

Dehydropeptide-based plasmonic magnetogels: a supramolecular composite nanosystem for multimodal cancer therapy

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Supplementary Information

Synthesis of Npx-L-Met-Z-ΔPhe-OH

Synthesis of *tert*-butoxycarbonylmethionine [Boc-L-Met-OH (2)]. A solution of di-*tert*-butyl dicarbonate (2.35 g, 0.011 mol, 1 equiv) in 3.75 mL of *tert*-butyl alcohol was added dropwise at room temperature (rt) to a stirring solution of L-methionine (1.58 g, 0.011 mol) in NaOH 1 M (10.6 mL, 1.1 equiv). A white precipitate was formed, and the resulted mixture was allowed to stir at rt overnight. The solution was acidified to pH 1-1.5 with a KHSO₄ solution 1 M. The turbid reaction mixture was extracted with ethyl acetate (2 x 20 mL), dried over magnesium sulfate anhydrous, filtered, and the solvent was removed under reduced pressure. Compound **2** was isolated as an oil (2.02 g; 81%). This procedure was adapted from a previously reported method.³⁵

¹H NMR (400 MHz, DMSO) δ: 1.37 (9H, s, 3 x CH₃ Boc); 1.79-1.872 (2H, m, β-CH₂ Met), 2.02 (3H, s, SCH₃ Met); 2.44-2.50 (2H, m, γ-CH₂ Methionine), 3.96-4.02 (H, m, α-CH Met), 7.11 (H, d J = 8 Hz, NH Met), 12.5 (1H, br s, CO₂H Met).

Synthesis of the methyl ester of β-hydroxyphenylalanine [H-D,L-Phe(β-OH)-OMe (3)]. The β-hydroxyphenylalanine [H-D,L-Phe(β-OH)-OH] (25 mmol, 4.52 g) was added to 40 mL of methanol in an ice bath. Thionyl chloride (3.4 equiv) was added dropwise and the reaction mixture was left stirring for 4 h at 40 °C. The solvent was removed under reduced pressure and ethyl ether was added. The process was repeated until a white solid of compound **3** was formed (98%, 5.68 g). This procedure was adapted from a method previously described.³⁶

¹H NMR (400 MHz, DMSO) δ: 3.59 (3H, s, OCH₃); 4.13-4.14 [1H, d, α-CH Phe(β-OH)]; 4.99-5.02 [1H, t, β-CH Phe(β-OH)]; 6.56 [1H, d (J = 4.4 Hz), OH Phe(β-OH)]; 7.31-7.38 [5H, m, ArH Phe(β-OH)]; 8.48 [3H, s, NH₃⁺ Phe(β-OH)].

Synthesis of the methyl ester of *N*-*tert*-butoxycarbonyl-L-methionyl-β-hydroxyphenylalanine [Boc-L-Met-D,L-Phe(β-OH)-OMe (4)]. Boc-L-Met-OH (2.02 g, 8.10 mmol) was dissolved in acetonitrile (20 mL) and put in an ice bath. HOBt (1.00 equiv, 1.24 g, 8.10 mmol), DCC (1.00 equiv, 1.67 g, 8.10 mmol), H-D,L-Phe(β-OH)-OMe (1.00 equiv, 1.88 g, 8.10 mmol), and triethylamine (2.00 equiv, 2.25 mL, 16.21 mmol) were added with 2 min between each addition. The mixture was left stirring at rt overnight. The urea was filtered, and the solvent removed under reduced pressure. Acetone was added, and the mixture was stored in the freezer for 2 h. The urea was filtered again. Evaporation at reduced pressure gave a residue that was partitioned between ethyl acetate (50 mL) and KHSO₄ (50 mL, 1M). The organic phase was thoroughly washed with KHSO₄ (1M, 2 x 50 mL), NaHCO₃ (1M, 2 x 50 mL) and brine (3 x 50 mL) and dried with MgSO₄. Removal of the solvent afforded compound **4** as a diastereomeric mixture (2.12 g, 84.2%).

¹H NMR (400 MHz, DMSO) δ: 1.32-1.38 (9H, s, 3 x CH₃ Boc); 1.60-1.65 (2H, m, β-CH₂ Met); 2.165-2.26 (2H, m, γ-CH₂ Met); 2.49 (3H, s, SCH₃ Met); 3.64 (3H, s, OCH₃), 4.535-4.58 (1H, m, α-CH Met); 5.10 [1H, s, OH Phe(β-OH)]; 5.13-5.15 [1H, m, β-CH Phe(β-OH)]; 5.93-5.96 [1H, s, α-CH Phe(β-OH)]; 6.90-6.92 and 7.04-7.06 (1H, dd (J = 8.4 Hz), NH Met); 7.19-7.38 [5H, m, ArH Phe(β-OH)]; 7.78-7.80 and 7.92-7.94 (H, dd (J = 8.8 Hz and 9.2 Hz), NH Phe(β-OH)).

Synthesis of the methyl ester of *N*-tert-butyloxycarbonyl-L-methionyl-Z-dehydrophenylalanine [Boc-L-Met-Z-ΔPhe-OMe (5)]. To a solution of compound **4** in dry acetonitrile (10 mL, 1 M), DMAP (0.1 equiv, 0.082 g, 0.68 mmol) and Boc₂O (1 equiv, 1.49 g, 6.83 mmol) were added under rapid stirring at rt. The mixture was monitored by ¹H NMR until all reactant was consumed. *N,N,N',N'*-tetramethylguanidine (2% in volume, 0.2 mL) was added under continuous stirring. The mixture was left stirring at rt and monitored by ¹H NMR until all reactant was consumed. Evaporation at reduced pressure gave a residue that was partitioned between ethyl acetate (50 mL) and KHSO₄ (1 M, 30 mL), washed with KHSO₄ (1 M, 2 x 30 mL), NaHCO₃ (1 M, 3 x 30 mL), water (1 x 30 mL) and brine (2 x 30 mL) and dried with MgSO₄. Removal of the solvent afforded compound **5** (1.67 g, 60%).

¹H NMR (400 MHz, DMSO) δ: 1.38 (9H, s, CH₃ Boc); 1.85-1.92 (2H, m, β-CH₂ Met), 1.91-1.94 (2H, m, γ-CH₂ Met); 2.07 (3H, s, CH₃ Met); 3.69 (3H, s, OCH₃ ΔPhe); 4.14-4.16 (H, m, α-CH Met); 7.11 (H, d (J = 7.6 Hz), NH Methionine); 7.25 (H, s, β-CH ΔPhe); 7.31-7.38 (3H, m, ArH ΔPhe), 7.70 (2H, d (J = 3.6 Hz), ArH ΔPhe); 9.64 (1H, s, NH ΔPhe).

Synthesis of the methyl ester of L,D-methionyl-Z-dehydrophenylalanine [H-D,L-Met-Z-ΔPhe-OMe,TFA (6)]. TFA (3 mL mmol⁻¹) was added to Boc-L-Met-Z-ΔPhe-OMe (1.65 g, 4.04 mmol), and the mixture was left stirring 4 h at rt. The solvent was removed under reduced pressure and ethyl ether was added, storing in the freezer for 1 h. The solvent was removed under reduced pressure. Precipitation from ethyl ether afforded compound **6** as a white solid (1.63 g, 95.4%).

¹H NMR (400 MHz, CDCl₃) δ: 1.38 (3H, s, CH₃ Met); 1.59-1.62 (2H, m, β-CH₂ Met); 1.74-1.83 (2H, m, γ-CH₂ Met); 3.07-3.14 (1H, m, α-CH Met); 3.92 (3H, s, OCH₃ ΔPhe); 6.92 (1H, s, β-CH ΔPhe); 7.11-7.16 (1H, m, ArH ΔPhe), 7.37-7.45 (2H, m, ArH ΔPhe), 7.69-7.72 (2H, m, ArH ΔPhe).

Synthesis of the methyl ester of *N*-naproxen-L-methionyl-Z-dehydrophenylalanine [Npx-L-Met-Z-ΔPhe-OMe (7)]. Compound **6** was dissolved in dry DCM (5 mL mmol⁻¹) in an ice bath. Triethylamine (3 equiv; 0.42 mL) was added, followed by (S)-(-)-naproxen (1 equiv, 0.25 g), being left stirring overnight in an ice bath. A white precipitate was formed. The mixture was filtered and the solid was identified as compound **7** (0.15 g, 29%).

¹H NMR (400 MHz, CDCl₃) δ: 1.61 (3H, d (J = 7.2 Hz), CH₃ Npx); 1.95- 2.10 (2H, m, β-CH₂ Met), 2.00 (3H, s, CH₃ Met); 2.53-2.56 (2H, m, γ-CH₂ Met); 3.72-3.79 (1H, m, CH Npx); 3.74 (1H, s, OCH₃ ΔPhe); 3.91 (3H, s, OCH₃ Npx); 4.77 (1H, q (J = 6.4 Hz), α-CH Met); 6.47 (1H, d (J = 6.8 Hz), NH Met); 7.08 (1H, s, β-CH ΔPhe); 7.12-7.15 (1H, m, ArH); 7.25-7.42 (7H, m, ArH); 7.63-7.66 (3H, m, ArH); 7.85 (1H, s, NH ΔPhe).

¹³C NMR (100.6 MHz, CDCl₃) δ: 15.03 (SCH₃); 18.29 (CH₃ Npx); 29.68 (β-CH₂ Met); 29.97 (γ-CH₂ Met); 46.96 (α-CH Npx); 52.56 (OCH₃ ΔPhe); 52.60 (α-CH Met); 55.31 (OCH₃ Npx); 105.61 (CH Npx); 119.19 (CH Npx); 123.53 (C ΔPhe); 125.94 (CH Npx); 126.19 (CH Npx); 127.71 (CH Npx); 128.59 (CH ΔPhe); 128.98 (C Npx); 129.24 (CH Npx); 129.59 (CH ΔPhe); 129.70 (CH ΔPhe); 133.29 (C ΔPhe); 133.36 (CH ΔPhe); 133.82 (C Npx); 135.61 (C Npx); 157.78 (C Npx); 165.09 (C=O ΔPhe); 169.83 (C=O Met); 174.92 (C=O Npx).

Synthesis of the *N*-naproxen-L-methionyl-Z-dehydrophenylalanine [Npx-L-Met-Z-ΔPhe-OH (1)]. The (S)-(+)-naproxen-dehydrideptide (0.120 g, 0.23 mmol) was dissolved in acetone (10 mL mmol⁻¹) and a solution of NaOH 1 M (6 equiv) was added. The reaction was followed by TLC until no starting material was detected. The organic solvent was removed under reduced pressure, and the reaction mixture was acidified to pH 2-3 with KHSO₄ (1 M). The solid was filtered and washed with ethyl ether. The solid was identified as Npx-L-Met-Z-ΔPhe-OH, **1** (0.115 g, 98.9%).

¹H NMR (400 MHz, DMSO) δ: 1.42 (3H, d (J = 7.2 Hz), CH₃ Npx); 1.83-2.05 (2H, m, CH₂ Met), 2.05 (3H, s, CH₃ Met); 2.29-2.32 (2H, m, CH₂ Met); 3.83-3.85 (1H, m, CH Npx), 3.83 (3H, s, OCH₃ Npx); 4.44-4.54 (1H, m, α-CH Met); 7.08-8.26 (12H, m, ArH); 8.27 (1H, d = 8 Hz, NH Met); 9.52 (1H, s, NH ΔPhe).

¹³C NMR (100.6 MHz, DMSO) δ: 14.67 (SCH₃); 19.03 (CH₃ Npx); 29.41 (β-CH₂ Met); 31.76 (γ-CH₂ Met); 44.61 (CH Npx); 52.01 (α-CH Met); 55.16 (OCH₃ Npx); 105.69 (CH Npx); 118.45 (CH Npx); 125.40 (CH Npx); 126.39 (C ΔPhe); 126.50 (CH Npx); 126.66 (CH Npx); 128.37 (C Npx); 128.39 (CH ΔPhe); 129.09 (CH Npx); 129.12 (CH ΔPhe); 129.92 (CH ΔPhe); 132.14 (CH ΔPhe); 133.11 (C Npx); 133.43 (C ΔPhe); 137.21 (C Npx); 156.96 (C Npx); 166.11 (C=O ΔPhe); 170.98 (C=O Met); 173.65 (C=O Npx). HR-MS (ESI) *m/z*: [M + H]⁺ calcd for C₂₈H₃₁N₂O₅S⁺ 507.19; found, 507.19. Mp: 189-192 °C.

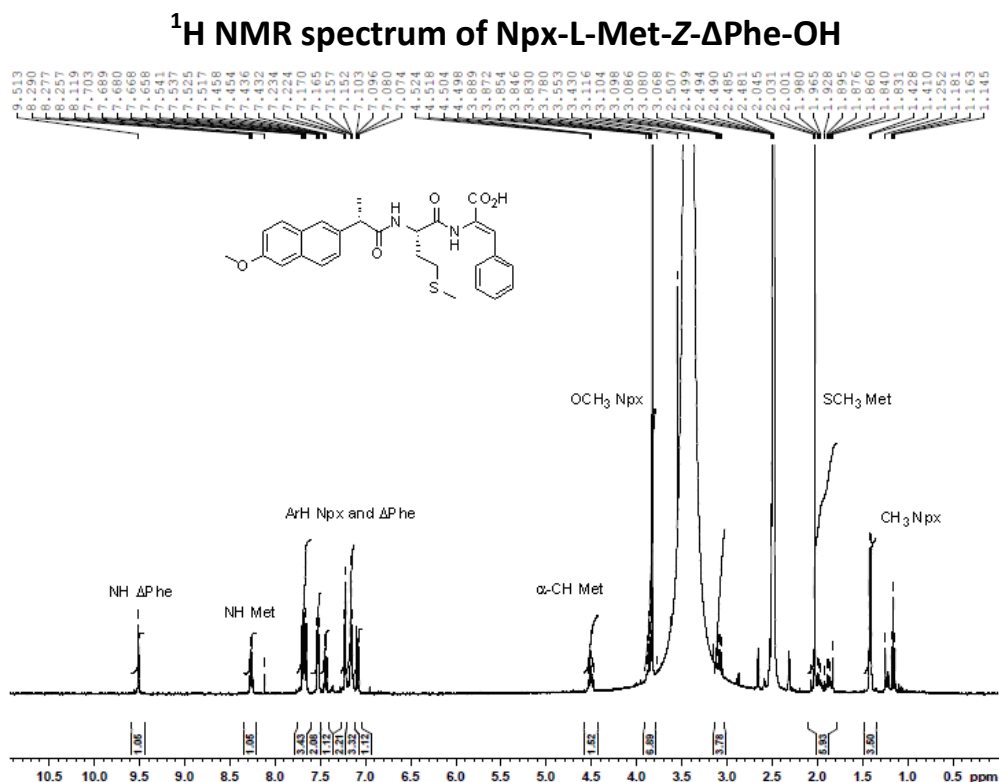


Figure S1. ^1H NMR spectrum of Npx-L-Met-Z- Δ Phe-OH **1** in $\text{DMSO}-d_6$.

In ^1H NMR spectrum of Npx-L-Met-Z- Δ Phe-OH, **1** in DMSO (Figure S1), it is possible to observe a doublet at 1.41 ppm due to the methyl protons of naproxen, two singlets at 2.05 ppm and 3.83 ppm assigned to the SCH_3 and OCH_3 of methionine and naproxen, respectively, and a multiplet between 4.44 ppm and 4.54 ppm due to the $\alpha\text{-CH}$ proton of methionine and between 7.08 ppm and 8.26 ppm due to the aromatic protons of naproxen and dehydrophenylalanine. The two signals of the NH protons appear as doublet at 8.27 ppm and as a singlet at 9.52 ppm.

UV-Visible absorption spectrum of Npx-L-Met-Z- Δ Phe-OH

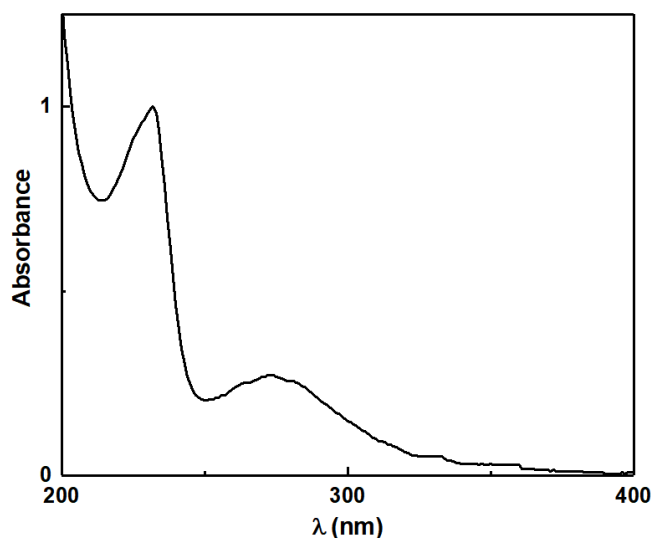


Figure S2. UV-Visible absorption spectrum of Npx-L-Met-Z- Δ Phe-OH in ethanol.

Hydrogel and hydrogelator critical aggregation assay

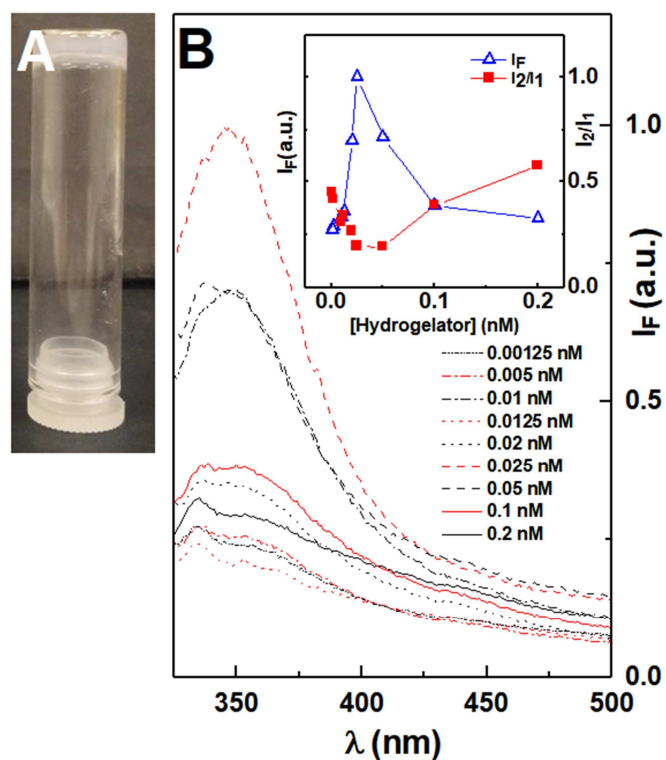


Figure S3. (A) Image of the glass vial flipping test to demonstrate hydrogel formation. (B) Fluorescence emission assay for determination of critical aggregation concentration at pH=6 (λ_{exc} = 290 nm).

Direct docking of flurbiprofen in cyclooxygenase-1

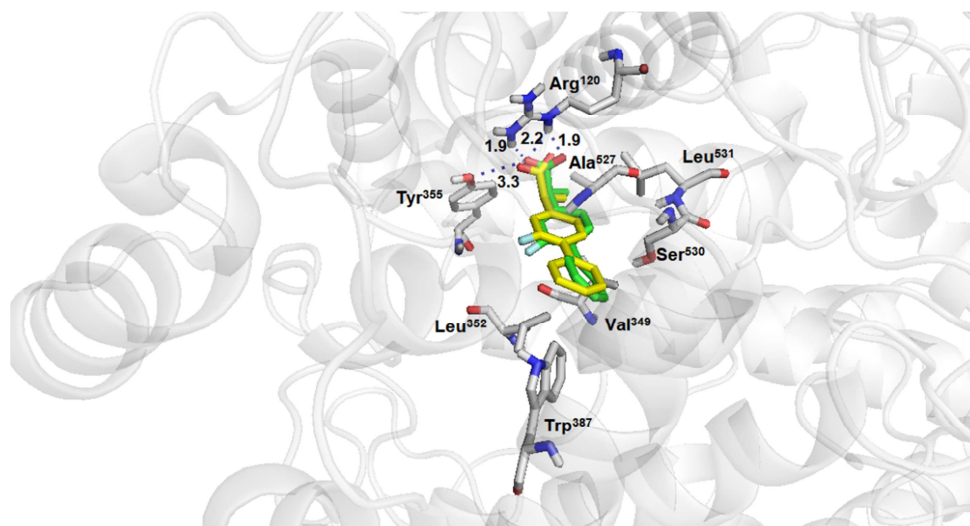


Figure S4. Representation of the crystallographic (green) and direct docking (yellow) flurbiprofen conformation in the active site of cyclooxygenase-1 and polar interactions distances (Å) obtained from PyMOL software.

Details on Rietveld Analysis

Rietveld analysis was carried out using either a 5th-order polynomial (Figure 5B) or a background defined by linear interpolation between a set of points at constant scattering angles and refinable intensities (Figures 5A and 5C) using FullProf.⁶⁸ In the case of gold decorated manganese ferrite, the amorphous contribution was first removed using a linear interpolation of a set of background points automatically determined by WinPLOTR software (background threshold = 0.05; number of smoothing iterations = 3; noisy data).⁶⁸

Rietveld analysis of the gold-decorated nanoparticles is affected by the procedure used to remove the amorphous background due to the organic coating contribution, as evidenced by the larger obtained R_f values. In this case, the size of the MnFe_2O_4 phase was fixed, as from the mild coupling procedure, it is not expected a particle size variation.

Mechanical spectra of magnetogels

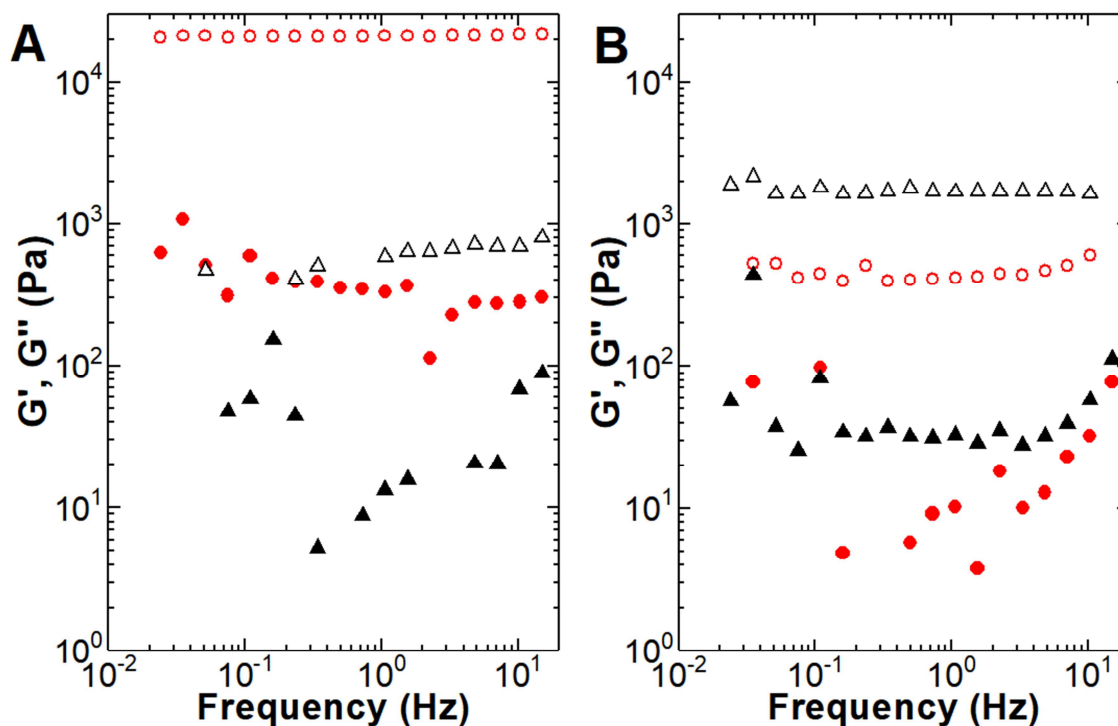


Figure S5. Mechanical spectra (G' , empty symbols; G'' , solid symbols) of the gels formulated with 10 m/m% (circles) and 20 m/m% (triangles) of (A) gold-decorated nanoparticles and (B) core/shell nanoparticles.

Core/shell nanoparticles hydrogelator-induced aggregation

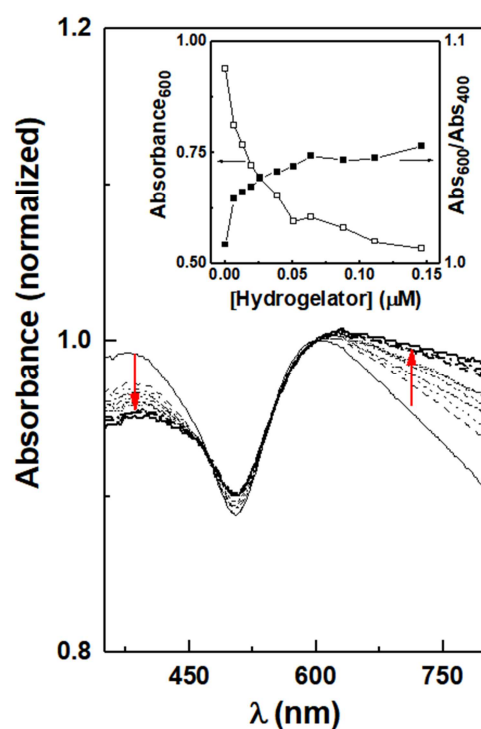


Figure S6. Normalized (at 600 nm) absorption spectra of core/shell manganese ferrite/gold nanoparticles (0.018 wt%) titrated with the hydrogelator. Inset: Absorbance at 600 nm and ratio of absorbance at 600 nm to 400 nm.

Considering the bare surface of the core/shell nanoparticles, the titration with the hydrogelator induced a reduction of global absorbance (absorbance at 600 nm), a higher gold plasmon to manganese ferrite absorbance ratio, a redshift and a widening of the gold plasmon band, thus suggesting that the hydrogelators promoted the aggregation of the core/shell nanoparticles.

FTIR determination of secondary structures

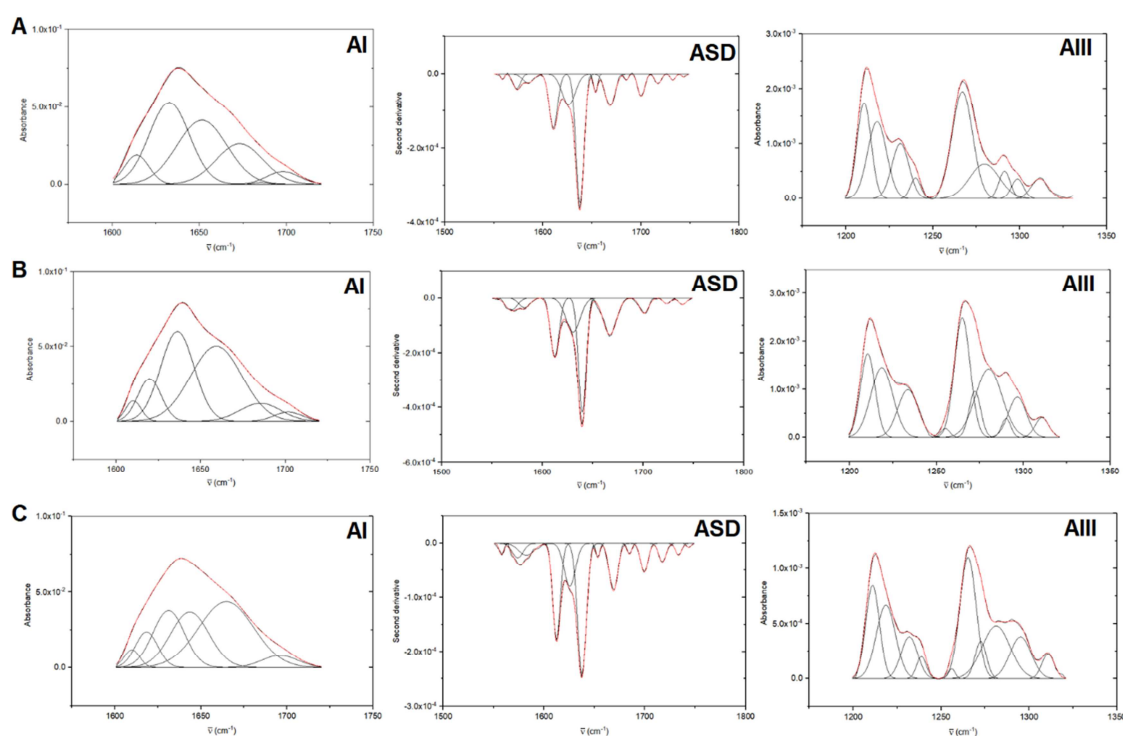


Figure S7. FTIR spectra of hydrogel (A), magnetogels containing 10 m/m% core-shell nanoparticles (B), and magnetogels containing 10 m/m% decorated nanoparticles (C). Legend: AI is amide I band; ASD is second derivative of amide I band; AIII is amide III band.

Particles size histograms from TEM

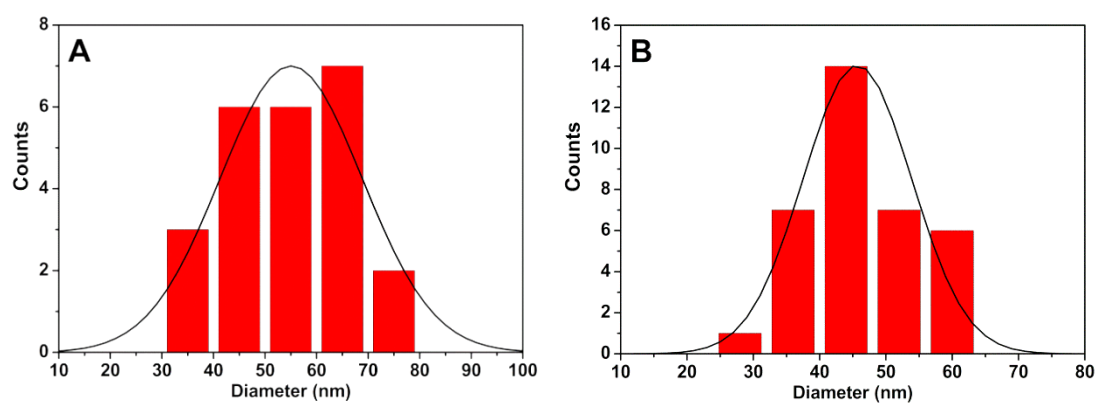


Figure S8. Size histograms of the nanoparticles obtained from TEM. (A) Core/shell manganese ferrite/gold nanoparticles (from image A1 of Figure 10); (B) gold-decorated manganese ferrite nanoparticles (from image B1 of Figure 10).

Electron Dispersive X-ray spectra

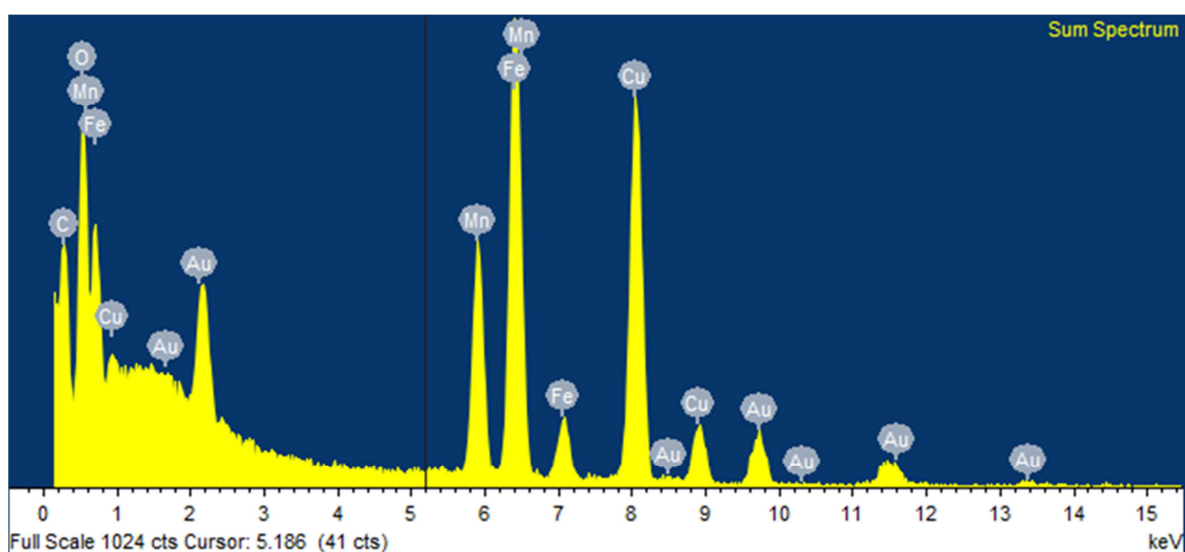


Figure S9. Electron dispersive X-ray (EDX) spectrum of core/shell manganese ferrite/gold nanoparticles.

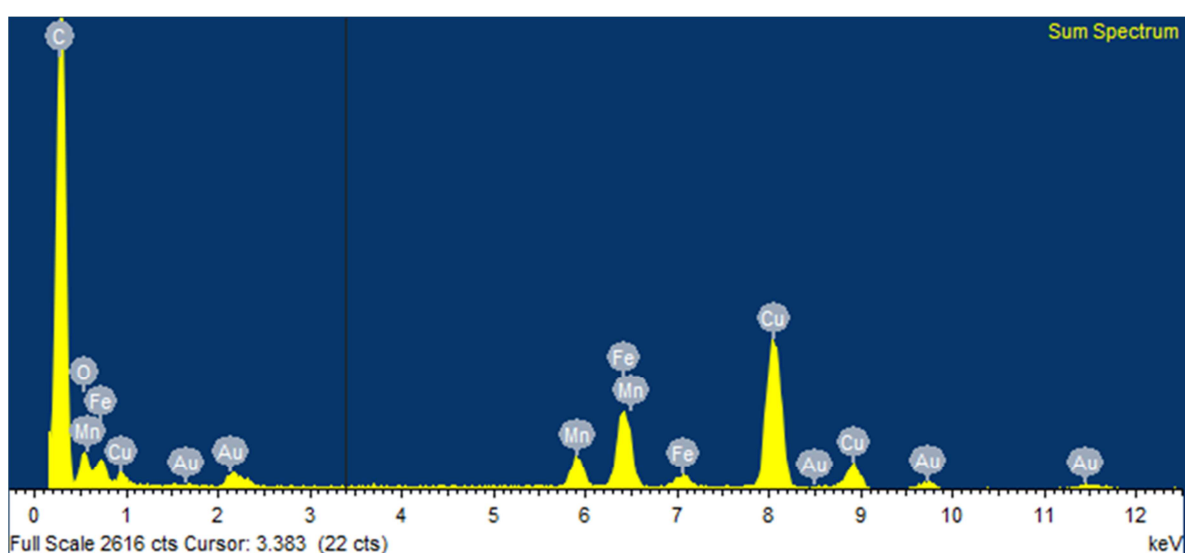


Figure S10. Electron dispersive X-ray (EDX) spectrum of gold-decorated manganese ferrite nanoparticles.

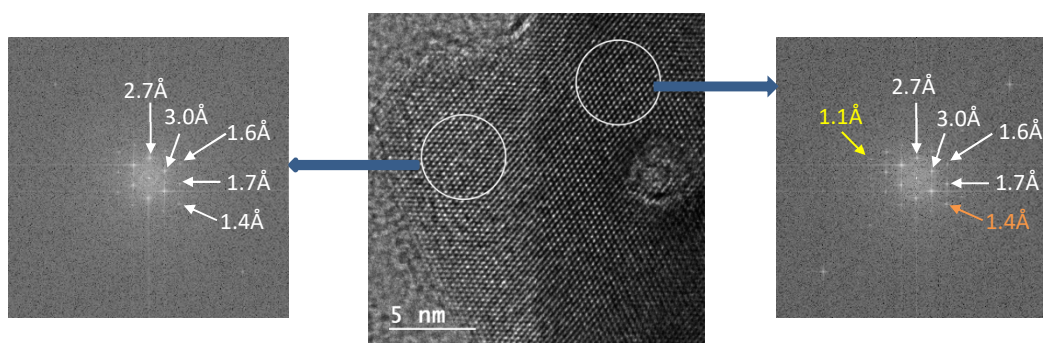


Figure S11. Fast Fourier Transform (FFT) of selected regions of TEM image (corresponding to Figure 10-A3).

Spectral overlap for FRET assays

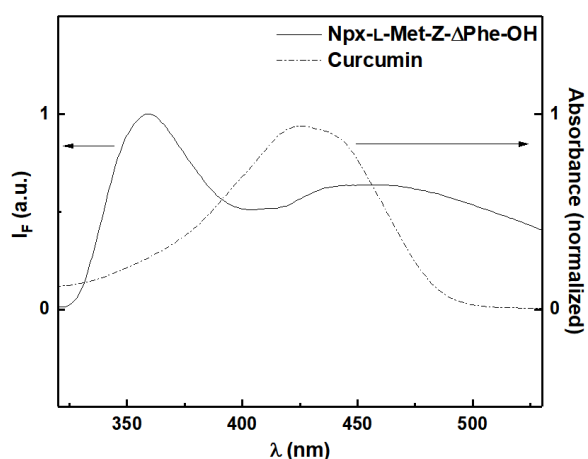


Figure S12. Overlap between fluorescence emission spectrum of the hydrogel and absorption spectrum of curcumin (spectra are normalized).

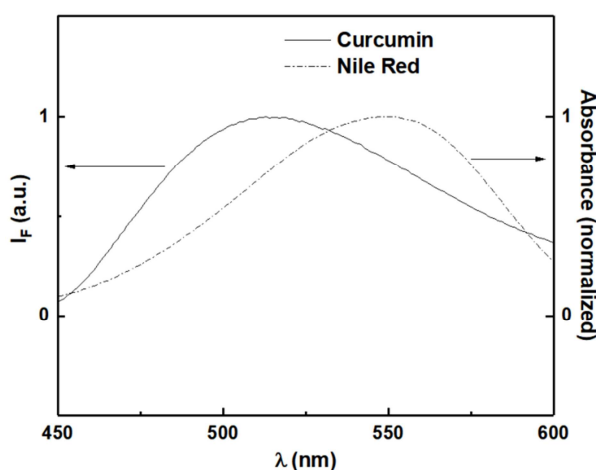


Figure S13. Overlap between fluorescence emission spectrum of curcumin and absorption spectrum of the dye Nile Red (spectra are normalized).

Fluorescence intensity dependence on temperature (eqn. (7)) in irradiation assays

Assuming an Arrhenius behaviour for the rate constant, k_{nr} , of non-radiative processes,⁹⁰ it is possible to determine the temperature in each irradiation assay,

$$k_{nr} = k_0 e^{-\frac{E_a}{RT}}$$

where k_0 is the preexponential factor; E_a is the activation energy, R the gas constant and T the absolute temperature.

The fluorescence quantum yield, Φ_F , is given by

$$\Phi_F = \frac{k_F}{k_F + k_{nr}} \Rightarrow \frac{1}{\Phi_F} - 1 = \frac{k_{nr}}{k_F}$$

where k_F is the fluorescence rate constant.

Taking as reference the fluorescence quantum yield at room temperature, Φ_F^0 , it is obtained

$$\frac{\Phi_F^0}{\Phi_F} - \Phi_F^0 = \Phi_F^0 \frac{k_{nr}}{k_F}$$

or, similarly,

$$\frac{I_F^0}{I_F} - \Phi_F^0 = \Phi_F^0 \frac{k_{nr}}{k_F}$$

where I_F represents the fluorescence intensity at a given wavelength.

Therefore, we obtain

$$\frac{I_F^0}{I_F} - \Phi_F^0 = \Phi_F^0 \frac{k_0}{k_F} e^{-\frac{E_a}{RT}}$$

Considering, as an approximation, that the fluorescence quantum yield of curcumin near the nanoparticles is negligible, it is obtained that

$$\frac{I_F^0}{I_F} \approx \Phi_F^0 \frac{k_0}{k_F} e^{-\frac{E_a}{RT}}$$

or

$$\frac{I_F^0}{I_F} \propto A e^{-\frac{E_a}{RT}} \quad (\text{eqn. 7})$$

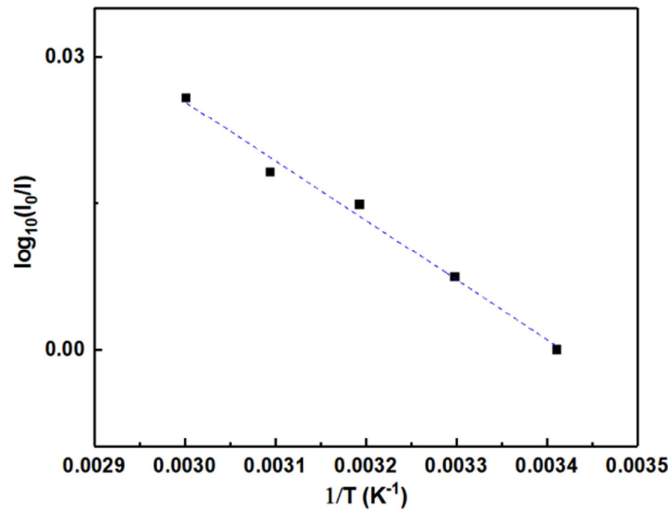


Figure S14. Curcumin fluorescence intensity variation at increasing temperature for Npx-L-Met-Z-ΔPhe-OH hydrogel 0.3 wt% and 4 μM of curcumin; $\log_{10}(I_0/I) = -60.966(1/T) + 0.2083$ ($R^2 = 0.989$).

Drug release assays

Table S1. Coefficients of determination (R^2) obtained for the release of curcumin (20 μ M) in hydrogel, magnetogel containing 10% core/shell manganese ferrite/gold nanoparticles (CS), magnetogel with 10% gold-decorated manganese ferrite nanoparticles (D), and magnetogel containing 10% non-coated manganese ferrite nanoparticles (MnFe_2O_4), with (IR) and without incidence of radiation, for a contact area of 3.8 cm^2 .

	First-order	Hixson-Crowell	Higuchi	Korsmeyer-Peppas	Gompertz
Hydrogel	-0.24	-0.38	0.76	0.92	0.99
CS	-0.36	-0.10	0.90	0.98	0.99
CS_IR	-0.36	-0.62	0.77	0.90	0.99
D	-0.36	-2.06	0.29	0.95	0.99
D_IR	-0.36	-2.07	0.01	0.93	0.98
MnFe_2O_4	-0.36	-0.77	0.72	0.98	0.99

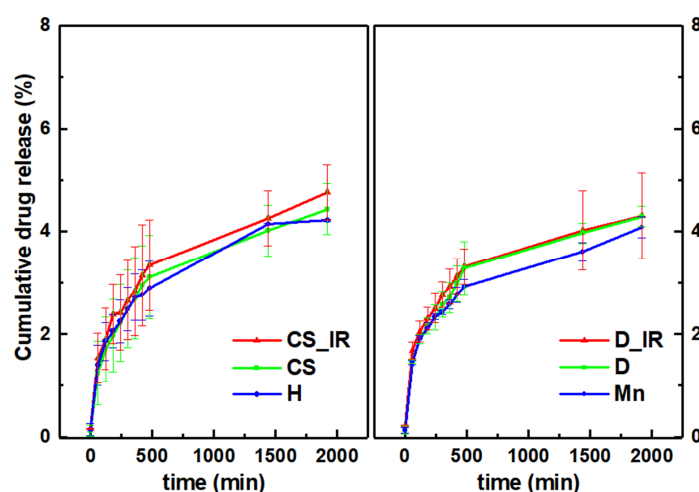


Figure S15. Cumulative release profiles (3 replicates) of hydrogelator in hydrogel (H), magnetogel containing 10% core/shell manganese ferrite/gold nanoparticles (CS), magnetogel with 10% gold-decorated manganese ferrite nanoparticles (D), and magnetogel containing 10% non-coated manganese ferrite nanoparticles (Mn) nanoparticles, with (IR) and without incidence of radiation, for a contact area of 3.8 cm^2 .

Table S2. Coefficients of determination (R^2) obtained for the release of the hydrogelator in hydrogel, magnetogel containing 10% core/shell manganese ferrite/gold nanoparticles (CS), magnetogel with 10% gold-decorated manganese ferrite nanoparticles (D), and magnetogel containing 10% non-coated manganese ferrite nanoparticles (MnFe_2O_4), with (IR) and without incidence of radiation, for a contact area of 3.8 cm^2 .

	First-order	Hixson-Crowell	Higuchi	Korsmeyer-Peppas	Gompertz
Hydrogel	-0.24	-0.49	0.80	0.99	0.99
CS	-0.31	-0.31	0.84	0.99	0.99
CS_IR	-0.29	-0.51	0.80	0.99	0.99
D	-0.50	-0.72	0.73	0.99	0.99
D_IR	-0.74	-1.12	0.61	0.99	0.99
MnFe_2O_4	-0.89	-0.89	0.68	0.99	0.99

Table S3. Release coefficients of the Korsmeyer-Peppas and Gompertz models obtained for the hydrogelator release profiles (20 μ M) in hydrogel, magnetogel containing 10% core/shell manganese ferrite/gold nanoparticles (CS), magnetogel with 10% gold-decorated manganese ferrite nanoparticles (D), and magnetogel containing 10% non-coated manganese ferrite nanoparticles (MnFe_2O_4), with (IR) and without incidence of radiation, for a contact area of 3.8 cm^2 .

	Korsmeyer-Peppas		Gompertz		
	K_s	N	X_{max}	a	b
Hydrogel	0.42	0.3094	32.93	5.37	0.296
CS	0.38	0.3291	10.51	6.47	0.611
CS_IR	0.47	0.3079	24.63	5.22	0.350
D	0.50	0.2875	11.01	5.15	0.517
D_IR	0.62	0.2580	10.19	4.55	0.507
MnFe_2O_4	0.52	0.2710	21.43	4.64	0.309

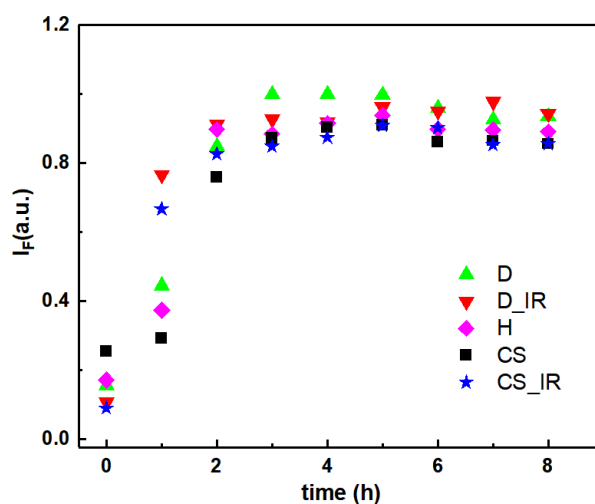


Figure S16. Time dependence of fluorescence emission of curcumin in SUVs of egg-PC/cholesterol 7:3, with excitation at 420 nm, during the interaction with drug-loaded hydrogel (H), core/shell (CS) and gold-decorated manganese ferrite (D) nanoparticle-containing magnetogels, with (IR) and without irradiation.