Supporting information

Enzyme-driven biodegradable nanomotor based on tubular-shaped polymeric vesicles

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Materials

For the synthesis of the PEG-PDLLA copolymers, methoxy-PEG_{22}-OH (1 kDa) was purchased from Creative PEG Works and N_3-PEG_{67}-OH (3 kDa) was purchased from Rapp Polymere. D,L-Lactide was purchased from Acros Organics. For the formation of polymersomes, ultra-pure MilliQ water was obtained from Labconco Water Pro PS purification system (18.2 ME). Dialysis membranes of MWCO 12-14000 g mol^{-1} Spectra/Por were used to remove the organic solvent. Sodium chloride was purchased from Merck to increase osmotic pressure for the shape transformation of spherical polymersomes. For the coupling of enzymes, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) from Alfa Aesar and N-Hydroxysuccinimide (NHS) from J&K Scientific was used. All other chemicals were supplied by Sigma-Aldrich.

Methods

Nuclear Magnetic Resonance (NMR) was measured on an Agilent 400 MHz INOVA spectrometer equipped with a dual-channel inverse probe and $CDCL_3$ as solvent. ^1H spectra were acquired using 16 scans and a relaxation delay of 2 s.

Dynamic Light Scattering (DLS) measurements were performed on a Malvern Instruments Zetasizer (ZEN 1600), using Zetasizer Software (Malvern Instruments) for analysis of the data. Samples were loaded in Malvern disposable capillary cells. The average of three size measurements with 10 scans of 10 seconds was taken.

Cryogenic Transmission Electron Microscopy (cryo-TEM) pictures were taken on a JEOL TEM 2100 microscope (JEOL Japan). Analysis and processing of the data was performed using ImageJ (a free program developed by NIH and available at imagej.nih.gov/ij/).
Protocol: EM Science TEM grids were glow discharged with a 208 carbon coater (Cressington). On each grid 3 µL sample was added, blotted and immediately vitrified through freeze plunging into liquid ethane at 100% humidity using an automatic vitrification robot, FEI Vitrobot™ Mark IV (blot time 1 s, blot force 3). Samples were loaded in a 914 High tilt cryoholder (Gatan, Munich, Germany) and inserted into a JEOL Transmission Electron Microscope 2100 (Japan) at 200 kV. Images were

taken with a 4096 x 4096 pixel CCD camera (Gatan). The average dimensions and membrane thickness of each sample were obtained from different regions (images) and analyzed with plot profile tools of ImageJ.

Fourier-Transform-Infared Spectroscopy (FTIR) measurements were performed on a Bruker TENSOR 27.

Confocal imaging was done on a Leica (Wetzlar, Germany) SP8 confocal microscope equipped with a 63x/1.30 GLYC immersion objective. Detectors used were HyD (480nm - 541nm) Standard mode, HyD (596nm - 688nm) Standard mode.

Nanoparticle Tracking Analysis (NTA) was performed on a nanosight LM10 at 20x magnification. This technique combines laser light scattering with a CCD camera (30 fps) to track individual particles between 30-1000 nm size in real time. It uses the Stokes-Einstein equation ($D = \frac{T * kB}{3\pi\eta d}$, where D is the particle diffusion coefficient, kB the Boltzmann constant, η the viscosity, T the temperature and d the hydrodynamic diameter) to correlate the tracking coordinates obtained from the displacement of the particles with their size (5). In this experiment we analyzed the movement of catalase functionalized nanotubes with addition of three concentrations of hydrogen peroxide (0.07%, 0.18% and 0.35% v/v H_2O_2).

Protocol: nanomotors were diluted in 1 mL PBS buffer (0.05 M, pH 7) so that single motors were visualized (Video S1). To these samples 0, 2, 5 or 10 µL of a H_2O_2 stock solution (35 wt%) were added before injection into the sample cell. Videos of 60 seconds were recorded and processed by the NTA2.2 software. By analyzing the video, x and y coordinates of each particle were determined as a function of time intervals. Mean square displacements obtained for 100 frames by averaging over 15-20 particles were plotted versus the time intervals (Figure S3). The diffusion coefficient (D) of the nanotube motors at these concentrations were extracted from the linear fit of the MSD ($\langle r2 \rangle$) versus the time (t) according to the equation $\langle r2 \rangle = 4D \cdot \Delta t$. The diffusion coefficients of the nanomotors at 0, 0.07, 0.18, and 0.35% v/v H_2O_2 were determined to be 9.2, 10.3, 15.4, and 22.0, respectively.

Experimental procedures

Synthesis of poly(ethylene glycol)–poly(D,L-lactide) block copolymer:

Poly(ethylene glycol)–poly(D,L-lactide) (PEG-PDLLA) was synthesized by ring opening polymerization (ROP) as described in previous work [22]. For the formation of PEG_{22}-$PDLLA_{45}$, 0.2 mmol methoxy-PEG-OH macroinitiator (194 mg) was mixed with 9 mmol D,L-Lactide (1.3 g) in a round bottom flask (13 wt% PEG in total). For the azide functionalized polymer the amounts were adjusted to obtain N_3-PEG_{67}-$PDLLA_{90}$ (150 mg N_3-PEG-OH, 634 mg D,L-Lactide). First, the reagents were dried by adding dry toluene and evaporating the solvent completely. Then, 15 mL dry DCM with 0.1 mmol DBU (0.5 equivalents of initiator = 15 µL) was added to the dried material under argon. The reaction was left to proceed for 4 hours at 30 °C. After finishing the polymerization, the mixture was washed thrice with 1M $KHSO_4$, after which it was dried with NA_2SO_4 and filtered off. The polymer was concentrated by evaporating most solvent (~4ml) and then precipitated in ice cold diethyl ether (100 ml). The waxy

substance was dried under nitrogen, dissolved in 1,4 dioxane (4 ml) and lyophilized to yield a white powder (~80% yield). Polymerization was checked with NMR, using the protons of PEG (3.65-3.7 ppm), terminal methyl unit (singlet at 3.40 ppm, in case of methoxy-PEG), lactide CH_3 (multiplet at 1.55-1.65 ppm) and CH (multiplet at 5.15-5.25 ppm) (Figure S1).

Preparation of shape transformed PEG_{22}-$PDLLA_{45}$ polymersomes:

In total four different polymer blends were made; 0, 5, 10, and 30 wt% of N_3-PEG_{44}-$PDLLA_{67}$ mixed with PEG_{22}-$PDLLA_{45}$. The different ratios of PEG_{22}-$PDLLA_{45}$ and N_3-PEG_{44}-$PDLLA_{67}$ were weighed such that total amount was 10 mg. The mixtures were dissolved in 1 mL of organic solvent (4:1, THF:Dioxane v/v) in a 15 mL glass vial with a stirring bar. The vial was closed with a rubber septum and the mixture was stirred for 30 minutes at 800 rpm. Subsequently, 1 mL MilliQ water (50 wt%) was added via a syringe pump at 1 mL per hour (1 hours in total) until a cloudy suspension was obtained. The suspension was transferred to a pre-hydrated membrane (Spectra/Por, molecular weight cut-off: 12-14 kDa) and dialyzed against 1L of MilliQ to obtain spherical polymersomes or 50 mM NaCl to obtain tubular shaped polymersomes for 24 hours in a fridge at 4 °C, with a solution change after 1 hour. Samples were stored in the fridge at 4 °C.

Incorporation of fluorescent dyes inside polymersomes:

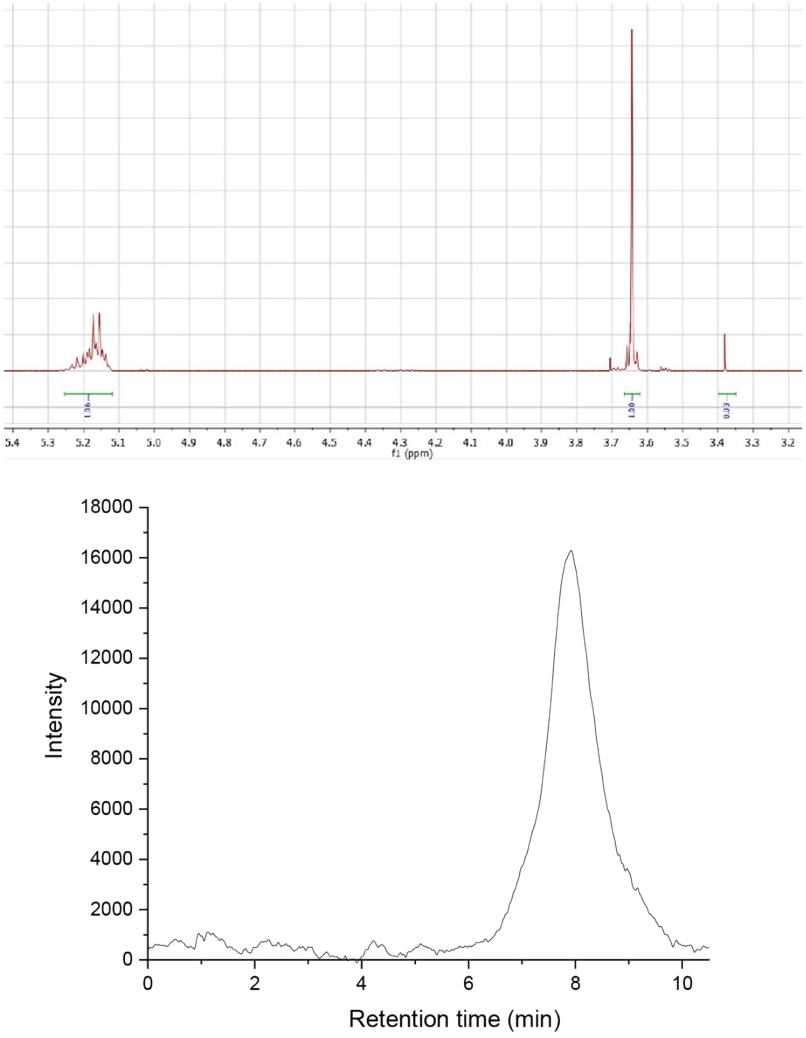
Ten mg of PEG_{22}-$PDLLA_{45}$ was dissolved in 1 mL of organic solvent (4:1 THF:Dioxane v/v) in a 15 mL glass vial with a stirring bar. Ten mg fluorescein sodium salt and 15 µl of a 1 mM Nile red solution in MilliQ were added to the mixture and the vial was covered in aluminium foil. The standard protocol for the preparation of polymersomes was continued. All samples were stored in the dark at 4 °C.

Attachment catalase to azide functionalized nanotubes:

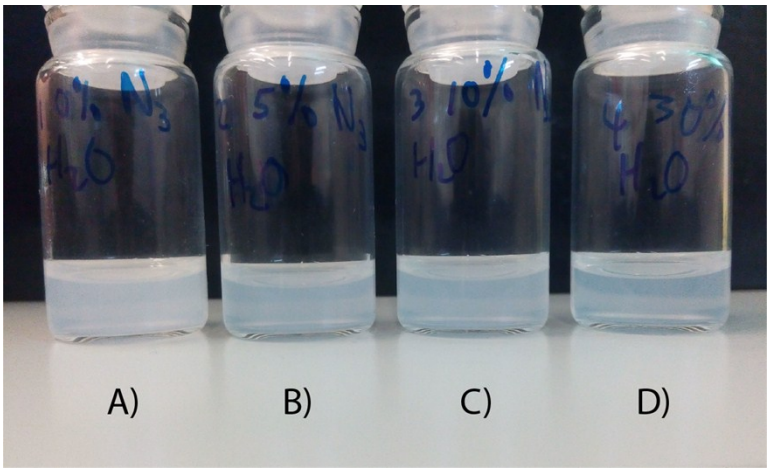
An excess of DBCO-COOH linker (0.1 mg,) in 0.3 mL of MilliQ was added to 0.2 mL of 5 wt% N_3-PEG_{67}-$PDLLA_{90}$ nanotubes in a 5 mL glass vial with a stirring bar. The solution was stirred at 100 rpm for 12 hours at 4 °C to allow the DBCO to completely dissolve and the click-reaction to proceed. Afterwards, 0.5 mL of a 400 mM EDC with 100 mM NHS solution in MilliQ was added. The mixture was stirred at 100 rpm for 1 hour, after which it was centrifuged in 0.22 µm Ultrafree-MC centrifugal filters (Merck, Durapore PVDF) for 5 min. at 5000 rpm, 6 °C. Several washing steps with MilliQ were performed to remove all unbound reactants. The nanotubes were dissolved in 1 mL PBS buffer (0.01 M, pH 7.4) and then mixed with 0.5 mL catalase solution (2mg, >20.000 units/mg protein) in PBS in a 5 mL glass vial. The coupling reaction was performed overnight at 4 °C with stirring at 100 rpm. After the coupling the nanotubes were centrifuged in 0.22 µm filters for 5 min. at 5000 rpm, 6 °C and subsequently washed several times with MilliQ.

Supplementary Table 1: Overview of copolymer composition

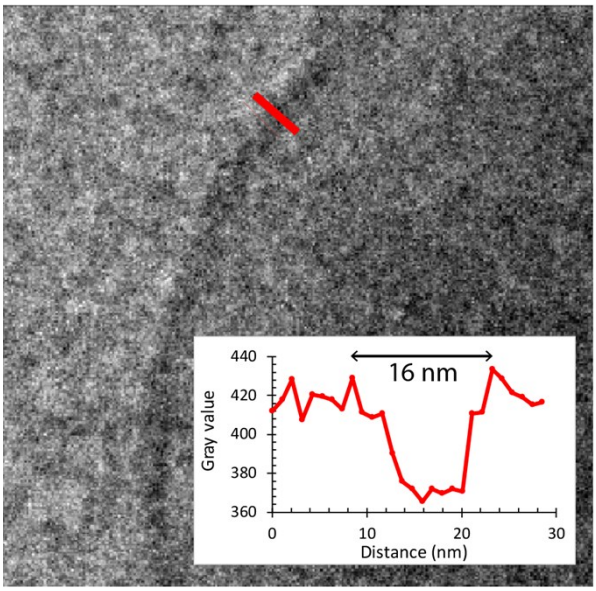
Polymer	Polymer composition (NMR)	PDI (GPC)
PEG22-PDLLA45	PEG22-PDLLA46	1.09
N3-PEG67-PDLLA75	N3-PEG67-PDLLA77	1.10



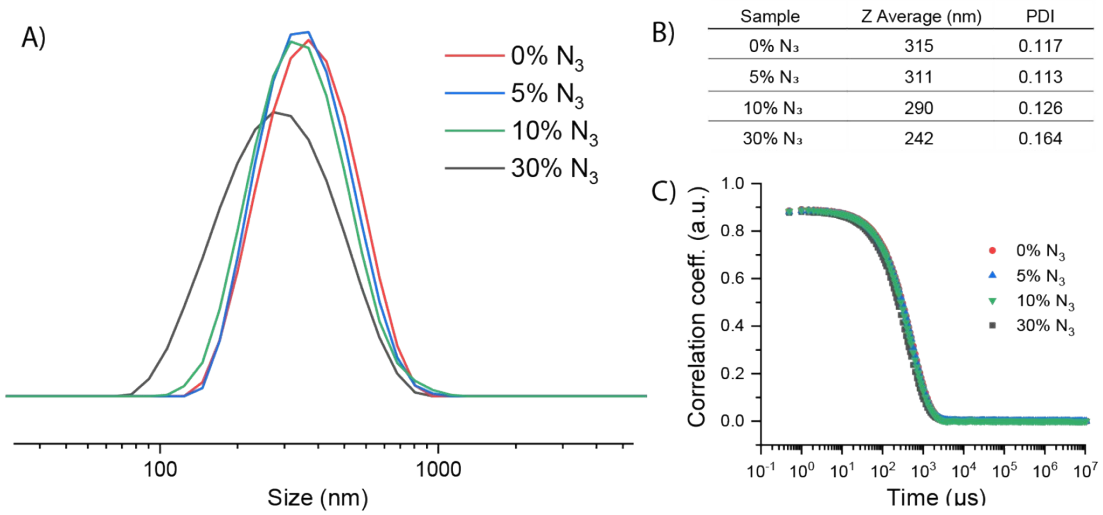
Supplementary Figure 1: Characterisation of PEG-PDLLA polymers. A) NMR spectrum of PEG_{22}-$PDLLA_{45}$. B) GPC chromatogram of PEG_{22}-$PDLLA_{45}$.



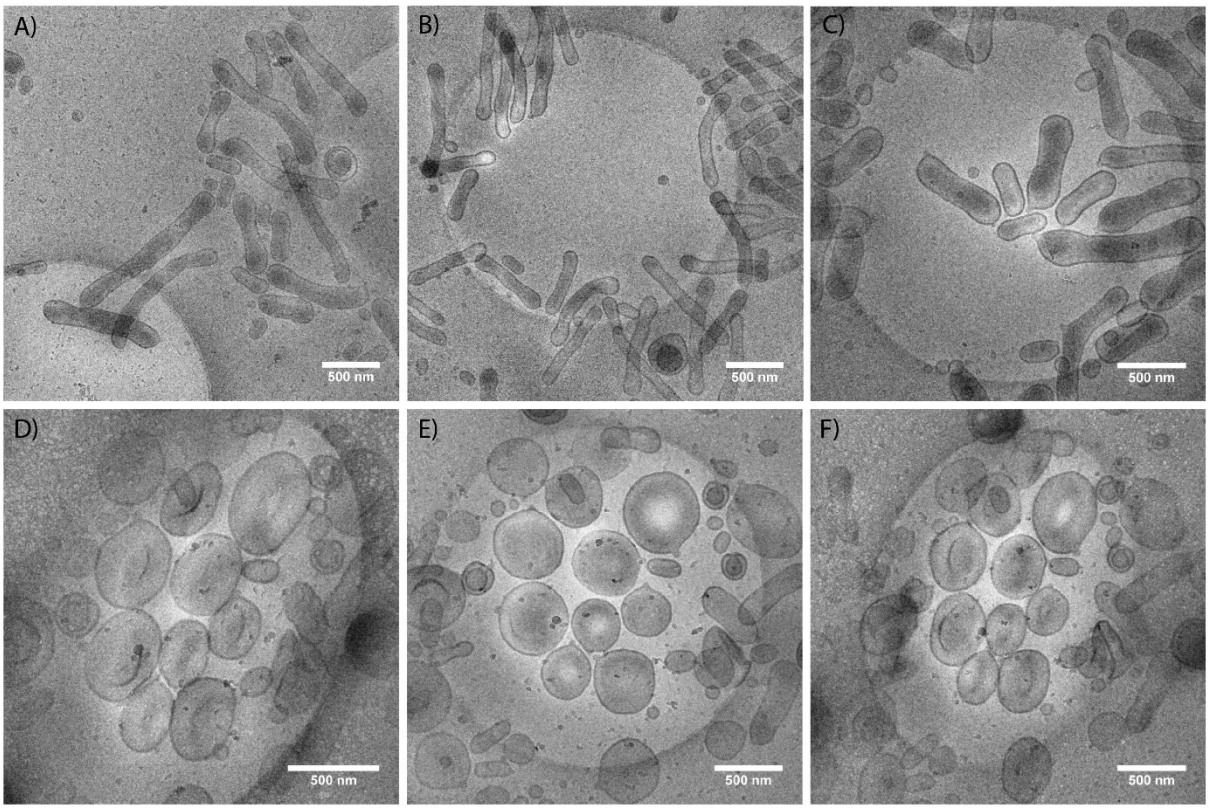
Supplementary Figure 2: Formation of spherical PEG_{22}-$PDLLA_{45}$ polymersomes with different wt% N_3-PEG_{44}-$PDLLA_{67}$ dialyzed against MilliQ A) 0% N_3-PEG_{44}-$PDLLA_{67}$ B) 5% N_3-PEG_{44}-$PDLLA_{67}$ C) 10% N_3-PEG_{44}-$PDLLA_{67}$ D) 30% N_3-PEG_{44}-$PDLLA_{67}$.



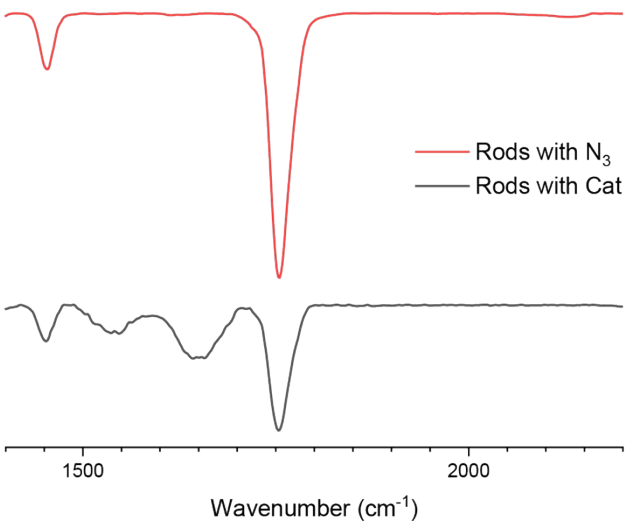
Supplementary Figure 3: Membrane thickness of spherical PEG_{22}-$PDLLA_{45}$ polymersomes, visualized with plot profile function of ImageJ.



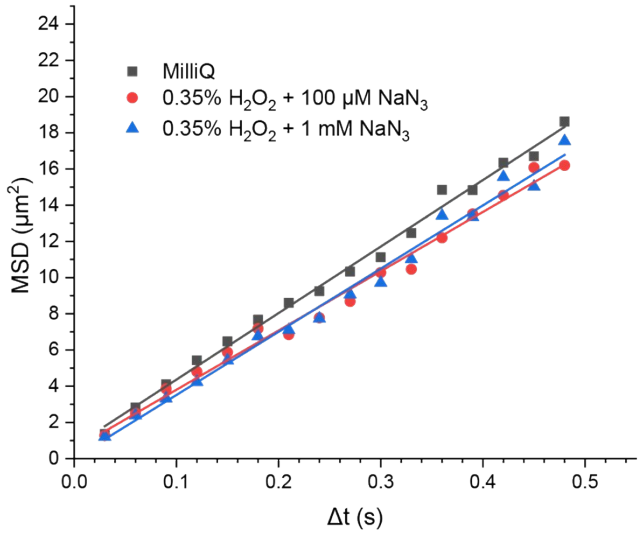
Supplementary Figure 4: Size overview of spherical PEG-PDLLA polymersomes measured by DLS. A) Size distribution by intensity. A decrease in size was measured at higher ratios of N_3-PEG_{67}-$PDLLA_{75}$. B) Overview of the average size and polydispersity. C) Correlation coefficients of all samples.



Supplementary Figure 5: Cryo-Transmission Electron Microscopy (cryo-TEM) images of PEG-PDLLA polymersomes dialyzed against 50 mM NaCl A) PEG_{22}-$PDLLA_{45}$ B) PEG_{22}-$PDLLA_{45}$ + 5 wt% N_3-PEG_{44}-$PDLLA_{67}$ C) PEG_{22}-$PDLLA_{45}$ + 10 wt% N_3-PEG_{44}-$PDLLA_{67}$ D-F) PEG_{22}-$PDLLA_{45}$ + 30 wt% N_3-PEG_{44}-$PDLLA_{67}$ D) -45° angle tilt E) 0° angle F) +45° angle tilt.



Supplementary Figure 6: FTIR spectra of PEG_{22}-$PDLLA_{45}$ + 5 wt% N_3-PEG_{44}-$PDLLA_{67}$ polymersomes before (upper red line) and after (bottom grey line) coupling of catalase. Before coupling a small peak of N_3 around 2100 cm^{-1} is present. After coupling, typical peaks of CO stretch absorption from catalase between 1600-1700 cm^{-1} are present.



Supplementary Figure 7: Mean squared displacement (MSD) values of nanotubes obtained at various H_2O_2 concentrations with inhibitor.