Supporting Information

Brushing the surface: cascade reactions between immobilized nanoreactors

Dalin Wu,[a] Serena Rigo,[a] Stefano Di Leone,[a] Andrea Belluati,[a] Edwin C. Constable,[a] Catherine E. Housecroft,*[a] and Cornelia G. Palivan*[a]

Chemicals

Hexamethylcyclotrisiloxane (D3) (98%), calcium hydride (95%), benzophenone (≥99%), n-butyllithium (2.0 M solution in cyclohexane), platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution (in xylene, Pt ~2 %), chlorodimethylsilane (98%), 2-allyloxyethanol (98%), trifluoromethanesulfonic anhydride (≥99%), biotin (≥99%), N,N'-dicyclohexylcarbodiimide (99%), 4-(dimethylamino)pyridine (≥99%), streptavidin atto488, lactoperoxidase from bovine milk (LPO) (≥150 U/mg), uricase from bacillus fastidious (~9 U/mg), horseradish peroxidase (~150 U/mg), uric acid (≥99%), melittin from honey bee venom (≥85%), trimethylamine (TEA) (≥99%), 2-methyl-2-oxazoline (99%), (3-aminopropyl)trimethoxysilane (97%), tetrahydrofuran (inhibitor-free, ≥99.9%), toluene (anhydrous, 99.8%), α-bromoisobutyryl bromide (98%), anhydrous copper(I) bromide (99.999% trace metals basis), anhydrous copper(II) bromide (99%), *N,N,N′,N″,N‴*-pentamethyldiethylenetriamine (PMDETA) (99%), 2-hydroxyethyl methacrylate (HEMA) (97%), 2-aminoethyl methacrylate hydrochloride (2-AEMA.HCl) (97%), cyclohexane (≥99.9%), *N,N*-dimethylformamide (anhydrous, 99.8%), ethanol (≥99.9%) were purchased from Sigma. Amplex® Red was purchased from life technologies. BodiPy630/650 was purchased from Thermo Scientific Inc. Trimethylamine and hexamethylcyclotrisiloxane were distilled over calcium hydride before use. Tetrahydrofuran and cyclohexane were freshly distilled from sodium and benzophenone before use. Other chemicals were used directly as received. Atto 655 modified uricase and and atto635 modified LPO were synthesized and purified according to the published procedure.[1]

Characterization methods

Contact Angle Measurement

The water contact angles of the surfaces were measured on a Drop Shape Analyzer (KRÜSS Company, Germany) with a constant water drop volume (4 μL). The average values of measured static contact angles were calculated from at least three different position of the measured surfaces.

Ellipsometry

The thickness of the PHEMA-co-poly(2-aminoethyl methacrylate hydrochloride) polymer layer on the silicon wafer surface was determined by spectroscopic ellipsometry (SE 850, Sentech instruments GmbH, Germany) with a laser wavelength of 658 nm (Accurion, Cauchy model) under air at room temperature (ca. 22 °C). Before each measurement, the surface was thoroughly rinsed with water and ethanol, then dried under a gentle air stream to remove any dust on the surface. During the measurement, the power of the laser was set to 2% and the objective magnification was 10X. The data were processed using software with EP4 model. The measured data were averaged over three separate positions on the functional surfaces.

Atomic Force Microscopy (AFM)

The topography of the functional silicon wafer and chip surfaces were characterized by AFM using a JPK NanoWizard 3 AFM (JPK Instruments AG). The measurements were taken in the AC mode in air, using silicon cantilevers (Tap150 Al-G, Budget Sensors) with a nominal spring constant of 10−130 N m^{-1} and a resonance frequency of 150 kHz. The images were collected with a resolution of 512 x 512 pixels at a scanning rate 0.2 line s^{-1}. First, the dried specimen surface containing immobilized biotin-polymersomes was fixed on the bottom of the well (1 cm x 1cm), and then 2 mL PBS were added to rehydrate the surface prior to the AFM measurement. The images were analysed using JPK data processing software.

Confocal Laser Scanning Microscopy (CLSM)

The fluorescence of the surfaces was measured by CLSM (Zeiss LSM510 META/ConfocoCor 2 FCS microscope). The measurement was performed using an Argon 2 (λ = 488 nm) laser and a water immersion objective (C-Apochromat 40X/1.2 W). The pinhole diameter was set to 70 μm, and a main beam splitter (HFT488), a secondary beam splitter

(NFT 490), and a bandpass filter (BP 505-550) were used for all the measurement process. All images were recorded with a detector gain fixed to 700. The brightness was adjusted equally for all images using ImageJ.[1]

Fluorescence Correlation Spectroscopy (FCS)

FCS measurements were carried out using a Zeiss LSM880 instrument with a He-Ne laser (λ = 488 nm). The pinhole diameter was 90 µm and system was calibrated using streptavidin atto488 PBS solution (0.08 µg/mL). Each measurement represented an average of 30 recordings of 12 s duration. The measured autocorrelation curves were fit by a three-dimensional diffusion model (eq 1) ether for a single component, when analysing the free dye, or for two components when analysing the biotin modified polymersomes binding with streptavidin atto488 where the parameters for one component were obtained according to the recorded values of the free streptavidin atto488. The measured samples were prepared as follows. The 200 µL biotin modified polymersome (5 mg/mL) (formed by $PDMS_{21}$-$PMOXA_7$-OH and $PDMS_{28}$-$PMOXA_{10}$-biotin diblock copolymers) was mixed with 5 µL streptavidin atto 488 PBS solution (40 µg/mL). At the same time, 200 µL normal polymersome (5 mg/mL) (formed only by $PDMS_{21}$-$PMOXA_7$-OH) was also mixed with 2 µL streptavidin atto488 PBS solution (40 µg/mL) as control. The above mixtures were shaken for 2 h at 50 rpm at room temperature (ca.22 °C) in the dark before FCS measurement.

$$(\tau) = 1 + \frac{1}{N} \times G_t \times G_d G_d(\tau)$$

$$G_t(\tau) = 1 + \frac{T_t}{1 - T_t} \times e^{-\tau/\tau_t}$$

$$A = \sum_{i=1}^{n} \frac{\Phi_i}{\left(1 + \left(\frac{\tau}{\tau_{d,i}}\right)^{a_i}\right) \times \left(1 + \left(\frac{\tau}{\tau_{d,i}}\right)^{a_i}\right) \times \frac{1}{S^2}}$$

(1)

where N is the average number of particles in the confocal volume, $G_t(\tau)$ describes the molecules in the triple state, T_t represents the triplet fraction, and τ_t represents the triplet relaxation time. The term G_d (τ) describes the diffusion of one component (n=1) or of two components (n=2) in three dimensions. Finally, τ_d represents the diffusion time, ϕ represents the fractional intensities, S represents the structural parameter, and a represents the anomaly parameter.

The τ_t was fixed to 3 µs and S to 5. The τ_d of streptavidin atto488 was determined by applying the one-component system model of the streptavidin atto488 PBS solution (100 nM). And the determined values were used as parameters for the free streptavidin atto488 in the following measurements. The average τ_d of the nano-objects formed by biotin modified polymersomes with streptavidin atto488 was determined using the two-component system where the first component τ_d was fixed to the determined average value of the free streptavidin atto488.

Transmission Electron Microscopy (TEM)

TEM measurements were carried out using a Phillips CM100 instrument with an acceleration voltage of 80 kV. The samples in PBS solution (5 µL) was first deposited on a glow-discharged Formvar and carbon coated copper grid for 60 seconds. Afterwards, the solution on the grid was removed, washed twice with H_2O (5 µL) and once withuranyl acetate (5 µL, 2% in aqueous solution). Finally, the sample was negatively dyed using uranyl acetate (5 µL, 2%, aqueous solution) for 10 s before the TEM measurement.

Dynamic and Static Light Scattering (DLS and SLS)

DLS and SLS measurement were performed on an LS device (LS Instrument Switzerland) with 30 mW He-Ne laser at room temperature (ca. 22 °C). The measurement angle was from 30 degree to 135 degree with increasing 1 degree at each measurement. At each angle, three measurements (each of 60 s) were carried out.

Fluorescence spectroscopy

A fluorescence spectrophotometer (Spectramax 5) was used for measurements with λ_{exc} = 570 nm and λ_{em}^{max} = 595 nm.

Quartz crystal microbalance with dissipation (QCM-D)

QCM measurements were carried out using a Biolin Scientific instrument. The biotin modified silicon sensor chips (model no. QSX303) were placed in the QCM-D chamber. The frequency signal fluctuation was below ±1 Hz under

continuous buffer flow (250 µL/s) before pumping streptavidin atto488 stock PBS solution (40 µg/mL) and biotin-polymersomes (1 mg/mL) PBS solution. The pumping rate of streptavidin atto488 and biotin-polymersomes was kept 50 µL/s. The QCM-D measurements data were collected at 3rd, 5th, 7th, 9th and 11th odd overtones and the reported QCM-D data were all obtained at the 7th overtone. In order to calculate adsorbed mass of streptavidin atto488 and biotin-polymersomes on the surface of chip, the Sauerbrey equation (2) was applied to do the calculation.

$$\Delta m = -C\Delta f \qquad (2)$$

where Δm is the absorbed mass, C is the proportionality constant (17.7 ng cm^{-2} Hz^{-1}) and Δf is the frequency shift.

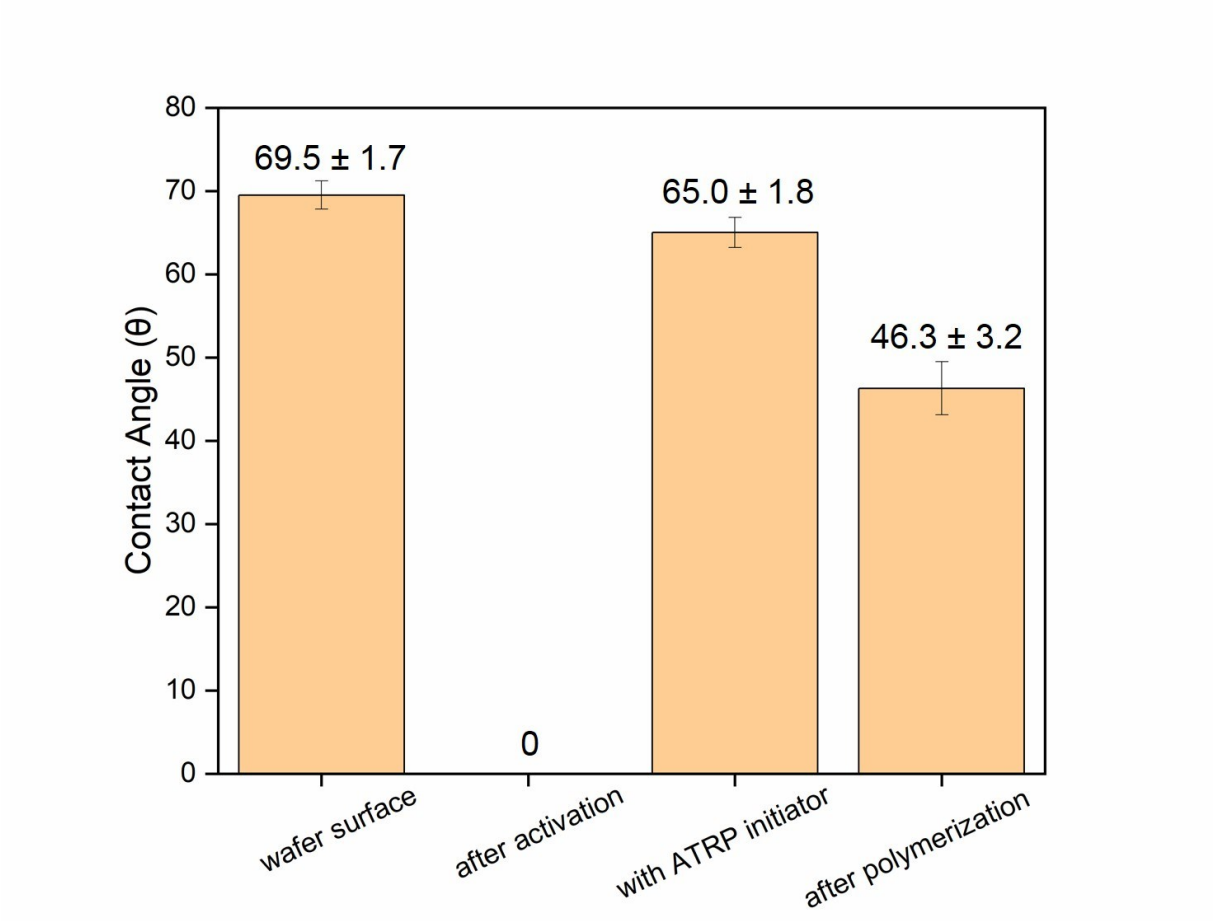

Figure S1. The contact angle values of the surface after different functionalization steps (n ≥ 6).

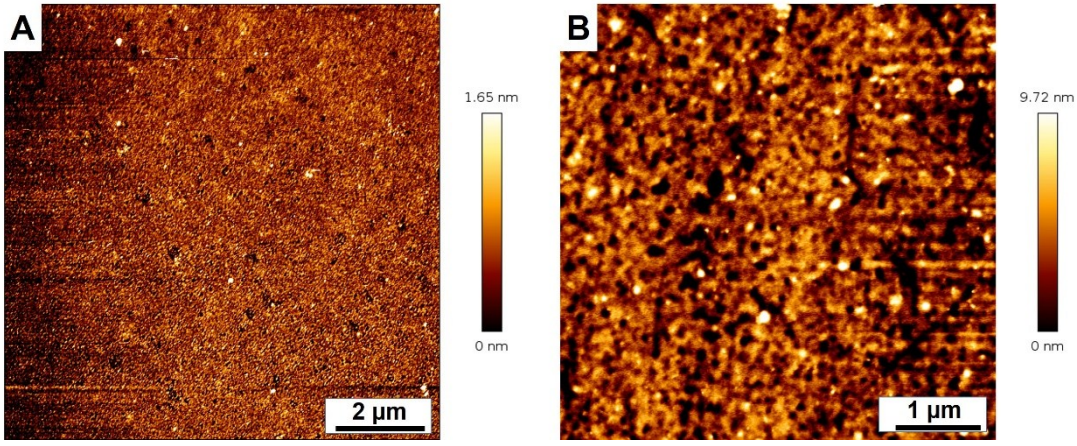

Figure S2. AFM images of the wafer surface. (A) Un-functionalized wafer surface and (B) PHEMA-co-P(2-AEMA.HCl) modified wafer surface.

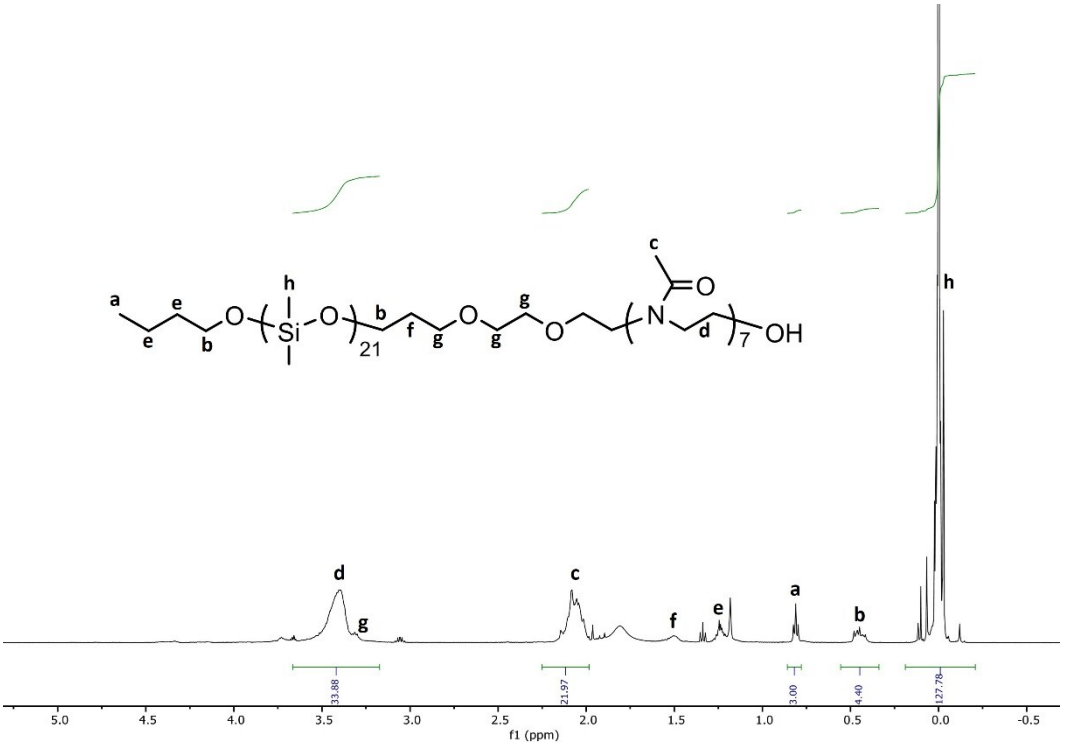

Figure S3. ^{1}H NMR spectrum of the $PDMS_{21}$-$PMOXA_{7}$-OH in chloroform-d.

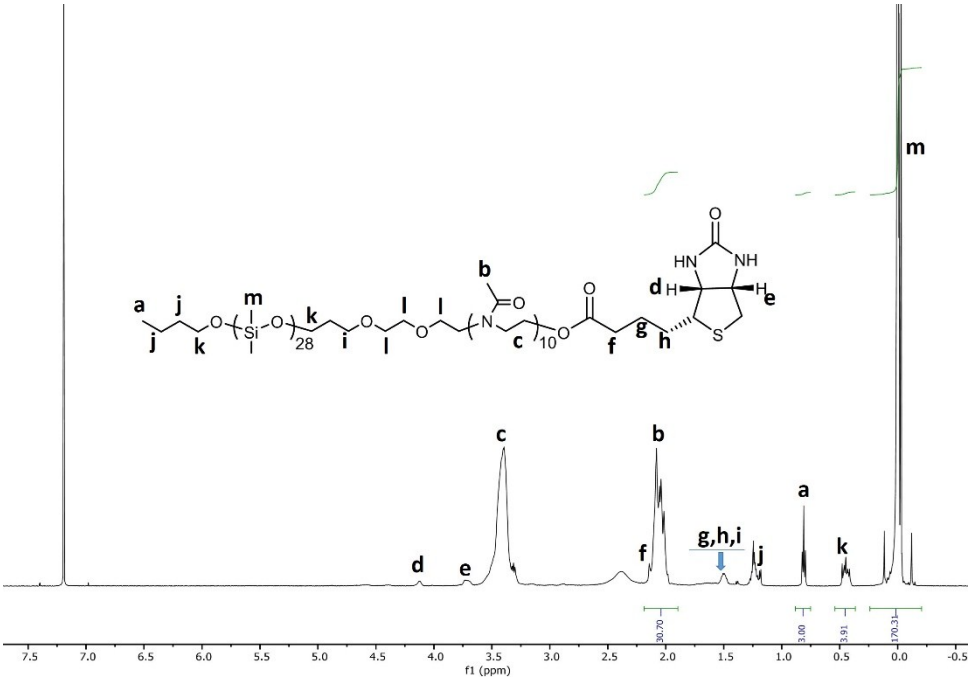

Figure S4. ^{1}H NMR spectrum of $PDMS_{28}$-$PMOXA_{10}$-biotin in chloroform-d.

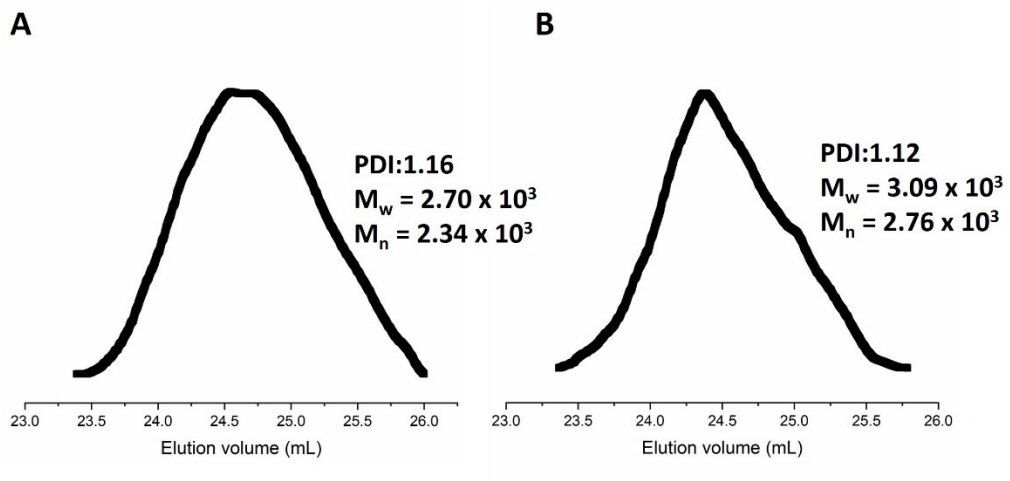

Figure S5. GPC plots of the $PDMS_{21}$-$PMOXA_7$-OH and $PDMS_{28}$-$PMXOA_{10}$-biotin with chloroform as the running phase. (A) $PDMS_{21}$-$PMOXA_7$-OH and (B) $PDMS_{28}$-$PMXOA_{10}$-biotin.

Table 1. The DLS and SLS data summary of prepared polymersome and nanoreactors in PBS buffer.

Sample	R_h (nm) (90 degree)	R_g (nm) (30-135 degree)	$\rho = \frac{R_g}{R_h}$
biotin-polymersomes	96 ± 5	93	0.97
Uricase-nanoreactors	114 ± 10	112	0.99
LPO-nanoreactors	100 ± 10	93	0.93

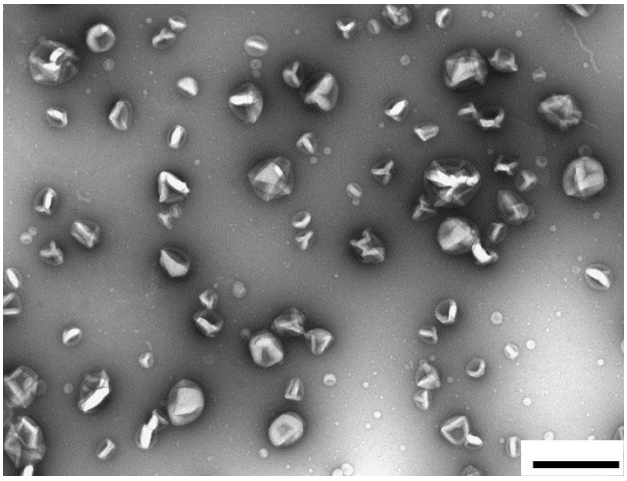

Figure S6. TEM image of the biotin modified polymersome formed by $PDMS_{21}$-$PMOXA_7$-OH and $PDMS_{28}$-$PMOXA_{10}$-biotin diblock copolymers (weight ratio 20:1). The length of the scale bar is 500 nm.

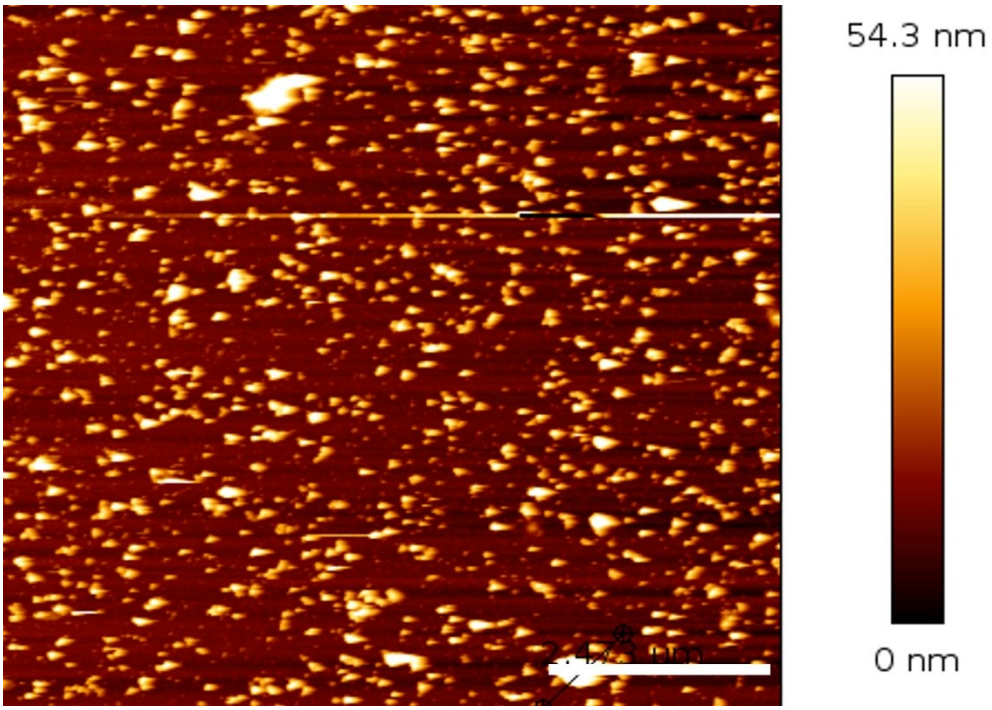

Figure S7. AFM image of the biotin-polymersomes anchored on surface of streptavidin modified surface measured in water at room temperature (c.a 22 °C).scale bar is 5 μm.

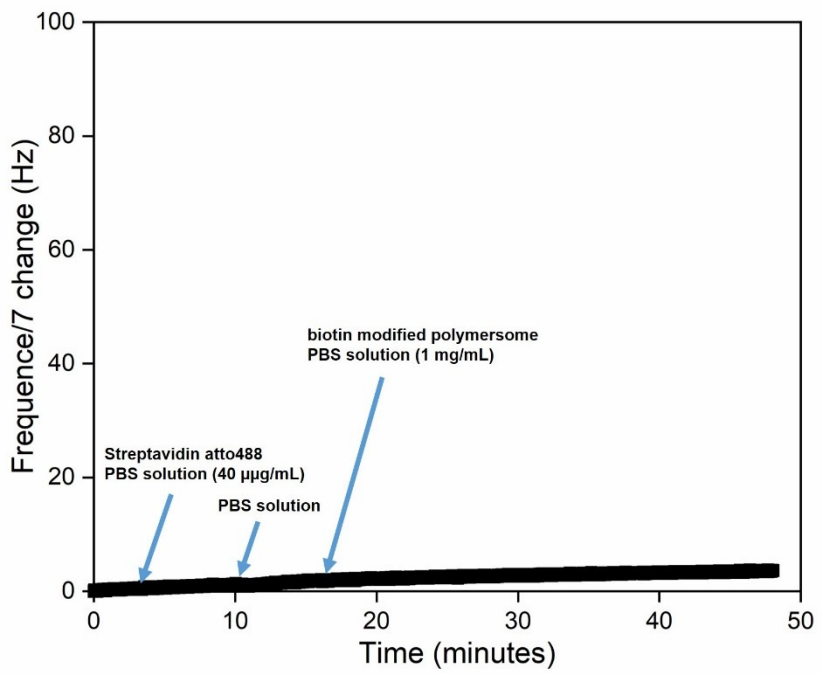

Figure S8. The QCM-D result of the PHEMA-co-P(2-AEMA.HCl) modified silicon chip treated by streptavidin atto488 PBS solution and biotin modified polymersome PBS solution.

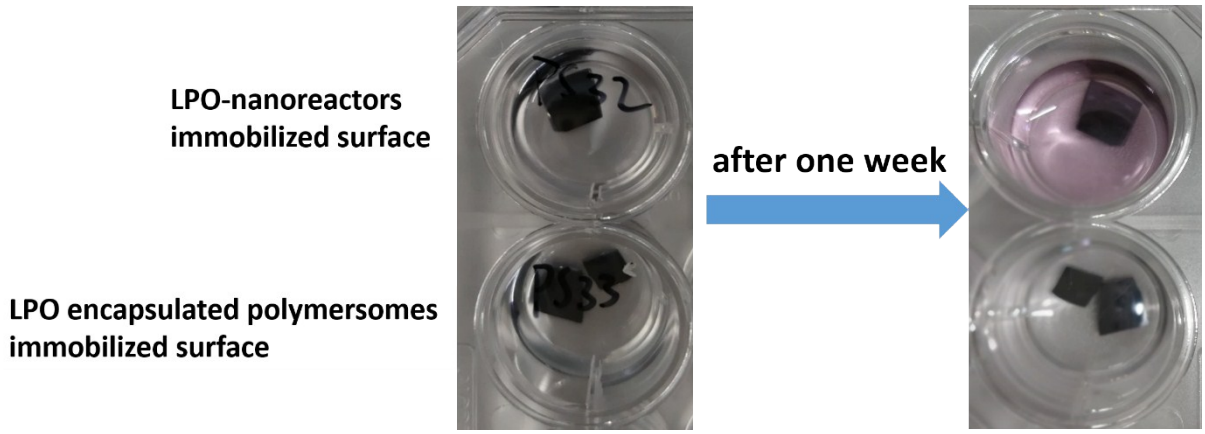

Figure S9. The digital images of the reaction medium of the LPO-nanoreactors immobilized surface in the PBS buffer with amplex red and H_2O_2.

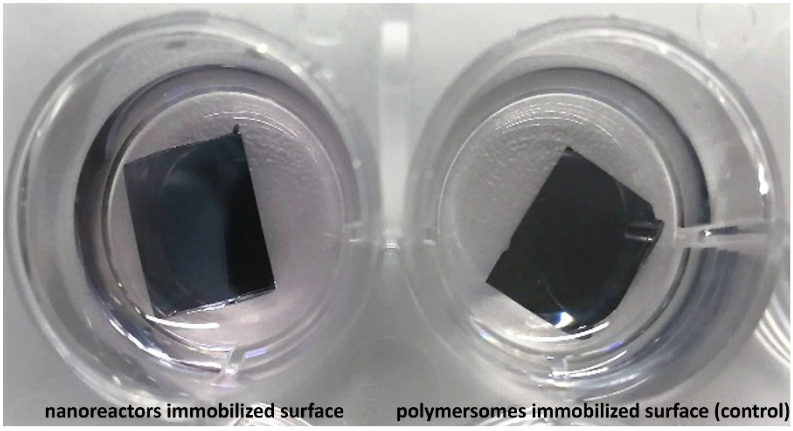

Figure S10. The digital image of the solvents after 9 days at room temperature (ca. 22 °C). Left is nanoreactors immobilized surface and right is enzymes encapsulated polymersomes immobilized surface.

References.

1. A. Belluati, I. Craciun, J. Liu and C. G. Palivan, Biomacromolecules, 2018, 19, 4023–4033.
2. S. Rigo, G. Gunkel-Grabole, W. Meier and C. G. Palivan, *Langmuir*, **2019**, 35, 4557-4565.