Supporting Information

Therapeutic nanosweepers promote β-Amyloid removal from brain for Alzheimer's disease treatment

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1. Materials

All chemicals and solvents were purchased from commercial suppliers and used as received unless specified. The water used in this work was ultrapure water.

N-acryloxysuccinimide (NAS), acrylamide (AAM), N-(3-aminopropyl) methacrylamide (APM), N,N'-methylenebisacrylamide (BIS), ammonium persulfate (APS), and tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich. Fluorescence dyes including Rhodamine B isothiocyanate (RhB), Fluorescein isothiocyanate (FITC), and Thioflavin T (ThT) were purchased from Sigma-Aldrich. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 99%), Traut's Reagent were purchased from Sigma-Aldrich. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP·HCl, 98%), Dimethyl sulfoxide (DMSO, 99.9%) was purchased from J&K (Beijing, China). Rink amide 4-methyl-benzhydrylamine (MBHA) resin (loading density: 0.436 mmol g^{-1}, 100-200 mesh, 1% DVB) and all Fmoc-protected amino acids used in solid-phase peptide synthesis were obtained from Bide Pharmatech Co., Ltd (China). $A\beta_{42}$ peptides (purity ≥ 98%) and TAT peptides (purity ≥ 98%) were purchased from GL Biochem Ltd. (Shanghai, China). Bovine serum albumin (BSA) and mouse peripheral blood neutrophil separation Kit were purchased from Solarbio Life Science Co. Ltd. (Beijing, China). Isoflurane and brain stereotaxic apparatus were purchased from RWD Life Science Co. Ltd. (Shenzhen, China). The cell line bEnd.3, BV2, and N2a were purchased from the American Type Culture Collection (ATCC). The TRITC phalloidin was purchased from YEASEN. Anti-β-Amyloid, 1-42 antibody (catalog no.805509) was purchased from BioLegend (USA). Cell lysis buffer, in situ terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis assay kit, and Alexa Fluor 488–conjugated goat anti-mouse immunoglobulin G (IgG) were purchased from Beyotime. DMEM cell culture medium, fetal bovine serum (FBS), and Penicillin-Streptomycin Solution were purchased from Gibco.

2. Instruments

The Mass Spectrometry (MS) analysis was performed using LCQ-Advantage. Transmission electron microscope (TEM) images were obtained on Talos F200C. The Dynamic Light Scattering (DLS) data was obtained using Malvern Panalytical Nano-ZS. The UV-vis absorption spectra were acquired with NanoDrop ND-8000 (Thermo Scientific, USA) and Shimadzu 1750. The fluorescence spectra were measured on Hitachi F4600. Quartz crystal microbalance (QCM) measurements were conducted using Q-Sence E4 system (Q-Sence, Västra Frölunda, Sweden). The confocal laser scanning microscopy (CLSM) was performed on Carl Zeiss LSM 800 with Airyscan. The fluorescence activated cell sorting (FACS) was measured by BD LSR Fortessa. The fluorescent images were scanned using IVIS Spectrum (PerkinElmer).

3. Peptides Synthesis

Peptides LT, LP, AT, and AP were synthesized via standard Fmoc solid-phase peptide synthesis (SPPS) method. Rink Amide MBHA resin (0.25 mmol) was swelled in anhydrous CH_2Cl_2 for 30 min. Fmoc deprotection was performed in the presence of piperidine in DMF (30 %) solution for 15 min. After washing by DMF, CH_3OH, and CH_2Cl_2, amino acid coupling reactions were carried out by dissolving a mixture of amino acid/HATU/DIEA (4:4:1.1 relative to the resin) in DMF and mixing with resin for 1.5 h. After the coupling reactions of all amino acids being completed, the acryloyl modification of the peptides was performed by sequentially adding 2.5 mL acrylic chloride, 2.5 mL trimethylamine, and 20 mL CH_2Cl_2 for 1.5 h. Peptides were cleaved from the resin using a cocktail of 95 % trifluoroacetic acid, 2.5 % triisopropyl silane, and 2.5 % H_2O for 1.5 h. The peptides solution was concentrated by rotary evaporation and precipitated by cold diethyl ether to obtain the crude peptides.

4. Preparation of nanosweepers (NS)

The preparation of nanosweepers (NS) was achieved via a three-step process. [1] For the imaging purposes, BSA was labeled with Fluorescein isothiocyanate (FITC). Firstly, BSA was dissolved in PBS buffer (10 mM, pH 7.4) and diluted to 5 mg mL^{-1} with PBS buffer (10 mM, pH 7.4). FITC was dissolved in DMSO to obtain 1% (m/v) solution. Subsequently, FITC solution was added into BSA solution with a molar ratio of 4:1 (FITC/BSA). The reaction was kept at 4°C for 12 h and then the solution was dialyzed against PBS buffer (10 mM, pH 7.4) to remove unreacted FITC and obtain FITC labeled BSA (FITC-BSA).

The BSA and FITC-BSA were first conjugated with N-acryloxysuccinimide (NAS) to attach acryloyl groups onto its surface, respectively. The amount of NAS (10% in DMSO, m/v) was at 300:1 (molar ratio, NAS/protein), and the conjugation was kept at 4 ^oC for 2 h. The solution was dialyzed against PBS buffer (10 mM, pH 7.4) to remove unreacted NAS.

Then, the concentration of the Acryloylated BSA (Acr-BSA) was tuned to 1 mg/mL by diluting with PBS buffer (10 mM, pH 7.4). The molar ratio of Acr-BSA/APM/AAm/Acr-LT/BIS/APS was 1:25:2000:25:300:450. The mass ratio of APS/TEMED was 1:2. Polymerization was initiated in situ by the addition of tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) and kept at 4 ^oC for 2 h. The N-(3-aminopropyl) methacrylamide (APM), acrylamide (AAm), and acryloyl LT peptides (Acr-LT) were used as the monomers, and the N,N'- methylenebisacrylamide (BIS) was used as the cross-linker. After the polymerization, the reaction mixture was dialyzed (MWCO = 10 kDa) against PBS buffer (10 mM, pH 7.4) to remove unreacted monomers and by-products.

Next, Mal-PEG-NHS (Mal-PEG-NHS/Acr-BSA, 30:1, n/n) was added and stirred for another 3 h at 4 ^oC. The mixture was dialyzed against PBS buffer to remove unreacted Mal-PEG-NHS. Finally, TCEP (TCEP/Acr-BSA, 200:1, n/n) and TAT peptides (TAT/Acr-BSA, 20:1, n/n) were added and stirred for

another 12 h at 4 °C. The mixture was dialyzed against PBS buffer to obtain the NS. The BSA content in the NS was determined by NanoDrop ND-8000.

Nanocapsules including NC-LP, NC-AT, and NC-AP were prepared in a similar method by replacing Acr-LT with Acr-LP, Acr-AT, and Acr-AP, respectively. The formulations without LT peptides (denoted as NS-wo LT) were prepared by replacing Acr-LT with acrylamide (AAM). The formulations without TAT peptides (denoted as NS-wo TAT) were prepared by replacing TAT with PBS.

5. Dynamic light scattering (DLS) analysis of nanosweepers

The particle size of nanosweepers was measured using the following method. Briefly, the obtained nanocapsules were dispersed in PBS buffer (10 mM, pH 7.4) and filtered through 0.22 μm Millipore filter to prepare the sample solution. The hydrodynamic diameter and zeta potential were determined with Malvern Panalytical Nano-ZS at room temperature.

6. Ultraviolent-visible (UV-Vis) spectra of NS and measurement of LT peptide amount on per NS

The UV-Vis spectra of NS-wo LT and NS were recorded using the following method. Briefly, NS and NS-wo LT were firstly prepared using FITC labeled BSA. Then, we normalized the concentrations of NS and NS-wo LT by measuring the ultraviolent-visible (UV-Vis) absorption of FITC (480-530 nm). Subsequently, the UV-Vis spectra of NS-wo LT and NS were recorded with a UV-Vis spectrometer.

Additionally, the amount of the LT peptides on per NS was measured using the following method. According to the absorbance increase at λ_{abs} = 279 nm, the concentration of LT peptides can be calculated. The number of LT peptides on per NS was calculated via the quantization equation shown below.

$$\frac{n_{LT}}{n_{NS}} = \frac{C_{LT}}{C_{NS}}$$

7. Aβ solution preparation

The lyophilized $A\beta_{42}$ peptides were initially dissolved in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) to obtain $A\beta_{42}$ solution (5 mg mL^{-1}). Then, the $A\beta_{42}$ solution was sonicated for 15~30 min and incubated with shaking at 25 °C for 2 h in a sealed vial. Subsequently, the obtained sample was separated into aliquots in sterile microcentrifuge tubes followed by the removal of HFIP under a gentle stream of nitrogen and the peptide film stored at -20 °C. Before use, the dry aliquot was resuspended in a small quantity of anhydrous dimethylsulfoxide (DMSO) followed by sonication for 10 min, then diluted to a final concentration of 40 μM with PBS buffer (10 mM, pH 7.4). This solution should be used immediately to avoid the oligomers assembly.

8. Measurement of binding affinities between NS and Aβ monomers

The binding affinities between NS and Aβ monomers were studied via Förster resonance energy transfer (FRET) analysis, fluorescence imaging, and quartz crystal microbalance (QCM) measurements.

8.1 FRET characterization

Briefly, the nanocapsules were labeled with RhB. Aβ was labeled with FITC. $A\beta_{42}$ solution (40 μM) was mixed with nanocapsules (200 μg mL^{-1}) by volume ratio of 1:1 in a sterile microcentrifuge tube, and then the fluorescent emission spectra were recorded with excitation at 450 nm. After incubating at 37 °C for 96 h, the fluorescence spectra of these solutions were recorded in the same method.

8.2 Fluorescence imaging of the mixture of Aβ and nanocapsule

Briefly, the nanocapsules were labeled with Cy5 (red). Aβ was labeled with FITC (green). The samples were prepared by mixing $A\beta_{42}$ solution (40 μM) and nanocapsules (200 μg mL^{-1}) by volume ratio of 1:1 in a sterile microcentrifuge tube. After incubating at 37 °C for 96 h, the sample was mixed with glycerin and dispersed in 35 mm plastic bottomed μ-dishes. The image was obtained on a confocal laser scanning microscopy.

8.3 QCM measurements

QCM measurements were conducted using a Q-Sence E4 system (Q-Sence, Västra Frölunda, Sweden). $A\beta_{42}$ monomer solution (20 μM) was obtained as described in section 7. The Au sensor chip was immersed in the solution of different nanocapsules (100 μg mL^{-1}), which is conjugated by Traut's Reagent and reduced by TCEP for 24 h at room temperature. Then the chip was rinsed with deionized water, dried with nitrogen gas, and put into the standard flow module before measurements. Each sensor chip surface with attached nanocapsules was washed with PBS buffer (10 mM, pH 7.4) for 1 h at a flow rate of 1 μL min^{-1} until the baseline was stable. Then, the flow buffer was replaced with $A\beta_{42}$ solution (20 μM) at a flow rate of 1 μL min^{-1} for 35 min. Finally, the flow buffer was replaced with PBS buffer (10 mM, pH 7.4) at a flow rate of 1 μL min^{-1} for 30 min. The binding affinities between $A\beta_{42}$ fibrils and different nanocapsules were measured in the same manner. All QCM experiments in this study were operated at room temperature.

To evaluate the specificity of NS in binding Aβ, the binding affinity of NS to proteins in neutrophil lysates was also measured by QCM. Neutrophil was collected, washed three times and homogenized in a lysis buffer with proteinase and phosphorylase inhibitor cocktail. After centrifuging for 15 min (12,000

rpm, 4°C), the supernatant was used as the flow buffer to measure the binding affinities of NS to proteins in neutrophil following above method.

9. Inhibition of Aβ fibrosis by NS

The inhibitory effect of NS on Aβ fibrosis was studied via thioflavin-T (ThT)-based fluorescence assay and TEM measurements.

9.1 ThT fluorescence assay

To evaluate the inhibition on $A\beta_{42}$ aggregation by different nanocapsules, the samples were prepared by mixing $A\beta_{42}$ solution (40 μM) and nanocapsules (200 μg mL^{-1}) with volume ratio of 1:1 and incubated at 37 °C in sterile microcentrifuge tubes. Then, the mixture (15 μL) was mixed with ThT solution (10 μM, 385 μL), and the fluorescence intensity was measured with excitation and emission at 440 nm (slit width = 5 nm) and 485 nm (slit width = 5 nm). Three replicates were performed, and the data were averaged.

9.2 TEM measurements

TEM measurements were performed with Talos F200C electron microscope at an acceleration voltage of 200 kV. $A\beta_{42}$ solution (40 μM) and nanocapsules (200 μg mL^{-1}) with volume ratio of 1:1 was mixed and incubated at 37 °C in sterile microcentrifuge tubes for 96 h. Then, the mixture (10 μL) or different nanocapsule solution was dropped onto a carbon-coated copper grid (Beijing Zhongjingkeyi Technology Co., Ltd, China) for 10~15 min and blotted with filter paper to remove excess liquid. Then the sample was negatively stained with phosphotungstic acid (5~10 μL) for 1~2 min, blotted again, and air-dried before analysis on TEM.

10. Cells culture

Murine neuroblastoma (N2a) cells, murine microglia (BV2) cells, and murine-brain microvascular endothelial (bEnd.3) cells were cultured in Dulbucco's modified Eagle medium (DMEM) medium containing 10% FBS, 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (complete DMEM) at 37 °C in humidified 5% CO_2. These cells were passaged every three days. The isolated neutrophils were cultured in 1640 medium containing 5% FBS, 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin at 37 °C in humidified 5% CO_2.

11. Cytotoxicity of NS to N2a cells and BV2 cells

The cytotoxicity of NS to N2a cells and BV2 cells was evaluated by CCK-8 assay. Briefly, N2a cells and BV2 cells were seeded at a density of 1×10^4 cells/well in 96-well plates containing 100 μL complete DMEM medium and grew for 24 h at 37 ^oC. Subsequently, the medium in the well was removed and different concentrations (18.75, 37.5, 75, 150, and 300 μg mL^{-1}) of NS was added together with 90 μL of complete DMEM medium (total 100 μL) into the wells. After being incubated for 24 h, the cells were washed with PBS and the fresh medium containing 10% CCK-8 was added to each plate. The cells were incubated for another 4 h. Finally, the plate was gently shaken for 1 min and the absorbance at 450 nm was recorded with a micro-plate reader.

12. Mitigation of Aβ-mediated neurotoxicity by NS and released NS

The capability of NS to mitigate the neurotoxicity of Aβ aggregates to N2a cells was also evaluated by CCK-8 assay. Briefly, N2a cells were incubated with Aβ (10 μM) in the absence or presence of NC-AP, NC-AT, NC-LP, and NS (0.075 mg mL^{-1}) for 24 h. Then, the cells were washed with PBS and the fresh medium containing 10% CCK-8 was added to each plate. The cells were incubated for another 4 h. Finally, the plate was gently shaken for 1 min and the absorbance at 450 nm was recorded with a micro-plate reader.

The capability of released NS from NS/NE to mitigate the neurotoxicity of Aβ aggregates to N2a cells was evaluated following similar procedure. N2a cells were incubated with Aβ (10 μM) in the absence or presence of PMA-treated neutrophil, NS/NE, NS, and released NS (0.075 mg mL^{-1}) for 24 h. Then, the cells were washed with PBS and the fresh medium containing 10% CCK-8 was added to each plate. The cells were incubated for another 4 h. Finally, the plate was gently shaken for 1 min and the absorbance at 450 nm was recorded with a micro-plate reader.

13. Aβ phagocytosis and removal mediated by NS and released NS

The capability of NS and released NS to promote the phagocytosis and removal of Aβ by microglia was investigated via fluorescence activated cell sorting (FACS) analysis and confocal laser scanning microscopy (CLSM) observation.

13.1 FACS analysis

For FACS analysis, BV2 cells were seeded into 6-well plates at a density of 1×10^5 cells per well and incubated overnight for cell attachment. The medium was replaced with fresh serum-free DMEM medium

and incubated for 1 h before the addition of nanocapsules. FITC-labeled $A\beta_{42}$, as marker of phagocytosis, was used in this study. FITC-Aβ (1.25 μM) and NC-AP, NC-AT, NC-LP, NS (0.15 mg mL^{-1}) were premixed for 1.5 h, and then incubated with BV-2 cells. After incubation for 3.5 h, the cells were washed three times with PBS buffer and harvested by 0.25% trypsin. Then the cells were fixed with 4% polyformaldehyde solution for FACS analysis.

The capability of released NS to promote the phagocytosis and removal of Aβ by microglia was also investigated following similar procedure. FITC-Aβ (1.25 μM) was firstly premixed with PMA-treated neutrophil, NS/NE, NS, and released NS (0.15 mg mL^{-1}) for 1.5 h, and then incubated with BV-2 cells. After incubation for 3.5 h, the cells were washed three times with PBS buffer and harvested by 0.25% trypsin. Then the cells were fixed with 4% polyformaldehyde solution for FACS analysis. All of these experiments were performed in triplicate.

13.2 CLSM observation

For CLSM observation, similar procedures were performed. BV2 cells were seeded into 35 mm plastic bottomed μ-dishes at a density of 1×10^5 cells per well and incubated overnight for cell attachment. In addition, the nanocapsules were labeled with Cy5. FITC-Aβ (1.25 μM) and NC-AP, NC-AT, NC-LP, NS (0.15 mg mL^{-1}) were premixed for 1.5 h, and then incubated with BV-2 cells. After incubation for 3.5 h, the cells were washed three times with PBS buffer, followed by fixing in 4% paraformaldehyde and staining with TRITC phalloidin and 4',6-diamidino-2-phenylindole (DAPI). After the staining, the cells were observed using CLSM.

The capability of released NS to promote the phagocytosis and removal of Aβ by microglia was also investigated following similar procedure. FITC-Aβ (1.25 μM) was firstly premixed with PMA-treated neutrophil, NS/NE, NS, and released NS (0.15 mg mL^{-1}) for 1.5 h, and then incubated with BV-2 cells. After incubation for 3.5 h, the cells were washed three times with PBS buffer, followed by fixing in 4% paraformaldehyde and staining with TRITC phalloidin and 4',6-diamidino-2-phenylindole (DAPI). After the staining, the cells were observed using CLSM.

14. Isolation of neutrophil from peripheral blood

The neutrophil was isolated from mouse peripheral blood with mouse peripheral blood neutrophil separation kit according to the manufacturer's protocol. The morphology of neutrophil was observed on TEM and confocal laser scanning microscopy.

15. Cytotoxicity of NS to neutrophil

The cytotoxicity of NS to neutrophils was evaluated by CCK-8 assay. Briefly, the isolated neutrophils were seeded at a density of 1×10^4 cells/well in 96-well plates with 1640 medium (90 μL) containing different concentrations (25, 50, 100, 200, and 400 μg mL^{-1}) of NS. After incubation for 1 h, 2 h, and 12 h, the CCK-8 (10 μL) was added to each well. The cells were incubated for another 4 h. Finally, the plate was gently shaken for 1 min and the absorbance at 450 nm was recorded with a micro-plate reader.

16. Preparation and characterization of NS/NE

To facilitate effective loading of NS into neutrophils, we optimized the co-incubation time and concentration of NS with neutrophils, as well as the amount of TAT peptides conjugated on NS, and then the loading of NS into neutrophils was analyzed via CLSM and FACS. Briefly, the isolated neutrophil (1×10^5 cells ml^{-1}) were seeded in a sterile tube. NS was labeled with Cy5, and then incubated with neutrophils at different concentrations (25, 50, 100, and 200 μg mL^{-1}) at 37 ^oC for different co-incubation time (15, 30, 60, 120, and 180 min). In addition, the ratio of TAT peptides (10:1, 20:1, 30:1, and 40:1, TAT:NS) conjugated on NS was adjusted. After washing three times with PBS buffer, the NS/NE was observed using CLSM and performed FACS analysis. Cy5 fluorescence signal was monitored at 670 nm via the APC-A channel. For following experiments, neutrophil was incubated with NS at the concentration of 200 μg mL^{-1} at 37 ^oC for 2 h. After washing with ice-cold PBS thrice, the NS/NE suspension was obtained.

Quantitative analysis of NS in the NS/NE was performed using the following method. Briefly, NS/NE was disrupted by RIPA cell lysis buffer to release NS from the neutrophil. The cell lysate was collected and centrifuged at 10,000 g for 5 min. The supernatant was recorded with a micro-plate reader. The loading capacity of NS in NS/NE was determined by the linear correlation between concentrations of NS and the fluorescence intensity of Cy5.

17. Chemotactic capability of NS/NE to the inflammatory factors

The chemotactic capability of NS/NE to the inflammatory factors was investigated using the transwell migration assay (transwell polyethylene terephthalate (PET) membrane: 3 μm pore size, 6.5 mm diameter and 0.33 cm^2 membrane surface area, Corning). [2] Briefly, original neutrophil and NS/NE (2×10^5 cells) were added to the upper chamber of the transwell. The lower chamber of the transwell was filled with FBS free culture medium containing different concentrations (0, 1, 10, 100, and 1000 nM) of inflammatory factors mimic formylmethionylleucylphenylalanine (fMLP). After 1 h of incubation, the cells in the lower chamber were recorded on the microscope, and counted by Image J software. The

chemotaxis index (($N_{fMLP} - N_{control}$)/$N_{control}$)) was calculated, where N_{fMLP} and $N_{control}$ are the counted numbers of neutrophil in the lower chamber after incubating with each formulation in the presence of fMLP and the original neutrophil in the absence of fMLP, respectively.

18. BBB penetration capability of NS/NE

BBB penetration capability of NS/NE was evaluated by using an in vitro BBB model established with bEnd.3 cell monolayer. [2] Briefly, bEnd.3 cells (1×10^5 cells/well) were seeded onto the upper chamber of the transwell in 24-well plates, and cultured with the medium containing 10% FBS. The integrity of the cell monolayer was evaluated by measuring the TEER values using a Millicell-ERS voltohmmeter (Millipore). The cell monolayers with TEER values higher than 300 Ω cm^2 were used as the BBB model for the transmigration studies. The NS was pre-labeled with Cy5. NS and NS/NE (2×10^5 cells, 200 μl) were added to the upper chamber, and the FBS free medium with or without fMLP (10 nM) was added to the lower chamber. After 3 h of incubation, the supernatant in upper chamber and the culture medium in lower chamber were sampled, and the bEnd.3 cell layers on the membrane of the transwells were harvested. The amounts of NS or NS/NE in the supernatant, intracellular and culture medium in lower chamber were determined using micro-plate reader by recording the fluorescence intensity of Cy5.

19. The release of NS from NS/NE

The release of NS from NS/NE was evaluated using the following method. Briefly, PBS was applied to simulate the normal physiological condition. fMLP and phorbol myristate acetate (PMA) were applied to simulate the microenvironment of chemotactic process and inflammatory brain, respectively. The NS was pre-labeled with Cy5. NS/NE (1×10^6 cells/well) were seeded in 24-well plates, and then incubated with the FBS free medium that contained PBS, fMLP (10 nM) or PMA (100 nM) for different periods (0, 2, 4, 6, and 8 h). The amounts of NS in NS/NE and released in the supernatant medium were determined using micro-plate reader by recording the fluorescence intensity of Cy5.

To demonstrate accelerated NS release from NS/NE with the formation of neutrophil extracellular traps (NET), the released DNA concentration in the supernatant of the release medium was determined by NanoDrop ND-8000.

20. Collection of released NS

NS/NE was firstly incubated with PMA (100 nM) at 37 °C in humidified 5% CO_2 for 8 h. After PMA stimulation, NS was released from NS/NE and dispersed into the culture medium. Then, the culture medium was subjected to ultrafiltration centrifugation (12,000 rpm, 30 min) with ultrafiltration centrifuge

tube (MWCO = 100,000 Da) to collect released NS dispersed in the culture medium. Afterward, released NS was washed with PBS buffer (10 mM, pH 7.4) three times. After washing away surface impurities, the released NS was obtained. A control experiment was performed under identical conditions with NE. The functions of released NS were studied as described in sections 12 and 13.

21. Stability of LT peptides on NS surface and integrity of NS in neutrophils

To study the stability of LT peptides on NS surface in neutrophils, the BSA core of NS was labeled with FITC and the carboxyl group at the end of LT peptides on NS surface was labeled with Cy5, respectively. Briefly, NS was firstly prepared using FITC labeled BSA as described in section 4. Then, the carboxyl of LT peptide on NS surface was pre-activated by adding a solution of N-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-dimethylaminopyridine (DMAP) (LT/NHS/EDC/DMAP, 1:1:1:0.1, n/n/n/n) and stirred for 2 h at 4 ^{o}C. Next, Cy5-NH_2 (LT/ Cy5-NH_2, 1:1, n/n) was added to the above solution and stirred for another 4 h at 4 ^{o}C. The mixture was dialyzed (MWCO = 10 kDa) against PBS buffer (10 mM, pH 7.4) to obtain the NS.

The released NS from NS/NE was collected as described in section 20. The fluorescence spectra of NS and released NS were recorded with excitation at 494 nm (slit width = 5 nm) and 649 nm (slit width = 5 nm), respectively. The fluorescence intensity was measured with emission at 526 nm (slit width = 5 nm) and 670 nm (slit width = 5 nm). Three replicates were performed, and the data were averaged.

To study the integrity of NS in neutrophils, the released NS from NS/NE was also collected as described in section 20 and dropped onto a carbon-coated copper grid for 15 min and blotted with filter paper to remove excess liquid. Then the sample was negatively stained with phosphotungstic acid (10 μL) for 1 min, blotted again, and air-dried before analysis on TEM.

22. Animals

All animals used for experiments were allocated blindly to treatment groups. All protocols were approved by the Animal Care and Use Committee of Laboratory Animal Center, Nankai University. The C57BL/6J male mice (6–8 weeks) were used for the normal mice. For therapeutic evaluation experiments, 9-month male APPswe/PSEN1dE9 (APP/PS1) transgenic mice were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). The 6–8 weeks and 9-month male C57BL/6J mice (littermates) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd.. All mice were directly transported to the animal facility at Nankai University. Mice were housed in a standard individual ventilation cages animal experimental system (Suzhou Fengshi Laboratory Animal Equipment Co. Ltd.) with corn cob bedding and a wire lid, providing climbing opportunities (APP/PS1 and 9-month male C57BL/6J mice were housed one mouse per cage). The housing condition of mice is the specific

pathogen-free (SPF) laboratory animal center with free access to food and water, the humidity keeps at 30–70% (20–26 ^{o}C), and keeps at 12 h of light and 12 h of dark cyclic condition.

23. Establishment of encephalitis mice model

The encephalitis mice model was established using the following method. Briefly, the normal C57BL/6J mice (6–8 weeks) were first anesthetized with 3.5% isoflurane through inhalation. Then the mice were fixed on a brain stereotaxic apparatus. The scalp was cut and a hole was drilled at certain position on the skull (1.0 mm lateral, 0.2 mm posterior from bregma). A 5 μL of Hamilton syringe with a needle was then inserted through the hole into the lateral ventricle at 2.5 mm below the horizontal plane of bregma. Lipopolysaccharide (LPS, 5 mg mL^{-1}, 2 μL) in PBS was infused at the right lateral ventricles. [3]

24. NS accumulation in brain of encephalitis mice and APP/PS1 mice

The capability of NS/NE to cross BBB and NS accumulation in brain of encephalitis mice and APPswe/PSEN1dE9 (APP/PS1) transgenic mice were investigated using *ex vivo* imaging and brain tissue sections observation.

24.1 *ex vivo* imaging

Briefly, NS was pre-labeled with Cy5 and then co-incubated with neutrophils to prepare NS/NE. At 8 h post-surgery, NS/NE (4.35×10^6 cells/mouse) were intravenously injected into the encephalitis mice. Liposome was used as the control to load NS (NS/Lip). Similar study was also performed on normal C57BL/6J mice for comparison. At 8 h post-injection, the mice were sacrificed and the major organs (brain, heart, liver, spleen, lung, and kidney) were harvested for *ex vivo* imaging using IVIS Spectrum. ROI was circled around different organs, and the fluorescence intensity of the Cy5 signal was analyzed using Living Image Software.

The NS accumulation in brain of APP/PS1 mice was investigated following similar procedure. Briefly, NS/NE (4.35×10^6 cells/mouse) were intravenously injected into the APP/PS1 mice. Liposome was used as the control to load NS (NS/Lip). Similar study was also performed on 9-month C57BL/6J mice as the wild-type (WT) control. At 8 h post-injection, the mice were sacrificed and the major organs (brain, heart, liver, spleen, lung, and kidney) were harvested for *ex vivo* imaging using IVIS Spectrum. ROI was circled around different organs, and the fluorescence intensity of the Cy5 signal was analyzed using Living Image Software.

24.2 Brain tissue sections observation

At 8 h post-injection, the encephalitis mice were sacrificed and the brain was harvested for frozen sectioning followed by DAPI staining. CLSM was used to visualize the accumulation of NS in the brain. The quantification of NS in brain tissue sections was performed by Image J software.

25. Therapeutic efficacy of NS in AD mice

To investigate the therapeutic efficacy of NS in AD mice, APP/PS1 mice were injected with NS/NE (4.35×10^6 cells/mouse, 100 μL) via tail vein injection every 3 days for 30 days. Similar study with NS/Lip was also performed for comparison. The APP/PS1 mice and 9-month wild-type (WT) mice injected with PBS were used as controls to demonstrate pathological dysfunction in AD mice. The body weight was recorded during the treatment. After treatment, the mice were euthanized. The therapeutic efficacy of NS in AD mice was investigated via immunofluorescence staining and TUNEL apoptosis staining.

25.1 Immunofluorescence staining

The Aβ deposition in the hippocampus and cortex of APP/PS1 mice after treatment was investigated via immunofluorescence staining. Briefly, the brain was harvested and fixed in 4% paraformaldehyde for 24 h, then dehydrated, embedded, and cut into 8 μm frozen slices. The sections were washed three times with PBS, blocked with normal goat serum for 1 h, and subsequently incubated with anti-β-Amyloid, 1-42 antibody (1:200, BioLegend) overnight at 4 ^{o}C. Sections were then washed three times with PBS, and the slices were incubated with Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G (IgG) (1:500, Beyotime) for 1 h at room temperature. Then, the slices were stained with DAPI for 10 min. The fluorescence images were obtained using CLSM and the fluorescence intensity of Alexa Fluor 488 was analyzed by Image J software.

25.2 TUNEL apoptosis staining

The neuronal damage in the hippocampus and cortex of APP/PS1 mice after treatment was studied via TUNEL apoptosis staining. Briefly, the frozen brain tissue sections were washed three times with PBS and stained by the TUNEL apoptosis assay kit according to the manufacturer's protocol. DAPI was used for nuclear staining. The stained brain sections were observed by CLSM.

26. Biocompatibility of NS/NE

The biocompatibility of NS/NE was investigated via pathological analysis, hematological, and biochemical. Briefly, the APP/PS1 mice and 9-month wild-type (WT) mice were given different formulations as described in section 25. After treatment, the blood samples of the mice were collected. Histological studies of the major organs, including heart, liver, spleen, lung, and kidney, were performed using hematoxylin and eosin (H&E) staining. The blood routine examinations including WBC (white blood cell), RBC (red blood cell), HGB (hemoglobin), PLT (platelets) count, and blood chemistry examinations including ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase), CRE (creatinine), BUN (blood urea nitrogen) were measured by detection kits according to manufacture' protocols.

27. Statistics analysis

All statistical analyses were carried out with GraphPad Prism software. Statistical comparisons were made by unpaired Student's t-test (between two groups) and one-way ANOVA (for multiple comparisons). All data are presented as mean ± s.d. (n = 3). Significant differences were expressed as asterisks. *P < 0.05 was considered significant, and **P < 0.01 ***P < 0.001 **** P < 0.0001 were considered highly significant.

28. Reference

1. Y. Zhao, J. Cai, Z. Liu, Y. Li, C. Zheng, Y. Zheng, Q. Chen, H. Chen, F. Ma, Y. An, L. Xiao, C. Jiang, L. Shi, C. Kang and Y. Liu, *Nano Lett.*, 2019, **19**, 674-683.
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3. J. Qi, L. Feng, X. Zhang, H. Zhang, L. Huang, Y. Zhou, Z. Zhao, X. Duan, F. Xu, R. T. K. Kwok, J. W. Y. Lam, D. Ding, X. Xue and B. Z. Tang, *Nat. Commun.*, 2021, **12**, 960.

29. Supplementary Figures and Tables

Table S1. Nanocapsule abbreviations and corresponding peptide sequences used in preparation.

Nanocapsule Abbreviation	Sequence of Peptides Used in Preparation
NS	Acr-KLVFFGGGGTKPR
NC-LP	Acr-KLVFFGGGGPTKR
NC-AT	Acr-KAAGGGGGGTKPR
NC-AP	Acr- KAAGGGGGGPTKR

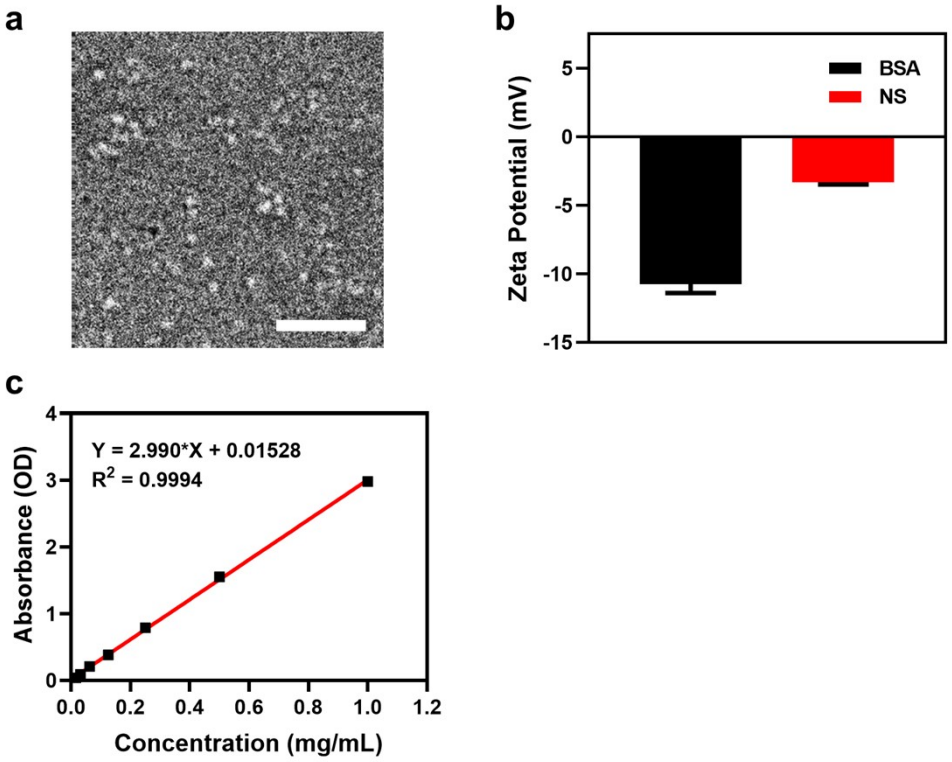

Figure S1. Characterization of NS. a) TEM image of BSA. Scale bar, 50 nm. b) Zeta potential of BSA and NS. c) Linear correlation between the absorption (λ_{abs} = 279 nm) and concentrations of peptides LT in a PBS solution (pH 7.4, 0.01 M) at 37 °C. Data are presented as mean ± s.d. (n = 3).

The concentration of LT peptides is 0.932 mg mL^{-1}.

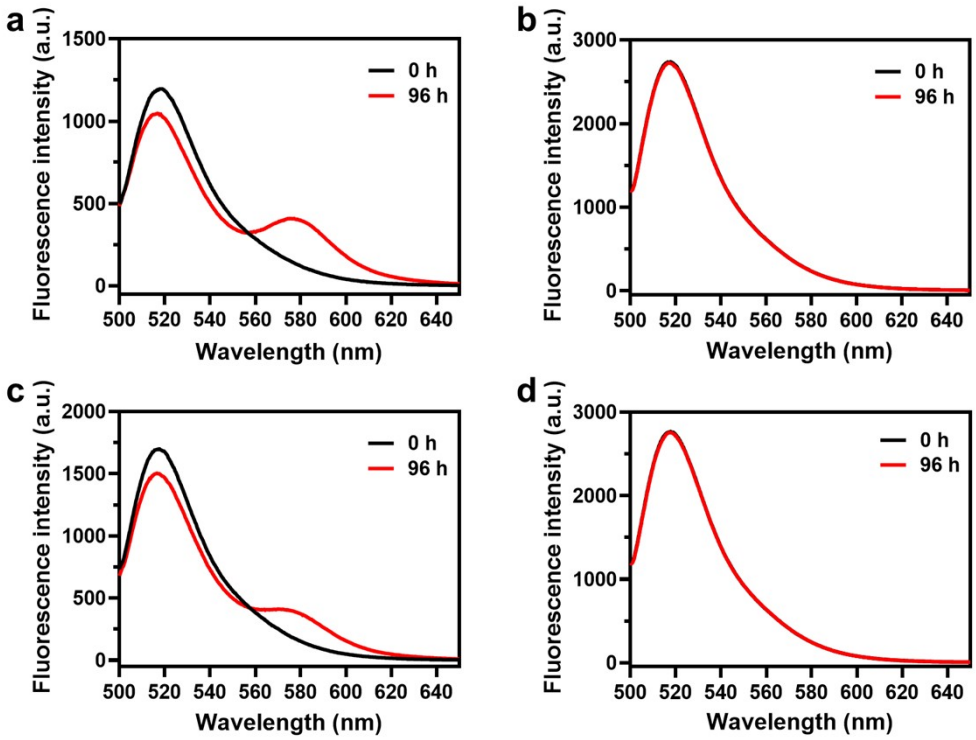

Figure S2. Fluorescence spectra of a) NS, b) NC-AT, c) NC-LP, and d) NC-AP incubated with Aβ monomers for 0 h and 96 h. The nanocapsules were labeled with RhB. Aβ was labeled with FITC. The spectra were recorded with excitation at 450 nm.

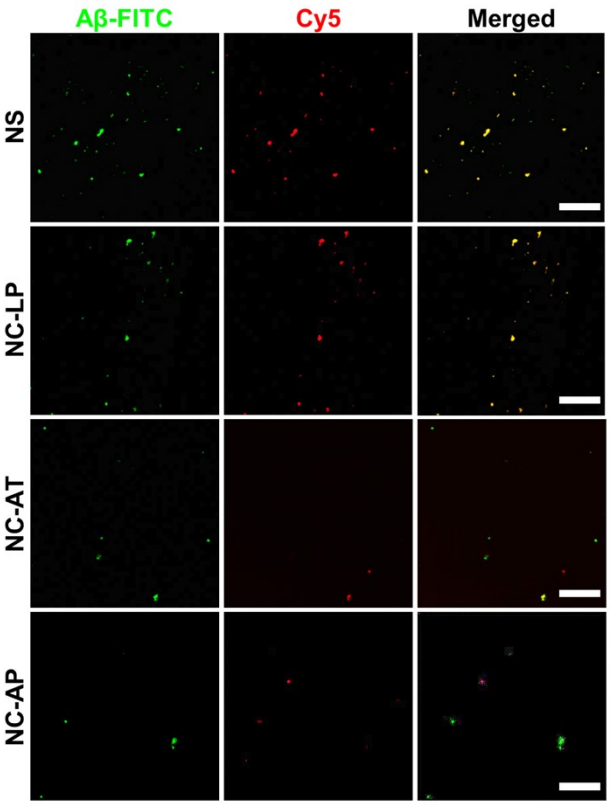

Figure S3. Fluorescence images of the mixture of Aβ and nanocapsules. The nanocapsules were labeled with Cy5 (red). Aβ was labeled with FITC (green). Scale bar, 20 μm.

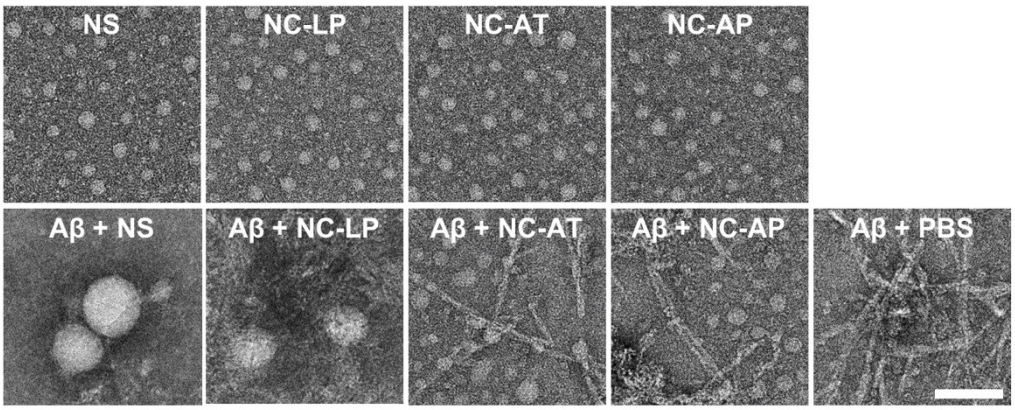

Figure S4. TEM images of NS, NC-LP, NC-AT, NC-AP, Aβ/NS, Aβ/NC-LP, Aβ/NC-AT, Aβ/NC-AP, and Aβ/PBS (Aβ monomers incubated with NS, NC-LP, NC-AT, NC-AP, and PBS for 96 h, respectively). Scale bar, 100 nm.

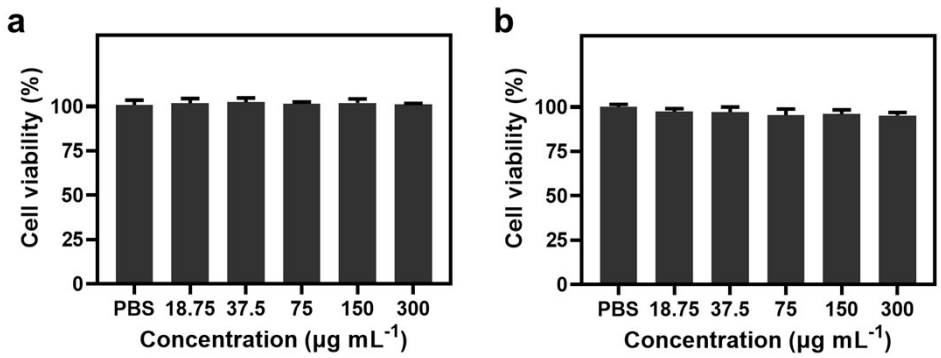

Figure S5. Cytotoxicity of NS to a) N2a cells, and b) BV2 cells incubated with different concentrations of NS for 24 h. Data are presented as mean ± s.d. (n = 3).

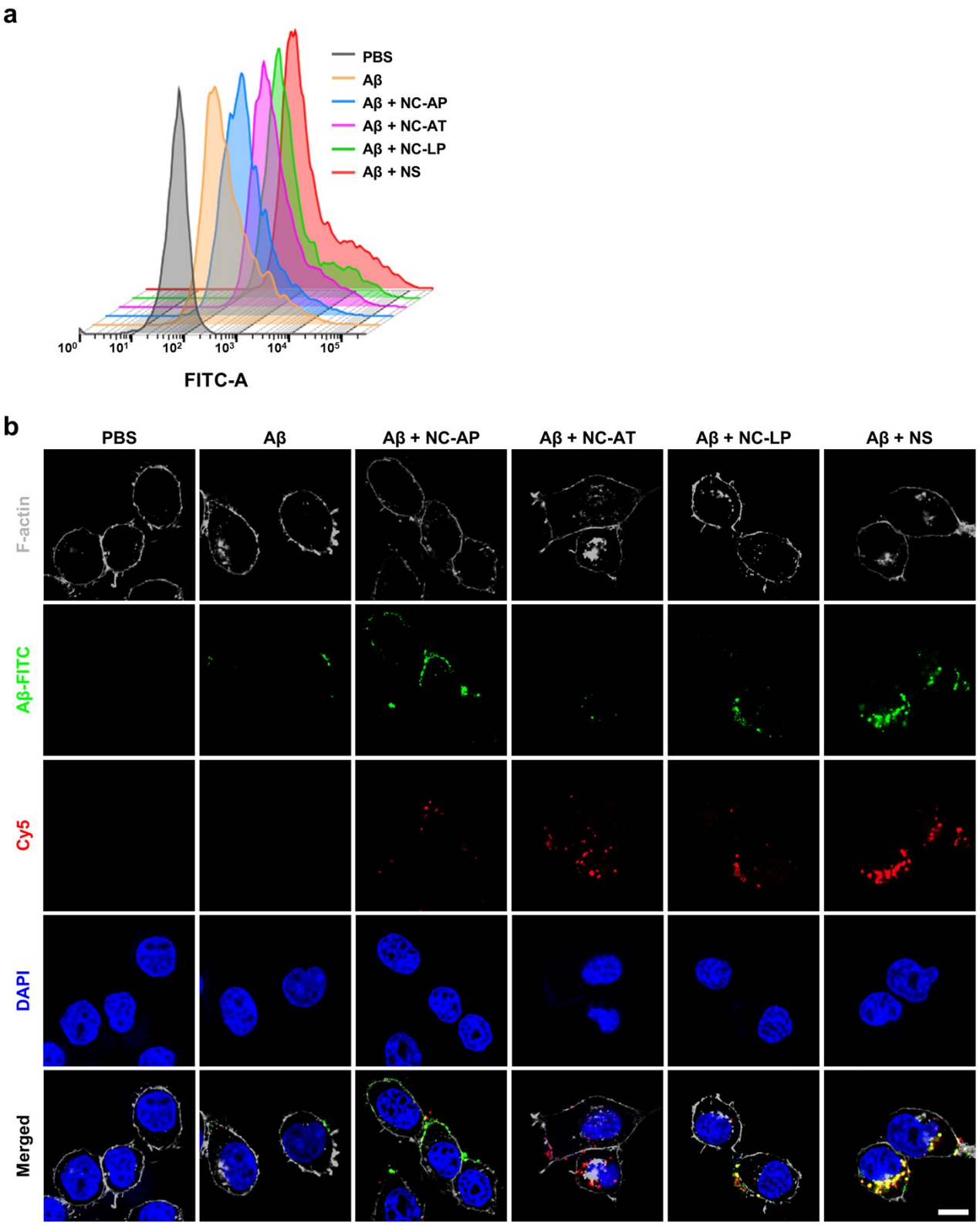

Figure S6. a) Flow cytometry analysis and b) CLSM images of the Aβ phagocytosis by BV2 cells incubated with NC-AP, NC-AT, NC-LP, and NS at 37 °C for 3.5 h. The cell membrane was stained by TRITC phalloidin (gray). Aβ was labeled with FITC (green). The nanocapsules were labeled with Cy5 (red). The nuclei were stained by DAPI (blue). Scale bar, 20 μm.

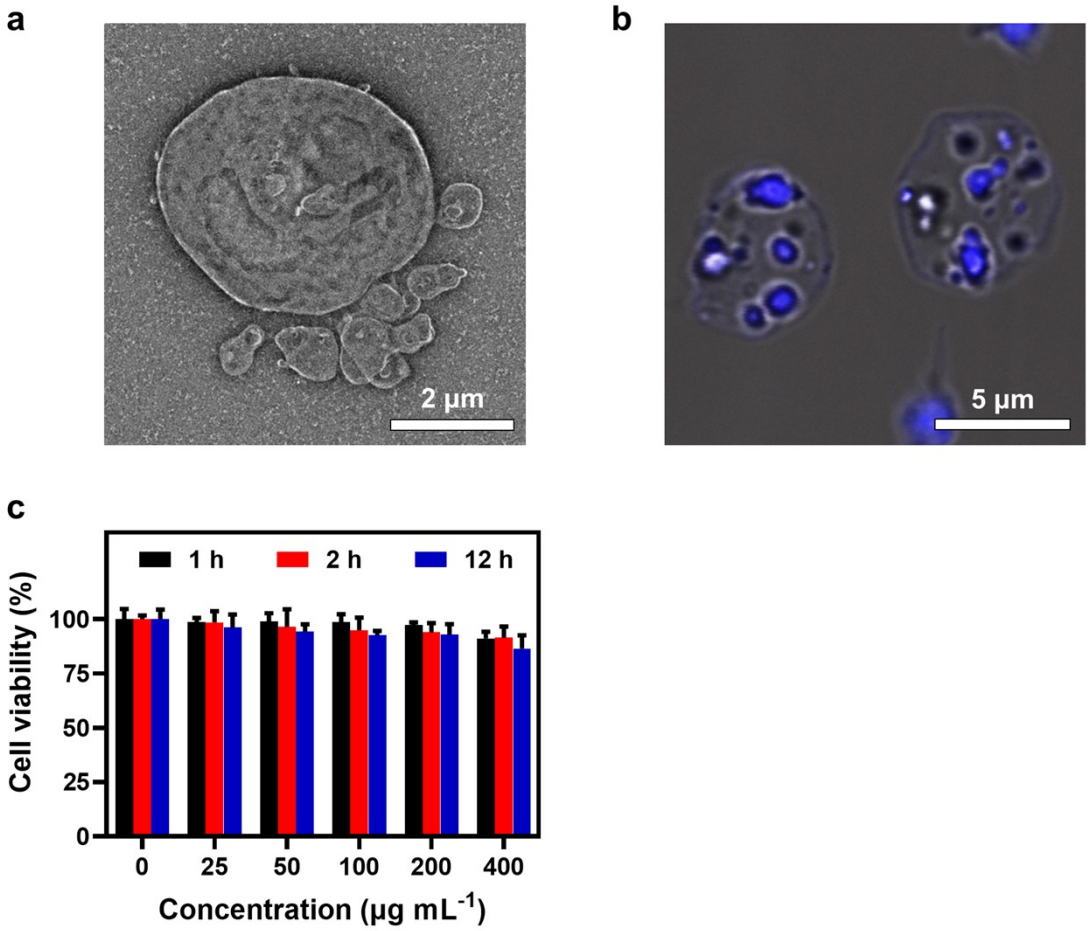

Figure S7. a) TEM image of isolated neutrophil (with phosphotungstic acid negative staining). b) The CLSM image of neutrophil. The nuclei were stained by DAPI (blue). c) Cytotoxicity of NS to neutrophil incubated with different concentrations of NS for 1 h, 2 h, and 12 h, respectively. Data are presented as mean ± s.d. (n = 3).

The neutrophil was isolated from peripheral blood of healthy mice via the neutrophil isolation kit with a yield of 8.69×10^6 cells/mouse.

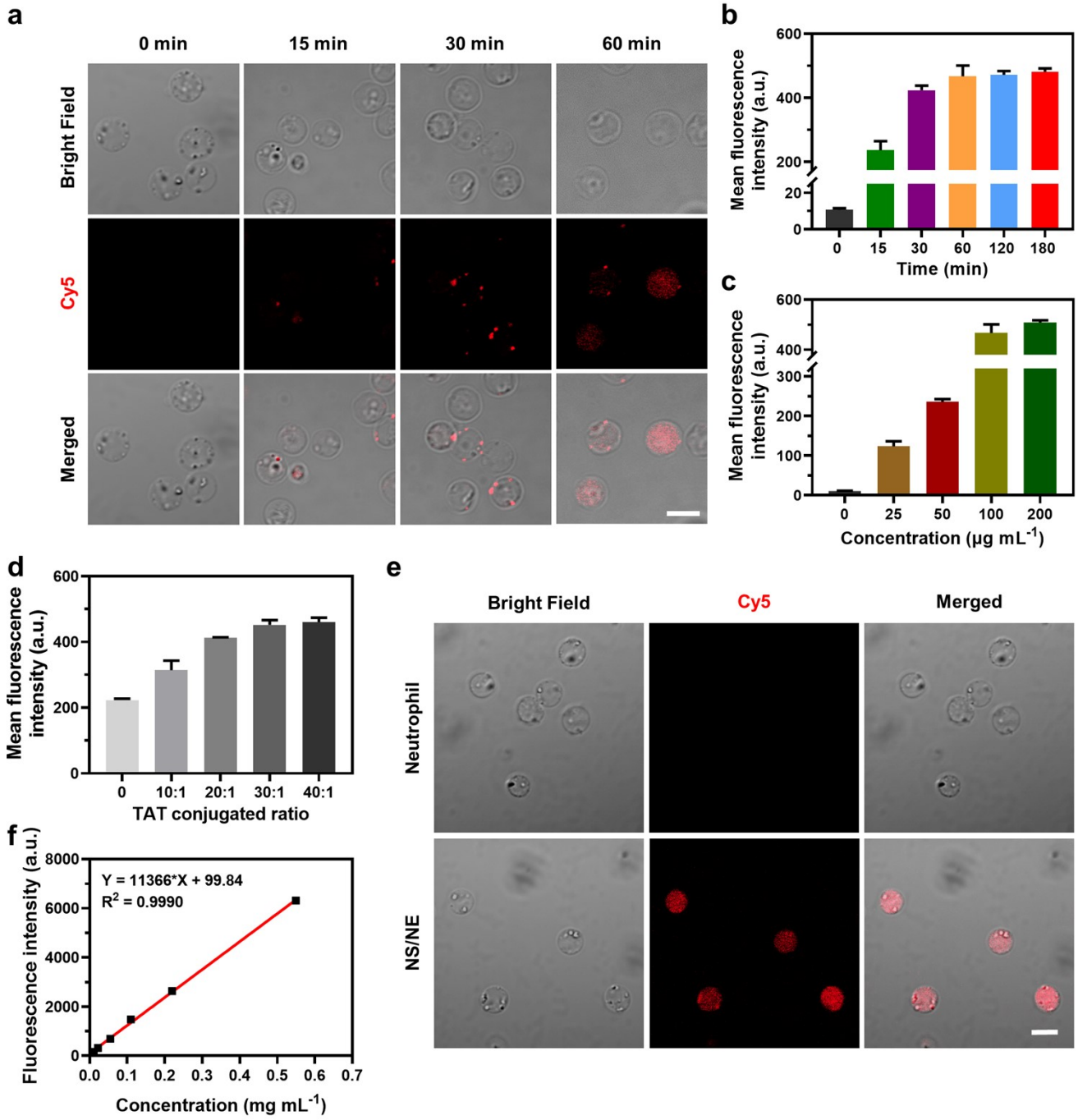

Figure S8. Characterization of NS/NE. a) CLSM images of neutrophil treated with NS (200 μg mL^{-1}) at 37 °C for different times. Scale bar, 5 um. b,c) The mean fluorescence intensity of neutrophil incubated with NS for (b) different times and (c) with different concentrations at 37 °C. d) The mean fluorescence intensity of neutrophil incubated with NS conjugated with various ratios of TAT peptide. e) CLSM images of neutrophil and obtained NS/NE. Scale bar, 5 μm. f) Linear correlation between concentrations of NS in PBS solution (pH 7.4, 0.01 M) at 37 °C and the fluorescence intensity of Cy5 (λ_{ex} = 601 nm, λ_{em} = 664 nm). The NS was pre-labeled with Cy5 (red). Data are presented as mean ± s.d. (n = 3).

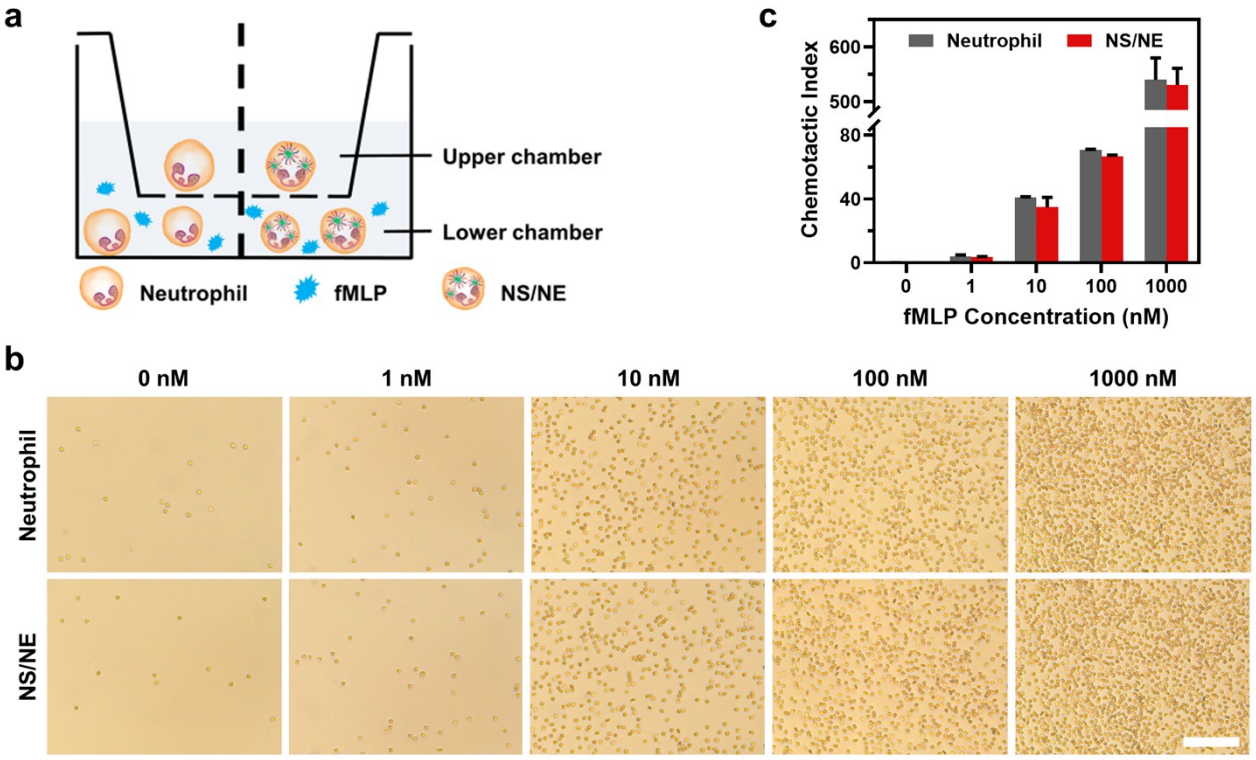

Figure S9. The chemotactic capability to the inflammatory factors of NS/NE. a) Schematic illustration of neutrophil and NS/NE migrated to the lower chamber of the transwell system in the presence of fMLP. b) The images of neutrophil and NS/NE migrated to the lower chamber of the transwell system in the presence of different concentrations of fMLP for 1 h. Scale bar, 50 μm. c) The chemotactic index of neutrophil and NS/NE in (b). Data are presented as mean ± s.d. (n = 3).

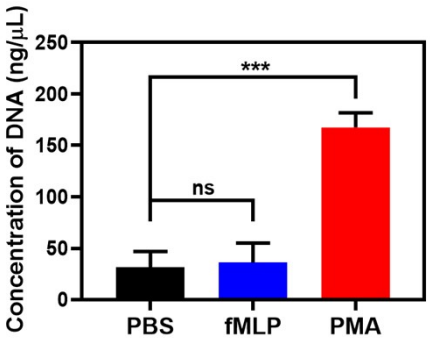

Figure S10. The DNA concentration in the supernatant of NS/NE incubated with PBS, fMLP (10 nM), and PMA (100 nM) for 8 h, respectively. Data are presented as mean ± s.d. (n = 3). Significant differences were expressed as asterisks: *** $P < 0.001$.

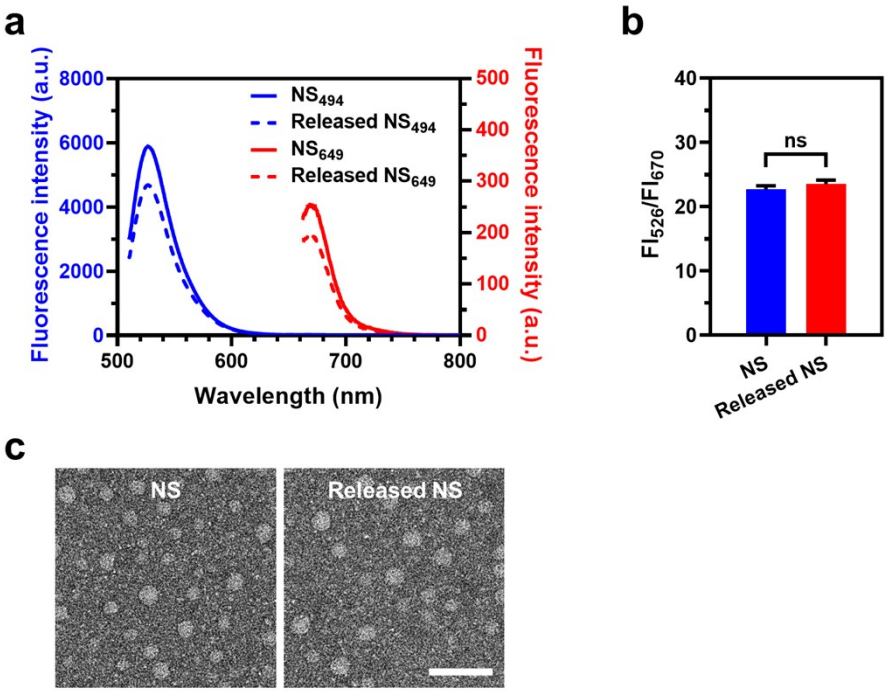

Figure S11. a) Fluorescence spectra of NS and released NS. The solid line and dotted line were recorded from NS and released NS with excitation at 494 nm and 649 nm, respectively. b) Fluorescence intensity (FI) ratio of NS and released NS at maximum emission wavelengths of 526 nm and 670 nm in (a). c) TEM images of NS and released NS. Scale bar, 100 nm.

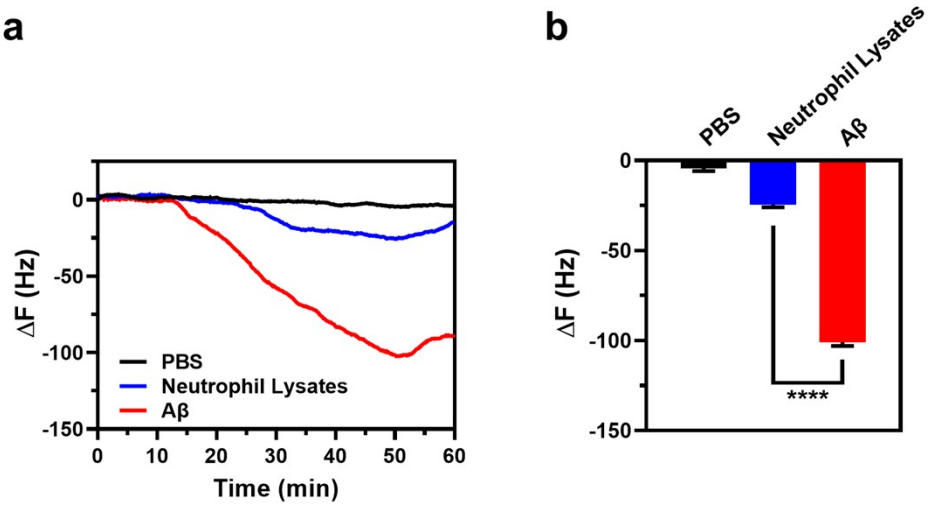

Figure S12. QCM measuring the binding affinity of NS to proteins in neutrophil lysates and Aβ monomers.

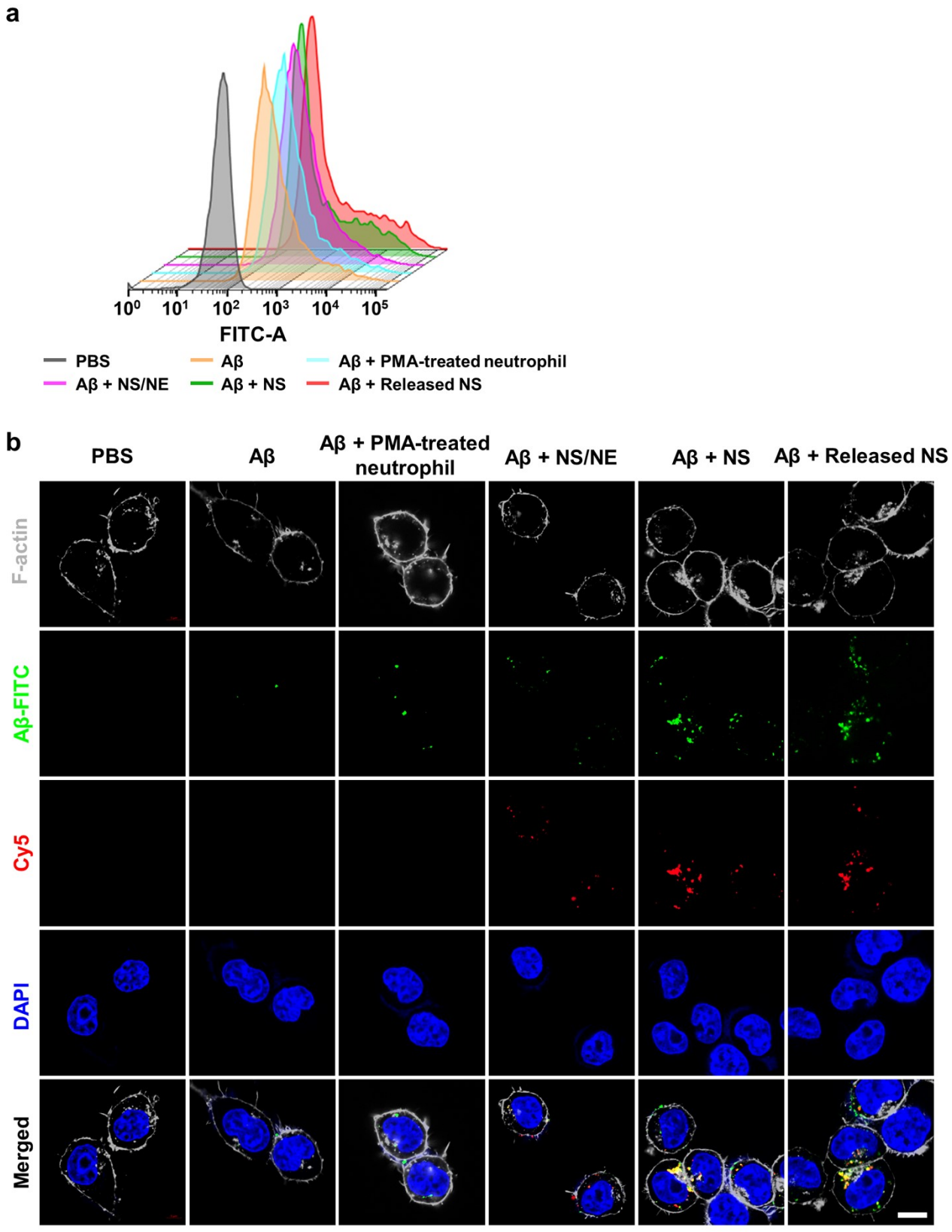

Figure S13. a) Flow cytometry analysis and b) CLSM images of the Aβ phagocytosis by BV2 cells treated with or without PMA-treated neutrophil, NS/NE, NS, and released NS at 37 °C for 3.5 h. The cell membrane was stained by TRITC phalloidin (gray). Aβ was labeled with FITC (green). The nanocapsules were labeled with Cy5 (red). The nuclei were stained by DAPI (blue). Scale bar, 10 μm.

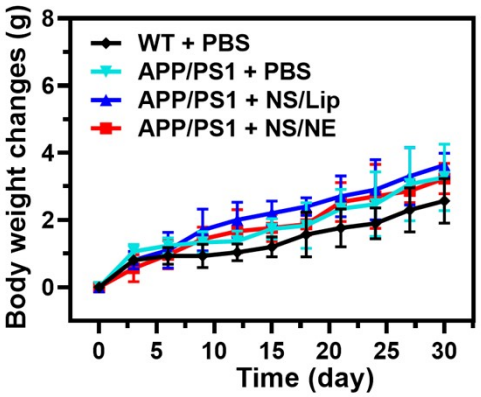

Figure S14. Body weight changes of the APP/PS1 mice treated with NS/Lip and NS/NE. Data are presented as mean ± s.d. (n = 3).

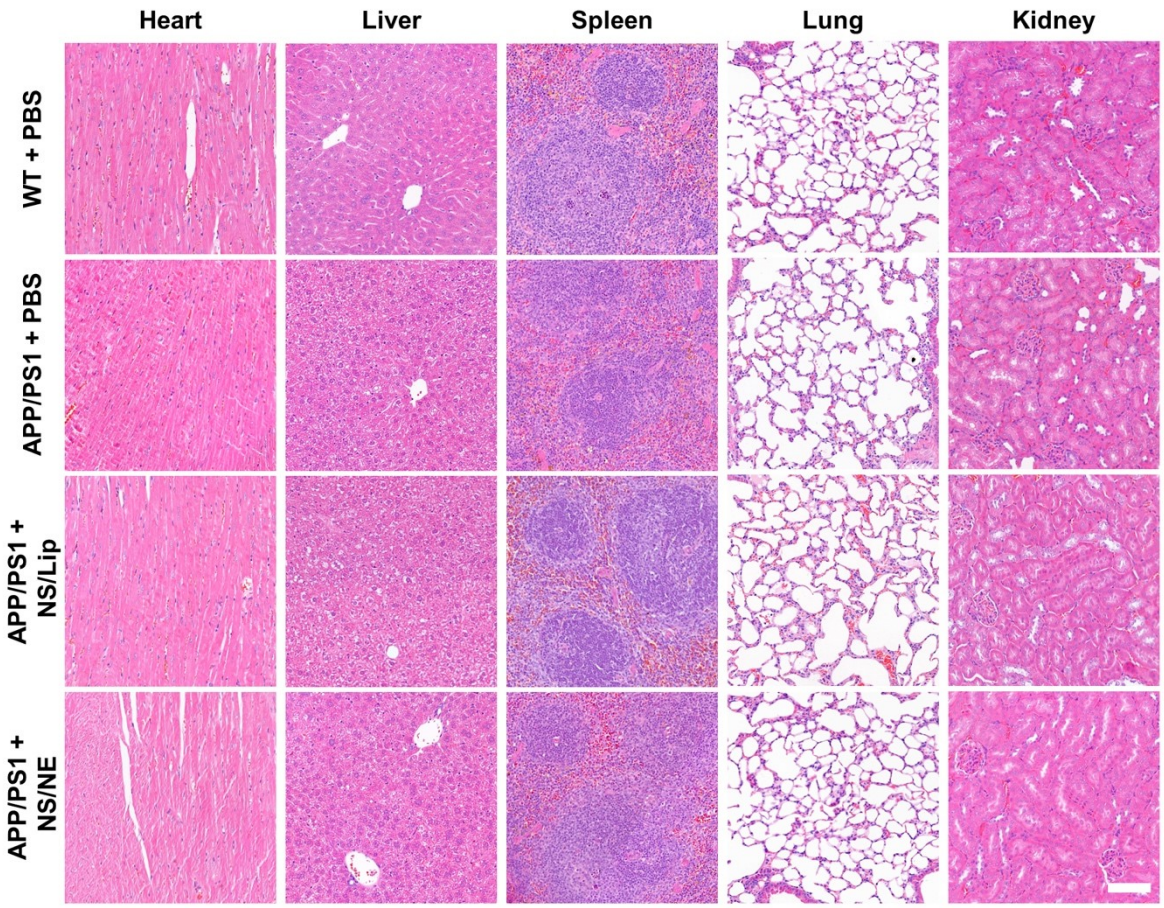

Figure S15. Hematoxylin and eosin (H&E) staining images of the heart, liver, spleen, lung, and kidney collected from the APP/PS1 mice treated with NS/Lip and NS/NE. Scale bar, 100 μm.

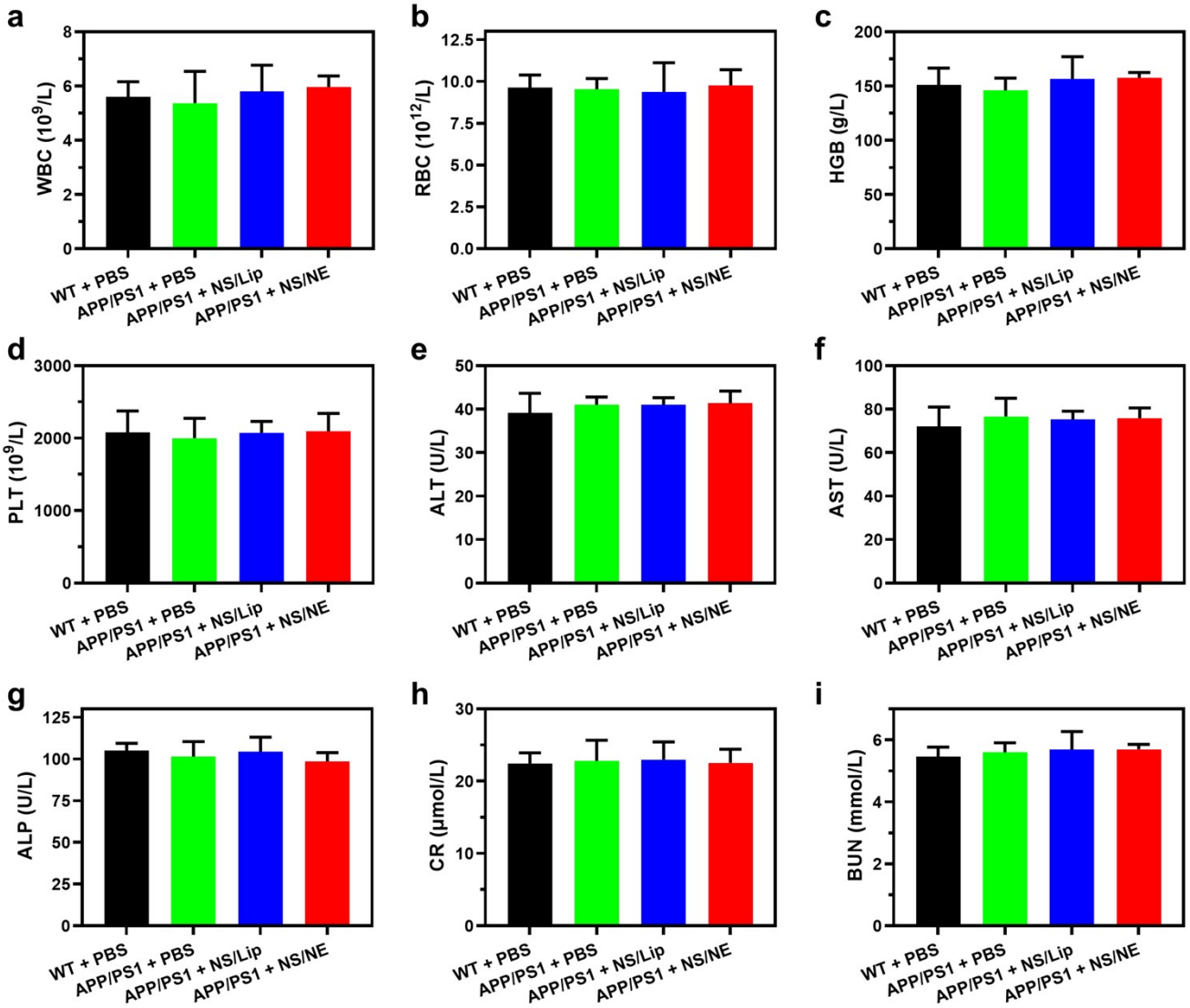

Figure S16. (a-d) Blood routine examinations and (e-i) blood chemistry examinations of the APP/PS1 mice treated with NS/Lip and NS/NE. a) WBC, white blood cell, b) RBC, red blood cell, c) HGB, hemoglobin, d) PLT, platelets, e) ALT, alanine transaminase, f) AST, aspartate transaminase, g) ALP, alkaline phosphatase, h) CR, creatinine, i) BUN, blood urea nitrogen. Data are expressed as mean ± s.d. (n = 3).