

Photodynamic Eradication of *Pseudomonas aeruginosa* with Ru-Photosensitizers Released from Enzyme Degradable Nanocarriers

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Supporting Information

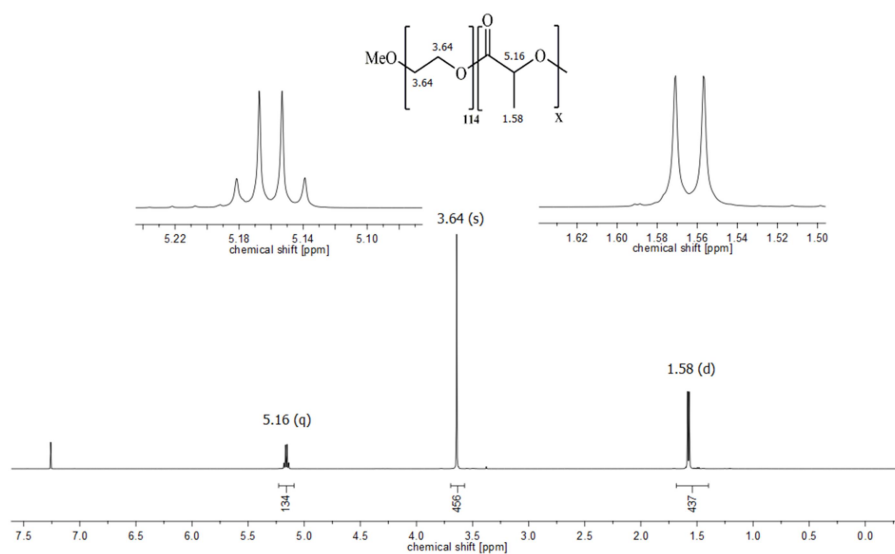


Figure S1: ^1H -NMR spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{143}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J = 7.1$ Hz, 134H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.64 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG- OMe), 1.58 (d, $^3J = 7.2$ Hz, 437H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 1.48 (d, $^3J = 7.2$ Hz, 6H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)).

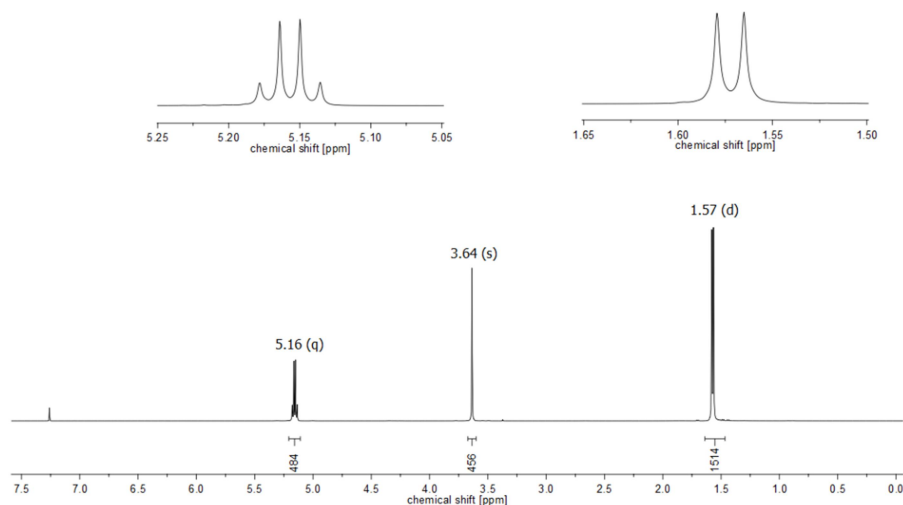


Figure S2: ^1H -NMR spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{499}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J = 7.1$ Hz, 484H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.64 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG-OMe), 1.57 (d, $^3J = 7.2$ Hz, 1514H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)).

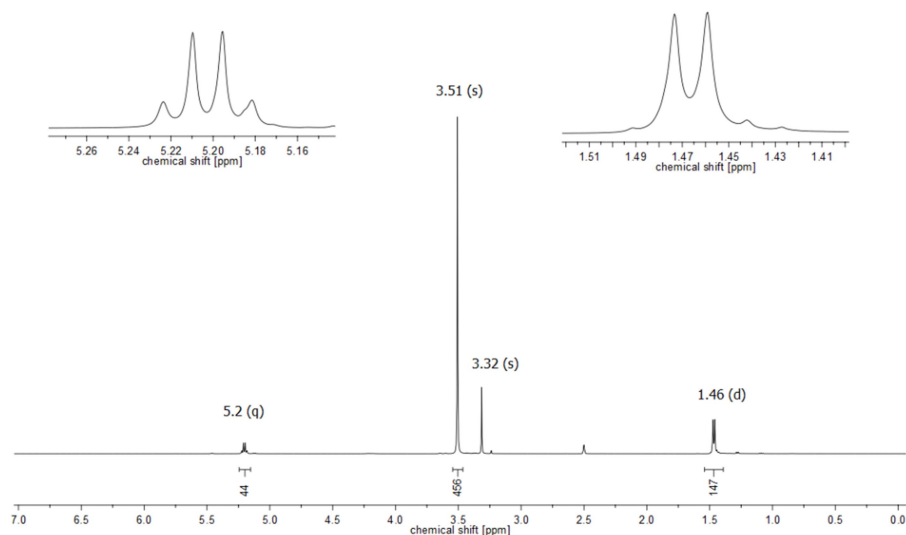


Figure S3: ^1H -NMR Spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{48}$ solvent ($\text{DMSO-}d_5$).

^1H -NMR (500 MHz $\text{DMSO-}d_6$) δ (ppm) = 5.20 (q, $^3J = 7.0$ Hz, 44H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 5.01 (q, $^3J = 7.1$ Hz, 1H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)), 3.51 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.32 (s, 78H, H_2O), 3.31 (s, 3H, OCH_3) (PEG-OMe), 1.46 (d, $^3J = 7.0$ Hz, 147 H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)).

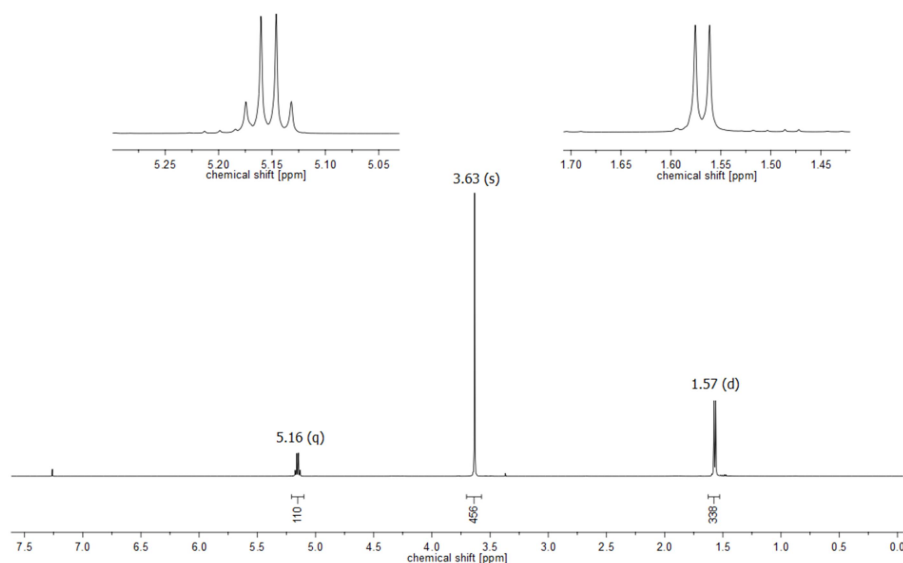


Figure S4: ^1H -NMR spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{112}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J = 7.1$ Hz, 110H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.63 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG-OMe), 1.57 (d, $^3J = 7.2$ Hz, 338H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 1.48 (d, $^3J = 7.2$ Hz, 6H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)).

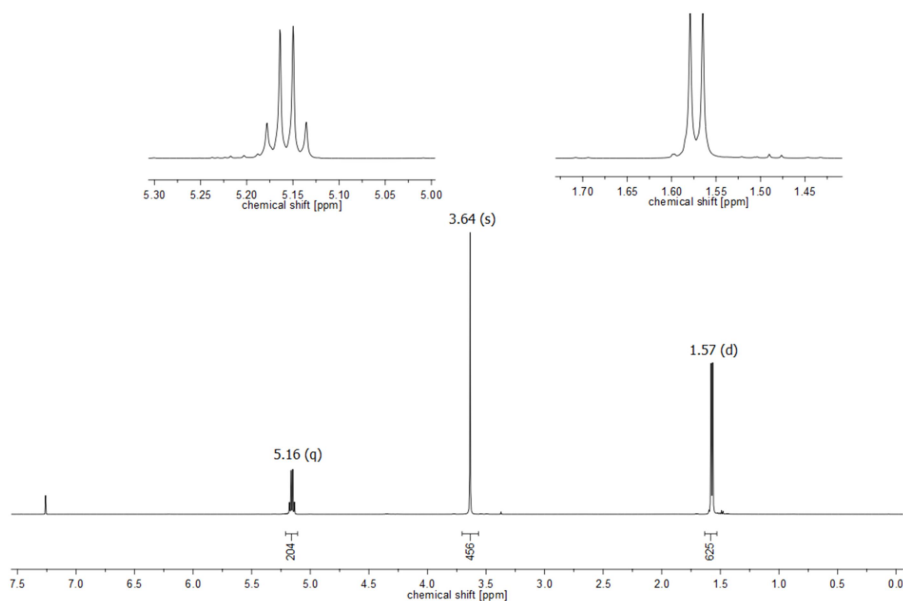


Figure S5: ^1H -NMR spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{207}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J = 7.1$ Hz, 204H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.64 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG-OMe), 1.57 (d, $^3J = 7.2$ Hz, 625H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 1.48 (d, $^3J = 7.2$ Hz, 10H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)).

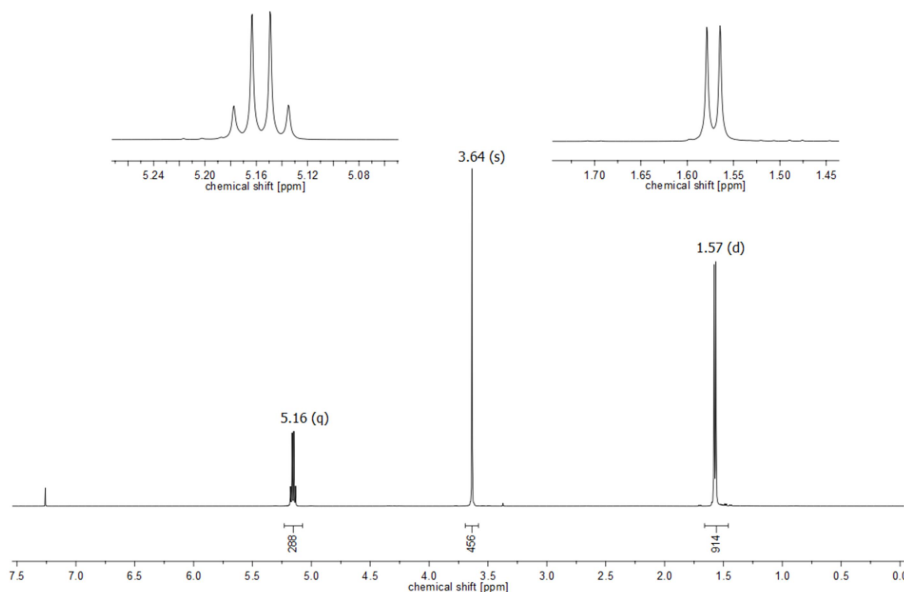


Figure S6: ^1H -NMR Spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{301}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J=J$ 7.1 Hz, 288H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.64 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG-OMe), 1.57 (d, $^3J=7.2$ Hz, 914H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 1.48 (d, $^3J=7.2$ Hz, 10H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)).

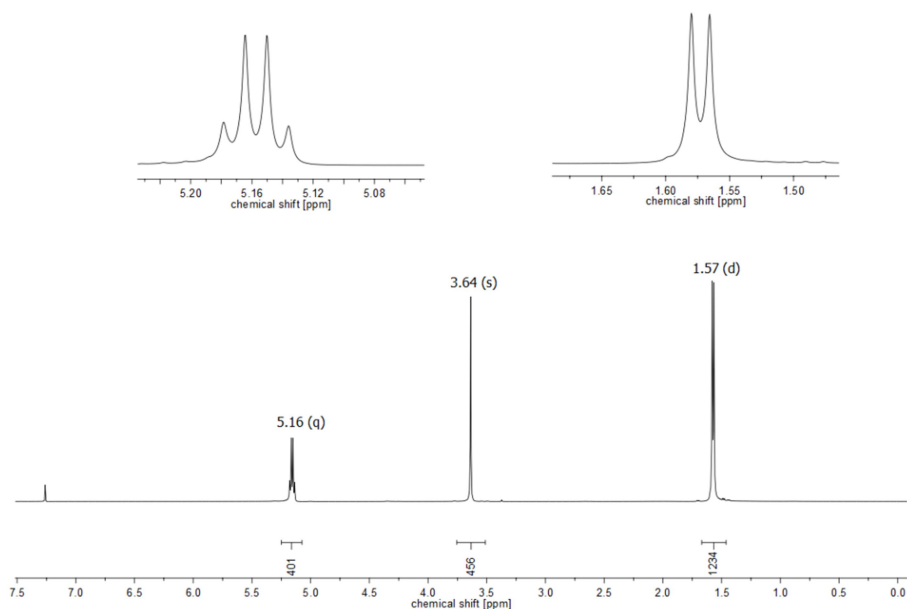


Figure S7: ^1H -NMR Spectrum of $\text{PEG}_{114}\text{-}b\text{-PLA}_{408}$ (solvent CDCl_3).

^1H -NMR (500 MHz, CDCl_3): δ (ppm) = 7.26 (s, CDCl_3): 5.16 (q, $^3J=J$ 7.1 Hz, 401H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 3.64 (s, 456H, $\text{OCH}_2\text{CH}_2\text{O}$ (PEG)), 3.36 (s, 3H, OCH_3) (PEG-OMe), 1.57 (d, $^3J=7.2$ Hz, 1234H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (PLA)), 1.48 (d, $^3J=7.2$ Hz, 16H, $\text{CH}_3\text{CH}(\text{C}=\text{O})\text{O}$ (monomer)).

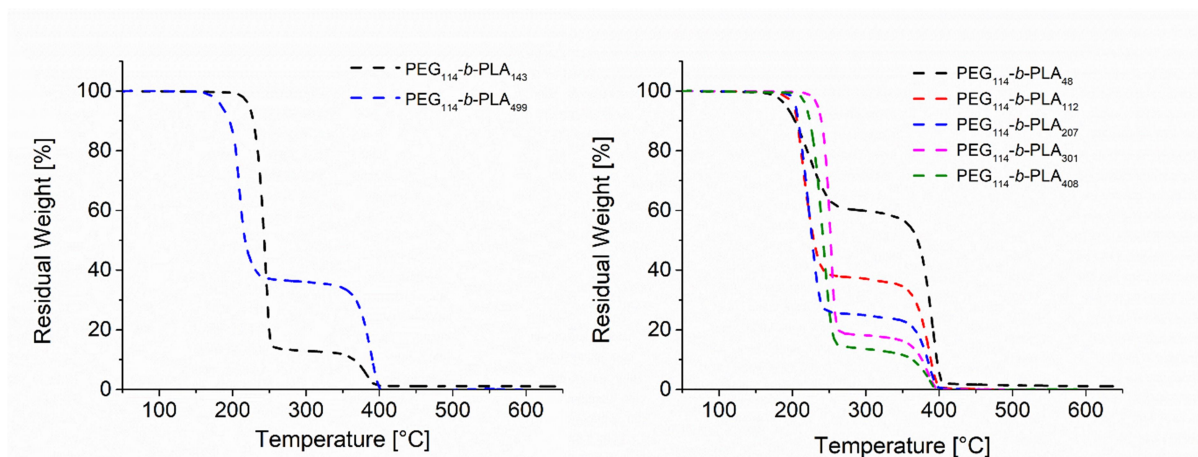


Figure S8: TGA traces of the synthesized $\text{PEG}_{114}\text{-}b\text{-PLA}_x$ polymers with a heating rate of $5^\circ\text{C}/\text{min}$ from 200 to 450°C under N_2 atmosphere.

Table S1: Values of degree of polymerization of the PLA block of the synthesized $\text{PEG}_{114}\text{-}b\text{-PLA}_x$ polymers using $^1\text{H-NMR}$ and by weight determined with thermogravimetric analysis (TGA)

Polymers and nominal degree of polymerization	Number average degree of polymerization ($^1\text{H-NMR}$ spectroscopy)	Approximate mass average degree of polymerization (TGA)
$\text{PEG}_{114}\text{-}b\text{-PLA}_{150}$	143	139
$\text{PEG}_{114}\text{-}b\text{-PLA}_{500}$	499	491
$\text{PEG}_{114}\text{-}b\text{-PLA}_{50}$	48	47
$\text{PEG}_{114}\text{-}b\text{-PLA}_{100}$	112	100
$\text{PEG}_{114}\text{-}b\text{-PLA}_{200}$	207	202
$\text{PEG}_{114}\text{-}b\text{-PLA}_{300}$	301	306
$\text{PEG}_{114}\text{-}b\text{-PLA}_{400}$	408	400

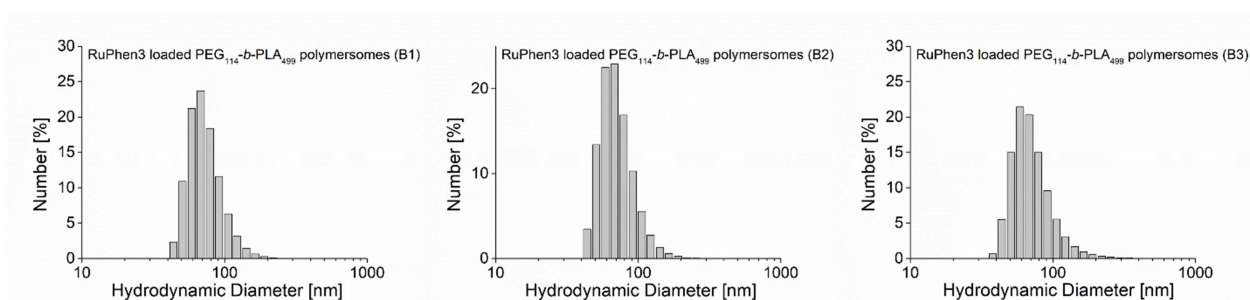


Figure S9: Size Distributions (DLS) of $\text{PEG}_{114}\text{-}b\text{-PLA}_{499}$ polymersomes in water (Batches 1-3).

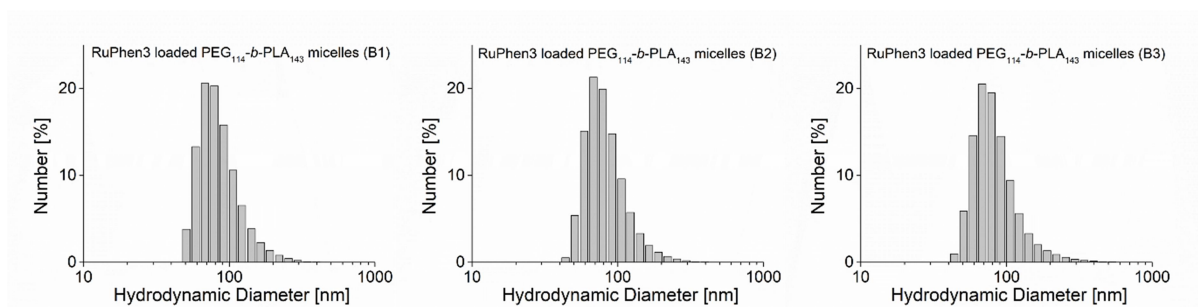


Figure S10: Size Distributions (by DLS) of PEG₁₁₄-*b*-PLA₁₄₃ micelles in water (Batches 1-3).

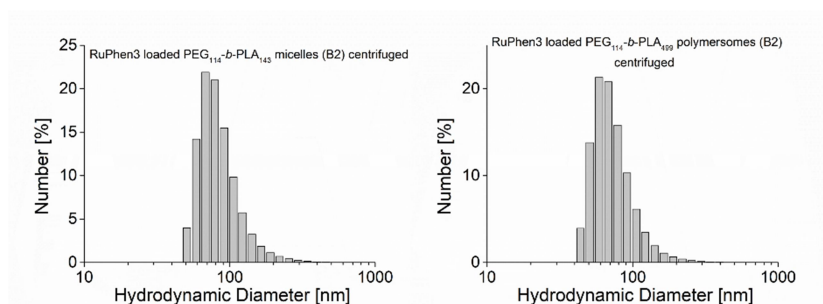


Figure S11: Size Distributions (by DLS) of PEG₁₁₄-*b*-PLA₁₄₃ micelles and PEG₁₁₄-*b*-PLA₄₉₉ polymersomes after 10x centrifugation and solvent exchange in water (Batch 2).

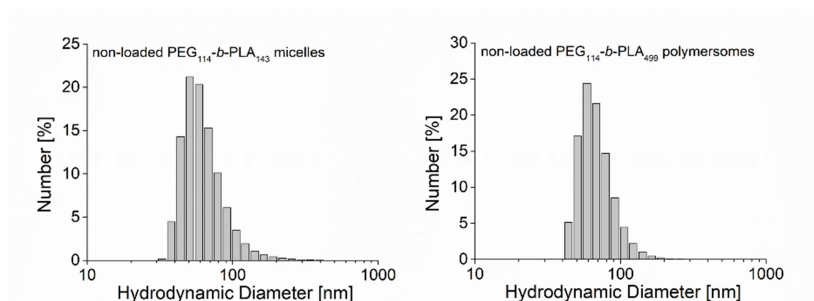


Figure S12: Size Distributions (by DLS) of non-loaded PEG₁₁₄-*b*-PLA₁₄₃ micelles and PEG₁₁₄-*b*-PLA₄₉₉ polymersomes in water.

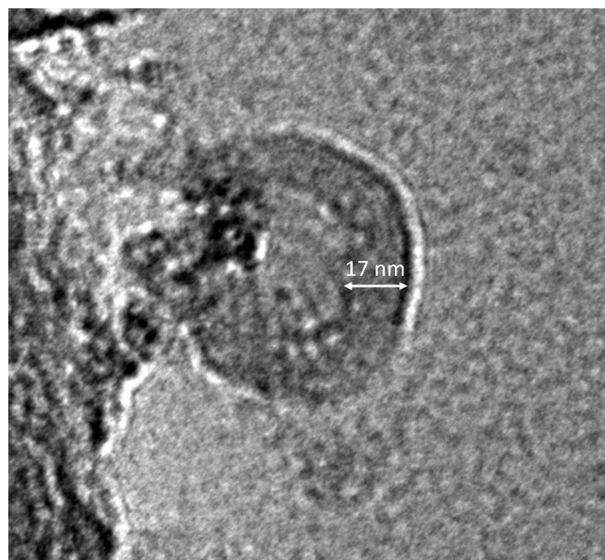


Figure S 13: TEM images of RuPhen3-loaded PEG₁₁₄-*b*-PLA₄₉₉ polymersomes as shown in Figure 3 with indicated wall thickness (compare arrow).

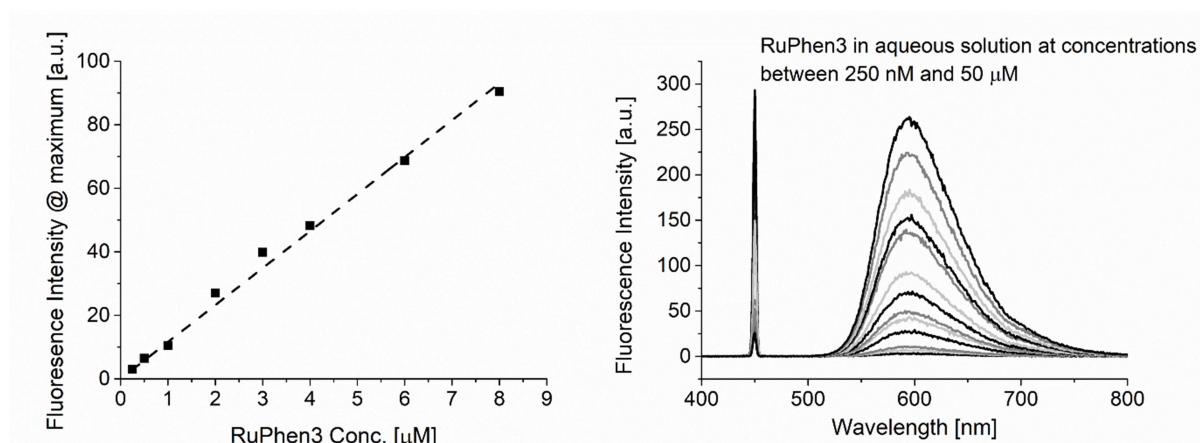


Figure S14: Fluorescence intensity at the maximum emission for different RuPhen3 concentrations in aqueous solution as a function of concentration (left) and fluorescence emission spectra of RuPhen3 in aqueous solution for concentrations between 250 nM and 50 μM (right) excited at 450 nm.

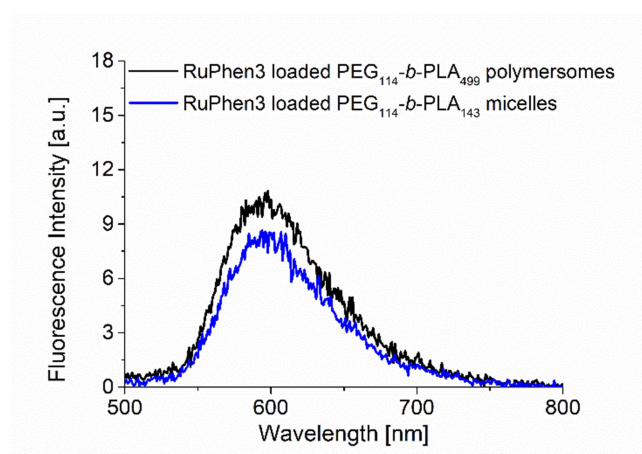


Figure S15: Fluorescence emission spectra of RuPhen3 loaded PEG₁₁₄-*b*-PLA₁₄₃ micelles and PEG₁₁₄-*b*-PLA₄₉₉ polymersomes in aqueous solution (Batch3) for upscaled batches excited at 450 nm.

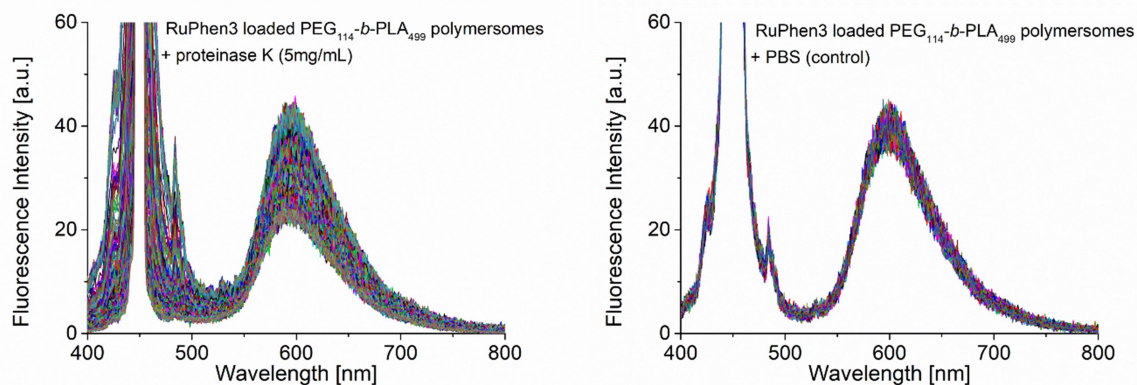


Figure S16: Fluorescence emission spectra of RuPhen3 (excited at 450 nm) encapsulated into PEG₁₁₄-*b*-PLA₄₉₉ polymersomes over minimum 12h in presence of proteinase K (5mg/mL) in PBS (left) and without addition of enzyme in PBS (right). The spectra show a slow increase followed by strong decrease in fluorescence intensity at around 600 nm (RuPhen3).

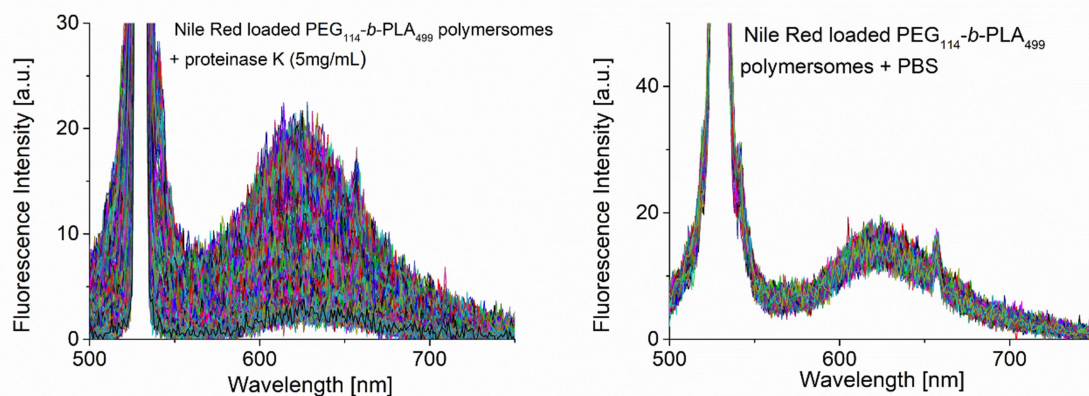


Figure S17: Fluorescence emission spectra of Nile Red (NR)(excited at 530 nm) encapsulated into PEG₁₁₄-*b*-PLA₄₉₉ polymersomes over minimum 12h in presence of proteinase K (5mg/mL) in PBS (left) and without addition of enzyme in PBS (right). The spectra show a slow increase followed by strong decrease in fluorescence intensity at around 620 nm (NR).

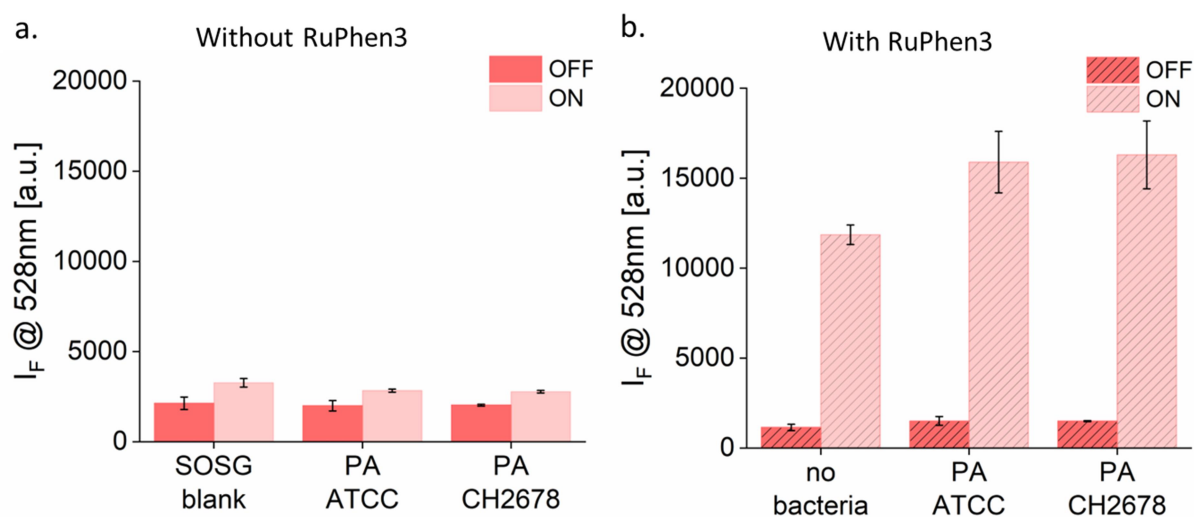


Figure S18: SOSG probe signal for detecting singlet oxygen production by a. SOSG probe and the used bacterial strains PA ATCC and PA CH2678, b. neat RuPhen3 incubated with and without bacteria strains. The samples were irradiated for 30 min (ON) or incubated in dark for 30 min (OFF).

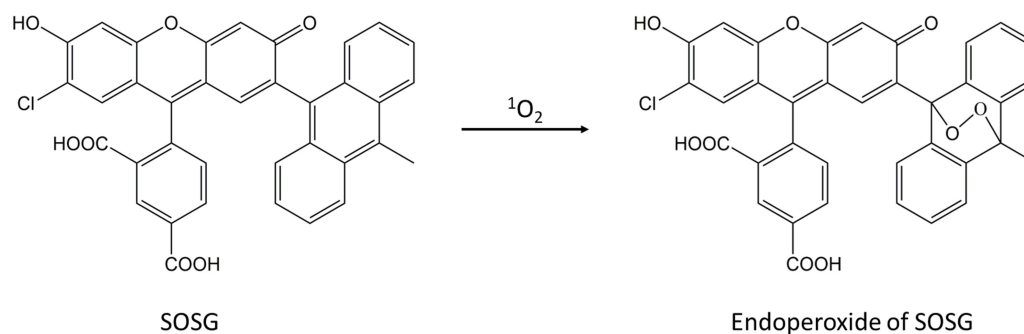


Figure S19: The conversion of the SOSG probe into its endoperoxide form in the presence of singlet oxygen.