pharmaceutics

# Supplementary Materials: Conjugation of Therapeutic PSD-95 Inhibitors to the Cell-Penetrating Peptide Tat Affects Blood-Brain Barrier Adherence, Uptake, and Permeation

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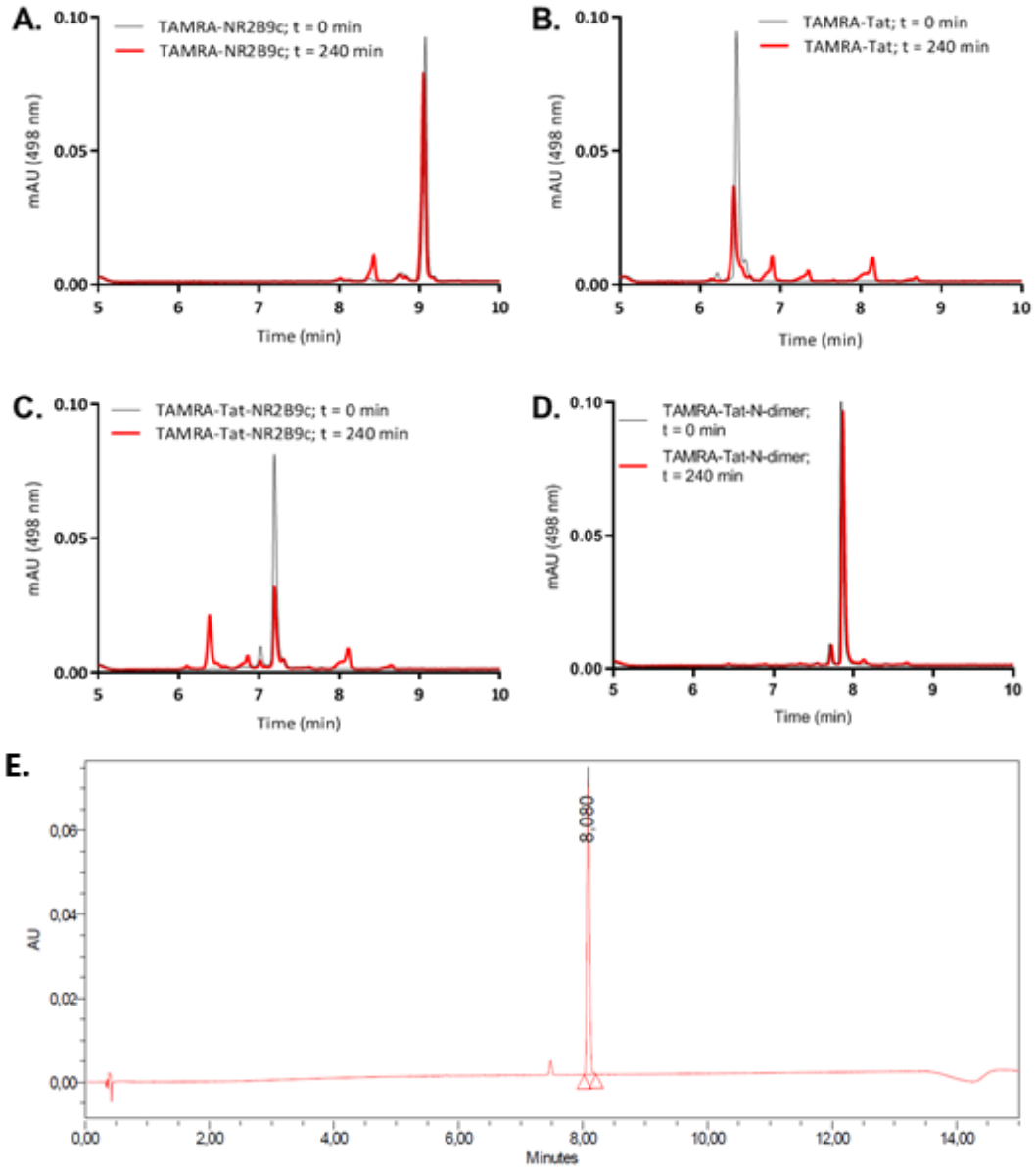


**Figure S1.** (**A–D**) Degradation profiles illustrated as chromatograms obtained at 498 nm (TAMRA detection) from UPLC analysis of TAMRA-NR2B9c, TAMRA-Tat, TAMRA-Tat-NR2B9c, and TAMRA-Tat-*N*-dimer before and after 4 h incubation in 37 °C media supplemented with 10% FBS. (**E**) UPLC chromatogram obtained at 498 nm of TAMRA as single entity.

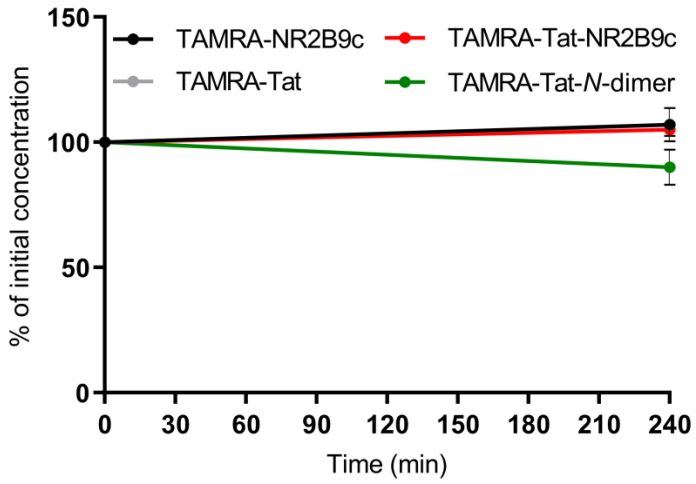


**Figure S2.** Remaining peptide after 4 h incubation in 37 °C PBS. Data are presented ± SD (N = 2).

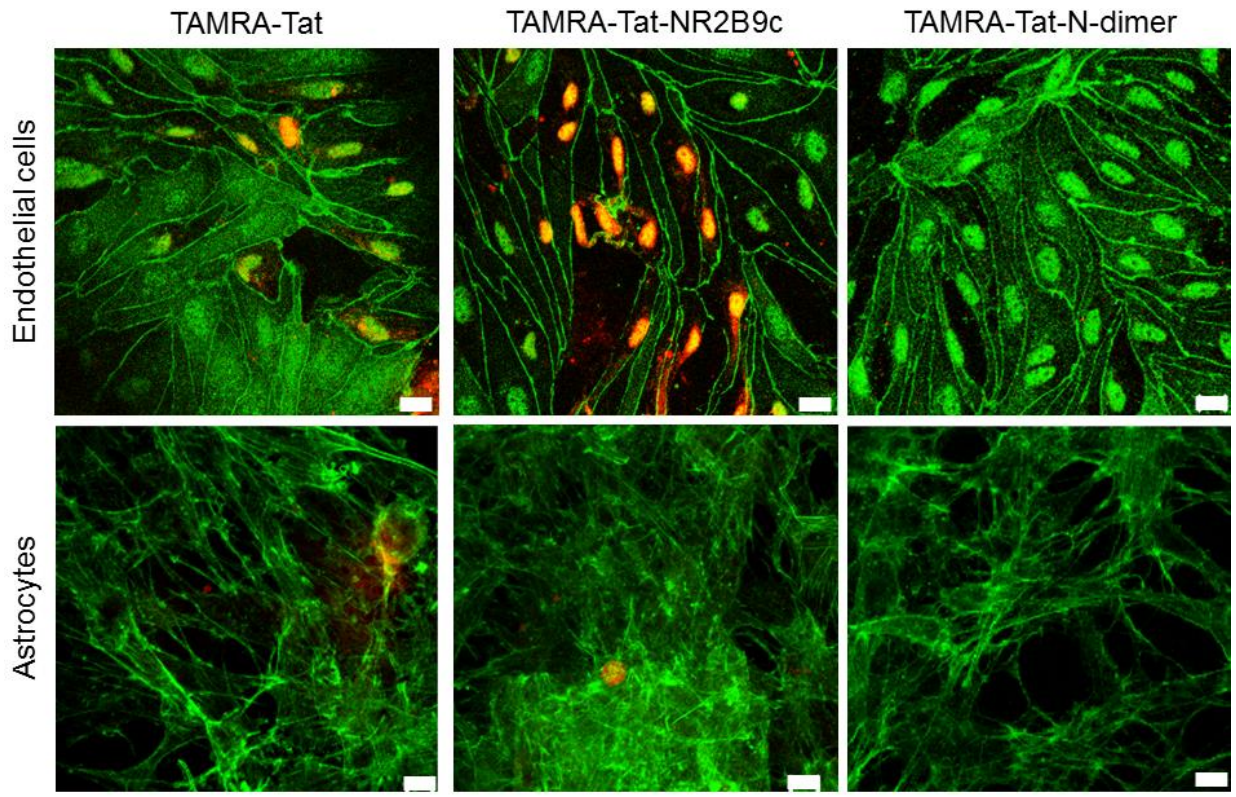


**Figure S3.** 100 μM TAMRA-Tat, TAMRA-Tat-NR2B9c, or TAMRA-Tat-*N*-dimer was applied to the *in vitro* bovine blood-brain barrier model for 15 min. Following cell fixation, peptide uptake into the endothelial cells and the astrocytes was inspected via TAMRA-fluorescence using confocal microscopy with co-staining of ZO-1 and β-actin, respectively. Z-stacks, scale bar: 10 μm.

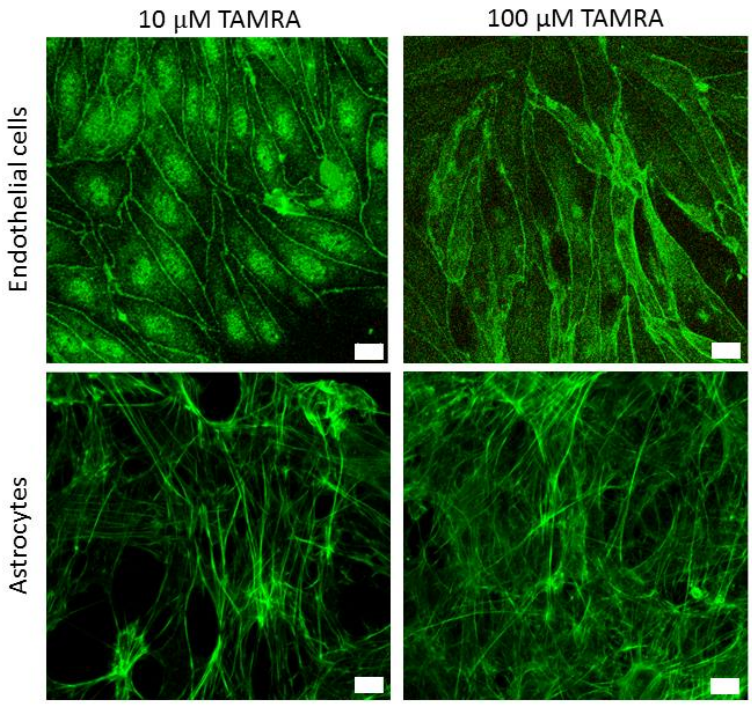


**Figure S4.** 10 µM or 100 µM TAMRA was applied to the *in vitro* bovine blood-brain barrier model for 3 h. Following cell fixation, potential TAMRA uptake into the endothelial cells and the astrocytes was inspected using confocal microscopy with co-staining of ZO-1 and β-actin, respectively. Z-stacks, scale bar: 10 µm.

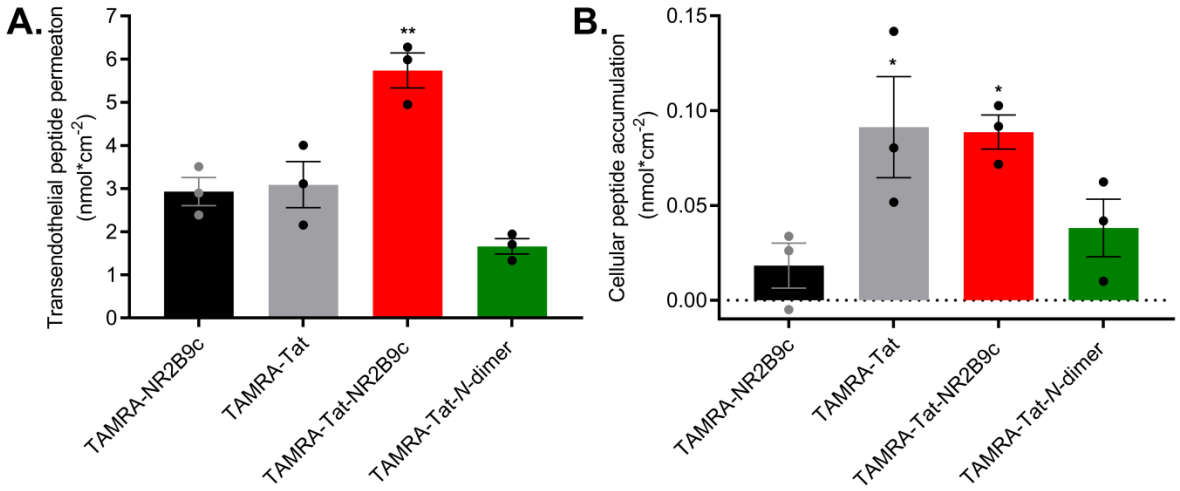


**Figure S5.** 100 µM TAMRA-NR2B9c, TAMRA-Tat, TAMRA-Tat-NR2B9c, or TAMRA-Tat-*N*-dimer was applied to an *in vitro* blood-brain barrier model composed of primary mouse endothelial cells in co-culture with primary rat astrocytes for 3 h. (**A**) Total peptide being transported across the barrier was quantified. (**B**) After the permeation study, the cells were washed with HBSS prior quantification of peptide accumulating in the cell fraction. Data are presented as mean ± SEM (N = 3, *n* = 3) Levels of significance are *: $p < 0.05$ and **: $p < 0.01$ when compared to TAMRA-NR2B9c (one-way ANOVA with Dunnett's multiple comparisons test).

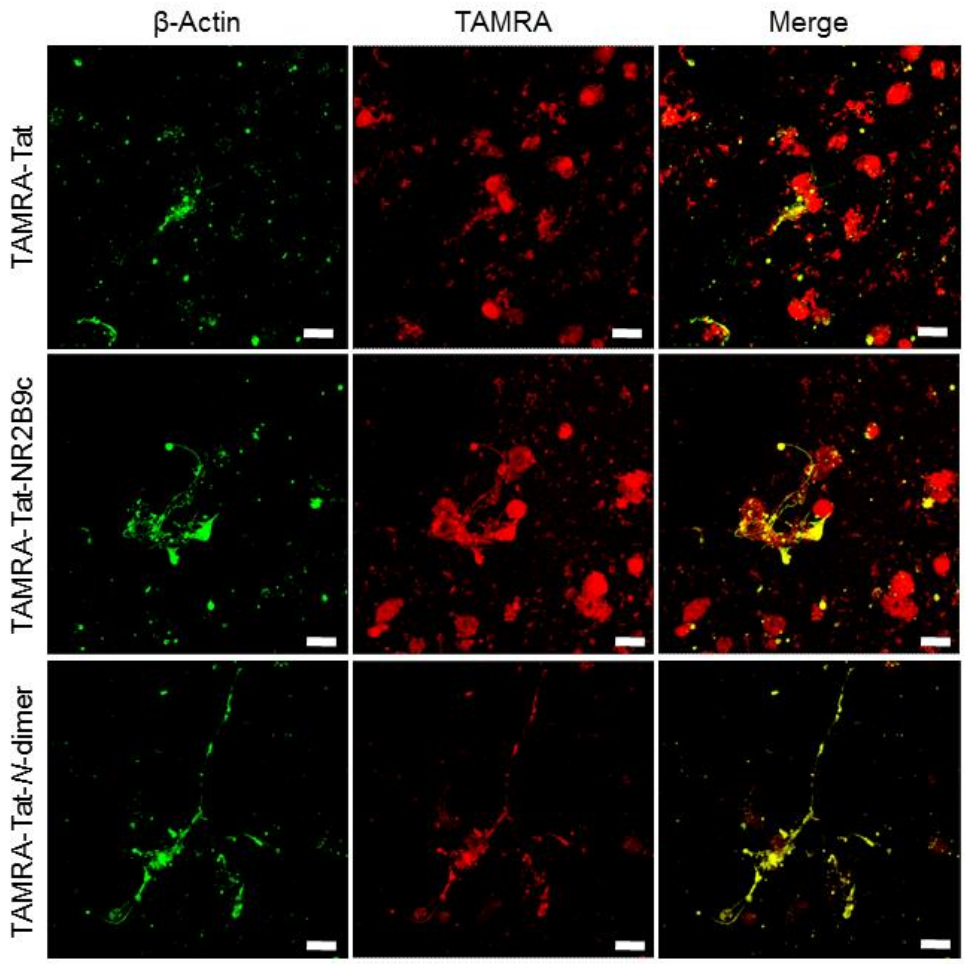


**Figure S6.** Primary mouse cortical neurons were incubated with 1 μM TAMRA-Tat, TAMRA-Tat-NR2B9c, or TAMRA-Tat-*N*-dimer for 1 h at 37 °C. Following cell fixation, peptide uptake was inspected by confocal microscopy via TAMRA fluorescence with co-staining of β-actin. Single xy section, scale bar: 10 μm.

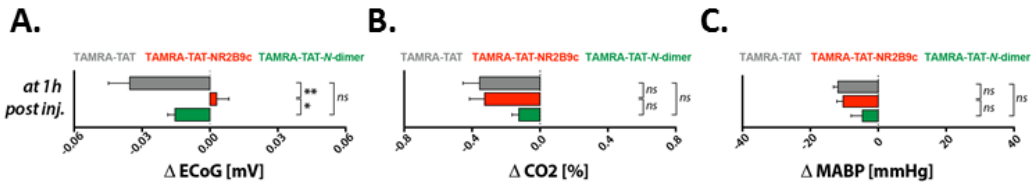


**Figure S7.** Physiological parameters after 1 h peptide circulation during two-photon imaging of peptide blood-brain barrier permeation in live mice: (**A**) Electrocorticographic activity (ECoG), (**B**) exhaled $CO_2$, and (**C**) mean arterial blood pressure (MABP). Data are presented as mean ± SEM (N = 5). Levels of significance are * $p < 0.05$; ** $p < 0.01$ (two-tailed unpaired *t*-test).