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Electronic Supplementary Information

Influence of surface charge on the formulation of elongated PEG-b-PDLLA nanoparticles

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S1- MATERIALS AND METHODS

S1.1- Materials

All chemicals were used as received unless otherwise stated. PEG initiators were purchased from Rapp Polymers and JenKem Technology USA. D,L-lactide was ordered from Sigma-Aldrich (Merck) as well as all other chemicals. Ultra-pure MilliQ water obtained from a Labconco Water Pro PS purification system (18.2 MΩ) was used for the aqueous solutions. ARPE-19 cells and related medium were ordered from ATCC (LGC Standards).

S1.2- Instrumentation

Nuclear Magnetic Resonance spectroscopy (NMR): Proton (^{1}H) NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer with $CDCl_3$ as a solvent and TMS as internal standard.

Size Exclusion Chromatography (SEC): SEC was conducted using a Shimadzu Prominence-i GPC system with a PL gel 5 µm mixed D column (Polymer Laboratories) with a differential refractive index detector and THF used as an eluent with a flow rate of 1 mL/min.

Dynamic light scattering & Zeta-potential measurements (DLS): Size and zeta-potential measurements were conducted using a Malvern Instruments Zetasizer Nano (ZSP), with Zetasizer Software (Malvern Instruments) used for processing and analyzing data. Samples were diluted 100 times in deionized water, with about 7.4 as a resulting pH, and measured in triplicates at room temperature. Size measurements were performed in disposable BrandTech transparent cuvettes with about 500 µL of sample. Zeta-potential measurements were conducted with automatic number of runs (minimum 12), count rates between 150-300 kcps and attenuation of 8- 9, in a specific Disposable Malvern Capillary Cell cuvette, containing 1 mL of sample.

Cryogenic transmission electron microscopy (cryo-TEM): Samples for Cryo-TEM measurements were prepared by first treating the grids (Quantifoil R2/2 Cu 200 mesh grids or Lacey carbon coated, R2/2, Cu, 200 mesh, EM sciences) in a Cressington 208 carbon coater for 40 seconds. Afterwards, 3 µL of sample solution was brought on the grid and blotted in a FEI

Vitrobot Mark III, at 100 % humidity for 3 seconds (offset -3) and directly plunged in liquid ethane. TEM imaging was performed using a FEI Titan (300 kV electron source) with a LaB6 filament and equipped with an autoloader station. Analysis and processing of the data was performed using ImageJ, a program developed by the NIH and available as public domain software at http://rsbweb.nih.gov/ij/.

S1.3- Experimental methods

Synthesis and characterization of poly(ethylene glycol)-poly(D,L-lactide) block copolymers: The synthesis of PEG-b-PDLLA was performed according to a modified literature procedure.[1] Monomethoxy-PEG-OH, tBoc-NH-PEG-OH or COOH-PEG-OH macroinitiator was weighed into a round bottom flask along with D,L-lactide in order to obtain around 13 wt% PEG in the final copolymer composition. In all cases, dry toluene (ca. 50 mL) was then added to the flask and the solvent evaporated in order to dry the contents before polymerization. The dried reagents were then re-dissolved in dry DCM and DBU was added (0.5 equiv. with respect to [initiator]) under Argon. The reaction was stored at RT for around 2 hours, until there was no evidence of the monomer from the ^{1}H-NMR spectra. After completion was confirmed by ^{1}H-NMR, the reaction mixture was diluted using DCM and washed twice with 1M $KHSO_4$ and once with brine before drying with Na_2SO_4, filtering and evaporating most of the solvent. The concentrated copolymer solution (in DCM) was then precipitated into ice cold diethyl ether and the remaining wax was partially dried under nitrogen before dissolving in dioxane and lyophilisation to yield a white powder (70-90% yield). In the case of tBoc-NH-PEG-b-PDLLA polymers, a standard TFA deprotection step was performed to finally obtained NH_2-PEG-b-PDLLA. Copolymer composition was calculated by using the protons of PEG (3.65-3.7 ppm), lactide CH_3 (multiplet at 1.55-1.65 ppm) and CH (multiplet at 5.15-5.25 ppm). All $Đ$ values were calculated to be less than 1.1 using polystyrene standards for calibration, demonstrating that the reaction conditions and base catalyst gave good control over polymerization.

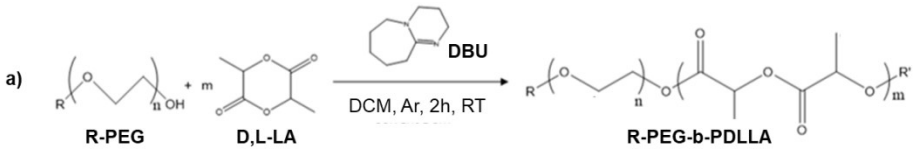
Polymersome and tube formulation: The synthesis of PEG-b-PDLLA was performed according to a modified literature procedure.[1] In a 15 mL vial, block-copolymer (20 mg total) was dissolved in 2 mL of organic solvent - a mixture of distilled THF and dioxane (4:1 v/v). A magnetic stirring bar was added and the vial was sealed with a rubber septum. The solution was let to stir for at least 30 minutes. Afterward, 2 mL of Milli-Q water was added via a syringe pump at a rate of 1 mL/h. After 2 hours, the resulting cloudy suspension was transferred into a dialysis

membrane (SpectraPor, molecular weight cut-off: 12,000-14,000 Da, flat width 25 mm), which was pre-hydrated. The polymersomes were dialyzed at 4 °C against precooled water (1 L) overnight with a water change after 1h. The replacement of the water with a [NaCl] solution at a pre-defined concentration (between 10 and 100mM) was performed in order to osmotically induce a shape change transformation of the spheres into tubes in some cases.

Cytotoxicity assays: ARPE-19 cells were cultured at 37 °C and 5 % CO_2 in DMEM:F12 medium unless otherwise stated. Cells were plated at 2.10^5 cells/well and treated with the 6 types of vesicles (pol 0, pol +, pol -, tub 0, tub +, tub -) at 3 different polymer concentrations (1.25, 0.625, 0.250 mg/mL) for 24h. After incubation with particles, cells were washed 3 times with DPBS, and 10% AlamarBlue solution was added. After 4h incubation, both absorbance at 570nm and fluorescence at 590nm were measured by spectrophotometry.

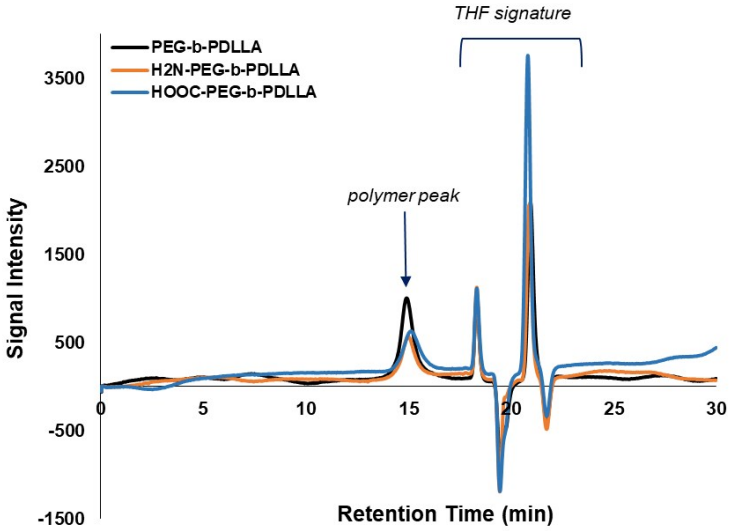
1 L. K. E. A. Abdelmohsen, D. S. Williams, J. Pille, S. G. Ozel, R. S. M. Rikken, D. A. Wilson and J. C. M. Van Hest, *J. Am. Chem. Soc.*, 2016, **138**, 9353–9356.

S1- Synthesis route of the 3 polymers

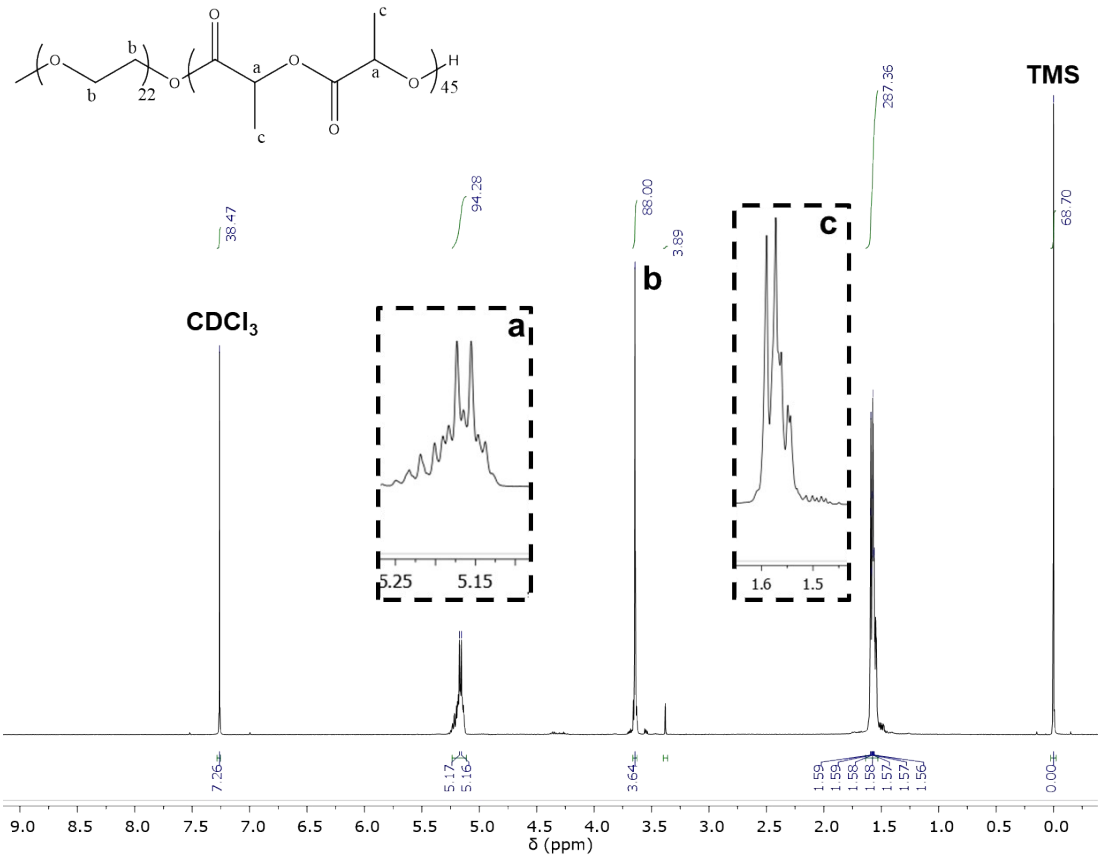


Polymer	Value of R in the synthesis
Unmodified	CH_3
Amino-modified	tBOC
Acido-modified	CO_2H

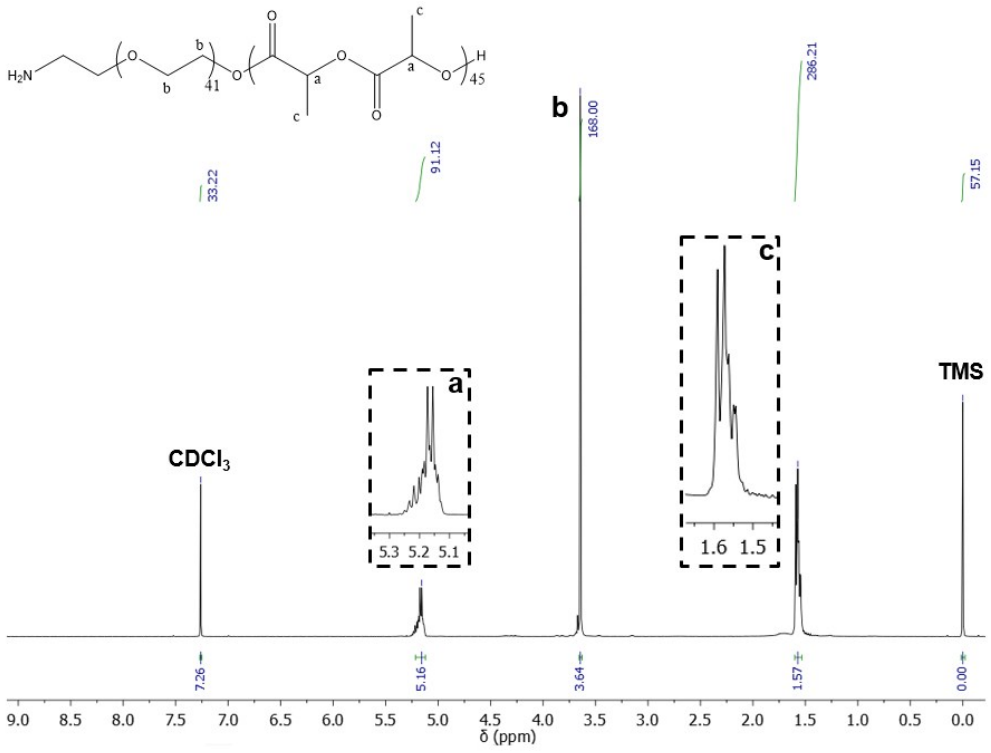
S2- SEC TRACES OF ALL POLYMERS



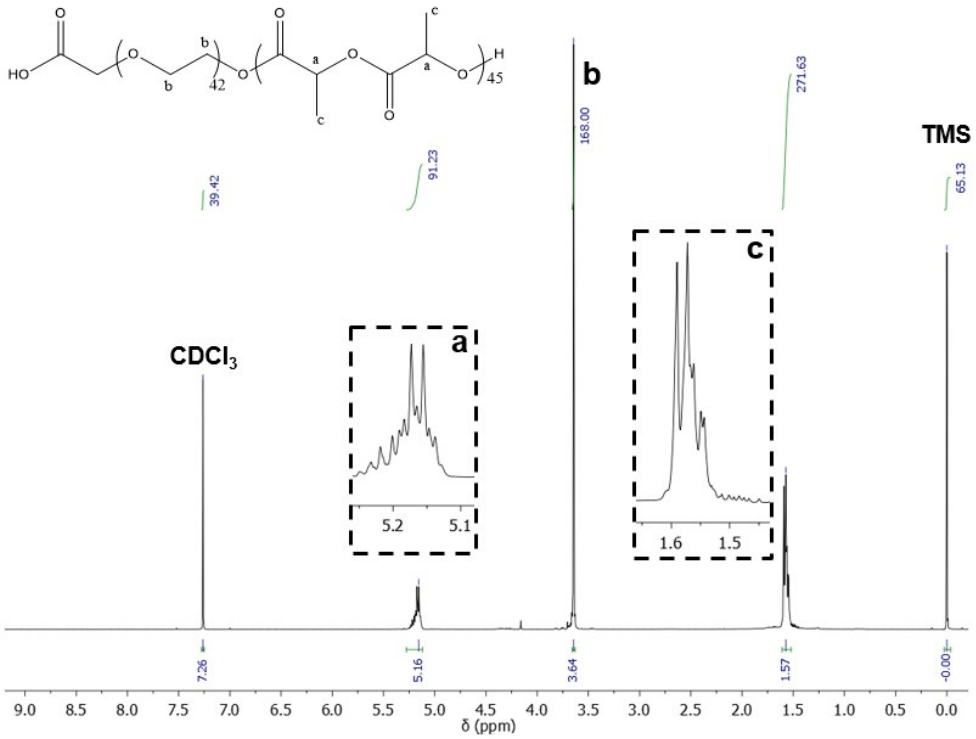
S3- ^{1}H NMR OF PEG-b-PDLLA



S4- ^{1}H NMR OF H_2N-PEG-b-PDLLA

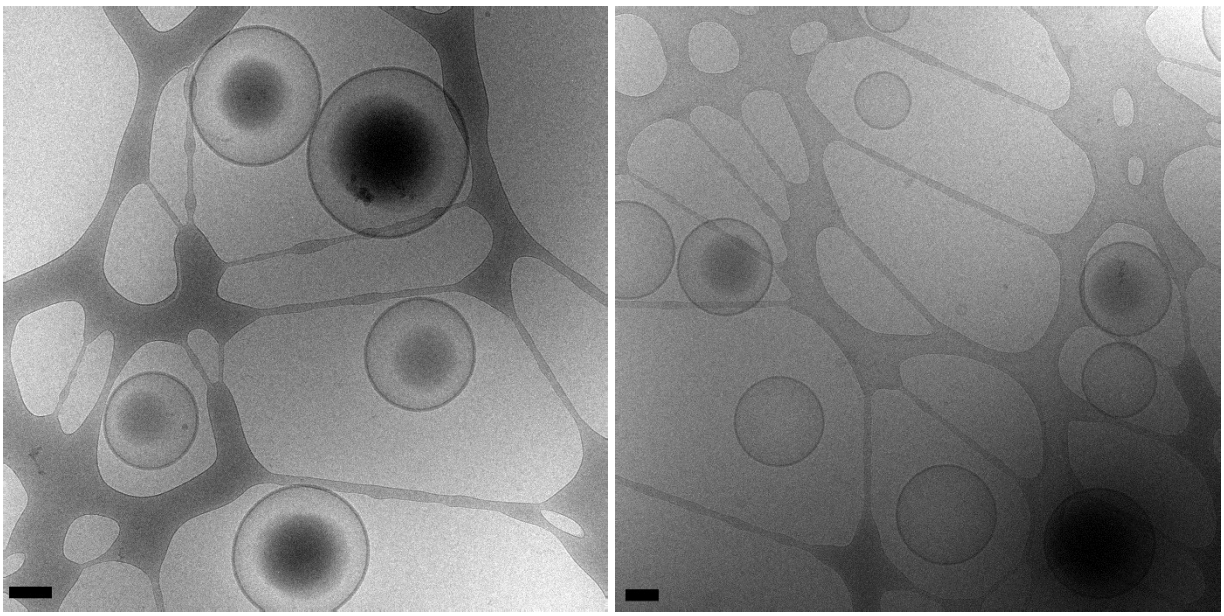


S5- ^{1}H NMR OF HO_2C-PEG-b-PDLLA

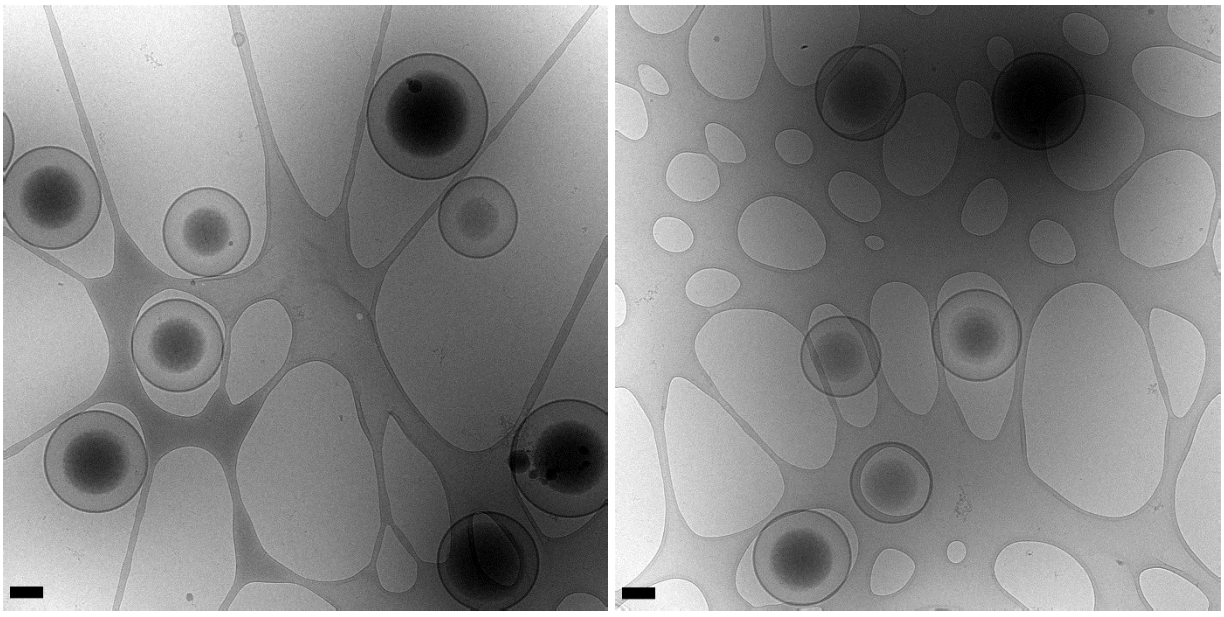


S6- Wide view CryoTEM images when [NaCl]=0 mM (scale bar 200 nm)

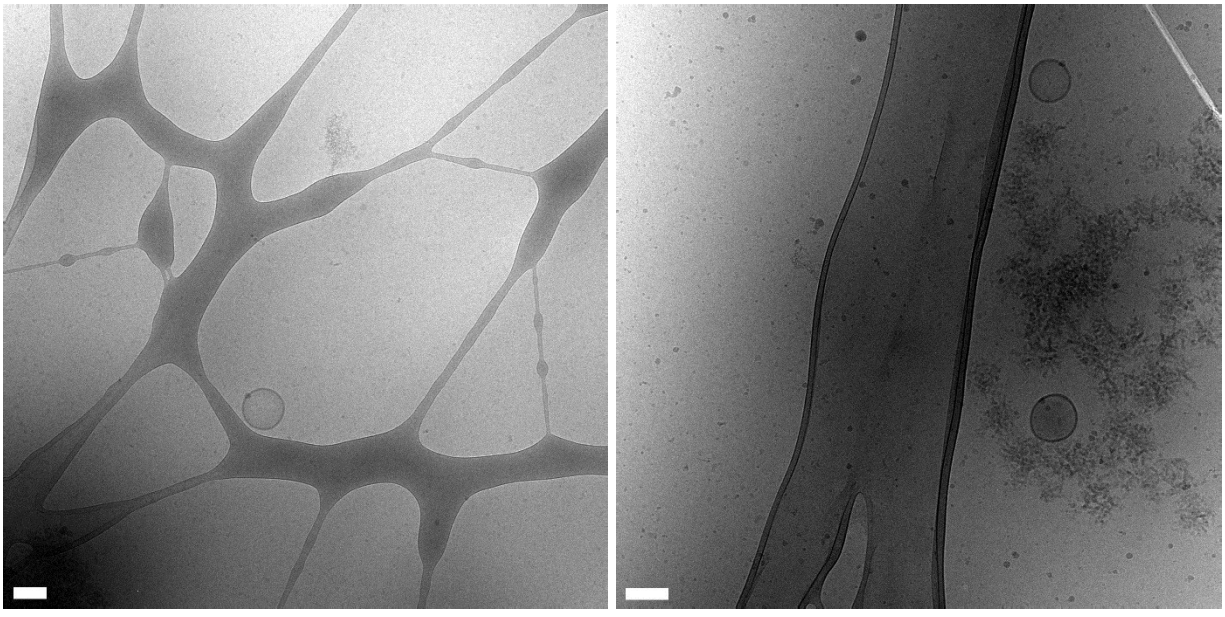
Unmodified



A-modified

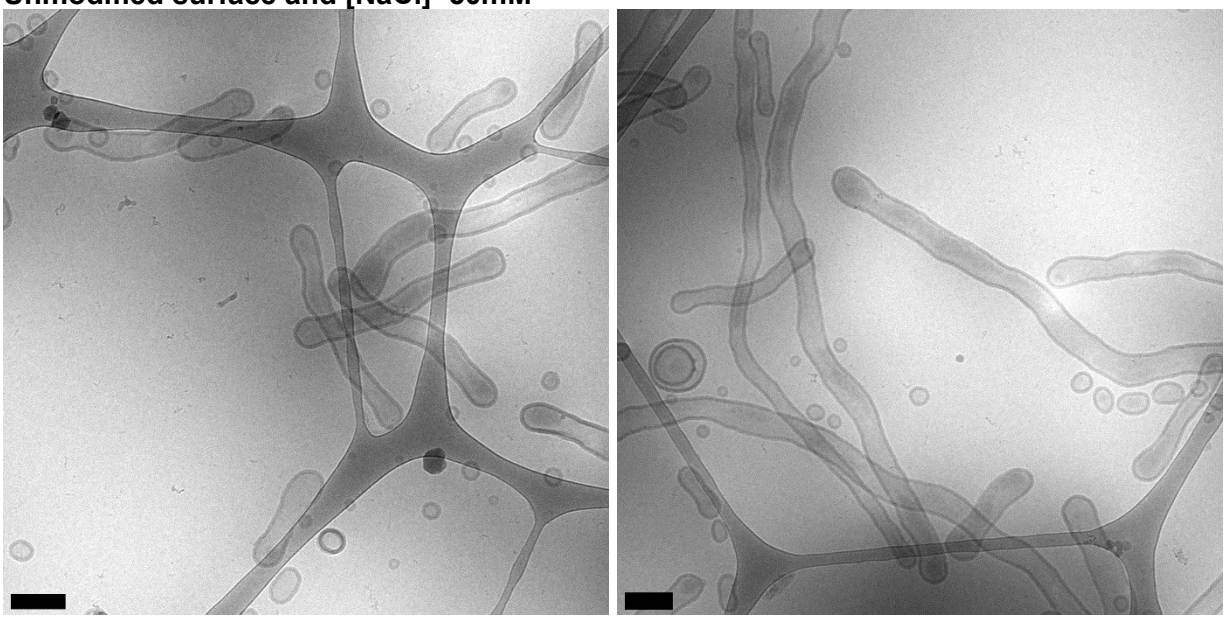


CA-modified



S7- Wide view CryoTEM images figure 3 (scale bar 200 nm)

Unmodified surface and [NaCl]=50mM



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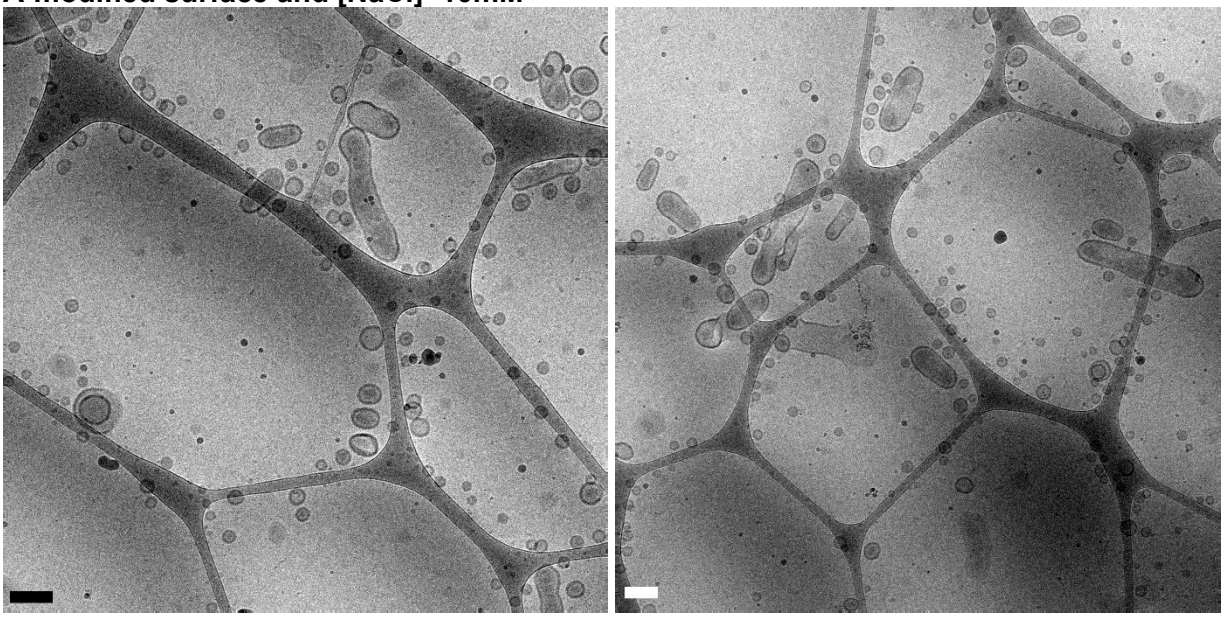

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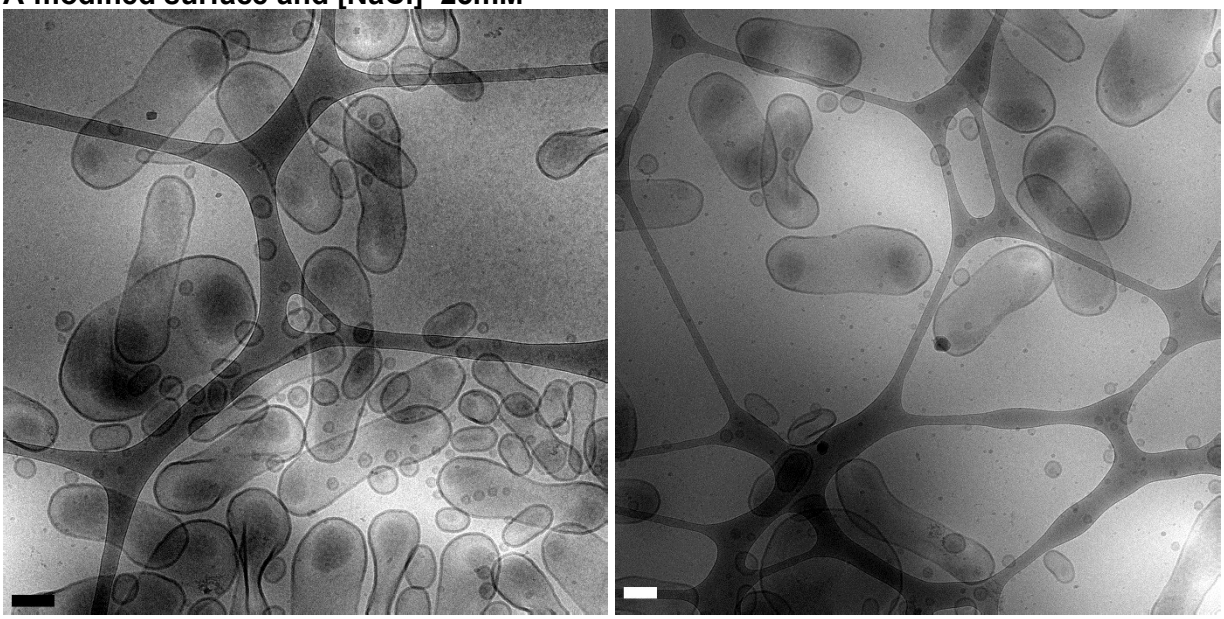
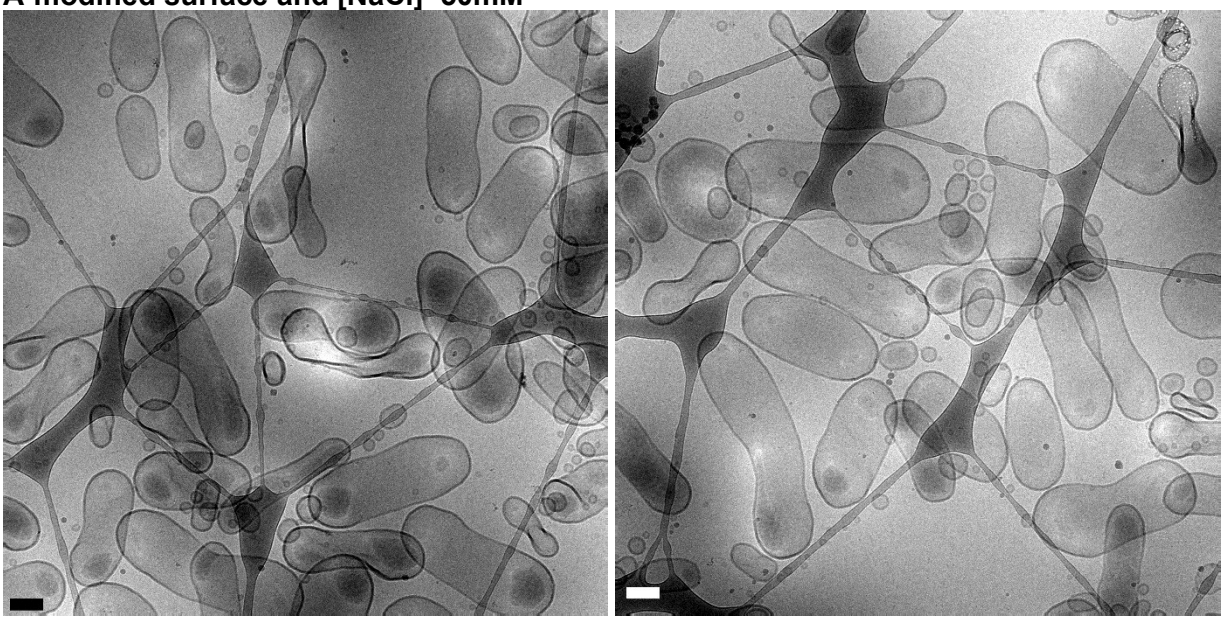


A-modified surface and [NaCl]=50mM



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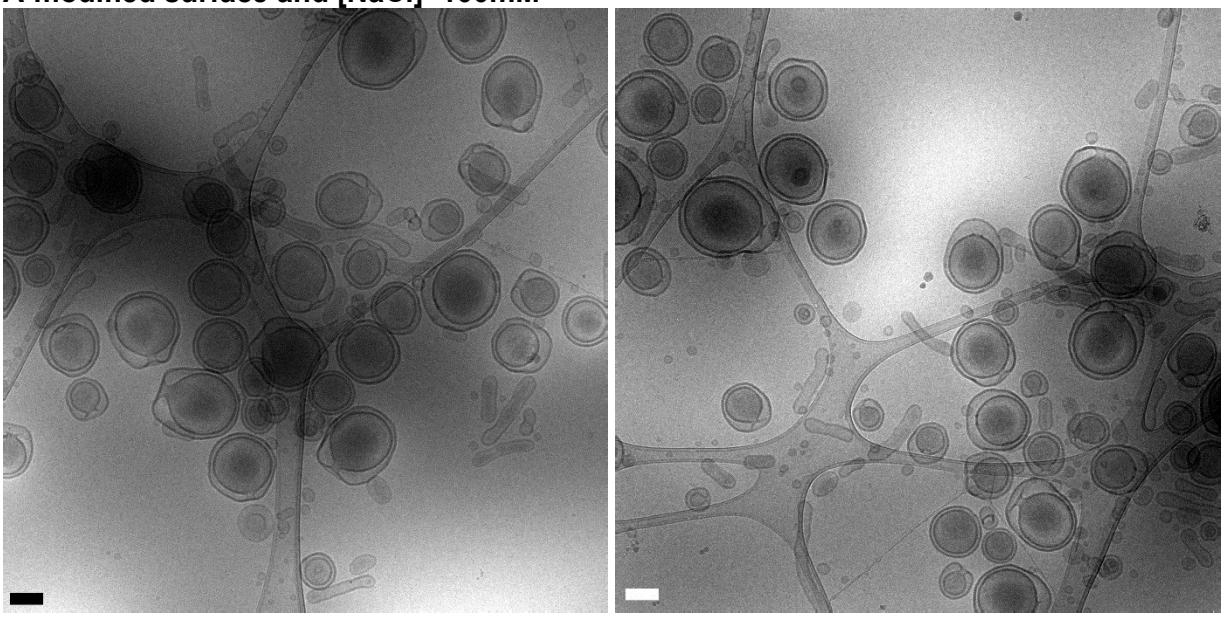

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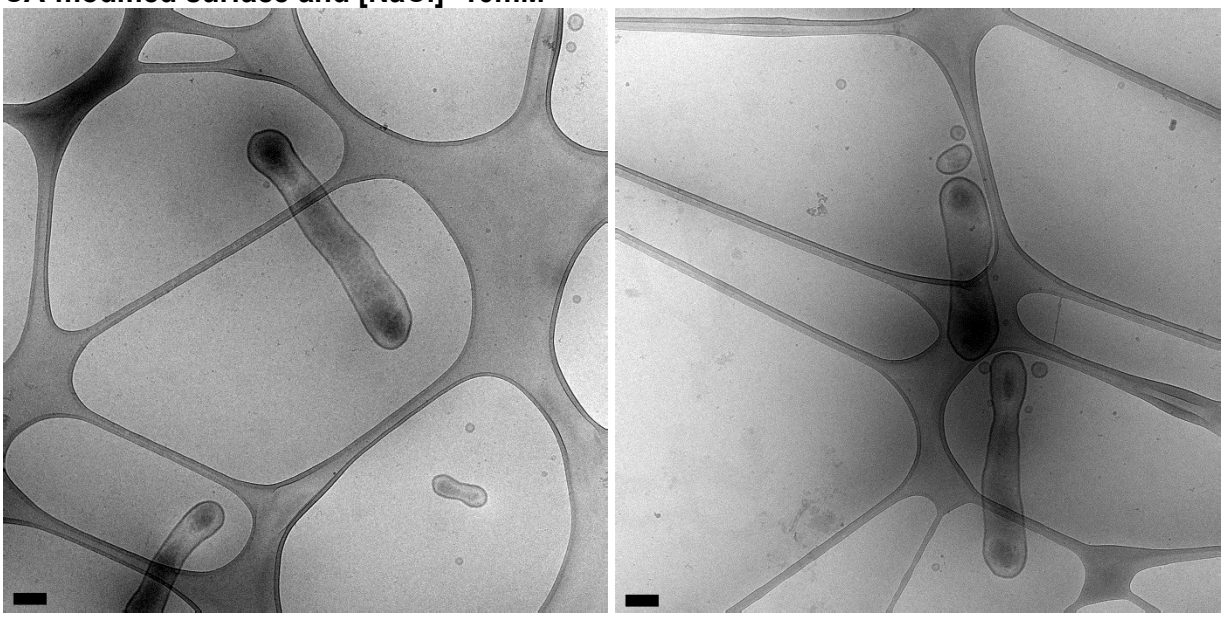

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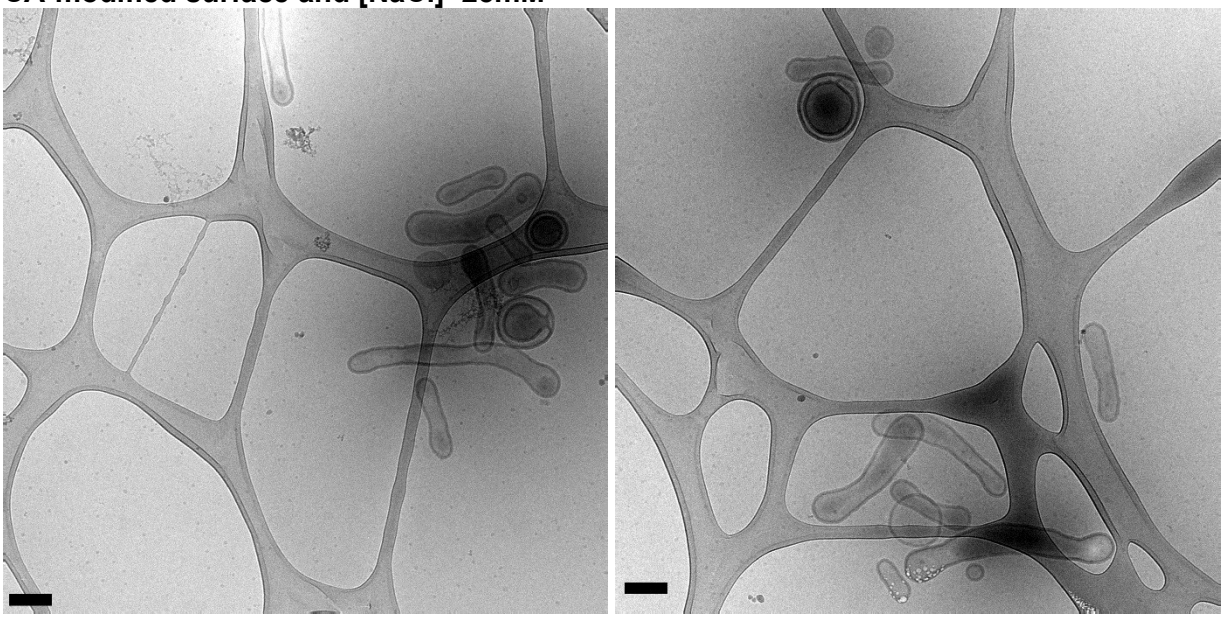

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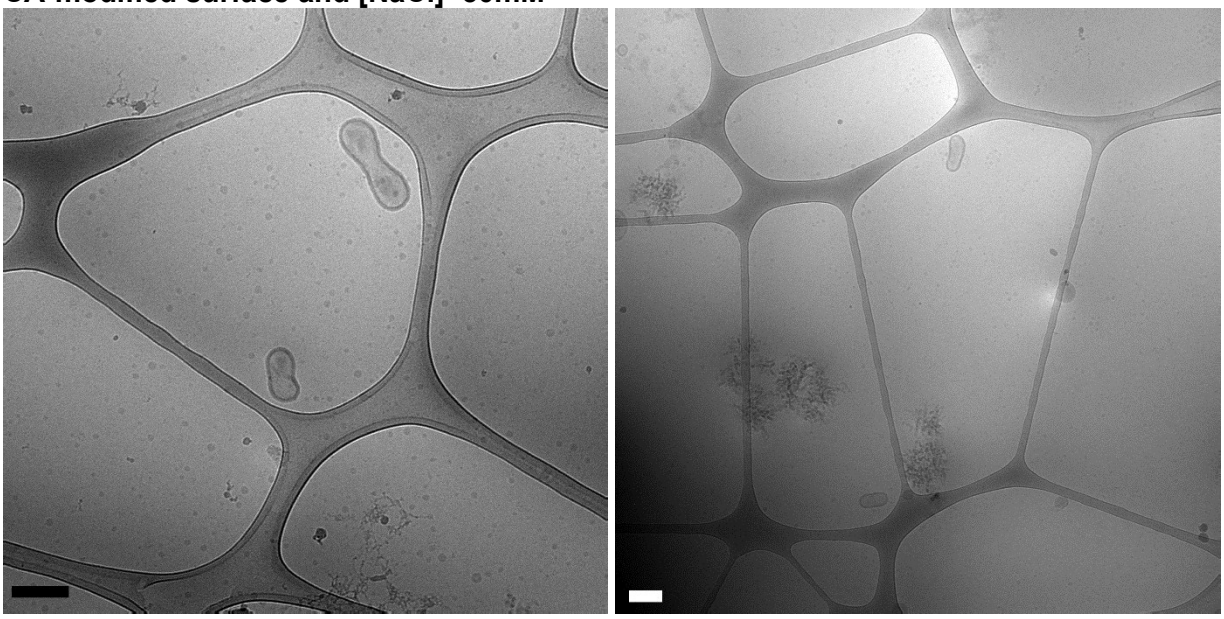

CA-modified surface and [NaCl]=100mM