# RGDS modifying ursolic acid: Getting insight into self-assembly, nano-property

### Supplementary Data



Features of UA-RGDS in the active pockets of ALP, TNF-a, IL-1β, and IL-10

**Supplementary Figure 1** The features of UA-RGDS in the active pockets of ALP (alkaline phosphatase), TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ), IL-1 $\beta$ (interleukin-1 $\beta$ ) and IL-10, (interleukin-10): The blue box showing the consistency evaluation score of the Ligandfit docking between UA-RGDS and ALP been 4; the red box showing the CDOCKER energy between UA-RGDS and TNF- $\alpha$  been -65.4kcal/mol; the yellow box showing the Libdock score between UA-RGDS and IL-1 $\beta$  been 162.1; the green box showing the Libdock score between UA-RGDS and IL-1 $\beta$  been 162.1; the green box showing the Libdock score between UA-RGDS and IL-1 $\beta$  been 162.1; the green box showing the Libdock score between UA-RGDS and IL-1 $\beta$  been 162.1; the green box showing UA-RGDS acting on 12 amino acid residues (Asn446, His277, Ala275, Asp273, Asn442, Tyr441, Tyr423, Gly224, Arg113, Ala221, ASN446, HIS277, ALA275, ASP273, ASN442, Tyr423, Ser223, Tyr222 and Arg153) of the active pocket of ALP; the red arrow showing UA-RGDS acting on five amino acid residues (Leu57, Leu120, Tyr119, Tyr59, Tyr151 and Leu57) of the active pocket of TNF- $\alpha$ ; the yellow arrow showing UA-RGDS acting on eight amino acid residues (Asp75, His277, Thr79, Leu80, Gln81, Tyr24, Phe133, Val132 and Ala127) of the active pocket of IL-1 $\beta$ ; the green arrow showing UA-RGDS acting on seven amino acid residues (Leu65, Phe56, Cys62, Phe111, Leu105, Leu23 and Leu19) of the active pocket.



FT-ICR-MS, qCID, NOESY and tetramer conformation of UA-RGDS

**Supplementary Figure 2** FT-ICR-MS, qCID, NOESY and tetramer conformation of UA-RGDS. (A) Full spectrum of FT-ICR-MS for UA-RGDS: the peak at 870.52989 been ion of UA-RGDS minus H (theoretical value: 870.53352), the peak of the divalent negative ion at 1306.79697 been the anion of [UA-RGDS]<sub>3</sub> minus H (theoretical value: 2613.6179), and the peak of the divalent negative ion at 1743.05182 been the anion of [UA-RGDS]<sub>4</sub> minus H (theoretical value: 3485.15974); (B) qCID of [UA-RGDS]<sub>4</sub> minus H: the peak at 870.52552 been the anion of UA-RGDS]<sub>4</sub>

RGDS minus H (theoretical value: 870.53352); (C) qCID of [UA-RGDS]<sub>3</sub> minus H: the peak at 870.52898 been the anion of UA-RGDS minus H (theoretical value: 870.53352), the peak at 1743.02558 been the anion of [UA-RGDS]<sub>2</sub> minus H (theoretical value: 1742.07541); (D) NOSEY 2D <sup>1</sup>H NMR: Cross peak 1 been from the interactions of the H of COOH of the Asp residue of one UA-RGDS with the H of NH<sub>2</sub> of the Arg residue of another two UA-RGDS, cross peak 2 been from the interactions of the H of the NH<sub>2</sub> of the Arg residue of the fourth UA-RGDS and the H of the NH<sub>2</sub> of the Arg residue of the second UA-RGDS; (E) According to NOSEY, the distances of the mentioned H been less than 4 Å UA-RGDS been energy minimized, and then four energy minimized UA-RGDS been manually operated to form a tetramer, this operation led the tetramer to having a bird-like conformation.



#### AFM images of UA-RGDS in the serum of OVX mice

**Supplementary Figure 3** AFM images of UA-RGDS in the serum of the OVX mice, existing as nano-particles: (A) AFM images of the serum alone; (B) At  $10^{-4}$  M of concentration in the serum UA-RGDS existing as dispersed nano-particles of 16 nm-156 nm in height; (C) At  $10^{-5}$  M concentration in rat serum UA-RGDS existing as dispersed nano-particles of 16 nm-137 nm in height; (D) At  $10^{-6}$  M concentration in the serum UA-RGDS existing as dispersed nano-particles of 26 nm-158 nm in height.

### In the femur of OVX mice UA-RGDS releasing UA and RGDS



**Supplementary Figure 4** FT-ICR-MS of the extracted homogenate of the femur of UA-RGDS treated OVX mice. (A) Full spectrum of the FT-ICR-MS of the extracted homogenate of the femur of UA-RGDS treated OVX mice; (B) Locally amplified peak at 870.52096 of UA-RGDS minus H (theoretical value: 870.53352); (C) Locally amplified peak at 455.35700 of UA minus H (theoretical value: 455.35197); (D) Locally amplified peak at 432.17703 of RGDS minus H (theoretical value: 432.18374).

### In the serum of OVX mice UA-RGDS stably existing



**Supplementary Figure 5** FT-ICR-MS spectrum of the extracted homogenate of the serum of OVX mice containing 1.2 mg of UA-RGDS (one hour): (A)Full FT-ICR-MS spectrum of the serum of OVX mice containing 1.2 mg of UA-RGDS; (B) Locally amplified peak giving no related peak at 455.35197(theoretical value of UA minus H); (C) Locally amplified peak giving no related peak at 432.17703(theoretical value of RGDS minus H); (D) Locally amplified peak giving a peak at 870.55220 of UA-RGDS minus H (theoretical value: 870.53352).



**Supplementary Figure 6** FT-ICR-MS spectrum of the extracted homogenate of the serum of OVX mice containing 1.2 mg of UA-RGDS (ten hours): (A)Full FT-ICR-MS spectrum of the serum of OVX mice containing 1.2 mg of UA-RGDS; (B) Locally amplified spectrum giving no related peak at 432.17703(theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no related peak at 455.35197(theoretical value of UA minus H); (D) Locally amplified spectrum giving a peak at 870.55014 of UA-RGDS minus H (theoretical value: 870.53352).

## In vivo UA-RGDS distributing towards the femur of OVX mice only



**Supplementary Figure 7** FT-ICR-MS of the extracted homogenate of the femur of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the femur of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving a peak of RGDS minus H at 432.17703(theoretical value: 432.18374); (C) Locally amplified spectrum giving a peak of RGDS minus H at 432.17703(theoretical value: 432.18374); (D) Locally amplified spectrum giving a peak of UA-RGDS minus H at 870.52096 (theoretical value: 870.53352).



**Supplementary Figure 8** FT-ICR-MS of the extracted homogenate of the serum of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the serum of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 9** FT-ICR-MS of the extracted homogenate of the heart of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the heart of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 10** FT-ICR-MS of the extracted homogenate of the liver of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the liver of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 11** FT-ICR-MS of the extracted homogenate of the spleen of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the spleen of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 12** FT-ICR-MS of the extracted homogenate of the kidney of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the kidney of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 13** FT-ICR-MS of the extracted homogenate of the lung of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the lung of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).



**Supplementary Figure 14** FT-ICR-MS of the extracted homogenate of the brain of UA-RGDS treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the brain of UA-RGDS treated OVX mice; (B) Locally amplified spectrum giving no ion peak at 432.18374 (theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no ion peak at 455.35197 (the theoretical value of UA minus H); (D) Locally amplified spectrum giving no ion peak at 870.53352 (the theoretical value of UA-RGDS minus H).

## In vivo UA non selectively distributing towards the organs of OVX mice



**Supplementary Figure 15** FT-ICR-MS of the extracted homogenate of the heart of UA treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the heart of UA treated OVX mice; (B) Locally amplified spectrum giving a peak of UA minus H at 455.35887(theoretical value: 455.35197).



**Supplementary Figure 16** FT-ICR-MS of the extracted homogenate of the femur of UA treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the femur of UA treated OVX mice; (B) Locally amplified spectrum giving a peak of UA minus H at 455.35721(theoretical value: 455.35197).



**Supplementary Figure 17** FT-ICR-MS of the extracted homogenate of the brain of UA treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the brain of UA treated OVX mice; (B) Locally amplified spectrum giving a peak of UA minus H at 455.36214(theoretical value: 455.35197).



**Supplementary Figure 18** FT-ICR-MS of the extracted homogenate of the lung of UA treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the lung of UA treated OVX mice; (B) Locally amplified spectrum giving a peak of UA minus H at 455.35957(theoretical value: 455.35197).



**Supplementary Figure 19** FT-ICR-MS of the extracted homogenate of the liver of UA treated OVX mice. (A) Full spectrum of FT-ICR-MS of the extracted homogenate of the liver of UA treated OVX mice; (B) Locally amplified spectrum giving a peak of UA minus H at 455.35486(theoretical value: 455.35197).

### In vitro release of UA-RGDS in pH 7.4 homogenate of the femur of OVX mice



**Supplementary Figure 20** FT-ICR-MS of the extracted homogenate of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice (30 minutes). (A) Full spectrum of FT-ICR-MS of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice; (B) Locally amplified spectrum giving no related peak at 432.17703(theoretical value of RGDS minus H); (C) Locally amplified spectrum giving no related peak at 455.35197(theoretical value of UA minus H); (D) Locally amplified spectrum giving a peak at 870.52170 of UA-RGDS minus H (theoretical value: 870.53352).



**Supplementary Figure 21** FT-ICR-MS of the extracted homogenate of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice (60 minutes). (A) Full spectrum of FT-ICR-MS of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice; (B) Locally amplified spectrum giving a peak at 432.18975 of RGDS minus H (theoretical value: 432.17703); (C) Locally amplified spectrum giving a peak at 455.35529 of UA minus H (theoretical value: 455.35197); (D) Locally amplified spectrum giving a peak at 870.53422 of UA-RGDS minus H (theoretical value: 870.53352).



**Supplementary Figure 22** FT- FT-ICR-MS of the extracted homogenate of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice (90 minutes): (A) Full spectrum of FT-ICR-MS of 1.2 mg of UA-RGDS in pH 7.4 homogenate the femur of OVX mice; (B) Locally amplified spectrum giving a peak at 432.19091 of RGDS minus H (theoretical value: 432.17703); (C) Locally amplified spectrum giving a peak at 455.35261 of UA minus H (theoretical value: 455.35197); (D) Locally amplified spectrum giving no related peak at 870.53352 (theoretical value of UA-RGDS minus H).

#### **UA-RGDS** without hepatorenal toxicity

To explore the effect of UA-RGDS on liver function the serum levels of ALT and AST of the OVX mice treated by CMC-Na (negative control) and the OVX mice treated by UA-RGDS (100µmol/kg/day, 28 consecutive days) were measured by using alanine aminotransferase ELISA kit (Servicebio) and aspartate aminotransferase ELISA kit

(Servicebio), while the results were showed in **Supplementary Figure 23**, which indicated that UA-RGDS exhibited no hepatorenal toxicity.



**Supplementary Figure 23** Effects of UA-RGDS on liver and kidney function: (A) UA-RGDS did not alter serum level of ALT in OVX mice; (B) UA-RGDS did not alter serum level of AST in OVX mice; (C) UA-RGDS did not alter serum level of Cr in OVX mice; (D) UA-RGDS did not alter serum level of BUN in OVX mice; n=8.