Photoresponsive polymersomes for nanoencapsulation of multiple cargo as a potential theranostic strategy

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Supporting information

Materials

Bovine albumin serum (BSA), poly(ethylene-*alt*-maleic anhydride) (PEMA, average MW 100 - 500 KDa), 2-nitrobenzyl alcohol (NBA, 97 %), N,N-dimethylformamide (DMF, 99 %), tetrahydrofuran (THF, 99.8 %), dimethyl sulfoxide (DMSO), rhodamine-b base (Rh-B, dye content 97 %), 5-fluorouracil (5-FU,) iron(III) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99.99 %), n-hexane, 2-propanol, and gold nanoparticles suspension stabilized in citrate buffer (AuNPs, 7.2 x 10 NPs/mL, 40.00 nm diameter) were purchased from Merck Millipore (Burlington, Massachusetts, United States). Ethanol (EtOH) was obtained from Bell Chem International S.A.S. (Longwood, Florida, United States). Methanol (MetOH) and sodium hydroxide (NaOH) were obtained from PanReac AppliChem (Darmstadt, Germany). 1-octadecene (90 %), cyclohexane, oleic acid (OA, 90 %), deuterated dimethyl sulfoxide (DMSO-d_6, with tetramethylsilane (TMS, 0.03 vol. %), deuteration degree min. 99.8 %), sodium citrate tribasic dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, ≥ 99 %), sodium chloride (NaCl), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle Medium (DMEM), and non-essential amino acids were purchased from Sigma-Aldrich (Saint Louis, Missouri, United States). EZ-Link hydrazide-LC-Biotin linker (Biotin linker, MW 371.50 g/mol, spacer arm length 24.7 Å) was purchased from Thermo Fisher Scientific Inc (Waltham, Massachusetts,

United States). Streptavidin-functionalized magnetic beads (Dynabeads™ MyOne streptavidin, strept-MBs, 10 mg/mL) were from Invitrogen (Carlsbad, California, United States). Streptavidin horseradish peroxidase (strep-HRP) and 3,3',5,5;-tetramethylbenzidine chromogen substrate solution (TMB) were acquired from Abcam (Cambridge Science Park, Cambridge, United Kingdom). Disodium hydrogen phosphate (Na_2HPO_4, 99 %) was supplied from PanReac AppliChem (Darmstadt, Germany). Potassium dihydrogen phosphate (KH_2PO_4, 99 %) and potassium chloride (KCl, 99.3 %) were supplied from J.T.Baker® (Xalostoc, Mexico). Hydrochloric acid (HCl, > 37 %) was supplied from Honeywell Fluka™ (Seelve, Germany). The female ovary Chinese hamster cell line (CHOK1), the large intestine colorectal cancer cell line (SW480), and the metastasis-derived cell line (SW620) were obtained from the American Type Culture Collection (ATCC, Manassas, Virginia, United States). Fetal bovine serum (FBS) was purchased from Microgen (Bogotá, Cundinamarca, Colombia), and fetal equine serum (FES) was purchased from Gibco (Waltham, Massachusetts, United States). All the chemicals were used as received without further purification.

All chemicals were used as received without further purification, and the aqueous solutions were prepared using deionized water (18.2 MΩ·cm).

Equipment and methods

For thermogravimetric (TGA) analysis, 12 mg of PEMA, NBA, and PEMA-*r*-NBA amphiphilic copolymer were weighed and placed under a 30 mL/min nitrogen atmosphere in a thermogravimetric analyzer (Mettler Toledo TGA/SDTA 851E) from 30 to 600 °C employing a heating rate of 10 °C/min with a final isotherm of 10 min. The onset (T_{onset}) was defined as the initial degradation temperature, calculated according to ATMS E2550 by a point on the TG curve with a deflection from the baseline established before the thermal event. Additionally, the maximum thermal degradation temperature (Tmax) was also recorded, along with the residual weight percentage at 600 °C.

Fourier-transform infrared (FT-IR) spectroscopy experiments were performed using an FT-IR spectrometer (Nicolet 6700 Series) equipped with a single-reflection ATR (ATR FT-IR) and a type IIA diamond crystal mounted in tungsten carbide. Dried precursors and copolymer samples were characterized. The diamond ATR had a sampling area of approximately 0.5 mm^2, with a consistent, reproducible pressure applied to every sample. The IR spectra were collected at 4 cm^{-1} resolution over 128 scans in the 4000-400 cm^{-1} range.

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded at 400 MHz on a Bruker Ascend TM600 NMR spectrometer. The ^{1}H-NMR chemical shifts were reported as δ in parts per million (ppm) relative to the TMS standard at ambient temperature in DMSO-d_6. NMR spectra were analyzed using MestReNova v14.0 software. The copolymer degree of substitution of PEMA-*r*-NBA and PEMA-*r*-NBA-Biotin was calculated from the ^{1}H-NMR spectra according to the following equations:

$$Degree\ of\ substitution\ (\%) = \frac{A_{aromatic\ H}/4}{A_{ethane\ H}/4} \times 100\ \%\quad (Eq.\ S1)$$

$$Degree\ of\ substitution\ of\ biotin\ (mol\ \%) = \frac{A_{biotin\ N-H}/2}{A_{ethane\ H}/4} \times 100\%\qquad (Eq.\ S2)$$

Where $A_{aromatic\ H}$ is the integral area of the protons of the aromatic ring, $A_{ethane\ H}$ is the area related to the integral of protons belonging to the -CH- groups in the anhydride backbone, and $A_{biotin\ N\text{-}H}$ is the area of the N-H in the aromatic ring of the biotin linker.[1]

For hydrodynamic diameter (D_H), dispersity (Đ), and the ζ-potential parameter, dynamic light scattering (DLS) and electrophoretic light scattering (ELS) were evaluated in a Malvern Zetasizer Lab-Blue equipment with 633 nm He-Ne laser (at 90 ° angle) from Malvern instruments. The measurements were performed using the DTS0012 or ZSU1002 cells for D_H and the DTS1070 cell for ζ-potential. The samples for the DTS1070 and DTS0012 were prepared at a 1:9 sample-to-water ratio, each at 1 mL. For the ZSU1002 cell, 20 µL of the pure sample was dispensed into the capillary tube.

The radius of gyration (R_g) was calculated using the Guinier plot obtained from the static light scattering (SLS). SLS studies were performed on a MultiFLow Field-Flow Fractionation system (FFF, AF2000) configured to work in size exclusion chromatographic (SEC) mode with an isocratic pump (PN1130), an autosampler (PN5300), and coupled to a 21-angle multiangle light scattering detector (MALS, PN3621) from Postnova Analytics (Landsberg, Germany). The same polymersome dispersions prepared for DLS studies were also used for SLS studies. Data were collected at 21 angles, maintaining the same solution concentration in all measurements. For the SLS studies, 20 µL of polymersome solution ([copolymer] ~ 0.15 mg/mL) was injected each time and eluted with a working flow rate of 0.2 mL/min of 0.01 M NaCl at 25 °C for 60 min using a Sepharose 4B column (Sigma-Aldrich, Saint Louis, Missouri, United States). The baseline calibration of the elugram was performed using a 90° distribution line. Using the equation below (Eq. S3), the R_g was estimated from the linear range of the Guinier plot, Kc/Rθ vs. sin² (θ/2).

$$R_g = \frac{8\pi^2 \Delta\rho}{\lambda^2 m} \qquad (Eq.\ S3)$$

Where, K is a constant, c is the concentration of polymersomes, R_θ is the Rayleigh ratio, λ is the wavelength of the X-rays, Δρ is the difference in electronic density between particles and the surrounding environment, and m is the slope of the Guinier plot.

Transmission electron microscopy (TEM) images were recorded using a Tecnai F20 super Twin TMP microscope operating at an accelerating voltage of 200 keV. For this purpose, 3 µL of polymersome dispersion (~0.5 mg/mL copolymer) was sonicated for 3 s before drop-casting onto Formvar/carbon-supported 200 mesh copper grids, and the solvent was allowed to air-dry overnight. All polymersome samples were stained with a 2 wt % aqueous solution of uranyl acetate (UA). Bright-field scanning transmission electron microscopy (STEM) images were acquired at 30 keV using a Thermo Scientific Apreo 2 S LoVac Field-Emission Scanning Electron Microscope (FESEM). Sample preparation was the same as described in the TEM images above. TEM and STEM micrographs were processed and analyzed using ImageJ (National Institutes of Health (NIH) and Laboratory for Optical and Computational Instrumentation (LOCI)) to determine the size distribution of control nanopolymersomes (CNP) and to calculate the corresponding TEM and STEM diameters (D_{TEM} and D_{STEM}) from 200 and 400 individual particles, respectively. Additionally, 120 measurements from TEM micrographs were used for determining membrane thickness.

For the Cryo-TEM image recording, 1.5 µL of polymersome dispersion (~1.5 mg/mL copolymer) was drop-casted onto a glow-discharged Lacey/carbon-supported 300-mesh copper grid (Ted Pella, USA). The sample was kept at 100% humidity

inside the Vitrobot Mark III chamber (FEI Company, Eindhoven, Netherlands). The excess liquid was automatically blotted onto filter paper, followed by cryo-immobilization by plunge-freezing in liquefied ethane. The plunge-frozen sample was transferred to a Tecnai F20 EM (FEI Company, Eindhoven, The Netherlands) in the Cryomicroscopy Unit at the Scientific and Technological Center of the Universitat de Barcelona using a cryo-holder (Gatan, Pleasanton, United States). The sample was examined in cryogenic conditions at 200 keV and using low-dose imaging conditions. Low-dose images were recorded with a 4096 × 4096 pixel CCD Eagle camera (FEI Company, Eindhoven, The Netherlands).

Polymersome nanoencapsulation and laser-stimulated release were evaluated using a Thermo Scientific Varioskan Lux in the 200-800 nm wavelength range, with 96-well plates or the Thermo Scientific µDrop Plate. Laser-stimulated release studies were conducted using a portable HTLD-4II UV LED spot-curing lamp with a 365 nm wavelength UV LED and an effective irradiation area of 12 mm.

A Physical Property Measurement System Model 6000 was used for magnetic susceptibility measurements, employing the Quantum Design VSM module at ±5 T (300 K) in a direct current (DC) configuration.

The centrifuge used for polymerosome purification was a HERMLE Z 36 HK (HERMLE Labortechnik). Christ Alpha 1-2 LSC basic lyophilization was implemented to dry off the polymersome samples and subsequently quantify the encapsulation efficiency and loading capacity. In addition, a holder with embedded neodymium magnets was used to separate and wash the modified Strep-MBs. A thermal mixer, Thermo LPG–mySPIN 6 Mini Centrifuges, and a vortex (Thermo Fisher Scientific, Waltham, Massachusetts, United States) were also used.

Synthesis of Fe_3O_4 MNPs

The Fe_3O_4 MNPs were synthesized according to the method described by Ding et al.2 via thermal decomposition. Briefly, 2 mmol $FeCl_2{\cdot}4H_2O$ was dissolved in 6 mL of H_2O with the subsequent addition of 8 mL of EtOH, 14 mL of hexane, and 1.90 mL of OA while stirred for 30 min. Then, 0.24 g of NaOH was added, and the mixture was stirred at 70 °C. The sticky $Fe(oleate)_3$ precursor was separated from the organic layer containing the $Fe(oleate)_3$ complex and dispersed in 0.32 mL of OA and 12.50 mL of 1-octadecene. Then, the mixture was degassed with N_2 for 30 min at room temperature, followed by heating at 320 °C for 30 min under N_2. Once the mixture was cooled to room temperature, it was precipitated with ethanol and separated via centrifugation. The resulting isolated solid was redispersed in hexane and precipitated with EtOH repeatedly to purify the Fe_3O_4 MNPs.

Synthesis of the amphiphilic random copolymer

PEMA-*r*-NBA amphiphilic random copolymer was synthesized by a substitution reaction in a 1:2 PEMA:NBA ratio with a 2.5 % w/v of PEMA in DMF. The substitution occurred between the cyclic anhydride of PEMA and the OH group from the NBA. The reaction conditions were 18 h at 70 °C and 700 rpm in dried DMF. The obtained copolymer was precipitated with 0.1 M HCl, centrifuged, diluted in DMSO, dialyzed against a 1:9 ethanol (70% v/v):water ratio, and further centrifuged at 21,000 rpm, 4 °C for 30 min, followed by drying in the oven for 24 h at 45 °C. The same reaction parameters were accomplished for the biotin functionalized copolymer (2.5, 5, 10, and 15 % of biotin-linker regarding moles of anhydride), employing the same concentration of 2.5 w/v % of PEMA as the unfunctional polymer, but with except the reaction solvent that was a DMSO:DMF mixture in a 1:9 ratio for the functionalized copolymers aiming to improve the solubility of the linker.

Self-assembly of empty nanopolymersomes

The empty polymersomes, referred to as control nanopolymersomes (CNP), were assembled via nanoprecipitation. Typically, 1 mL of a 5 mg/mL copolymer in THF, serving as the organic phase, was added dropwise to 1 mL of the aqueous phase at a rate of 0.016 mL/min for 1 h and 30 min, while stirring at 500 rpm and room temperature, using a CA08-100 syringe pump. The subsequent purification of polymersomes was accomplished via 24 h dialysis against water using Spectra/Por molecular porous membrane tubing with an MWCO of 12-14 kDa. The self-assembly process was the same for the biotin-functionalized polymersomes, except for the type of organic phase solvent. It was a mixture of THF and DMF at 12.5, 25, 50, and 70 % for assembling 2.5, 5, 10, and 15 % functionalized polymersomes, respectively, aiming to improve the solubility of the biotin-functionalized polymer. All organic solutions were filtered through a WhatmanTM syringe nylon filter with a 0.2 µm pore size before the nanoprecipitation process to remove polymer impurities and homogenize the solution.

Stability of polymersomes

The stability of CNP was evaluated in nine different media. Each medium was prepared at high concentrations of 5.0 M for citric acid (CA), 10X for the PB and PBS at different pH, and 1.0 M for NaCl and KCl. Then, the CNP solution was added to each medium until the defined concentration was reached. Hence, H_2O type II was the original media; therefore, the CNP solution was diluted to 0.5 M CA, 0.1 M CB pH 4.0, 1X PB pH 5.4, 1X PB pH 7.4, 1X PBS pH 5.4, 1X PBS 7.4, 0.1 M NaCl and 0.1 M KCl. Once the sample was prepared, it was allowed to settle for 15 minutes before DLS measurements were taken.

The stability of polymersomes over time was studied by measuring the size and dispersity of CNP in three media: water, 1X PBS pH 5.4, and 1X PBS pH 7.4 over 6 weeks. For this purpose, 20 µl of CNP solutions in each medium were measured using the ZSU1002 low-volume cell. Then, the obtained size and dispersity were plotted for further analysis.

Self-assembly of cargo-loaded bare and functionalized polymersomes

The process for encapsulating cargo of varying natures, properties, and uses into polymersomes was similar to that for assembling CNP, with a few variations. For example, for polymersomes containing 2 and 8 % w/v Rh-B (Rh-B-P), the aqueous phase consisted of 0.1 and 0.4 mg/mL Rh-B solutions, respectively. Furthermore, for encapsulating 10 and 20 % w/v 5-FU into the polymersomes, the organic phase solvent containing 5-FU was a 2:8 MetOH:THF ratio mixture. Therefore, 5-FU-polymersomes (5-FU-P) were purified by dialysis against water for 2 h, exchanging the media every 30 min, followed by solvent evaporation for 22 h at room temperature. The dialysis time was reduced to avoid the outflow of 5-FU from the nanopolymersomes' core to the aqueous medium.

The encapsulation efficiency (EE) and loading capacity (LC) were calculated considering the calibration curves shown in Figures S3-S4 using the following equations:

$$EE\ (\%) = \frac{Weight\ of\ cargo\ into\ the\ polymersomes}{Initial\ weight\ of\ cargo} \times 100\ \% \qquad (Eq.\ S4)$$

$$LC\ (\%) = \frac{Weight\ of\ cargo\ into\ the\ polymersomes}{Total\ weight\ of\ the\ formulation} \times 100\ \% \qquad (Eq.\ S5)$$

For encapsulating AuNPs into the polymersomes (AuNPs-P), the aqueous phase was replaced by a mix of 1:3 AuNPs solution:water ratio. For the assembly of magnetopolymersomes (MNPs-P), 1 mL of the hydrophobic Fe3O4 MNPs dispersed in hexane was dried overnight at room temperature to remove the hexane. Then, 1 mL of a 5 mg/mL copolymer solution in THF was added to the dried Fe3O4 MNPs, confirming their dispersion in the solution. Then, the subsequent nanoprecipitation process was accomplished. Polymersomes co-encapsulating Fe_3O_4 MNPs and 25% w/v 5-FU (MNPs/5-FU(25%)-P) were prepared by adding 5-FU and MNPs to the organic phase, followed by the nanoprecipitation process as detailed above. In all cases, the extent of encapsulated cargo was calculated according to the PEMA-*r*-NBA polymer concentration (5 mg/mL).

Release of 5-FU from polymersomes

The 5-FU release was achieved by dissolving 100 µL of 5-FU-P (20% w/v) in a Pur-A-LyzerTM Mini 12000 Dialysis kit and suspending the mixture in 800 µL of two media: water type II and 1X PB (pH 5.4). Then, the sample was irradiated for 0, 5, and 15 min at 365 nm using the HTLD-4II UV LED spot curing lamp at 2 cm from the light source. Once irradiation ended, 4 µL of the media solution was collected, and the maximum absorbance at 269 nm corresponding to 5-FU in both media was measured every 5 min for up to 60 min. An equal volume of the collected sample was replaced in each measure with the corresponding medium. Each measurement was in triplicate. The extent of drug release was calculated according to Eq. S6.

$$Drug\ release\ (\%) = \left(\frac{M_R}{M_L}\right) \times 100\ \% \qquad (Eq.\ S6)$$

M_L and M_R are related to the amount of the loaded and released drug, respectively, as quantified from the corresponding calibration curves in water type II and 1X PB at pH 5.4.

The extent of photocleavage was calculated for the CNP in three different media (pH 3.0 – 4.0 H_2O, 1X PB pH 5.4, and **C)** 1X PBS pH 7.4.) as is shown in the Eq. S7.

$$Photocleavage\ (\%) = \left(1 - \frac{A_t}{A_0}\right) \times 100\ \% \qquad (Eq.\ S7)$$

A_t corresponds to an absorbance at any time of UV irradiation, whereas A_0 is related to the absorbance before UV irradiation.

Surface-biotin test

The biotin on biotin-functionalized polymersomes (f-CNP) at four biotin-ligand proportions (2.5, 5, 10, and 15 %) was qualitatively determined using a homemade magneto-assay according to Quinchia et al. 2024[3] in 1X PB pH 5.4. Briefly, 20 µL of strep-MBs (1 mg/mL in 1X PB pH 7.4) were placed in a 1.5 mL microcentrifuge tube and washed twice with 50 µL of 1X PBS pH 7.4. After each washing step, the microcentrifuge tube was placed in the magnetic holder for 2 min before the supernatant was removed. The washed strep-MBs were resuspended in 50 µL of f-CNP (~ 0.0025 mg/mL copolymer), and the mixture was incubated for 30 min at 37 °C in a thermo-shaker under constant stirring at 1100 rpm. The f-CNP–MBs conjugate was washed thrice with 50 µL of washing buffer. Then, 50 µL of 50 ng/mL of strep-HRP in binding buffer was added and incubated for 30 min at 37 °C under constant stirring, followed by five washing steps. After removing the

supernatant, 50 µL of TMB was added, and the reaction was incubated for 30 min at 20 °C in the dark. Finally, the supernatant was separated from the strep-HRP-f-CNP–MBs magnetocconjugate and added to a 96-well plate spectrophotometer. The absorbance spectrum was collected from 300 to 800 nm. The magneto-assay was developed with CNP in 1X PB pH 5.4 as a negative control (C-), and AuNPs coupled to a probe with a biotin terminal strand were used as a positive control (C+). The experiment was performed in triplicate in independent experiments.

Cell line maintenance

Three cell lines corresponding to CHOK1, SW480, and SW620, with fewer than 17 passages, were established to test the photo-responsivity of functionalized polymersomes' potential performance. The cell lines were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum for CHOK1 and 10% (v/v) equine serum for SW480 and SW620, along with 50 µg/mL penicillin and 50 IU/mL streptomycin, in a humidified atmosphere containing 5% CO_2 at 37 °C.

In-vitro MTT-cytotoxicity and phototoxicity assays

The viability assays were performed at 80% cell confluence. Once the confluence was confirmed, 1 x 10^4 cells were seeded into 96-well plates with 100 µL of culture medium per well and incubated at 37 °C in a humid atmosphere containing 5 % CO_2 for 24 h. Then, the culture medium was removed, and cells were treated with seven concentrations (4.7, 9.4, 18.8, 37.5, 75, 150, and 300 µg/mL) of bare (CNP) and functionalized (f-CNP) polymersome dispersions to evaluate the effect of the empty polymersomes in the viability of the cell lines after 24 h.

Subsequently, 50 µL of MTT at 1 mg/mL was added, and the plate was incubated in the dark for 2 h. After incubation, the MTT solution was removed, and 100 µL of 2-propanol was added to each well to dissolve the formazan crystals formed in the metabolically active cells. The plates were gently swayed for 30 min before the absorbance wasmeasured at 570 and 650 nm (reference wavelength).

Absorbances were subtracted, and cell viability was determined using the following equation:

$$Viability\ (\%) = \frac{Absorbance\ treated\ cells}{Absorbance\ of\ control\ cells} \times 100\ \% \quad (Eq.\ S8)$$

Each assay was performed in duplicate. A 10 % v/v solution of DMSO in water was used as a positive control, and DMEM culture medium was used as a negative control.

Photo-triggered delivery of cargo into cells

The highest concentration of CNP and f-CNP, along with the longest UV exposure time (300 s), with a minimal effect on cell viability, was selected to evaluate the photo-triggered delivery of 5-FU into cells under UV light (λ = 365 nm). For this, 96-well plates seeded with 1 x 10^4 cells in 100 µL of supplemented culture medium were maintained at 37 °C, 5 % CO_2 between 22 h and 26 h. Then, the culture medium was removed, followed by the addition of 100 µL of each treatment (Treatment medium: CM, cells control: CC, CNP, 5-FUP, f-CNP, f-5FUP). The samples were evaluated at 0 s and 300 s of exposure to UV after 4 h of treatment. After 24 h of incubation, the culture medium was removed, followed by adding 50 µL of MTT solution, and the procedure continued as described above. The experiments were done in quintuplicate.

Statistical Analysis

The data analysis was performed using IBM SPSS Statistics version 23.0 software, released by IBM Corp. in 2012. Each experiment for each assay was conducted in three independent replicates. The normality of the quantitative variables in the study was assessed using the Kolmogorov-Smirnov test. Subsequently, bivariate analyses were conducted using the Kruskal-Wallis test with the Bonferroni correction to determine whether the treatments and their concentrations showed statistically significant differences compared to the negative control ($p < 0.05$).

Tables

Table S1. Thermal degradation onset, maximum weight loss rate temperature, and final residue extent of PEMA-*r*-NBA copolymer and its PEMA and NBA polymer precursors.

Sample	T_{Onset} (°C)	T_{Max} (°C)	Residue (%)
PEMA	262.75	298.41, 433.18	13.03
NBA	177.44	219.62	2.33
PEMA-*r*-NBA	114.05	122.64, 249.88, 419.17	14.70

Table S2. Mean D_H of CNP behavior dependency on the medium and pH surrounding the system.

Medium	D_H (nm)
H_2O pH 3.0-4.0	80.38 ± 1.57
0.5 M CA pH 1.4	491.20 ± 18.95
0.1 M CB pH 4.0	112.30 ± 1.88
1X PB pH 5.4	82.66 ± 1.27
1X PB pH 7.4	268.50 ± 8.57
1X PBS pH 5.4	108.20 ± 1.31
1X PBS 7.4	328.40 ± 15.09
0.1 M NaCl pH 3.0-4.0	130.10 ± 1.67
0.1 M KCl pH 3.0-4.0	333.70 ± 7.70
1.0 M NaCl pH 3.0-4.0	1048.00 ± 279.90
1.0 M KCl pH 3.0-4.0	7891.00 ± 5965.00

Table S3. Size, dispersity, ζ-potential, encapsulation efficiency, and loading capacity of Rh-B- and 5-FU-loaded NPs.

Encapsulated active component	D_H (nm)	Dispersity (Đ)	ζ-potential (mV)	EE (%)	LC (%)
Rh-B (2 %)	133.10 ± 12.75	0.28 ± 0.00	-59.46 ± 1.89	21.08	0.84
Rh-B (8 %)	135.00 ± 7.40	0.30 ± 0.03	-59.04 ± 5.94	21.84	3.08
5-FU (10 %)	83.30 ± 1.24	0.38 ± 0.00	-48.48 ± 2.65	7.31	2.29
5-FU (20 %)	80.17 ± 0.91	0.21 ± 0.00	-44.66 ± 5.11	14.20	5.32

Figures

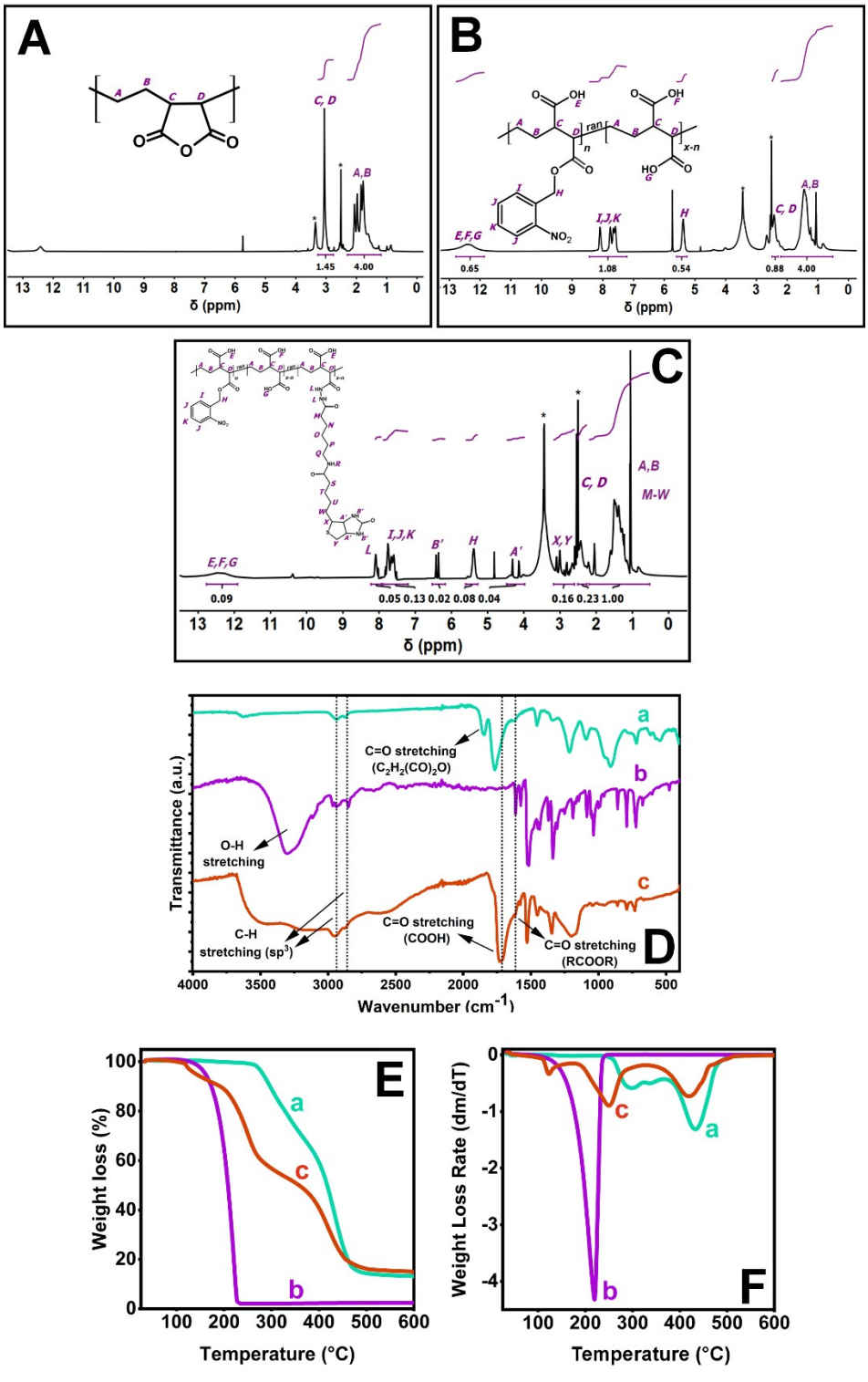

Figure S1. ^{1}H NMR of **A)** PEMA, **B)** PEMA-*r*-NBA and **C)** the biotin-functionalized PEMA-*r*-NBA copolymer. The chemical shift at δ 2.5 and δ 3.5 ppm corresponds to DMSO and water (labeled as *), respectively. The integral values for the area of

each peak are normalized. **D)** ATR FT-IR spectra, **E)** TGA, and **F)** DTG thermograms of a) PEMA, b) NBA, and c) PEMA-*r*-NBA. The dm/dT values were divided by a factor of 0.001.

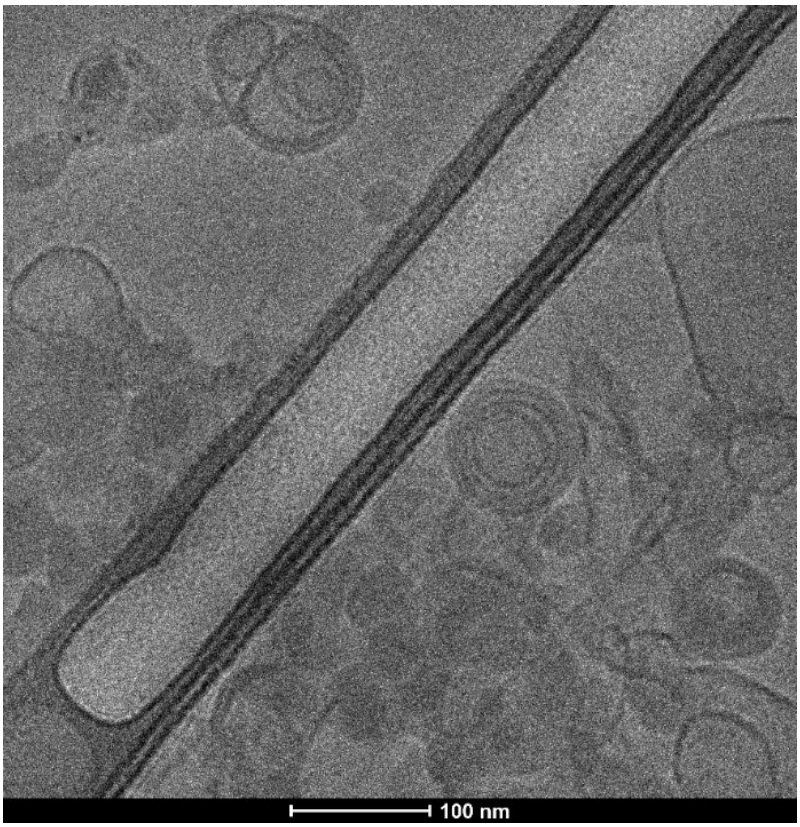

Figure S2. Cryo-TEM micrograph of CNP in water media after 6 weeks of assembly.

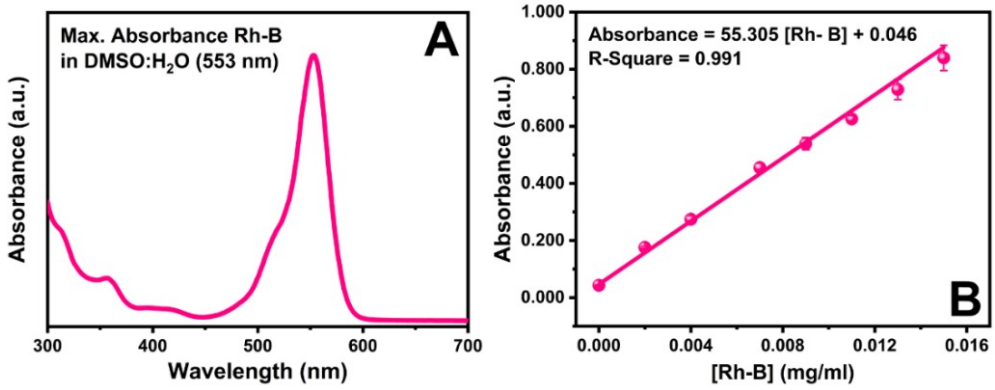

Figure S3. A) Rh-B UV-vis spectrum with maximum absorbance at 553 nm and **B)** resultant calibration curve in a 3:7 water: DMSO solution in a 96-well plate with 60 µl per well for Rh-B encapsulation efficiency and loading capacity determination.

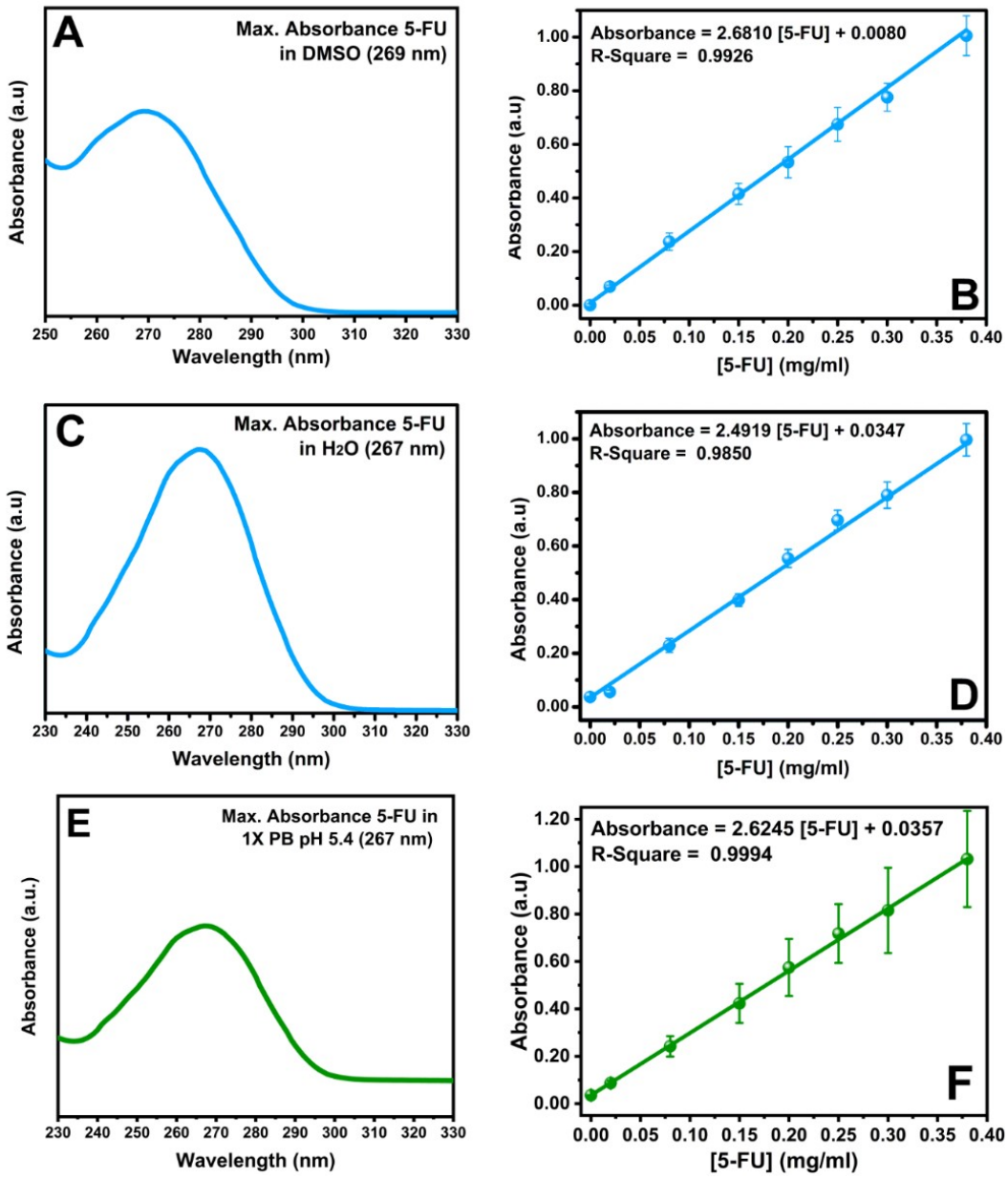

Figure S4. **A)** 5-FU UV-vis spectrum with maximum absorbance at 269 nm and **B)** resultant calibration curve in DMSO in a µDrop plate using 4 µl per cell for 5-FU encapsulation efficiency and loading capacity determination. **C)** 5-FU UV spectrum with maximum absorbance at 267 nm and **D)** resultant calibration curve in H_2O in a µDrop plate using 4 µl per cell for 5-FU release analysis. **E)** 5-FU UV spectrum with maximum absorbance at 267 nm and **F)** resultant calibration curve in a 1X PB pH 5.4 in a µDrop plate using 4 µl per cell for 5-FU release analysis.

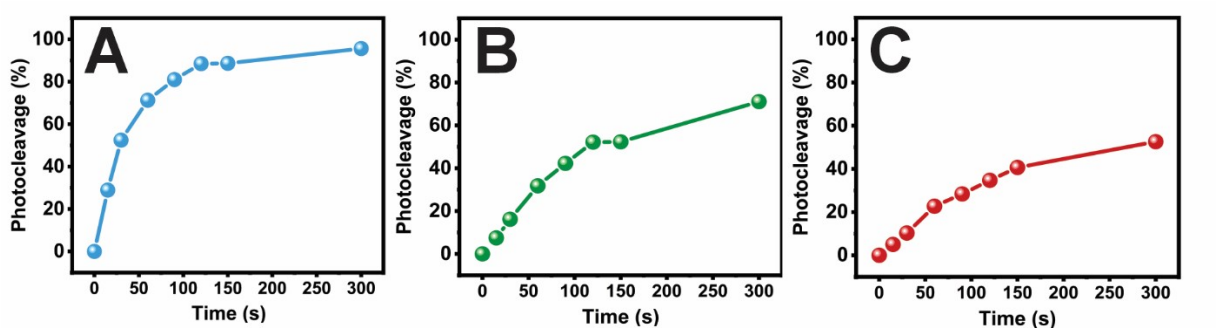

Figure S5. Photocleavage extent at 310 nm UV irradiation over time in three different media. **A)** pH 3.0 – 4.0 H_2O, **B)** 1X PB pH 5.4, and **C)** 1X PBS pH 7.4. The extent of photocleavage was calculated relative to the basal state (zero UV irradiation).

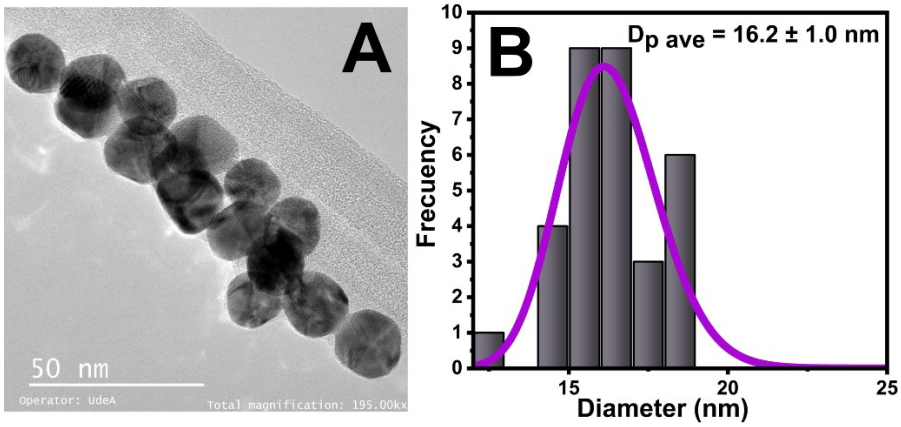

Figure S6. A) TEM micrograph and **B)** diameter histogram of AuNPs obtained from counting 32 particles. The size distribution corresponded to a Lognormal distribution.

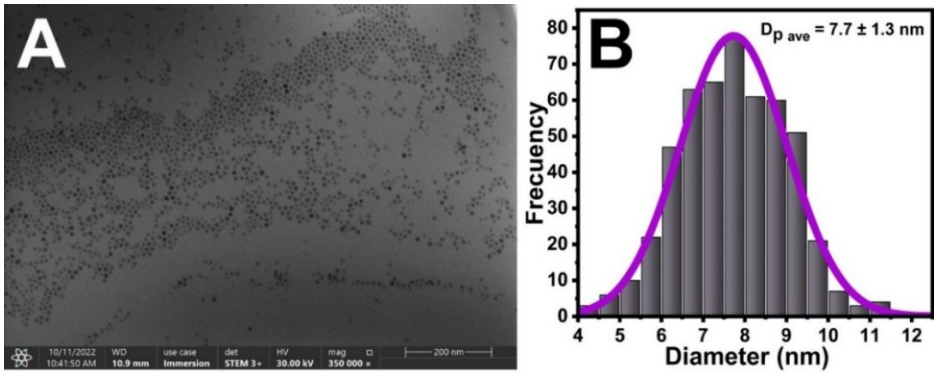

Figure S7. A) STEM micrograph and **B)** diameter histogram of Fe_3O_4 NPs obtained from counting 500 particles. The size distribution corresponded to a normal distribution.

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