Supporting Information

Biological Activation of Fenton Reaction in Polymeric Nanoreactors Driven by Ferrocene-Containing Membranes: A Microenvironment Dependent Study

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1. Apparatus

Characterization of the composition of BCPs

Nuclear Magnetic Resonance (NMR) spectroscopy. Studies were performed employing a Bruker Advance III 500 (Bruker Biospin, Germany) was used for recording ^{1}H-NMR (500.13 MHz) spectra using $CDCl_3$ as solvent at room temperature for BCP characterization (composition and molar mass of all block copolymers). The chemical shifts were referenced to corresponding solvent signals ($CDCl_3$: δ = 7.26 ppm for ^{1}H-NMR) and were expressed in ppm (**Table S1**).

Gel Permeation Chromatography (GPC). The molar mass distributions (Đ), weight average molar mass (Mw), and number average molar mass (Mn) of all block copolymers were measured using size exclusion chromatography (SEC) equipped with an Agilent 1260 Infinity variable wavelength detector (VWD, Agilent Technologies, Germany) a multi angle laser light scattering (MALLS) detector (MiniDAWN TREOS II, Wyatt Technology Europe, Germany), and a refractive index (RI) detector (K-2301 (KNAUER)). The column (high-performance liquid chromatography (HPLC) Column Plgel, Mixed-C, 300 × 7.5 mm, average bead size: 5 µm) and the pump (Isocratic pump, Agilent 1200 series) were from Agilent Technologies (Germany). DMAc in 3 g L^{-1} LiBr was used as the eluent with a flow rate of 1 mL min^{-1} at 25°C (**Table S1**).

Characterization of Empty-FcPsomes and GOx-FcPsomes

Dynamic Light Scattering. Measurements of aqueous Empty-FcPsomes and Enzyme-FcPsomes solutions (≤ 1 mg mL^{-1}) were carried out using a Zetasizer Nano-series instrument (Malvern Instruments, UK) equipped with Dispersion Technology Software (version 5.00). The measurements were carried over a range of pH at 20 °C. The equilibration time was 30 s and the acquisition time was 75 or 125 s (15 or 25 runs, 5 s/run) per three measurements. The data was collected using the NIBS (non-invasive backscatter) method using a Helium–Neon laser (4 mW, l = 632.8 nm) and a fixed angle of 173°. The data was analysed using Malvern Software 7.11.

Zeta potential (ζ). ζ measurements were carried out on Empty-FcPsomes (1 mg mL^{-1} in buffer) at 25°C using a Zetasizer Nano-series instrument (Malvern Instruments, UK) through electrophoretic light scattering. Data evaluation was carried out using Malvern Software 7.11.

Cross-linking. An EXFO Omnicure 2000 (Lumen Dynamics GroupInc., Canada) UV lamp equipped with a high-pressure mercury lamp as UV source was used for crosslinking.

Microplate reader. (enzyme assays) TECAN infinite PRO microplate reader equipped with I-control 1.10 software was used for the determination of the enzymatic activities. Measurements to validate the H_2O_2 production or consumption were done by using Amplex Red Assay by fluorescence spectroscopy (λex = 534 nm, λem = 580 nm, 1 h every 10 min). (MTT studies) Multiskan (Thermo Fisher Scientific) by UV-VIS spectroscopy.

Hollow Fiber Filtration (HFF). Hollow fiber filtration (HFF) was carried out using KrosFlo Research Iii System (SpectrumLabs, USA), equipped with a polysulfone-based separation module (MWCO: 500 kDa, SpectrumLabs, USA). Conditions: 1 mM PBS at pH 8, 200 mL waste.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). The cryo-TEM measurement was performed on the Libra 120 microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany), equipped with a charge coupled device (CCD) camera at an acceleration voltage of 120 kV. The samples were prepared by dropping 2 µL of Psomes solution, prepared as described below, on a copper grid modified with holey carbon foil (Lacey type). After 1 min sedimentation, the excess water was removed at the method of blotting with a filter paper, followed by plunging into liquid ethane (-178 ℃). Then, the frozen grids were transferred into electron microscope using a Gatan 626

cryo-holder. All images were recorded in bright field at -173 °C. The average diameter and membrane thickness of FcPsomes were calculated by analyzing 50-100 particles from cryo-TEM images.

Electron Paramagnetic Resonance (EPR). Continuous wave (CW) EPR spectra were recorded on an EMX-plus spectrometer (Bruker Biospin) operating at X-band, equipped with the high-sensitivity resonator ER 4119 HS-W1, and the variable temperature unit ER4141VT. Acquisition parameters were microwave power of 10 mW, receiver gain of 1×10^4, modulation frequency of 100 kHz, modulation amplitude of 1 G, sweep width of 100 G, time constant of 10.24 ms, conversion time of 40.96 ms, 16 scans, and 1024 data points. The temperature was controlled within 294±1 K. The liquid samples were loaded into glass capillaries (50 µL) which were sealed with wax. For the measurements the capillaries were inserted in standard quartz ESR tubes (i.d. = 3 mm).

pH meters. FiveEasyPlus pH-meter from Mettler Toledo and the SevenEasy pH Meter S20 from Mettler Toledo. The routine is the storage of the Electrodes in 3M KCl (for long and short time), minimum every 2 months a calibration with pH buffer packs 4.01/7.00/10.01 - 3points calibration.

Inductively coupled plasma - optical emission spectrometer (ICP-OES). ICP-OES 720 (Varian-Agilent) with automatic injector SPS3 and Milestone microwave digester (Ethos D).

Asymmetrical flow field-flow fractionation (AF4). The general AF4 protocol for the separation of FcPsomes is still described in a previous paper.[1,2] AF4 measurements were performed with an Eclipse DUALTEC system (Wyatt Technology Europe GmbH, Dernbach, Germany) using 1 mM PBS solution at pH 7.5, 6.5 and 5.5 as carrier liquid, containing 200 mg L^{-1} NaN_3 to prevent bacteria or algae contamination. The channel spacer, made of poly(tetrafluoroethylene), had a thickness of 490 µm. The dimensions of the channel were 26.5 cm in length and from 2.1 to 0.6 cm in width. The membranes used as accumulation walls were composed of regenerated cellulose with a molecular weight cut-off (MWCO) of 10 kDa (Microdyn-Nadir GmbH, Wiesbaden, Germany). Flow rates were controlled with an Agilent Technologies 1260er series isocratic pump equipped with vacuum degasser (Agilent Technologies, Santa Clara, CA, USA). The detection system consisted of a MALS detector (DAWN HELEOS II, Wyatt Technology Europe GmbH, Dernbach, Germany) operating at a wavelength of 659 nm with online DLS detector (QELS module, Wyatt Technology Corp., Santa Barbara, CA, USA) which is an add-on unit connected to the 99° angle of the MALS, a diode array detector SPD-M20A (Shimadzu Europa GmbH, Duisburg, Germany) and a refractive index (RI) detector (Optilab T-rEX, Wyatt Technology Europe GmbH, Dernbach, Germany) operating at a wavelength of 658 nm. All injections were performed with an autosampler (1260 series, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The data collection and calculation of molar masses and radii were performed by Astra 7.3.219 software (Wyatt Technology Corp., Santa Barbara, CA, USA). The cross-flow rate (F_x) profile was optimized to achieve optimal fractionation of FcPsomes.

The following protocol was applied: detector flow was set to 0.7 mL min^{-1}, focusing was performed with focus flow (F_f) 2.0 mL min^{-1} for 3 min followed by an isocratic elution step with a F_x of 2 mL min^{-1} for 3 min followed by an exponential F_x gradient from 2 to 0 mL min^{-1} within 40 min. The last step proceeds without F_x (0 mL min^{-1}) for 10 min. Three injections of 200 µL were performed for each sample (c = 0.5 mg mL^{-1}). M_w and radius of gyration (R_g) of FcPsomes were calculated from the MALS data of detectors 6 to 17 applying a Berry fit. The results of molar mass determination were previously carried out through the manual injection of various sample concentrations directly into the RI detector, resulting in a value of $\frac{d_n}{d_c}$ equal to 0.105 mL g^{-1}.[2] ($\frac{d_n}{d_c}$ ratio is also called the "specific refractive index increment", shows the change in the refractive index (d_n) of a BCP solution with respect to a change in BCP concentration (d_c)).

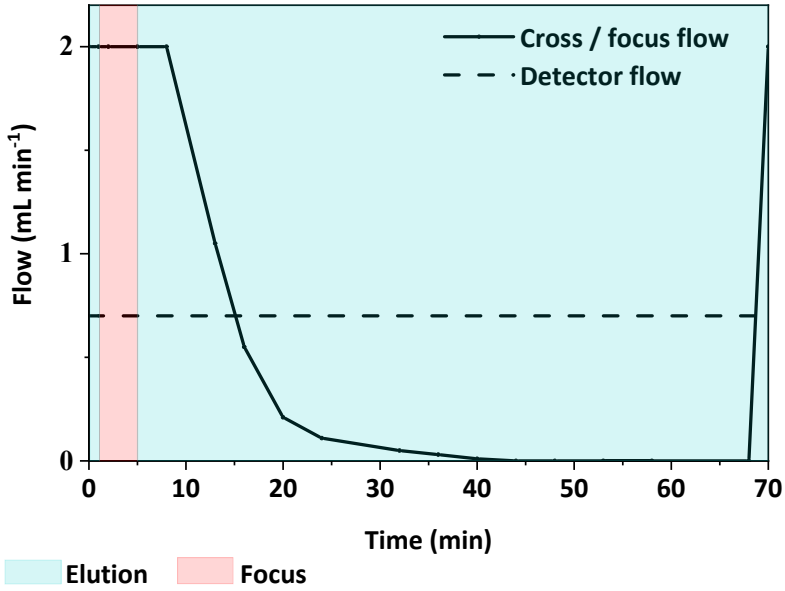

Figure S 1. AF4 separation flow profile for FcPsomes using 1 mM PBS at pH 5.5, pH 6.5 and pH 7.5.

Conformation studies: [1,3,4]

- ***Scaling parameter (ν)*:** by plotting logarithmic R_g vs M_w, ν can be determined by the slope of the plot; it gives information about the molecular shape in the used solvent. K is an empiric parameter comparable to Kuhn-Mark-Houwink-Sakurada.

$$R_g = K.M_w^{\nu} \qquad \text{Equation S1}$$

Some examples:[5]

ν = 0.33 → spheres

ν = 0.5-0.6 → random coil macromolecule

ν = 1 → rigid rod

- ***Apparent density ($d_{app,i}$)*:** gives information about molecular density, and it is calculated by R_g and M_w (with V as volume fraction, α as geometrical correction, N_A as Avogadro's number):

$$d_{app,i} = \frac{M_i}{V(R_g)_i \cdot N_A} \cdot \alpha \qquad \text{Equation S2}$$

2. Materials

Poly(ethylene glycol) methyl ether (MeO-PEG-OH; M_n = 2000 g mol^{-1}; M_w/M_n = 1.05), 2,2´-bipyridine, 4-aminobutanol, 2-(*N*,*N*´-diethylamino)ethyl methacrylate (DEAEMA), 2,3- dimethylmaleic anhydride

(98 %), 4-amino1-butanol (98 %), n-hexane, ethyl acetate, tetrahydrofurane (THF, 98 %), methacryloyl chloride (97 %), 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (RAFT, 98%, HPLC), 2,2'-azobis(2-methylpropionitrile) (AIBN, 98 %), 2-(*N*,*N*´-dimethylamino)ethyl methacrylate (DMAEMA), *n*-butyl methacrylate (nBMA), methacryloylic chloride, 2-bromoisobutyryl bromide, N,N'-dicyclohexylcarbodiimide (DCC, 99 %), 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS), 4-(dimethylamino)pyridine (DMAP, 99 %), 4-amino-1- butanol, 2-aminoethanol, ferrocene carboxylic acid (FcA, 97.0%), 2-hydroxyethyl methacrylate (HEMA, ≥ 99%, contains ≤ 50 ppm monomethyl ether hydroquinone as an inhibitor), copper (I) bromide (CuBr), aluminum oxide (neutral, activated), phosphate buffered saline (tablet), sodium hydroxide, dimethyl sulfoxide (DMSO), fluorescein 5(6)-isothiocyanate, myoglobin from equine skeletal muscle (Myo, essentially salt-free, lyophilized powder), Glucose oxidase obtained from aspergillus niger (GOx, 100000 - 250000 units g^{-1}), D-(+)-glucose (Glc, ≥ 99.5%), hydrogen peroxide solution (30%), 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) were purchased from Sigma-Aldrich. Toluene, THF, ethyl acetate and chloroform-$_d$ were purchased from Acros Organics. *n*-Hexane, hydrochloric acid (37%) and silica gel were purchased from Merck (Germany). Anhydrous 2-butanone (Fluka), triethylamine (Fluka) and anhydrous tetrahydrofuran (THF, Sigma-Aldrich) were stored over a molecular sieve. Amplex red was purchased from ThermoFisher. Syringe filters and dialysis membrane were purchased from Carl Roth (Germany). Hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Fluka (München, Germany). 0.2 µm nylon filter and 0.8 µm cellulose mixed ester (CME) filter were purchased from Carl Roth (Karlsruhe, Germany). All dialysis membranes (M_{WCO} 5 kDa, 8-14 kDa, 100 kDa) were ordered from Carl Roth (Karlsruhe, Germany). Cyanine 5 NHS-ester (Cy5-NHS, Lumiprobe). Milli-Q water was employed to prepare all the solutions in this work.

3. Biological Materials and Protocols

Cell Culture. Biological tests were performed to evaluate the anticancer capacity of the nanosystem using PC-3 and RWPE-1 cell lines from the American Type Culture Collection ATCC (Rockwell, MD, USA). PC-3 cell line represents a grade IV (metastatic) prostate adenocarcinoma that does not express androgen receptors. RWPE-1 cells are responsive to androgens and exhibit numerous characteristics typical of normal cells. PC-3 cells were routinely cultured in RPMI-1690 (Gibco, RBL), containing 10% fetal bovine serum (FBS). RWPE-1 cells were maintained in complete K-SFM medium containing bovine pituitary extract (50 µg mL^{-1}) and epidermal growth factor (5 ng mL^{-1}). The cell cultures were maintained with 1% penicillin/streptomycin/amphotericin B at 37 °C and 5% CO_2. When the cells reach 70-80% confluency, they are washed with phosphate-buffered saline (PBS), stripped with 0.25% trypsin, and spread into a new culture batch at a density of 30,000 to 40,000 cells per square centimeter. The medium should be routinely renewed every three days by replacing it with an equal volume of fresh medium.

Cell viability and cytotoxicity assay. Cell viability assays were carried out with the empty-FcPsomes A, C and D, and additionally GOx-FcPsomes D, at different concentrations (0.1, 1, 10, 25 and 50 µg mL^{-1}). Cells were seeded in 96-well plates (approximately 1.5×10^4 cells per well) and incubated at 37 °C with 5% CO_2 for 24 h in complete medium. They were subsequently treated with the different compounds mentioned and incubated for 24 h. Cytotoxicity was determined using an MTT (3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay. MTT (5.0 mg mL^{-1} solution) was added to the cells and further incubated for 1.5 h. The culture medium was then removed, and the purple formazan crystals formed by the mitochondrial dehydrogenase and reductase activity of vital cells were dissolved in DMSO. The absorbance, directly proportional to the number of surviving cells, was quantified at 570 nm using a microplate reader (Multiskan, Thermo Fisher Scientific). The fraction of live cells was calculated from the absorbance of untreated control cells (**Figure 3A**).

Statistical analysis. Statistical analysis was performed using GraphPad Prism Version 8 software. Results were expressed as the mean ± standard error of the mean (SEM). The statistical significance

is evaluated using the Bonferroni Test for multiple comparisons after an analysis of variance of one or two factors (ANOVA). Differences were considered statistically significant *p < 0.05; **p < 0.01; ***p < 0.001.

4. Preparation of body fluids[4,6]

Simulated Blood Plasma (extracellular fluid) (SBP, V_F = 50 mL). The body fluid was prepared by adding 401.8 mg of sodium chloride, 17.8 mg of sodium bicarbonate, 11.3 mg of potassium chloride, 11.3 mg of dipotassium hydrogen phosphate, 15.6 mg of magnesium chloride, 11.00 mg of calcium chloride, 3.6 mg of sodium sulphate, 305.9 mg of Tris(hydroxymethyl)aminomethane, 3.5 mg of bovine serum albumin (BSA), 70.0 mg of glucose and 0.5 mg of L-Lysine. Adjust the pH to 7.4. The body fluid will be filtrated using 0.2 µm filter (before to use) and stored at 4 °C.

Simulated Cytoplasm (intracellular fluid) (SCC, V_F = 50 mL). The body fluid was prepared by adding 5.0 mg of sodium chloride, 45.0 mg of sodium bicarbonate, 11.3 mg of potassium chloride, 850 mg of dipotassium hydrogen phosphate, 60.0 mg of magnesium chloride, 0.5 mg of calcium chloride, 3.6 mg of sodium sulphate, 305.9 mg of Tris(hydroxymethyl)aminomethane, 10 mg of bovine serum albumin (BSA), 70.0 mg of glucose and 0.5 mg of L-Lysine. The body fluid will be filtrated using 0.2 µm filter (before to use) and stored at 4 °C. Adjust the pH to 7.4.

5. Synthesis and Characterization of the block copolymer (BCPs)

BCP-A (AS255). Amphiphilic block copolymers $mPEG_{45}$-b-P($DEAEMA_{89}$-co-$DMIHMA_{21}$) (BCP) were successfully synthesized by atom transfer radical polymerization (ATRP).[4,7] The block copolymer features a methoxy end group at the hydrophilic poly(ethylene glycol) (PEG) segment, while the hydrophobic part consists of pH-sensitive 2-(diethylamino)ethyl methacrylate (DEAEM) and photo-crosslinker, 3,4-dimethyl maleic imidohexyl methacrylate (DMIHMA). The composition, molar mass and dispersity (Đ) were well characterized by ^{1}H nuclear magnetic resonance spectroscopy (NMR) and size exclusion chromatography (SEC) (**Table S1 and Figure S2**). Used educts for the synthesis of BCP-A is presented in **Table S2**.

BCP-C (AS103). Amphiphilic block copolymers $mPEG_{45}$-b-P($DEAEMA_{46}$-co-$DMAEMA_{26}$ co-$DMIBMA_{23}$) (BCP) were successfully synthesized by ATRP.[4] The block copolymer features a methoxy end group at PEG segment, while the hydrophobic part consists of DEAEMA, 2-(*N*,*N´*-dimethylamino)ethyl methacrylate (DMAEMA) and photo-crosslinker, 3,4-dimethyl maleic imidobutyl methacrylate (DMIBMA). The composition, molar mass and dispersity (Đ) were well characterized by ^{1}H nuclear magnetic resonance spectroscopy (NMR) and size exclusion chromatography (SEC) (**Table S1 and Figure S2**). Used educts for the synthesis of BCP-C is presented in **Table S2**.

BCP-D (AS281). Amphiphilic block copolymers $mPEG_{45}$-b-P($DEAEMA_{55}$-co-$nBMA_{21}$ co-$DMIBMA_{24}$) (BCP) were successfully synthesized by ATRP.[7,8] The block copolymer exhibits a methoxy end group at PEG segment, whereas the hydrophobic component comprises of pH-sensitive DEAEM, n-butyl methacrylate (nBMA), and photo-crosslinker, DMIBMA. The composition, molar mass and dispersity (Đ) were well characterized by ^{1}H nuclear magnetic resonance spectroscopy (NMR) and size exclusion chromatography (SEC) (**Table S1 and Figure S2**). Used educts for the synthesis of BCP-D is presented in **Table S2**.

FcBCP (AS262). Amphiphilic block copolymers $mPEG_{45}$-b-P($DEAEMA_{59}$-co-$FcMA_{20}$ co-$DMIBMA_{28}$) (FcBCP) were successfully synthesized by Reversible addition fragmentation chain-transfer polymerization (RAFT).[2] This block copolymer features a methoxy end group at PEG-RAFT segment, while the hydrophobic part consists of pH-sensitive DEAEM, *2*-(methylacryloyloxy)ethyl ferrocenecarboxylate (FcMA) and photo-crosslinker, DMIBMA. The composition, molar mass and

dispersity (Đ) were well characterized by ^{1}H nuclear magnetic resonance spectroscopy (NMR) and size exclusion chromatography (SEC) (**Table S1 and Figure S2**). Used educts for the synthesis of FcBCP is presented in **Table S2**.

Atom transfer radical polymerization (ATRP) for synthesizing BCPs, BCP-A, BCP-D, and BCP-D.
Solid DMIBMA or DMIHMA (both stored in ethyl acetate) was dried in a vacuum oven for 3-5 h, and fluid DEAEMA was filtrated through a neutral aluminum oxide column to remove the inhibitor. In a dried 25 mL Schlenk flask, macroinitiator (PEG-Br), 2,2'-bipyridine, DEAEMA, DMIBMA, the BCP specific monomer, and dry solvent were added under inert gas atmosphere. After the reaction flask was degassed via two freeze-pump-thaw cycles, CuBr was added. The reaction mixture was degassed via two freeze-pump-thaw cycle again, flushed with inert gas and stirred in an oil bath, preheated, at 50 °C for 20 h under protection from light. The reaction mixture was diluted with 3 mL THF and passed through a neutral aluminum oxide column to remove the catalysts, which was then washed with 3 times THF (3 mL). The quenched reaction mixture was dialyzed for two days against acetone using a dialysis membrane (MWCO 6-8 kDa) to remove macroinitiator and/or monomer. After removing the solvent by rotary evaporation and vacuum drying (high vacuum for complete solvent removal), (sticky) block copolymer was collected.

Reversible addition−fragmentation chain-transfer (RAFT) polymerization for synthesizing FcBCP
The FcBCP was synthesized by RAFT polymerization. Solid DMIBMA (stored in ethyl acetate) was dried in a vacuum oven for 3-5 h, and fluid DEAEMA was filtrated through a neutral aluminum oxide column to remove the inhibitor. In a dried 25 mL Schlenk flask, PEG-RAFT-reagent, DEAEMA, DMIBMA, the BCP specific monomer (FcMA) and AIBN were dissolved 1,4-dioxane. Then the solution was degassed using at least four freeze−pump−thaw cycles, back-filled with argon and stirred at 70 °C for 24 h. The crude product was purified twice by precipitation in cooled n-hexane, using acetone for dissolving BCP again. After vacuum drying (high vacuum for complete solvent removal), a sticky block copolymer was collected.

6. Fabrication and Characterization of Empty-FcPsomes

Empty-FcPsomes (30 wt% FcBCP). 1 mg·mL^{-1} of each BCPs solution were prepared using HCl 0.01 M, all solutions were filtered using 0.2 µm Nylon filters independently. Subsequently, three different solutions were prepared by mixing 30 wt% of FcBCP (3 mL of FcBCP stock solution) with 70 wt% of the rest of the BCPs (7 mL of BCP-A, BCP-C or BCP-D). The pH of the three solutions was increased until 9 and stirred in dark condition for 3 days. Afterwards, the solutions were filtered using 0.8 CME filter and photo irradiated (crosslinking process) for 45 s. Final HFF purification was carried out in 10 mM NaCl. Finally, different batches of FcPsomes A, FcPsomes C and FcPsomes D solutions in 10 mM NaCl were studied by DLS (**Table S4**).

Empty-FcPsomes (40 wt% FcBCP). 1 mg·mL^{-1} of each BCPs solution were prepared using HCl 0.01 M, all solutions were filtered using 0.2 µm Nylon filters independently. Subsequently, three different solutions were prepared by mixing 40 wt% of FcBCP (4 mL of FcBCP stock solution) with 60 wt% of the rest of the BCPs (6 mL of BCP-A, BCP-C or BCP-D). The pH of the three solutions was increased until 9 and stirred in dark condition for 3 days. Afterwards, the solutions were filtered using 0.8 CME filter and photo irradiated (crosslinking process) for 45 s**.** FcPsomes A, FcPsomes C and FcPsomes D solutions were studied by DLS (**Table S4**).

Swelling-shrinking properties by DLS. Cyclic pH switches in 10 mM NaCl of Empty-FcPsomes A, C and D using 30 wt% or 40 wt% BCP were performed at pH 8.0 (collapsed state) and 5.0 (swollen state) for 5 cycles also proving no disassembly and membrane rupture as well as no aggregation (**Figures S3-S5**).

Determination of the critical pH value (pH*). pH titration of Empty-FcPsomes (30 wt% BCP) was also studied by DLS (0.5 mg mL^{-1}) in 10 mM NaCl and 1 mM PBS. Following, the vesicles were titrated

from basic to acidic conditions while simultaneously measuring their size by DLS by adding 0.1 M HCl to determine the pH* (transition point from swelling to shrinking and vice versa) and swelling point of membrane for each FcPsomes (**Tables S5**). pH titration was carried out by duplicate using two different batches for each sample. The pH* was determined by applying a Boltzmann fit and identifying the inflection point.

Stability studies by DLS. The size and polydispersity index (PdI) of the samples were evaluated by DLS under different simulated body fluids (SBP, SCC) after a short incubation time in order to determine the post-incubation appearance of Empty-FcPsomes and the homogeneity of the solutions (**Figure 1C**). To achieve reproducible therapeutic function, it is best to use the same batches of nanocontainers. For this purpose, their stability under storage at -20 °C was evaluated. 500 µL of batches of Empty-FcPsomes A, C and D were frozen at -20 °C. The samples were measured by DLS freshly prepared (as reference) and after being frozen for 1, 2, 3 and 4 weeks (**Figure 1D**).

7. Trapping of radicals formed by Fenton reaction by DMPO

Stock solutions. $FeSO_4 \times 7H_2O$ (0.2 mM) in 10 mM NaCl at different pH values; FcBCP (0.3 mg mL^{-1}, 0.21 mM of Fc containing) in 10 mM NaCl at different pH values; FcPsomes A, B and C (1.0 mg mL^{-1}, 0.21 mM of Fc containing) in 10 mM NaCl at different pH values; H_2O_2 (10 mM in H_2O); DMPO (100 mM in H_2O) in water. pH 5.0, 5.5, 6.0,6.5, 7.0 and 7.5 were the pH values studied.
Spin-trapping experiment followed by EPR measurements. Reaction Mixture, 40 µL of corresponding sample ($FeSO_4 \times 7H_2O$, FcBCP or FcPsomes) at different pH values, 5 µL of DMPO (100 mM) and 5 µL of H_2O_2 (10 mM). All spectra were recorded immediately after the preparation of the reaction mixture (**Figure 2**). Before conducting the experiments with FcPsomes, several experiments with $FeSO_4 \times 7H_2O$ and FcBCP were performed to ensure protocol optimisation and reproducibility (**Figure S7**).

8. Characterization of Empty-FcPsomes by AF4-LS measurements: pH dependency

AF4-LS method is well-known key tool for understanding the structure and morphology of Empty-Psomes due to the possibility to obtain different and complementary structural information at once, such as the scaling exponent (ν), the parameter ρ (R_g/R_h), and the apparent density.[1,2,4] The scaling exponent ν is the slope from logarithmic plot of R_g vs. M (M is the molar mass of the aggregate). In theory, ν = 0.33 is assigned for an ideal hard-sphere with a fractal surface, and $0.33 < \nu < 0.5$ for objects with lower density than a solid sphere and a less homogeneous surface. In general, values of ρ = 0.78 and 1.00 are expected for homogeneous and smooth spheres, and soft/hollow spheres with a rough surface, respectively. Values in the range from $1.00 < \rho \leq 2.00$, indicate a continuously increasing in the swelling degree of the sphere or aggregation up to the formation of anisotropic structures with elongated conformation. Herein, the investigation of pH dependence of all the ferrocene-containing polymeric vesicles was pursued. Three key pH values were chosen: pH 7.5, all are collapsed; pH 5.5, all are swollen; pH 6.5, different states are observed depending on the composition of the polymeric vesicle (**Figure 1, Figure S8**). Conditions: 0.5 mg mL^{-1} BCP in all cases and 1 mM PBS at pH 7.5, pH 6.5 or pH 5.5 as eluents for AF4.

9. Fenton Reaction by Amplex Red Assay: pH and environmental dependency

Stock solutions: 0.02 mg mL^{-1} of Amplex Red in Milli-Q water; 20 µM H_2O_2; 0.2 mg mL^{-1} of Myo in 1 mM PBS; 0.25 mg mL^{-1} of FcPsomes in 1 mM PBS at pH 5; 0.25 mg mL^{-1} of FcPsomes in 1 mM PBS at pH 6; 0.25 mg mL^{-1} of FcPsomes in 1 mM PBS at pH 7; 0.25 mg mL^{-1} FcPsomes in 1 mM PBS at pH 8; 10 mM PBS at pH 7.4.

Sample mixture: 100 µL of the samples (0.25 mg mL^{-1} of FcPsomes under different buffers). 10 µL of H_20_2 (20 µM) are added, the sample is stirred and incubated for 30 min. Afterwards, 108 µL of

reaction solution are added (It contains, 100 µL of 10 mM PBS at pH 7.4 + 3.4 µL of Amplex Red (0.02 mg mL^{-1}) + 5 µL of Myo (0.2 mg mL^{-1}). Fluorescence intensity measurements were recorded for 30-60 min every 10 min (λ_{Ex} 534 nm; λ_{Em} 585 nm). Samples without FcPsomes were taken as reference (100%, H_20_2, Amplex Red and Myo).

10. Fabrication and Characterization of GOx loaded-FcPsomes D

GOx-FcPsomes D. The GOx loading into the FcPsomes was carried out *in situ* approach, previously well- established.[4,9,10] 1 mg·mL^{-1} of each BCP-D solution (HCl 0.01 M as solvent), 1.2 mg·mL^{-1} of each FcBCP solution (HCl 0.01 M as solvent) and 2 mg mL^{-1} of GOx solution (1 mM PBS as solvent). Later, all solutions were filtered using 0.2 µm Nylon filters independently. Subsequently, 0.6 mL of Gox solution, 8.4 mL of BCP-D solution and 3 mL of FcBCP solution. The pH of the BCP solution must be increased until 5 before adding the enzyme solution was added. After the addition of the enzyme solution the pH was increased until 9. *Final conditions:* 1 mg· mL^{-1} FcPsomes D + 0.1 mg· mL^{-1} GOx. The final solution was stirred in dark condition for 3 days. Afterwards, the solution was filtered using 0.8 CME filter and photo irradiated (crosslinking process) for 3 min. Hollow Fiber Filtration (HFF) was used to purify the GOx-Psomes (500 kDa cut-off, V_{waste} = 150-200 mL, 130 mbar, 1 mM PBS).

Determination of the critical pH value (pH*). pH titration of GOx-FcPsomes D was also studied by DLS (0.5 mg mL^{-1}) in 10 mM NaCl and 1 mM PBS. Following, the vesicles were titrated from basic to acidic conditions while simultaneously measuring their size by DLS by adding 0.1 M HCl to determine the pH* (**Figure 3B**). pH titration was carried out by duplicate using two different batches for each sample. The pH* was determined by applying a Boltzmann fit and identifying the inflection point.

Preparation of FITC-GOx.[10] 25 mg of GOx were dissolved in 5 mL of sodium carbonate buffer. Then, 25 µL of the FITC isothiocyanate stock solution (10 mg·mL^{-1}, 2 mg in 200 µL DMSO) were added gradually into the GOx solution. The reactions were performed overnight under dark conditions. The solution was cleaned by dialysis (MWCO 20 kDa or 50 kDa) against PBS (1 mM, pH 7.4) for 2 days and freeze-dried afterwards.

Preparation of FITC-GOx-FcPsomes D. The same protocol was followed as for GOx-FcPsomes. As observed in **Figure S9**, self-assembled polymeric vesicles loaded with GOx-FITC were obtained; however, some disassembly occurred after HFF purification. For this reason, the loading efficiency could not be estimated. Furthermore, they may promote additional aggregates, which could overestimate the loading efficiency of GOx.

11. Synthesis of Cy5-labelled β-Cyclodextrin (Cy5-β-CD)

1 mg of 6-monodeoxy-6-monoamino-β-cyclodextrin hydrochloride (β-CD-NH_2•HCl) and 1 mg of Cy5-NHS were dissolved in 300 µL of anhydrous DMSO, and the mixture was stirred in the dark at room temperature overnight. The resulting solution was transferred into dialysis bag (MWCO: 100 - 500 Da) and dialyzed against Milli-Q water for 48 h. The final solution was kept from light at 4 °C for subsequent study.[11]

12. Host-Guest Breakdown Study: Cy5-βCD-GOx-FcPsomes D and Cy5-βCD-FcPsomes D

Fabrication

Cy5-βCD-GOx-FcPsomes D. The GOx and βCD-Cy5 loading into the FcPsomes was carried out *in situ* adopted approach, previously established.[4,9,10] 1 mg·mL^{-1} of each BCP-D solution (HCl 0.01 M as solvent), 1.2 mg mL^{-1} of each FcBCP solution (HCl 0.01 M as solvent), 2 mg mL^{-1} of GOx solution (1 mM PBS as solvent) and 1 mg mL^{-1} of βCD-Cy5 solution (Millipore as solvent). Later, all solutions were filtered using 0.2 µm Nylon filters independently. Subsequently, 0.6 mL of GOx solution, 0.3 mL

of βCD-Cy5 solution, 8.4 mL of BCP-D solution and 3 mL of FcBCP solution. The pH of the BCP solution must be increased until 5 before adding the enzyme and βCD-Cy5 solution were added. After the addition of the enzyme solution the pH was increased until 8. *Final conditions:* 1 mg mL $^{-1}$ FcPsomes D + 0.1 mg mL $^{-1}$ GOx + 0.05 mg· mL $^{-1}$ βCD-Cy5. The final solution was stirred in dark condition for 3 days. Afterwards, the solution was filtered using 0.8 CME filter and photo irradiated (crosslinking process) for 3 min. Dialysis for 48 h (1000 kDa cut-off, 1 mM PBS) was used to purify. The self-assembly of BCPs in requested vesicular structure was studied by DLS before purification (**Figure S10**). The fluorescence intensity was scanned by fluorescence spectrometer (λ_{ex}: 580 nm, λ_{em}: 550 - 750 nm) before and after dialysis (**Figure S10**) (Conditions: 0.25 mg mL^{-1} FcPsomes D in 1 mM PBS at pH 5).

Cy5-βCD-FcPsomes D. The βCD-Cy5 loading into the FcPsomes was carried out *in situ* adopted approach, previously established.[4,9,10] 1 mg mL^{-1} of each BCP-D solution (HCl 0.01 M as solvent), 1.2 mg mL^{-1} of each FcBCP solution (HCl 0.01 M as solvent) and 1 mg mL^{-1} of βCD-Cy5 solution (Millipore as solvent). Later, all solutions were filtered using 0.2 µm Nylon filters independently. Subsequently, 0.3 mL of βCD-Cy5 solution, 8.4 mL of BCP-D solution and 3 mL of FcBCP solution. The pH of the BCP solution must be increased until 5 before adding the βCD-Cy5 solution were added. After the addition of the enzyme solution the pH was increased until 8. *Final conditions:* 1 mg mL $^{-1}$ FcPsomes D + 0.05 mg mL $^{-1}$ βCD-Cy5. The final solution was stirred in dark condition for 3 days. Afterwards, the solution was filtered using 0.8 CME filter and photo irradiated (crosslinking process) for 3 min. Dialysis for 48 h (1000 kDa cut-off, 1 mM PBS) was used to purify. The self-assembly of BCPs in requested vesicular structure was studied by DLS before purification (**Figure S10**). The fluorescence intensity was scanned by fluorescence spectrometer (λ_{ex}: 580 nm, λ_{em}: 550 - 750 nm) before and after dialysis (**Figure S10**) (Conditions: 0.25 mg mL $^{-1}$ FcPsomes D in 1 mM PBS at pH 5).

Release experiments (Proof of concept)

For the Cy5-GOx-FcPsomes samples, two types of biological-like media were evaluated: (i) Cytosolic-like conditions, 1 mM glucose in 1 mM PBS at pH 7.4; (ii) Extracellular-like conditions, 10 mM glucose in 1 mM PBS at pH 7.4 (1.9 mL of Cy5-GOx-FcPsomes D (1.0 mg mL^{-1}, pH 7.4) + 2.7 µL of 0.7 M glucose (final 1 mM); 1.9 mL of Cy5-GOx-FcPsomes D (1.0 mg mL^{-1}, pH 7.4) + 27 µL of 0.7 M glucose (final 10 mM). To evaluate the βCD-Cy5 release from the GOx-FcPsomes D samples, they were placed in a dialysis tube (MWCO 100 kDa cut-off) in the aforementioned environments without solvent change (no disturbance of applied conditions over 24 h). The samples were measured by fluorescence spectroscopy before and after incubation (24 h). After incubation in both systems, samples are observed to be less turbid, which ensures a decrease in pH. The fluorescence intensity was scanned by fluorescence spectrometer (λ_{ex}: 580 nm, λ_{em}: 550 - 750 nm) before and after dialysis (**Figure S10**) (Conditions: 1.0 mg mL $^{-1}$ FcPsomes D in 1 mM PBS at pH 5).

For the Cy5-FcPsomes samples, two conditions were evaluated in presence of H_2O_2: (i) 1 mM PBS at pH 7.4; (ii) 1 mM PBS at pH 5.0 (1.9 mL of Cy5-FcPsomes D (1.0 mg mL^{-1}, pH 7.4) + 10 µL of 20 mM H_2O_2 (final 100 µM); 1.9 mL of Cy5-FcPsomes D (1.0 mg mL^{-1}, pH 5.0) + 10 µL of 20 mM H_2O_2 (final 100 µM)). To evaluate the βCD-Cy5 release from these FcPsomes D samples, they were placed in a dialysis tube (MWCO 100 kDa cut-off) in the aforementioned environments without solvent change (no disturbance of applied conditions over 24 h). The samples were measured by fluorescence spectroscopy before and after incubation (24 h). The fluorescence intensity was scanned by fluorescence spectrometer (λ_{ex}: 580 nm, λ_{em}: 550 - 750 nm) before and after dialysis (**Figure S10**) (Conditions: 1.0 mg· mL $^{-1}$ FcPsomes D in 1 mM PBS at pH 5).

13. Control experiment of dropping pH in the glucose-containing RMPI culture medium

The pH value of three different samples (GOx-FcPsomes D and GOx free) at three different concentrations (10 μg mL^{-1}, 1 μg mL^{-1}, 0.1 μg mL^{-1}) for 24 h were measured (**Figure S11**). *Stock solutions:* GOx-FcPsomes D (1, 1 mg mL^{-1}), GOx-FcPsomes D (2, 10 μg mL^{-1}); FcPsomes D (control, 1 mg mL^{-1}); GOx free (1, 0.02 mg mL^{-1}), GOx free (2, 10 μg mL^{-1}).

GOx-FcPsomes D: 10 μg mL^{-1} (3.5 mL of medium + 35 μL of GOx-FcPsomes D 1 mg mL^{-1}); 1 μg mL^{-1} (2.7 mL of medium + 300 μL of GOx-FcPsomes D 10 μg mL^{-1}); 0.1 μg mL^{-1} (3 mL of medium + 30 μL of GOx-FcPsomes D 10 μg mL^{-1}). *FcPsomes D (control):* 10 μg mL^{-1} (3.5 mL of medium + 35 μL of FcPsomes D 1 mg mL^{-1}); *GOx free:* 0.2 μg mL^{-1} (3.5 mL of medium + 35 μL of 0.02 mg mL^{-1}); 0.02 μg mL^{-1} (2.7 mL of medium + 300 μL of GOx 0.2 μg mL^{-1}); 0.002 μg mL^{-1} (3 mL of medium + 30 μL of GOx 0.2 μg mL^{-1}).

14. Enzyme activity of GOx loaded FcPsomes D at different pH values with the proper control experiments

Stock solutions needed: 0.25 mg mL^{-1} of GOx-FcPsomes D at pH 7.5 and pH 5 (1 mL of each condition); 0.25 mg mL^{-1} of FcPsomes D at pH 7.5 and pH 5 (1 mL of each condition); 0.25 mg mL^{-1} of FcPsomes D in presence of GOx (1.25 μg mL^{-1}) at pH 7.5 and pH 5 (1 mL of each condition); 0.25 mg mL^{-1} of Psomes D at pH 7.5 and pH 5 (1 mL of each condition); 10 mM PBS at pH 7.4; Glucose solution (0.2 mg mL^{-1}); Amplex Red (0.02 mg mL^{-1}); Myo (0.2 mg mL^{-1}). **Reaction solution:** 1.380 μL of 10 mM PBS at pH 7.4 + 450 μL of Amplex Red + 750 μL of Myo.

Sample mixture: 100 μL of the samples (0.25 mg mL^{-1} of X-Psomes under different conditions). 10 μL of Glucose solution (0.2 mg mL^{-1}) are added, the sample is incubated for 30 min. Afterwards, 100 μL of reaction solution are added. Fluorescence intensity measurements were measured after 60 min (λ_{Ex} 534 nm; λ_{Em} 585 nm, make a range). The experiment was repeated three times, each time using a triplicate of the sample (**Figure S13**).

15. Measurement of hydrogen peroxide generation under medium conditions (4500 mg $^{L-1}$ glucose)

Stock solution: 50 μg mL^{-1} of in 1000 μL of GOx-FcPsomes D, FcPsomes and Psomes D in presence of GOx (0.25 μg mL of GOx) at pH 7.4 in PBS and in presence of 4.5 mg of glucose. After 24 h incubation at 37ºC, hydrogen peroxide generation measurements were conducted by using Amplex Red Assay.

Sample mixture: 100 μL of the samples. 100 μL of reaction solution are added (It contains, 100 μL of 10 mM PBS at pH 7.4 + 3.4 μL of Amplex Red (0.02 mg mL^{-1}) + 5 μL of Myo (0.2 mg mL^{-1})). Fluorescence intensity measurements were recorded for after 60 min (λ_{Ex} 534 nm; λ_{Em} 585 nm) (**Figure S14**).

16. Additional Figures and Tables

Table S1. Composition, block ratio, molecular weight, dispersity of different batched of BCPs.[1]NMR data. [2]SEC-MALS data.

BCP-A	DEAEMA	Crosslinker	PEG	Block ratio	M^1 [g mol^{-1}]	M_w^2 [g mol^{-1}]	M_n^2 [g mol^{-1}]	Đ
AS255	89	21	45	1:2.44	24800	40200	35500	1.13
BCP-D	**DEAEMA/n-BMA**	**Crosslinker**	**PEG**	**Block ratio**	**M^1 [g mol^{-1}]**	**Mw^2 [g mol^{-1}]**	**Mn^2 [g mol^{-1}]**	**Đ**
AS281	55/21	24	45	1:2.22	21700	32700	37400	1.19
BCP-C	**DEAEMA/DMAEMA**	**Crosslinker**	**PEG**	**Block ratio**	**M^1 [g mol^{-1}]**	**Mw^2 [g mol^{-1}]**	**Mn^2 [g mol^{-1}]**	**Đ**
AS103	46/26	23	45	1:2.11	20900	31700	28800	1.10
FcBCP	**DEAEMA/ FcMA**	**Crosslinker**	**PEG**	**Block ratio**	**M^1 [g mol^{-1}]**	**Mw^2 [g mol^{-1}]**	**Mn^2 [g mol^{-1}]**	**Đ**
AS262	59/20	28	45	2.38	27500	154900	64500	2.40

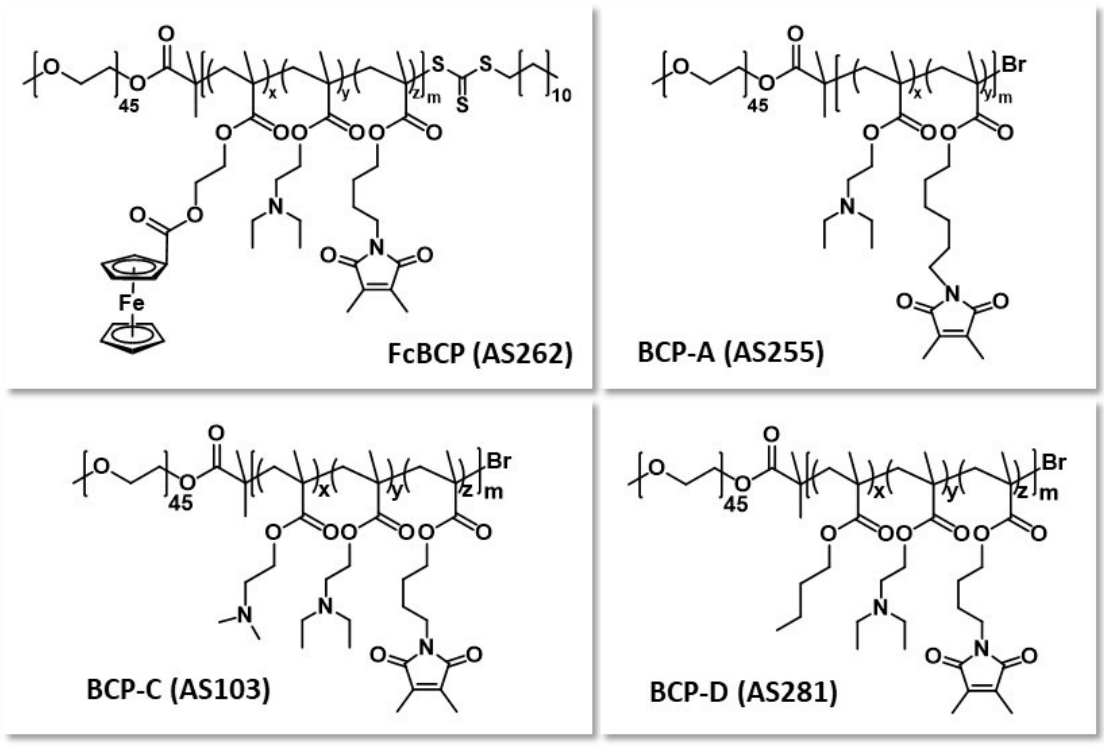

Figure S2. Chemical structure of different BPCs. BCP-A (AS255): $mPEG_{45}$-b-P($DEAEMA_{89}$-co-$DMIHMA_{21}$); **BCP-D (AS281):** $mPEG_{45}$-b-P($DEAEMA_{55}$-co-$nBMA_{21}$-co-$DMIBMA_{24}$); **BCP-C (AS103):** $mPEG_{45}$-b-P($DEAEMA_{46}$-co-$DMAEMA_{26}$-co-$DMIBMA_{23}$); **FcBCP (AS262):** $mPEG_{45}$-b-P($DEAEMA_{59}$-co-$FcMA_{20}$-co-$DMIBMA_{28}$).

Table S2. Used educts for the synthesis of block copolymers using ATRP and RAFT polymerization.

BCP-A	**PEG_{45}-Br**	**DEAEMA**	**DMIHMA**		**2,2´-Bipyridyl**	**CuBr**	**EMK**
	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]		eq. [mol] [mg]	eq. [mol] [mg]	[ml]
AS 255	1	88	22		2	1	4
	$4.979*10^{-5}$	$4.382*10^{-3}$	$1.095*10^{-3}$		$9.958*10^{-5}$	$4.979*10^{-5}$	
	107	0.88	321.1		15.6	7.1	
BCP-C	**PEG_{45}-Br**	**DEAEMA**	**DMIBMA**	**DMAEMA**	**2,2´-Bipyridyl**	**CuBr**	**EMK**
	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]	eq. [mol] [mg]	[ml]
AS 103	1	49	24	27	2	1	4,5
	$4.979*10^{-5}$	$2.44*10^{-3}$	$1.195*10^{-3}$	$1.344*10^{-3}$	$9.958*10^{-5}$	$4.979*10^{-5}$	
	107	0.49	317.0	0.227	15.6	7.1	
BCP-D	**PEG_{45}-Br**	**DEAEMA**	**DMIBMA**	**nBMA**	**2,2´-Bipyridyl**	**CuBr**	**EMK**
	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]	eq. [mol] [mg]	[ml]
AS 281	1	54	22	20	2	1	4
	$4.979*10^{-5}$	$2.689*10^{-3}$	$1.095*10^{-3}$	$0.996*10^{-3}$	$9.958*10^{-5}$	$4.979*10^{-5}$	
	107	0.54	290.6	0.159	15.6	7.1	
FcBCP	**PEG_{45}-RAFT**	**DEAEMA**	**DMIBMA**	**FcMA**	**AIBN**		**1,4-Dioxane**
	eq. [mol] [mg]	eq. [mol] [ml]	eq. [mol] [mg]	eq. [mol] [mg]	eq. [mol] [mg]		[ml]
AS 262	1	54	25	18	0.5		5
	$4.261*10^{-5}$	$2.301*10^{-3}$	$1.065*10^{-3}$	$7.670*10^{-4}$	$2.13*10^{-5}$		
	100	0.463	282.6	262.4	3.5		

Table S3. Sizes of different Empty-FcPsomes (30 wt% FcBCP) determined by AF4-LS at pH 5.5; 6.5 and 7.5.

Samples	pH	R_g [nm]	R_h [nm]
Fc-Psomes A	5.5	141	55.5
	6.5	72.5	44.9
	7.5	52.8	37.8
Fc-Psomes C	5.5	205	65.8
	6.5	123	53.4
	7.5	79.3	44.3
Fc-Psomes D	5.5	327	71.6
	6.5	194	64.0
	7.5	166	73.2

Table S4. DLS measurements of different batches of photo-crosslinked Empty-FcPsomes (30 wt% FcBCP) 1 mg mL^{-1} (collapsed state) in 10 mM NaCl. DLS measurements of one batch of photo-crosslinked Empty-FcPsomes (40 wt% FcBCP) 1 mg mL^{-1} (collapsed state) in 10 mM NaCl.

Batch 1 (30 wt%)	**Z-avg (nm)**	**PdI**
Empty-FcPsomes A	68.1 ± 1.0	0.182 ± 0.010
Empty-FcPsomes C	92.5 ± 1.2	0.180 ± 0.010
Empty-FcPsomes D	127.2 ± 0.4	0.162 ± 0.017
Batch 2 (30 wt%)	**Z-avg (nm)**	**PdI**
Empty-FcPsomes A	75.2 ± 1.0	0.155 ± 0.003
Empty-FcPsomes C	83.0 ± 2.0	0.142 ± 0.031
Empty-FcPsomes D	162.5 ± 0.2	0.169 ± 0.021
Batch 3 (30 wt%)	**Z-avg (nm)**	**PdI**
Empty-FcPsomes A	86.3 ± 0.9	0.172 ± 0.005
Empty-FcPsomes C	97.8 ± 1.2	0.184 ± 0.008
Empty-FcPsomes D	157.4 ± 3.4	0.135 ± 0.008
Average of 30 wt%	**Z-avg (nm)**	**-**
Empty-FcPsomes A	76 ± 9	-
Empty-FcPsomes C	91 ± 7	-
Empty-FcPsomes D	149 ± 19	-
Batch 1 (40 %wt, CryoTEM samples)	**Z-avg (nm)**	**PdI**
Empty-FcPsomes A	96.6 ± 1.4	0.168 ± 0.02
Empty-FcPsomes C	115.3 ± 1.1	0.179 ± 0.02
Empty-FcPsomes D	154.7 ± 1.3	0.198 ± 0.02

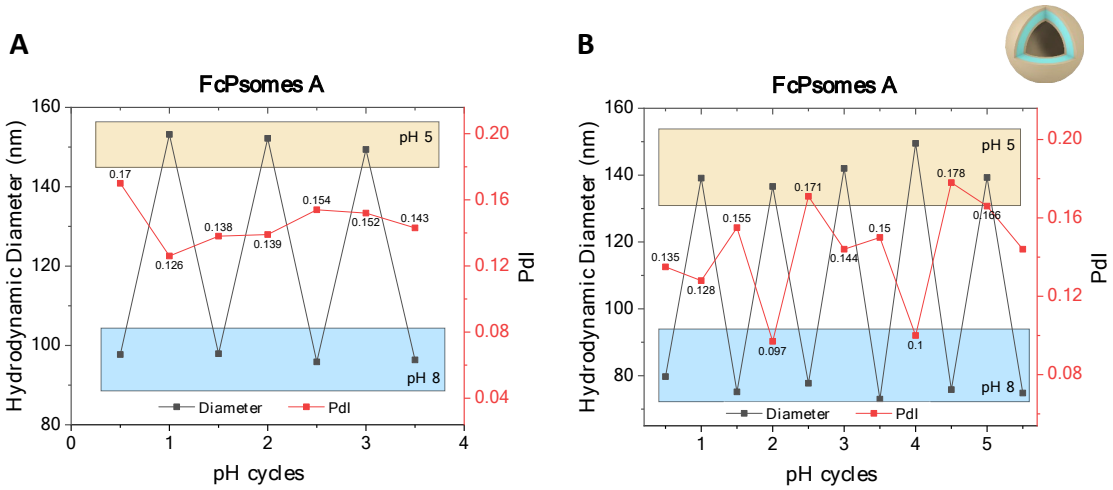

Figure S3. Characterization of Empty-FcPsomes A by DLS. Size determination of swelling-shrinking cycles between pH 5 and pH 8 in 10 mM NaCl by DLS (**A**) 40 wt% FcBCP (1.0 mg mL^{-1}) and (**B**) 30 wt% FcBCP (0.5 mg mL^{-1}).

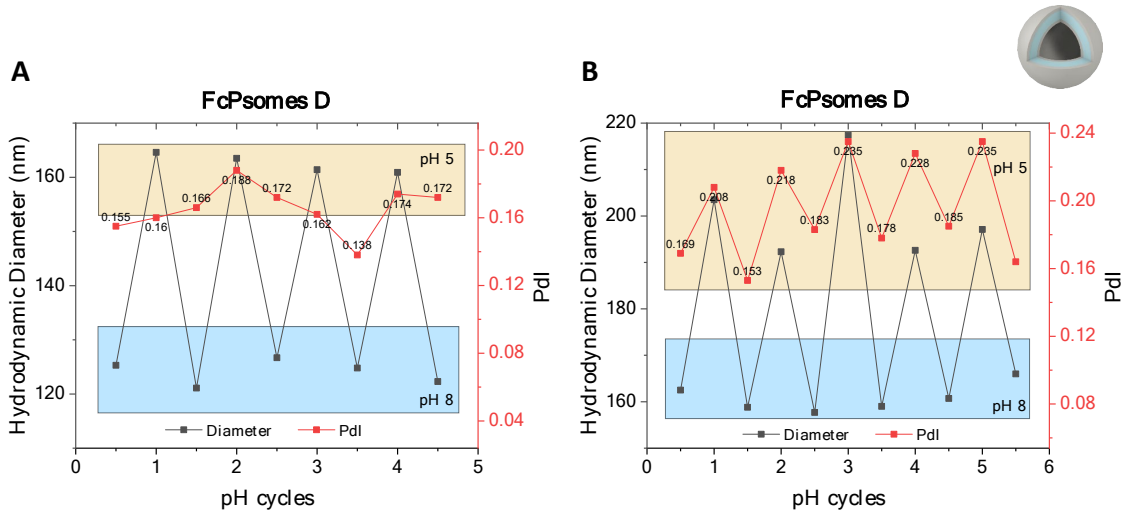

Figure S4. Characterization of Empty-FcPsomes D by DLS. Size determination of swelling-shrinking cycles between pH 5 and pH 8 in 10 mM NaCl by DLS (**A**) 40 wt% FcBCP (1.0 mg mL^{-1}) and (**B**) 30 wt% FcBCP (0.5 mg mL^{-1}).

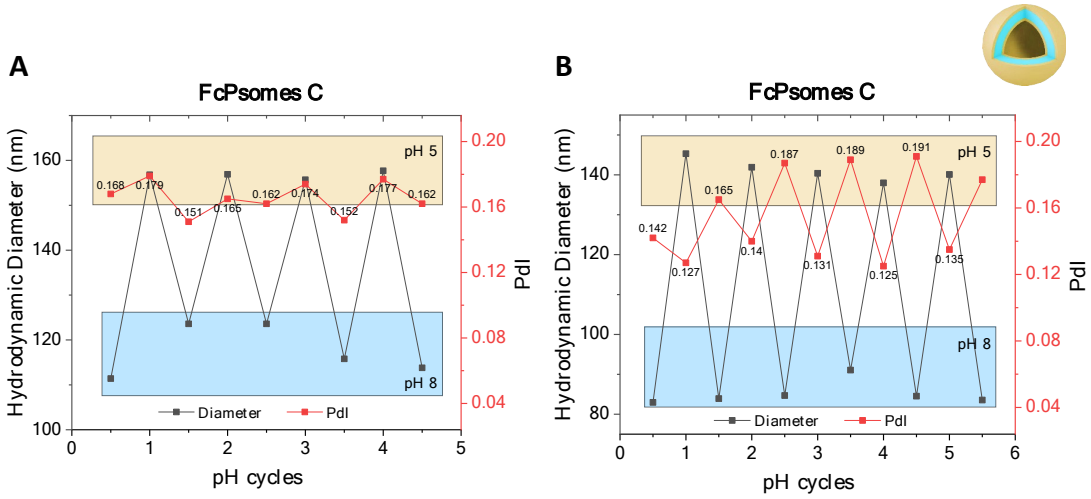

Figure S5. Characterization of Empty-FcPsomes C by DLS. Size determination of swelling-shrinking cycles between pH 5 and pH 8 in 10 mM NaCl by DLS (**A**) 40 wt% FcBCP (1.0 mg mL^{-1}) and (**B**) 30 wt% FcBCP (0.5 mg mL^{-1}).

Table S5. Zeta measurements of different photo-crosslinked Empty-FcPsomes (30 wt% FcBCP) 1 mg mL^{-1} under different microenvironments and pH values.

ζ (mV) 10 mM NaCl	ζ (mV) pH 5 (swollen state)	ζ (mV) pH 9 (collapsed state)
Empty-FcPsomes A	27.1 ± 2.0	12.7 ± 0.8
Empty-FcPsomes D	23.4 ± 2.0	5.6 ± 3.0
Empty-FcPsomes C	17.0 ± 3.5	-1.3 ± 3.1
ζ (mV) 1 mM PBS	**ζ (mV) pH 5 (swollen state)**	**ζ (mV) pH 9 (collapsed state)**
Empty-FcPsomes A	18.1 ± 5.3	-1.2 ± 0.6
Empty-FcPsomes D	28.3 ± 0.4	3.1 ± 0.9
Empty-FcPsomes C	16.0 ± 2.1	1.6 ± 1.6

Table S6. pH dependent size determination of different batches of photo-crosslinked Empty-FcPsomes (30 wt% FcBCP) 1 mg mL^{-1} under different microenvironments.

FcPsomes A	pH* in 10 NaCl / Swelling point	pH* in 1 mM PBS /Swelling point
Empty-FcPsomes A (1)	6.9/7.2	6.7/7.0
Empty-FcPsomes A (2)	6.2/6.6	6.2/6.7
Empty-FcPsomes A (3)	-	6.5/6.7
FcPsomes D	**pH* in 10 NaCl**	**pH* in 1 mM PBS**
Empty-FcPsomes C (1)	6.9/7.5	7.3/7.9
Empty-FcPsomes D (2)	6.4/7.1	6-5/7.2
Empty-FcPsomes D (3)		6.7/7.0
FcPsomes C	**pH* in 10 NaCl**	**pH* in 1 mM PBS**
Empty-FcPsomes C (1)	6.0/6.5	5.5/5.9
Empty-FcPsomes C (2)	5.8/6.2	5.7/6.3
Empty-FcPsomes C (3)		6.0/6.4

Table S7. Stabiliy of GOx-FcPsomes D after some long-term storage at -20°C by DLS (1 mg mL^{-1} in 10 mM NaCl).

GOx-FcPsomes D	Z-average (nm)	PDI
Fresh sample	176	0.168
Storage sample (3 months)	160 nm	0.218

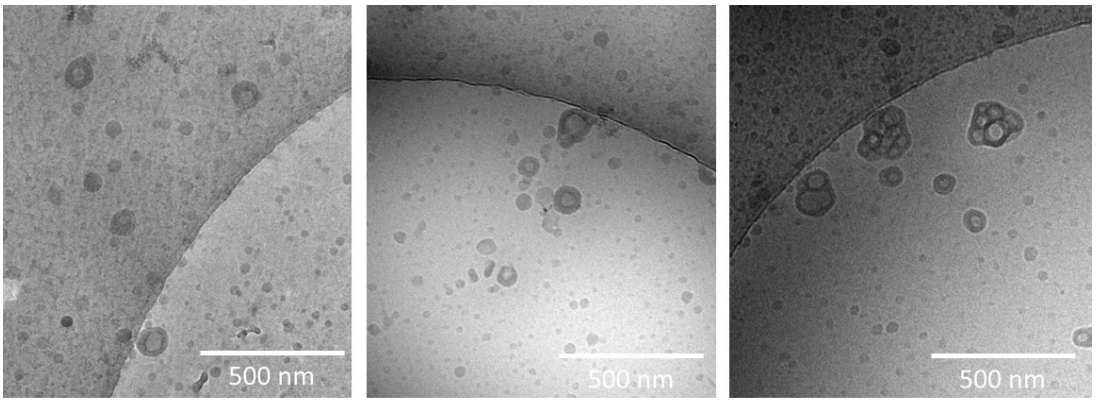

Figure S6. Cryo-TEM images and data analysis of FcPsomes (40 wt% of FcBCP). Diameter (⌀) and membrane thickness (MT) of Empty-FcPsomes by Cryo-TEM at pH 8. Mean diameters (⌀) for FcPsomes (⌀ FcPsomes A 79 ± 16, ⌀ FcPsomes C 86 ± 25, ⌀ FcPsomes D 86 ± 23) and membrane thickness (MT) (MT FcPsomes A 27 ± 8, MT FcPsomes C 24 ± 9, MT FcPsomes C 24 ± 9).

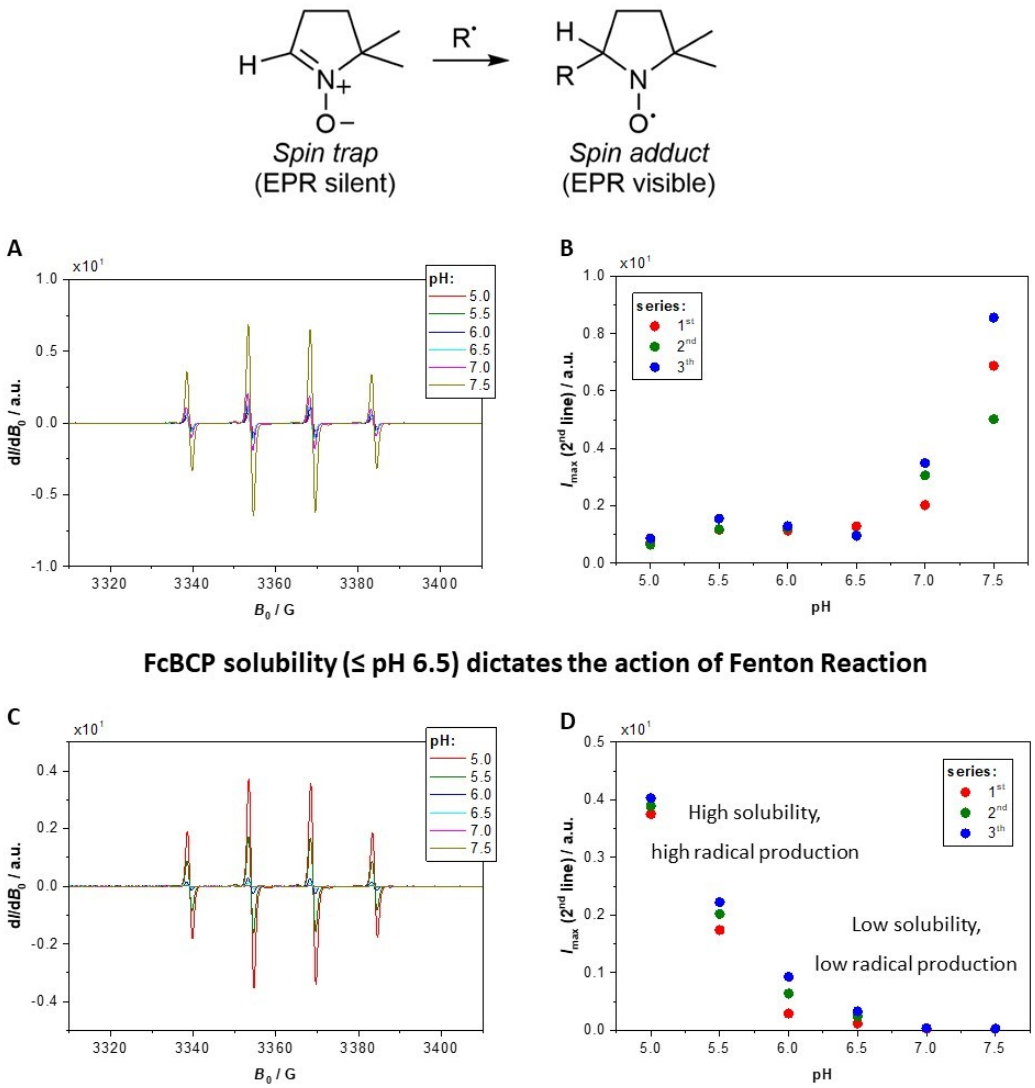

Figure S7. EPR study: The spectra (A and C) show a signal of 4 lines with a 1:2:2:1 intensity pattern and a hyperfine line splitting of about 14.9 G. It is the distinctive EPR signal of the DMPO adduct formed with a hydroxyl radical. A) Spectra of the spin trapping experiments with $FeSO_4 \times 7H_2O$ at different pH recorded immediately after the preparation of the reaction mixture (*The intensity of the signal increases with increasing pH*). B) The measurements using $FeSO_4 \times 7H_2O$ were repeated three times being in the reproducible satisfactory. C) Spectra of the spin trapping experiments with FcBCP (100%) at different pH recorded immediately after the preparation of the reaction mixture (*The intensity of the signal decreases with increasing pH*). D) The measurements using FcBCP (100%) were repeated three times being in the reproducible satisfactory.

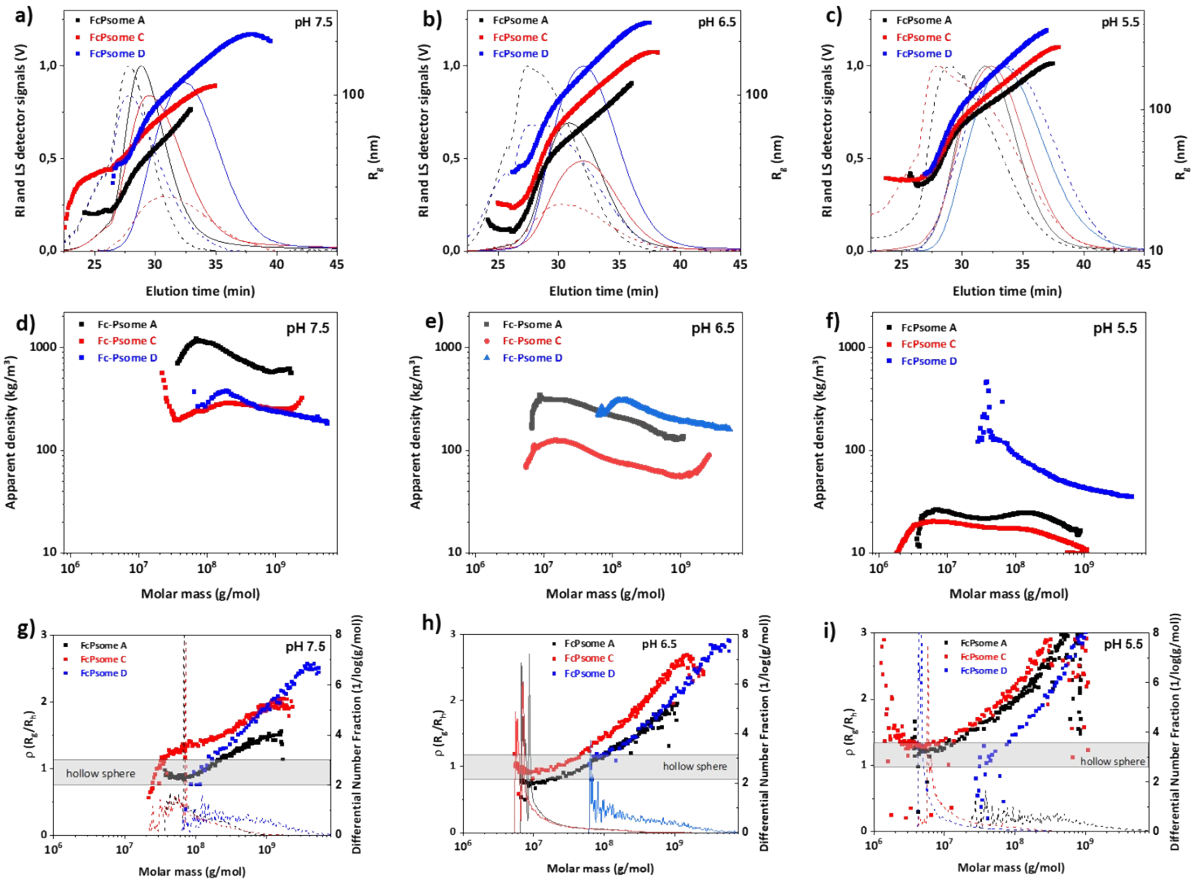

Figure S8. AF4 fractograms (RI and LS detector signals vs. R_g), apparent densities and rho parameter ($\rho = R_g/R_h$) vs. molar masses of different Empty-FcPsomes using 1 mM PBS at pH 7.5, pH 6.5 and pH 5.5.

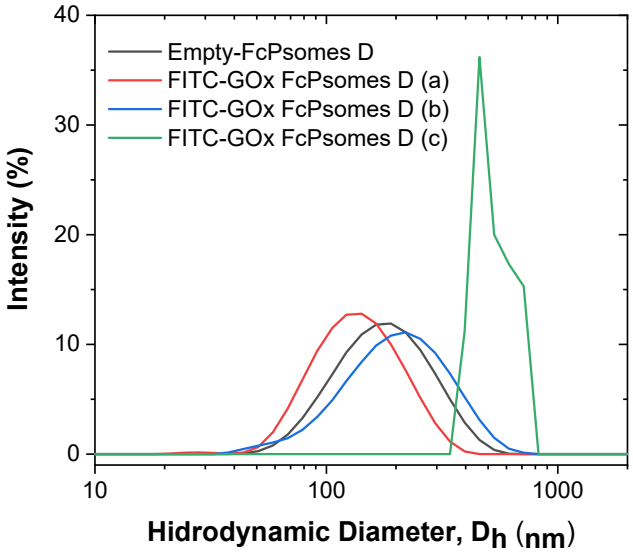

Figure S9. Monitoring the self-assembly process of FITC-GOx FcPsomes D by DLS (1 mg mL^{-1} in 10 mM NaCl): (a) after self-assembly and filtering; (b) after photo-crosslinking; (c) after HFF purification. It can be seen that there are no vesicles after purification, loading efficiency could not be estimated.

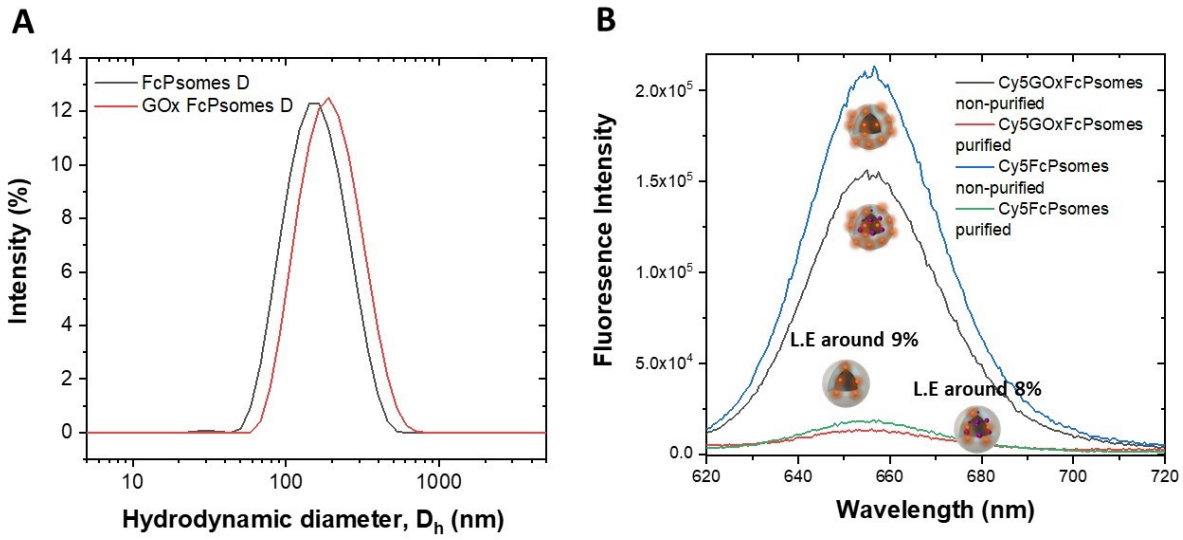

Figure S10. Diameter and loading efficiency of β-CD-Cy5 loaded FcPsomes D and GOx-FcPsomes D. (A) β-CD-Cy5 loaded GOx-FcPsomes D (Cy5-GOx) and FcPsomes D (Cy5) before dialysis by DLS (1 mg mL^{-1} in 10 mM NaCl, crosslinked); (B) β-CD-Cy5 loaded GOx-FcPsomes D (Cy5-GOx) and FcPsomes D (Cy5) before and after dialysis by fluorescence spectroscopy (0.25 mg mL^{-1} in 1 mM PBS at pH 5, 5 min

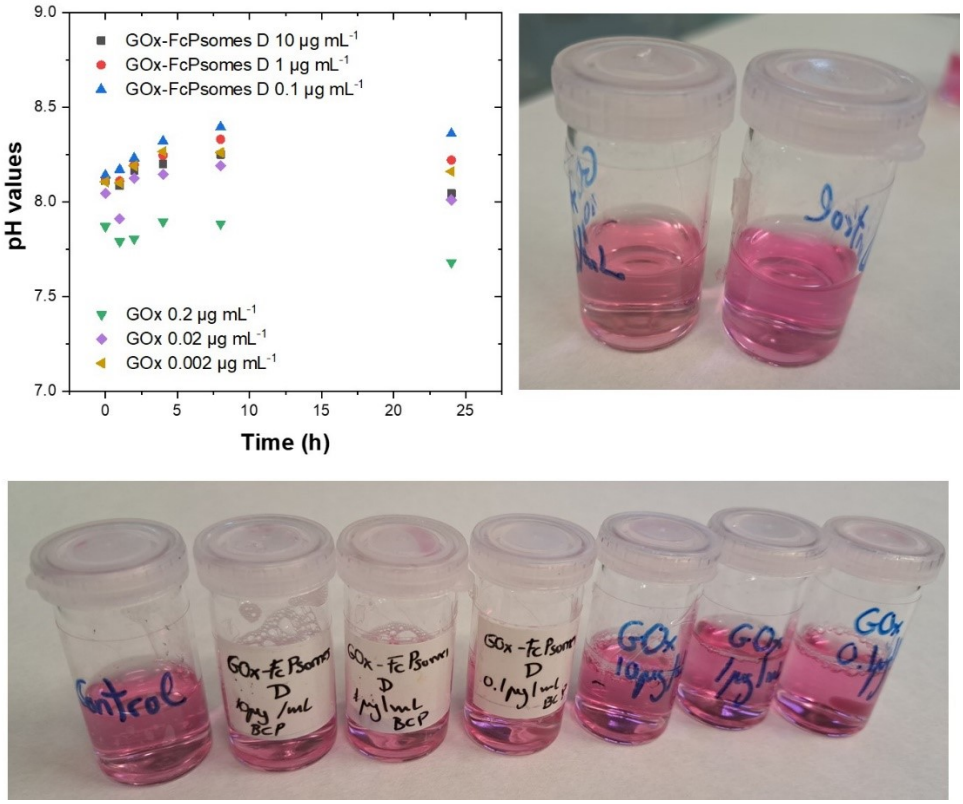

Figure S11. Control experiment of dropping pH in the glucose-containing RMPI culture medium. GOx-FcPsomes D, and GOx free at three different concentrations (10 μg mL^{-1}, 1 μg mL^{-1}, 0.1 μg mL^{-1}) for 24 h were measured. Control is FcPsomes D at pH 8.2 at the below picture.

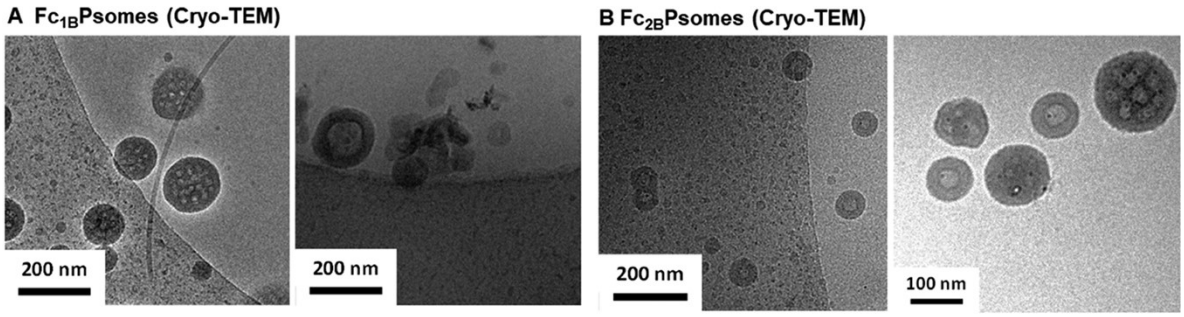

Figure S12. Images sourced from the article *Biomacromolecules* 2022 (DOI: 10.1021/acs.biomac.2c00901) showcase structures exhibiting phase separation besides vesicles structures, which are detailed in the supplementary section.

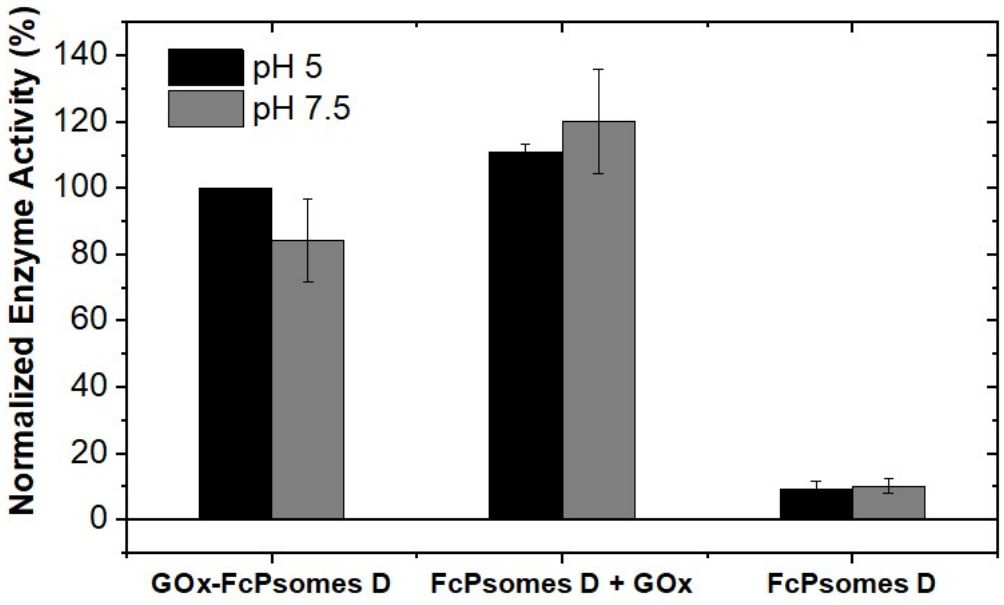

Figure S13. The enzyme activity of GOx-loaded FcPsomes D in the presence of glucose was evaluated at different pH values using the Amplex Red Assay, with GOx-Psomes D at pH 5 serving as the reference. Fluorescence intensity was measured after 60 min (λ_{Ex}: 534 nm; λ_{Em}: 585 nm).

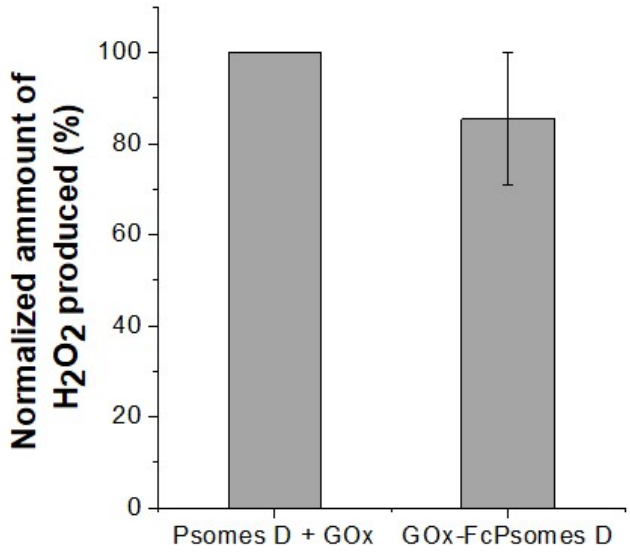

Figure S14. The enzyme activity of GOx-loaded FcPsomes D and Psomes D in presence of GOx was evaluated at glucose concentration using the Amplex Red Assay after 24 h incubation. Psomes D in presence of GOx serving as the reference (100%). Fluorescence intensity was measured after 60 min (λ_{Ex}: 534 nm; λ_{Em}: 585 nm).

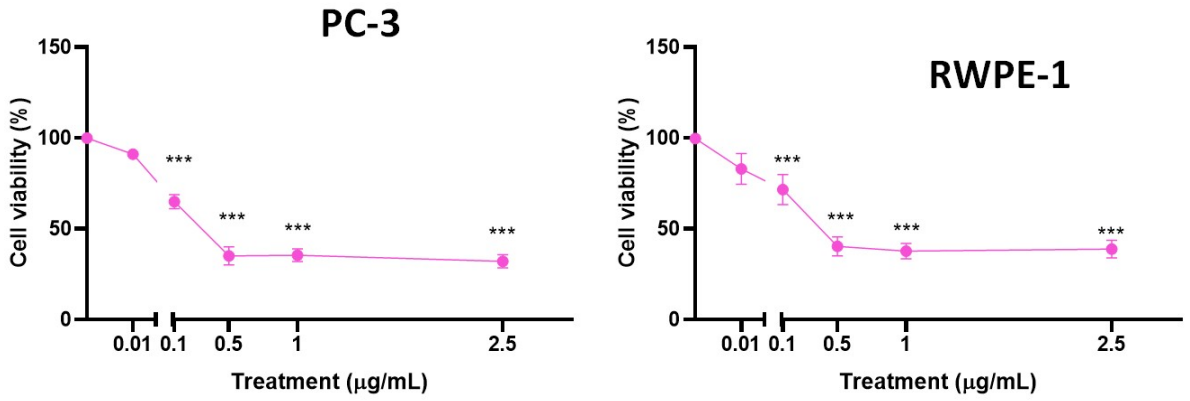

Figure S15. Control experiment by using naked GOx. Tumoral activity by using naked GOx assuming around 10 % of GOx loaded into FcPsomes D in PC3 and RWPE-1 cell line after 24 h incubation.

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