NANO MICRO small

Supporting Information

for *Small,* DOI: 10.1002/smll.202107126

Brain Targeting, Antioxidant Polymeric Nanoparticles for Stroke Drug Delivery and Therapy

*Haoan Wu, Bin Peng, Farrah S. Mohammed, Xingchun Gao, Zhenpeng Qin, Kevin N. Sheth, Jiangbing Zhou, and Zhaozhong Jiang**

Supporting Information

Brain targeting, antioxidant polymeric nanoparticles for stroke drug delivery and therapy

Haoan Wu, Bin Peng, Farrah S. Mohammed, Xingchun Gao, Zhenpeng Qin, Kevin N. Sheth, Jiangbing Zhou, Zhaozhong Jiang**

Keywords: blood-brain barrier, stroke, shrinkable nanoparticles, antioxidant, anti-edema

***Materials*:** Methoxy poly (ethylene glycol) maleimide [mPEG-MAL, molecular weight (MW): 2000] and maleimide poly (ethylene glycol) maleimide (MAL-PEG-MAL, MW: 2000) were purchased from Jenkem Technology. Thrombin-cleavable peptide (NH_2-norleucine-TPRSFL-C-SH) was purchased from AnaSpec. AMD3100 tetrahydrochloride was purchased from Santa Cruz Biotechnology. 3,3'-thiodipropionic acid (TDA), 2,2'-thiodiethanol (TDE), MeO-PEG2K-OH were purchased from Sigma-Aldrich. All the chemicals were used as obtained without any further purification.

***In vitro drug release*:** In vitro release study was carried out in a dialysis bag (molecular weight cutoff, 3 kDa) against 30mL PBS with or without 100 nM thrombin under gentle shaking at 37°C. At selected time intervals from 0.5 to 48 h, 500 μL buffer was withdrawn and replaced with the same volume of fresh medium. In the collected fractions, the released glyburide content in the PBS was detected by a high performance liquid chromatography instrument (SIL-10A, Shimadzu) with an Ascentis C18 separation column. The analysis was investigated at 254 nm according to the calibration curve of glyburide through a UV detector (Shimadzu RF-10A, Kyoto, Japan) with mobile phase of acetonitrile/ $NH_4H_2PO_4$ buffer (55:45 v/v) at a flow rate of 0.5 mL/min.

***Fluorescent Imaging*:** Immediately after MCAO surgery, mice were randomly assigned into experimental groups (n = 3). IR780-loaded PEG-PTT-T-PEG NPs were administered intravenously through the tail vein. Each mouse received the same amount of dye according to the fluorescence intensity. 24h post-operation, mice were sacrificed to isolate the brain and other organs for IVIS imaging (Xenogen) with excitation wavelength of 745 nm and emission wavelength of 820. Then, the brains were sliced coronally, the IR780 signal were also captured with an IVIS imaging system. Fluorescence intensity in each brain and slice was quantified using Living Image 3.0 (Xenogen).

***Immunofluorescence staining*:** Mice were received different treatments after MACO surgery as described above. After 72 h, the brains were harvested and fixed for 4 h in 4% paraformaldehyde. After that, the brains were transferred into 15% sucrose in 1×PBS at 4°C until tissue sinks, then transferred into 30% sucrose in 1×PBS at 4°C until tissue sinks. Brain tissue were cut into 30 μm sections with a Leica CM1950 Cryostat, and stained with primary antibodies, anti-ZO-1 mouse polyclonal antibody (Invitrogen) and anti-claudin 5 mouse polyclonal (Abcam) separately, and then secondary antibodies, A488-conjugated duck anti-mouse IgG (ThermoFisher Scientific). Images were captured using a laser scanning confocal microscope (Leica TCS SP8).

***TTC Staining*:** The brain infarction area was evaluated by TTC (2, 3, 5-triphenyltetrazolium chloride) staining. The brains were isolated and frozen on dry ice for 10 min. Then, the brains were cut into 6 coronal slices with the thick of 2 mm and incubated at 37 °C with 2% TTC in 1× PBS solution. After 15min incubation, the slices were transferred to the fixing solution with 4% paraformaldehyde. The infarct area in each slice was quantified using ImageJ.

Supplementary Figures

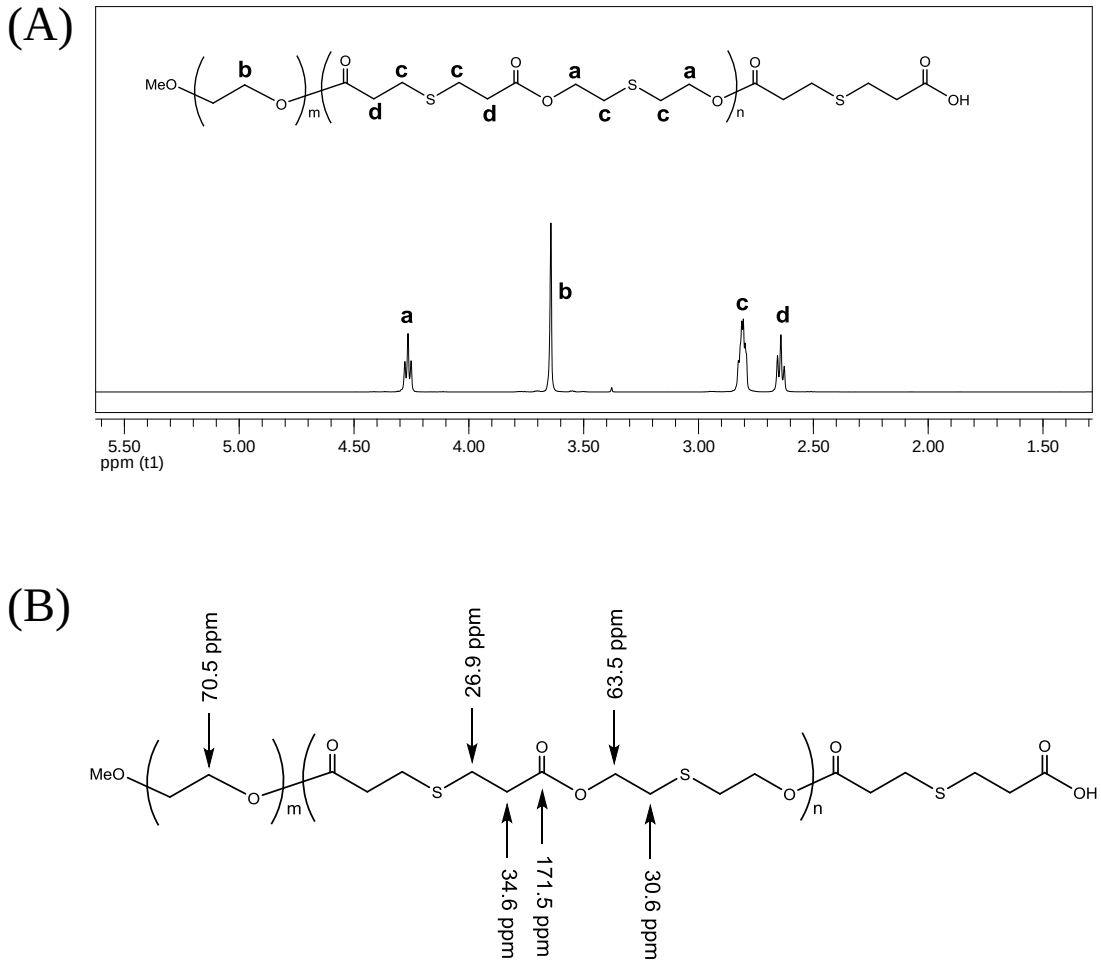


Figure S1. Structural assignments for (A) the proton and (B) carbon-13 NMR resonance absorptions of PEG-PTT block copolymer. Chloroform-*d* was used as the solvent.

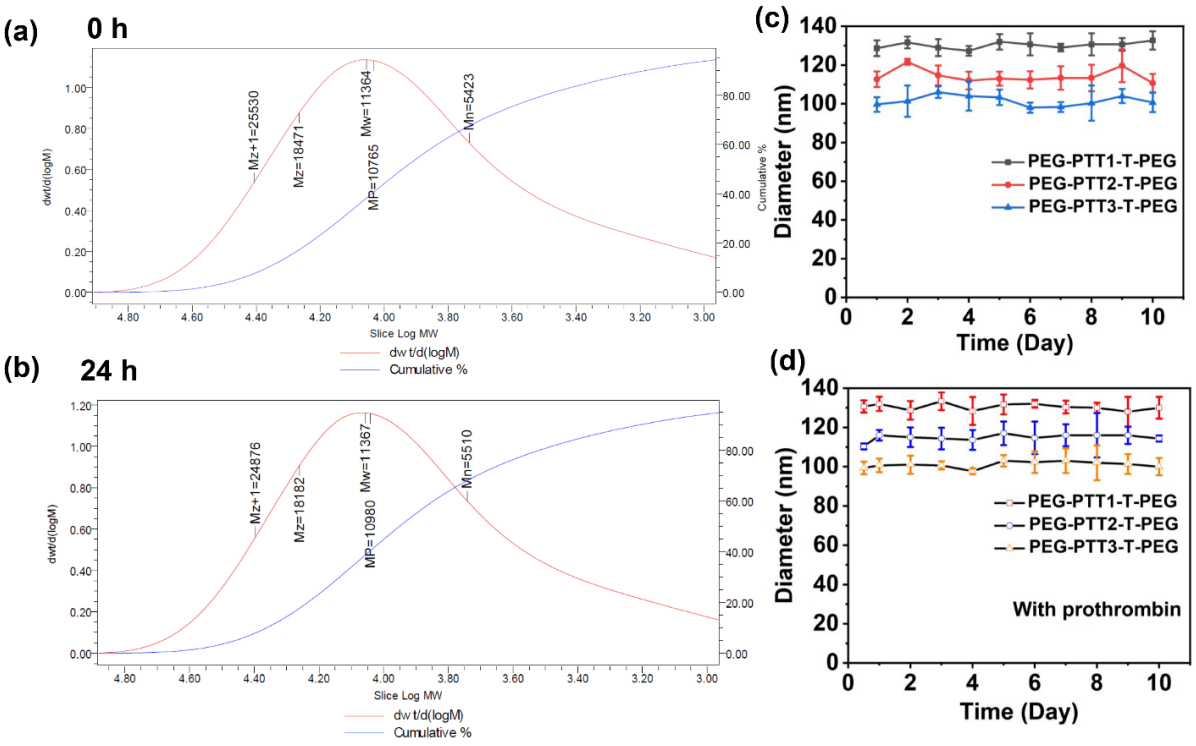
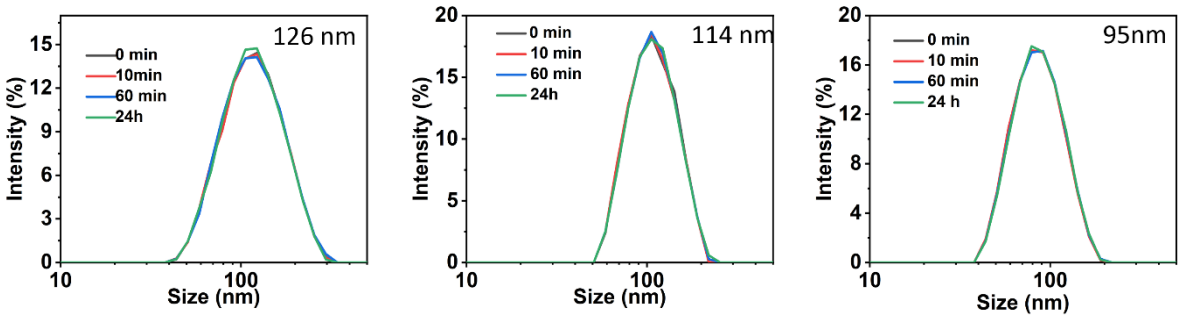


Figure S2. Characterization of the stability of PEG-PTT-T-PEG polymer and PEG-PTT-T-PEG NPs. (a, b) GPC analysis of the molecular weight of PEG-PTT3-T-PEG before and after 24 hours incubation in PBS using GPC. (c, d) Stability of PEG-PTT-T-PEG polymeric NPs without thrombin and with prothrombin in ten days.



PEG-PTT1-PEG PEG-PTT2-PEG PEG-PTT3-PEG

Figure S3. DLS changes of PEG-PTT-PEG NPs with and without thrombin.

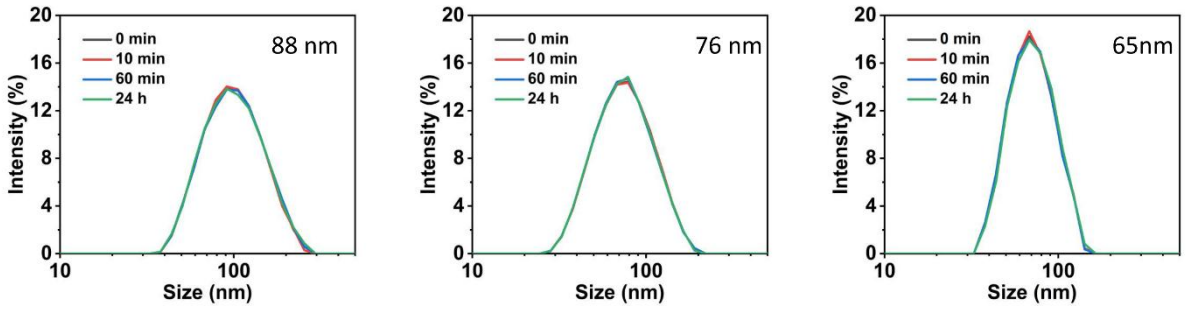


Figure S4. DLS changes of PEG-PTT NPs with and without thrombin.

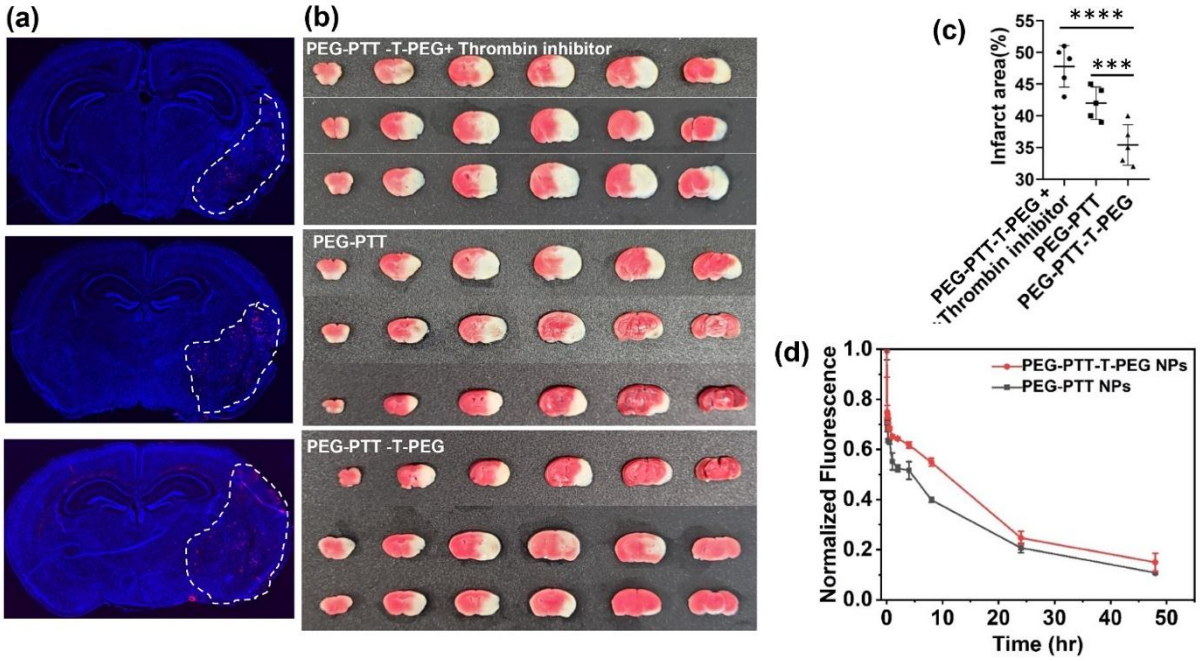


Figure S5. Characterization of PEG-PTT3-T-PEG NPs and PEG-PTT3 NPs for brain penetration and blood circulation. (a) Representative fluorescence images of CY5.5-loaded NPs (red in dotted line circle) in the stroke regions. (b) Representative images and (c) quantification of cerebral infarction in MCAO mice receiving treatment of the indicated NPs. (d) Blood circulation of PEG-PTT3-T-PEG NPs and PEG-PTT3 NPs in stroke mice

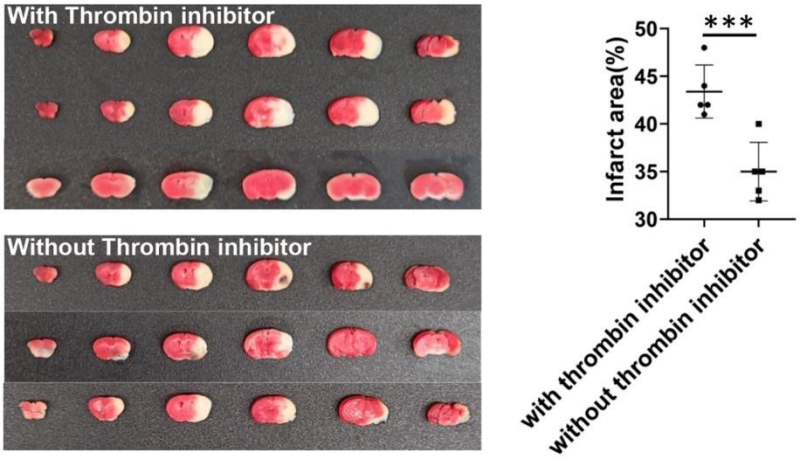


Figure S6. Representative images and quantification of cerebral infarction in MCAO mice receiving treatment of the indicated NPs with and without pre-treatment with heparin.

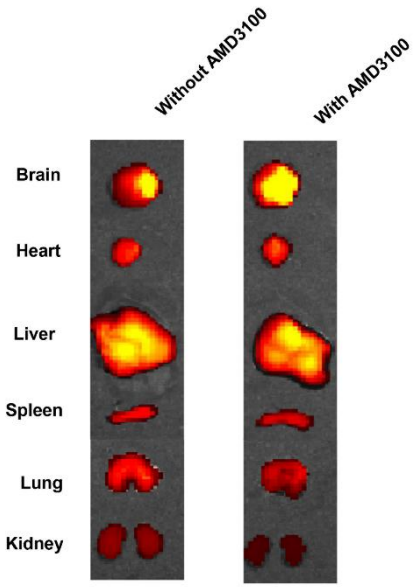


Figure S7. Ex vivo IVIS imaging showing the organs of stroke mice after intravenous injection of IR780-loaded PEG-PTT-T-PEG micelles with/without AMD3100 targeting for 24 h.

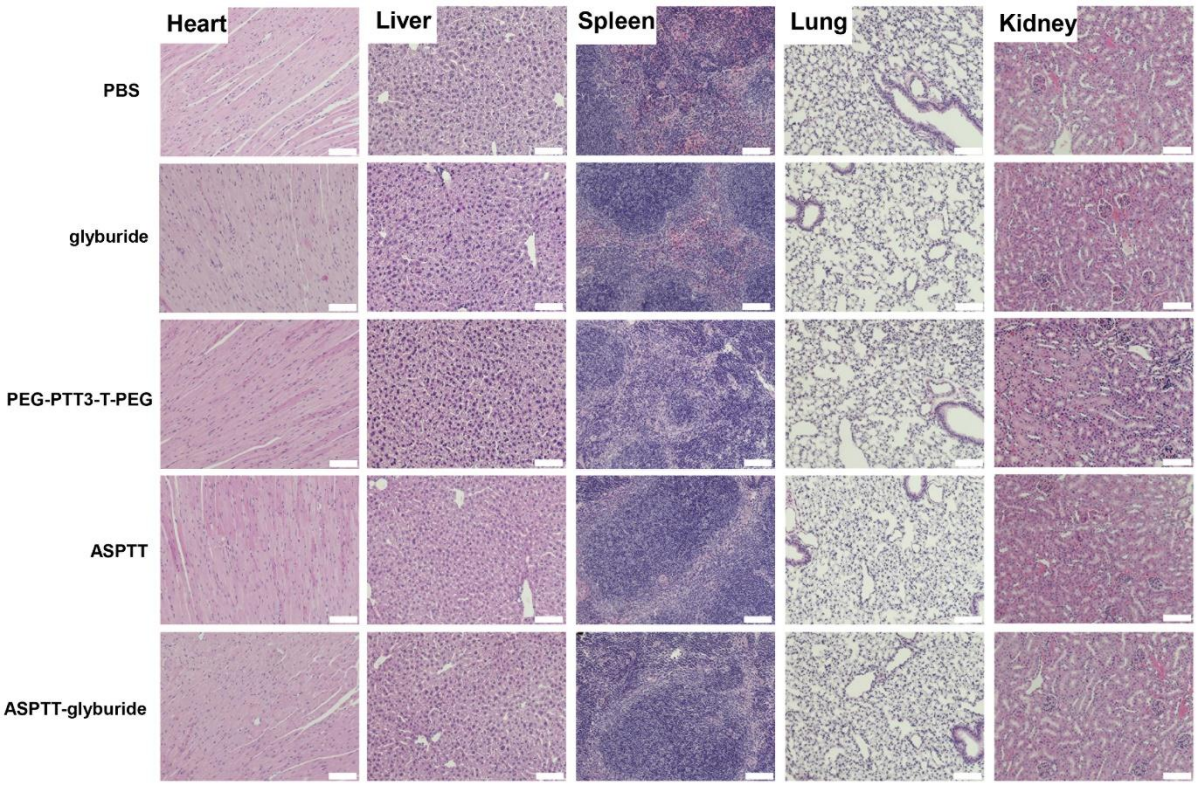


Figure S8. HE staining of main organs of different groups at day 4 post stroke, scare bar 100μm.

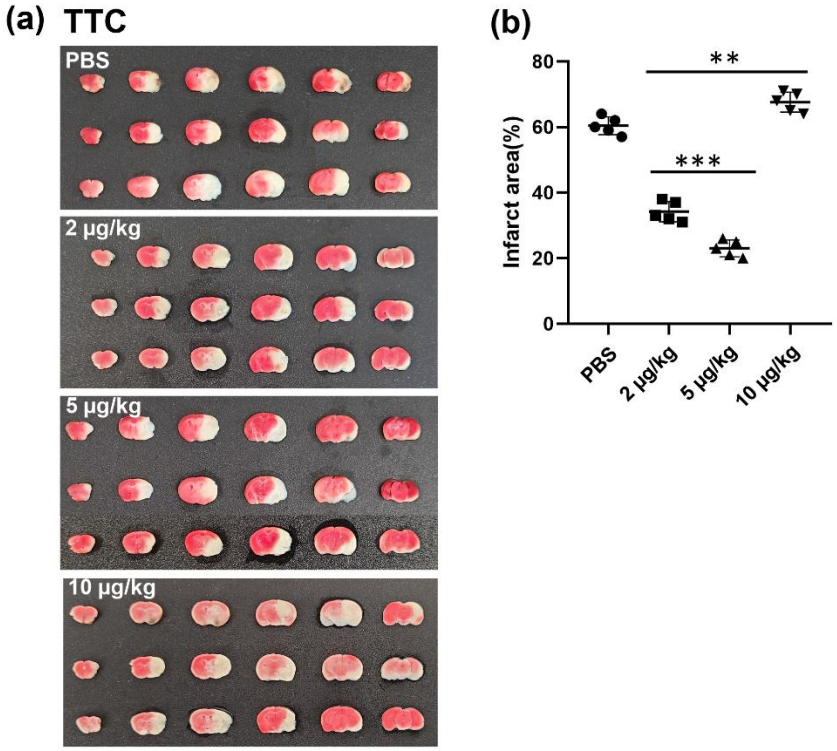


Figure S9. Representative images and quantification of brain infarction in MCAO mice received treatment of glyburide-loaded PEG-PTT3-T-PEG NPs at the indicated doses.

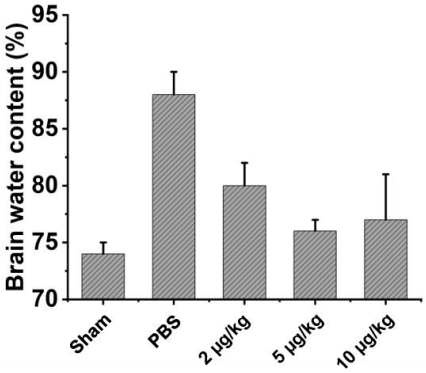


Figure S10. Tissue water content in MCAO mice received treatment of glyburide-loaded ASPTT NPs at the indicated dose.

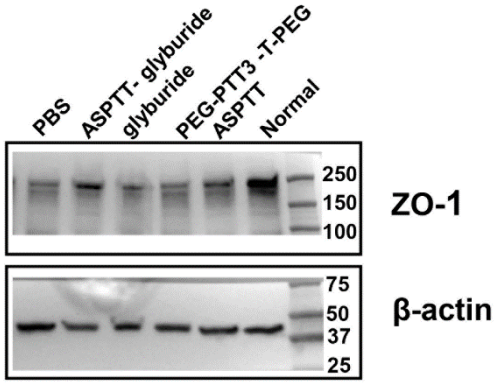


Figure S11. Western Blot analysis of ZO-1 protein of ischemic brain tissues isolated from mice received treatment with the indicated NPs.

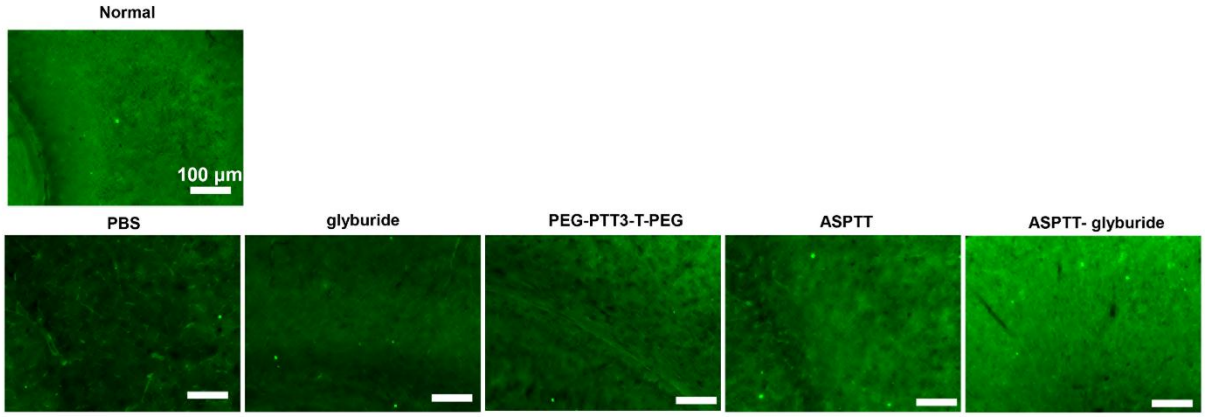


Figure S12. Representative images of ZO-1 immunofluorescence of ischemic brain tissues isolated from mice received treatment with the indicated NPs. Scale bar: 100 μm.

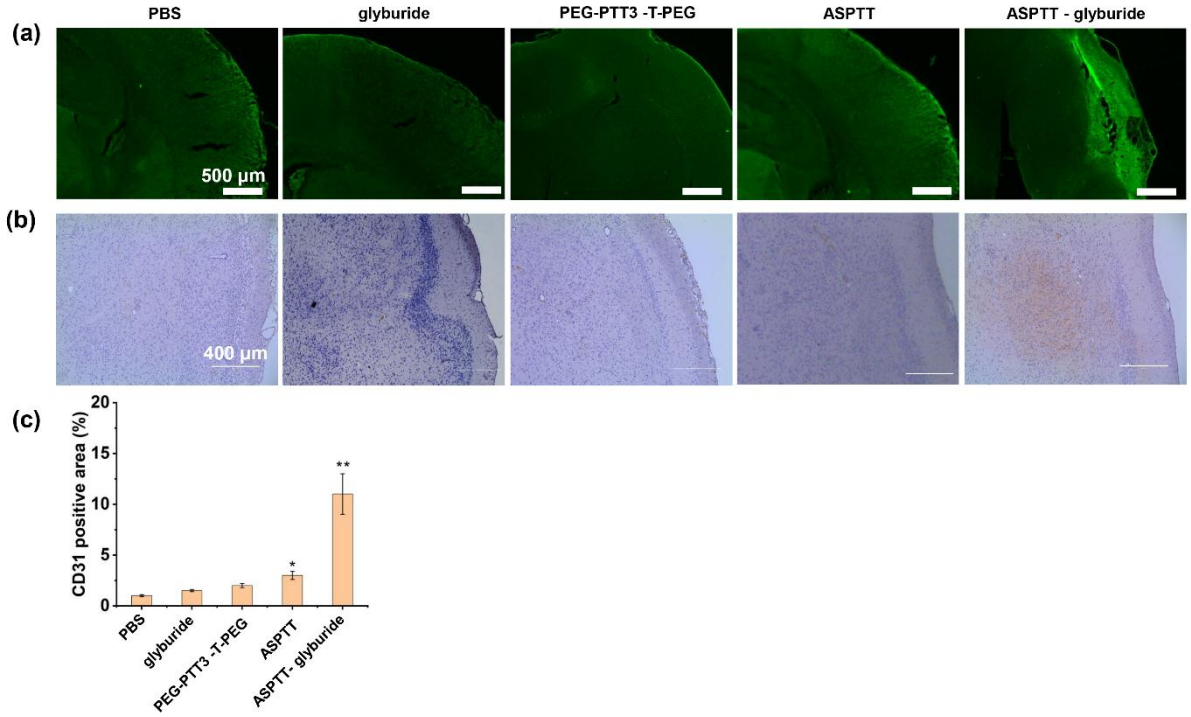


Figure S13. (a, b) CD31 immunofluorescence and immunohistochemistry assay of ischemic brain tissues isolated from mice received treatment with the indicated NPs at 15 days after stroke. (c) quantification of CD31 positive area at all groups.

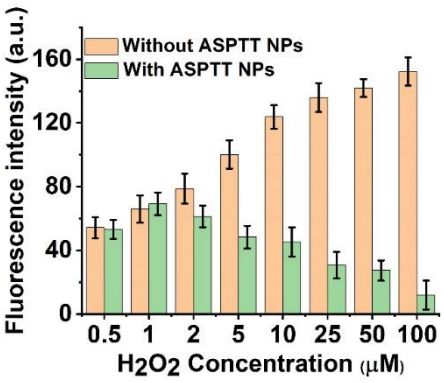


Figure S14. Characterization of the ROS response concentration for ASPTT NPs. ASPTT NPs (0.1mg/mL) were incubated with H_2O_2 at the indicated concentrations with 50 μM freshly prepared 2',7'-dichlorofluorescin diacetate (DCFDA) at 37°C. Thirty minutes later, the fluorescence signal of oxidation product 2', 7' –dichlorofluorescein (DCF) was detected at 535 nm by the multi-mode microplate reader (Spectramax M5, Molecular devices, USA) using a 488 nm excitation laser.

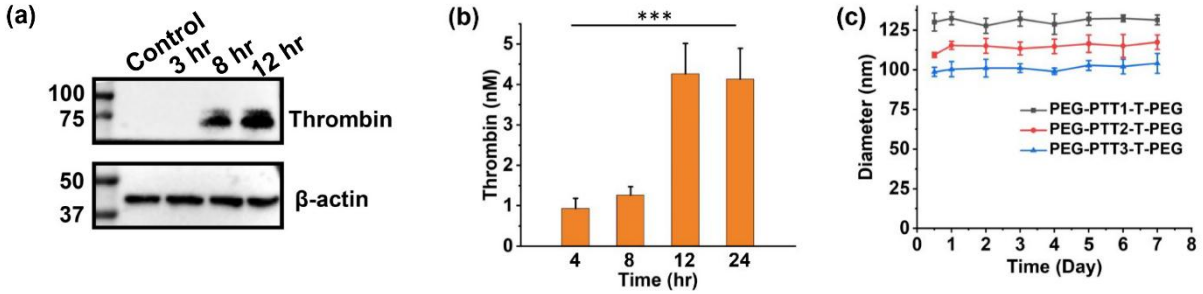


Figure S15. Characterization of thrombin expression in the brain and stability of PEG-PTT-T-PEG NPs. (a) Western Blot analyses of the expression of thrombin in the normal brain (control) and the ischemic brain at the indicated time points after stroke. (b) Quantification of the concentration of thrombin in the blood after stroke at the indicated time points using a SensoLyte FAC Thrombin Activity Assay Kit (AnaSpec, US). (c) Change of the size of PEG-PTT-T-PEG NPs with time after incubation in PBS buffer with 4.2 nM thrombin.

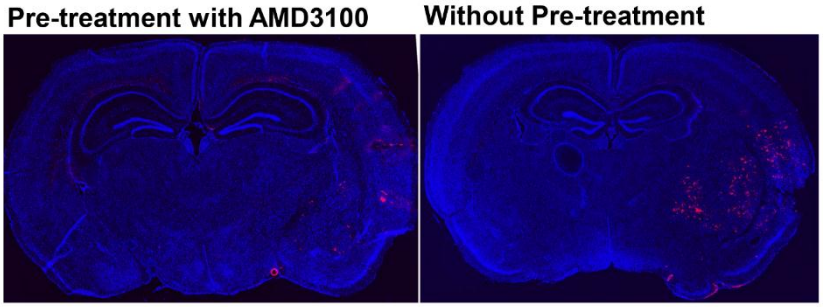


Figure S16. Pre-treatment of free AMD3100 inhibited brain penetration of ASPTT NPs.