Photosynthetic reaction center in ABA triblock polymersomes: highlights on the protein localization

R. R. Tangorra,[a] A. Operamolla,[a] F. Milano,[b] O. Hassan Omar,[c] J. Henrard,[d] R. Comparelli,[b] F. Italiano,[b] A. Agostiano,[a,b] V. De Leo,[a] R. Marotta,[e] A. Falqui,[f] G. M. Farinola,[a,c*] and M. Trotta[b*]

[a] Dipartimento di Chimica, Università degli Studi "Aldo Moro", Bari (Italy); [b] CNR-IPCF, Istituto per i processi Chimico-Fisici, UOS Bari (Italy); [c] CNR-ICCOM, Istituto di Chimica dei Composti Organometallici, Bari (Italy) and [d] University of Namur (FUNDP), Belgium, [e] Electron Microscopy Lab, Nanochemistry Department, Istituto Italiano di Tecnologia (IIT), Genova (Italy), [f] King Abdullah University of Science and Technology, Biological and Environmental Sciences and Engineering Division (Thuwal Kingdom of Saudi Arabia)

Supporting information

S1. Inhibition of Q_B-functionality assay in RC-ABA polymersomes

Inhibition of the final electron acceptor Q_B in photosynthetic reaction centers[1] reconstituted in ABA block copolymers.

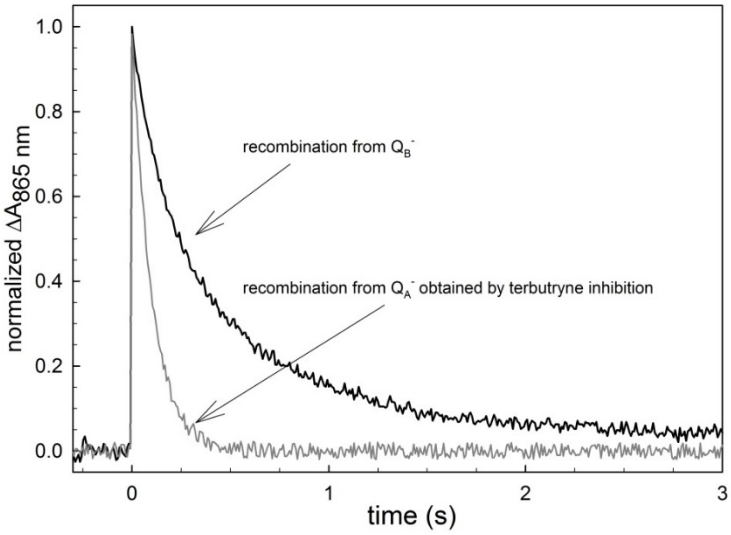


Figure S1.1. Normalized charge recombination kinetics of RC in ABA polymersomes. Charge recombination reaction from the final electron acceptor Q_B^- (black line) and from Q_A^- obtained by inhibiting the Q_B functionality by the herbicide terbutryn presence (grey line). Conditions: 5 mM phosphate buffer at pH 7, KCl 10 mM, 100 μM terbutryn.

S2. Hydrophilic dye probe entrapment within the internal aqueous core of the polymersomes

Effective formation of closed ABA vesicles was checked by the entrapment of the hydrophilic dye pyranine (8-hydroxypyrene-1,3,6-trisulfonatepyranine – Sigma Aldrich) according to a previously published protocol[2] and briefly outlined here.

A thin ABA film on an Eppendorf wall was hydrated with 0.5 ml of 4 % w/w cholate in phosphate buffer (5 mM potassium phosphate, 10 mM KCl, 1 mM pyranine, pH 7.2) and sonicated with titanium tip probe of Branson sonicator. To this suspension, a 30 μL aliquot of the RC 70 stock μM solution was added. The suspension was vigorously mixed and hence loaded onto a Sephadex G-50 gel filtration column, previously equilibrated with 1 mM pyranine and KCl 10 mM in phosphate buffer at pH 7.2 and eluted with the same medium. This allows the transition from micelle to vesicle ensuring the entrapment

of the dye. Removal of not incorporated pyranine was obtained by running a second Sephadex G-50 gel filtration column equilibrated with the same solution lacking pyranine.
Fractions collected after the second column may containing pyranine only if the MVT forms closed vesicles. The contemporary elution of RC, pyranine and ABA furthermore indicates that vesicles have an aqueous core in which the dye is solubilized and also carry the protein (see figure S2.1).
An elution profile was recorded to check the dye entrapment in ABA vesicles, collecting 0.18 mL fractions after a void volume of one mL. In figure S2.1, the obtained elution profile is reported: the effective RC presence is detected by recording the absorbance at 802 nm corresponding to the protein absorption peak (Cary Varian 5000 spectrometer), ABA polymer is evaluated by recording the IR absorption of the Si–CH_3 stretching band at 1260 cm^{-1} (Varian 670-IR spectrometer) and, finally, pyranine is reported as emission intensity at 510 nm with an excitation wavelength of 460 nm (Eclipse Varian spectrofluorometer).

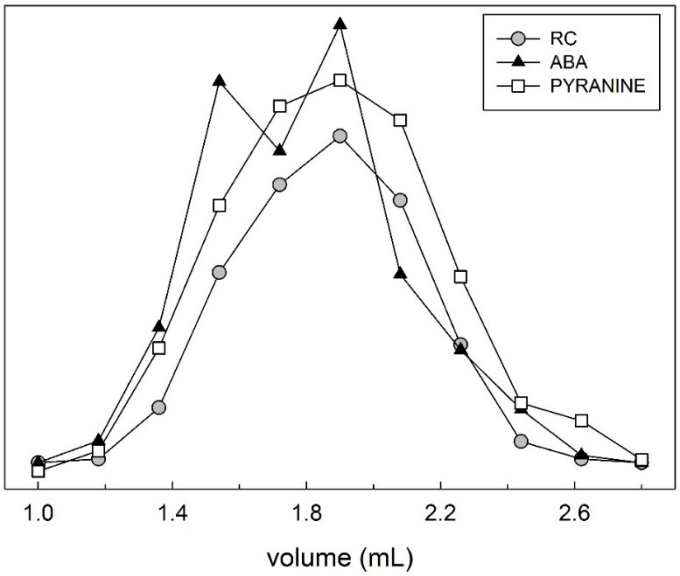


Figure S2.1. Elution profile of a Sephadex G-50 gel filtration column run on RC- $A_{22}B_{61}A_{22}$ polymersomes prepared by MVT technique. The following fingerprints were used: a) RC absorption is recorded at 802 nm; b) ABA absorption is recorded at 1260 cm^{-1} in correspondence of Si–CH_3 stretching band, c) pyranine is excited at 460 nm and the fluorescence recorded at 510 nm. Collected fraction contain 180 µL.

S3. ABA vesicles size distribution determination

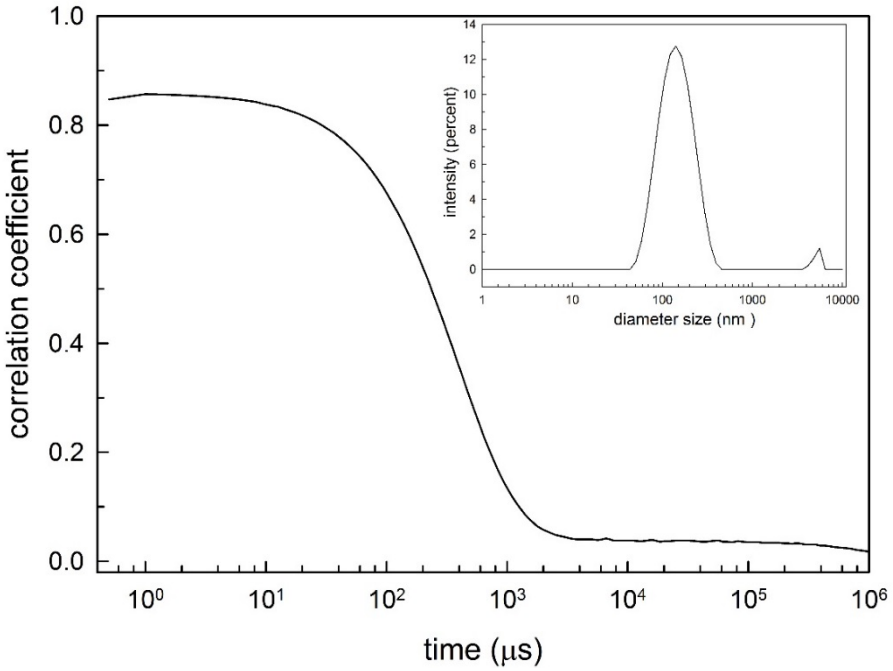


Figure S3.1. Correlation coefficient curve and dynamic size distribution obtained by light scattering intensity of ABA vesicles obtained by MVT technique. Measurements performed by the Malvern Zetasizer Nano 2590 DLS.

S4. Photocycle of RC embedded in A BA-polymersomes and in PMOXA-aggregates

In Figure S4.1 and S4.2 are reported RC photocycle rate of a solution containing 1 μM RC entrapped, respectively, in $A_{22}B_{61}A_{22}$ polymersomes and in $PMOXA_{22}$ aggregates, in presence of 10 μM reduced cyt c in phosphate buffer at pH 7.
The photocycle rate was measured by continuously illuminating the solution with a red-filtered light and cyt absorbance change was followed at 551 nm. As electron acceptor the synthetic dQ was employed with a final concentration of 20 μM, in the "dQ confined" and in the "dQ free" (dQ added in the bulk solution) conditions, as reported in the material and methods. Discussion about photocycle rate determination is reported in the main text.

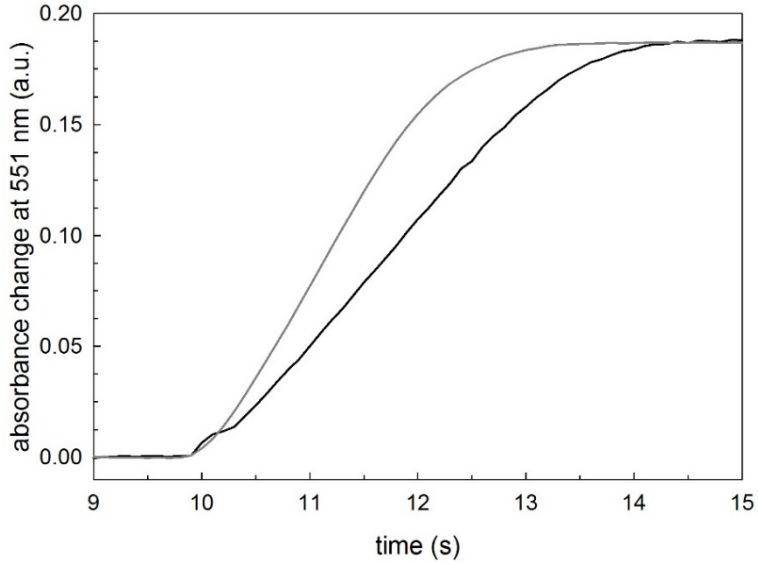


Figure S4.1. RC photocycle of RC embedded in ABA polymersomes with confined dQ (black trace) and free dQ (gray trace).

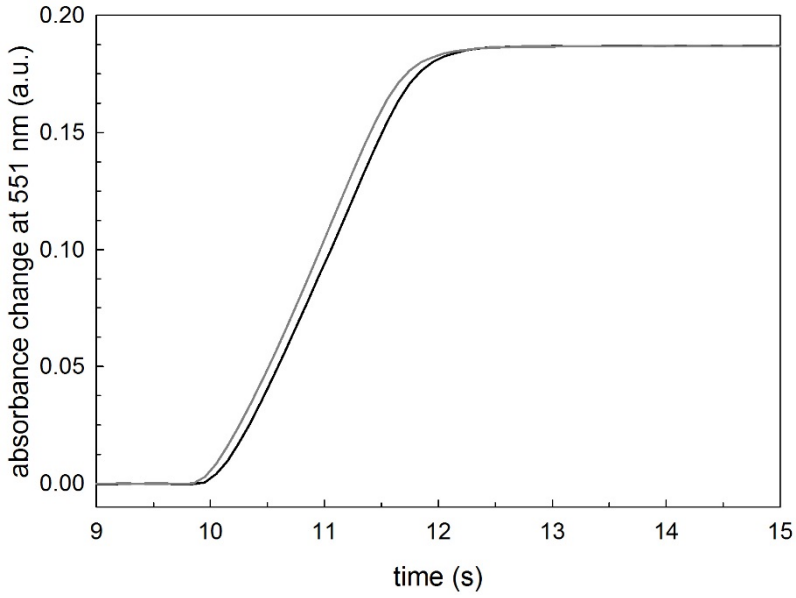
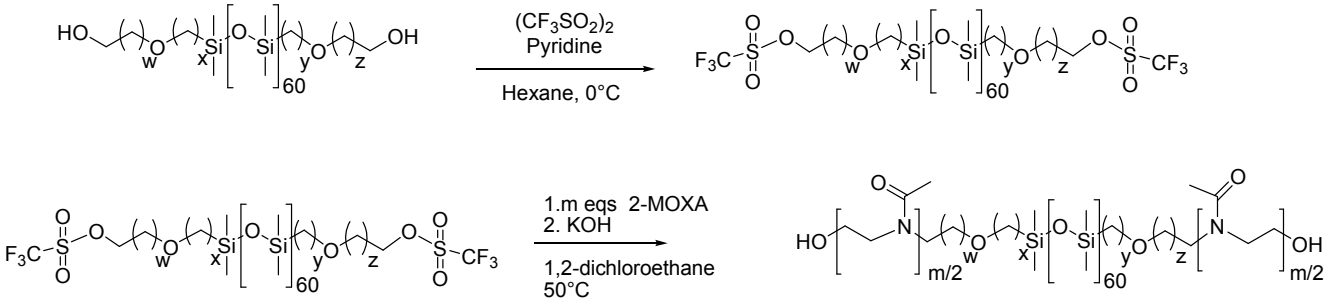


Figure S4.2. RC photocycle of RC in PMOXA-aggregates with confined dQ (black trace) and free dQ (gray trace).

S5 Synthesis of ABA triblock co-polymer and PMOXA polymer

The tri-block copolymers PMOXA-PDMS-PMOXA were synthesized according to a previously reported synthetic route[3] as illustrated in the Scheme 5.1.



Scheme S5.1. General procedure for the synthesis of PMOXA-PDMS-PMOXA. w+x+y+z=10, according to ^{1}H-NMR of commercial PDMS)**.**

PDMS ^{1}H-NMR

The commercial PDMS was carefully analyzed by ^{1}H-NMR. The ^{1}H-NMR spectrum recorded at 500 MHz in $CDCl_3$ (Figure 5.1) reveals the presence of chain terminations consisting of at least 6 methylene units, three of them linked to oxygen (signals between 3.40 and 3.75 ppm). In addition, the comparison between the integrals for the protons of the methyl units linked to silicon (366H) and the integrals for the protons of chain termination methylene units (given the value 4H) allows us to infer that the polymer has, on the average, 61 dimethylsiloxane repeating units. Therefore we hypothesized for PDMS the structure sketched in the Scheme S5.1. Trifluoromethansulfonation of peripheral hydroxyl terminations of the commercial PDMS proceeds with 100% efficiency, as judged from the complete disappearance of the proton signals centered at 3.72 ppm and 2.00 ppm, attributed to the terminal methylenes bonded to hydroxyl group, and to the alcoholic protons respectively, and from the appearance of a new signal, whose integral is 4, at 4.62 ppm. From the spectrum of PDMS@Triflate it is also clear that the signal centered at 1.6 ppm integrates for 8 aliphatic protons.

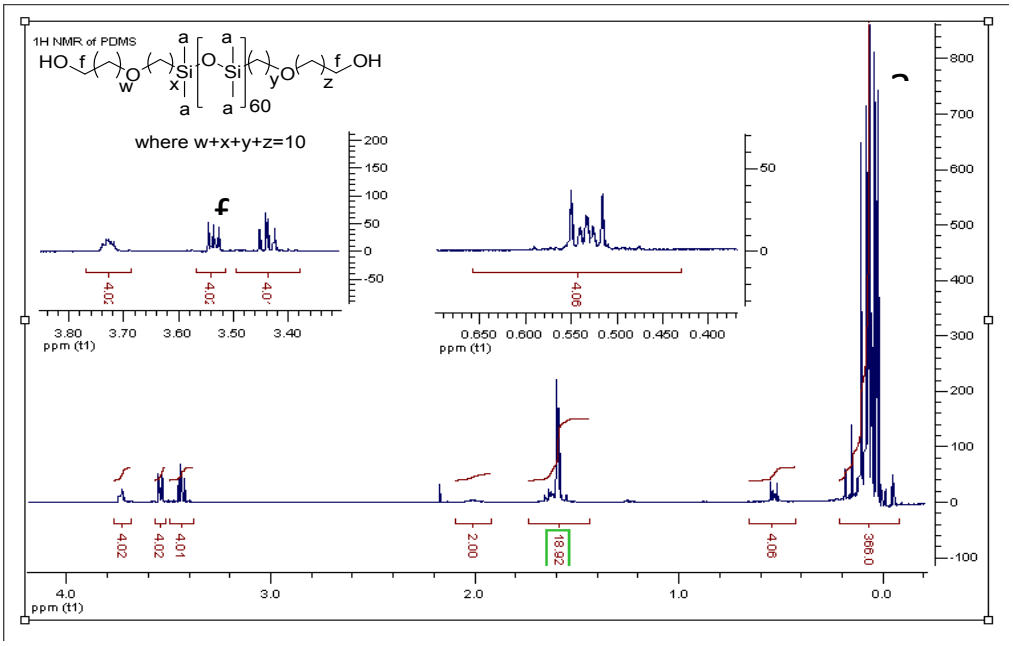


Figure S5.1. ^{1}H-NMR of commercial PDMS in $CDCl_3$ at 500 MHz**.**

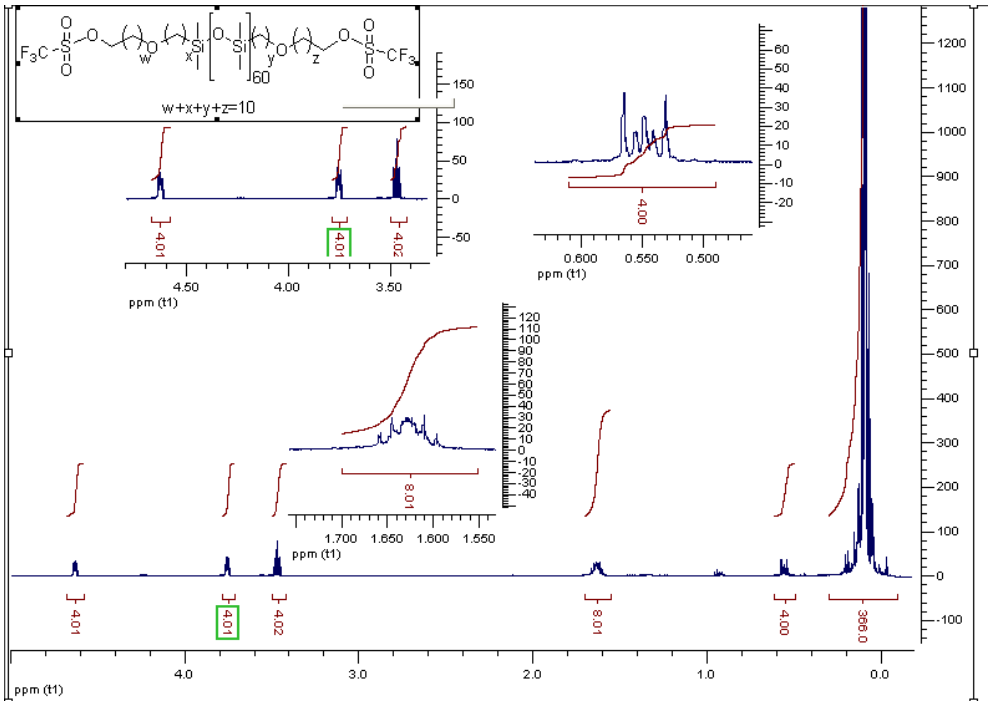


Figure S5.2. ^{1}H-NMR at 500 MHz in $CDCl_3$ of PDMS@triflate

Triblock-copolymers ^{1}H-NMR

Estimation of triblock copolymers molar weights was done from ^{1}H-NMR spectra, assuming for the PDMS block proton signals an integral of 366 (61 monomeric units), as was assessed from the proton spectrum of PDMS. We tentatively performed a GPC experiment in THF with polystyrene standards 4 and MALDI-TOF experiments but it was not possible to derive consistent data, considering the aggregation behavior of the materials.

The spectra of $A_{22}B_{61}A_{22}$ and $A_{10}B_{61}A_{10}$ are reported in the Figure S5.3 and S5.4 respectively.

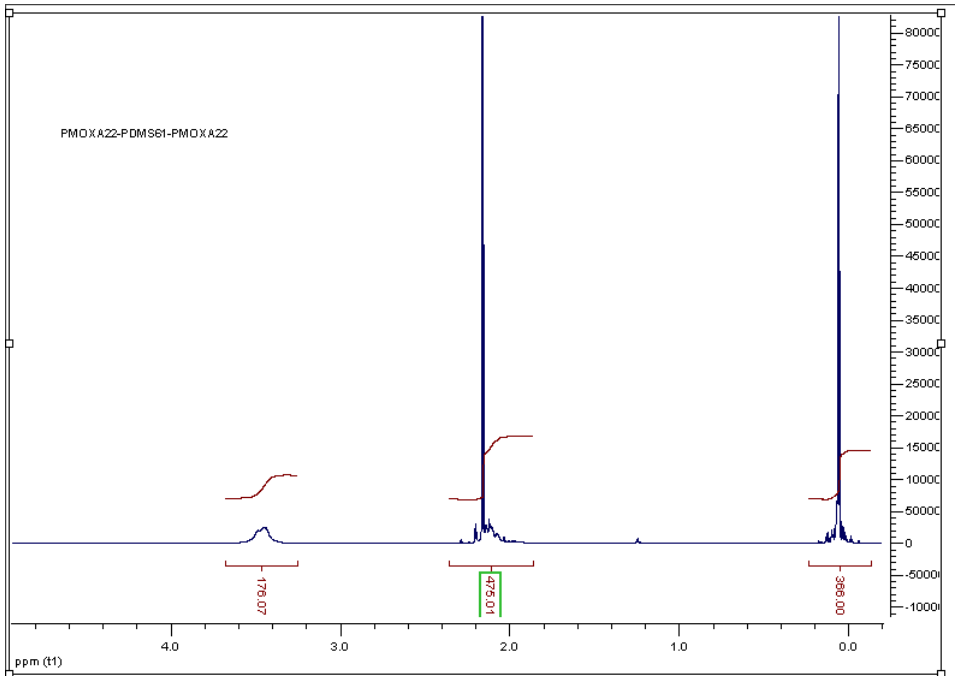


Figure S5.3. 500 MHz ^{1}H-NMR spectrum of $PMOXA_{22}$-$PDMS_{61}$-$PMOXA_{22}$ triblock copolymer in $CDCl_3$. (Acetone impurity covers methyl signals)

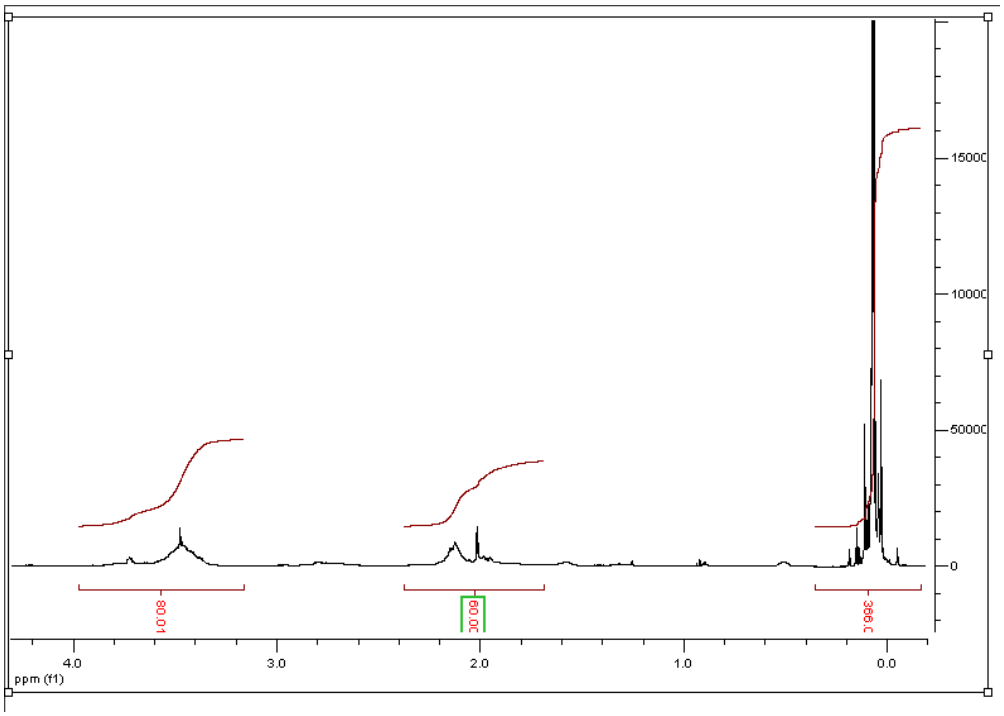


Figure S5.4. 500 MHz ^{1}H-NMR spectrum of $PMOXA_{10}$-$PDMS_{61}$-$PMOXA_{10}$ triblock copolymer in $CDCl_3$.

Synthetic details

All reactions were carried out under a nitrogen atmosphere in oven-dried glassware, using dry solvents. A Milestone Micro Synth microwave oven and a Teflon tube were used for the polymerization of 2-methyloxazoline. Hexane was dried anhydrous by distillation over molecular sieves for 12 hours immediately prior to use.
Compounds were characterized by ^{1}H-NMR, UV-Vis spectroscopy and, were possible, by MALDI-TOF spectrometry. MALDI-TOF spectra of ABA and commercial PDMS polymers are omitted in this paper, because they did not allow to reveal analytically useful information on molecular weights and dispersity, though we tested several matrixes and ionization additives.
^{1}H-NMR spectra were recorded at 500MHz, using the residual proton peak of $CDCl_3$ at 7.26 ppm as reference. MALDI-TOF spectra were recorded on a Microflex mass spectrometer (Bruker Daltonics) in the linear and positive mode at a laser frequency of 20 Hz and using a 96 spots ground steel target. The spectra were calibrated using peptide Standard II kit, purchased from Bruker-Daltonics, and processed according to the suggested protocol. The average molecular weights and polymerization degree were calculated with the software Polytools. The samples were prepared as follows, using a layer by layer deposition method: 2µl of a saturated solution of MALDI matrix a-cyano-4-hydroxycinnamic acid (4HCCA; ACROS Organics) in water:acetonitrile 1:1 were deposited on a steel target. After that, 1 µl of a solution of NaI (10 mg/ml) in water was deposited on top of the matrix layer, and finally 1µl of PMOXA water solution (10 mg/ml) was deposited as third layer. For each deposition the solvent was allowed to dry slowly in air.

Bis(trifluoromethanesulfonate) terminated poly(dimethylsiloxane) (PDMS@Triflate)

A 250 mL three-necked round-bottom flask equipped with a Soxhlet extractor containing a thimble filter filled with 4 Å molecular sieves, and a condenser was conditioned with nitrogen. Then 15g (2.77 mmol) of commercial PDMS and 50 mL of freshly distilled hexane were introduced and the solution was refluxed for 16 hours. The solution was then cooled down to 0°C and dry pyridine (1.55 g, 19.39 mmol) was added. Then, trifluoromethanesulfonic acid anhydride (5.46 g, 19.39 mmol) was added over 15 minutes. The resulting solution was stirred at 0°C for three hours and 10 mL of dry chloroform were added. A white precipitate was formed (complex between pyridine and anhydride), that was separated from the solution by centrifugation (6000 rpm for 10 minutes) in nitrogen filled tubes. The orange organic solution was recovered in a round bottom flask and stirred with active charcoal for 20 minutes. The solution was filtered, and the solvent evaporated under reduced pressure. 2.78g of colorless oil were isolated. ^{1}H-NMR($CDCl_3$, 500 MHz): -0.06-0.24 ppm(m, 366H, C$\underline{H}_3$-Si), 0.51-0.59 ppm (m, 4H, -CH_2-C$\underline{H}_2$-Si), 1.58-1.67 ppm (m, 8H, -CH_2-C$\underline{H}_2$-CH_2-), 3.43-3.50 ppm (m, 4H, -C$\underline{H}_2$-O-), 3.72-3.77 ppm (m, 4H, -C$\underline{H}_2$-O-), 4.60-4.65 ppm (m, 4H, $CF_3SO_3C\underline{H}_2$-CH_2-).

Poly(2-methyloxazoline)-block-poly(dimethylsiloxane)-blockpoly(2-methyloxazoline) ($PMOXA_{22}$-$PDMS_{61}$-$PMOXA_{22}$)

A Schlenk tube equipped with a water condenser was evacuated and backfilled with nitrogen (three times). Then it was charged with freshly distilled 2-methyl-2-oxazoline (217 mg, 2.56 mmol), 300 mg (≈0.06 mmol) of PDMS bistriflate and 3 mL of anhydrous 1,2-dichloroethane. The solution was stirred for 1 h at room temperature and then heated at 50 °C for 48 hours. After that, the reaction mixture was cooled to room temperature and 0.5 mL of a solution 0.5M of KOH in ethanol were added. After stirring the resulting mixture for 1 h, the solvents were evaporated under reduced pressure. The residual, a white oil, was resuspended in water:ethanol 5:1 v:v mixture (10mL) and purified by ultracentrifugation using mass filters with a cut off of 10 kDa (7000 rpm, 40 minutes). The higher molecular weight fraction was recovered from the filter and isolated after evaporation of the solvents under reduced pressure, yielding a white solid (300 mg). ^{1}H-NMR ($CDCl_3$, 500 MHz): -0.06-0.20 ppm (m, 366H, CH_3-Si), 2.00-2.21 (m, Integral not detected due to overlap with acetone impurities, -C(O)CH_3), 3.31-3.63 ppm (m, 176H, -O-CH_2-CH_2-N-). UV-Vis: l_{max} 276 nm.

Poly(2-methyloxazoline)-block-poly(dimethylsiloxane)-blockpoly(2-methyloxazoline) ($PMOXA_{10}$-$PDMS_{61}$-$PMOXA_{10}$)

The reaction procedure was the same as above, with the following quantities: 2-methyl-2-oxazoline (102 mg, 1.2 mmol), 300 mg (≈0.06 mmol) of PDMS bistriflate and 3 mL of anhydrous 1,2-dichloroethane. The residual, a colorless oil, was resuspended in absolute ethanol (10mL) and purified by ultracentrifugation using mass filters with a cut off of 3 kDa (7000

rpm, 30 minutes). The higher molecular weight fraction was recovered from the filter and isolated after evaporation of the solvents under reduced pressure, yielding a colorless oil (185 mg). ^{1}H-NMR ($CDCl_3$, 500 MHz): -0.06-0.20 ppm (m, 366H, CH_3-Si), 2.00-2.21 (m, 60H, -C(O)CH_3), 3.31-3.63 ppm (m, 80H, -O-CH_2-CH_2-N-).

Poly(2-methyloxazoline) ($PMOXA_{22}$)

A teflon tube was conditioned with nitrogen. Then freshly distilled 2-methyl-2-oxazoline (2.000 g, 1.99 mL, 23.5 mmol), ethylmesylate (0.139 g, 115 µL, 1.12 mmol) and 3 mL of dry acetonitrile were introduced. The tube was sealed and heated to 100°C for 1 hour in a microwave oven with a maximum power of 120 kW. After, the mixture was allowed to cool to room temperature and quenched with 3 mL of distilled water. The solvents were distilled off at reduced pressure, and the residue was resuspended in ethanol. The polymer was purified by precipitation from ethanol adding slowly cold diethyl ether. A white powder was separated by centrifugation and dried under vacuum. 1.130g of pure PMOXA were isolated with a yield of 57%. ^{1}H NMR ($CDCl_3$): δ 3.45 (m, 4H), 2.11 (m, 3H); MALDI-TOF: Mn =1981 Da; Mw=2048 Da, polydispersity 1.03; DP 23.
MALDI-TOF calculation of average polymerization degree of PMOXA initiated by ethylmesylate, corresponding to the monomer/initiator molar ratio, was performed with the Polytool program. Analysisis of MALDI-TOF spectrum gave a polymerization degree of 23 that is very close to the molar ratio value of 22 between monomer and initiator adopted for the cationic polymerization.

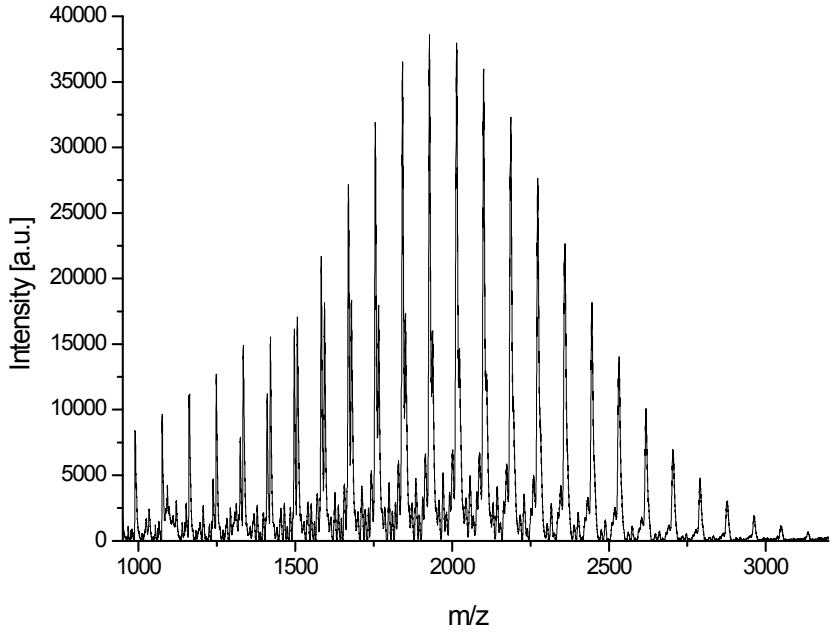


Figure S5.5. MALDI-TOF spectrum obtained from PMOXA synthesized by microwave assisted cationic polymerization of 2-methyl-2-oxazoline with a molar ratio of 22 between MOXA and ethylmesylate. Matrix HCCA.

References

1. A. Agostiano, F. Milano and M. Trotta, *Eur J Biochem*, 1999, 262, 358-364.
2. F. Milano, M. Trotta, M. Dorogi, B. Fischer, L. Giotta, A. Agostiano, P. Maroti, L. Kalman and L. Nagy, *J Bioenerg Biomembr*, 2012, 44, 373-384.
3. C. Nardin, T. Hirt, J. Leukel and W. Meier, *Langmuir*, 2000, 16, 1035-1041.
4. Graff, A.; Fraysse-Ailhas, C.; Palivan, C. G.; Grzelakowski, M.; Friedrich, T.; Vebert, C.; Gescheidt, G.; Meier, W., Amphiphilic Copolymer Membranes Promote NADH:Ubiquinone Oxidoreductase Activity: Towards an Electron-Transfer Nanodevice. *Macromolecular Chemistry and Physics* 2010, *211*, 229-238.