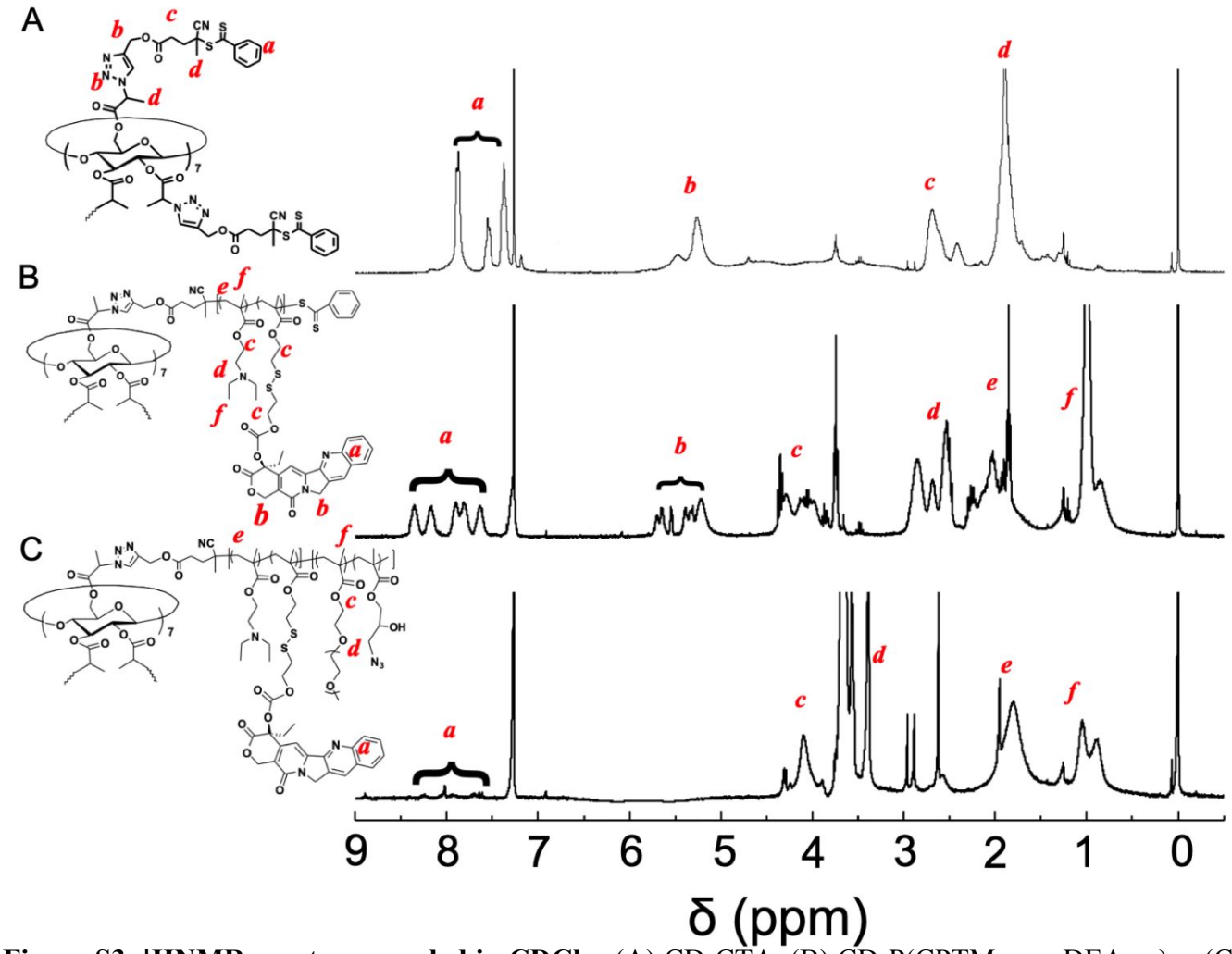


Figure S3. ^{1}HNMR spectra recorded in $CDCl_3$. (A) CD-CTA. (B) CD-P($CPTM_{20}$-*co*-$DEA_{18.5}$)$_{11}$. (C) CD-[P($CPTM_{20}$-*co*-$DEA_{18.5}$)-*b*-P($OEGMA_{85}$-*co*-$GMA(N_3)_{15}$)]$_{11}$.

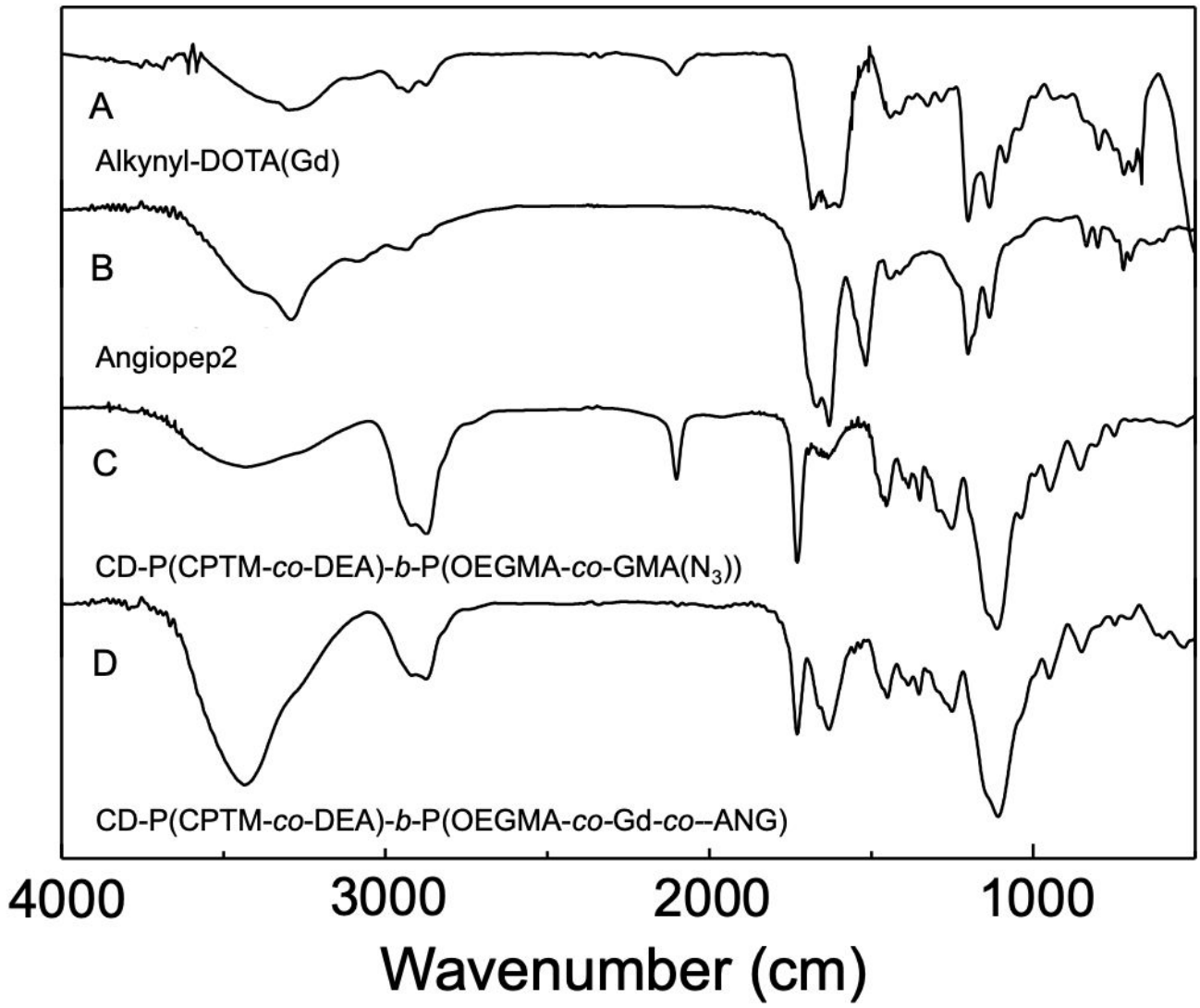


Figure S4. FT-IR spectra. (A) alkynyl-DOTA(Gd). (B) Angiopep-2. (C) CD-P(CPTM-*co*-DEA)-*b*-P(OEGMA-*co*-GMA(N_3)). (D) CD-P(CPTM-*co*-DEA)-*b*-P(OEGMA-*co*-Gd-*co*-ANG).

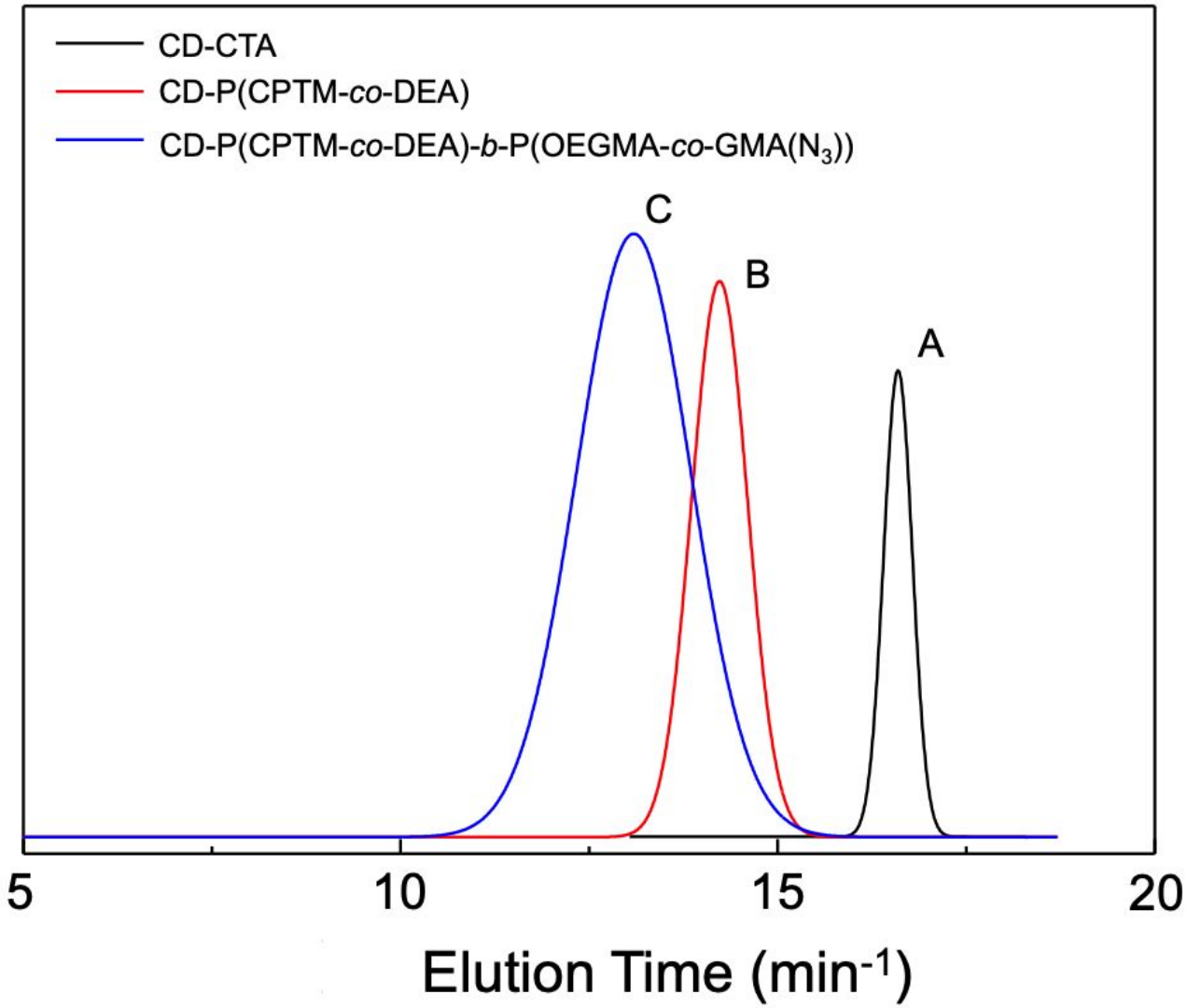


Figure S5. DMF GPC traces. (A) CD-CTA. (B) CD-P(CPTM-*co*-DEA). (C) CD-P(CPTM-*co*-DEA)-*b*-P(OEGMA-*co*-GMA(N_3)).

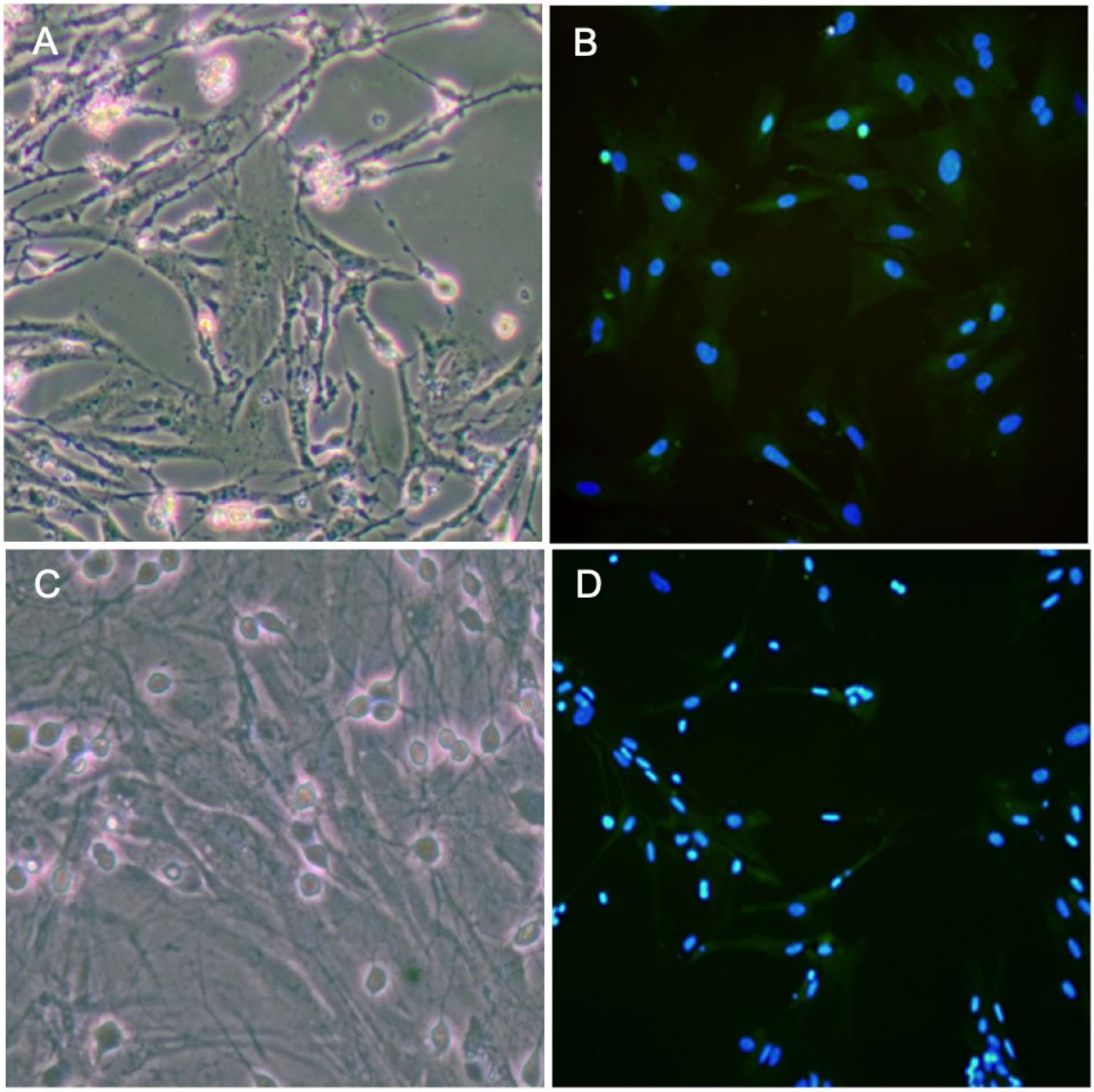


Figure S6. Representative inverted fluorescence microscope imaging of BCECs or Astrocyte. (A) BCECs incubated for 7 days. (B) Fluorescent identification of Factor VIII-related antigen on BCECs. (C) Astrocyte incubated for 7 days. (D) Fluorescent identification of glial fibrillary acidic protein (GFAP) on astrocyte (magnification, ×200)

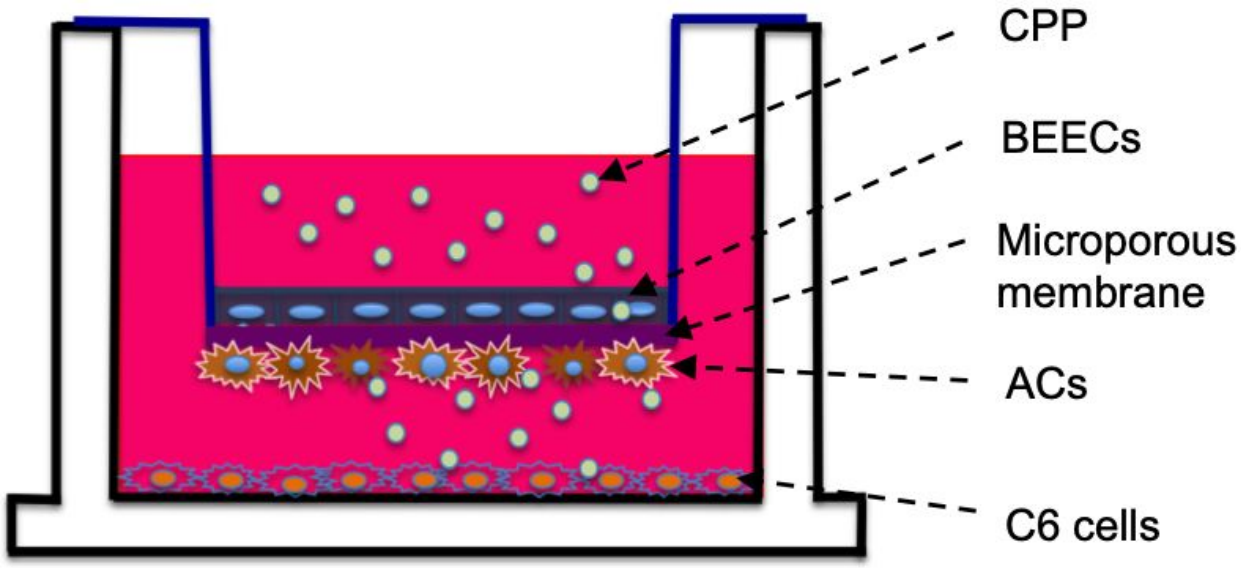


Figure S7. Schematic illustration of BBB transwell model.

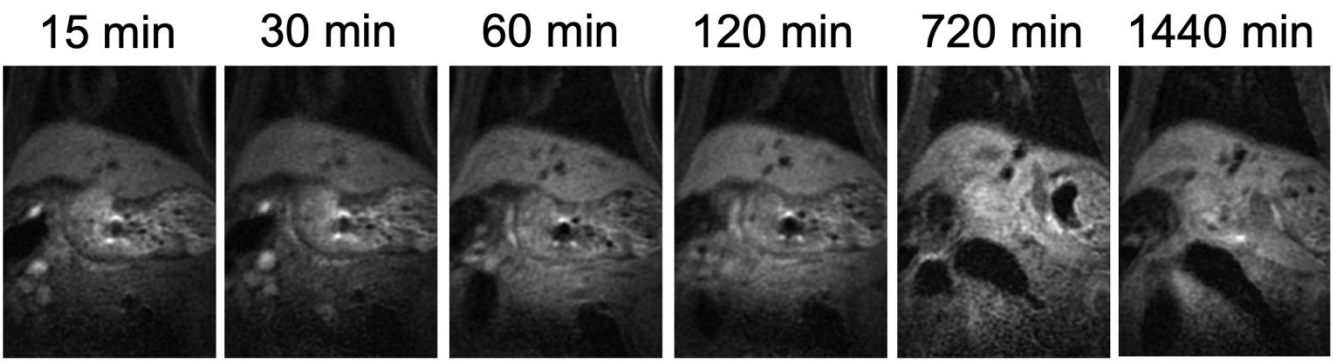


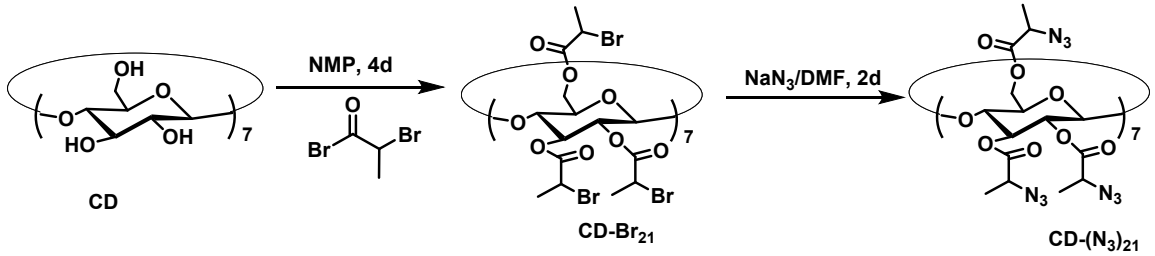
Figure S8. MR images recorded for liver of glioma-bearing rats at 15 min, 30 min, 60 min, 120 min, 720 min and 1440 min after intravenous injection of the aqueous solution of CPP-2.

Table S1. Detailed structural parameter of precursors and amphiphilic multiarm star block copolymers used in this study

Samples	Mn (g/mol)[a]	Mw/Mn[a]	Mn (g/mol)[b]
CD-CTA	5760	1.18	6659
CD-P(DEA-*co*-CPTM)	159900	1.26	175400
CD-P(DEA-*co*-CPTM)-*b*-P(OEGMA-*co*-Gd-*co*-ANG)	511000	1.35	673400

[a]Molecular weights and molecular weight distributions, M_W/M_n, were evaluated by GPC/MALS.

[b]Calculated from ^{1}HNMR results.



Scheme 1S. Synthetic routes employed for the preparation of CD-$(N_3)_{21}$.

REFERENCES

(1) Gaillard, P. J.; Voorwinden, L. H.; Nielsen, J. L.; Ivanov, A.; Atsumi, R.; Engman, H.; Ringbom, C.; de Boer, A. G.; Breimer, D. D. Establishment and Functional Characterization of an In Vitro Model of the Blood-Brain Barrier, Comprising a Co-culture of Brain Capillary Endothelial Cells and Astrocytes. *Eur. J. Pharm. Sci.* **2001**, 12, 215-222.