SUPPLEMENTAL INFORMATION

Peptide Adhesive Specifically Induces Microtubule Condensation

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Materials and Methods

Materials

All reagents and solvents for organic synthesis were obtained from commercially available sources and used without further purification. O-(benzotriazol-1-yl)N,N,N,N'-tetramethyluronium hexafluorophosphate (HBTU), piperidine, 4-methylmorpholine (NMM), and trifluoroacetic acid (TFA) were purchased from Sigma Aldrich Chemical Co. All Fmoc-protection 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 Biotechnology, China. Tubulin proteins (HiLyte Fluor™ 647 labeled, from porcine brain) were obtained from Cytoskeleton, Inc. The EJ cell lines were purchased from the cell culture center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Cell culture medium and fetal bovine serum (FBS) were purchased 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 purchased from Corning (Corning, New York, USA). All the other solvents used in the research were purchased from Beijing Solarbio Science & Technology Co.,Ltd.

Methods

Synthesis of the peptides

The peptides T1 and T2 were synthesized manually by standard Fmoc-chemistry solid-phase peptide synthesis. Wang-resin was used as solid phase support. Peptides were determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS, Brook Dalton, USA).

The preparation and characterization of T1-NPs and T2-NPs

The peptides T1 and T2 were respectively dissolved into DMSO to form a mother liquid (40 mM). Then the solution was dilute containing DMSO and water (the DMSO content is 10%, 30%, 50%, 70%, 90% respectively) to make the final concentration 40 μM. The UV-vis absorption and fluorescence spectra of the T1-NPs and T2-NPs solutions were measured to verify the formation of T1-NPs and T2-NPs. T1-NPs and

T2-NPs for other experiment including morphology and function in solution, *in vitro*, *in vivo*, were used in PBS with DMSO 1%.

Morphological transformation in solution and *in vitro*.

TEM characterization.

The prepared T1-NPs and T2-NPs with the concentration of 40 μM in water (1% DMSO) cultured for 0, 0.5, 4, and 24 h, were drip-coated onto the carbon-coated copper mesh and settled for 10 minutes. The excess liquid is removed with filter paper. The uranyl acetate was then dripped on the copper mesh for 3 minutes, removed, and dried under vacuum for measurement.

DLS characterization in solution.

The average sizes of nanoparticles with concentration of 40 μM in water (1% DMSO) were calculated based on triple results. The measurement condition was 25 °C and a detection angle of 90°, and the raw data was analyzed by the Zeta Sizer software package.

Zeta size characterization in solution.

The average sizes of nanoparticles with concentration of 40 μM in water (1% DMSO) were calculated based on triple results. The measurement condition was 25 °C. The detailed introduction to zeta sizer, and Table 1 provides detailed instrument information.

Table 1. Instrument details for Zetasizer.

Instrument model	Zetasizer Nano ZS
Parameters measured	Size, Zeta potential, Molecular weight, A2
Temperature control range	0 °C to 90 °C +/- 0.1 °C
Condensation control	Purge using dry air
Standard laser	4 mW, 633 nm
Correlator	25 ns to 8000 s, max 4000 channels

***In vitro* and *in vivo* confocal imaging of nanomaterials**.

The tubulins labeled by HiLyte Fluor™ 647 were collected by briefly centrifuge and were resuspended in G-PEM buffer (80 mM PIPES, pH 6.9, 2 mM $MgCl_2$, 0.5 mM EGTA, 1 mM GTP plus 10% glycerol, 5 μL) on ice. Then the tube was placed at 37°C for 20 min. 2 μL of taxol (TXD01, Cytoskeleton, Inc) was added into 18 μL of G-PEM plus 10% glycerol buffer at room temperature to obtain taxol stock (300 μM). Then, taxol stock was incubated with the microtubule reaction, at 37 °C for 5 min, to obtain microtubule stock which was kept in dark place at room temperature. For microscopic observation, 1 μL of microtubule stock was diluted into 200 μL in warm G-PEM buffer plus 30% glycerol and 20 μM taxol. Finally, 100 μL above diluted microtubule solution was cultured with 10 μL T1-NPs (40 μM), T2-NPs (40 μM), PBS, respectively, for 1, 12, 24 h, which was dripped on the glass slide for observation. The fluorescence of T1-NPs and T2-NPs were detected by 405 nm channel, and microtubules were detected by 640 nm channel.

For immunofluorescence assay of cells, bladder cancer cells (EJ) were incubated with T1-NPs and T2-NPs, respectively overnight in confocal dishes and fixed with 4% paraformaldehyde. After a brief wash with PBS, bladder cancer cells were permeabilized with 0.5% Triton X100 in PBS and blocked with 10% goat serum for 2 h at room temperature. Cells were then incubated overnight with anti-β-tubulin antibody at 4 °C followed by 1 h incubation with fluorescence conjugated secondary antibody at room temperature. After washing with PBST, confocal dishes were then examined under confocal microscope (LSM710, Zeiss, Germany).

T1-NPs and T2-NPs were respectively administered into EJ cell-xenografted BALB/c nude female mice. Tumours were harvested and sectioned. The sections were incubated by primary antibody overnight at 4 °C. Then, primary antibody working solution was removed and the sections were washed once with TBST buffer for 5 min 3 times. Then the secondary antibody labeled with Alexa Fluor 647 was added at room temperature in dark. After 1 h, the secondary antibody work solution was removed and the sample was wash with TBST for three times, each time for 5 min. Anti-fluorescence attenuation sealant was added and the sections were observed under confocal laser scanning microscope (LSM710, Zeiss, Germany).

Western blot text

The total proteins were extracted from EJ cells with RIPA lysis buffer containing protease inhibitor. A BCA protein assay kit (Solarbio, Beijing, China) was performed to quantify total proteins concentrations. The protein samples (50 μg) were loaded into each holes and separated using a 10% SDS–PAGE gel, and then transferred to a nitrocellulose membrane. The membranes were incubated with primary antibody against β-tubulin (1:1000 dilution, Solarbio) overnight at 4 °C after blocking at room temperature in 5% free-fat dried milk in Tris buffer solution for 2 h. Finally, the membranes were rinsed with TBS-T for 30 minutes before being incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. ECL imaging method was used to capture protein bands.

Flow cytometer

500 μL of EJ cells (1×10^5 cells) were added in each tube with pretreatment of TI-NPs or T2-NPs (40 μM, 24 h) which was then filled with an additional 5 μL of Annexin V-FITC, following by gently stir away from light for 10 minutes at room temperature. 5 μL of propidium Iodide was added and incubated at room temperature for 5 minutes, shielded from light. Flow cytometry analysis of EJ cells was carried out on BD FACSAriaIII.

***In vivo* therapy**

Animal conservation and euthanasia were carried out under the approval of the Laboratory Animal Conservation Administrative Group of the National Center for Nano Science and Technology. Ethics No. NCNST21-2103-0605.

A total of 2×10^6 EJ cells resuspended in PBS were mixed with equal volumes of Matrigel (BD Bioscience) which was injected subcutaneous into the flanks of 6-week-old nude mice (BALB/c). When the tumour volume reached approximately 60 mm^3, the BALB/c nude mice were randomly divided into 3 groups (n = 4 for each group) and treated intravenously with PBS, T1-NPs (400 μM, 200 μL), T2-NPs (400 μM, 200 μL), respectively. The mice were injected every other day during first 2 weeks. Tumour size and body weights were measured every other day. Tumour volume was calculated as follows:

V_{tumour} = length × width × width/2

Biological safety experiment

Hematoxylin-Eosin (H&E) staining and biochemical index test were used to study the safety of T1-NPs, T2-NPs and PBS to various tissues (heart, liver, spleen, lung, kidney) and blood taken 72 h after the i.v. injection of T1/2 NPs and PBS solution (200 μL, 400 μM). These tissues were fixed in 4% paraformaldehyde PBS buffer for 12 h, then embedded in paraffin and sectioned. The sections were further stained with hematoxylin-eosin.The blood was layered at room temperature, and the supernatant was obtained to test the biochemical indexes. Routine blood examination were performed using the automatic hematology analyzer (BC-5100, Mindray, China) with whole blood samples.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). The comparison between groups was analyzed 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 ***$p < 0.001$.

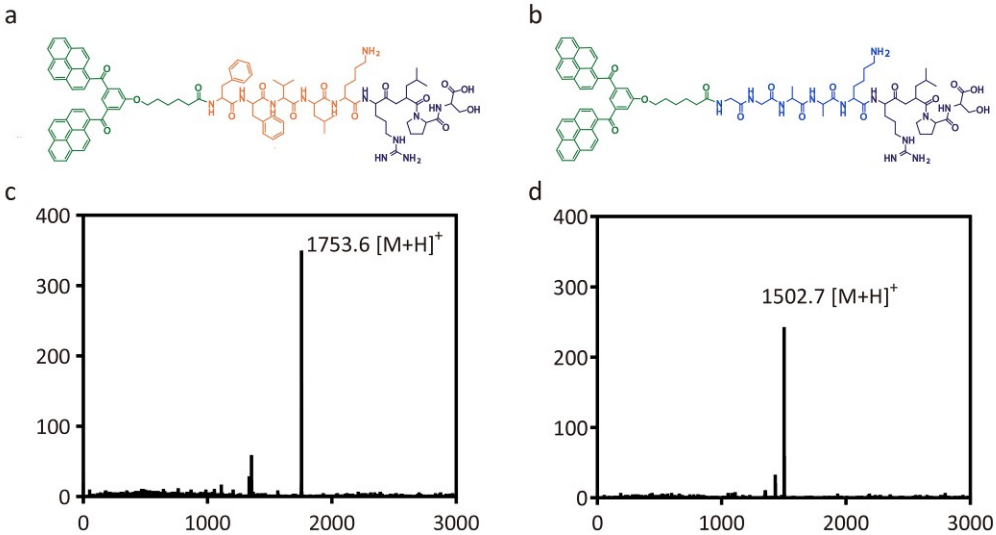


Figure S1. The MALDI-TOF-MS spectrum of Microtubule Trapping Peptide a) T1, BP-FFVLK-RLPS, b) T2, BP-GGAAK-RLPS.

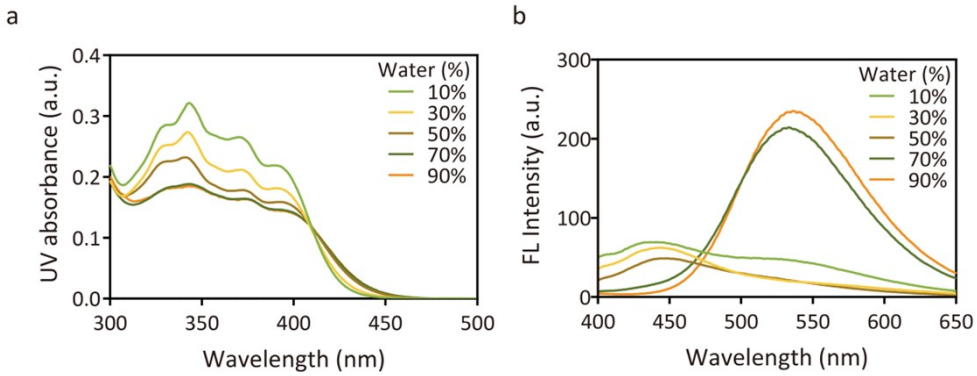


Figure S2. a) The UV-vis absorption spectrum of T2 in mixed solvents with increasing ratio of water in DMSO (10%~90%). b) Fluorescence spectrum of T2-NPs in mixed solvents with increasing ratio of water in DMSO (10%~90%) at the excitation wavelength of 380 nm.

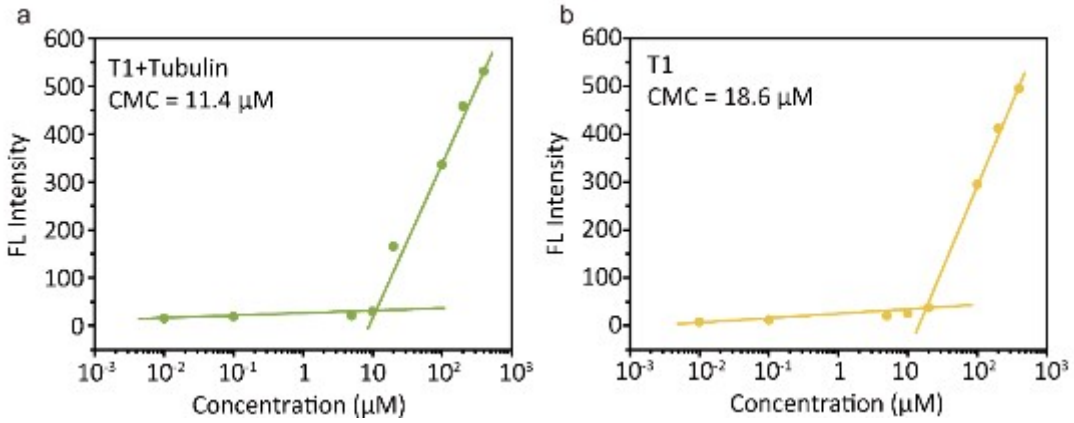


Figure S3. The critical aggregation concentration assay for T1 + Tubulin and T1 in PBS with 1% DMSO using concentration-dependent fluorescence assay.

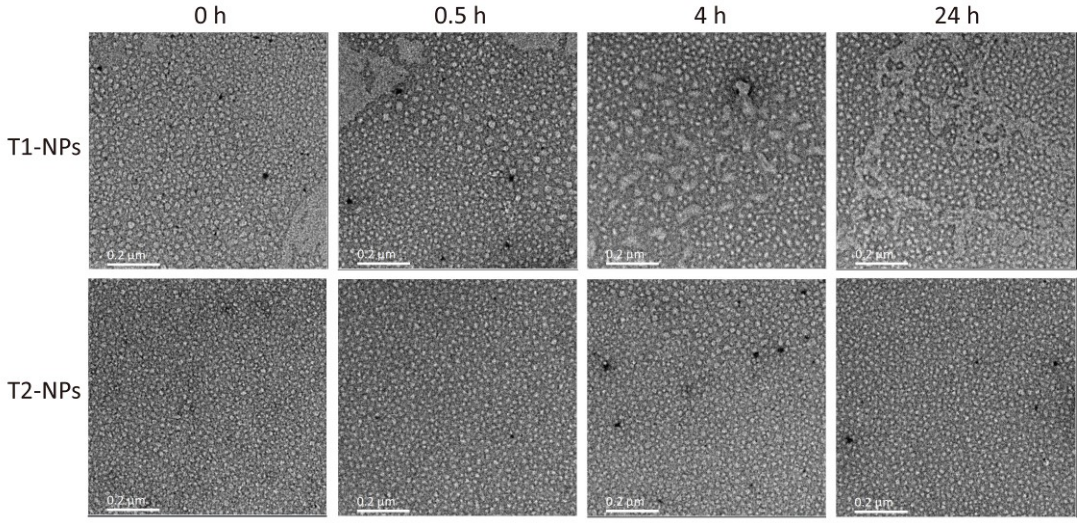


Figure S4. The time-dependent TEM images (0, 0.5, 4, 24 h) of T1-NPs and T2-NPs. The scale bar is 200 nm.

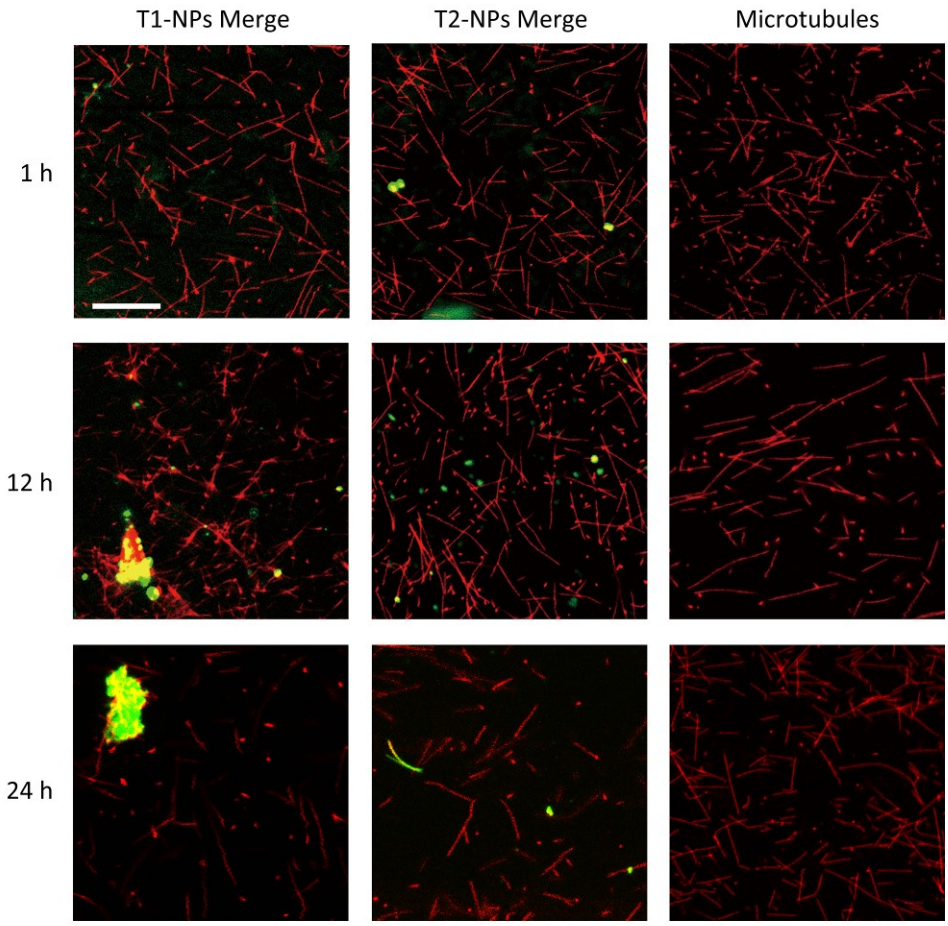


Figure S5. The interaction between HiLyte Fluo^{TM} 647 labelled microtubules (red) and T1-NPs or T2-NPs (green) were verified by confocal laser scan microscopy after culturing microtubules with nanoparticles for 1, 12, 24 h. The scale bar is 10 μm.

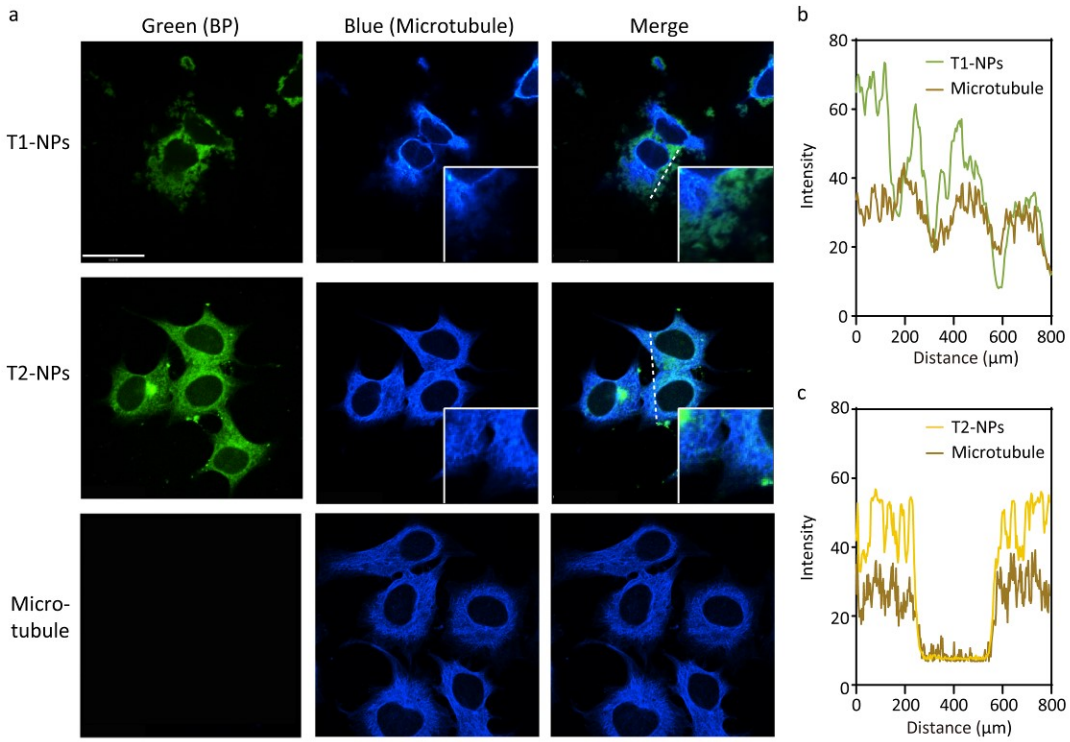


Figure S6. T1-NPs targeted and entangled microtubule in MCF-7 cell. a) CLSM image of T1-NPs and T2-NPs cultured with MCF-7 cells for 24 h. The scale bar is 10 μm. The white dash lines indicate co-localization region for quantitative analysis. The inset images are enlarged images. b) and c) Co-localization curvs of T1-NPs or T2-NPs and microtubules, respectively.

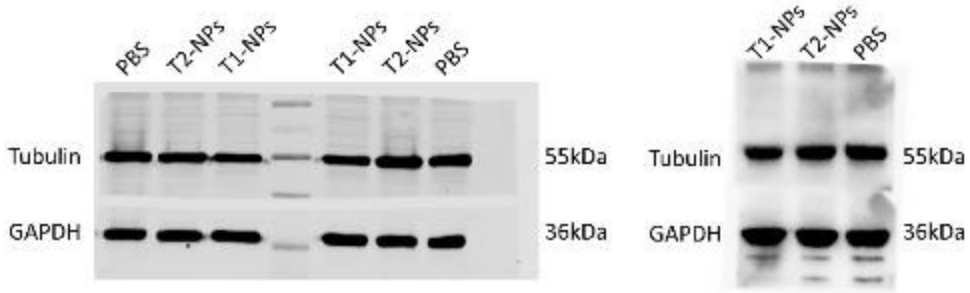


Figure S7. The full-size blot for Figure 3d.

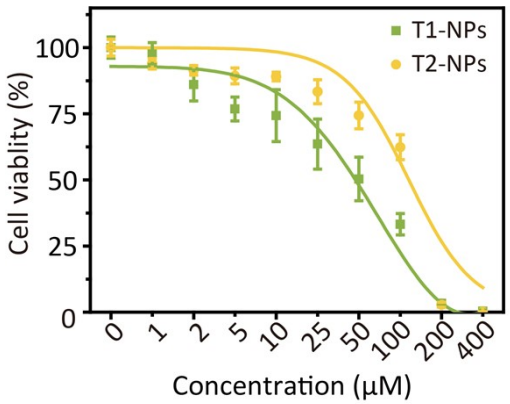


Figure S8. Cell viability of EJ cells treated with T1-NPs and T2-NPs by CCK-8 assay for 24 h.

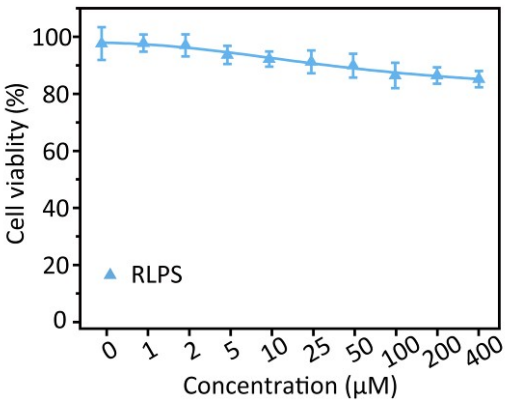


Figure S9. Cell viability of EJ cells treated with RLPS by CCK-8 assay for 24 h.

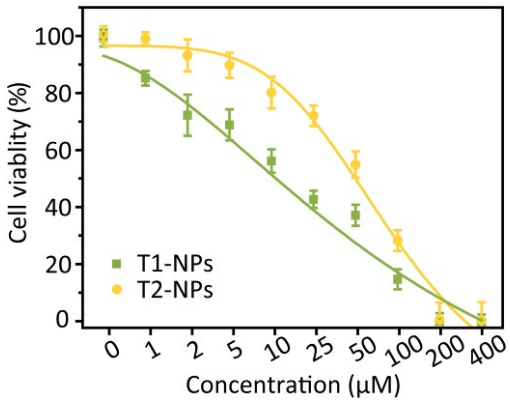


Figure S10. Cell viability of EJ cells treated with T1-NPs and T2-NPs by CCK-8 assay for 48 h.

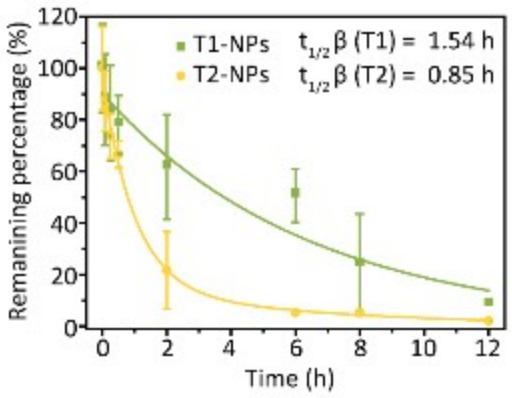
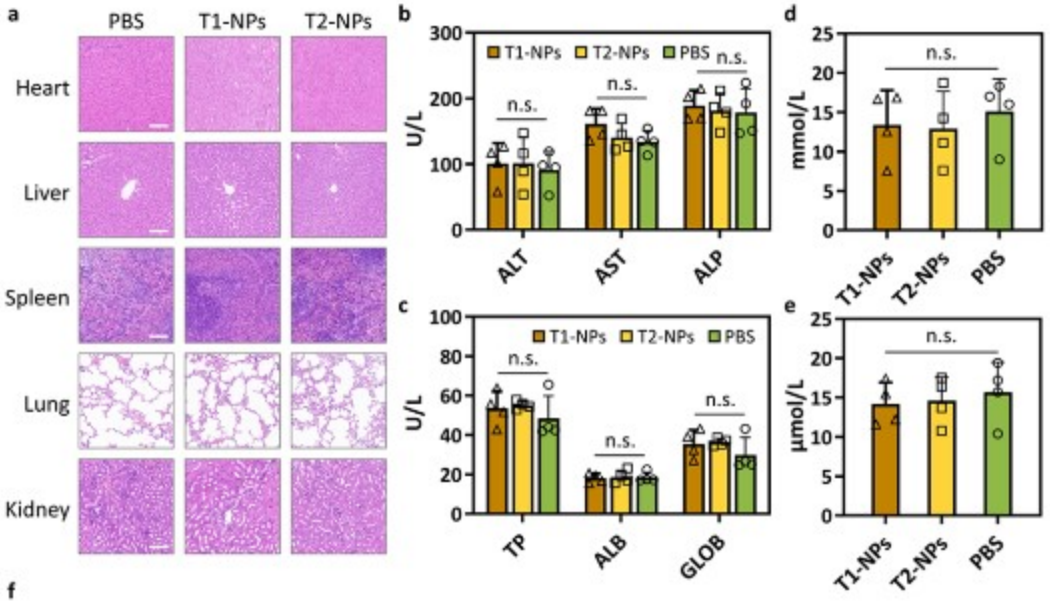


Figure S11. Blood circulation profile of T1-NPs and T2-NPs. The data are presented as means ± s.d., n = 3.



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Group	WBC 10^9/L	Neu %	Lym %	Mon %	RBC 10^{12}/L	HGB g/L	PLT 10^9/L
PBS	3.39±1.50	62.03±12.55	18.30±6.77	8.65±8.45	6.06±0.95	144.25±20.32	603.75±110.31
T1-NPs	5.00±3.06	69.23±3.37	13.58±2.84	6.23±5.16	6.29±0.40	151.75±10.56	545.50±184.56
T2-NPs	4.06±2.35	60.78±9.65	22.58±11.36	9.15±2.98	5.79±1.17	144.00±21.82	602.75±182.35

Figure S12. The biocompatibility evaluation of peptide based nanofibers. a) H&E staining of organ (Heart, Liver, Spleen, Lung, Kidney) after treatment with PBS, T1-NPs and T2-NPs (200 μL, 400 μM). Scale bar = 100 μm. b) Serum levels of ALT, AST and ALP, the results are shown as means ± s.d. n = 3, n.s. represents no significance. c) Serum levels of TP, ALB and GLOB, the results are shown as means ± s.d. n = 3, n.s. represents no significance. d) and e) Serum levels of BUN and CREA related with glomerular filtration rate, the results are shown as means ± s.d. n = 3, n.s. represents no significance. f) Blood routine examination results of WBC, Neu, Lym, Mon, RBC, HGB, PLT, the results are shown as means ± s.d. n = 3.