Electronic Supplementary Material (ESI) for RSC Advances.

Construction of biomimetic long-circulation delivery platform encapsulated by zwitterionic polymers for enhanced penetration of blood-brain barrier

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Experimental details

Characterizations

Dynamic light scattering (DLS) was used to measure the size, size distribution and zeta potential of particles in aqueous solution carried out with a Malvern Zetasizer Nano S instrument (Malvern Instruments Ltd) equipped with a 4.0 mW He-Ne laser operating at λ = 633 nm. All samples (0.5 $mg \cdot mL^{-1}$ of BSA) were measured at a scattering angle of 90° in 20 mM phosphate buffer (PB) at room temperature (25 °C).

Transmission electron microscopy (TEM) studies were performed with a Tecnai G2spirit Biotwin instrument at a voltage of 120 kV (FEI, USA). Samples were prepared by drop-casting solutions onto carbon-coated copper grids, and then air-drying at room temperature. And then the samples were negative stained by 1% phosphotungstic acid (pH = 7.0) before measurement.

^{31}P nuclear magnetic resonance spectroscopy (NMR) spectra were recorded using a Varian Mercury Plus 400 MHz spectrometer to confirm the attachment of PMPC onto the nanoparticles. Samples were prepared by dissolving in 50% H_2O/50% D_2O at 0.25 $mg \cdot mL^{-1}$ and pH was adjusted to 7.4.

Flow cytometry (FCM) was assessed using flow cytometer (Accuri C6, Becton, Dickinson and Company, Shanghai, China). TAT was labeled with FITC in pH = 8.5, 20 mM PB. Non-TAT modified (used as control to draw the gate) and FITC-labeled TAT-modified nanoparticles were recorded in flow cytometer to affirm the attachment of TAT onto the system.

SDS-PAGE gel electrophoresis was carried out to determine the molecular weight of the nanoparticles. Native BSA, nBSA, TAT-modified three systems were used as samples and Protein Ladder (purchased from Beyotime, Shanghai, China) was used as the maker. Precast gel was purchased from Willget

Biotech Co., Ltd, Shanghai, China. After stained and decolourization for three days, the result was shown in BIO-RAD Gel Doc XR+ (BIO-RAD, USA).

Supplementary Figures and Table

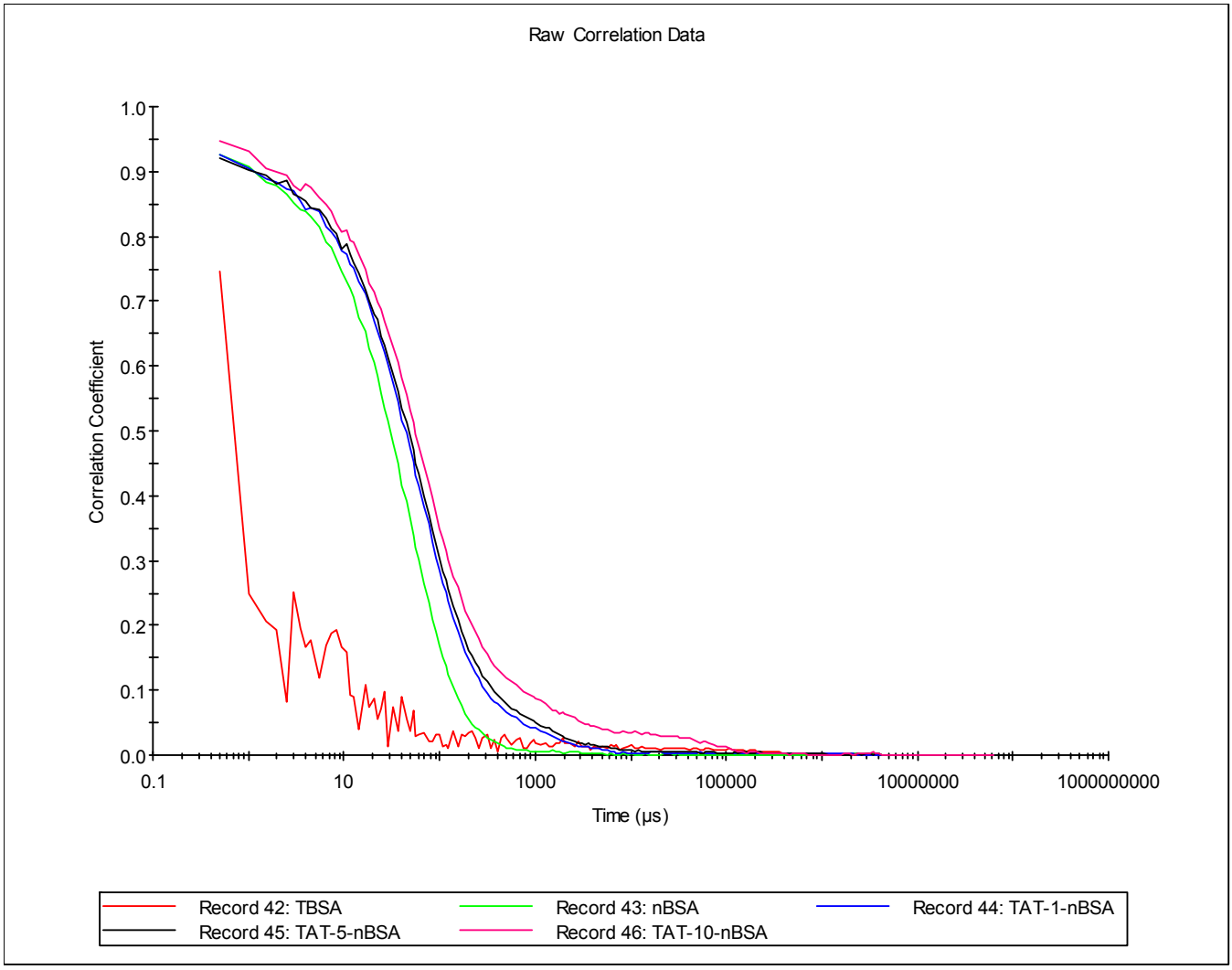

Figure S1. Correlation curves of DLS results for all nanoparticles prepared.

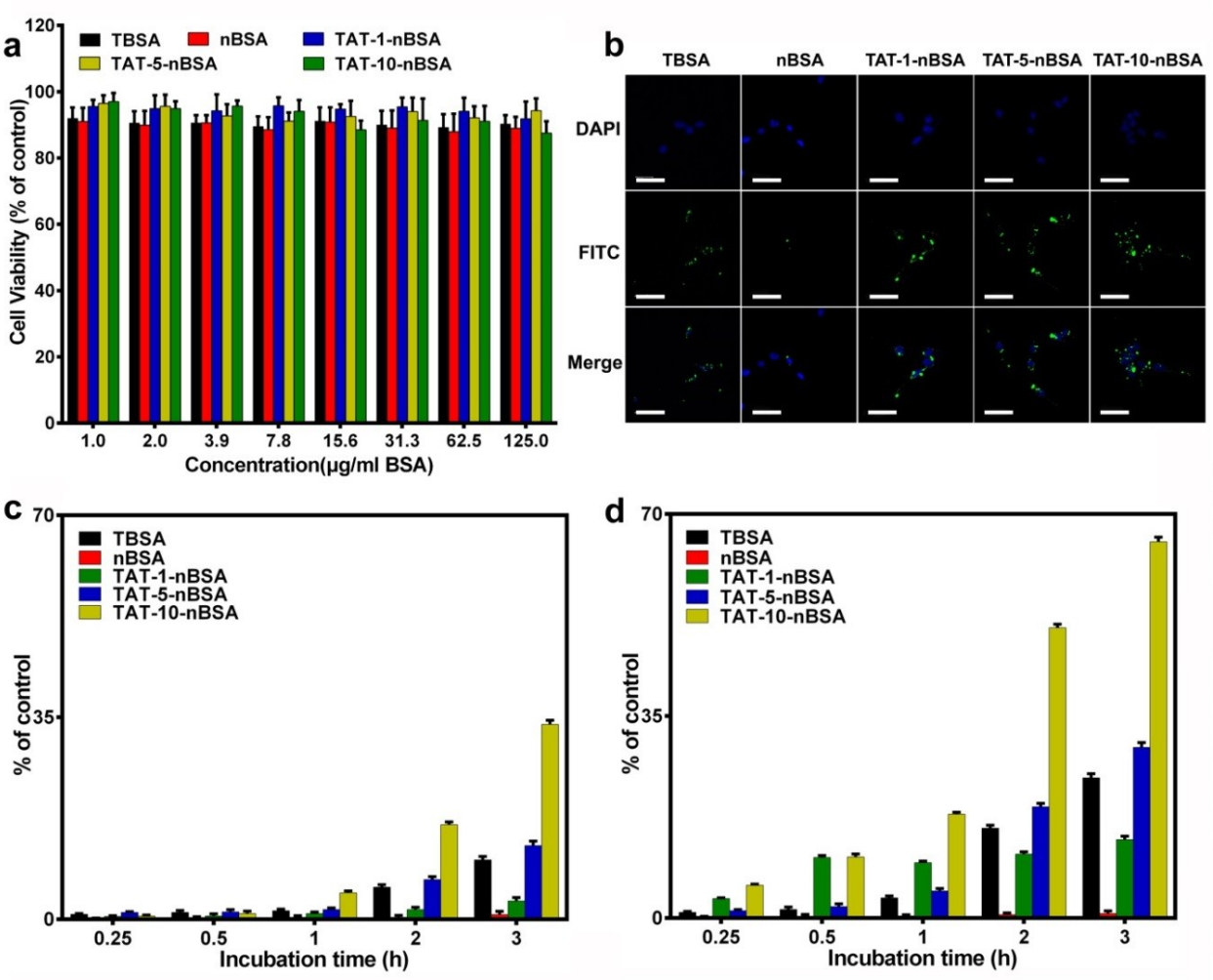

Figure S2. *In vitro* cell study. (a) Cell viability of L929 cells treated with TBSA, nBSA and TAT-nBSA for 24 h, respectively. (b) Confocal images of C6 (mouse glioma cells) cells treated for 3 h. Blue represented FL of DAPI, the nucleus, and green represented FL of nanoparticles, scale bar is 20 μm. Flow cytometric analysis (n = 10,000 cells) in (c) L929 (mouse fibroblast cells) and (d) C6 (mouse glioma cells) treated for 0.25, 0.5, 1, 2 and 3 h.

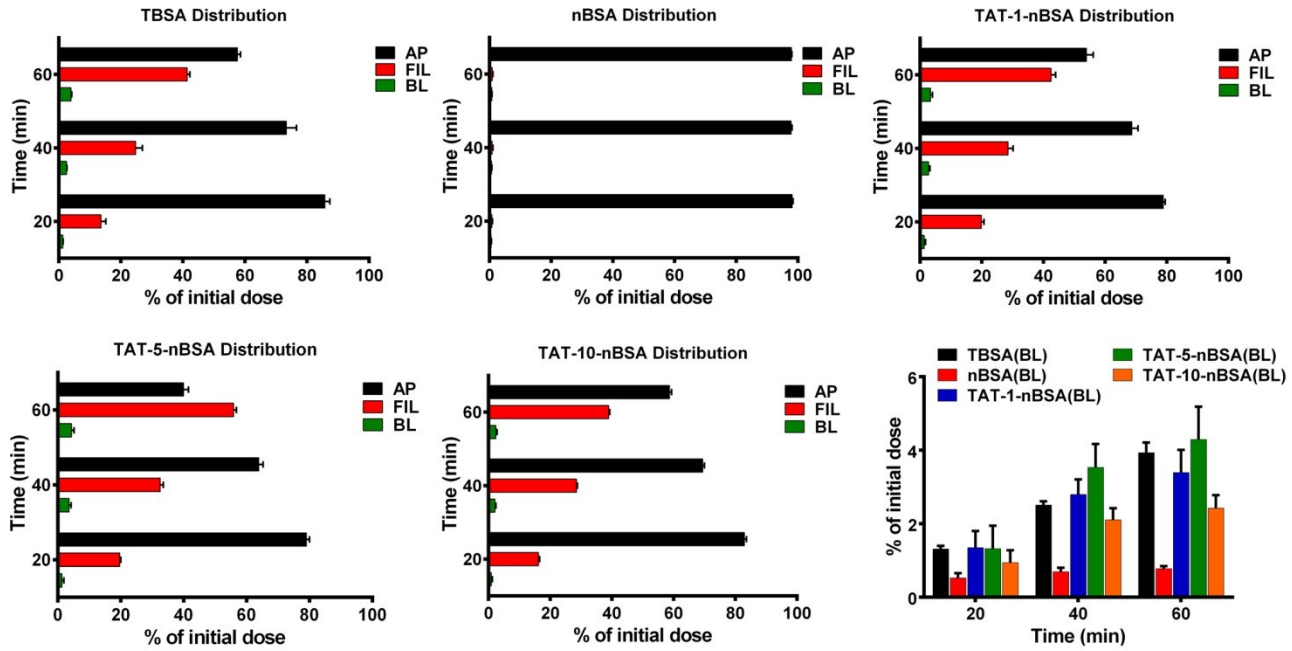

Figure S3. Distribution of the nanoparticles in the *in vitro* BBB model at time points of 20 min, 40 min and 60 min.

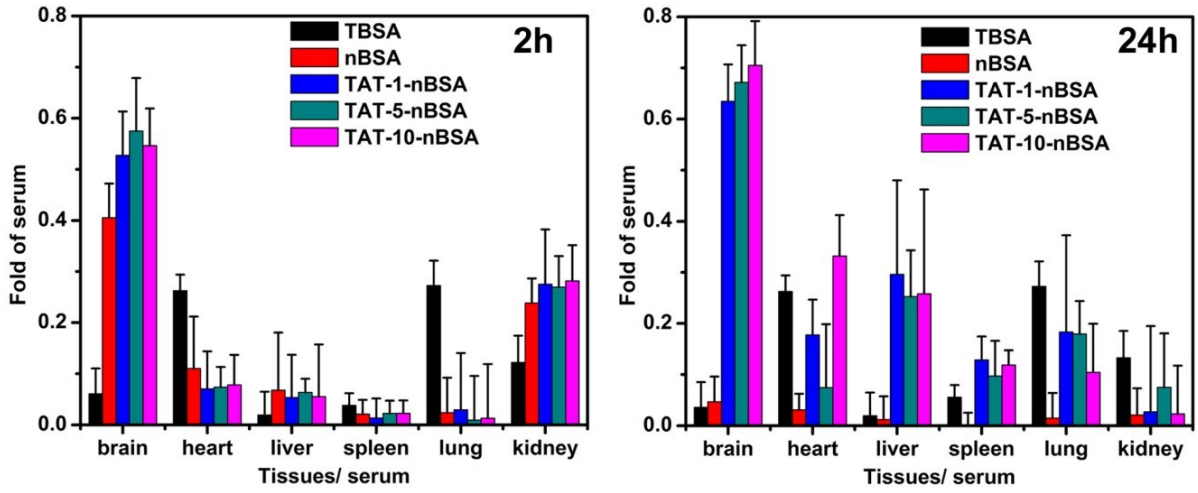

Figure S4. 2 h and 24 h post-injection biodistribution in brain, heart, liver, spleen, lung and kidney (fold of serum).

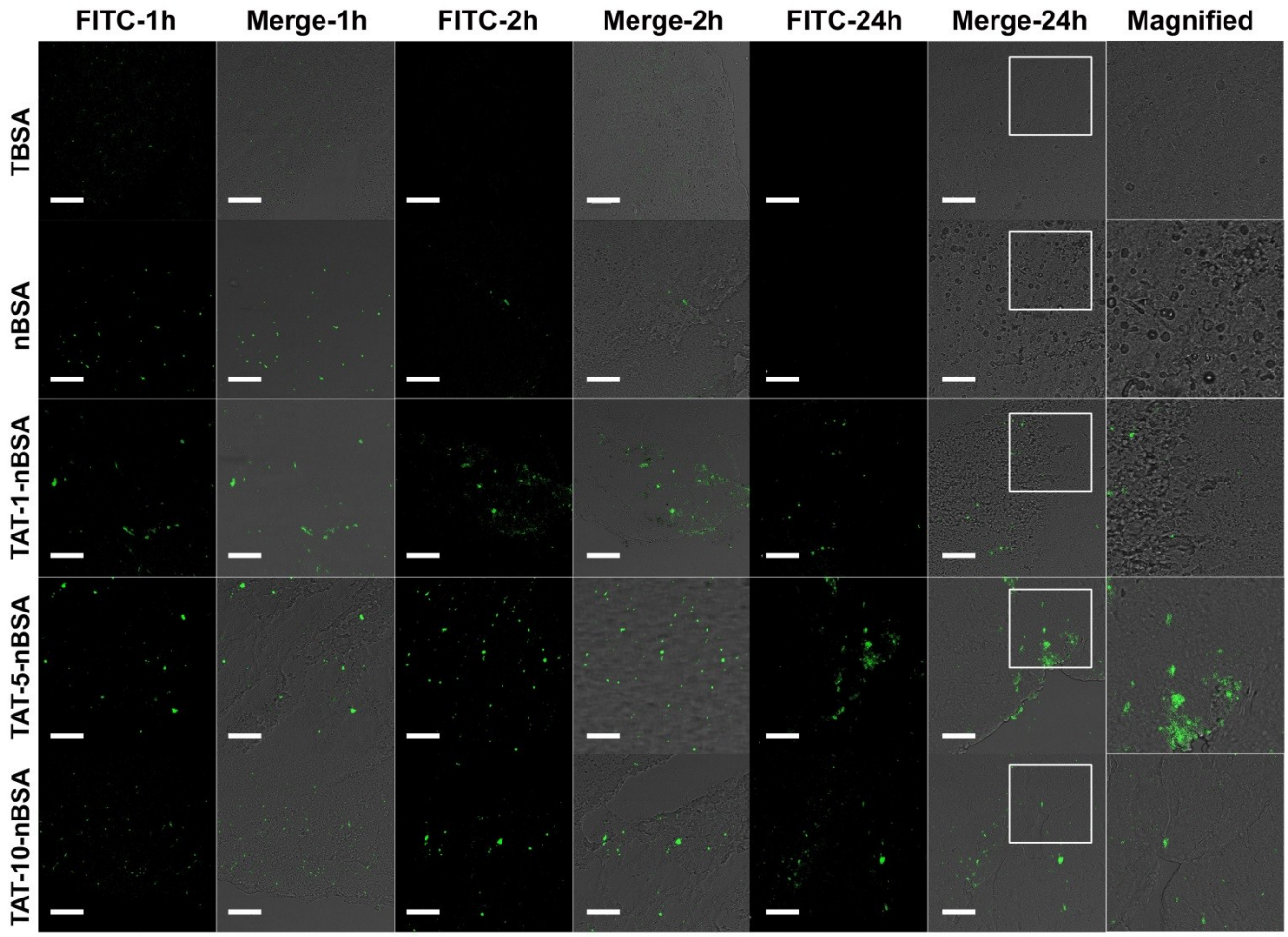

Figure S5. *Ex vivo* laser scanning confocal microscopy imaging of mouse brain sections (10 μm) from animals injected with FITC-labeled TBSA, nBSA and TAT-nBSA 1 h, 2 h and 24 h post-injection of the mice, respectively. Nanoparticles appear as green dots. Scale bar = 20 μm. Last column stands for the magnified version of white box part in "Merge-24h".