Supporting Information

Self-assembled RGD dehydropeptide hydrogels for drug delivery applications

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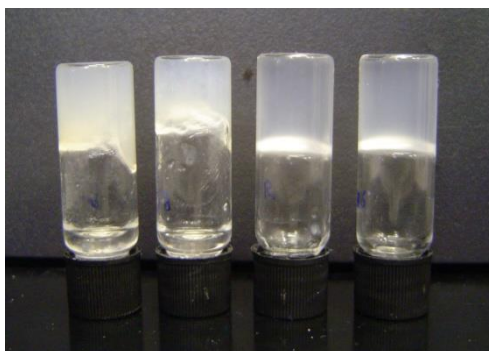
Figure S1. Images of hydrogels (phosphate buffer 0.1 M, pH 6.0) prepared with different concentrations of dehydrodipeptide **4**; concentrations from left to right: 0.50, 0.46, 0.40 and 0.32 wt%.
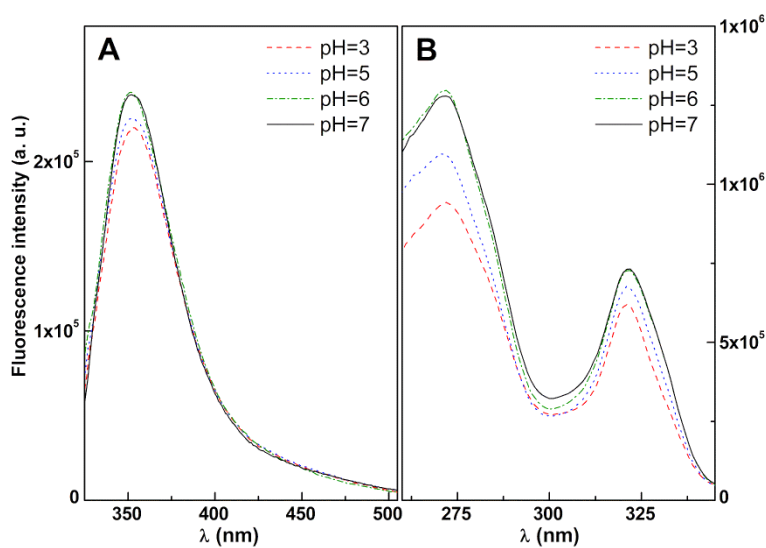

Figure S2. pH dependence of: (**A**) fluorescence emission (λ_{exc}= 290 nm); (**B**): Excitation spectra (λ_{em}= 350 nm), for peptide construct **4** (2×10^{-6} M, phosphate buffer 0.1 M).
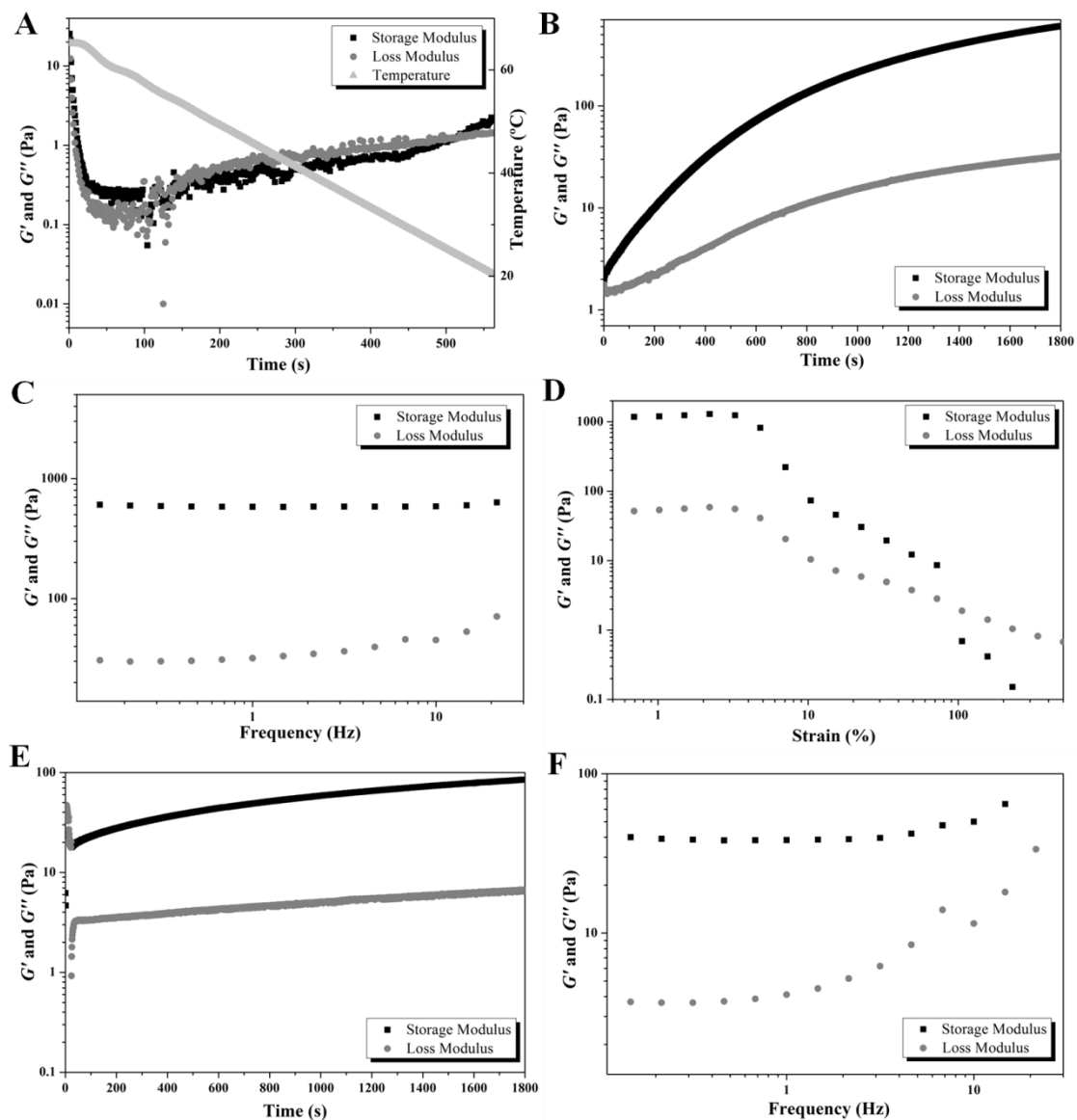
Figure S3. A) Temperature sweep (cooling 5 ºC min^{-1}, f = 1 Hz, γ = 0.5%); B) Structural build-up for 30 minutes (f = 1 Hz, γ = 0.5%, T = 20 ºC); C) Frequency sweep (γ = 0.5%, T = 20 ºC); D) Strain sweep (f = 1 Hz, T = 20 ºC); E) Structural build-up for 30 minutes (f = 1 Hz, γ = 0.5%, T = 20 ºC); F) Frequency sweep (γ = 0.5%, T = 20 ºC); for the hydrogel of peptide **4** (0.5 wt%; phosphate 0.1 M, pH 6.0).
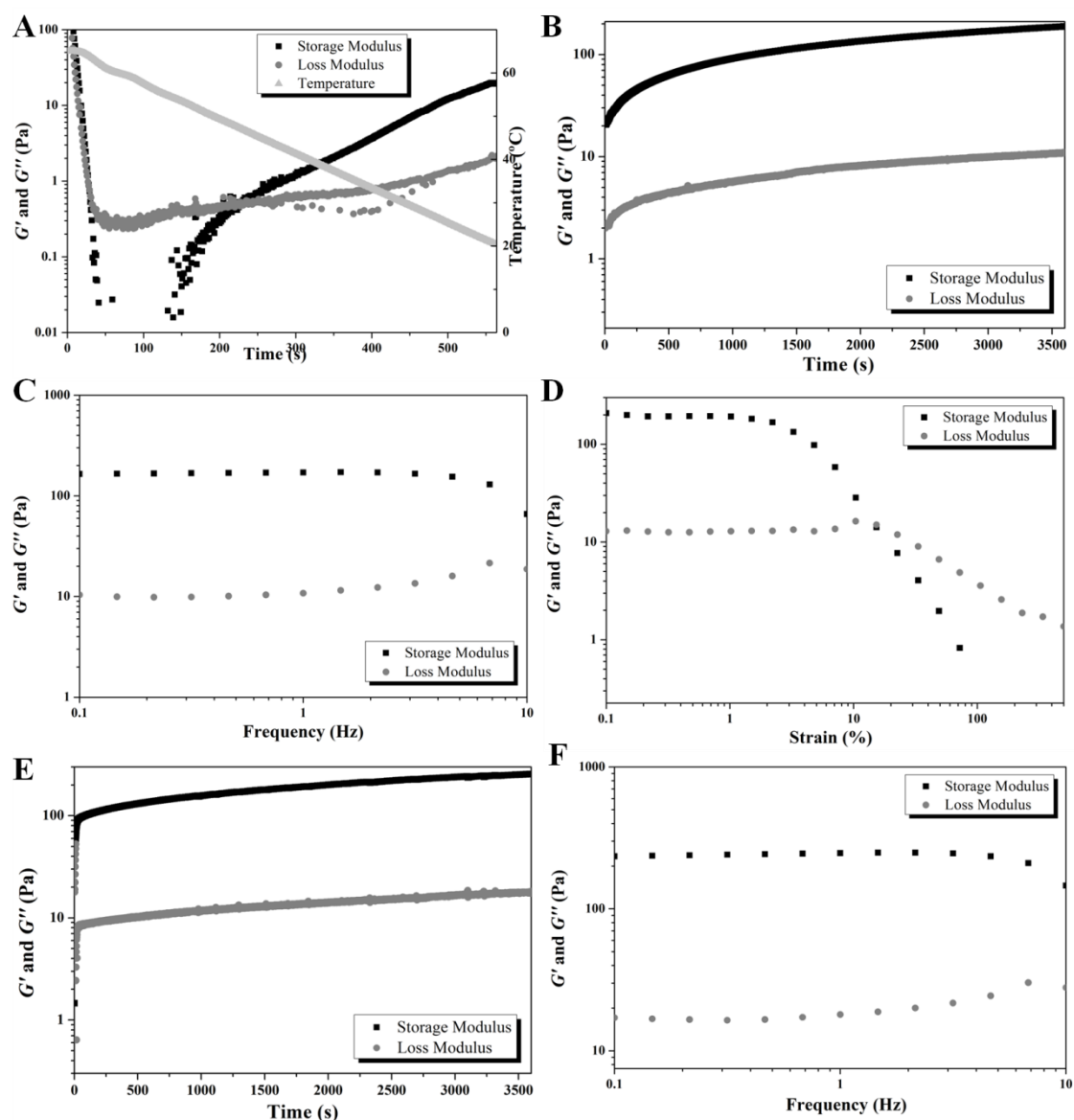
Figure S4. A) Temperature sweep (cooling 5 °C min^{-1}, $f = 1$ Hz, $\gamma = 0.5\%$); B) Structural build-up for 60 minutes ($f = 1$ Hz, $\gamma = 0.5\%$, T = 20 °C); C) Frequency sweep ($\gamma = 0.5\%$, T = 20 °C); D) Strain sweep ($f = 1$ Hz, T = 20 °C); E) Structural build-up for 60 minutes ($f = 1$ Hz, $\gamma = 0.5\%$, T = 20 °C); F) Frequency sweep ($\gamma = 0.5\%$, T = 20 °C); for hydrogel of peptide **4** (0.5 wt%; phosphate buffer 0.1 M, pH 6) after re-heating to 65 °C.
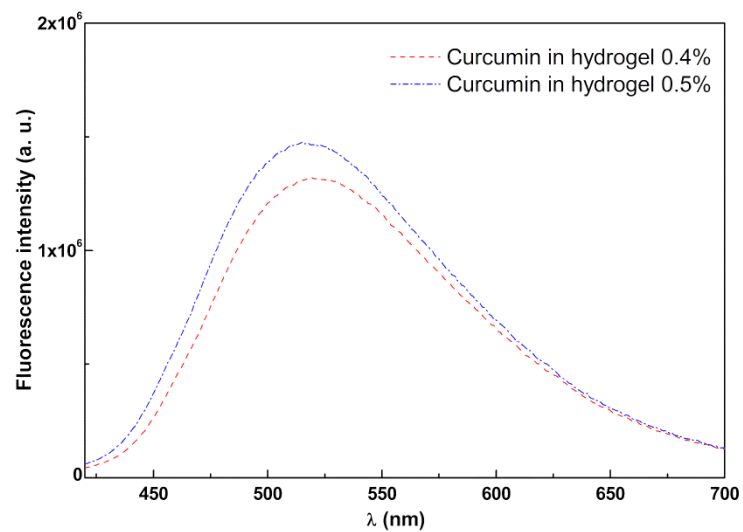
Figure S5. Fluorescence spectra (λ_{exc}= 410 nm, direct excitation) of curcumin (3×10^{-6} M) incorporated in hydrogel of dehydrodipeptide **4** (0.4% and 0.5% wt%, phosphate buffer 0.1 M, pH 6.0).
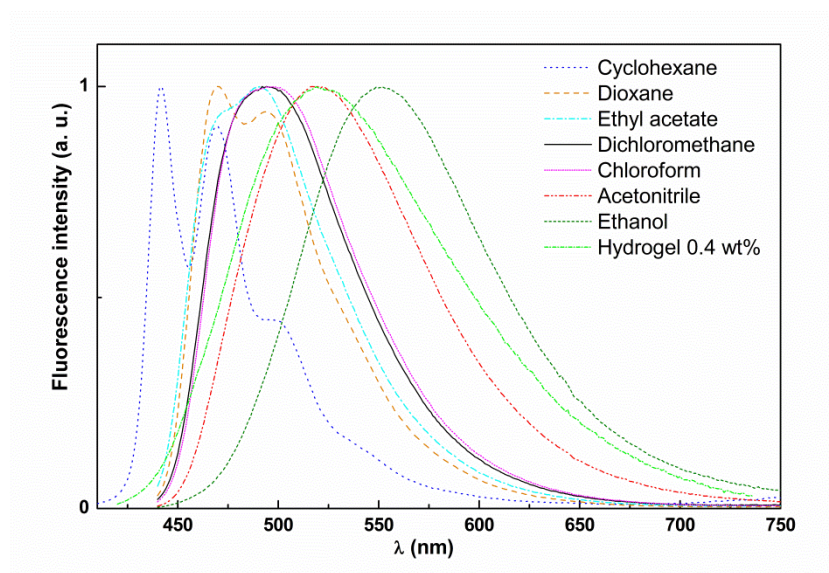
Figure S6. Normalized fluorescence spectra (at peak of maximum emission) of curcumin solutions (3×10^{-6} M, λ_{exc}= 410 nm) in several solvents and of curcumin incorporated into hydrogel of dehydrodipeptide **4** (0.4wt%, phosphate buffer 0.1 M, pH 6.0).
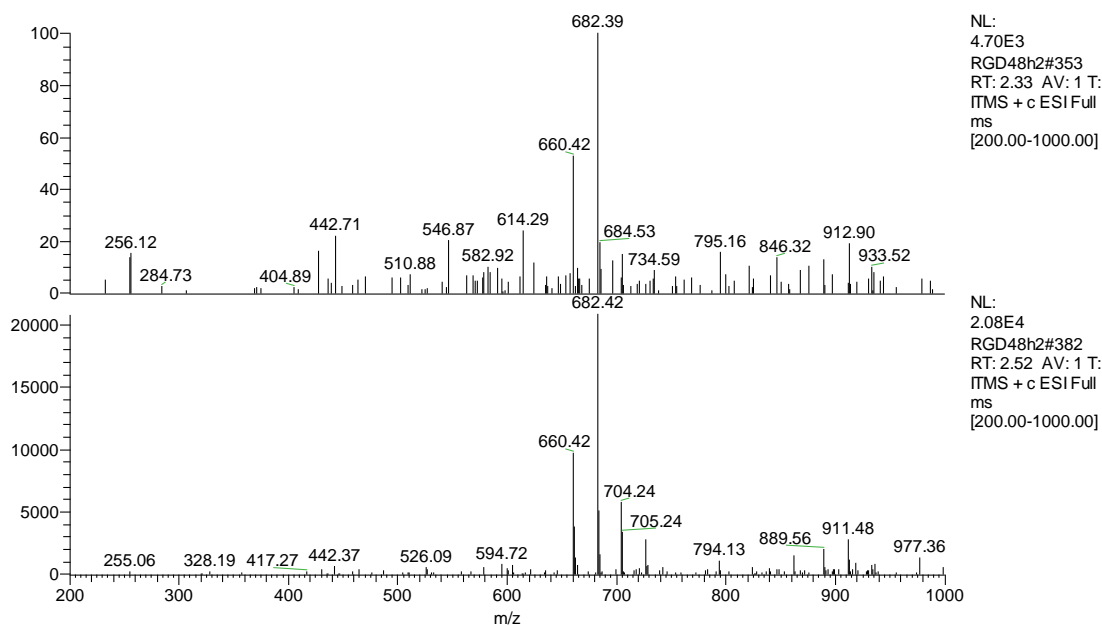
Figure S7. HPLC-MS spectrum of peptide **4** (0.25 mg mL^{-1}, phosphate buffer 0.1 M, pH 7.5) after 48 hours incubation with α-chymotrypsin (26.6 U.mL^{-1}) at 37 ºC.

Molecular Docking

The X-ray structure of the extracellular fragment of the α$_v$β$_3$ integrin, pdb id 1L5G, was used in this study.[1] Hydrogens were added and the charges of the protein residues at pH 7.0 were set with MolProbity with Reduce.[2-3] Coordinated ligands were removed and Manganese ions were set to net charge 2+. Nonpolar hydrogens were merged with AutoDock Tools (ADT).[4,5] Molecular docking was performed with AUTODOCK 4.2. A grid box with 90 × 90 × 90 points was created in order to contain the binding pocket for the cyclo(RGDf[N-Me]V) ligand, found in the experimental structure. The grid for probe target energy calculations, with a spacing of 0.25 Å, was placed with its center at the integrin-binding site (x =19.50 Å, y = 43.96 Å, z = 44.15 Å). Grid potential maps were calculated for the experimental cyclo(RGDf[N-Me]V) ligand and for peptide construct **4** using the module AutoGrid 4.0. The most provable conformation of peptide construct **4** was obtained by MD simulations. The RGD sequence of peptide construct **4** was fited to the RGD segment on the experimental cyclo(RGDf[N-Me]V) ligand. The stochastic search algorithm Lamarckian Genetic Algorithm (LGA),[6] which combines global search (Genetic Algorithm alone) to local search, was used to calculate the theoretical free energy of binding of the peptides to the integrin binding site. In the docking calculations, all possible torsions of the peptide side chains were set flexible, except for the guanidine group of arginine. Torsions in the

main chain of the peptides were constrained. Each docking consisted of 300 independent runs, with an initial population of 350 individuals, a maximum number of $25e^5$ energy evaluations, and a maximum number of 27,000 generations. A tolerance of 1.0 Å in root-mean-square deviation (rmsd) was used to group structures into clusters. Each cluster is represented by the energetically most favorable conformation. The number of individuals to survive to the next generation was set to 5. Default values were applied to the remaining parameters.

References

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