Supporting Information

The chelate-functionalized poly(2-oxazoline) for the destruction of bacterial cell membranes

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Measurements:

The conversion of monomers was determined by gas chromatography (GC) using a VARIAN 3400 gas chromatograph with a J&W Scientific DB-5 (30 m × 0.32 mm) column. Size exclusion chromatography (SEC) system with a multiangle laser light scattering detector (DAWN EOS, Wyatt Technologies, Santa Barbara, CA, USA, λ = 658 nm) and a refractive index detector (Dn-1000 RI WGE DR Bures, Dallgow, Germany, λ = 620 nm) was used for determination of the molar mass and dispersity (*Đ*) of the copolymers. DMF (POCH, Gliwice, Poland) with 5 mmol/L of LiBr was used as eluent. 100 Å, 1000 Å and 3000 Å GRAM columns (Polymer Standards Service, Mainz, Germany) were applied in the system. The composition of the copolymers was analyzed by ^{1}H and ^{13}C NMR spectrometry. The spectra were recorded for samples dissolved in $CDCl_3$ or D_2O using a Bruker Ultrashield spectrometer (Bruker, Billerica, MA, USA) operating at 600 MHz. Specord 200 plus ultraviolet-visible spectrophotometer (Analytik Jena, Jena, Germany) was applied for turbidimetric measurements of copolymer solutions. The cloud point temperature (T_{CP}) value was defined as the temperature at which the transmittance

of the copolymer solutions reached 50 % of its initial value. Dynamic light scattering (DLS) measurements were performed with the use of a Brookhaven BI-200 goniometer with a digital autocorrelator (BI-9000 AT, Brookhaven Instruments, New York, USA) and vertically polarized laser light (Brookhaven Instruments, New York, USA) operating at 35 mW and λ = 637 nm. The autocorrelation functions were analyzed using the constrained regularized algorithm CONTIN. The measurements were made at 90^{o} angle at 25^{o}C in at least triplicate. The average hydrodynamic diameter $D_{h\ aver.}$ was calculated from all the particle populations present in the solution. The particle size evaluation based on light scattering intensity was applied. Before DLS analysis, solutions were passed through membrane filters with the nominal pore size of 0.22 μm (ABLUO, GVS North America). Zeta potential (ζ) measurements were performed for sample solutions with the use of a Zetasizer Nano ZS 90 (Malvern Instruments, Malvern, UK) in a disposable folded capillary cell. ζ was calculated from the electrophoretic mobility, *u*, employing the Helmholtz–Smoluchowski equation ($u = \varepsilon\zeta/\eta$, where ε is the dielectric constant of the solvent and η is the viscosity of the solvent). Ca ions trapping ability was studied by complexometric titration in the presence of murexide as an indicator. Samples were dissolved in water (c=5 mg/mL), then $CaCl_2$ was added (an equimolar amount of Ca ions relative to the DOTA molecules in the conjugate) and stirred for 24 hours. After this time, 0.15 mL of NaOH (c=5 mol/L) and 0.15 mL of murexide solution in a mixture of water:ethanol 1:1 v/v (c=2.5 mg/mL) were added to the solution. Titrations were performed with 0.01 M EDTA solution until the colour changed from slightly pink to light purple, according to the complexometric titration procedure for the determination of Ca ions [1,2]. Based on the volume loss of an EDTA solution of known concentration, the amount of free Ca ions in the solution was calculated. The titration error was 4 % and it was determined by titrating reference solution of $CaCl_2$ of known concentration, without the addition of polymers and chelating compounds. The presence of Ca ions trapped by POx-DOTA was confirmed using X-ray photoelectron spectroscopy (XPS). The samples for XPS were prepared as follows: POx-DOTA was dissolved in water (pH~5), then $CaCl_2$ was added (an equimolar amount of Ca ions relative to the DOTA molecules in the conjugate) and stirred for 24 hours. Then, the solution was dialyzed

against water for 24 hours (MWCO: 1 kDa), changing the solvent several times. After this time, 0.05 mL was taken from the dialyzed solution and placed on a silicon wafer (Cemat Silicon S.A, 3 nm of SiO_2 layer), previously washed and degreased. The wafer with POx-DOTA was left in the air for 12 hours and then dried under reduced pressure for another 12 hours. The obtained films were analysed using the PHI 5700/660 Physical Electronics spectrometer with monochromatic Al Kα x-ray source with energy 1486.6 eV. The energy resolution was 0.3 eV. All photoelectron spectra have been calibrated against the peaks of Au 4f7/2 at 83.98 eV, Ag 3d5/2 at 368.27 eV, and Cu 2p3/2 at 932.67 eV of binding energy. The measurements were carried out at a take-off angle of 45^o in the detector direction. To compensate of the positive charge, which may appear on the study films during measurements, the float gun of free electrons was used. The survey spectra and the O1s, C1s, N1s and Ca2p core lines, were recorded at the pass energy of 187.85 eV and 23.5 eV, respectively. The binding energy E=285 eV of the C1s peak related to presence of the C-C bonding was chosen as the reference. An atomic concentration calculation and fitting procedure were performed by using MULTIPAK software (ver. 9.8.0.19 Ulvac-phi, Inc.). The Shirley background was applied to the deconvolution of Ca2p photoemission line. Cryogenic Transmission Electron Microscopy (cryo-TEM) images were obtained using a Tecnai F20 X TWIN microscope (FEI Company, Hillsboro, Oregon, USA) equipped with field emission gun, operating at an acceleration voltage of 200 kV. Images were recorded on the Gatan Rio 16 CMOS 4k camera (Gatan Inc., Pleasanton, California, USA) and processed with Gatan Microscopy Suite (GMS) software (Gatan Inc., Pleasanton, California, USA). Samples preparation was done by vitrification of the aqueous solutions on grids with holey carbon film (Quantifoil R 2/2; Quantifoil Micro Tools GmbH, Großlöbichau, Germany). Prior to use, the grids were activated for 15 seconds in oxygen plasma using a Femto plasma cleaner (Diener Electronic, Ebhausen, Germany). Cryo-samples were prepared by applying a droplet (3 µL) of the suspension to the grid, blotting with filter paper and immediate freezing in liquid ethane using a fully automated blotting device Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, Massachusetts, USA). After preparation, the vitrified samples were kept under liquid

nitrogen until they were inserted into a cryo-TEM-holder Gatan 626 (Gatan Inc., Pleasanton, USA) and analyzed in the TEM at -178°C.

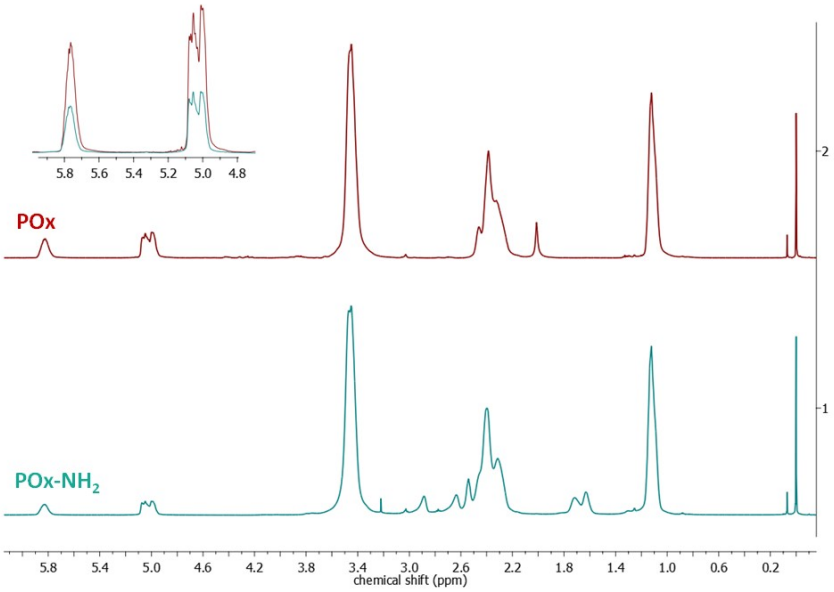

Fig. S1. ^{1}H NMR of POx and POx-NH_2 ($CDCl_3$).

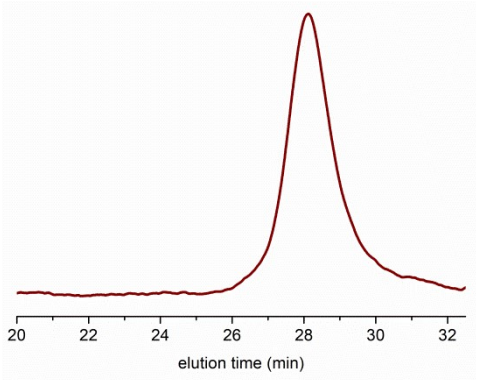

Fig. S2. SEC RI trace of POx.

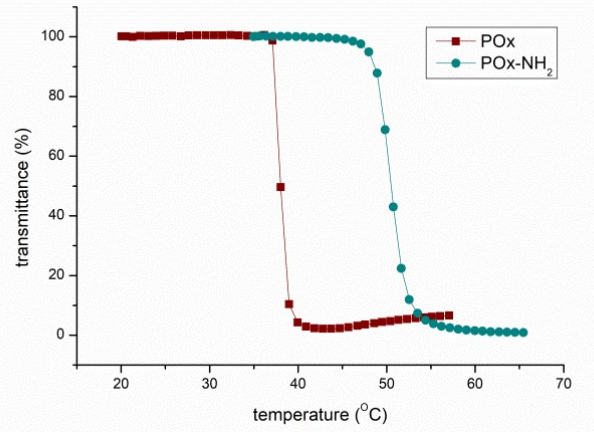

Fig. S3. Transmittance *vs* temperature curve for POx and POx-NH_2 aqueous solutions (c=5 mg/mL).

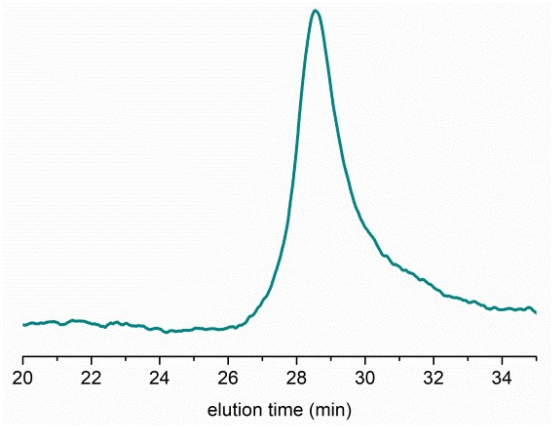

Fig. S4. SEC RI trace of POx-NH_2.

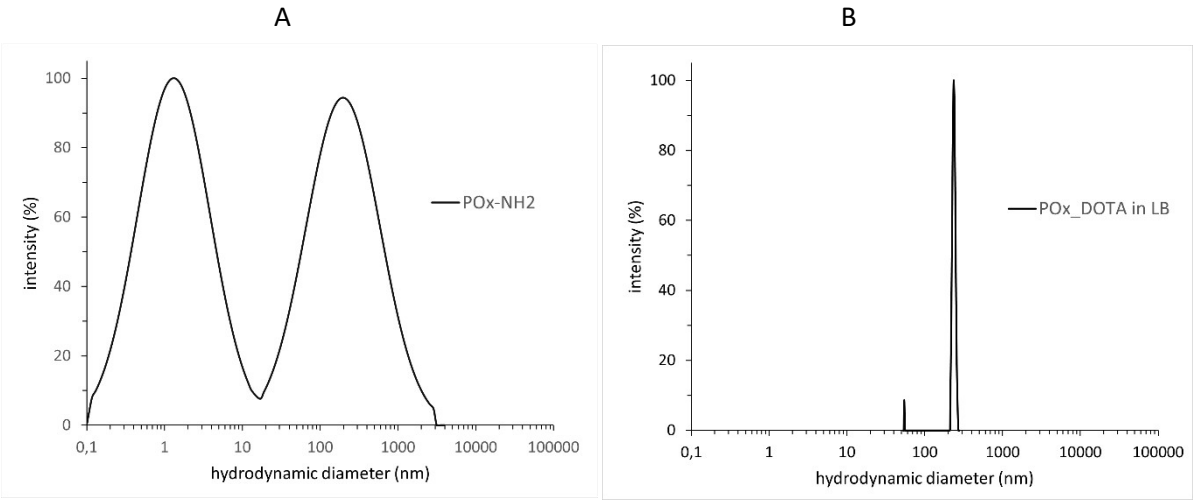

Fig. S5. Distributions of the hydrodynamic diameter (intensity weighted distribution) measured by DLS for POx-NH_2 in water (A) and POx-DOTA in LB (B).

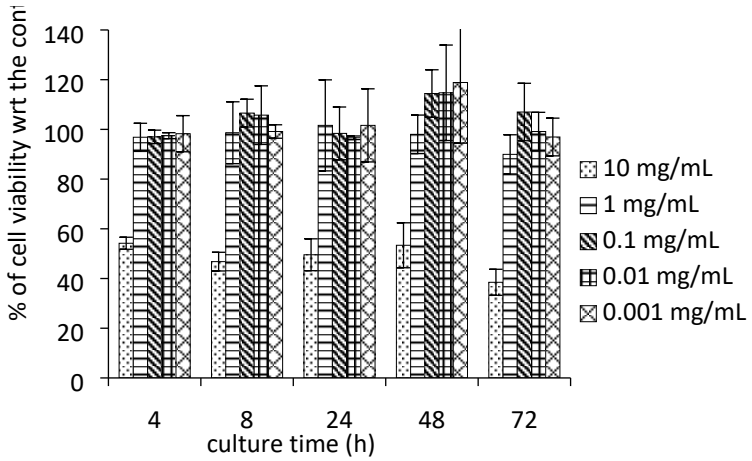

Fig. S6. The cytotoxicity assay of POx-DOTA at increasing concentrations, with the use of human fibroblasts. The results are shown as a percentage of the control, where untreated cells constituted 100 %.

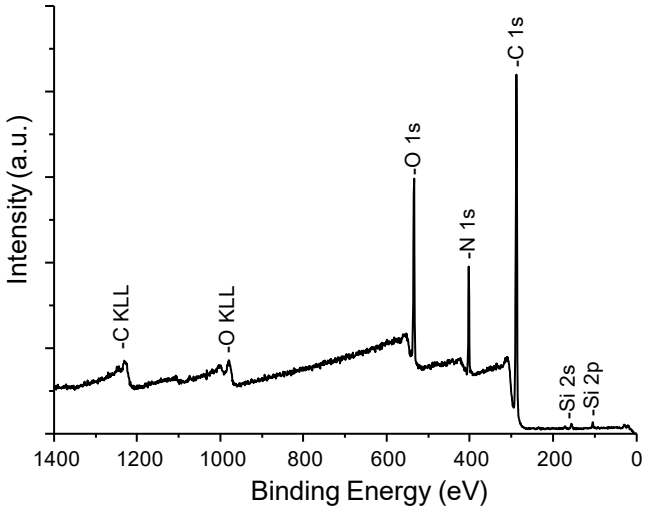

Fig. S7. Survey photoemission spectrum of PEtOx.

References:

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2 J. C. H. Van Schouwenburg, *Anal. Chem.*, 1960, **32**, 709–711.