

## Supporting Information

### **Anticancer Activity of Structural Analogs of a Phytochemical Peptide from *Trichoderma longibrachiatum* and Related Peptide-Decorated Gold Nanoparticles**

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## 1. Table S1: plant protection properties of trichogin analogs

Acronym	PPP
TRIC <sup>b</sup>	poor activity against plant pathogens [22]
Api8-NH2	new sequence. [21] <sup>b</sup>
Leu4-NH2	not yet studied (new sequence)
K2569-Lol	active against Gram- bacterial plant pathogens [23]
K259-NH2	active against Gram- bacterial plant pathogens [23]
K259-Lol	active against <i>Pyricularia oryzae</i> [22]
K25-Lol	active against <i>B. cinerea</i> [21]
K56-Lol	active against <i>B. cinerea</i> and <i>Plasmopara viticola</i> [21,25]
K6-NH2	active against <i>B. cinerea</i> and <i>P. oryzae</i> [22,24]
K2-NH2	active against <i>P. oryzae</i> [22]
Api8-FITC	not yet studied (new sequence)
Leu4-FITC	not yet studied (new sequence)
Api8-SH	not yet studied (new sequence)
Leu4-SH	not yet studied (new sequence)

## 2. HPLC profiles and ESI-MS spectra of the newly synthesized peptides

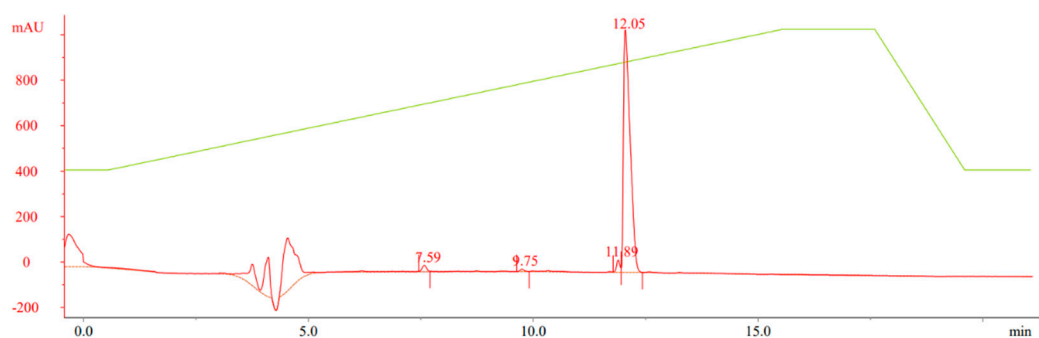
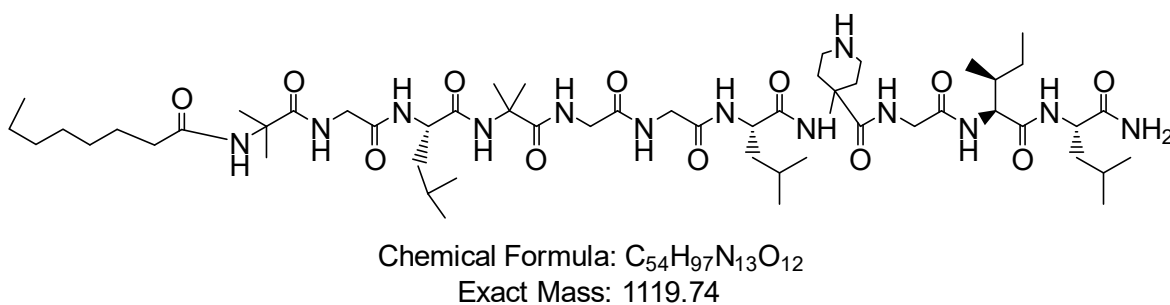


Fig S1. Api8-NH2. Phenomenex C18 column; gradient: 40%-100%B in 15 min, flux 1 mL/min, 216nm. Purity 95%

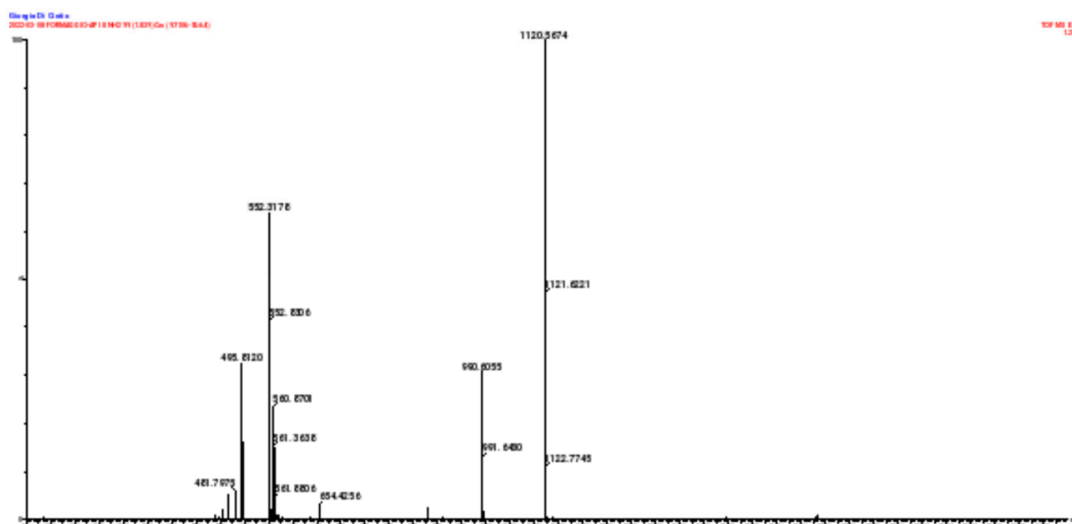


Fig. S2. HR-ESIMS Api8-NH<sub>2</sub>. [MW+H]<sup>+</sup><sub>found</sub> = 1120.5674 g/mol ; [MW+2H]<sup>++</sup><sub>found</sub> = 560.6701 m/z; [MW-Oct+H]<sup>+</sup> = 990.6055 m/z; [MW-Oct+2H]<sup>++</sup> = 495.8120 m/z.

### Api8-FITC

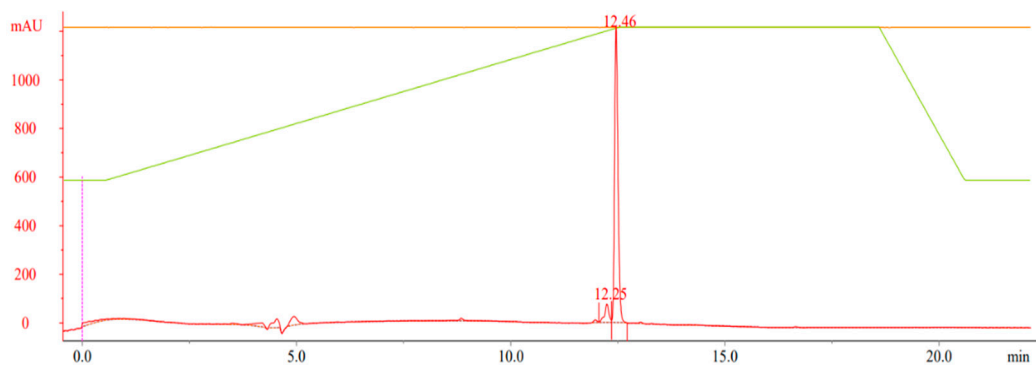
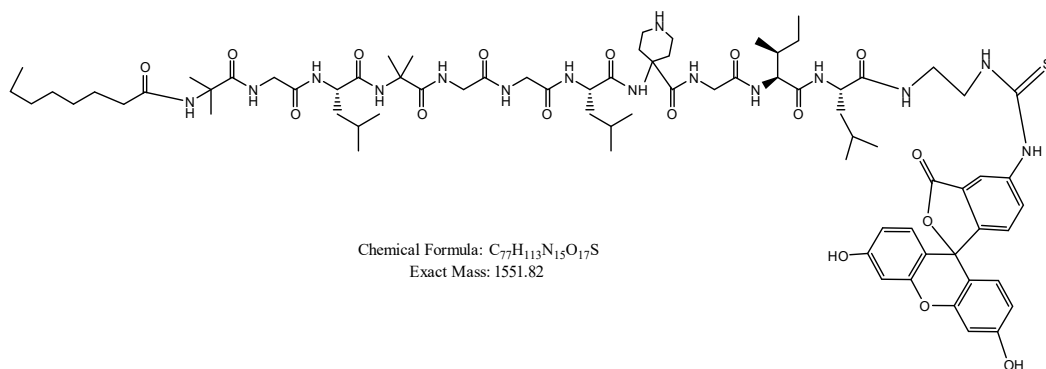


Fig S3. HPLC Chromatogram Api8-FITC. Purity: 95%

ESI-HRMS

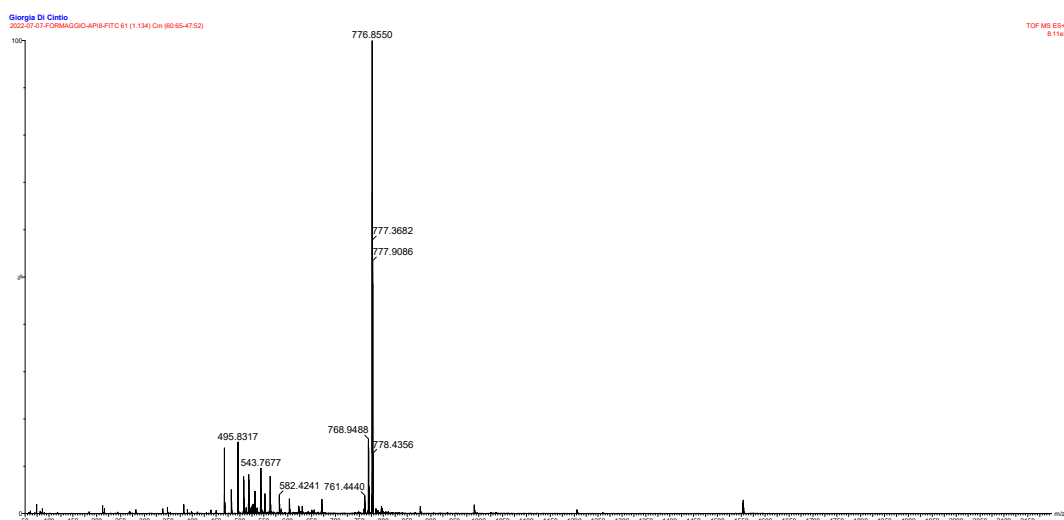


Fig. S4. ESI-HRMS Api8-FITC.  $[MW+H]^+_{\text{found}} = 1552.8671 \text{ m/z}$ ;  $[MW+2H]^{2+}_{\text{found}} = 776.8550 \text{ m/z}$ .

## Api8-SH

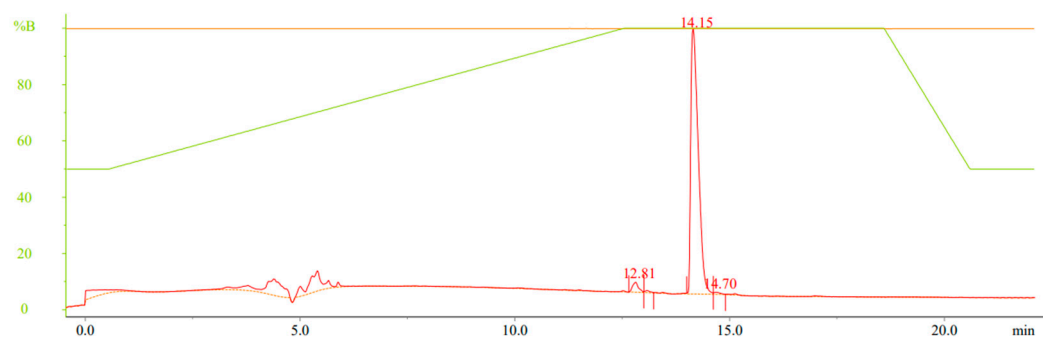
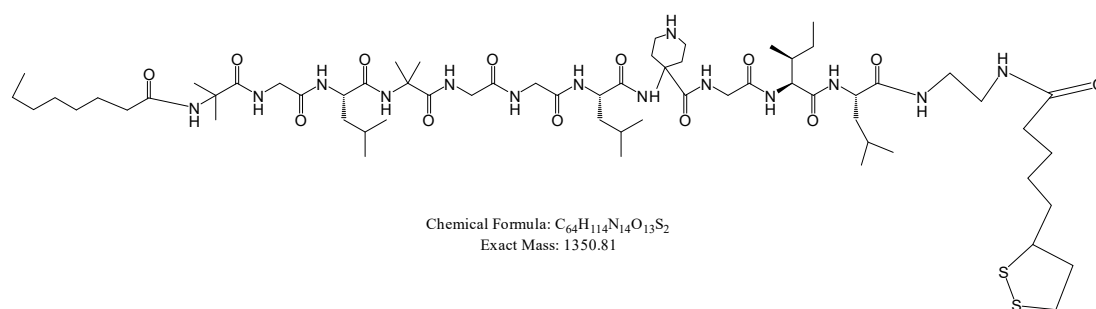


Fig. S5. HPLC chromatogram Api8-SH. Phenomenex C4 40-100%B in 13 min, flux 1 ml/min, wavelength 216 nm. Purity: 96%,

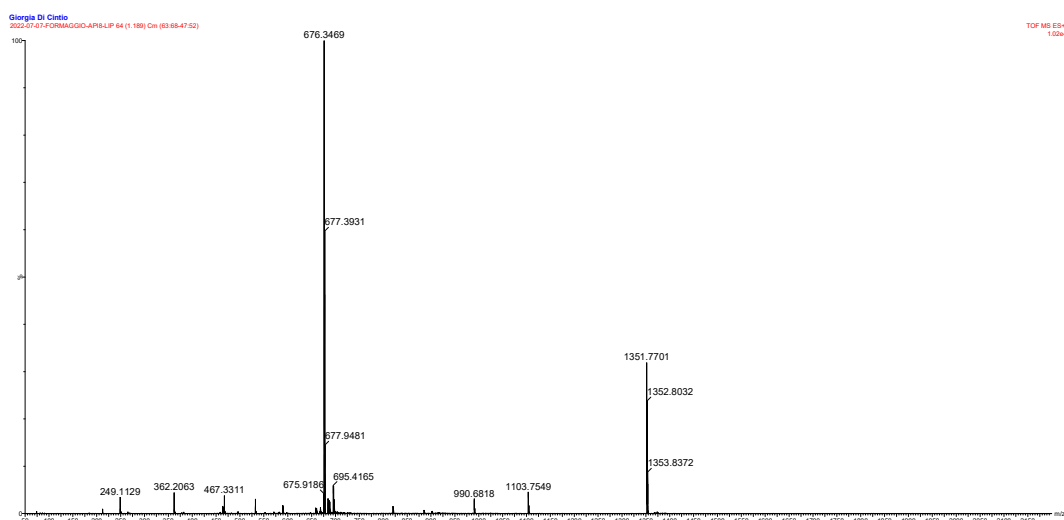
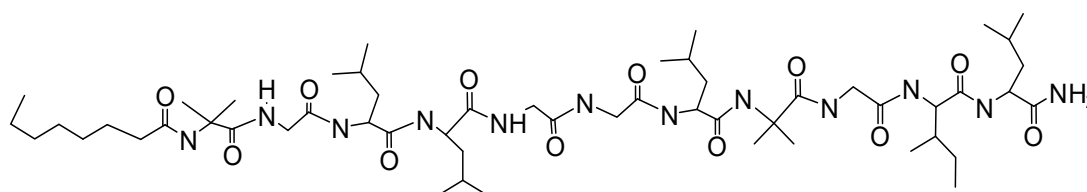


Fig. S6. ESI-HRMS Api8-SH.  $[MW+H]^+$ <sub>found</sub> = 1351.7701 m/z ;  $[MW+2H]^{++}$ <sub>found</sub> = 676.3469 m/z

## Leu4-NH2



Chemical Formula:  $C_{54}H_{98}N_{12}O_{12}$

Exact Mass: 1106.74

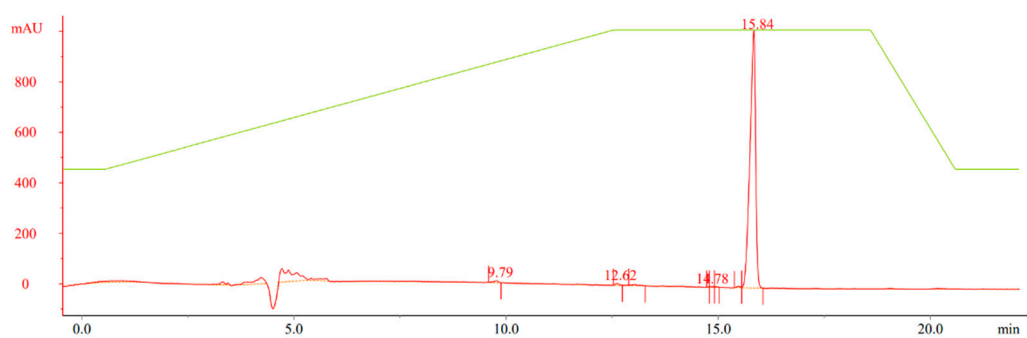


Fig. S7. HPLC Chromatogram Leu4-NH2. Phenomenex C18 50-100%B in 13 min, flux 1 ml/min, wavelength 216nm. Purity 98%.

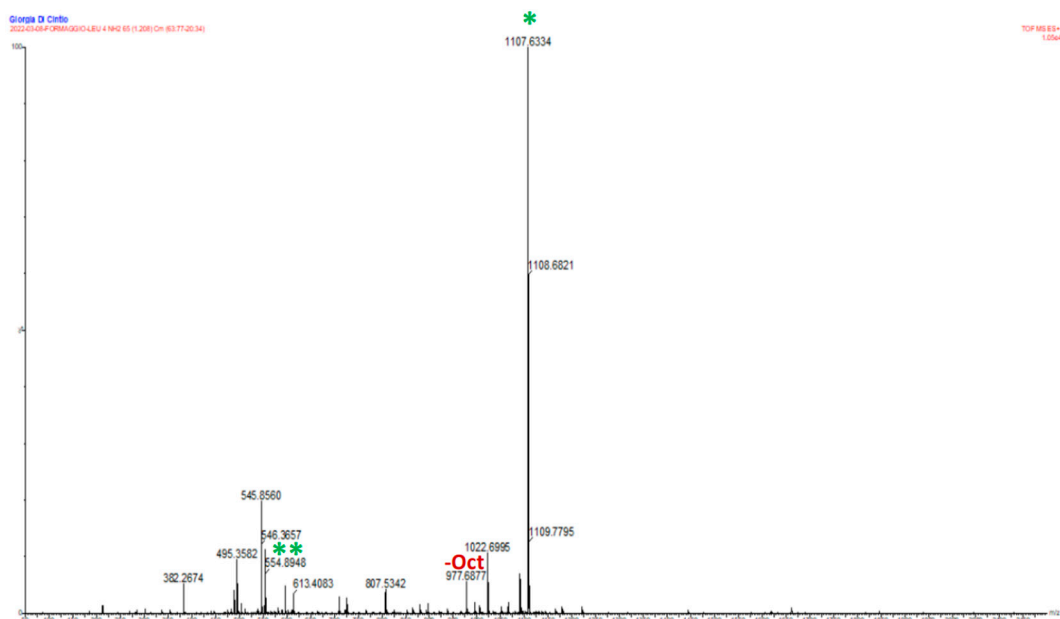


Fig. S8. ESI-HRMS Leu4-NH<sub>2</sub>. [MW+H]<sup>+</sup><sub>found</sub> = 1107.6334 m/z; [MW+2H]<sup>++</sup><sub>found</sub> = 554.8948 m/z; [MW-Oct+H]<sup>+</sup><sub>found</sub> = 977.6877 m/z.

## Leu4-FITC

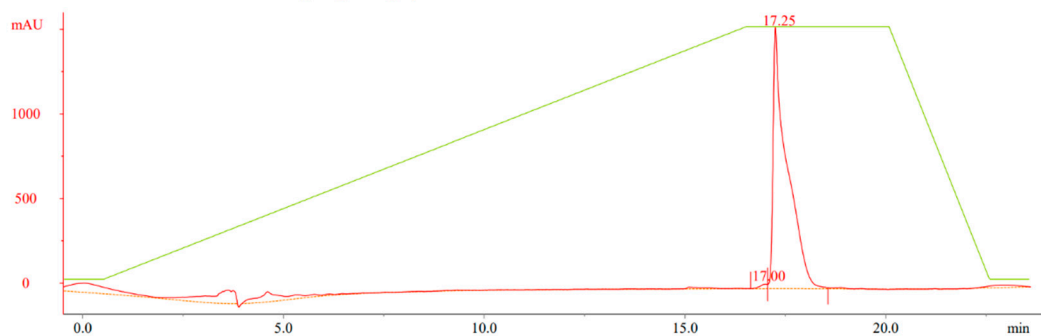
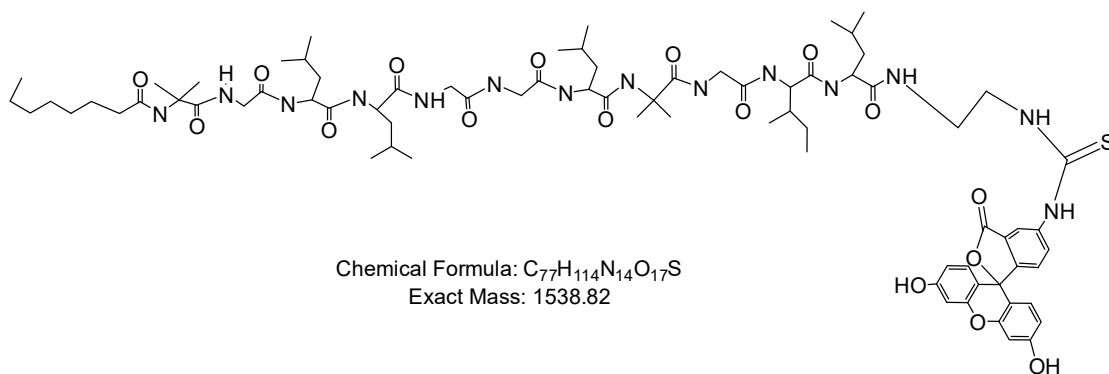


Fig S9. HPLC Chromatogram Leu4-FITC. Phenomenex C18 10-100%B in 15 min, flux 1 ml/min, wavelength 216nm. Purity 98%.

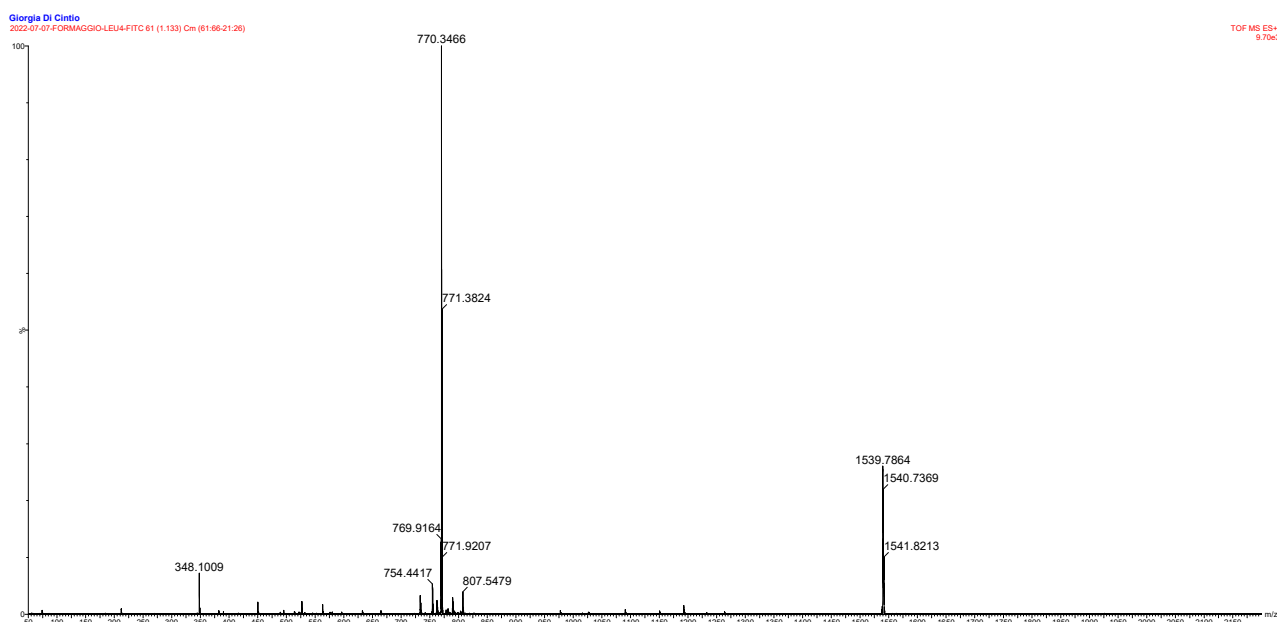


Fig. S10. ESI-HRMS Leu4-FITC.  $[\text{MW H}]^+_{\text{found}} = 1539.7864 \text{ m/z}$ ;  $[\text{MW}+2\text{H}]^{++}_{\text{found}} = 770.3466 \text{ m/z}$

## Leu4-SH

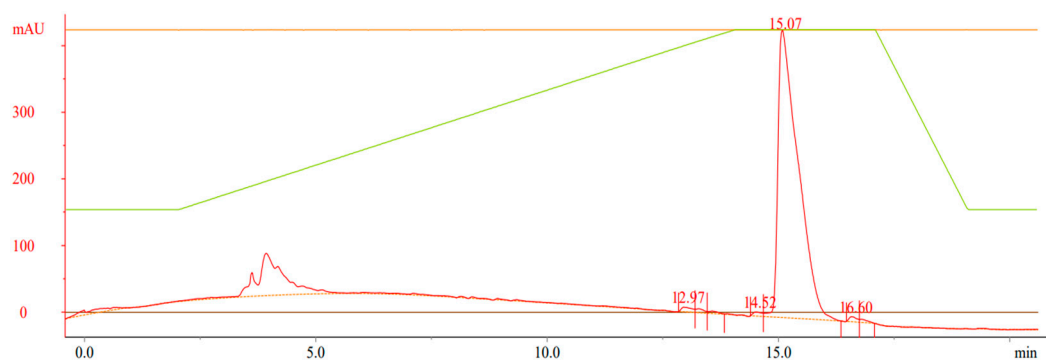
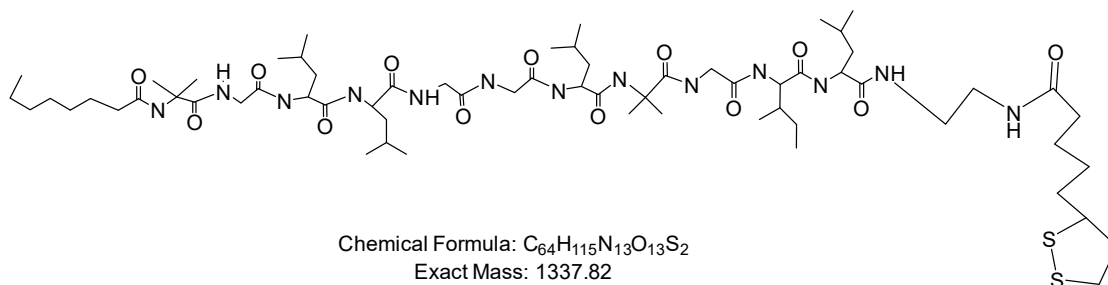


Fig. S11. HPLC chromatogram Leu4-SH. Phenomenex C4 40-100%B in 10 min, flux 1 ml/min, wavelength 216 nm. Purity 96%.

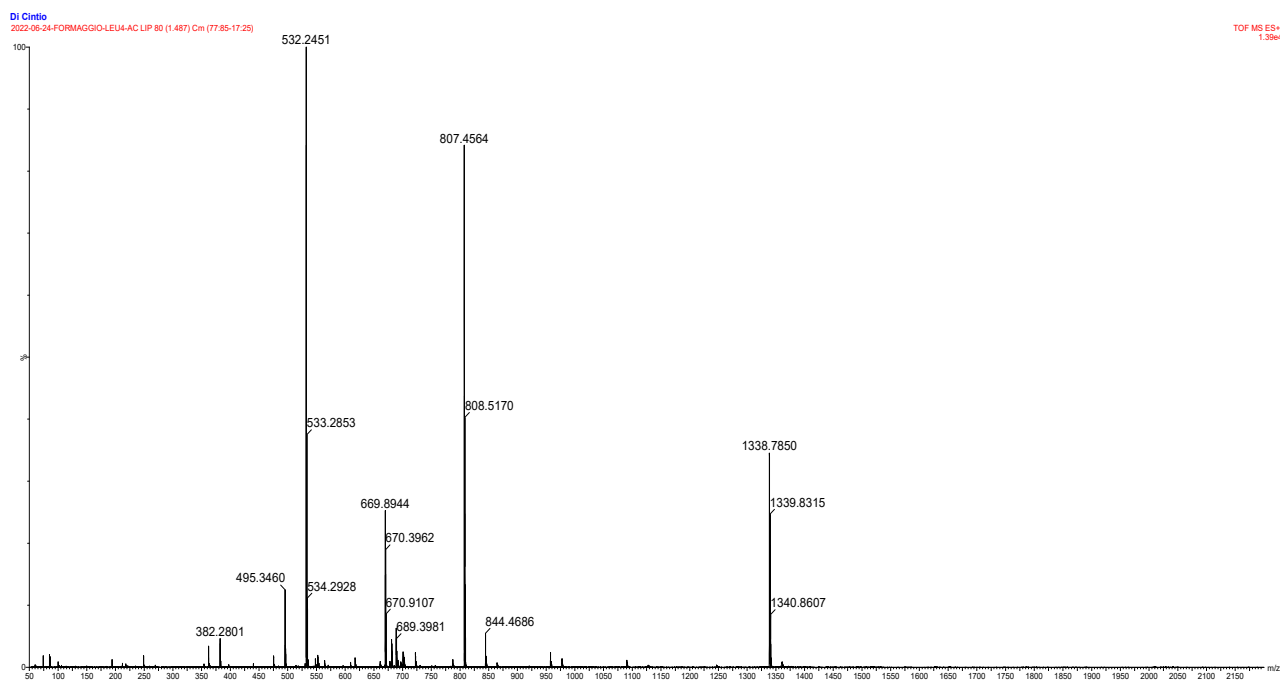


Fig. S12. ESI-HRMS Leu4-SH.  $[MW+H]^+_{\text{found}} = 1338.7850 \text{ m/z}$ ;  $[MW+2H]^{++}_{\text{found}} = 669.8944 \text{ m/z}$ ; mass fragmentation:  $532.25+807.46 = 1339.71$



## 2. GNP characterizations

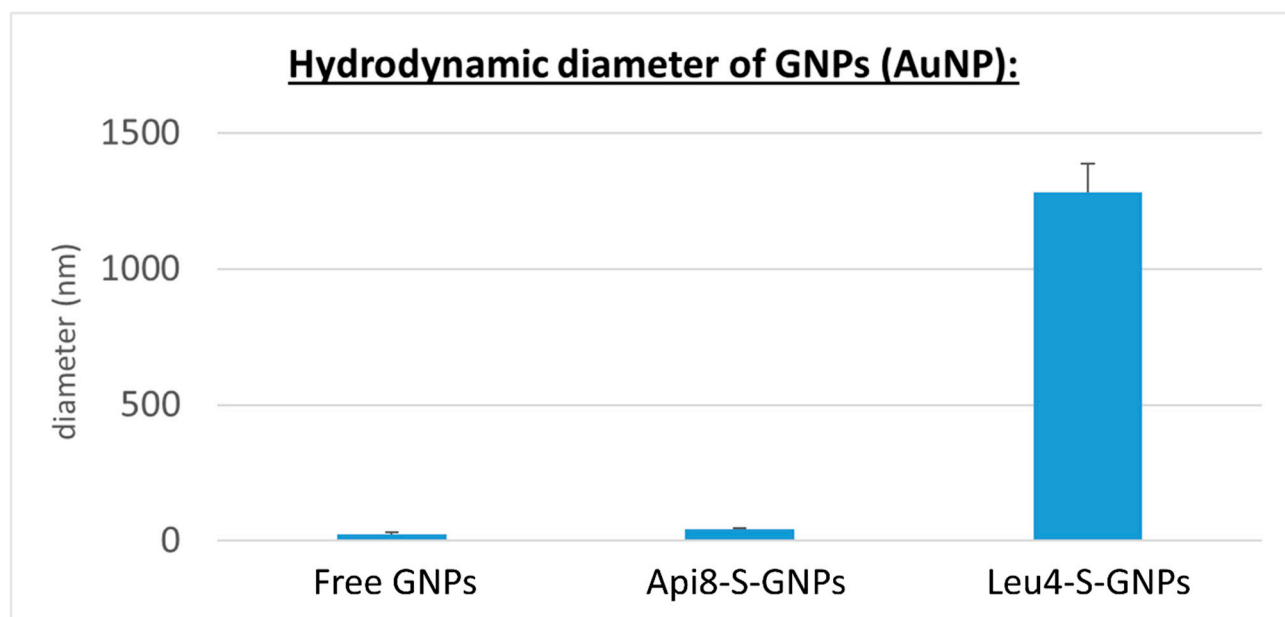


Figure S13. Average diameter of the GNPs obtained by dynamic light scattering.

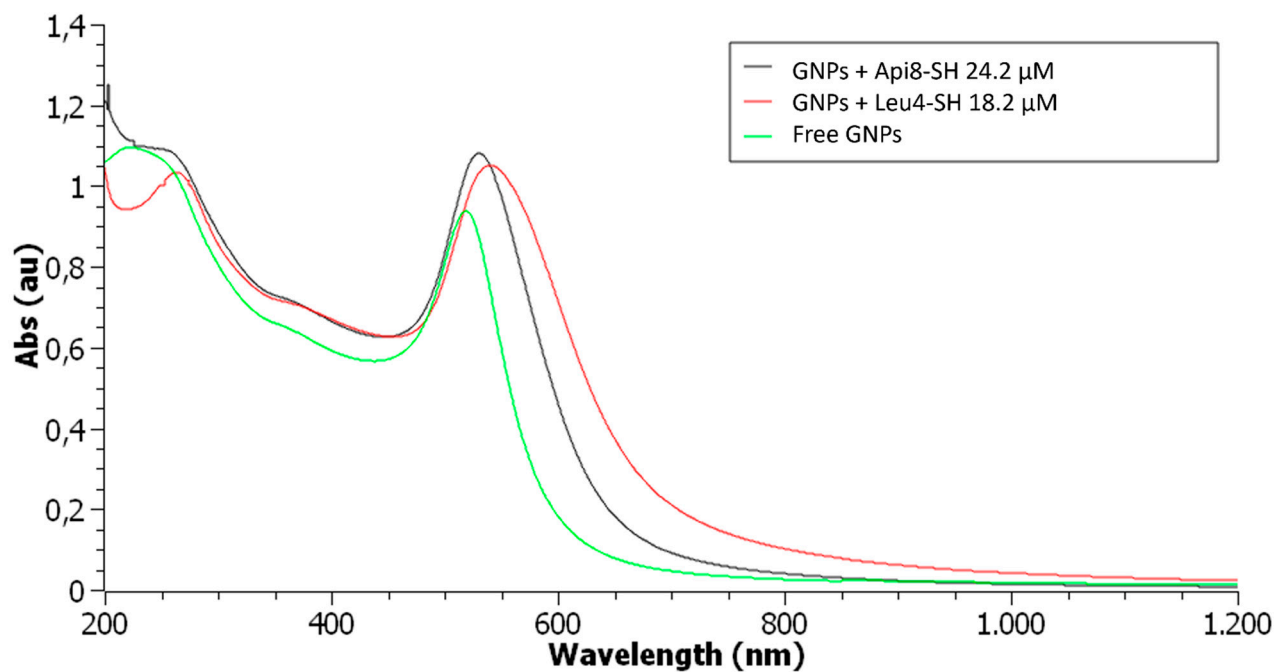


Figure S14. Absorption spectra of GNPs before (green curve) and after five-hour incubation in the presence of Leu4-SH 18.2  $\mu$ M (red curve) or Api8-SH 24.2  $\mu$ M (black curve).

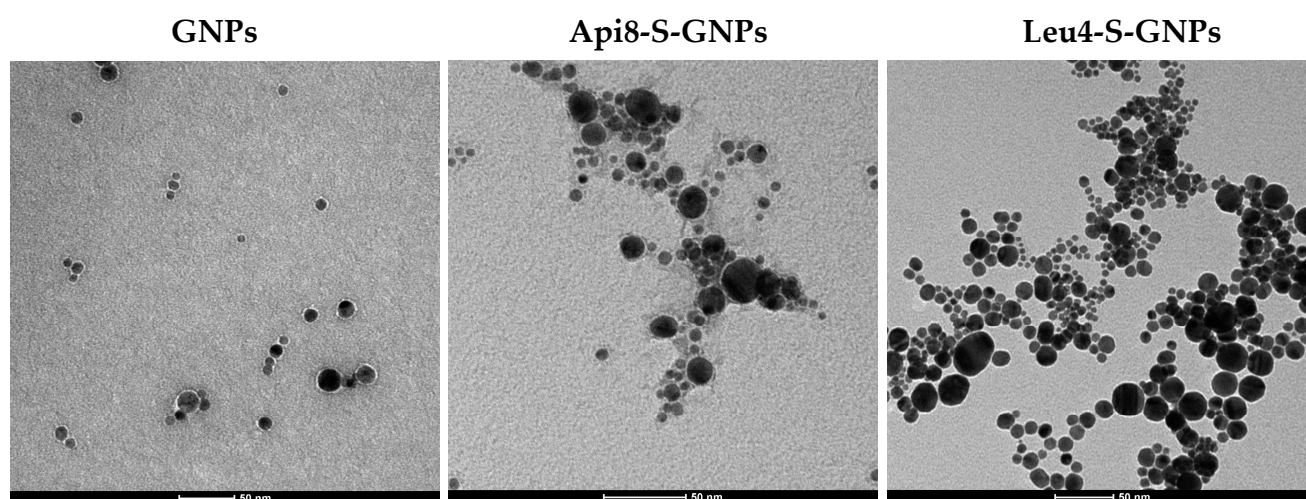


Figure S15. TEM images of GNPs suspensions before (left column) and after incubation with Api8-SH (central column) or Leu4-SH (right column).

### 3. CD spectra of the new peptide sequences

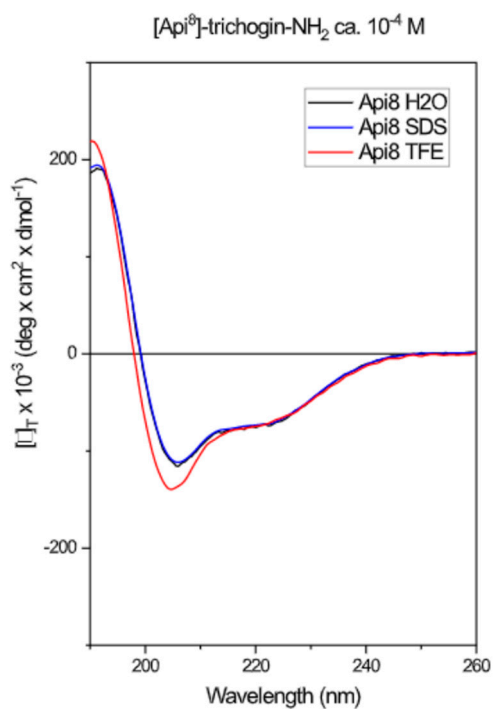


Figure S16. CD Spectra of Aπi8-NH<sub>2</sub> in sodium dodecyl sulfate (SDS) 100mM (blue), H<sub>2</sub>O (black) and 2,2,2-trifluoroethanol (TFE) (red). Peptide concentration 0.1mM.

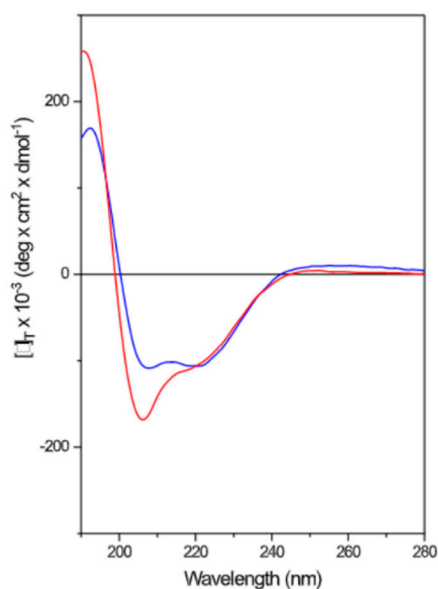


Figure S17. CD Spectra of Aπi8-FITC in SDS 100mM (blue) and TFE (red). Peptide concentration 0.1mM.

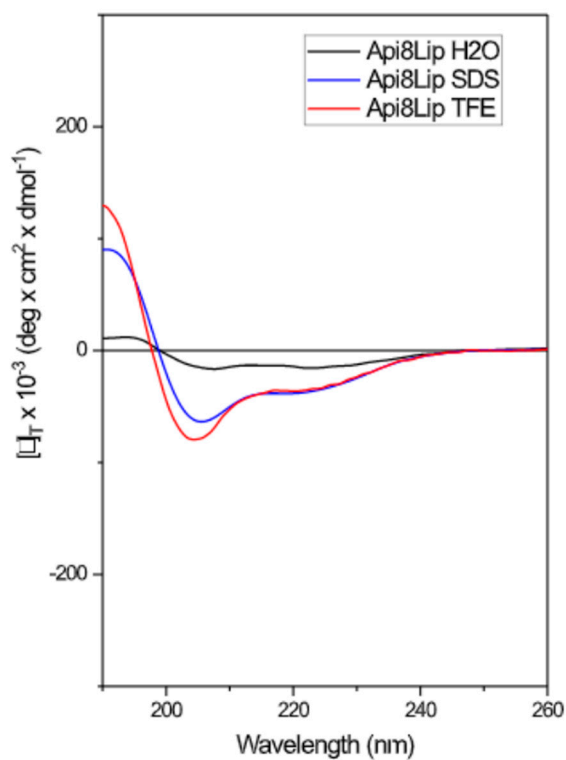


Figure S18. CD Spectra of Api8-SH in SDS 100mM (blue), H<sub>2</sub>O (black) and TFE (red). Peptide concentration 0.1mM.

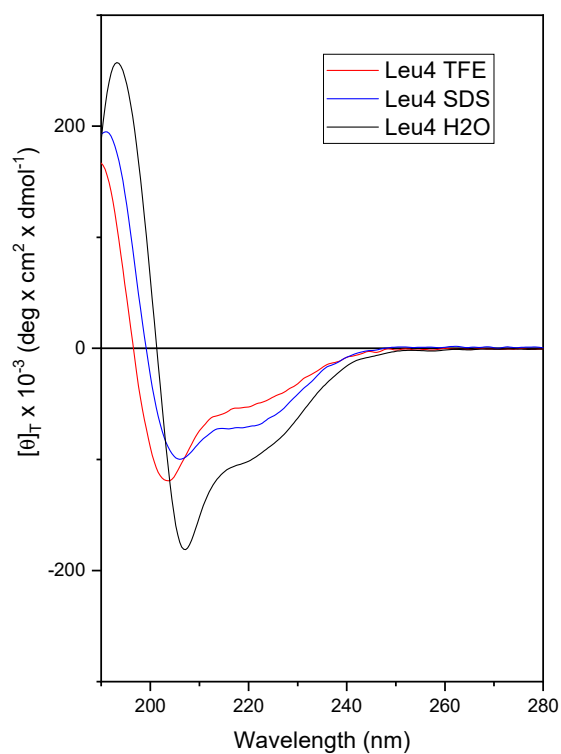


Figure S19. CD Spectra of Leu4-NH<sub>2</sub> in SDS 100mM (blue), H<sub>2</sub>O:CH<sub>3</sub>OH 9:1 (black) and TFE (red). Peptide concentration 0.1mM. Methanol was added to improve solubility

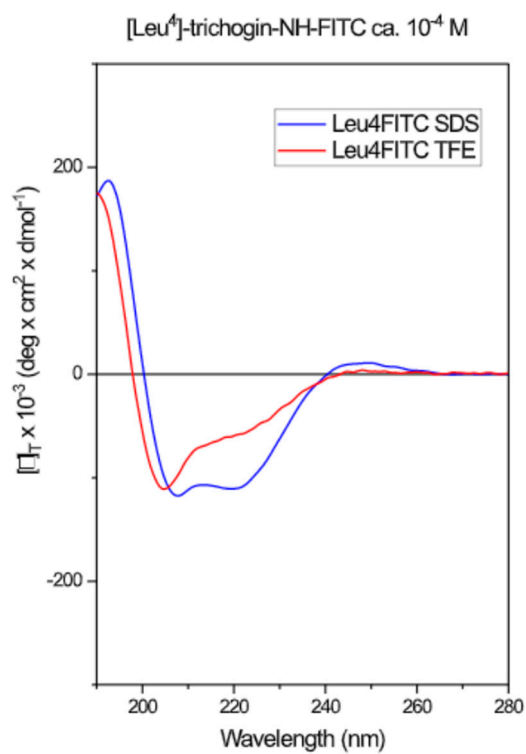


Figure S20. CD Spectra of Leu4-FITC in SDS 100mM (blue) and TFE (red). Peptide concentration 0.1mM.

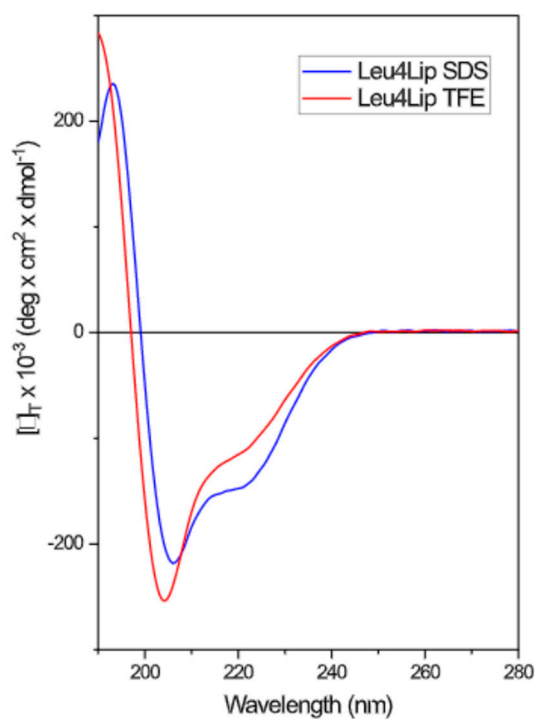


Figure S21. CD Spectra of Leu4-SH in SDS 100mM (blue) and TFE (red). Peptide concentration 0.1mM.

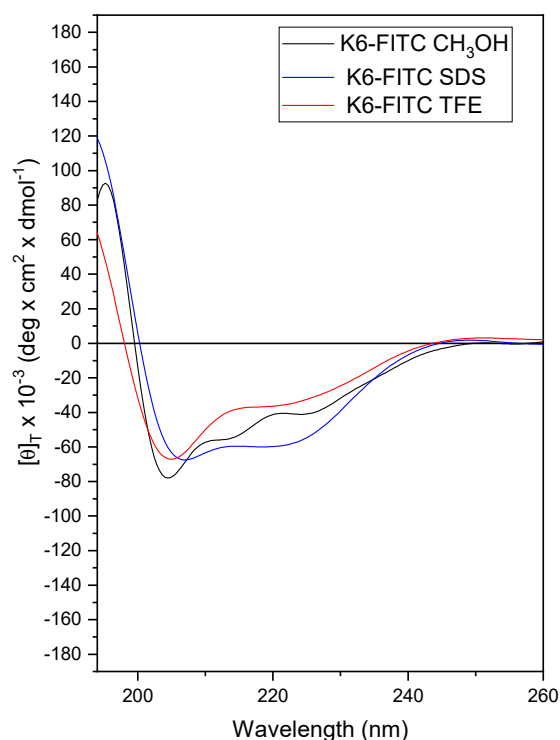


Figure S22. CD Spectra of K6-FITC in SDS 100mM (blue) and TFE (red). Peptide concentration 0.1mM.

#### 4. *In vitro* analysis

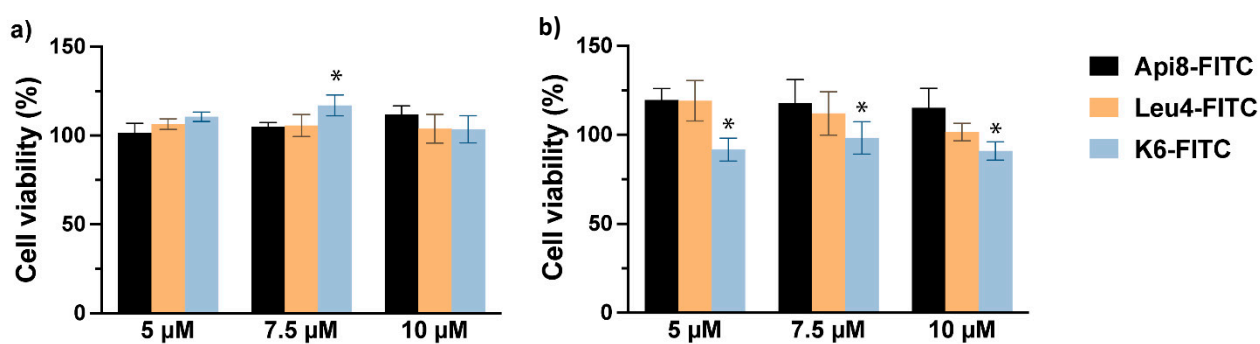


Figure S23. Cytotoxicity of FITC-conjugated peptaibols toward triple negative breast cancer cells MDA-MB-231 (a) and normal breast cells MCF-10A (b). Cells were exposed to increasing concentrations of peptaibols for 24 h and viability was measured with the MTS assay at the end of the incubation time. Data are expressed as mean percentage  $\pm$  SD of at least two independent experiments, carried out in triplicate. \*:  $p < 0.001$ , significantly different from controls (t-test).

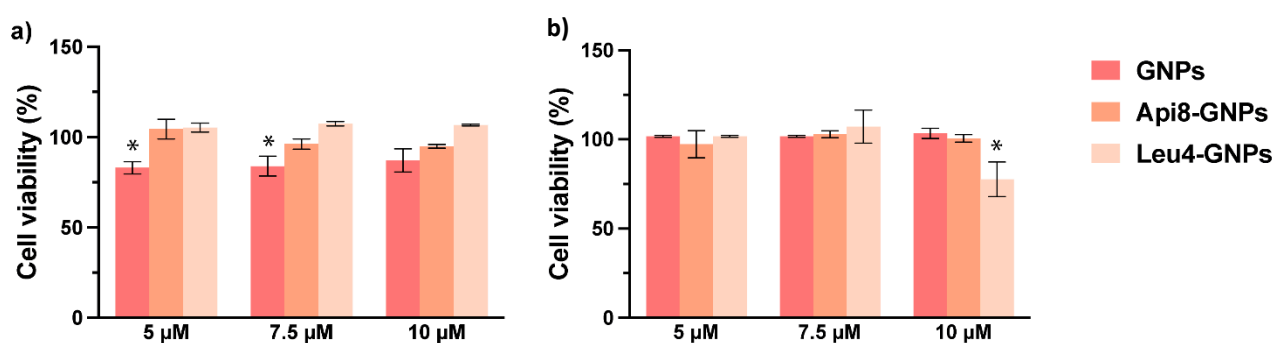


Figure S24. Cytotoxicity of GNP formulations toward triple negative breast cancer cells MDA-MB-231 (a) and normal breast cells MCF-10A (b). Cells were exposed to increasing concentrations of GNPs for 24 h and viability was measured with the MTS assay at the end of the incubation time. Data are expressed as mean percentage  $\pm$  SD of at least two independent experiments, carried out in triplicate. \*:  $p < 0.001$ , significantly different from controls (t-test).

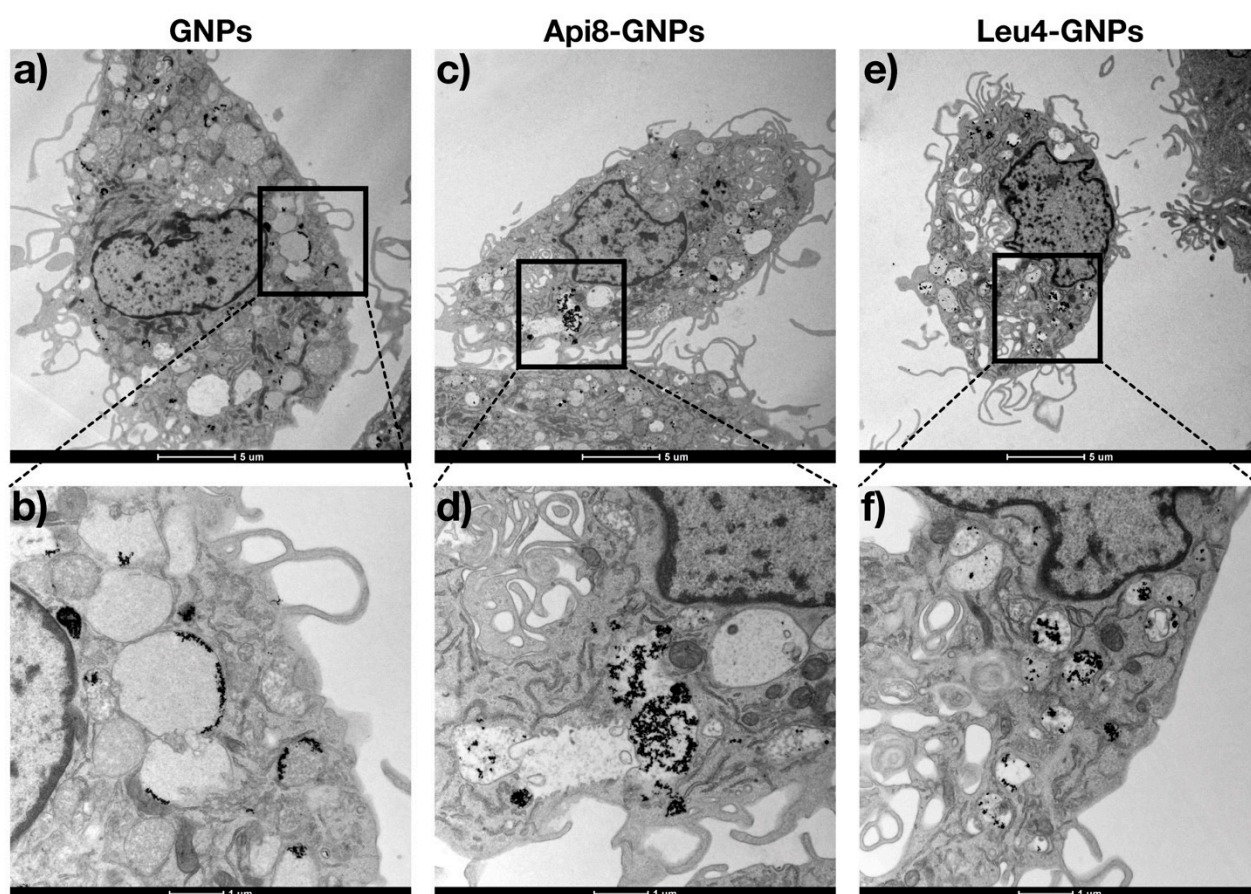


Figure S25. Representative TEM images of the interaction of different GNP formulations with human macrophages, derived from monocytes isolated from buffy coats. Scale bars: 5  $\mu$ m (a,c,e); 1  $\mu$ m (b,d,f).