Electronic Supplementary Material (ESI) for Chemical Communications

Polymer nanoreactors shown to produce and release antibiotics locally

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Supplementary Information

Poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) triblock copolymer was synthesised by cationic ring opening living polymerization (CROP), according to the method described in [1] with some modifications, by growing 2-methyl-2-oxazoline onto activated poly(dimethylsiloxane) (PDMS) macroinitator. The polymer contained 72 dimethylsiloxane units and 32 2-methyloxazoline units according to ^{1}H-NMR (Supplementary Information, Fig. S. 1.). A *PDI* of 1.83 was calculated for the block copolymer based on GPC measurements. This slightly higher value than the values previously published for PMOXA-*b*-PDMS-*b*-PMOXA block copolymers [1] is explained by the relatively high *PDI* value of the used pre-polymer (*PDI* = 1.62).

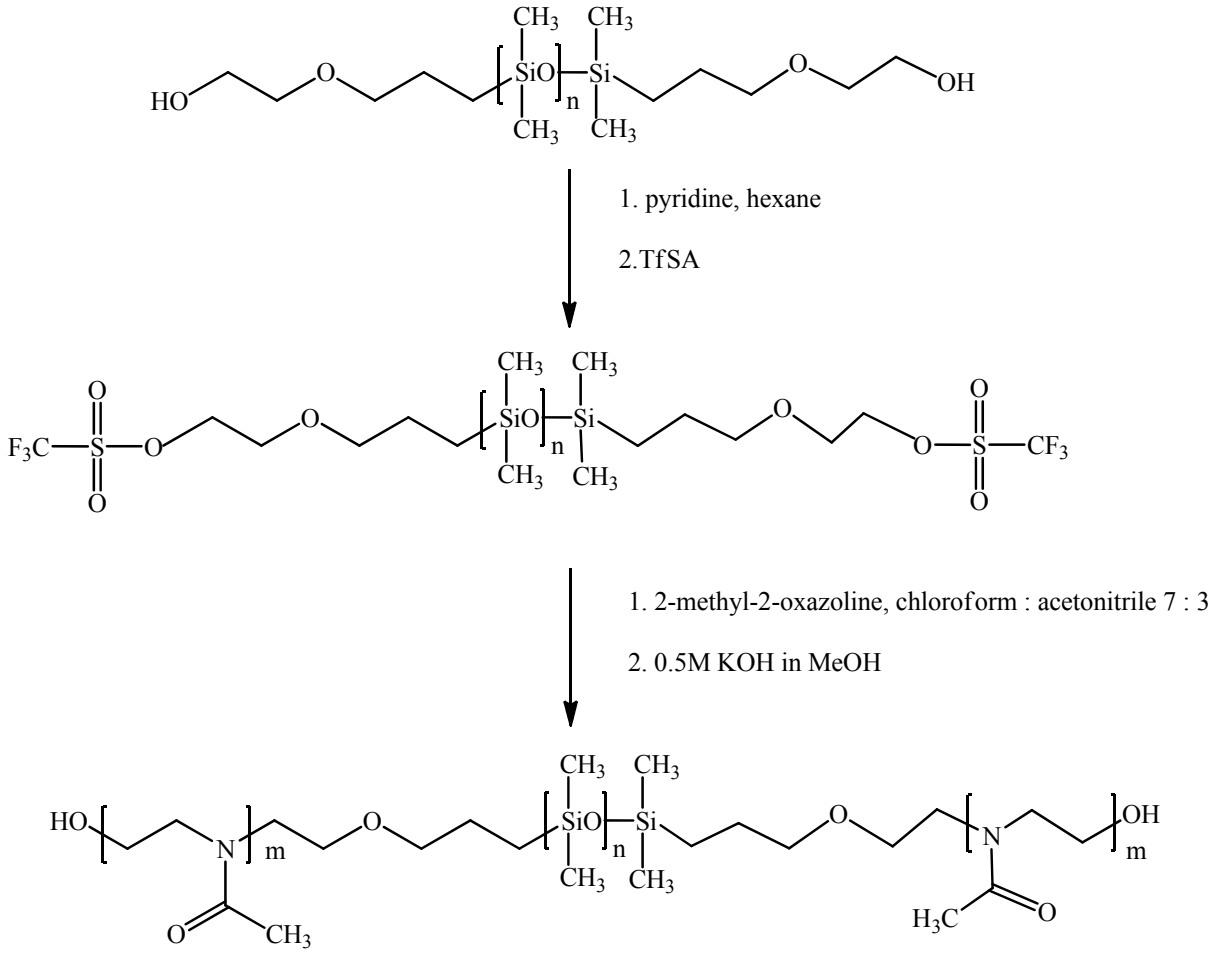

Figure S. 1. Reaction scheme of the poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) triblock copolymer synthesis.

^{1}H-NMR

^{1}H-NMR spectra were recorder with a Bruker DPX-400 spectrometer (Bruker, Switzerland). Deuterated chloroform was used as solvent (99.8% D, 0.1 % TMS). Spectra were analyzed with MestReNova 7.0.3 software (Mestrelab Research SL, Spain). The molecular weight of the polymer obtained from ^{1}H-NMR was 8323 g/mol.

^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm] = 0 ppm (Si-***CH*$_3$**, 6H), 0.5 ppm (Si-***CH*$_2$**-CH_2, 4H), 1.5-1.6 ppm (Si-CH_2-***CH*$_2$**-CH_2, 4H), 2.0-2.2 ppm (***CH*$_3$**-CON<, 6H), 3.3-3.5 ppm (>N-***CH*$_2$**-***CH*$_2$**-N<, 8H).

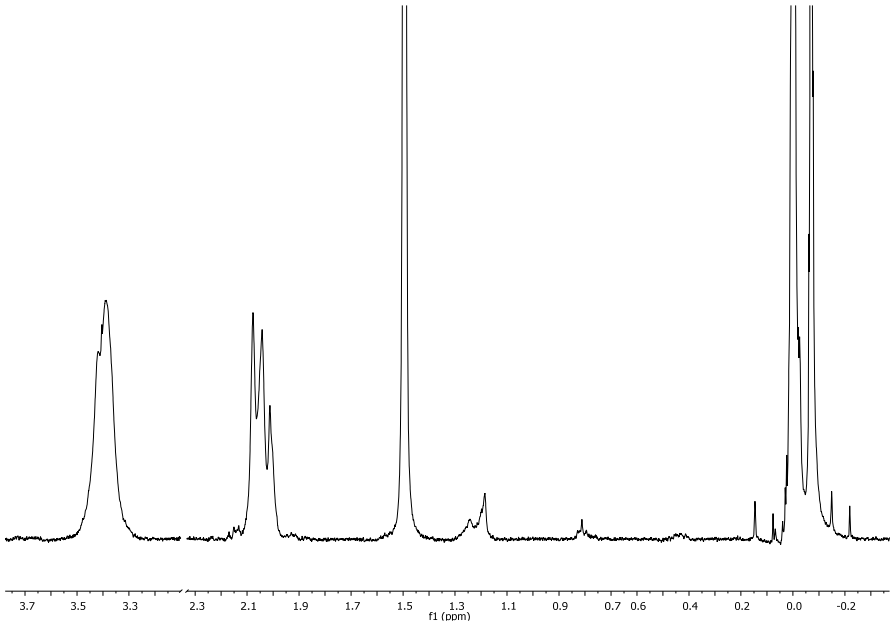

Figure S. 2. ^{1}H-NMR spectrum of poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline).

Gel permeation chromatography (GPC)

The molecular weight of the block copolymer was determined by GPC using Viscotek GPCmax system equipped with a PLgel 10 µm column, with THF as eluent (flow rate: 1 mL/min) at 40 °C and recorded by refractive-index (RI) and UV detector. Narrow polystyrene standards (PSS Polymer Standards Service, Germany) were used to calculate *Mn*, *Mw*, and the polydispersity index (*PDI*) of the block copolymer and the pre-polymer. The molecular weight of the polymer used was *Mn* = 7988 g/mol and the *PDI* = 1.83 .

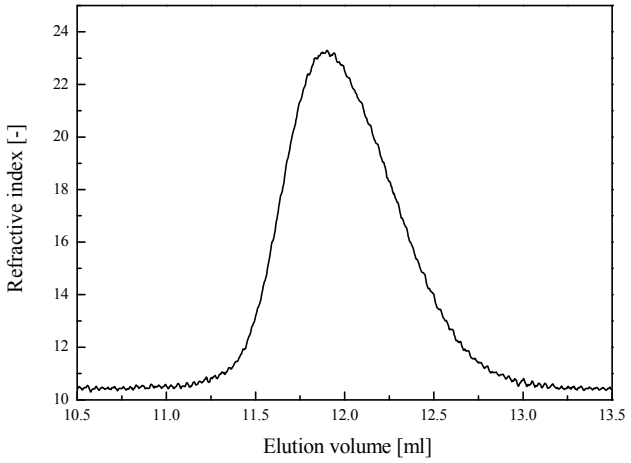

Figure S. 3. Gel permeation chromatogram of PMOXA-*b*-PDMS-*b*-PMOXA dissolved in THF. Refractive index detector.

Dynamic and static light scattering (DLS and SLS)

Dynamic and static light scattering experiments were performed at 20 °C ± 0.05 °C (293 °K ± 0.05 K) using an ALV-Langen Goniometer equipped with a frequency-doubled He-Ne laser (wavelength 633 nm). The apparatus allows measurement at scattering angles between 30° and 150°. Samples were measured in 10 mm diameter cylindrical quartz cells. Cells were then mounted in a thermostated optical matching bath (toluene bath). Vesicle samples were prepared according to the film rehydratation method. A series of dilutions (a minimum of 5) was then prepared from the original solution of vesicles. Typically, polymer concentrations ranged from 1 mg/ml to 0.1 mg/ml. An ALV-5000/E Multiple Tau Digital Correlator was used to calculate the photon intensity, and an autocorrelation function was used to calculate vesicle size. DLS data were analysed *via* the CONTIN algorithm.

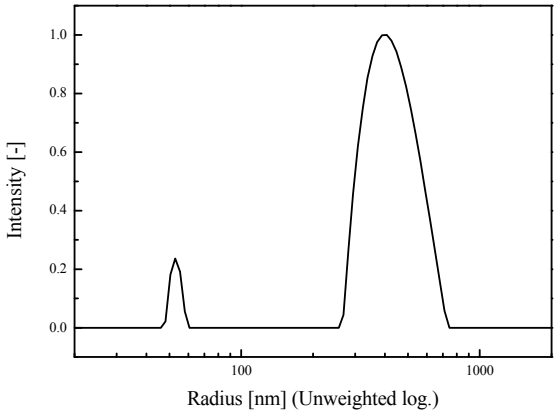

Figure S. 4. Size distribution obtained by DLS for 90°.

The size distribution obtained by dynamic light scattering (DLS) indicates two populations of objects: a major population with an average hydrodynamic radius of around 255 nm, and a minor one with around 50 nm. This relatively high value of R_H can be explained by the fact that samples measured by DLS were not, as is conventional, sequentially extruded through a membrane filter. Extrusion leads to a more homogenous size distribution and prevent aggregates formation. We did not extrude the samples, to prevent the loss of material during this step and thereby avoid lower efficiency in terms of final concentration of antibiotic. In our case, the system efficiency took priority over homogeneity. Similar broad size distribution and high R_H have been reported for non-extruded samples [2]; in our case, the system efficiency took priority over homogeneity. The major population of spherical objects has a hollow-sphere morphology (Rg/Rh=1.09) [3], which was also confirmed by TEM are not R_H obtained by cumulant analysis was 255 nm.

Encapsulation efficiency

The concentration of the encapsulated enzyme was estimated and the results are presented in Table 1. Despite a number of papers describing polymersomes for encapsulation, few discuss the quantification of encapsulation efficiency [2, 4, 5], typically 10% – 15% of the initial amount used for the encapsulation with film rehydration. Lower (4.5%) [6] and higher values (46%) [7] can be found in literature. Heretofore; encapsulation of catalyst has been limited to random entrapment during vesicle formation. However, the encapsulation efficiency assay, which yields what we call the apparent value, is based on evaluation of the amount of enzyme that is not encapsulated. In this regard it is important to emphasize that a certain fraction of enzyme can be encapsulated in polymersomes that are non-permeable to substrate, thereby lowering the efficiency of the system [2, 4, 5].

Transmission electron microscopy (TEM)

TEM images were taken on a Philips EM 400 (Philips Electronics, Netherlands) operated at 80 kV, equipped with a Megaview III charge-coupled device camera (CCD) and controlled with Morgagni 268D control and image acquisition software. The sample was deposited on a glow-discharged, parlodion-and carbon-coated, 200 mesh copper grid and incubated for 1 min, before the droplet was blotted on filter paper. Afterwards, the samples were negatively stained with a 2% uranyl acetate solution.

Cephalexin synthesis and spectrophotometric assay

Cephalexin synthesis was carried out in PBS buffer pH 7.4 at 37 °C ± 1 °C for seven days. The concentrations of PGME and 7-ADCA in the reaction mixture were 81.4 mM and 46.7 mM, respectively. The concentration of cephalexin was estimated using UV-Vis spectroscopy by the modified method described in [8]. The method involves conversion of penicillins to the corresponding highly absorbing piperazine-2, 5-dione derivatives by heating in an alkaline (carbonate buffer pH 9.2) sorbitol-zinc ion solution, and followed by incubation with 1 M NaOH solution. Since an intact ß-lactam ring and a free amino group in the side-chain of the drug molecule are required, no interference due to 7-ADCA and PGME was observed. Changes in absorbance were followed at 388 nm. The procedure of the assay in general was as follows: 100 µL of analysed sample was mixed with 100 µL sorbitol-zinc ion solution (in carbonate buffer pH 9.2) and heated, with the heating block at 60 °C, for 10 min. Then 100 µL of 1 M NaOH was added and mixed properly. A sample of PBS buffer was treated exactly as the analysed sample and used as the reference. The cephalexin concentration in analysed samples was calculated by a standard curve ($R^2 = 0.998$).

Nanoreactor stability and activity

To ensure that the enzyme was not leaking from the nanoreactors after long term storage (14 days at 37 °C), we combined TEM with activity assays of the enzyme. A purified nanoreactor solution was compared to a free enzyme solution in PBS buffer (at a concentration of around that of enzyme encapsulated in vesicles). For both solutions the penicillin acylase activity was assayed spectrophotometrically. The activity was assessed by spectroscopic assay based on a secondary reaction of cephalexin with sorbitol solution containing zinc ions, which results in highly absorbing derivatives [8].No activity was recorded for the enzyme encapsulated in non-permeable polymersomes, because the substrate did not penetrate the vesicle membranes. As expected, free enzyme in solution did show activity. After three weeks of storage at room temperature and at 37 °C no significant morphological changes appeared in TEM micrographs, suggesting that nanoreactors are mechanically stable.

The significant difference in absorbance between nanoreactors prepared at room temperature and those prepared at 8 °C indicates that temperature plays a major role in their final efficiency in terms of the final activity of the overall system. To verify this deduction, a free enzyme activity assay based on

penicillin G hydrolysis catalysed by penicillin acylase was performed. The activity of the enzyme stored at different temperatures, 8 °C and room temperature, was measured over time (five days) (Supplementary Information, Fig. S. 5.).

After five days, the retained activity of the enzyme was 79% for the sample stored at 8 °C, and 65% for the sample stored at room temperature. This corroborates the difference in enzymatic activity of the nanoreactors prepared at RT compared to those prepared at 8 °C.

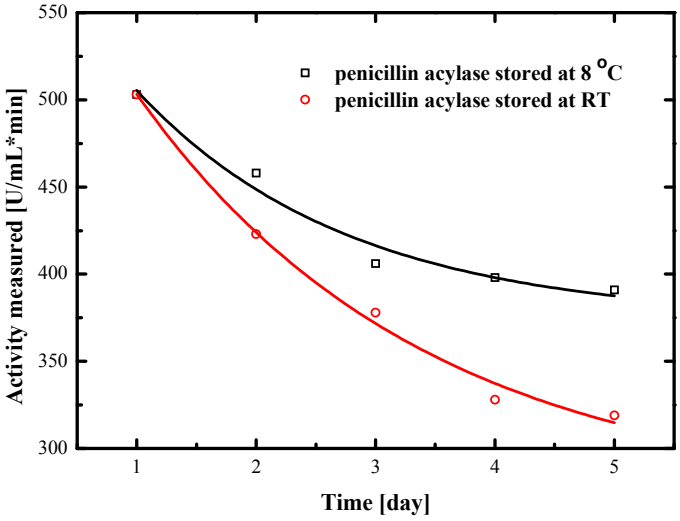

Figure S. 5. Penicillin acylase deactivation profiles in bulk solution during storage at 8 °C and at RT, respectively.

References

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