Supplementary Information

In situ Construction of Nano-networks from Transformable Nanoparticles for Anti-angiogenic Therapy

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1. Materials and Methods

1.1. Materials

***Materials*:** All reagents and solvents for organic synthesis were purchased from commercially available sources and used without further purification unless otherwise stated. O-(benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (HBTU), piperidine, 4-methylmorpholine (NMM), 2, 5-dihydroxybenzoic acid (DHB), trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Chemical Co. All F_{moc}-amino acids and Wang-resins were obtained from GL Biochem. (Shanghai) Ltd. Cell counting kit-8 assay (CCK-8) was obtained from Beyotime Institute of Biothechnology, China. The human breast cancer cell line MDA-MB-231 was purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Cell culture medium and fetal bovine serum (FBS) were from Wisent Inc. (Multicell, Wisent Inc., St. Bruno, Quebec, Canada). 0.25% Trypsin-EDTA and antibiotic solution (penicillin and streptomycin) were purchased from Invitrogen (Invitrogen, Carlsbad, CA). Culture dishes and plates were from Corning (Corning, New York, USA). Dichloromethane (DCM) and N, N-dimethylformamide (DMF) were distilled over CaH_2 and stored under Ar. Silica gel (200-300 mesh). Distilled and deionized water was used throughout the work.

1.2. Methods

***Mass spectrometry (MS) Measurement*:** Mass spectra were acquired on a MALDI-TOF-MS using a Microflex LRF System spectrometer (Bruker Daltonics) under positive-ion mode.

***UV-vis spectroscopy*:** UV-vis absorption spectra were recorded on a Shimadzu 2600 UV-vis spectrophotometer. Spectroscopic studies of solvents were spectroscopic grade and used as received. The spectra were recorded in quartz glass cuvettes and according to Lambert-Beer's law the extinction coefficients were calculated.

***Fluorescence spectroscopy*:** A F-280 spectrofluorometer was used for fluorescence spectroscopic studies. These samples (1 mL) were added in a quartz cuvette (1 cm path length) and the measurements were carried out at room temperature. The emission spectra (λ_{ex} = 340

nm) were recorded between 370 and 675 nm.

***CD spectroscopy*:** The CD spectra were recorded by J-810 Circular Dichroism Spectrometer (Jasco, Japan).

***Scanning electron microscope (SEM)*:** High resolution scanning electron microscopy images were acquired on Tecnai G2 F20 U-TWIN under an acceleration voltage of 10.0 kV, a working distance of 5.0 mm.

***Transmission electron microscopy (TEM)*:** The measurements were performed on a Tecnai G20 S-TWIN electron microscope operating at an acceleration voltage of 200 kV. For the observation of aggregates, a drop of sample solutions was placed on carbon-coated copper grids. The surface-deposited nanoparticles were negatively stained with 2% uranyl acetate for 40 s before the TEM studies.

***Cell culture*:** MDA-MB-231 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (penicillin and streptomycin) at 37 °C in an atmosphere containing 5% CO_2.

***Confocal laser scanning microscope (CLSM)*:** The TNPs and C-TPN**s** treated MDA-MB-231 cells were investigated on a Zeiss LSM710 confocal laser scanning microscope (Jena, Germany).

***Cell cytotoxicity assay*:** Cells were seeded in a 96-well plate and incubated for 24 h. Then cells were treated with materials with a serial of concentrations at 37 °C in a humidified environment containing 5% CO_2 for another 24 h. Cell Counting Kit (CCK-8) was utilized to detect cell survival rate.

***Wound healing assay*:** In monolayer culture, scratches produce callus area between cells, and then monitor the cells growth to scratch to reflect the migration phenomenon. The area of

wound healing was photographed under a microscope (Leica DMI6000B, Germany).

***Migration assays and Invasion assays*:** Transwell chambers (Corning, China) were placed in the corresponding culture plate. Upper and lower culture was apart by polyethylene terephthalate (PET) membrane apart. As a result of PET membrane permeability, the lower the composition in the culture can affect the interior of cells. This method can study the lower the culture influence on cell growth, migration and invasion.

***Statistical analysis*:** Data are presented as the mean ± standard deviation (SD). Comparison between groups was analysis with the Student's t-test. Differences were considered statistically significant when the p values were less than 0.05 ($p < 0.05$). The level of significance was defined at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

1.3 Supporting Figures

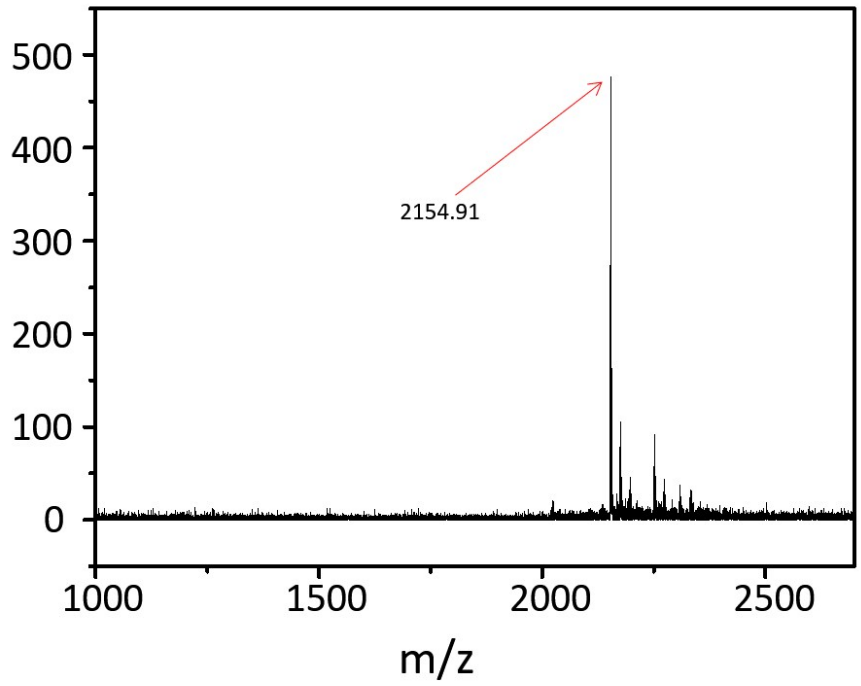

Figure S1. MALDI-TOF mass spectrum of TNP peptide.

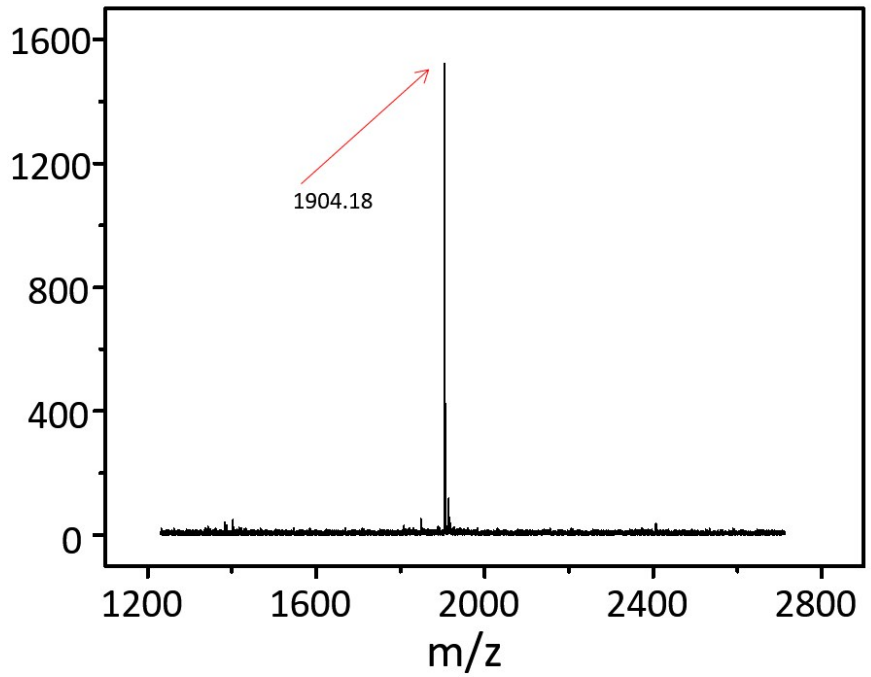

Figure S2. MALDI-TOF mass spectrum of C-TNP peptide.

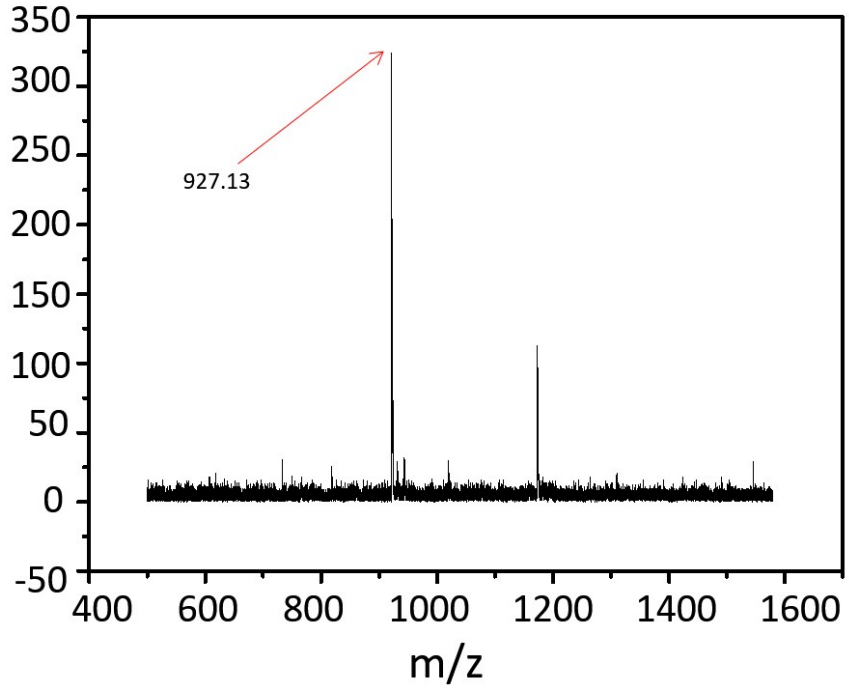

Figure S3. MALDI-TOF mass spectrum of RRRKRR peptide.

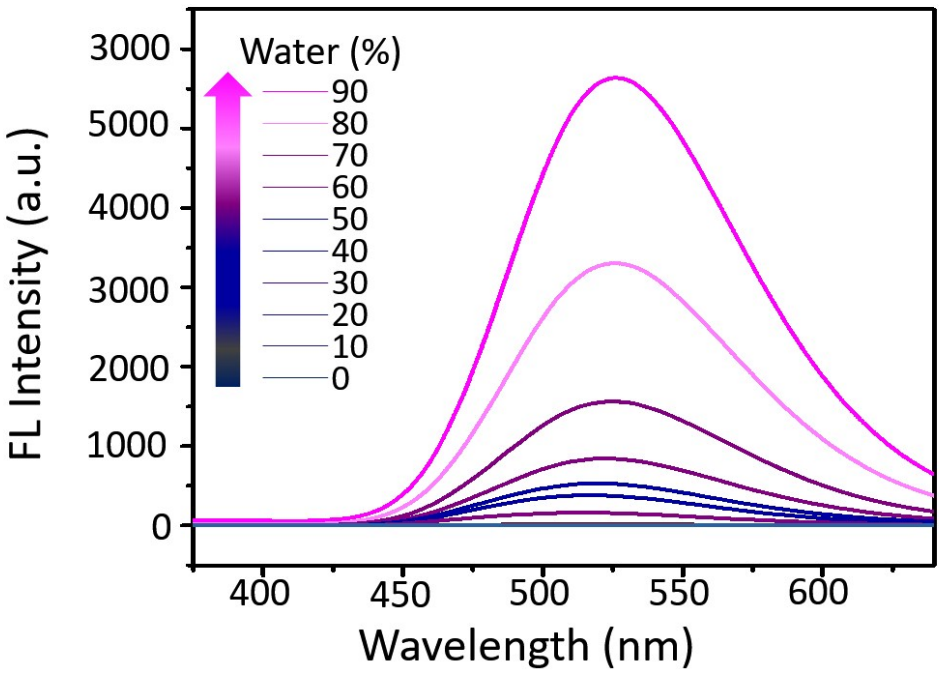

Figure S4. Fluorescence spectra characteristics of self-assembly process to form C-TNPs (c = 3.0×10^{-5} M) in mixed H_2O/DMSO solutions.

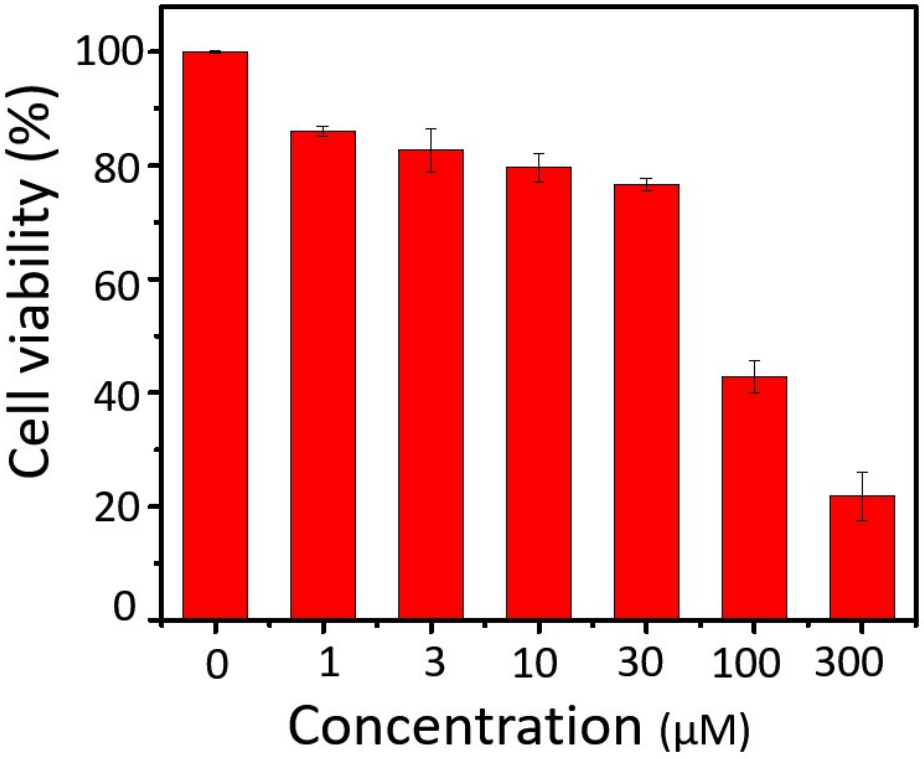

Figure S5. Cell cytotoxicity assay of TNP peptide.

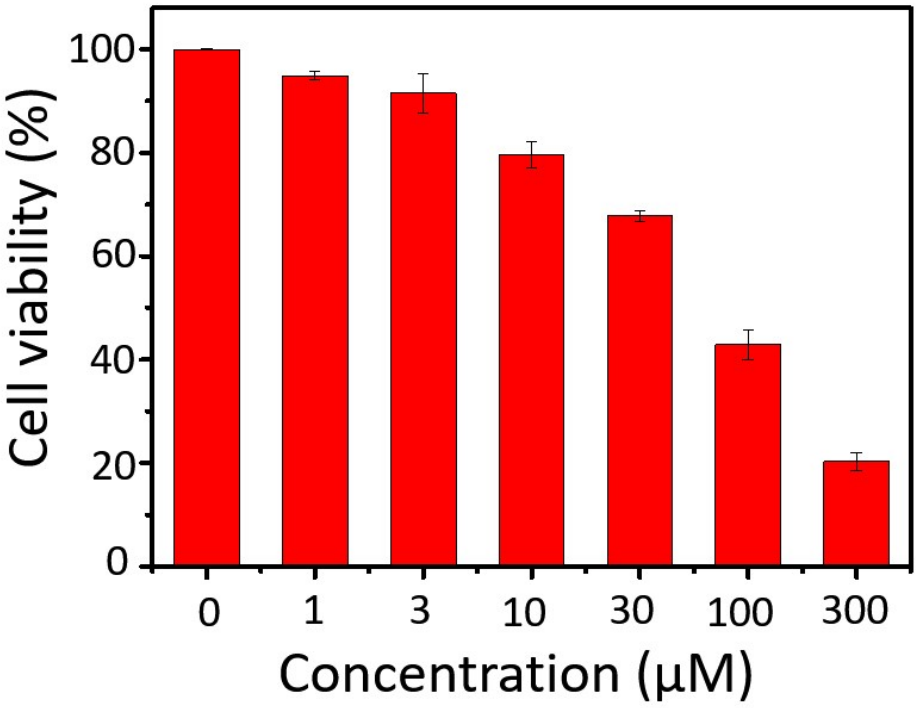

Figure S6. Cell cytotoxicity assay of C-TNP peptide.

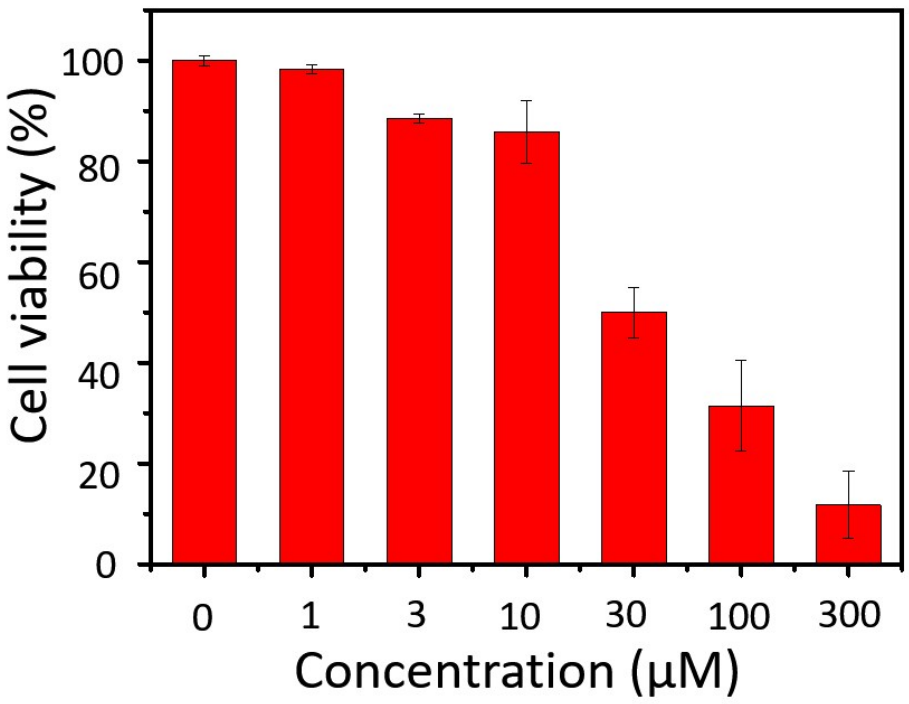

Figure S7. Cell cytotoxicity assay of RRRKRR peptide.

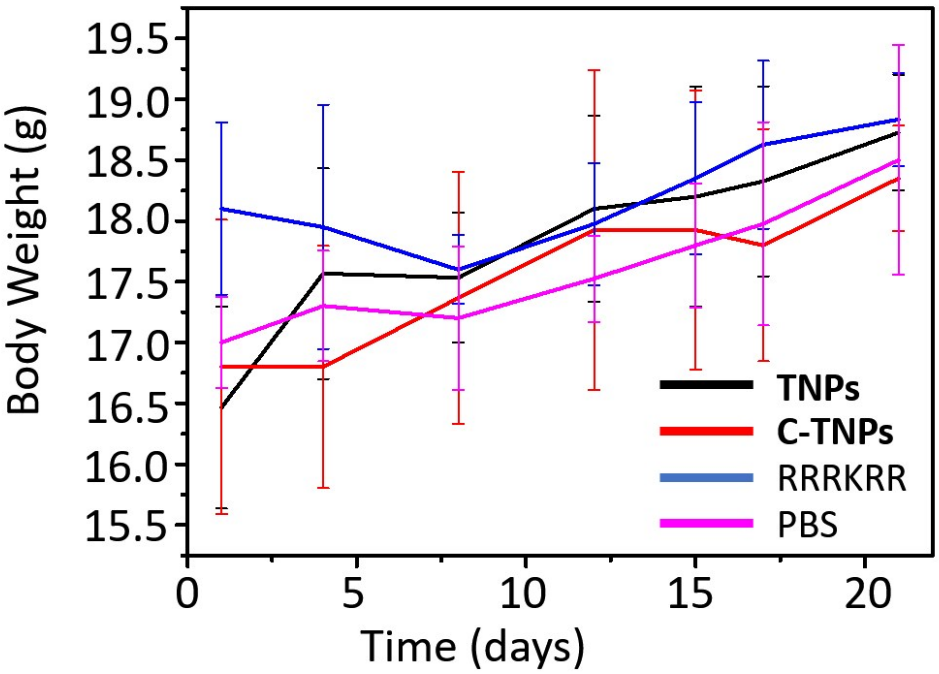

Figure S8. Body weight of mice treated with TNPs, C-TNPs, RRRKRR and PBS, respectively.

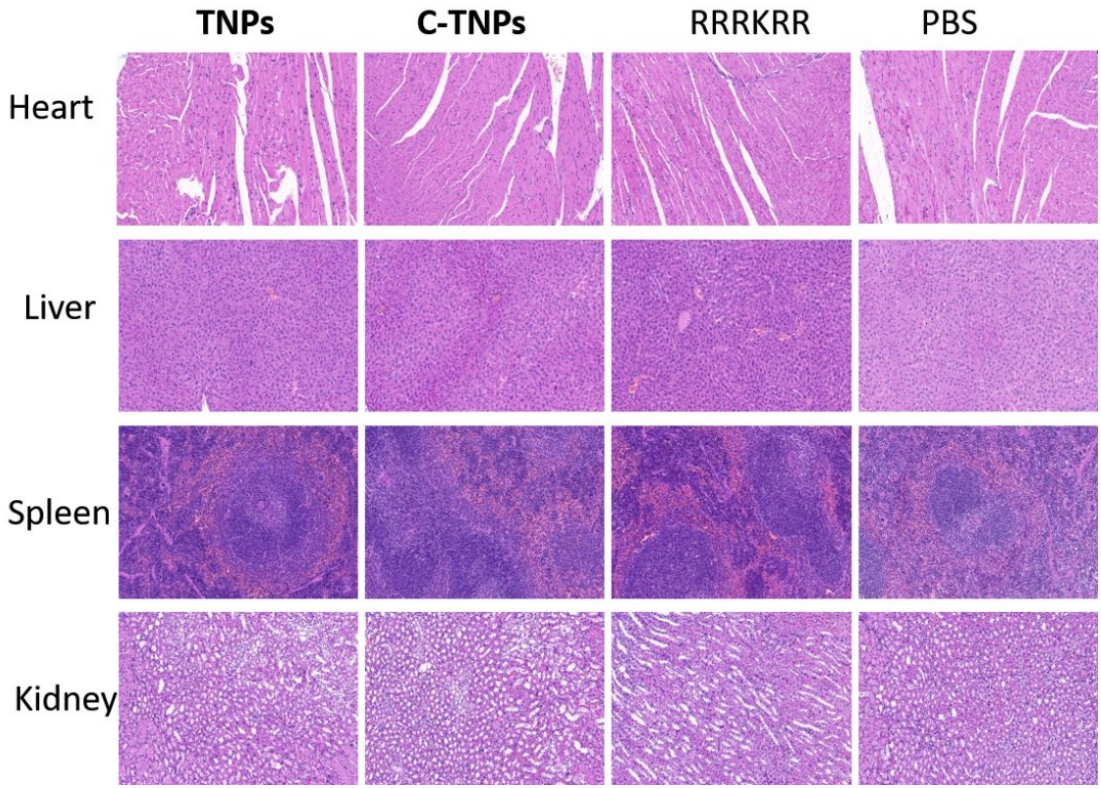

Figure S9. H&E staining images of Heart, Liver, Spleen, Kidney of MDA-MB-231 tumor bearing mice treated with TNPs, C-TNPs, RRRKRR and PBS, respectively.