Electronic Supplementary Information

A ROS-Responsive Supramolecular Peptide Hydrogel Attenuates Rheumatoid Arthritis by Modulating Synoviocyte Activity and Inflammatory Microenvironments

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Experimental section

Reagents and equipment

2-chlorotrityl chloride resin (Loading density: 0.58 mmol/g) and all Fmoc-protected amino acids used in solid-phase peptide synthesis were obtained from GL Biochem Co., Ltd (Shanghai, China) or Bide Pharmatech Co., Ltd (Shanghai, China). (S)-(+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid (Naproxen) was purchased from Meryer Biochemical Technology Co., Ltd (Shanghai, China). (9H-Fluoren-9-yl) methyl (2-aminoethyl) carbamate (Fmoc-$NHNH_2$) was purchased from Bide Pharmatech Co., Ltd (Shanghai, China). O-benzotriazol-1-yl-tetramethyluronium hexafluorophosphate (HBTU) was purchased from Shanghai Titan Scientific Co., Ltd (China). Piperidine and hydrogen peroxide (H_2O_2) were bought from Tianjin DaMao Chemical Reagent Factory. Trifluoroacetic acid (TFA) was purchased from Tianjin Kermel Chemical Reagent Co., Ltd. All other organic solvents were bought from Tianjin Jindong Tianzheng Fine Chemical Reagent Factory (China). Commercially available reagents were used without further purification, unless noted otherwise.

Dulbecco's Modified Eagle Medium (DMEM) were purchased from Thermo Fisher scientific (Beijing, China). Fetal bovine serum (FBS) were purchased from VivaCell Biosciences (Shanghai, China). Phosphate buffered saline (PBS) were bought from Beijing Solarbio Science&Technology Co., Ltd (China). Penicillin-streptomycin solution, 0.25% Trypsin-EDTA were purchased from Beijing Labgic Technology Co., Ltd. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT) was bought from HEOWNS Biochemical Technology Co., Ltd.

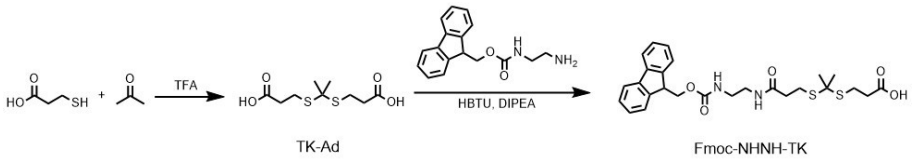
High performance liquid chromatography (HPLC) analysis was performed on Waters 2695 separations Module. Mass spectra (MS) were analyzed by LTQ-Orbitrap XL mass spectrometer (Thermo Fisher scientific, San Jose, CA, USA). The cellular fluorescence images were captured using AE2000 inverted fluorescence microscope (Motic, China). Transmission electron micrograph (TEM) images were obtained on a Tecnai G2 F20 transmission electron microscope (FEI, USA). Circular dichroism spectra were obtained on Jasco J-815 circular dichroism spectrometer (JASCO, Japan).

Rheology test was conducted by Discovery HR-20 TA Instruments.

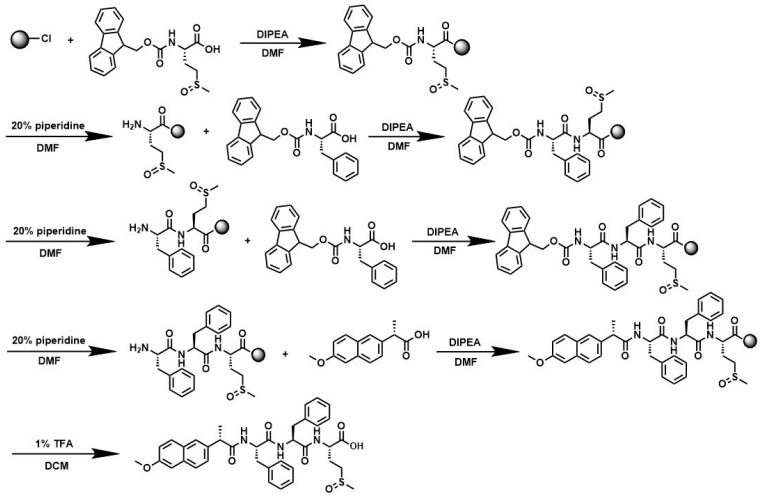
Cells and animals

Human umbilical vein endothelial cells (HUVEC) were purchased from the Shanghai Institutes of Biological Science, Chinese Academy of Science. Human Fibroblast-Like Synoviocyte Rheumatoid Arthritis (HFLS-RA) cells were purchased from Jennio Biotech Co.,Ltd (GuangZhou, China). Immunization Grade Bovine Type II Collagen and Incomplete Freund's adjuvant (IFA) were obtained from Chondrex (Washington DC, USA). CD®(SD) IGS Rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal experiments were approved by the Animals Experimentation Ethics Committee of Tianjin Medical University and carried out in accordance with the institutional guidelines.

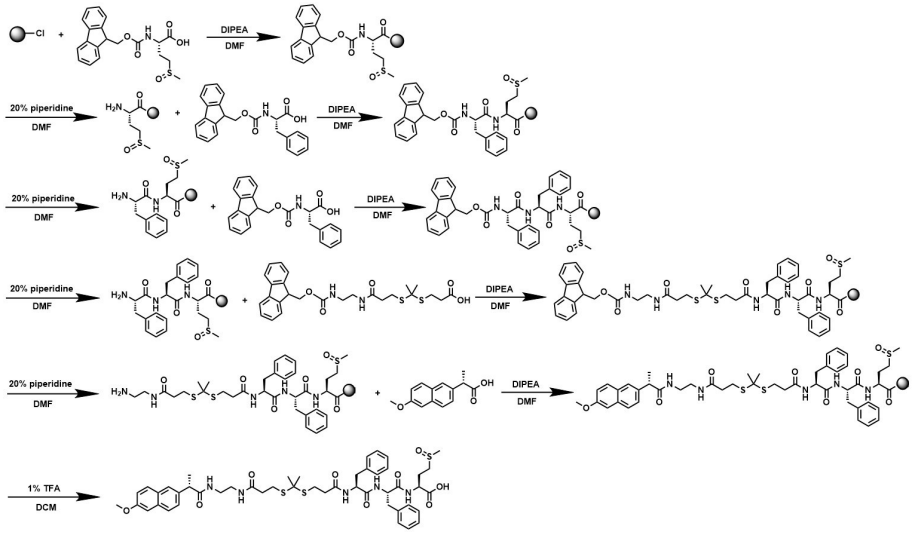
Supplementary figures



Scheme S1. The synthetic route for Fmoc-NHNH-TK.



Scheme S2. The synthetic route for **Nap-FFM^{O}**.



Scheme S3. The synthetic route for **Nap-TK-FFM^{O}**.

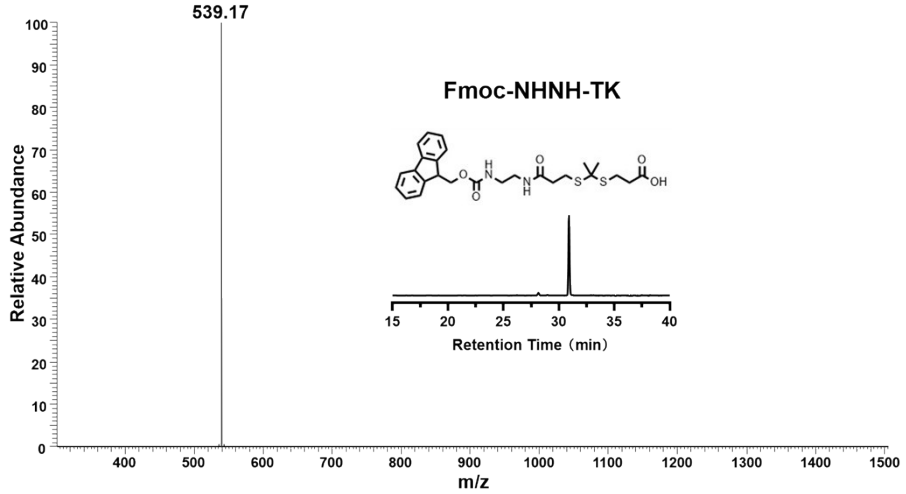


Fig. S1. MS spectrum and HPLC chromatogram (inset) of **Fmoc-NHNH-TK**. Wavelength for HPLC detection: 254 nm. MS calculated for **Fmoc-NHNH-TK** [(M+Na)$^+$]: 539.17; obsvd. [(M +Na)$^+$]: m/z 539.17.

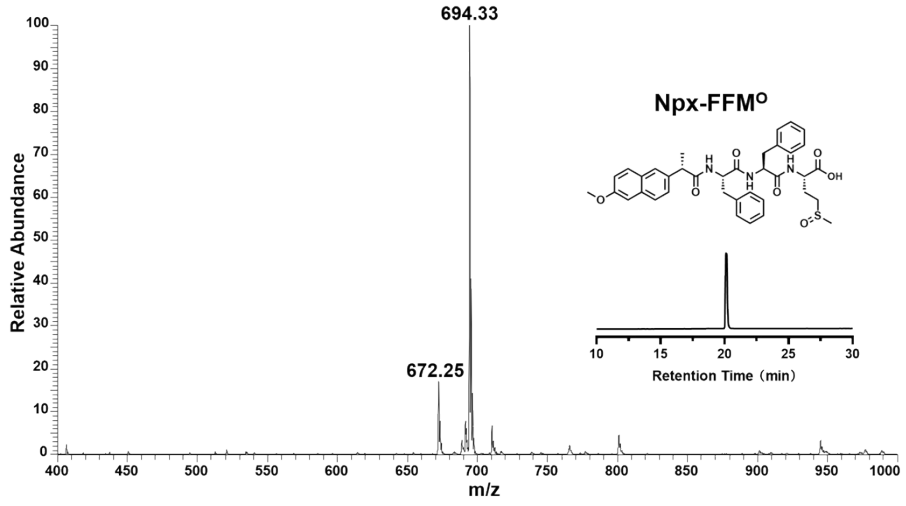


Fig. S2. MS spectrum and HPLC chromatogram (inset) of **Npx-FFM^O**. Wavelength for HPLC detection: 220 nm. MS calculated for **Npx-FFM^O** [(M+H)$^+$]: 672.27, [(M+Na)$^+$]: 694.26; obsvd. [(M+H)$^+$]: m/z 672.25, [(M +Na)$^+$]: m/z 694.33.

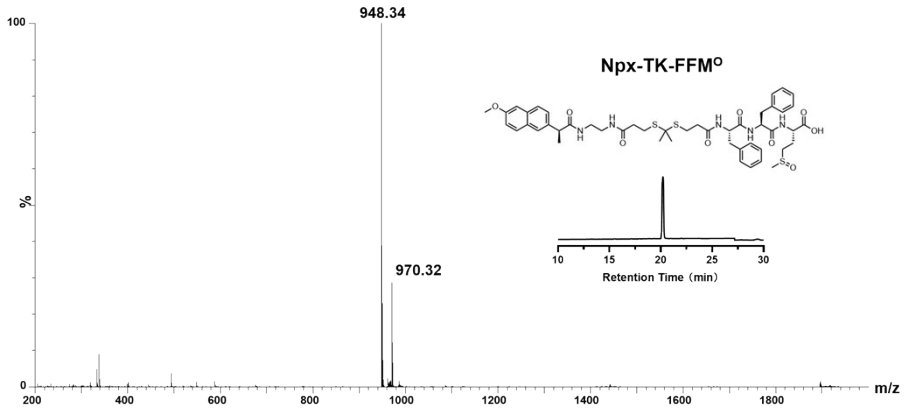


Fig. S3. MS spectrum and HPLC chromatogram (inset) of **Npx-TK-FFM^{O}**. Wavelength for HPLC detection: 220 nm. MS calculated for **Npx-TK-FFM^{O}** [(M+H)$^{+}$]: 948.37, [(M+Na)$^{+}$]: 970.35; obsvd. [(M+H)$^{+}$]: m/z 948.34, [(M +Na)$^{+}$]: m/z 970.32.

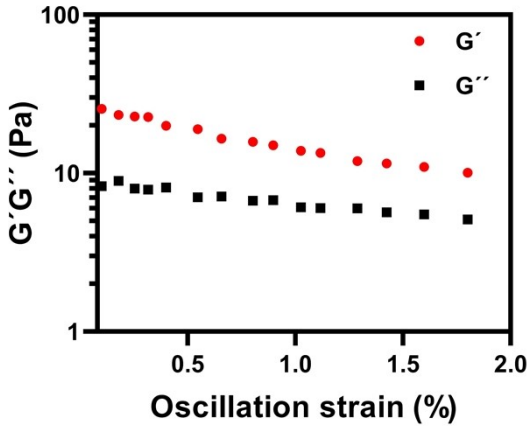


Fig. S4. Strain dependence of the dynamic storage modulus (G′) values and the loss modulus (G″) values of 4 wt% **Gel NTF** in PB at 25 °C (frequency: 1 rad/s).

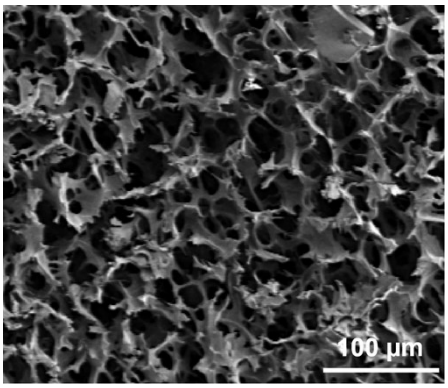


Fig. S5. SEM micrograph of **Gel NTF**.

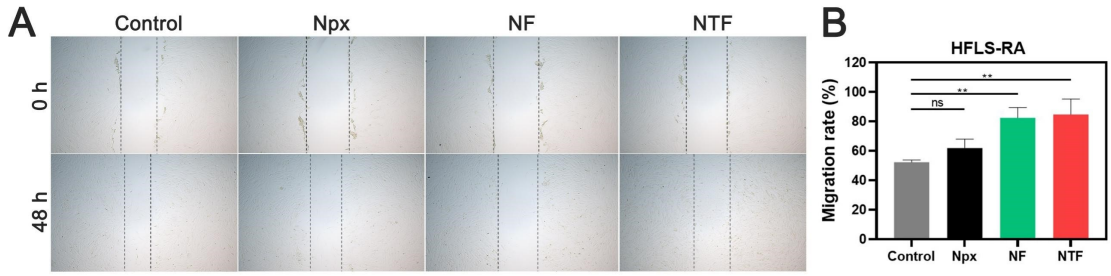


Fig. S6. (A) Wound-healing assay of HFLS-RA treated with different chemicals at 0 and 48 h. (B) Quantitative analysis of wound healing rate derived from the bright-field image of A. Data are presented as mean ± SD (n = 3). ***$p < 0.001$, ****$p < 0.0001$ compared with the control group.

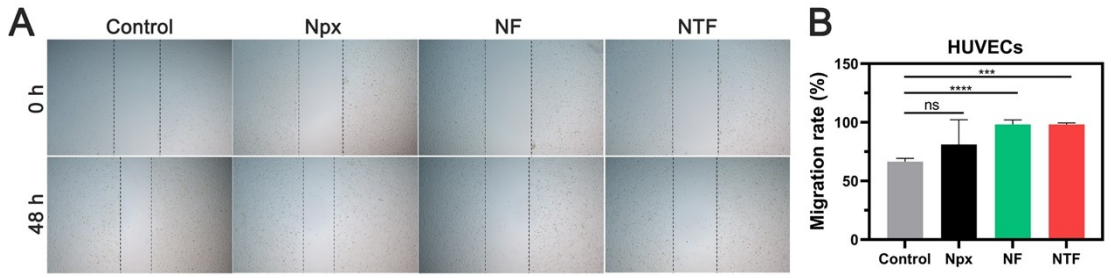


Fig. S7. (A) Wound-healing assay of HUVECs treated with different chemicals at 0 and 48 h. (B) Quantitative analysis of wound healing rate derived from the bright-field image of A. Data are presented as mean ± SD (n = 3). ***$p < 0.001$, ****$p < 0.0001$ compared with the control group.

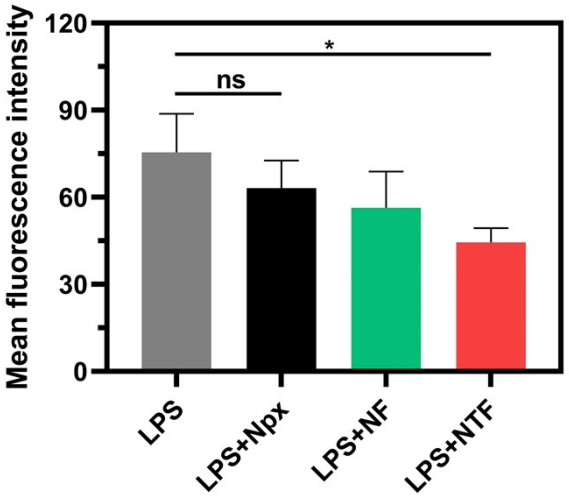


Fig. S8. Quantitative analysis of the fluorescence signal in Figure 2F. Data are presented as mean ± SD (n = 3). *$p < 0.05$ compared with the control group.

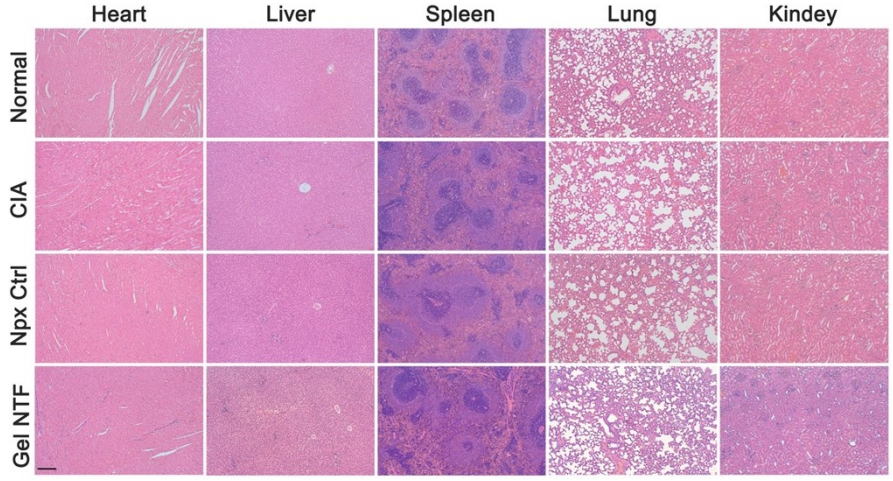


Fig. S9. H&E staining of major organs of CIA rats with different treatments. Scale bar: 100 μm.