Electronic Supplementary Material (ESI) for ChemComm.


# Supporting Information

## Selective Photodimerization of Acenaphthylene in Polymersome Nanoreactors

Sjoerd J. Rijpkema, Sam Vissers and Daniela A. Wilson*

*Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands*

**e-mail:* d.wilson@science.ru.nl

# Table of contents

# 1. Materials, instrumentation and methods

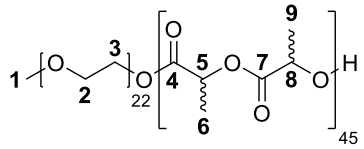
All PEG polymers were obtained from Rapp Polymere or AV Chemistry. All lactide monomers were provided free of charge by Corbion. All other reagents were obtained from commercial sources and were used without purification unless otherwise stated. Solvents were dried by passing over activated alumina columns in a MBraun MB SPS800 under a nitrogen atmosphere and stored under argon. Reactions were carried under a dry atmosphere of argon unless stated otherwise. Standard syringe techniques were applied for the transfer of dry solvents and air- or moisture sensitive reagents. Ultrapure water was obtained from a QPOD MilliQ system. Dialysis membranes of MWCO 12-14000 Dalton Spectra/Por were used to remove organic solvent.

Nuclear Magnetic Resonance (NMR) characterization was carried out on a Bruker AVANCE HD nanobay console with a 9.4 T Ascend magnet (400 MHz) and a Bruker AVANCE III console with a 11.7 T UltraShield Plus magnet (500 MHz) equipped with a Bruker Prodigy cryoprobe, in chloroform ($CDCl_3$). NMR spectra were recorded at 298 K unless otherwise specified. Chemical shifts are given in parts per million (ppm) with respect to tetramethylsilane (TMS, δ 0.00 ppm) as internal standard for $^1H$ NMR. Coupling constants are reported as J-values in Hz. Peak assignment is based on 2D gDQCOSY, $^1H$-$^{13}C$ gHSQCED, and $^1H$-$^{13}C$ gHMBC spectra. Side group and end of chain signals separated from the bulk polymer $^1H$ signal are only reported when observed with clear s/n ratio and no overlap with polymer peaks, and may be (in)visible on other NMR spectrometers or with different concentrations. Gel permeation chromatography (GPC) equipped with PL gel 5 µm mixed D column calibrated for polystyrene (580 to 377,400 g/mol) was carried out on a Shimadzu instrument with THF as eluent using differential refractive index and UV (254 nm) detectors. Transmission electron microscopy (TEM) was carried out on a JEOL TEM 1400 equipped with CCD camera at 60 kV. Carbon coated Cu TEM grids (200 mesh) were glow discharged with a Cressington 208 glow discharge device. Within 30 minutes of glow discharging 5 µL of sample was dispersed on a TEM gird and removed using paper tissue after 1 minute. The samples were stained by adding 5 µL of uranyl acetate which was removed after 1 minute using paper towel. The samples were then left to dry for 3 more hours. Malvern Zetasizer nano S was used for dynamic light scattering (DLS) measurements equipped with He-Ne laser of wavelength 633 nm. All images analysis was carried out using ImageJ, available in a public domain http://fiji.sc/ [1]. 300 W xenon light source was purchased from Asahi Spectra, Japan (MAX-303) with a wavelength range of 250-1050 nm. Filters were used to narrow the range of the light source when required.

# 2. Experimental Procedures

### 2.1 α-methoxy poly(ethylene glycol)-*b*-polylactide block copolymers using LLA or DLA

Poly(ethylene glycol)-*b*-polylactide (PEG-*b*-PLA) was synthesized by ring opening polymerization (ROP). Methoxy-PEG-OH macroinitiator (194 mg, 0.2 mmol, 1 eq.) was mixed with lactide (1.30g, 9.0 mmol, 45 eq.). First, the reagents were dried by addition of dry toluene and removing the solvent under reduced pressure. Then, dry toluene (15 mL) was added to the dried material under argon. Subsequently, $Sn(oct)_2$ (4.3 µL, 0.013 mmol, 0.065 eq.) was added and the mixture was degassed for 30 minutes with argon. The reaction then refluxed for 16 h at 111 °C. Afterwards, the mixture was concentrated under reduced pressure and subsequently precipitated in ice cold diethyl ether (2x). The polymer was then dissolved in 1,4-dioxane (5 ml) and lyophilized to yield a white powder (1.18 g, 79% yield). **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.19 (q, *J* = 7.11 Hz, 90H, 5-C*H* + 8-C*H*), 3.64 (br s, 88H, 2-C$H_2$ + 3-C$H_2$), 3.38 (s, 3H, 1-C$H_3$), 1.58 (d, *J* = 7.11 Hz, 270H, 6-C$H_3$ + 9-C$H_3$). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.6 (4-*C* + 7-*C*), 71.1 (2-*C*$H_2$ + 3-*C*$H_2$), 68.8 (5-*C*H + 8-*C*H), 59.0 (1-*C*$H_3$), 16.8 (6-*C*$H_3$ + 9-*C*$H_3$). **$M_w/M_n$** 1.15




### 2.2 α-methoxy poly(ethylene glycol)-*b*-polylactide block copolymers using DLLA

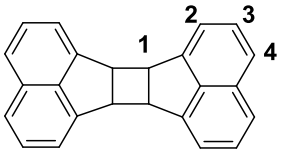
Poly(ethylene glycol)-*b*-polylactide (PEG-*b*-PLA) was synthesized by ring opening polymerization (ROP). Methoxy-PEG-OH macroinitiator (194 mg, 0.2 mmol, 1 eq.) was mixed with lactide (1.30 g, 9.0 mmol, 45 eq.). First, the reagents were dried by addition of dry toluene and removing the solvent under reduced pressure. Then, dry DCM (15 mL) was added to the dried material under argon. Subsequently, DBU (15 µL, 0.1 mmol, 0.5 eq.) was added to the mixture. The reaction then stirred for 2 h at 30 °C. Afterwards, the mixture was washed with 1M $KHSO_4$ (3x), concentrated under reduced pressure and subsequently precipitated in ice cold diethyl ether (2x). The polymer was then dissolved in 1,4-dioxane (5 ml) and lyophilized to yield a white powder (0.84 g, 56% yield). **$^1$H NMR** (400 MHz, $CDCl_3$) δ 5.30−5.13 (m, 90H, 5-C*H* + 8-C*H*), 3.64 (br s, 88H, 2-C$H_2$ + 3-C$H_2$), 3.38 (s, 3H, 1-C$H_3$), 1.66−1.50 (m, 270H, 6-C$H_3$ + 9-C$H_3$). **$^{13}$C NMR** (101 MHz, $CDCl_3$) δ 169.6 (4-*C* + 7-*C*), 71.1 (2-*C*$H_2$ + 3-*C*$H_2$), 68.6 (5-*C*H + 8-*C*H), 59.0 (1-*C*$H_3$), 16.3 (6-*C*$H_3$ + 9-*C*$H_3$). **$M_w/M_n$** 1.09

### 2.3 General preparation of polymersomes

In total, 10 mg PEG-*b*-PLA polymer was dissolved in a mixture of THF and 1,4-dioxane (1 mL, 4:1 v/v) in a glass vial with stirring bar. After dissolving the polymer for 0.5 h at 21 °C, a syringe pump equipped with a syringe and a needle was used to deliver ultrapure water with a rate of 1 mL/h for 1 h via a rubber septum, while vigorously stirring the mixture (900 rpm). Upon finishing the water addition, 9.0 mL of ultrapure water was then added to quench the polymersomes. The polymersomes were spun down using a centrifuge (10 min, 13.000 rpm) and washed with ultrapure water a total of three times to finally be resuspended in 1 mL ultrapure water.

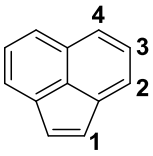
### 2.4 6b,6c,12b,12c-tetrahydrocyclobuta[1,2-a:3,4-a']diacenaphthylene (acenaphthylene dimer)

In a glass vial (~5 mL) acenaphthylene (0.5 mg, 3.28 µmol) was added to an mPEG-*b*-PLA polymersome solution (10 mg) in a mixture of water and methanol (1 mL). The mixture was stirred for 30 min and bubbled through with Ar for 5 min after which the vial was capped. The mixture was irradiated (300 W, 250-385 nm, ~10 mm from light source) for 30 minutes. The solvent was removed by lyophilisation, and the solid was re-dissolved in $CDCl_3$ for NMR analysis. **Syn-product: $^1$H NMR** (400 MHz, $CDCl_3$) δ 7.12 (dd, *J* = 8.2, 1.0 Hz, 4H, 4-C*H*), 7.07 (dd, *J* = 8.2, 6.7 Hz, 4H, 3-C*H*), 6.95 (dd, *J* = 6.6, 1.0 Hz, 4H, 2-C*H*), 4.77 (s, 4H, 1-C*H*). **Anti-product: $^1$H NMR** (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.1 Hz, 4H, 4-C*H*), 7.54 (dd, *J* = 8.1, 6.8 Hz, 4H, 3-C*H*), 7.47 (d, *J* = 6.8 Hz, 4H, 2-C*H*), 4.02 (s, 4H, 1-C*H*).




### 2.5 Acenaphthylene

**$^1$H NMR** (500 MHz, $CD_3OD$) δ 7.86 (d, *J* = 8.0 Hz, 2H, 4-C*H*), 7.73 (d, *J* = 6.4 Hz, 2H, 2-C*H*), 7.60 (dd, *J* = 8.0, 6.4 Hz, 2H, 3-C*H*), 7.12 (s, 2H, 1-C*H*). **$^1$H NMR** (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.1 Hz, 2H, 4-C*H*), 7.68 (d, *J* = 6.8 Hz, 2H, 2-C*H*), 7.54 (dd, *J* = 8.1, 6.8 Hz, 2H, 3-C*H*), 7.08 (s, 2H, 1-C*H*).



# 3. Supplementary Tables and Figures

**Table S1.** Summary of control reactions for the photodimerization of ACE. *ACE did not fully dissolve.

| Solvent | Concentration (μmol / mL) | Time (min) | Conversion (%) | Syn:anti |
|---|---|---|---|---|
| **Benzene** | 150.1 | 180 | 61.7 | 65:35 |
| **Benzene** | 65.7 | 180 | 3.0 | 64:36 |
| **$H_2O$*** | 32.9 | 180 | 0 | - |
| **MeOH** | 13.2 | 30 | 15.5 | 76:24 |
| **90:10 $H_2O$:MeOH** | 13.2 | 30 | 26.1 | 77:22 |

**Table S2.** Summary of photodimerization results for various solvent mixtures in the presence of $mPEG_{22}$-*b*-$PDLLA_{45}$ polymersomes, irradiated for 15 minutes. *ACE did not fully dissolve.

| $H_2O$:MeOH | Conversion % | Anti-product % | Syn-product% |
|---|---|---|---|
| **100:0*** | 0 | - | - |
| **90:10** | 43.2 | 47.7 | 52.3 |
| **75:25** | 55.3 | 56.4 | 43.6 |
| **50:50** | 56.7 | 65.5 | 34.5 |
| **25:75** | 55.7 | 95.7 | 4.3 |
| **0:100** | 15.5 | 23.5 | 76.5 |

**Table S3.** Summary of photodimerization results for various solvent mixtures without self-assembled structures, irradiated for 15 minutes. *ACE did not fully dissolve.

| $H_2O$:MeOH | Conversion % | Anti-product % | Syn-product% |
|---|---|---|---|
| **100:0*** | 0 | - | - |
| **90:10** | 26.1 | 22.1 | 77.9 |
| **75:25** | 17.9 | 20.3 | 79.7 |
| **50:50** | 32.2 | 27.6 | 72.4 |
| **25:75** | 30.8 | 26.9 | 73.1 |
| **0:100** | 15.5 | 24.0 | 76.0 |

**Table S4.** Summary of photodimerization results over time in the presence of $mPEG_{22}$-$PDLLA_{45}$ polymersomes in 25:75 $H_2O$:MeOH .

| Time (min) | Conversion % | Anti-product % | Syn-product% |
|---|---|---|---|
| **5** | 51.3 | 98.3 | 1.7 |
| **15** | 65.2 | 95.8 | 4.2 |
| **20** | 70.8 | 98.1 | 1.9 |
| **30** | 82.0 | 85.1 | 14.9 |
| **40** | 82.2 | 94.1 | 5.9 |

**Table S5.** Summary of photodimerization results for different morphologies in 25:75 $H_2O$:MeOH, irradiated for 30 minutes.

| Polymer/morphology | Conversion % | Anti-product % | Syn-product% |
|---|---|---|---|
| **mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes** | 82.2 | 94.1 | 5.9 |
| **mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets** | 78.0 | 98.2 | 1.8 |
| **mPEG$_{22}$-*b*-PDLA$_{45}$ crystalline sheets** | 82.0 | 94.1 | 5.9 |
| **mPEG$_{22}$-*b*-PDLLA$_{45}$ stomatocytes** | 73.2 | 91.3 | 8.7 |
| **mPEG$_{22}$-*b*-PDLLA$_{45}$ nanorods** | 77.6 | 92.6 | 7.4 |
| **PEG$_{44}$-*b*-PS$_{176}$ polymersomes** | 31.2 | 20.6 | 79.4 |

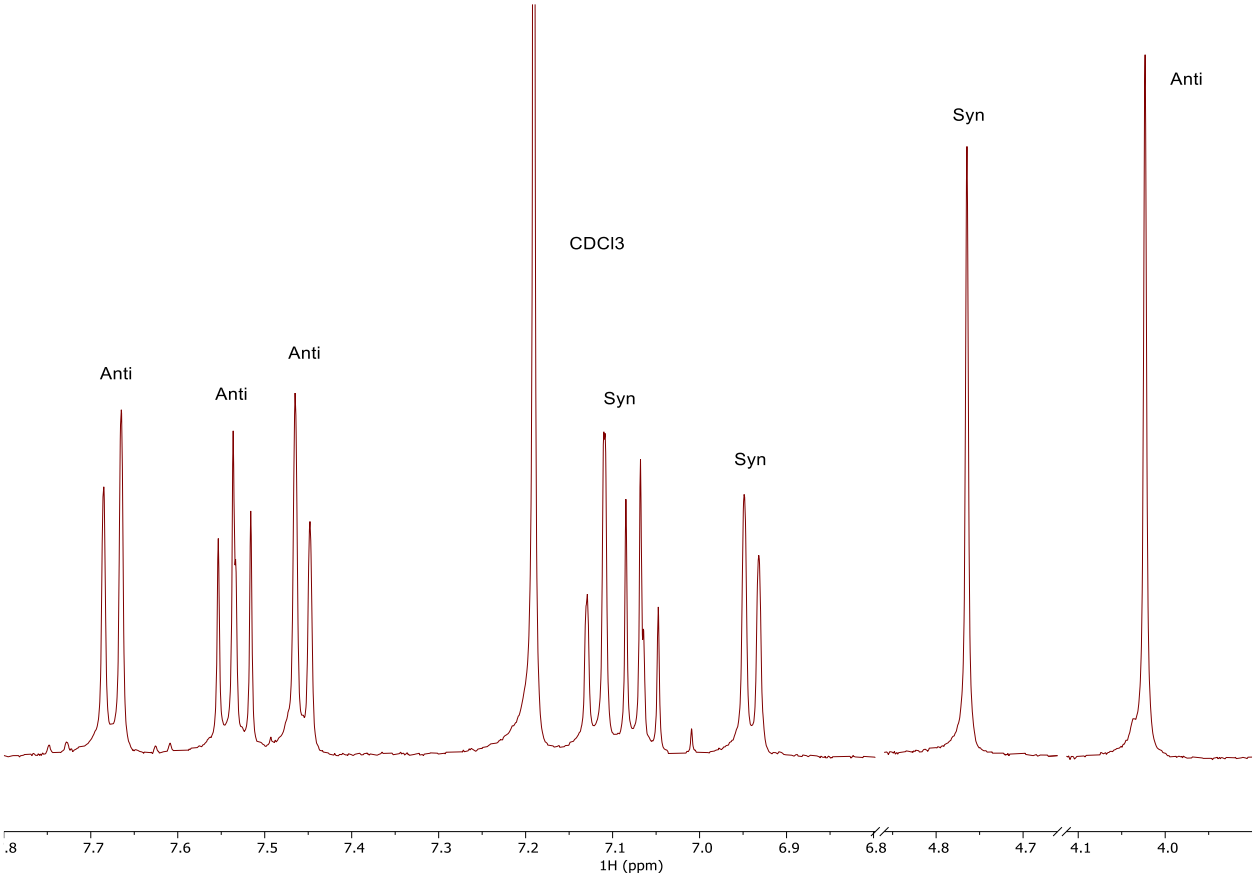



**Figure S1.** Typical $^{1}$H NMR spectrum in $CDCl_3$ of the *syn* and *anti* dimers of the [2+2] photodimerization reaction of acenaphthylene. Ratio of *syn*/*anti* was determined via relative integration of the singlets. Yield was determined by quantitative comparison to the internal standard 1,3,5-trimethoxy benzene.

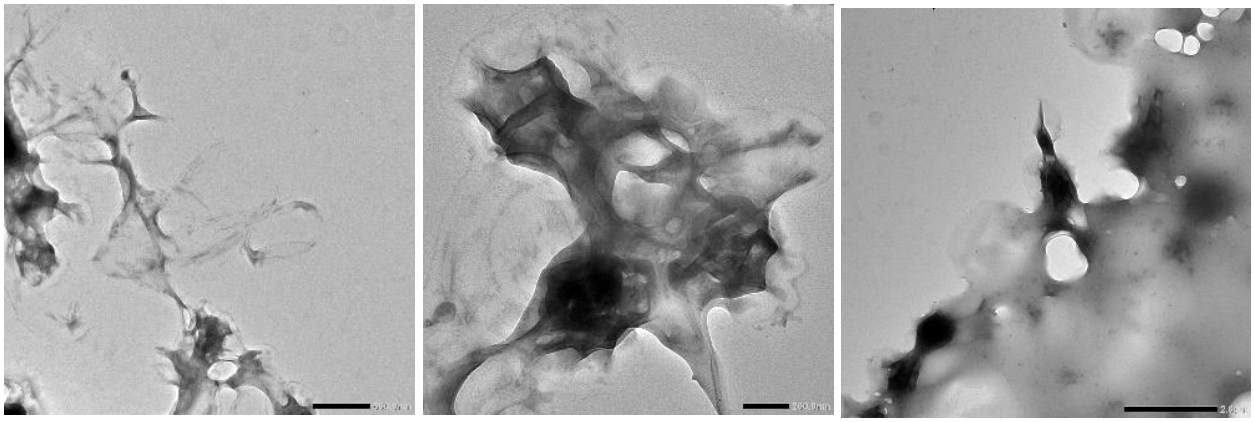
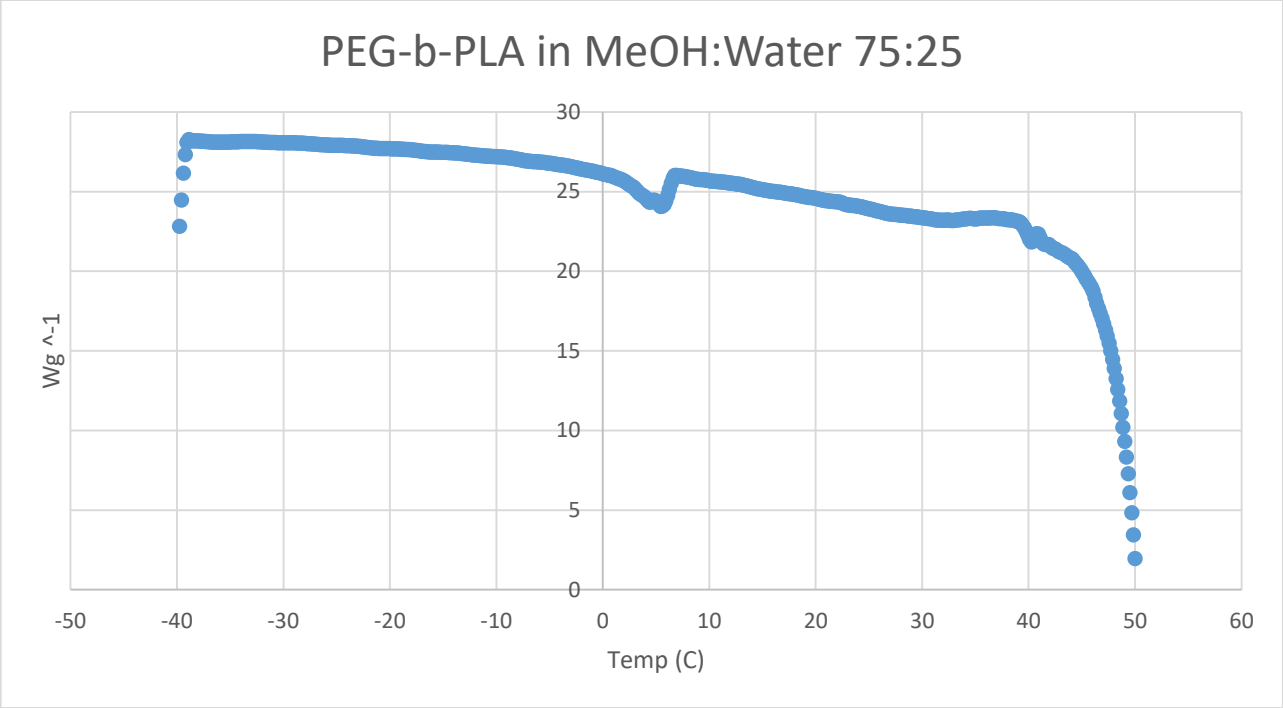


**Figure S2.** TEM images of $mPEG_{22}$-*b*-$PDLLA_{45}$ polymersomes from water/THF 50/50 (left), water/dioxane 50/50 (middle) and water/acetone 50/50 (right) after 16 h. A clear change in morphology was observed, as the structures are slowly breaking down. Scale bar: 500 nm (left), 200 (middle), 2000 nm (right).



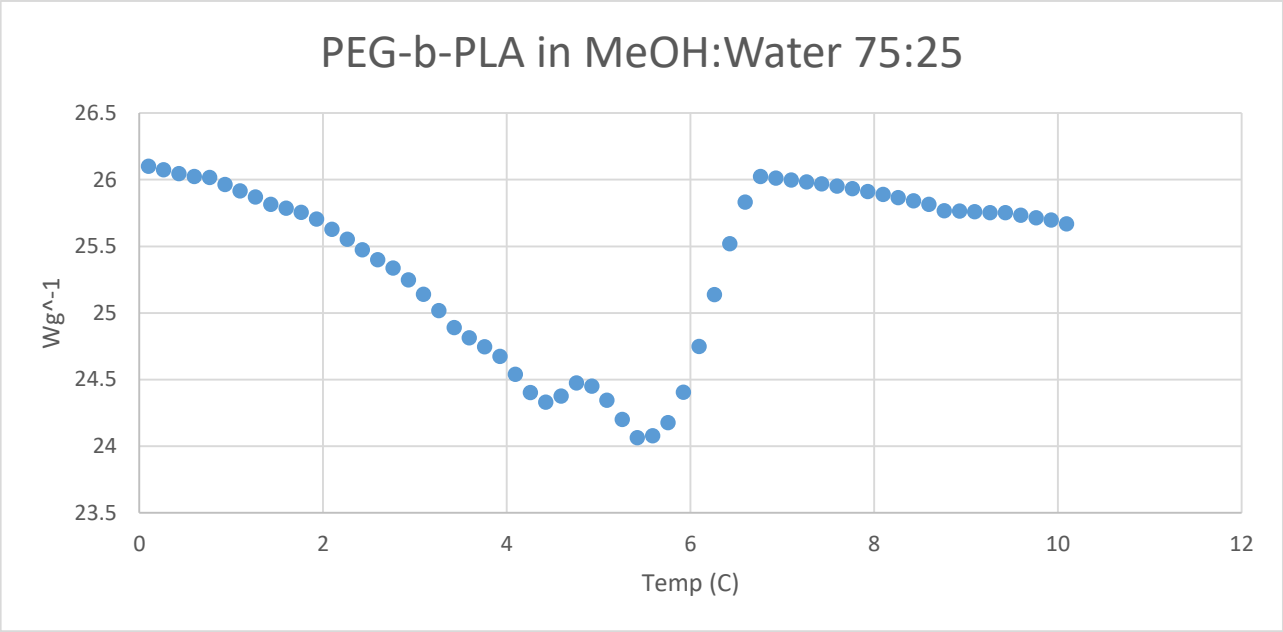




**Figure S3.** DSC measurement of mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes in water/MeOH 25/75 from -40 to 50 °C. The glass transition that is around 24 °C in water cannot be observed, instead a $T_g$ of around 5 °C is observed. Peak onset at 0.8 °C, peak offset at 6.8 °C, two peaks at 4.4 °C and 5.4 °C. Full measurement (top) and zoom-in (bottom). Cycle 2 (cooling) is shown of 3 total cycles performed. Suspension of 57.8 mg polymersome in 200 µL 1:3 v/v water/MeOH.

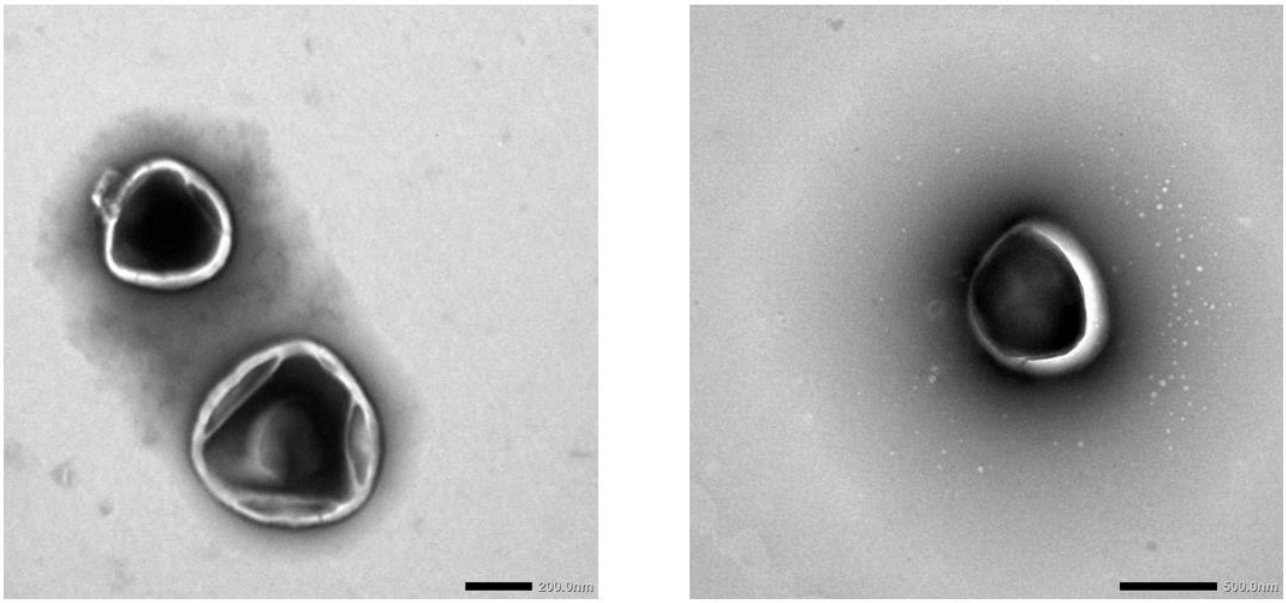



**Figure S4.** TEM images of mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes from MeOH before (left) and after (right) irradiation. No change in morphology was observed. Scale bar: 200 nm (left), 500 nm (right).

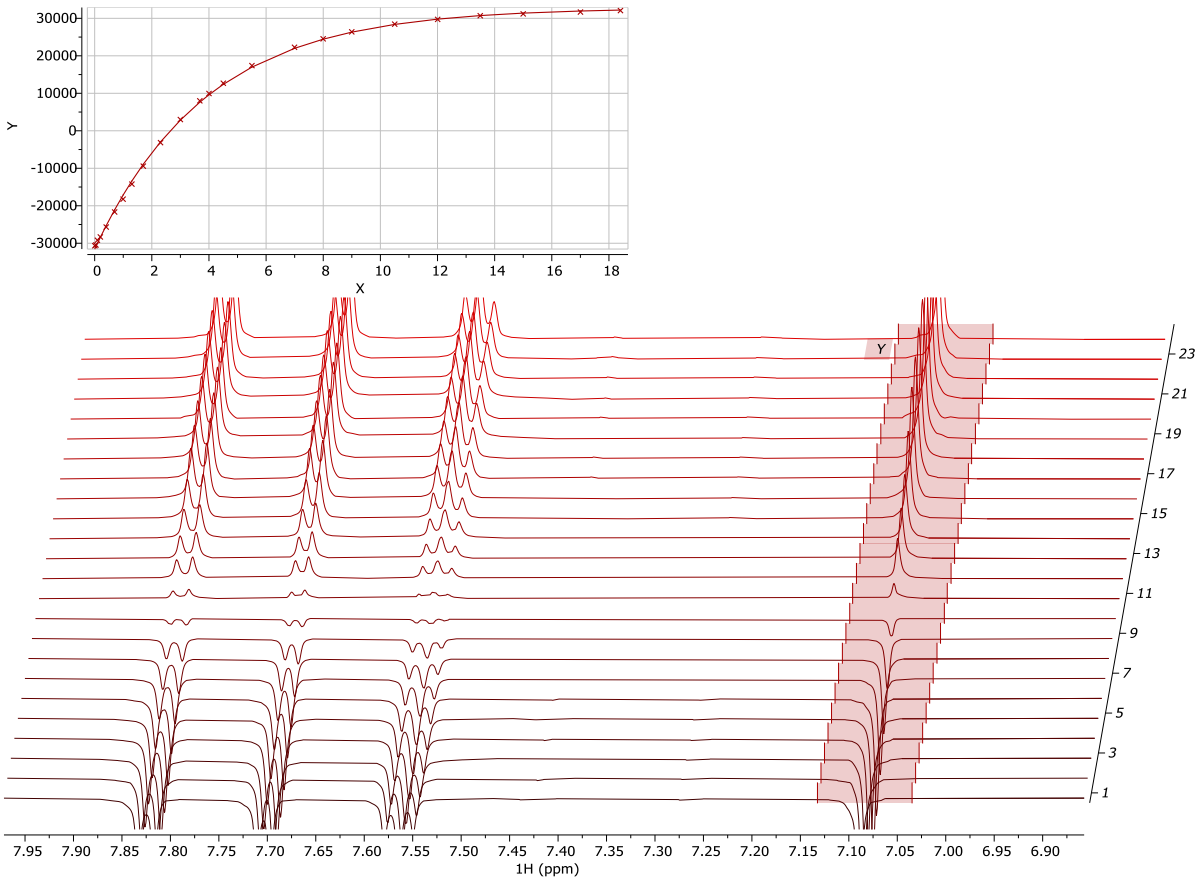



**Figure S5.** Typical $T_1$ determination $^1$H NMR spectrum in $D_2O$ at 40 °C of acenaphthylene. The singlet was used to determine $T_1$.

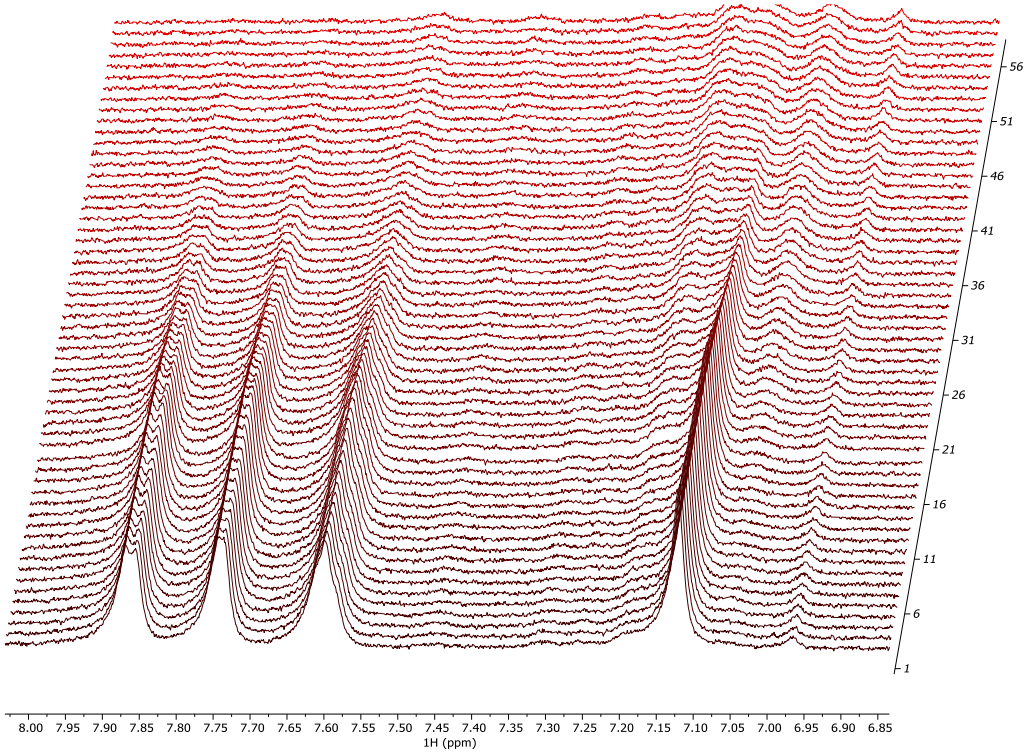



**Figure S6.** $^{1}$H NMR timecourse (60 min) in MeOD at 40 °C of the conversion of acenaphthylene in the presence of PEG-*b*-PLA polymersomes. A clear decrease of ACE is observed. Note: The conversion of ACE is slower compared to our other experiments, as a lower intensity lightsource was used which could be inserted into the NMR.

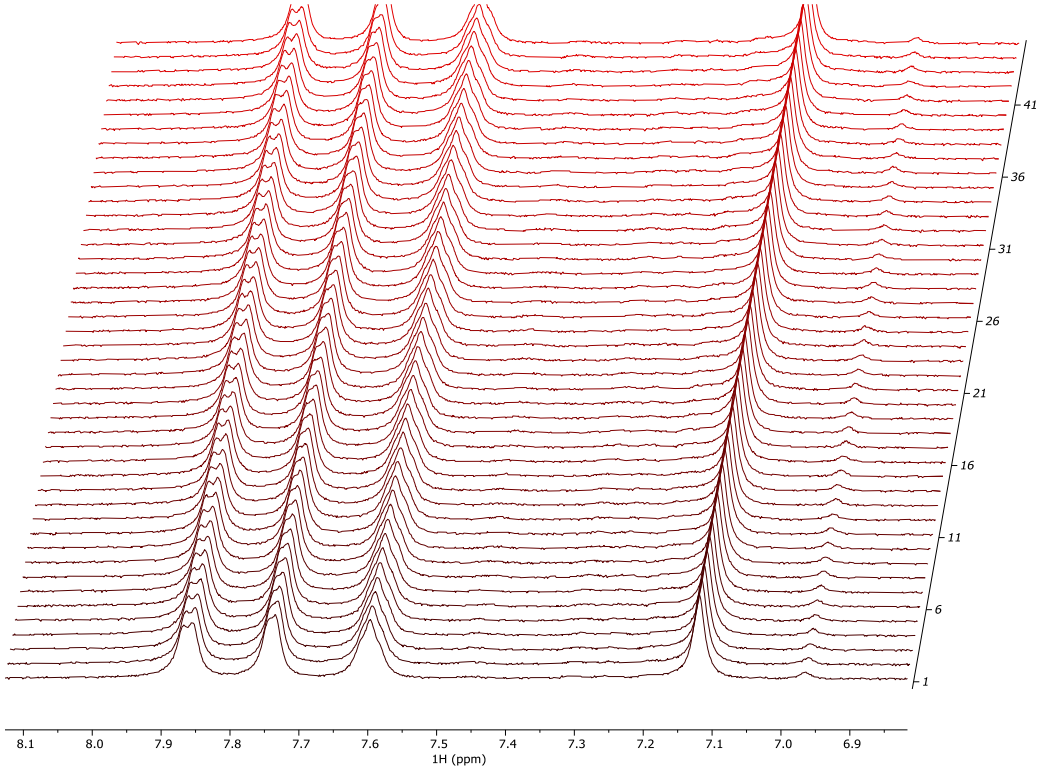
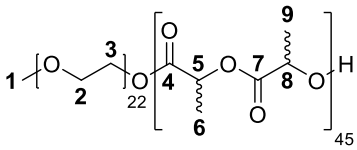


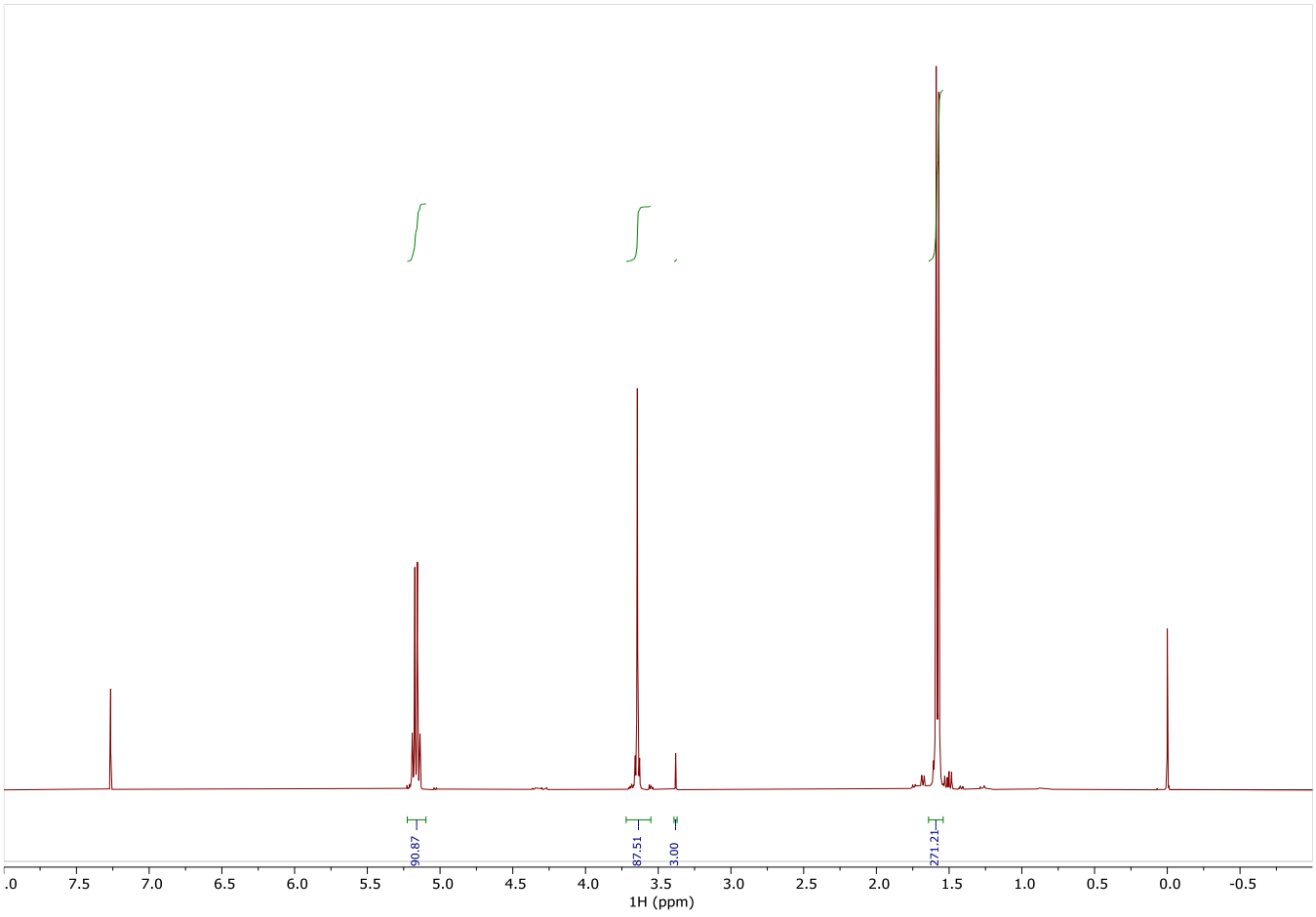

**Figure S7.** $^{1}$H NMR timecourse (45 min) in MeOD at 40 °C of the conversion of acenaphthylene in the presence of PEG-*b*-PS polymersomes. No decrease of ACE is observed during this time.

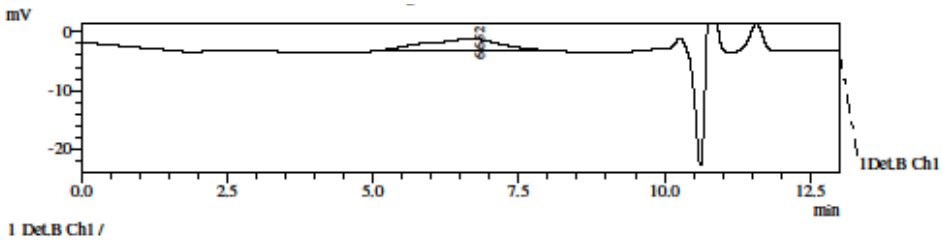
# 4. NMR and GPC spectra of polymers

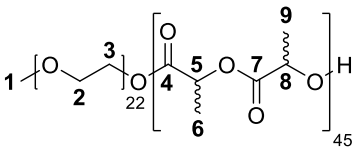




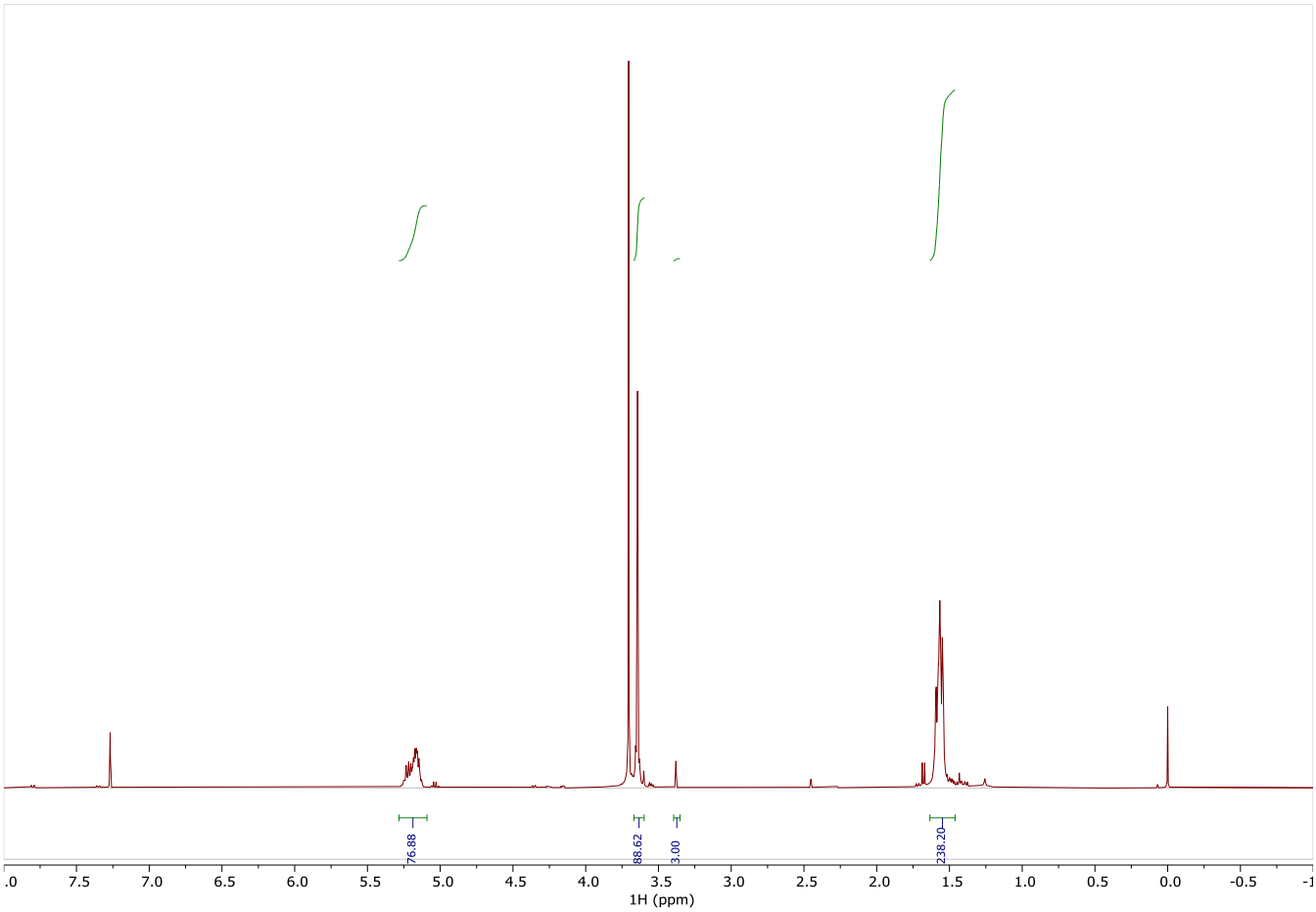

mPEG$_{22}$-*b*-PLLA$_{45}$

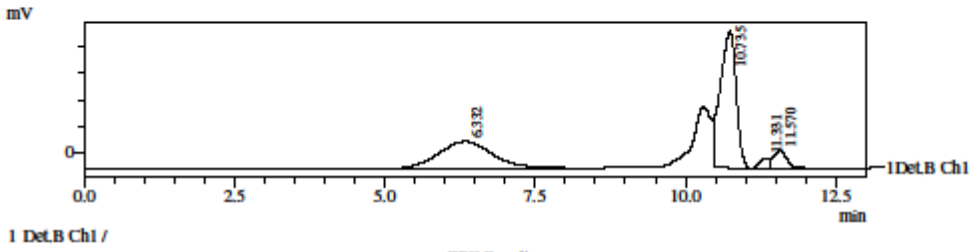


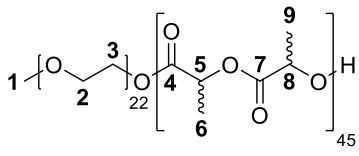





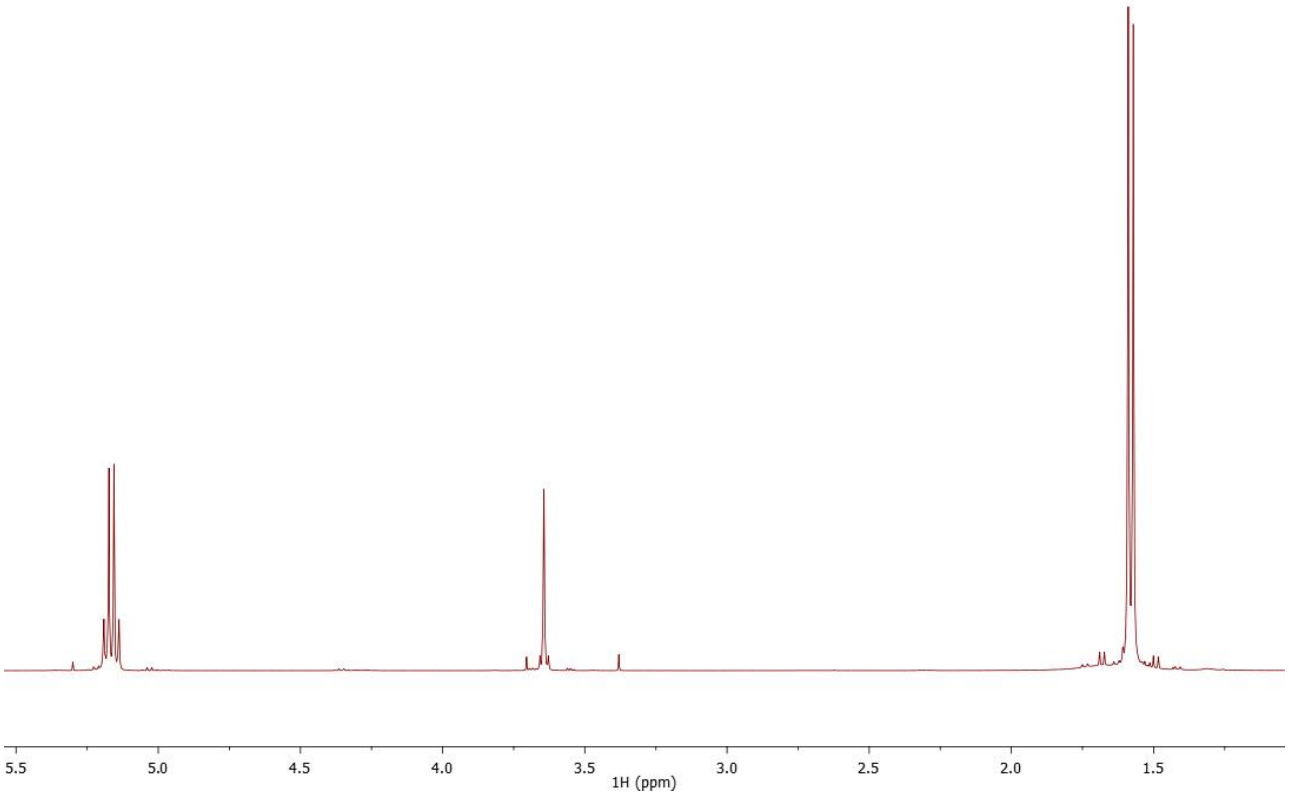

mPEG$_{22}$-*b*-PDLLA$_{45}$

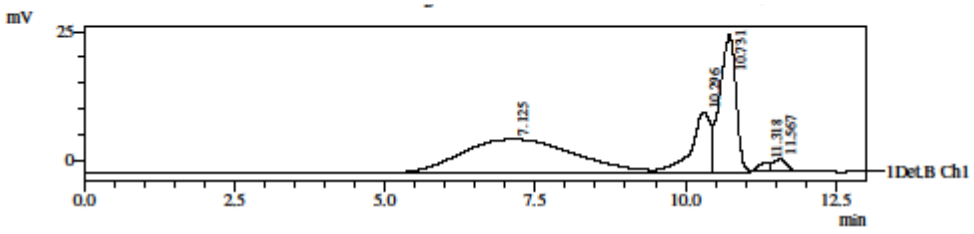




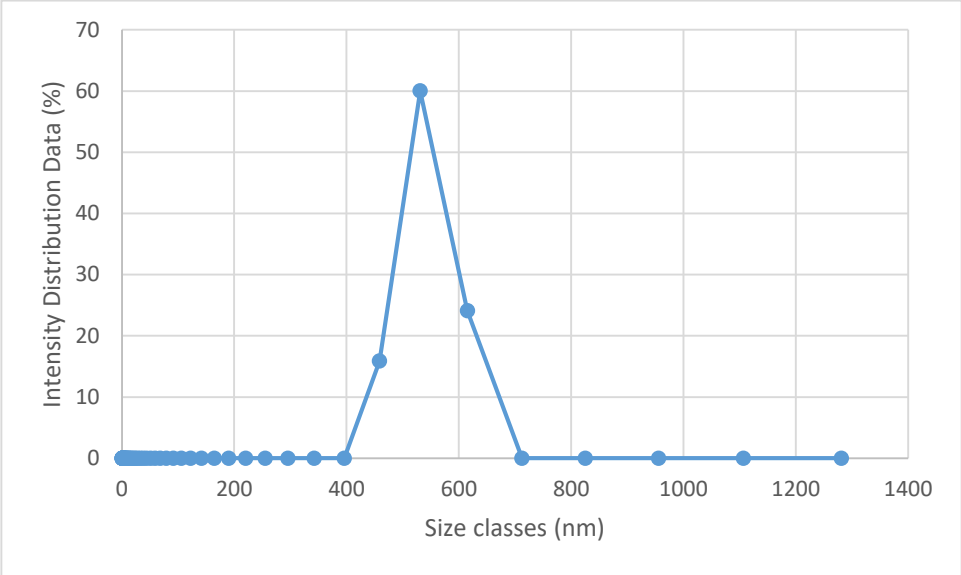

mPEG$_{22}$-*b*-PLLA$_{45}$



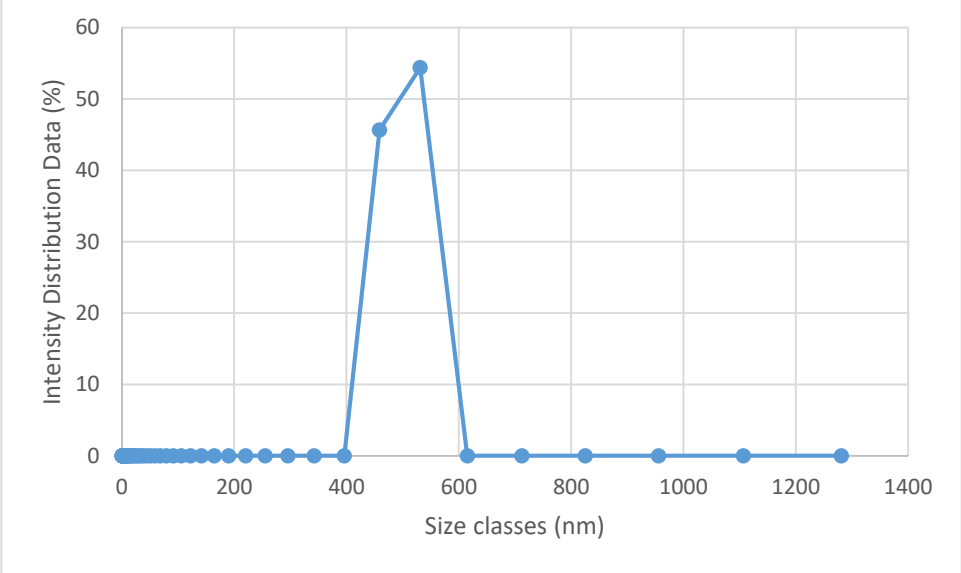



# 5. DLS Data of Nanostructures

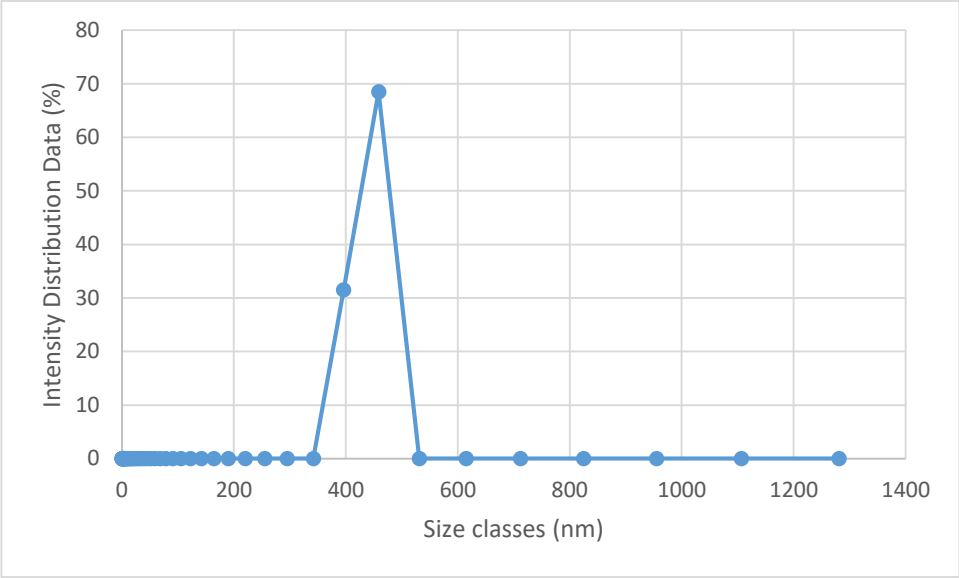
mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes in $H_2O$



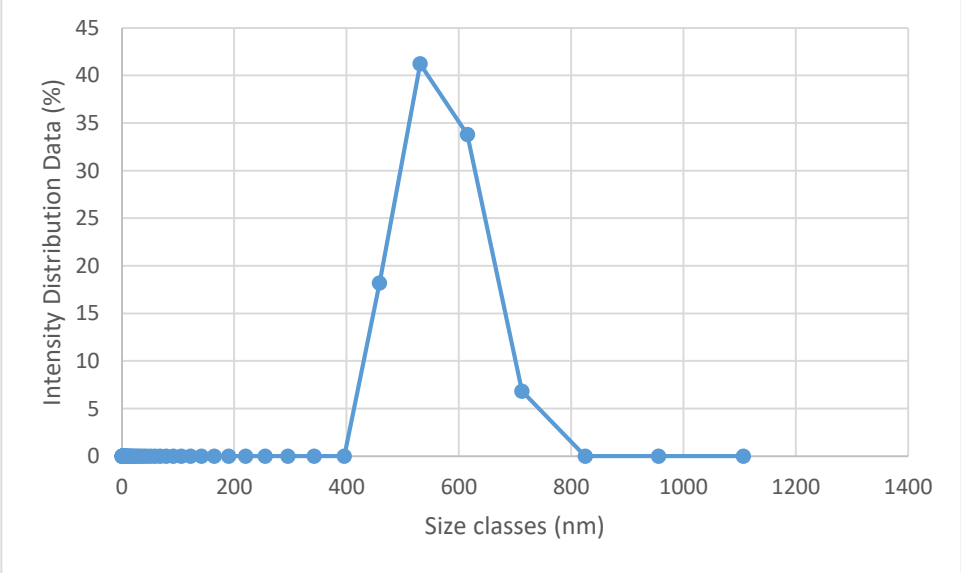

mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes in 50:50 MeOH:$H_2O$



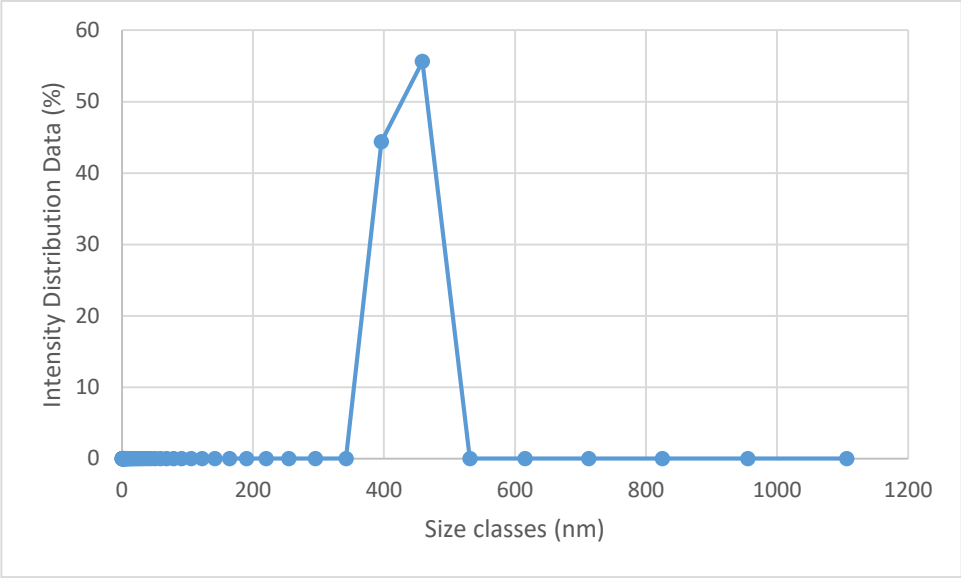
mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes in MeOH




mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets

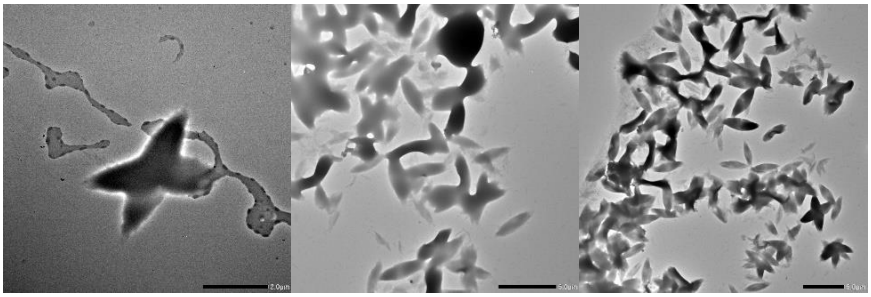


mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets + ACE (1 mg/ 10 polymersomes)

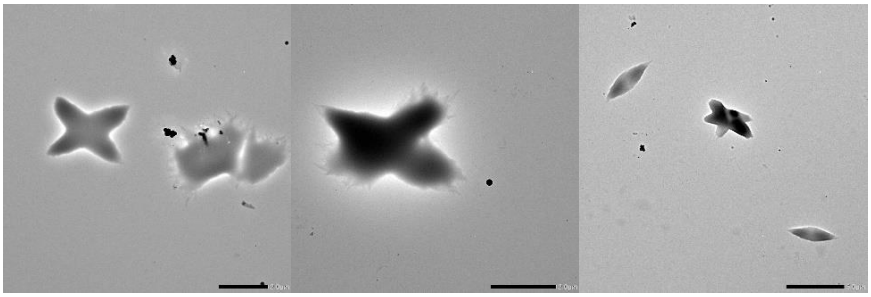



## 6. Additional TEM Images

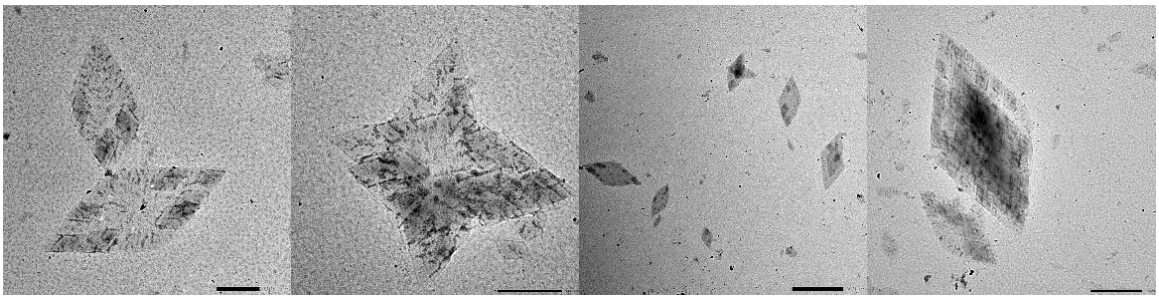
mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets in 50:50 MeOH:$H_2O$



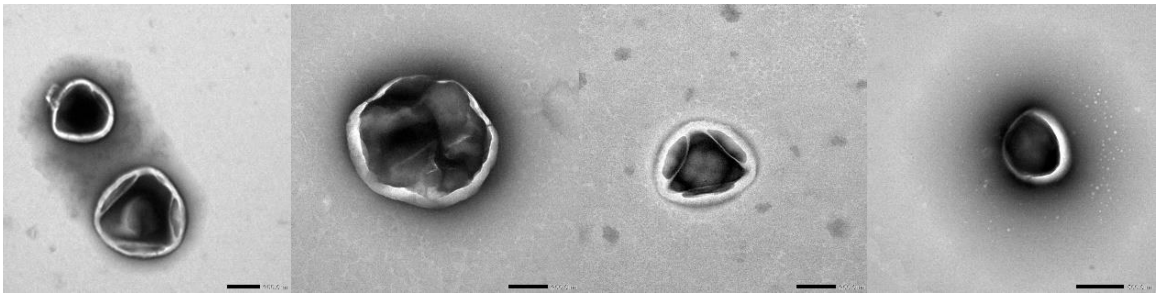
mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets in methanol



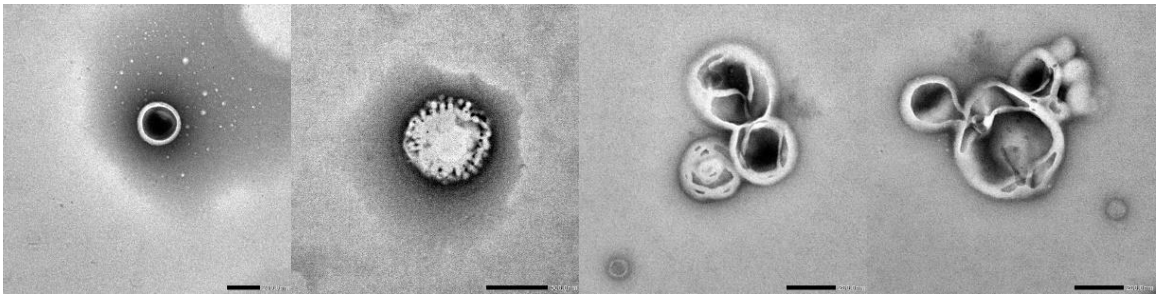
mPEG$_{22}$-*b*-PLLA$_{45}$ crystalline sheets before irradiation



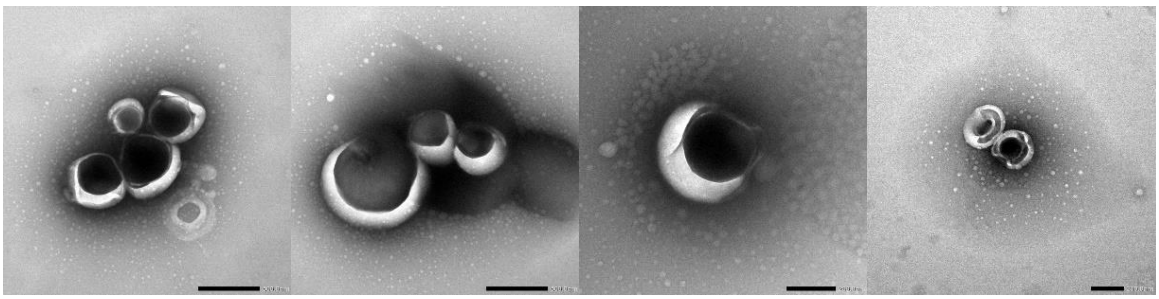
mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes before irradiation



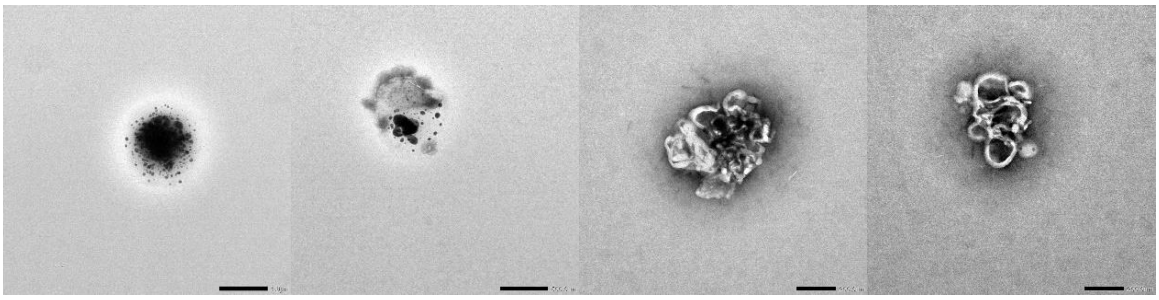
mPEG$_{22}$-*b*-PDLLA$_{45}$ polymersomes after irradiation



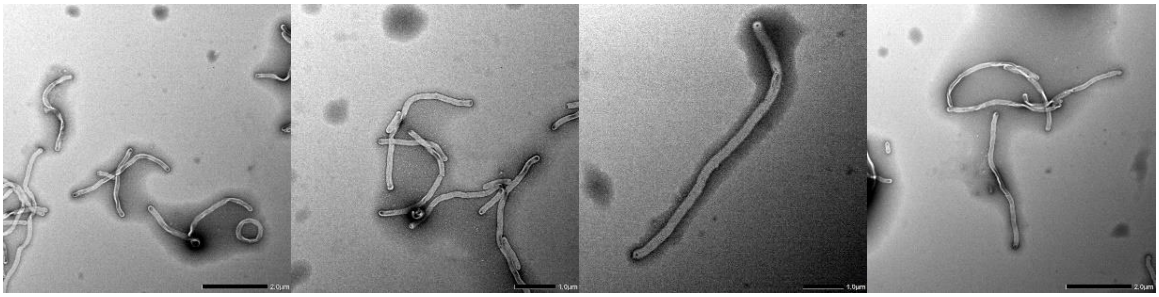
mPEG$_{22}$-*b*-PDLLA$_{45}$ stomatocytes before irradiation



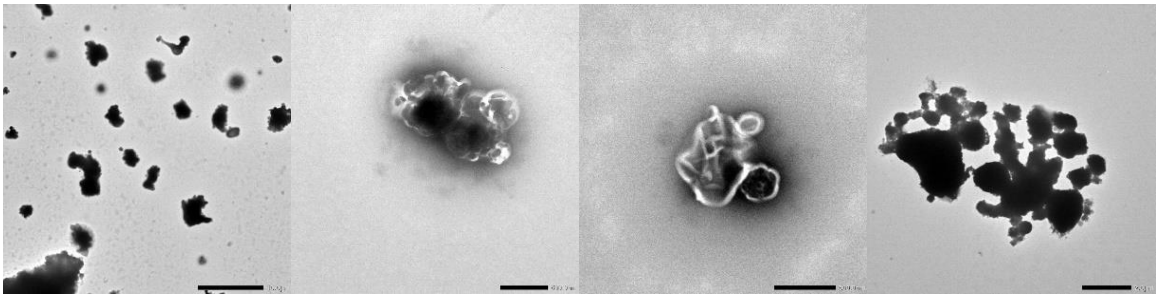
mPEG$_{22}$-*b*-PDLLA$_{45}$ stomatocytes after irradiation



mPEG$_{22}$-*b*-PDLLA$_{45}$ rods before irradiation



mPEG$_{22}$-*b*-PDLLA$_{45}$ rods after irradiation



## 7. Acknowledgements

The authors acknowledge support from the Ministry of Education, Culture and Science (Gravitation program 024.001.035). We would like to thank Corbion for providing us with LL, DD and LD lactide monomers. We would like to thank Paul B. White for his help with the $T_1$ determinations and Jiabin Luan and Danni Wang for their help with the DSC measurements.

## 8. References

[1] Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.-Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A., Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **2012,** *9*, 676.