Supplementary information

Early diagnosis of fibrogenic remodeling in idiopathic pulmonary fibrosis by targeting matrix metalloproteinase-2 with CT imaging†

Qingwen Xu,[‡a,b] *Bowei Li,*[‡b,d,e], *Chaochao Zhang,*[b,d,e] *Yuyao Sun*,[b,d,e] Mengli Song[b,d,e], Ying Li[b,d,e], *Lihua Li,*[*b] *Yan Wang,*[*a] *and Chenggong Yu*[*b,c,d,e]

[a]*The First Affiliated Hospital of Wannan Medical University (Yijishan Hospital of Wannan Medical University), Wuhu 241001, China*

E-mail: wangyan_scu@163.com (Y. Wang)

[b]*School of Pharmacy, Wannan Medical University, Wuhu 241002, China*

Email: llh05530226@126.com (L. Li)

[c]*Organoid Innovation Center, CAS Key Laboratory of Nano-Bio Interface, Division of Nanobiomedicine, Suzhou Institute of Nano-Tech and Nano-bionics, Chinese Academy of Sciences, Suzhou 215123, China*

E-mail: cgyu2018@sinano.ac.cn (C. Yu)

[d]*Anhui Provincial Engineering Research Center for Polysaccharide Drugs, Wannan Medical University, Wuhu 241002, China*

[e]*Anhui Innovative Center for Drug Basic Research of Metabolic Diseases, Wannan Medical University, Wuhu 241002, China*

‡ These authors contributed equally to this study.

† Electronic supplementary information (ESI) available.

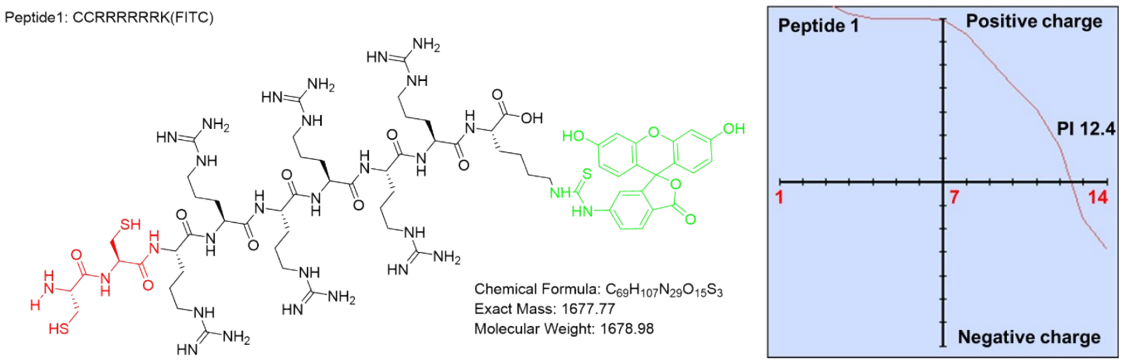
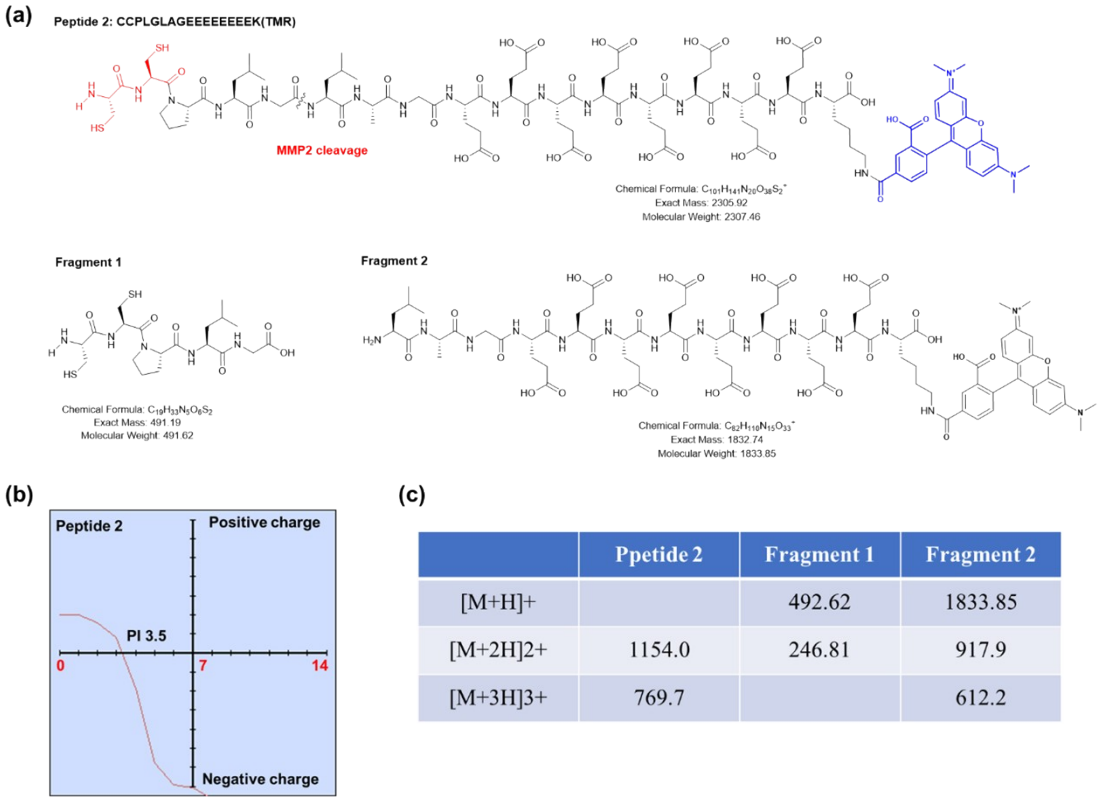


Fig. S1 Chemical structure and pH-dependent charge profile of Peptide 1. Molecular structure of Peptide 1 (CCRRRRRRK(FITC)) (left). Predicted net charge of Peptide 1 as a function of pH (right).



(c)

	Ppetide 2	Fragment 1	Fragment 2
[M+H]+		492.62	1833.85
[M+2H]2+	1154.0	246.81	917.9
[M+3H]3+	769.7		612.2

Fig. S2 Characterization of the MMP2-activatable Peptide 2. (a) Schematic of the Peptide 2 structure {CCPLGLAGEEEEEEEEE(Lys(TMR))} and its predicted cleavage fragments. (b) Predicted net charge of Peptide 2 as a function of pH. (c) Expected molecular ion peaks for the intact Peptide 2 and its MMP2-derived cleavage fragments.

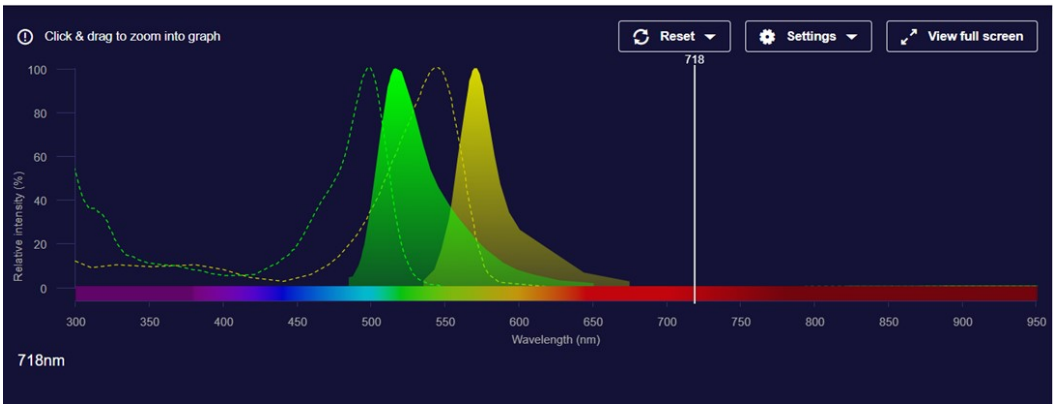
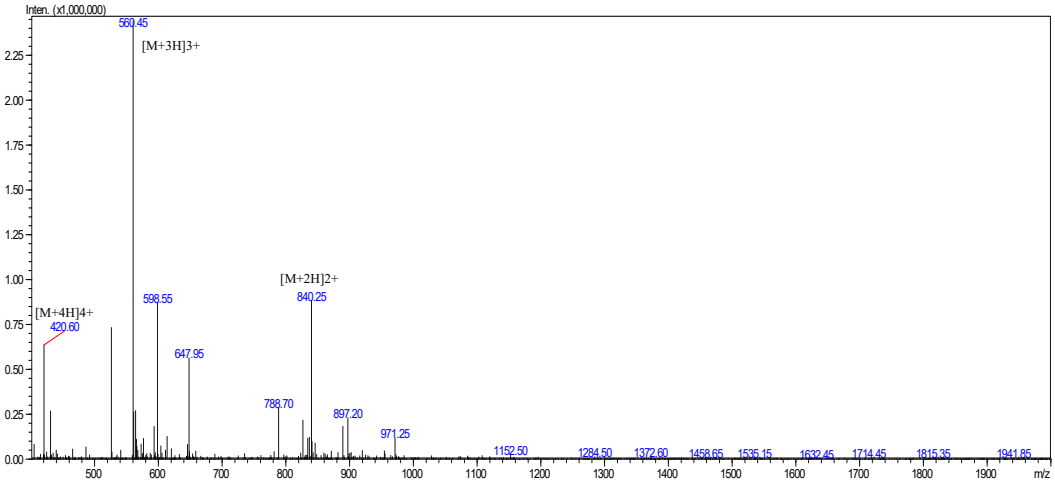


Fig. S3 Fluorescence emission spectra of the constituent fluorophores. Normalized emission spectra of FITC (labeling Peptide 1, green line) and TMR (labeling Peptide 2, yellow line).

MASS SPECTROMETRY REPORT



Sample Description		Instrument	SHIMADZU LCMS-2020		
Analyzed date:	2022/1/28	Probe:	ESI	Probe Bias:	＋4.5kv
Analyst:	Shen	Nebulizer Gas Flow:	1.5L/min	Detector:	1.2kv
Sample:	CK-9	CDL:	-20.0v	T. Flow:	0.2ml/min
M.W.:	1678.96	CDL Temp.:	250 °C	B. Conc.:	50%H2O/50%ACN
Lot. No.:	P220105-LL963236	Block Temp.:	400 °C		

Fig. S4 Mass spectrometry analysis of peptide 1.

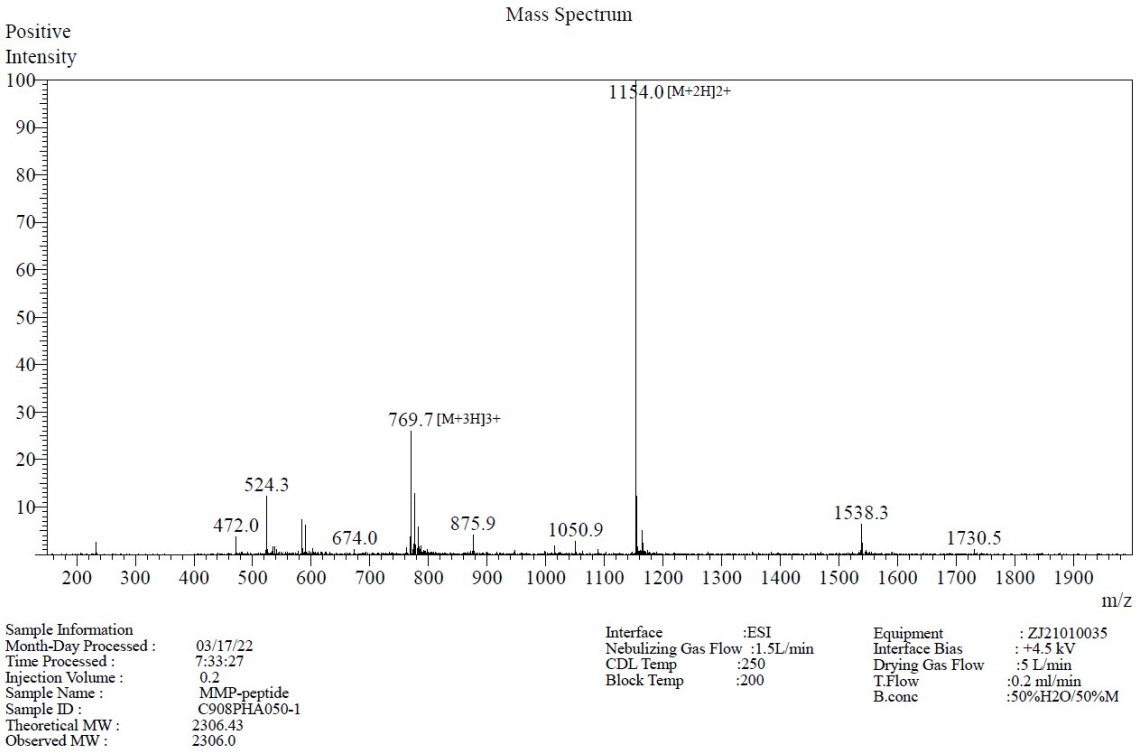


Fig. S5 Mass spectrometry analysis of peptide 2.

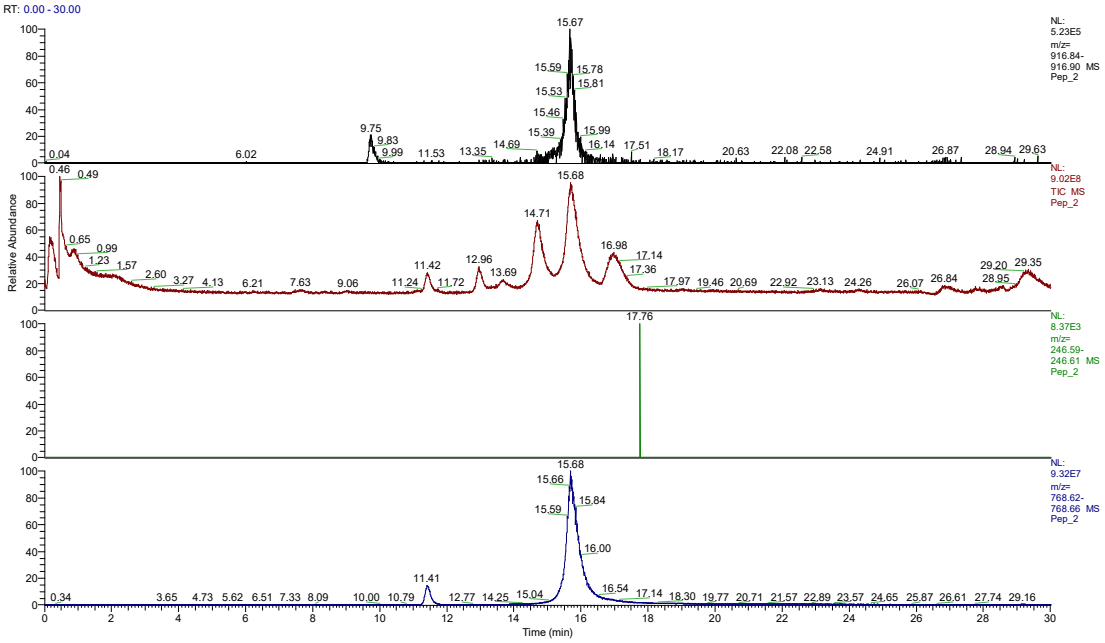


Fig. S6 Liquid chromatography and mass spectrometry analysis of the products resulting from MMP2 cleavage of peptide 2.

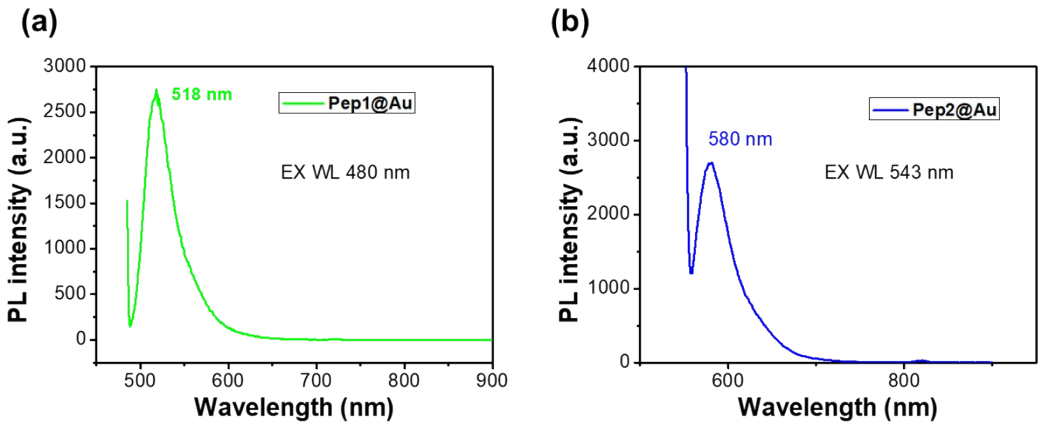


Fig. S7 Fluorescence emission spectra of pep1@Au and pep2@Au.

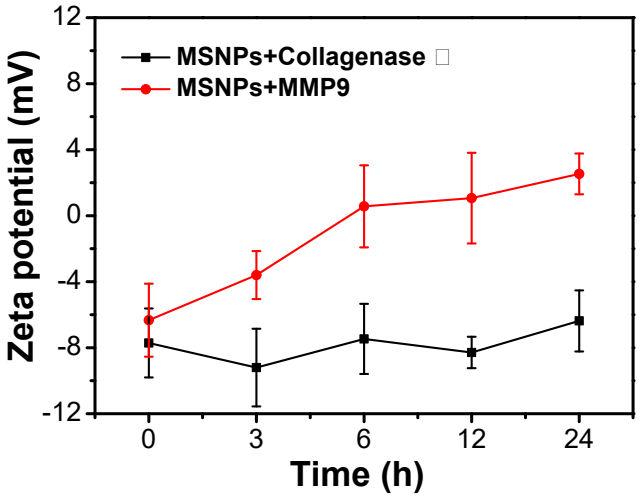


Fig. S8 Zeta potential of MSNPs treated with collagenase I or MMP9 for various durations. Data is presented as Mean ± SD, n ≥ 3.

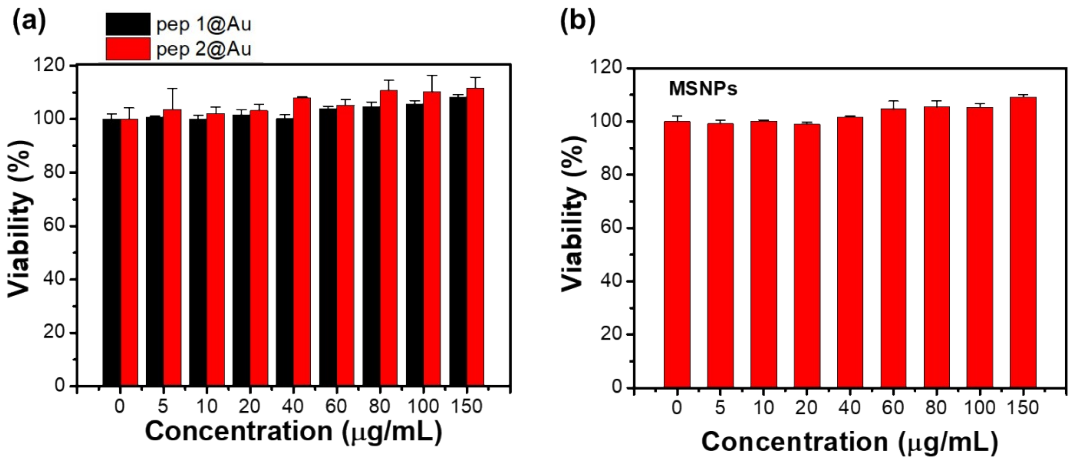


Fig. S9 (a) Cytotoxicity of pep1@Au and pep2@Au, and (b) MSNPs against A549 cells at varying concentrations. All data are presented as mean ± SD ($n \geq 3$).

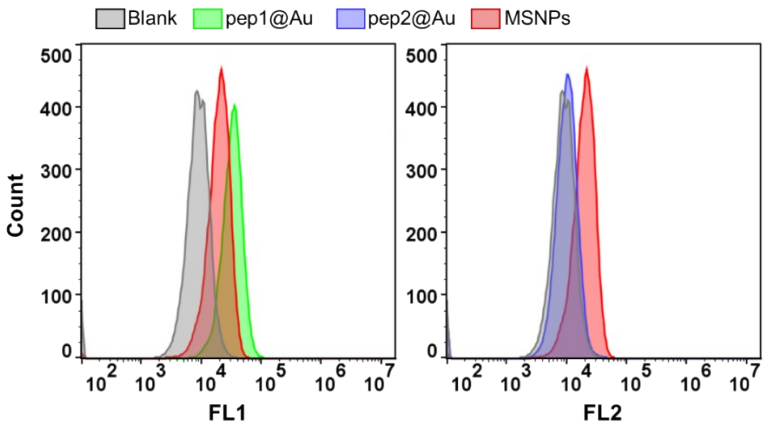


Fig. S10 Flow cytometry analysis of the mean fluorescence intensity in A549 cells after incubation with pep1@Au, pep2@Au, and MSNPs.

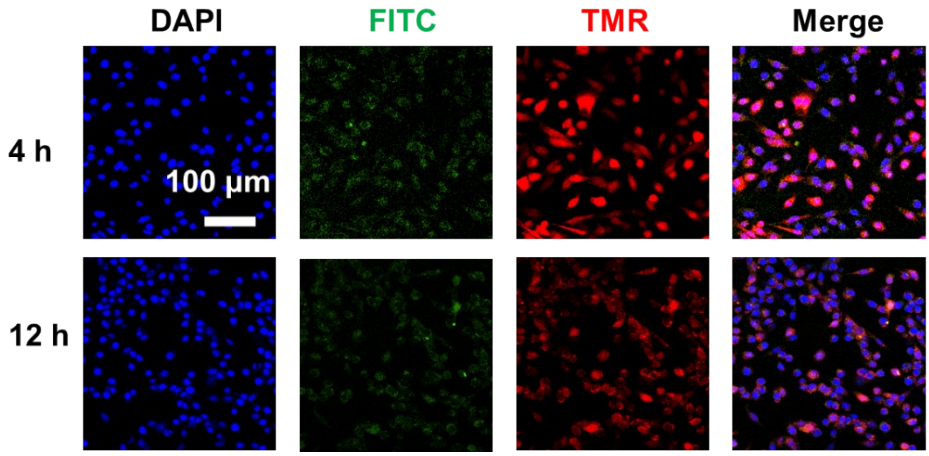


Fig. S11 LSCM images of A549 cells after co-incubation with MSNPs for 4 and 12 hours.

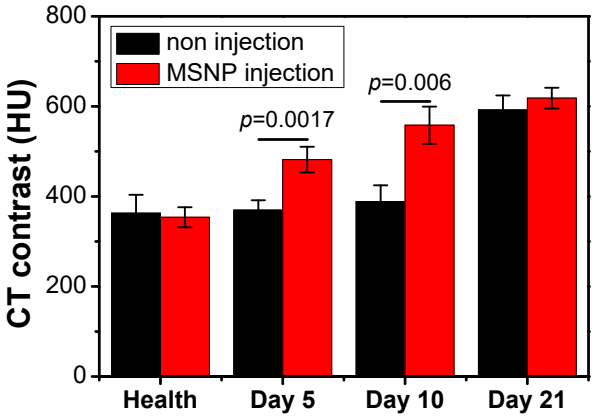


Fig. S12. CT intensity in the region of interest (ROI) before and after MSNP injection in healthy, and IPF model mice with 5, 10, and 21 days after BLM induction. Data are presented as mean ± SD, n = 3. A paired-samples t-test was used to compare signal intensity before and after contrast agent injection in mice, with the significance level set at $\alpha = 0.05$.

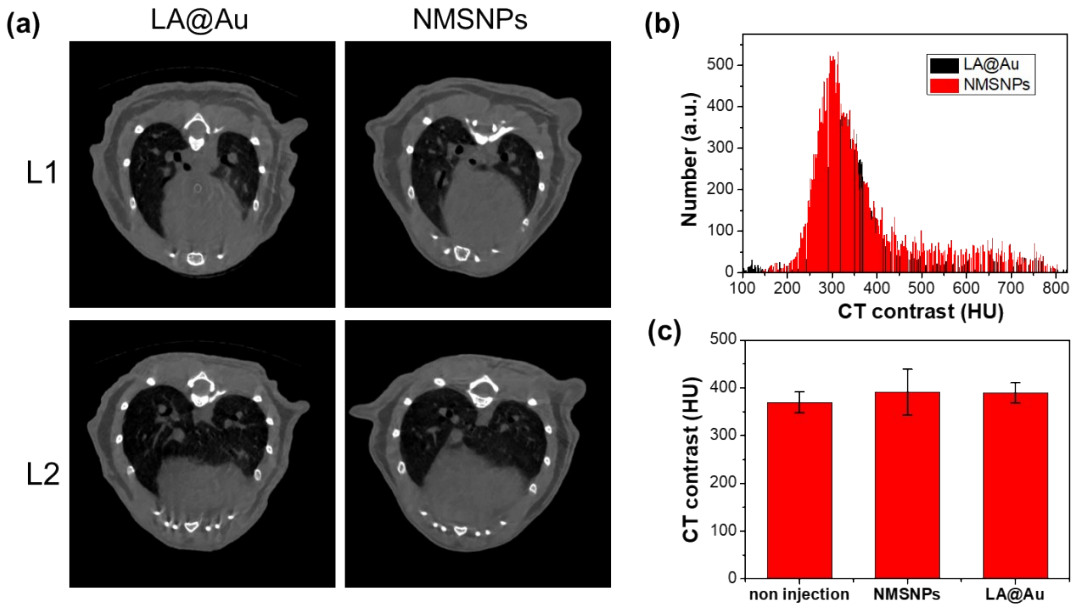


Fig. S13. (a) CT images and (b) the signal distribution of BLM model mice (day 5) after receiving injection of LA@Au or NMSNPs, respectively. (c) CT intensity of the ROI in the mentioned groups. Data is presented as Mean ± SD, n = 3.

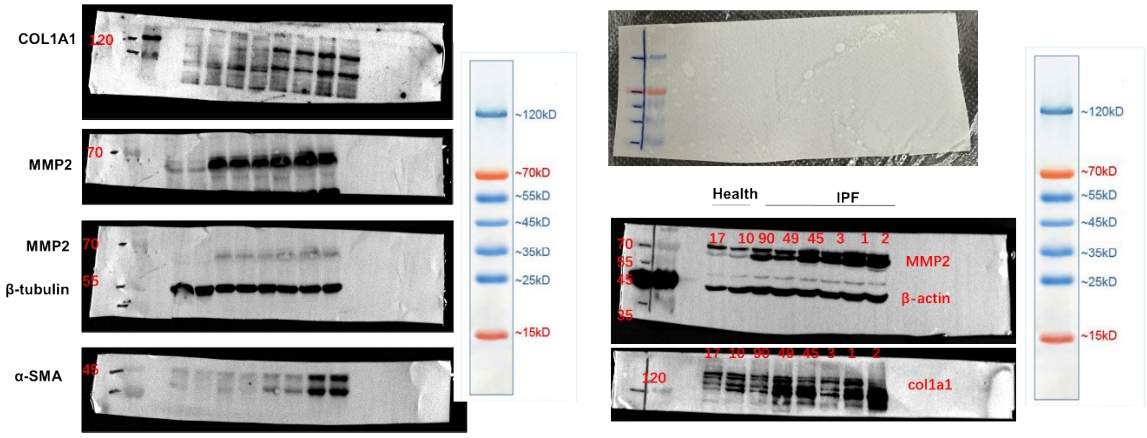


Fig. S14 Uncropped images of all blots shown in main figures.

Table S1. Zeta potential, DLS diameter, and PDI of prepared nanoparticles

Samples	Zeta potential (mV)	DLS dia. (nm)	PDI
AuNPs	-29.77±2.91	4.91±0.13	0.270
LA@Au	-35.57±2.40	5.78±0.32	0.275
pep2@Au	-35.08±1.04	17.62±3.67	0.345
Pep1@Au	36.32±1.17	22.35±1.84	0.305
MSNPs pep2:pep1=1:0.5	-14.22±0.32	45.72±3.51	0.204
MSNPs pep2:pep1=1:1	-3.28±0.62	56.88±5.80	0.225
MSNPs pep2:pep1=1:2	8.73±0.35	63.35±5.19	0.217