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Passerini chemistries for synthesis of polymer pro-drug and polymersome drug delivery nanoparticles

Alessandra Travanut,[a] Patrícia F. Monteiro,[a] Sean Smith,[b] Steven M. Howdle,[b] Anna M. Grabowska,[c] Barrie Kellam,[a] Michael A. R. Meier,[d] and Cameron Alexander.[a]*

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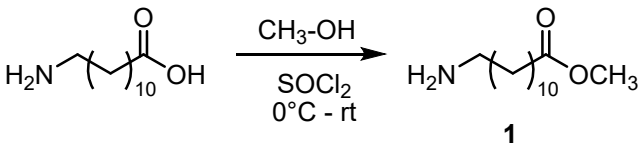
Materials

The following chemicals were used as received: trimethyl orthoformate (≥99%, Aldrich), thionyl chloride (≥97%, Aldrich), diisopropylamine (≥99%, Aldrich), phosphorus(V) oxidychloride (≥99%, Aldrich), 10-undecenal (≥90%, Aldrich), 3-mercaptopropionic

Synthetic procedures and spectroscopic data

Synthesis of methyl 12-isocyanododecanoate (3) [1]



1

Scheme S 1: methoxy protection of 12-aminododecanoic acid carboxylic group.

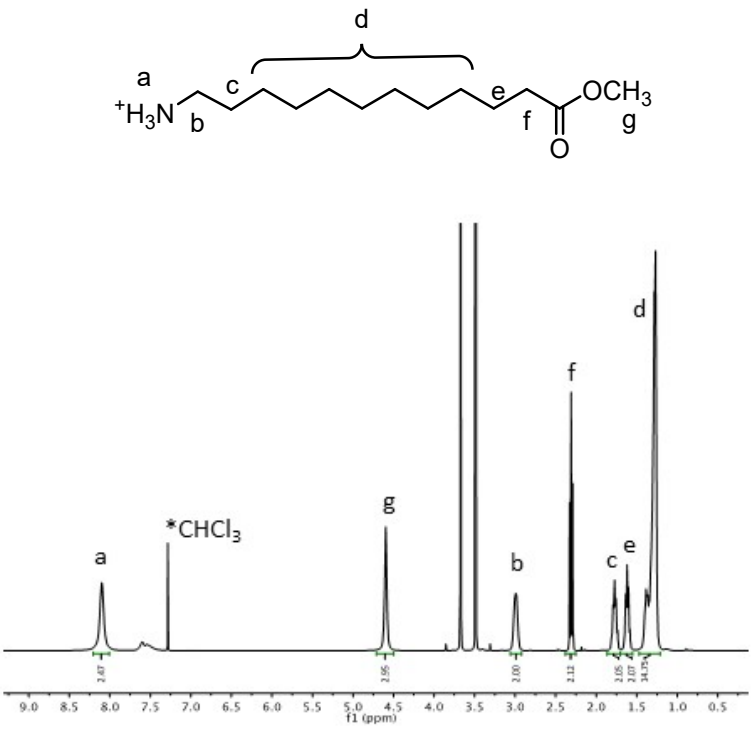
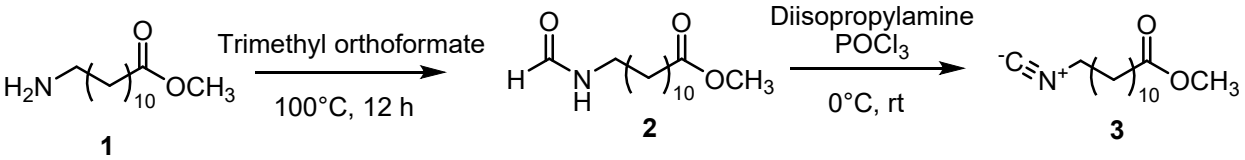
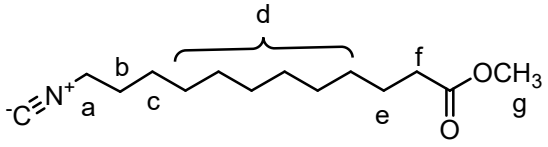


Figure S 1: ^{1}H NMR of 12-methoxy-12-oxododecan-1-aminium (1) in $CDCl_3$.



Scheme S 2: synthetic route of methyl 12-isocyanododecanoate (3): formylation of 12-methoxy-12-oxododecan-1-aminium amino group (1) and final dehydration to methyl 12-isocyanododecanoate (3).



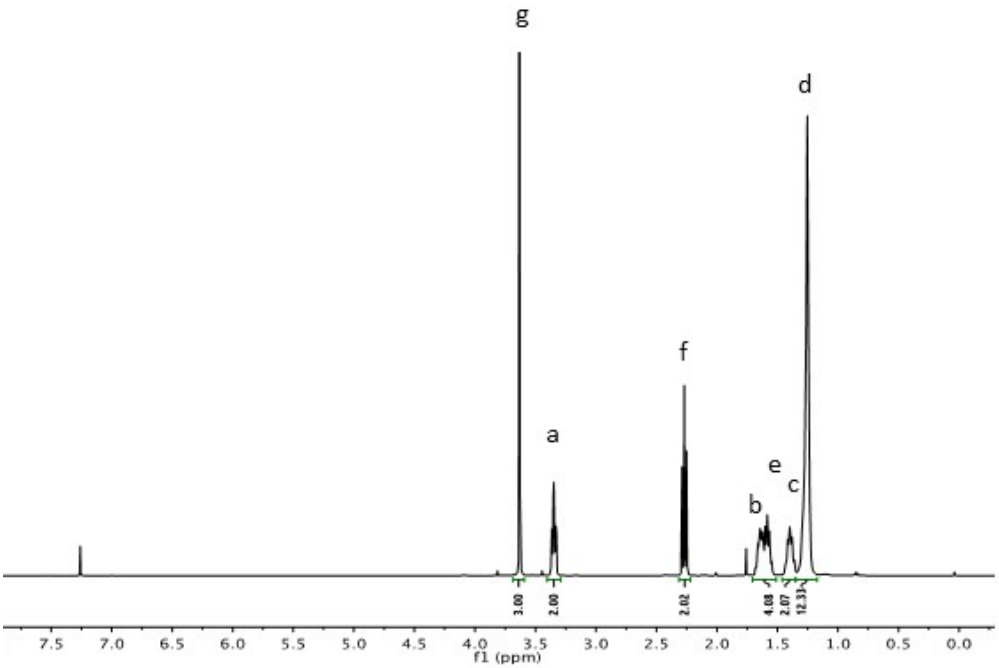


Figure S 2: ^{1}H NMR of methyl 12-isocyanododecanoate **(3)** in $CDCl_3$.

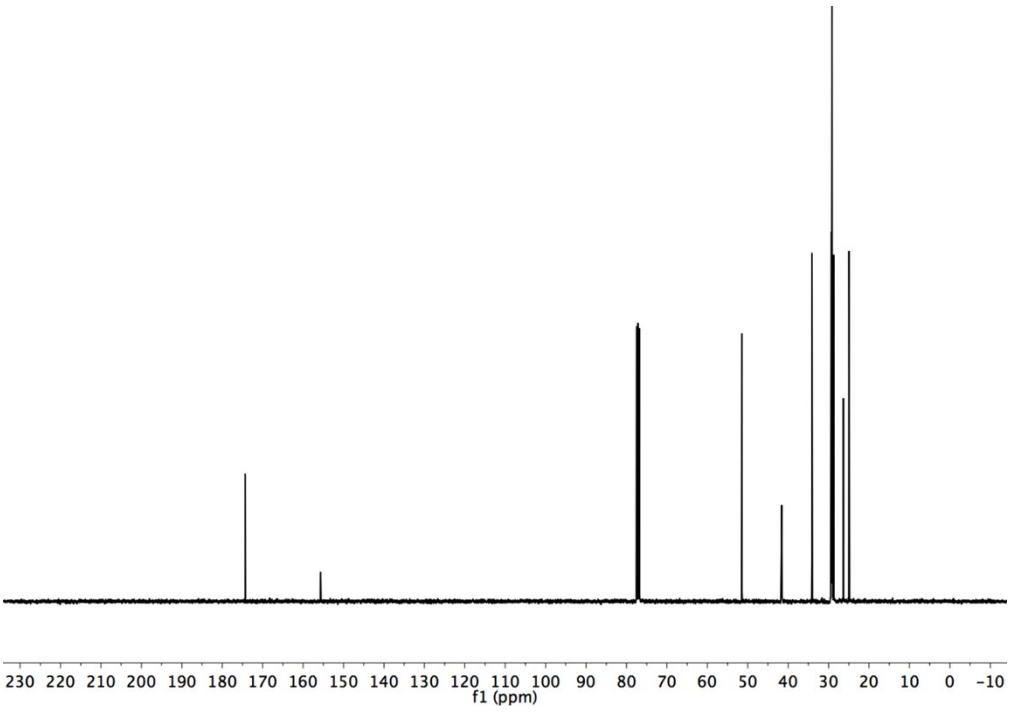
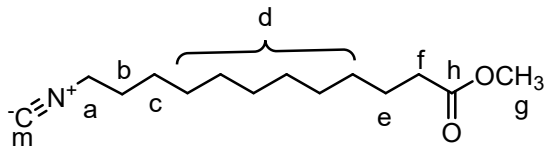


Figure S 3: ^{13} C NMR of methyl 12-isocyanododecanoate (3) in $CDCl_3$.



Synthesis of but-3-en-1-yl 12-isocyanododecanoate (4)

Scheme S 3: Synthesis of but-3-en-1-yl 12-isocyanododecanoate (4) starting from methyl 12-isocyanododecanoate (3).

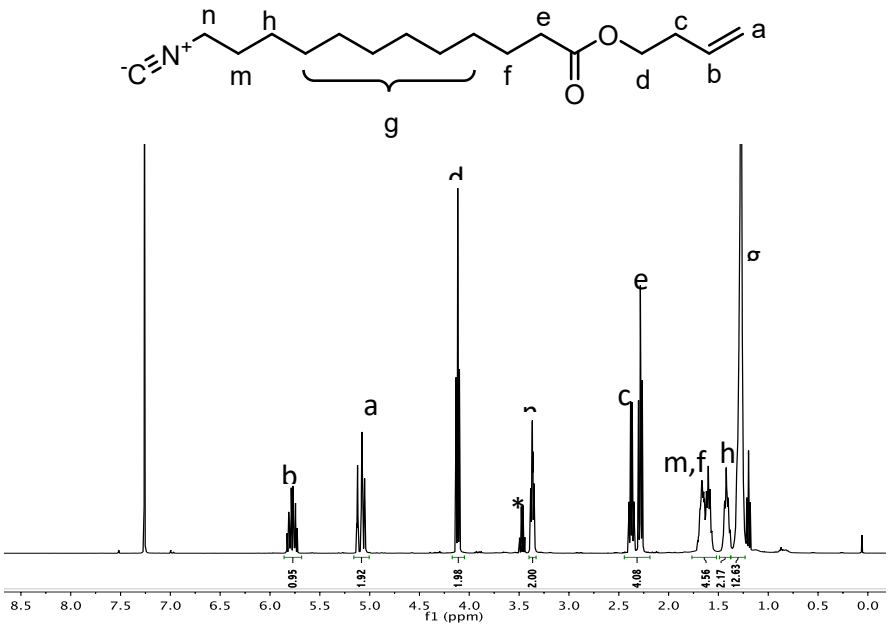


Figure S 4: ^{1}H NMR of but-3-en-1-yl 12-isocyanododecanoate (4) in $CDCl_3$; * diethyl ether.

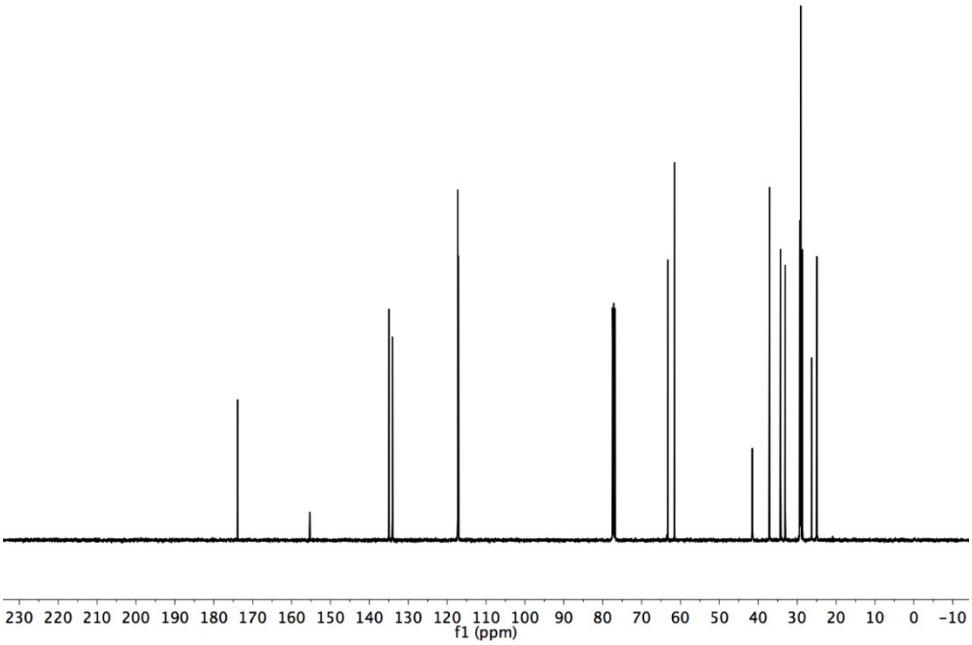
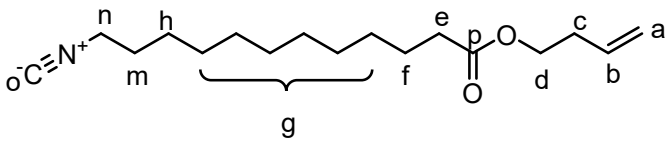
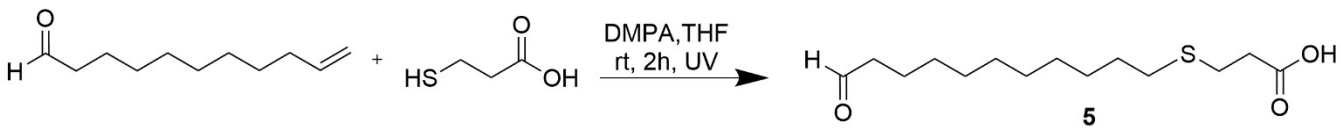


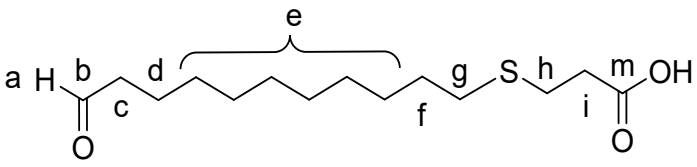
Figure S 5: ^{13} C NMR of but-3-en-1-yl 12-isocyanododecanoate (4) in CDCl3.



Synthesis of AB-Type monomer (5) [1]



Scheme S 4: synthesis of AB-type monomer (*5*) via thiol-ene reaction



Synthesis of P1 – Passerini-3CR polymerization[2]

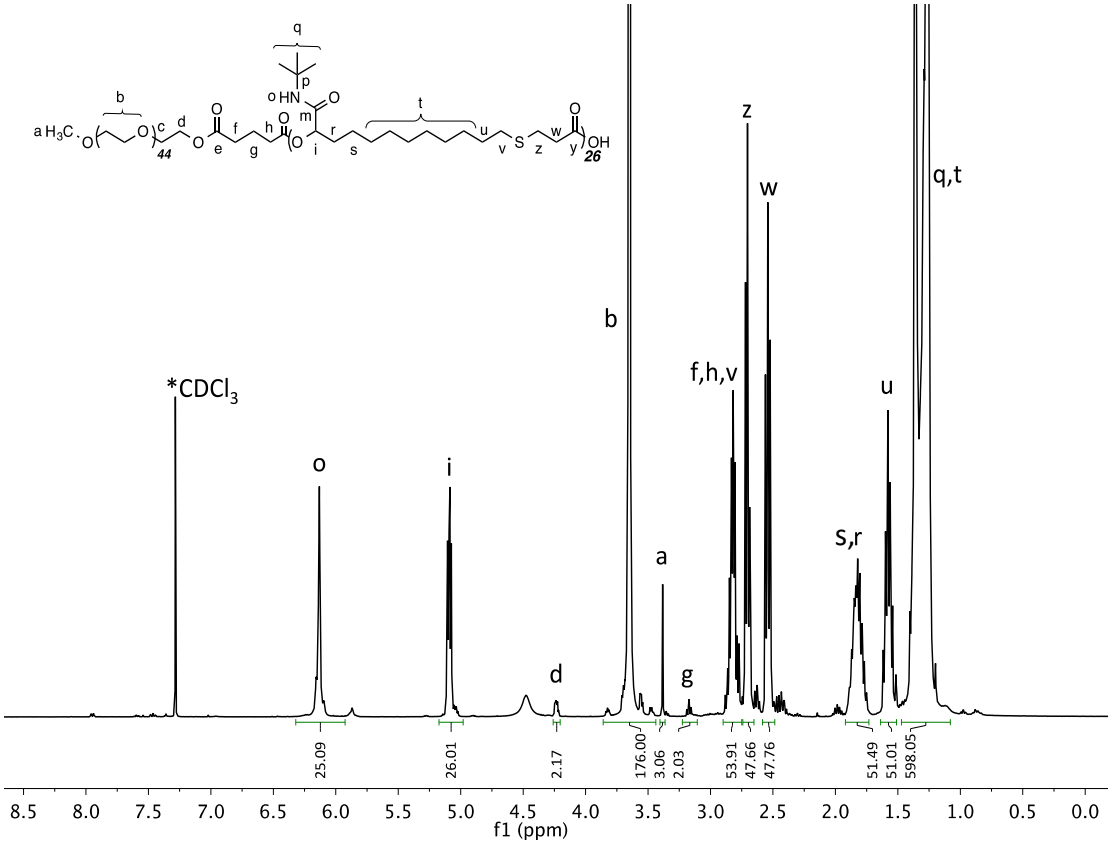
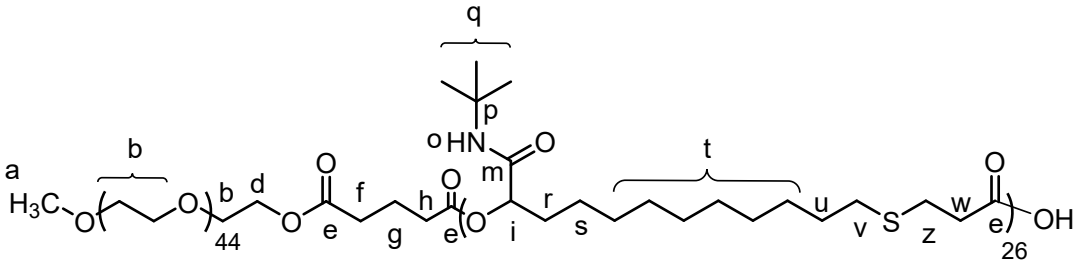
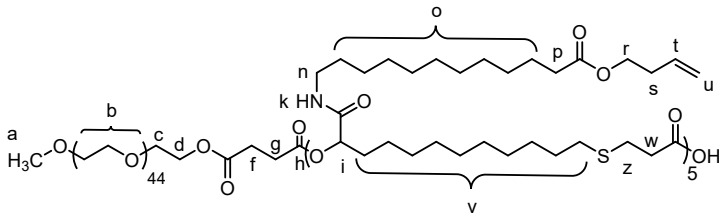


Figure S 6: Integrated ^{1}H NMR spectrum of Passerini diblock copolymer **P1** in $CDCl_3$



Synthesis of P2 – Passerini-3CR polymerization [2]



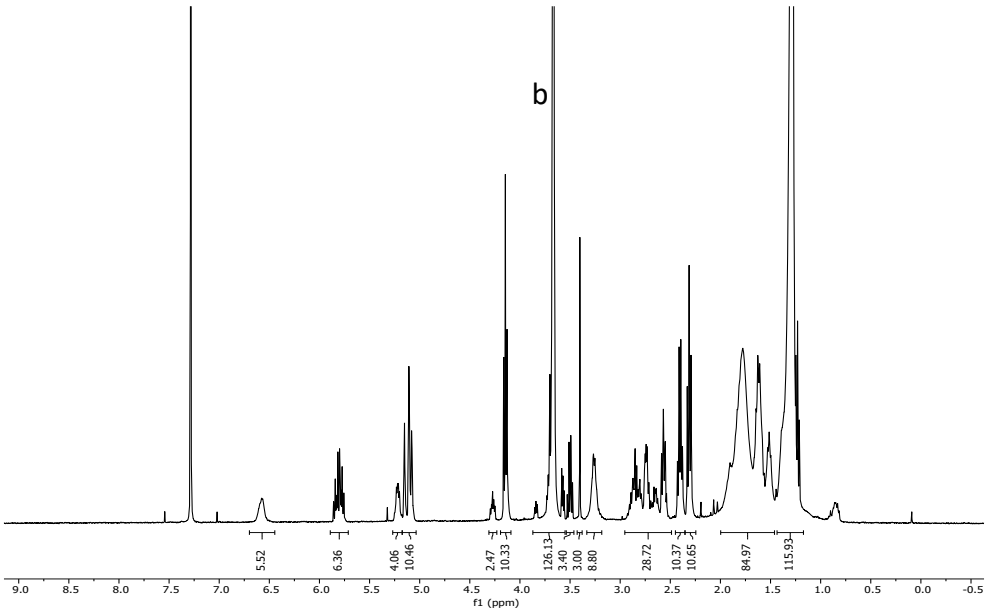


Figure S 7: Integrated ^{1}H NMR spectrum of Passerini diblock copolymer **P2** in $CDCl_3$

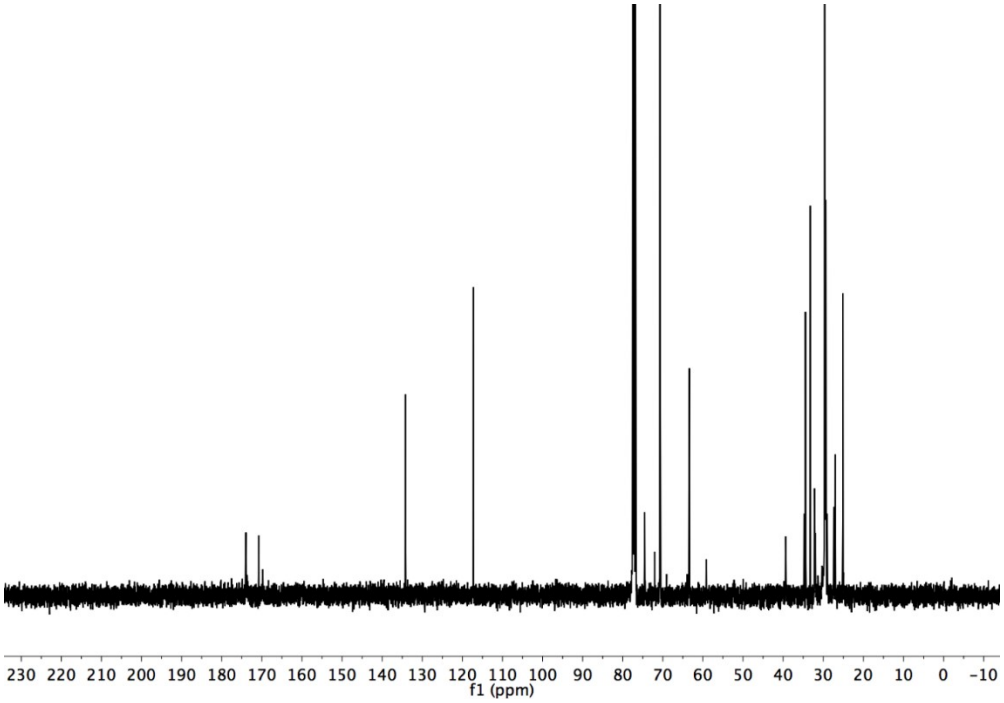
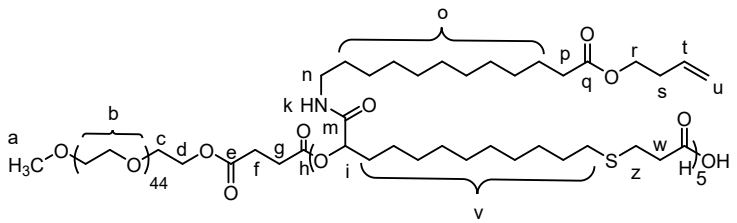
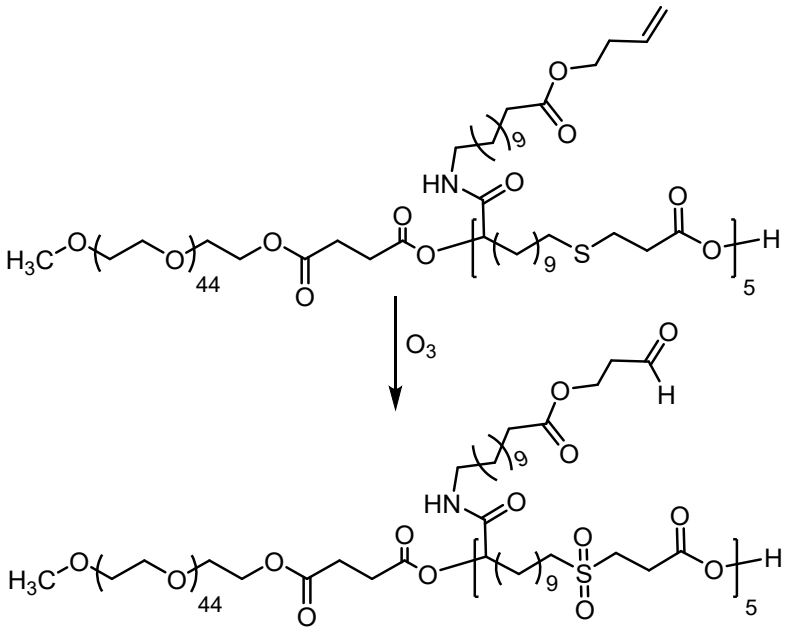


Figure S 8: ^{13} C NMR of P2 in $CDCl_3$.



P2 Oxidation to P3 via ozonolysis



Scheme S 5: Ozonolysis reaction scheme of **P2** Passerini diblock copolymer to yield **P3** Passerini diblock copolymer.

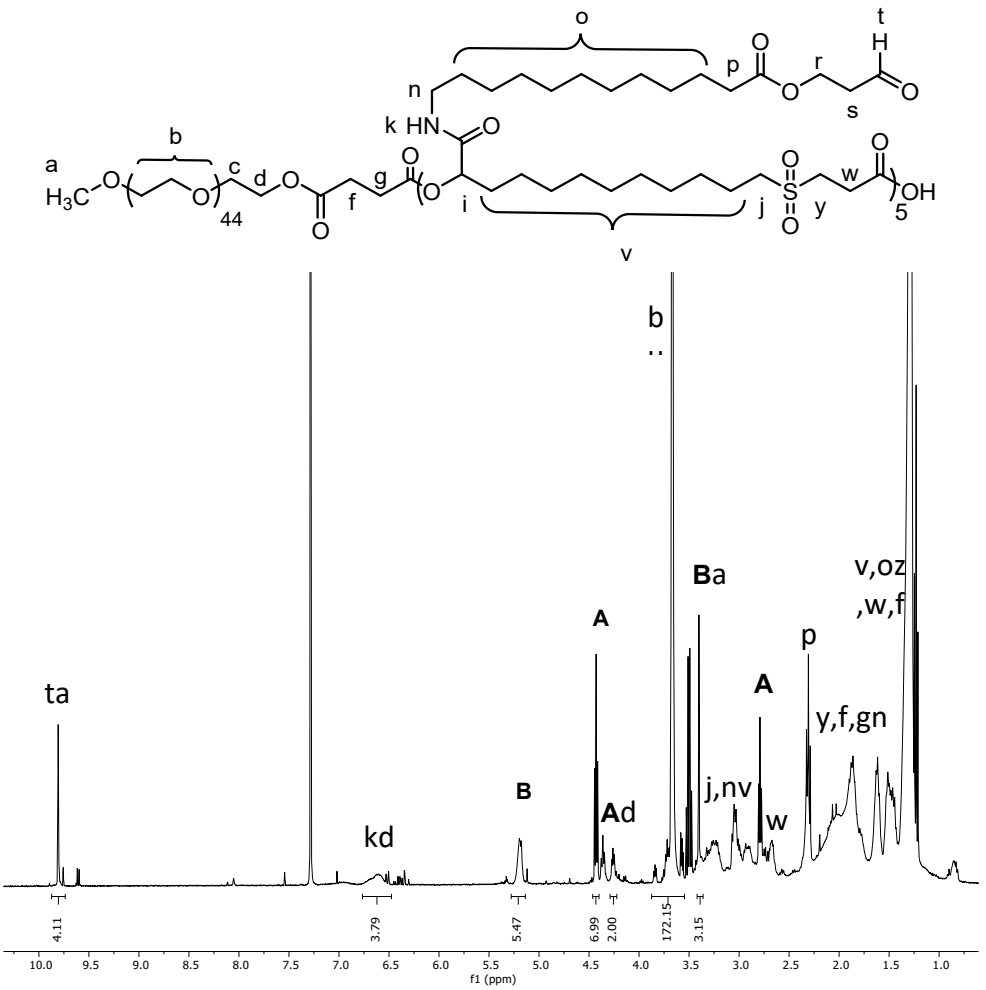


Figure S 9: Integrated ^{1}H NMR spectrum of Passerini diblock copolymer **P3** in $CDCl_3$.

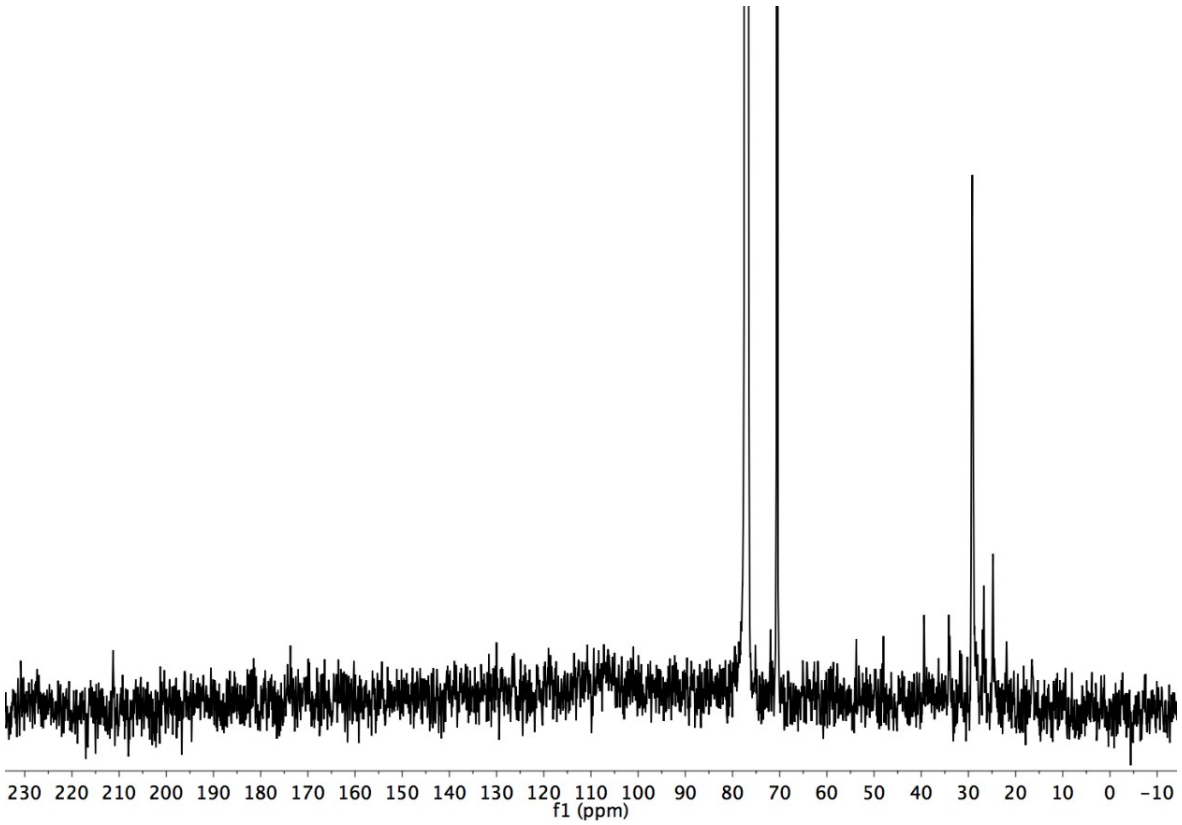
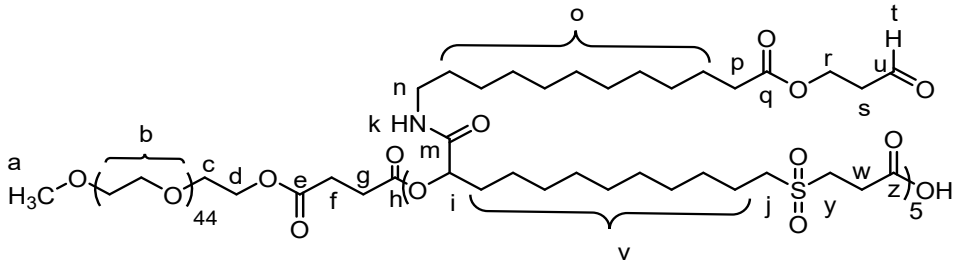


Figure S 10: ^{13} C NMR of P3 in $CDCl_3$.



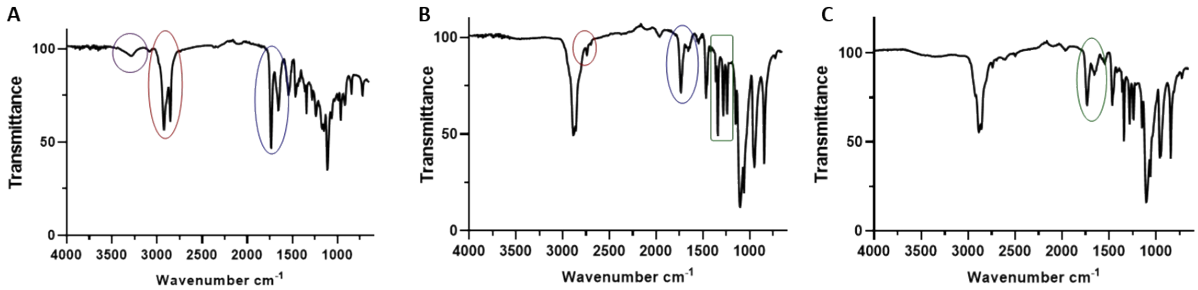
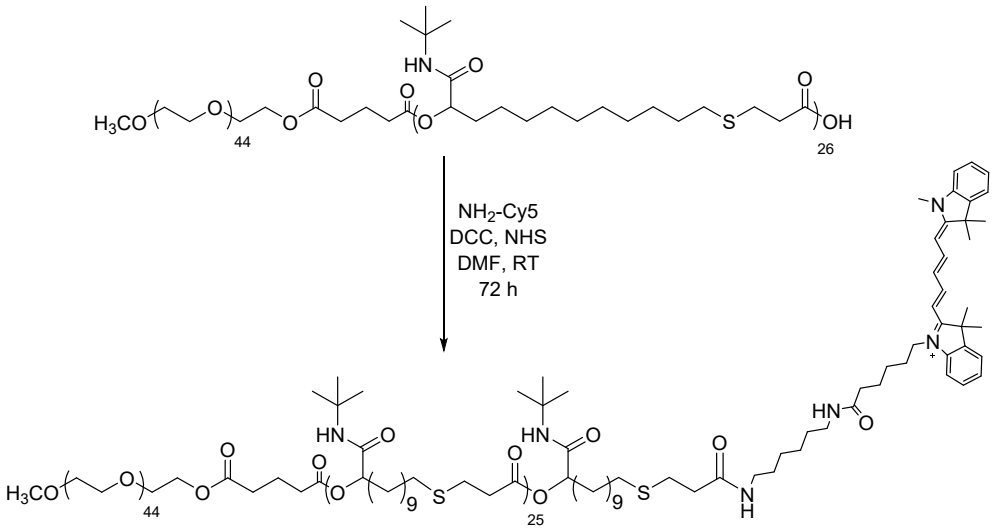
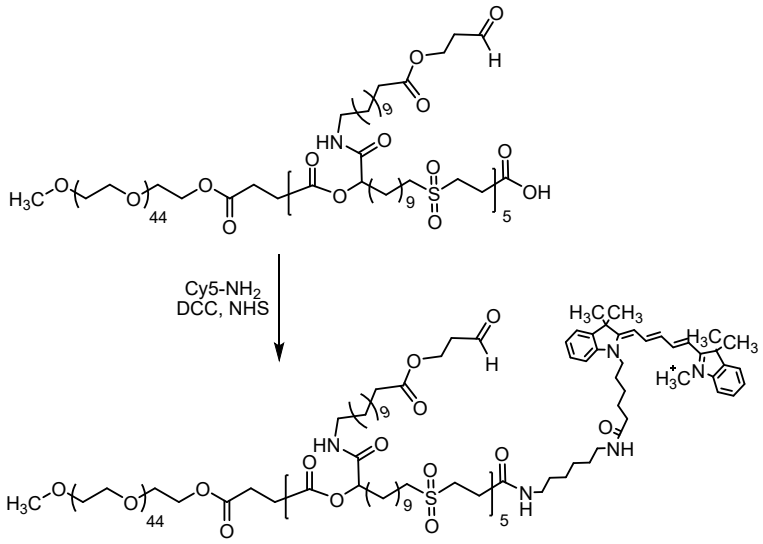


Figure S 11: (A) FT-IR characterization spectrum of Passerini P2 diblock copolymer, C-H stretches at 3280 and 2920 cm^{-1}, C=C stretch at 1653 cm^{-1}, C=O stretch at 1734 cm^{-1}. (B) FT-IR characterization spectrum of Passerini **P3** diblock copolymer, aldehyde group stretching at 2790 cm^{-1} and sulfone stretches at 1239, 1278 and 1339 cm^{-1}. (C) FT-IR characterization spectrum of Passerini **P4-Dox** prodrug, C=O stretch at 1734 cm^{-1} and imine stretch at 1654 cm^{-1}

Synthesis of P1-Cy5 and P3-Cy5



Scheme S 6: P1 Passerini diblock copolymer Cy5 conjugation reaction scheme.



Scheme S 7*:* Reaction scheme of P3 Passerini diblock copolymer conjugation reaction with amino Cy5.

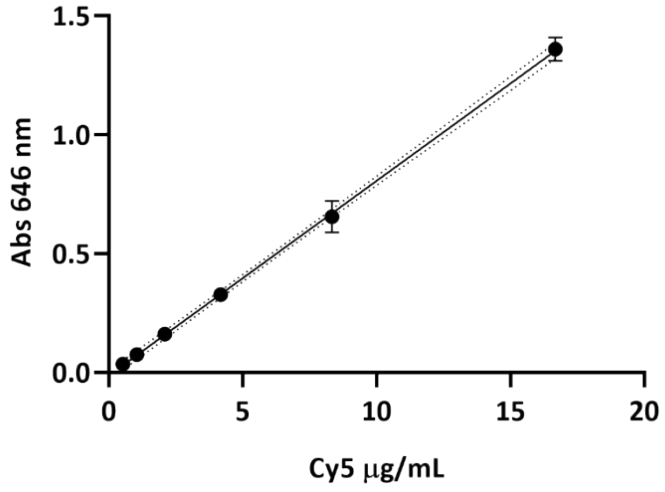


Figure S 12: Cy5-NH_2 calibration curve in water 10% DMF: Y= 0.08188X – 0.01184, R^2= 0.99.

Synthesis of P4-Dox

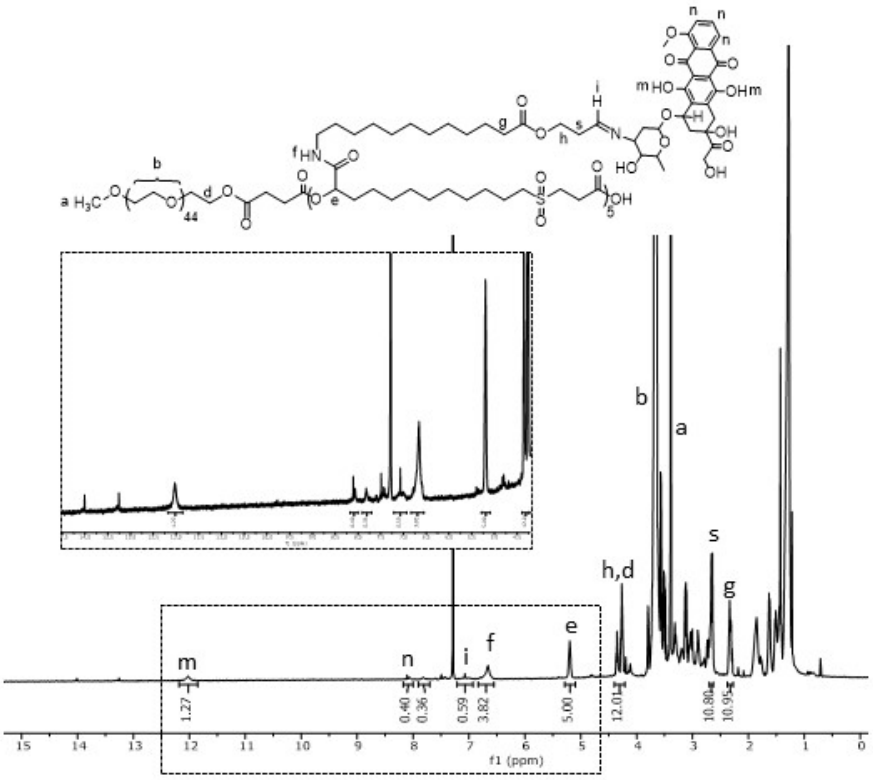


Figure S 13: Integrated ^{1}H NMR spectrum of Passerini diblock copolymer **P4-Dox** in $CDCl_3$.

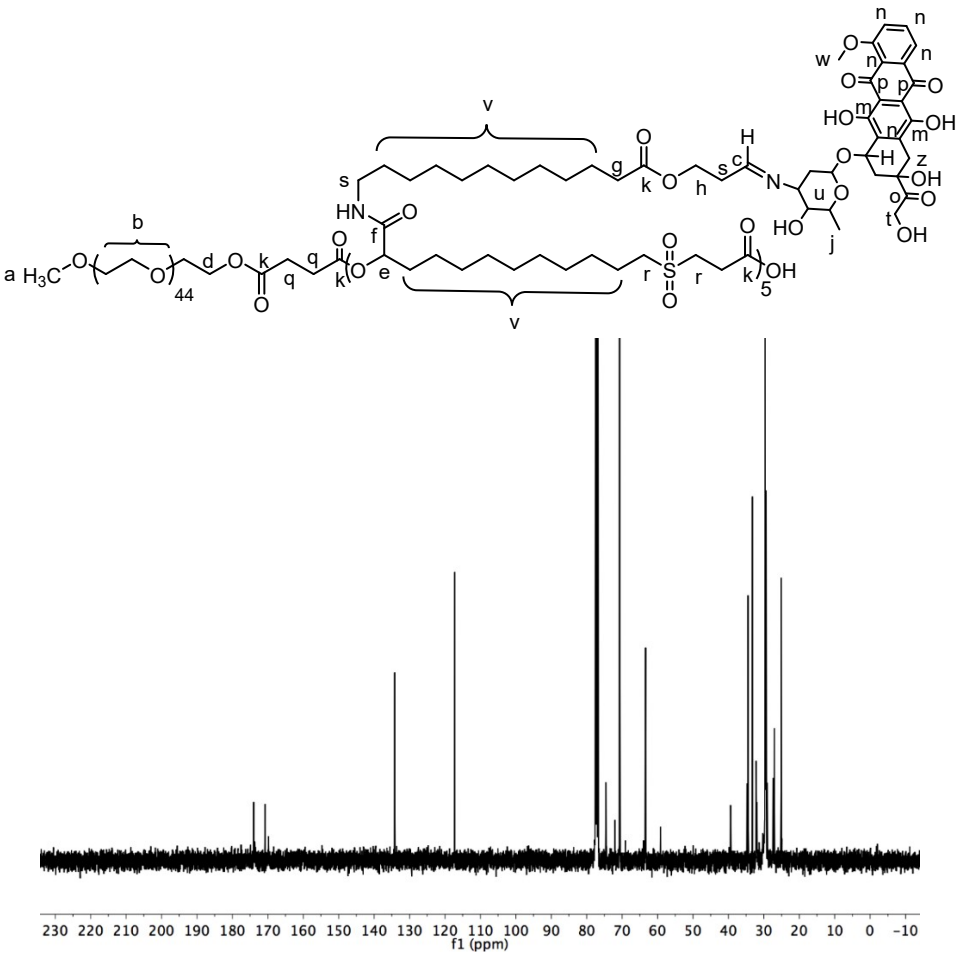


Figure S 14: ^{13} C NMR of **P4-Dox** in $CDCl_3$.

Polymer	Mn (g/mol)	Đ	^{1}H NMR Mn (g/mol)
P1	16000	1.55	11300
P2	3800	1.13	4900
P3	4700	1.11	5115
P4-Dox	7060	1.26	7000

Table S 1: SEC characterization data in THF and the molecular weight calculated by 1H NMR of the P1, P2, P3 and P4-Dox Passerini-3CR copolymers.

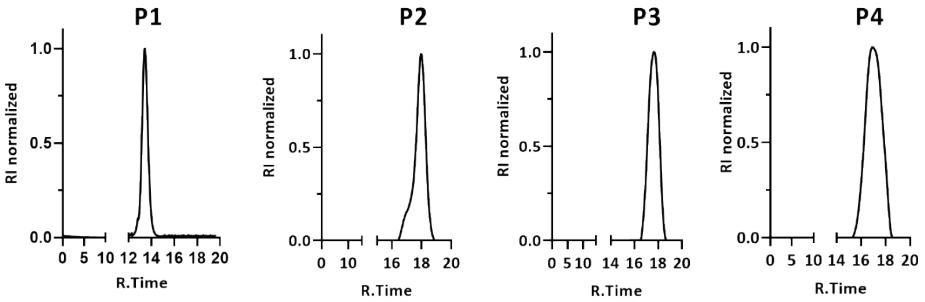


Figure S 15: SEC chromatogram in THF of of the P1, P2, P3 and P4-Dox Passerini-3CR copolymers.

Formulation and characterization

P1-Dox POLYMERSOMES FORMULATION

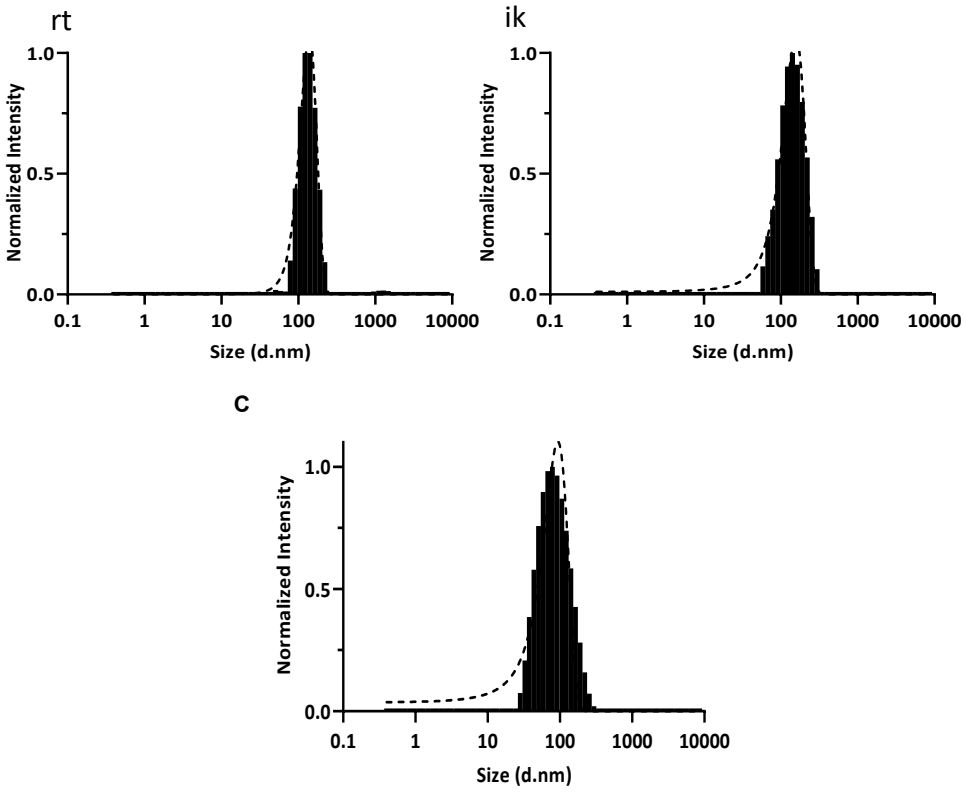


Figure S 16: DLS intensity data of polymersomes diameter (A) P1 135 nm± 33, (B) P1DOX 146 nm± 42 AND (C) P1-Cy5 91 ± 46 nm.

Formulation	diameter nm
P1 polymersome	139 ± 32
P1-Cy5 polymersome	91 ± 46
P1-Dox	146 ± 42

Table S 2: Characterization data of empty P1, P1-Cy5 and doxorubicin loaded polymersomes P1DOX.

P3-Cy5 AND P4-Dox NANOPARTICLES FORMULATION

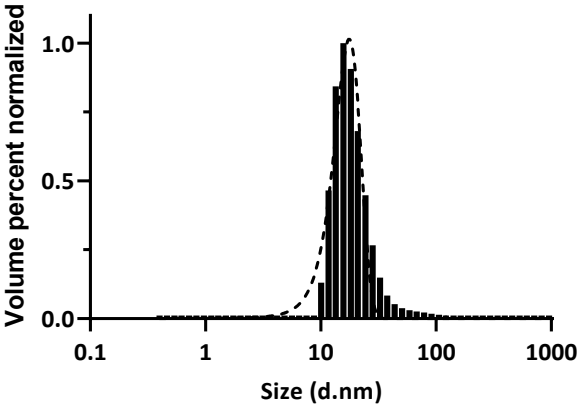


Figure S 17: DLS volume data of P3-Cy5 21 nm± 7.

Formulation	diameter nm
P3-Cy5 nanoparticles	21 ± 7
P4-Dox	16 ± 6

Table S 3: Characterization data of P3-Cy5 and P4-Dox nanoparticles.

P1-Dox AND P4-Dox POLYMERSOMES DRUG LOADING AND ENCAPSULATION EFFICIENCY

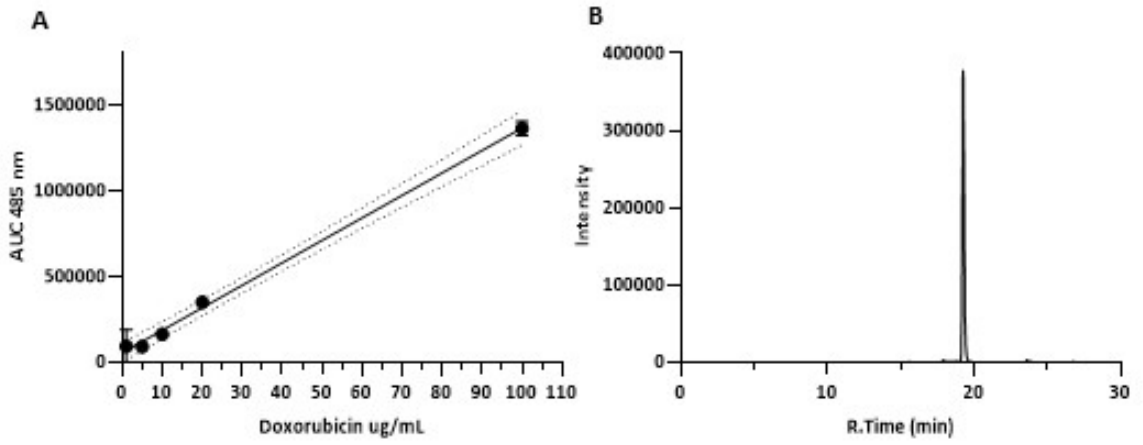


Figure S 18 (A) Doxorubicin hydrochloride calibration curve, UV detector 485 nm: Y= 13100*X, R^2=0.997. (B) Doxorubicin hydrochloride chromatogram UV detector 485 nm, RT=19.4 min

The drug loading and encapsulation efficiency were calculated using the following equations:

$$Drug\ loading\ (\%) = \frac{Weight\ of\ loaded\ drug}{Total\ weight\ of\ polymersomes} X\ 100 \quad \textbf{Eq 1}$$

$$Drug\ encapsulation\ \%\ (w/w) = \frac{Total\ amount\ of\ drug - Unloaded\ drug}{Total\ amount\ of\ drug}\ X\ 100 \quad \textbf{Eq 2}$$

P1-Dox AND P4-Dox IN VITRO DRUG RELEASE

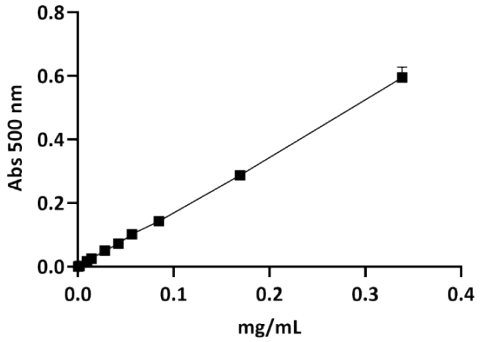


Figure S 19: Uv-Vis doxorubicin calibration curve in PBS pH 7.4: Y=1.7486x – 0.0006; R^2= 0.9996

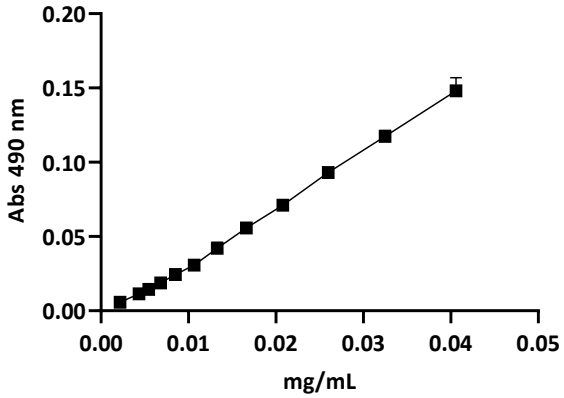


Figure S 20: Uv-Vis doxorubicin calibration curve in acetate buffer pH 5: Y=3.7889x – 0.0066; R^2= 0.9984

Cell culture experiments

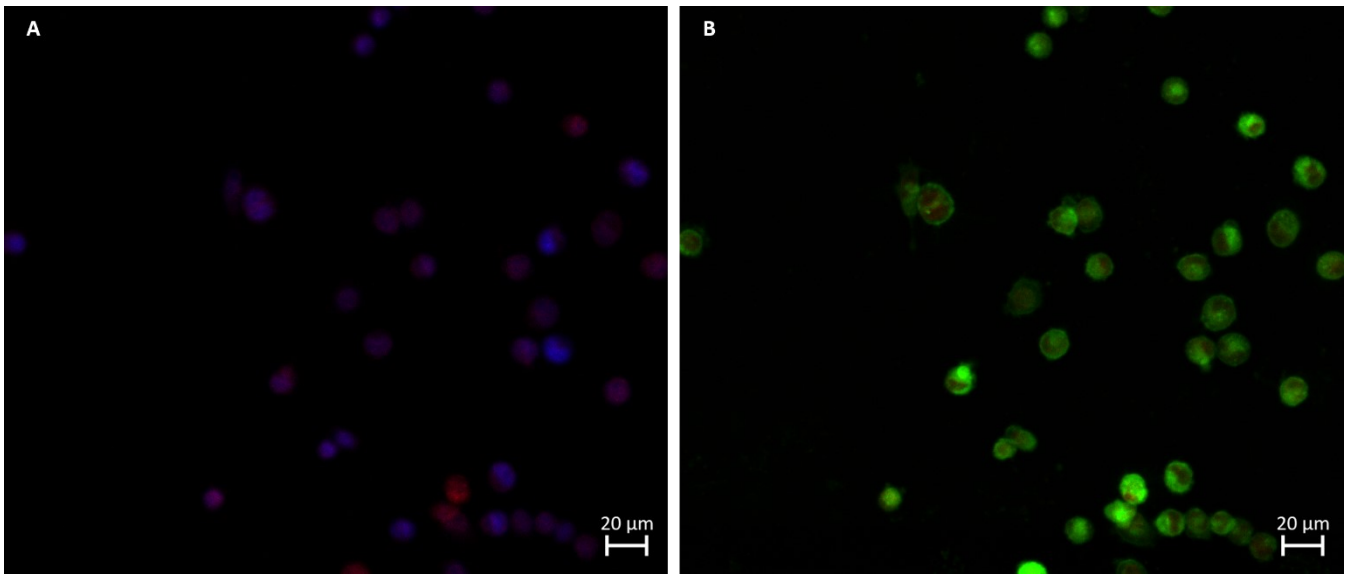


Figure S 21: Cellular uptake assessed by confocal microscopy of doxorubicin in MDA-MB-231 cells after 4 h incubation. (A) Superimposition of doxorubicin (Ex 480 nm/Em 590 nm and nuclei stained with Hoechst 33342 (Ex 350 nm/Em 461 nm), (B) Superimposition of doxorubicin (Ex 480 nm/Em 590 nm) and Cell membrane stained with Cell MaskTM Deep Red plasma membrane stain (Ex 649 nm/Em 666 nm). Scale bar 20 µm.

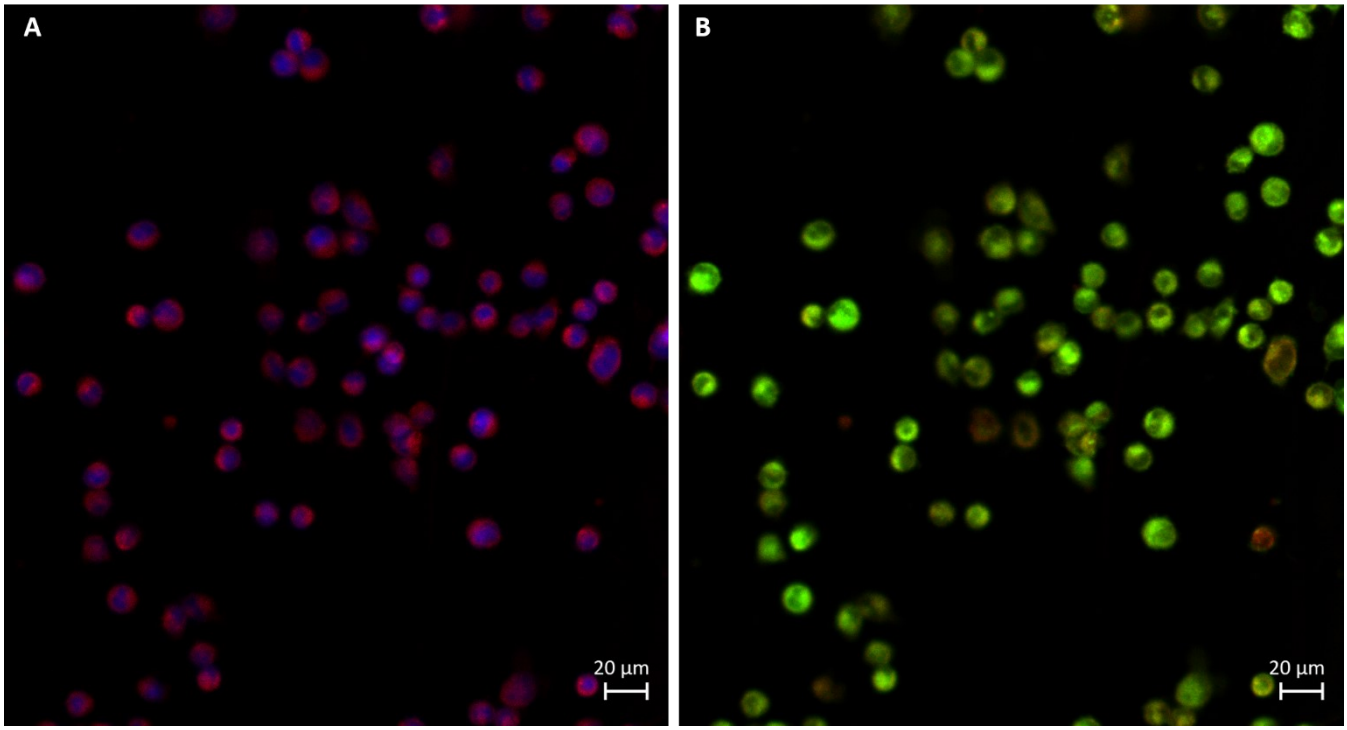


Figure S 22: Cellular uptake assessed by confocal microscopy of P4-Dox in MDA-MB-231 cells after 4 h incubation. (A) Superimposition of P4-Dox (Ex 480 nm/Em 590 nm and nuclei stained with Hoechst 33342 (Ex 350 nm/Em 461 nm), (B) Superimposition of doxorubicin (Ex 480 nm/Em 590 nm) and cell membrane stained with Cell MaskTM Deep Red plasma membrane stain (Ex 649 nm/Em 666 nm). Scale bar 20 µm.

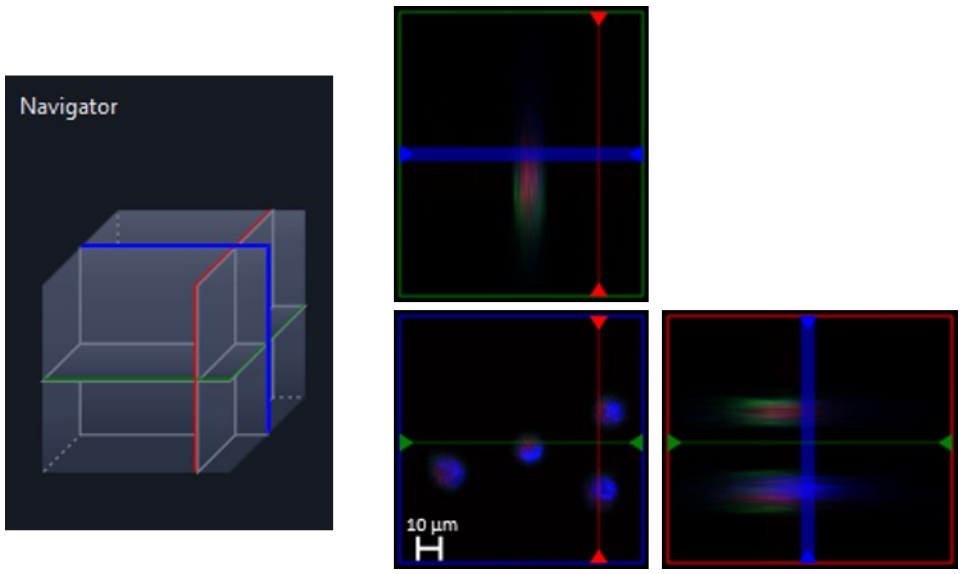


Figure S 23: Cellular uptake assessed by confocal microscopy of P1-Cy5 in MDA-MB-231 cells after 4 h incubation. Zeta-stack picture with x-y-z sections. Nuclei are stained blue (Hoechst 33342), membranes green (CellMask-Green) and polymers red (Cy5). Scale bar 10 µm. From the navigator: blue square X-Y, green square X-Z and red square Y-Z.

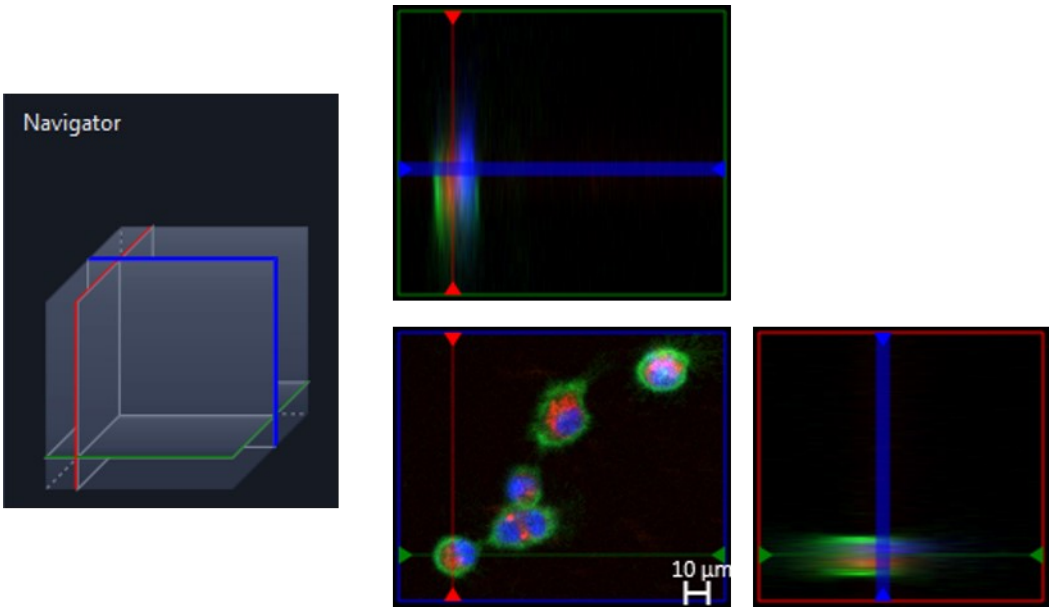


Figure S 24: Cellular uptake assessed by confocal microscopy of P3-Cy5 in MDA-MB-231 cells after 4 h incubation. Zeta-stack picture with x-y-z sections. Nuclei are stained blue (Hoechst 33342), membranes green (CellMask-Green) and polymers red (Cy5). Scale bar 10 µm. From the navigator: blue square X-Y, green square X-Z and red square Y-Z.

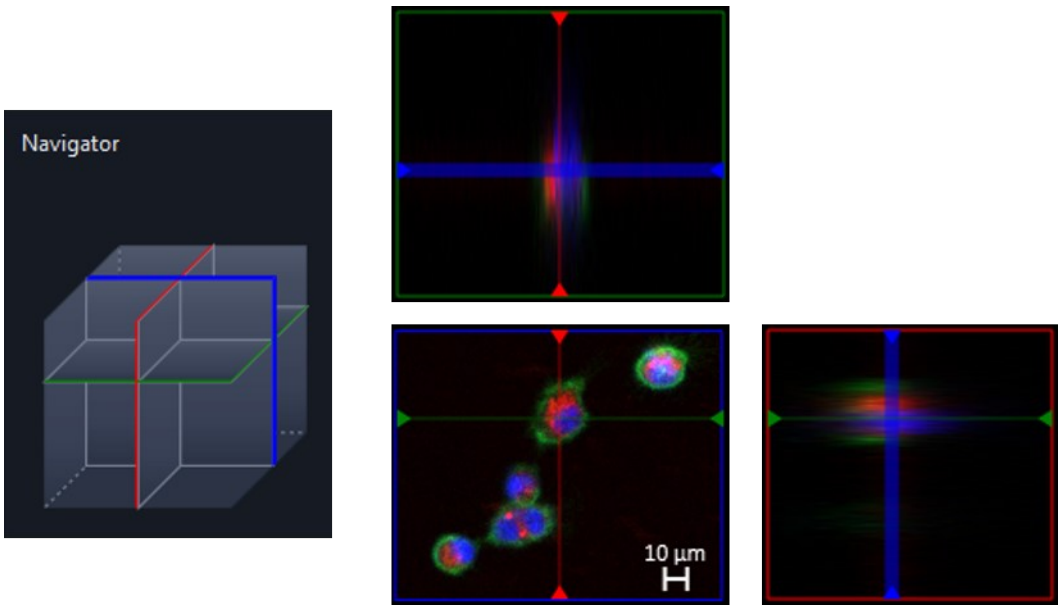


Figure S 25: Cellular uptake assessed by confocal microscopy of P3-Cy5 in MDA-MB-231 cells after 4 h incubation. Zeta-stack picture with x-y-z sections. Nuclei are stained blue (Hoechst 33342), membranes green (CellMask-Green) and polymers red (Cy5). Scale bar 10 µm. From the navigator: blue square X-Y, green square X-Z and red square Y-Z.

CELLULAR UPTAKE STUDIES WITH P1-Cy5 AND P3-CY5 NANOPARTICLES

Flow cytometry

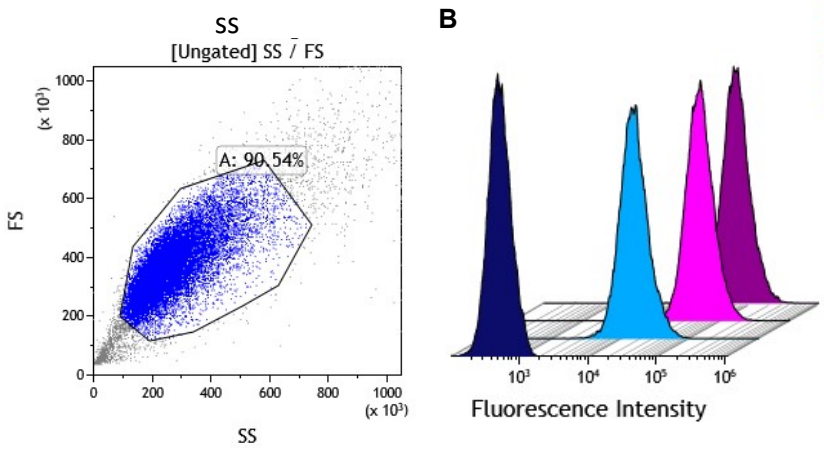


Figure S 26: Cellular uptake of Cy5-labelled **P1-Cy5** polymersomes by flow cytometry in MDA-MB-231 triple negative breast cancer cells after 4 h of incubation. (A) Gate showing negative control MDA-MB-231 cells - an example of the cell population taken for the uptake experiments (B) FACS uptake histograms for **P1-Cy5** polymersomes: in blue the negative control, in light blue P1-Cy5 2.29 µM, in pink P1-Cy5 22.93 µM and in purple P1-Cy5 47.85 µM.

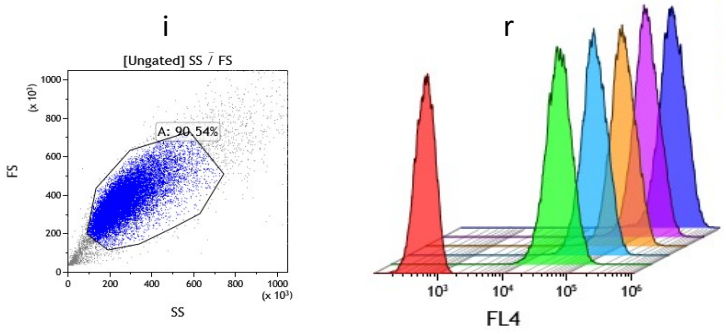


Figure S 27: Cellular uptake of **P2-Cy5**-labelled nanoparticles by MDA-MB-231 triple negative breast cancer cells after 4 h of incubation. (A) Gate showing negative control MDA-MB-231 cells - an example of the cell population taken for the uptake experiments. (B) FACS uptake histograms for **P3-Cy5**-labelled nanoparticles: 5µg/mL (green), 10µg/mL (light blue), 15µg/mL (orange), 20µg/mL (purple), 25µg/mL (blue); untreated cells (red). Data are representative of three experiments (n=3) (*p<0.05, t-test).

3D tumor TNBC spheroids

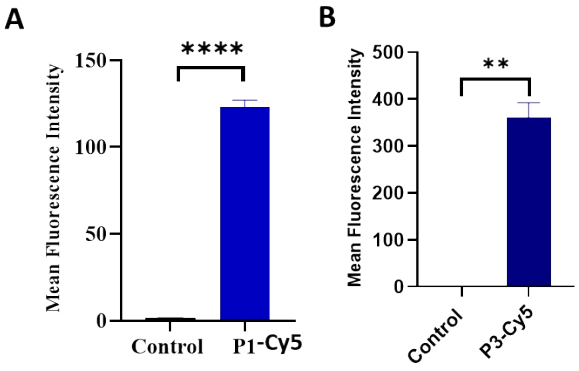


Figure S 28: Quantification of Mean Fluorescence Intensity of the untreated cells (control) and cells treated with P1-Cy5 (A) and P3_Cy5 (B) labelled nanoparticles in TNBC spheroids. Data are representative of three experiments (*p<0.05, t-test).

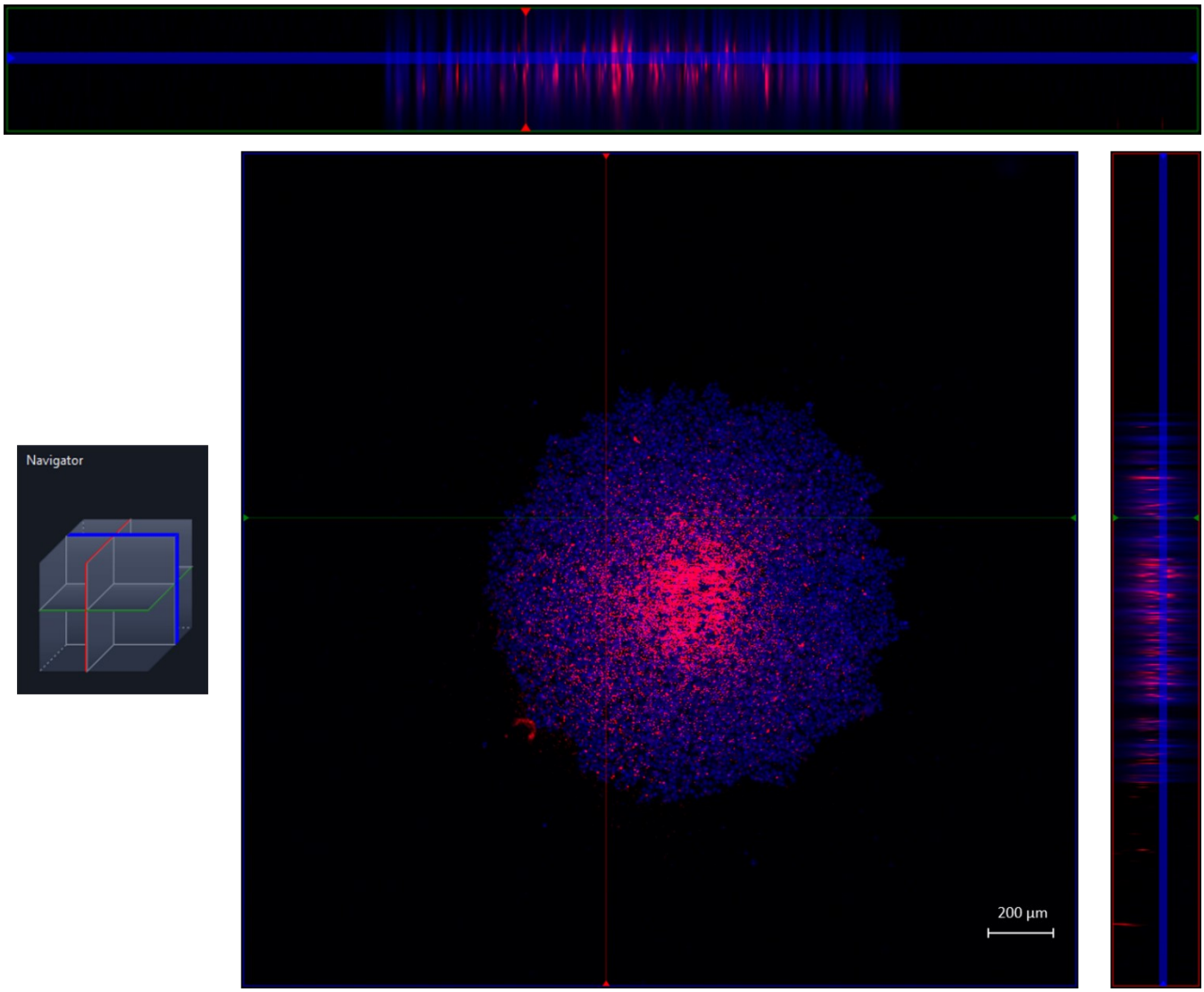


Figure S 29: Cellular uptake assessed by confocal microscopy of P1-Cy5 in MDA-MB-231 3D spheroids. Zeta-stack picture with x-y-z sections. Nuclei are stained blue (Hoechst 33342) and polymers red (Cy5). Scale bar 200 m. From the navigator: blue square X-Y, green square X-Z and red square Y-Z.

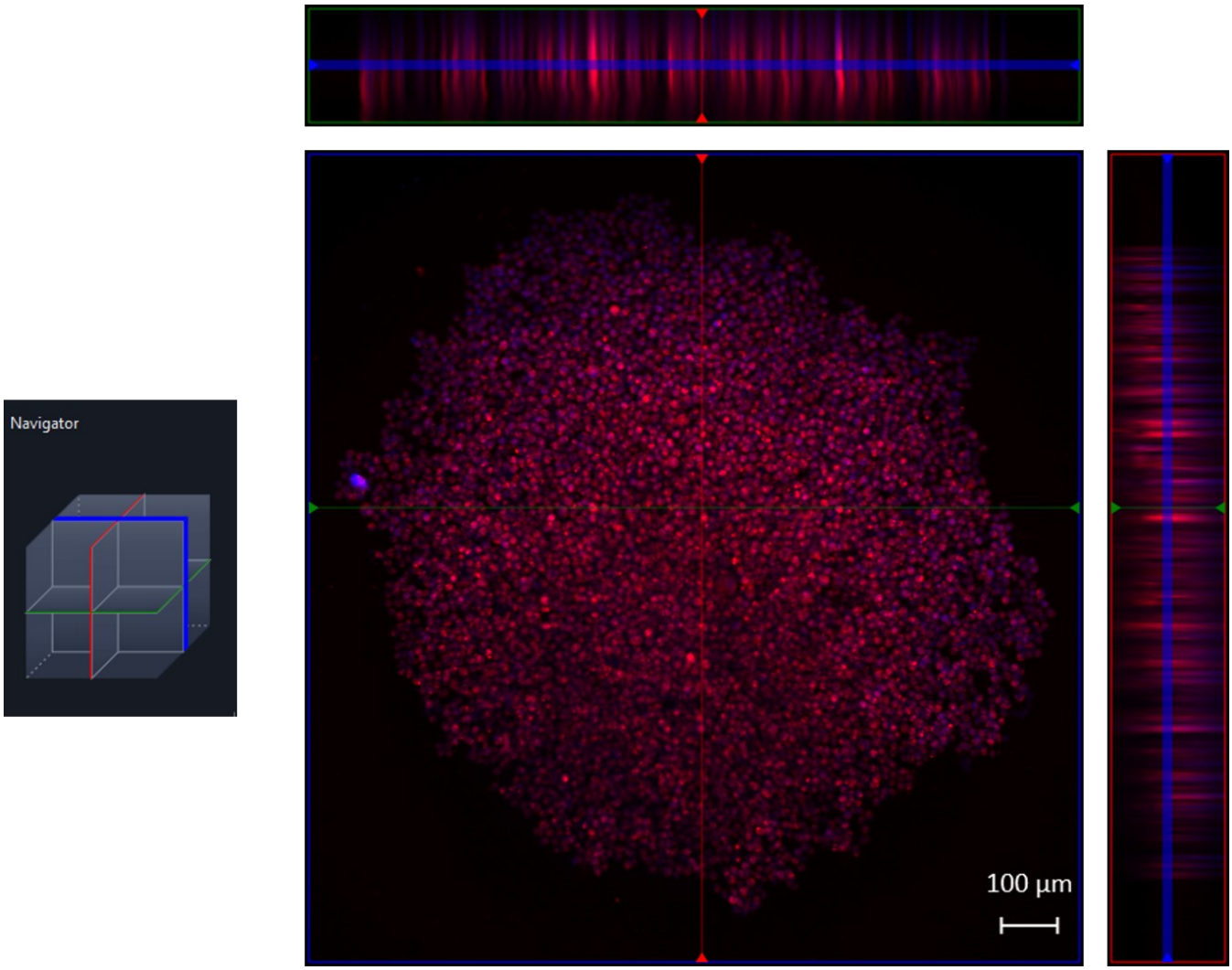


Figure S 30: Cellular uptake assessed by confocal microscopy of P3-Cy5 in MDA-MB-231 3D spheroids. Zeta-stack picture with x-y-z sections. Nuclei are stained blue (Hoechst 33342) and polymers red (Cy5). Scale bar 200 µm. From the navigator: blue square X-Y, green square X-Z and red square Y-Z.

Cell metabolic activity assays

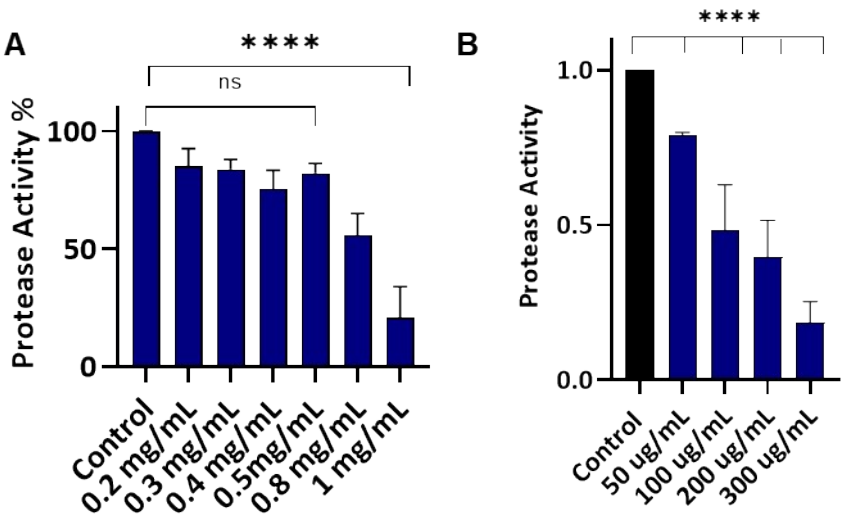


Figure S 23: Protease activity of MDA-MB-231 cells treated with P1- (A) and P3 (B) polymers. Data are representative of three experiments (*p<0.05, t-test).

Statistical analysis

Unless otherwise stated, all data are shown as mean ± standard deviation (SD). Two way analysis of variance (ANOVA) was applied for comparison of three or more group means (Tukey's multiple comparisons test). A P value of < 0.05 was considered statistically significant. ****, ***, **, and * display $p < 0.0001$, $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. GraphPad Prism 8.1 software was used for data analysis.

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Author Contributions

Alessandra Travanut: data curation, formal analysis, investigation, project administration, validation, writing of original draft and lead.
Patrícia F. Monteiro: data acquisition, analysis and method development support,
Sean Smith: ozonolysis reaction
Steven M. Howdle: project administration support, supervision
Anna M. Grabowska: project administration support, supervision
Barrie Kellam: project administration support
Michael A. R. Meier: project administration support, draft revision and editing
Cameron Alexander: project administration, validation, funding acquisition, draft revision and editing, lead.