Multicomponent copolymer planar membranes with nanoscale domain separation

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Supporting Information

1. Materials

Chemicals. Chloroform (analytical reagent grade) was acquired from JT Baker. Toluene (anhydrous, reagent grade) and trichloro(octadecyl) silane (reagent grade) were purchased from Sigma Aldrich. Ethanol (technical grade) was purchased from Biosolve. Lissamine rhodamine B sulfonyl chloride was purchased from ThermoFisher Scientific. All chemicals were used as received. Doubly deionized ultrapure water (18.2 MΩ cm) from Millipore (Germany) was used in all experiments unless otherwise stated.

Synthesis of PEO\textsubscript{45}-b-PEHO\textsubscript{x20} copolymer. PEO\textsubscript{45}-b-PEHO\textsubscript{x20} diblock copolymer was synthetized via cationic ring-opening polymerization of EHOx in a microwave reactor using PEO-Nos as a macroinitiator, based on our previous work.\textsuperscript{1} In a glovebox, a stock solution containing the initiator PEO-Nos, the monomer EHOx and chlorobenzene was prepared. The monomer concentration was set to 1 M and a monomer-to-initiator ratio of 20 was used. The desired volume of the solution was transferred into the microwave vial. The vial was sealed in the glovebox under argon atmosphere prior to the transfer into the microwave reactor. The polymerization of EHOx was conducted at 140 °C for 25 min, reaching 100% monomer conversion.

The mixture was then dissolved in 50 mL of ethanol. To remove chlorobenzene, homopolymers and residual monomers, the solution was placed into an ultrafiltration reactor (from Millipore Corporation, 1 kDa RC membrane). To achieve a total purification, 5 cycles (30 min), with 50 mL of EtOH and under a pressure of 5 bars, were carried out. To dissolve the polymer, 30 mL + 2 x 10 mL of EtOH were added into the reactor for 10 min each without applying any pressure. The combined solutions were concentrated under reduced pressure. Products were characterized by \textsuperscript{1}H NMR spectroscopy and GPC. $M_n$(NMR) = 6000 Da. $D$(GPC) = 1.20. The block ratio was determined by integrating the PEO backbone peak at 3.60 ppm and peaks of
PEHOx side chain at 1.24 and 0.86 ppm. $^1$H NMR (500 MHz, CDCl$_3$, 295K, δ, ppm): 0.86 (m, 6H, CH$_3$), 1.24 (m, 9H, CH(CH$_2$CH$_3$)-CH$_2$CH$_2$CH$_3$), 1.54 (m, 2H, N(COCH$_2$CH$_2$)), 2.24 (m, 2H, N(COCH$_2$CH$_2$)), 3.38 (s, 3H, -OCH$_2$CH$_2$O-CH$_3$), 3.43 (m, 4H, N(COCH$_2$CH$_2$)-CH$_2$CH$_2$), 3.65 (m, 180H, OCH$_2$CH$_2$O).

**Synthesis of PMOXA$_{10}$-b-PDMS$_{25}$ copolymer.** All reactions were carried out in a glovebox under argon atmosphere and in dry glassware according to a previously described protocol. In the first step, the PDMS$_{25}$-H moiety was synthetized from hexamethylocyclotrisiloxane in dried cyclohexane with $n$-butyllithium added dropwise. Dimethylchlorosilane was then added to the mixture and left stirred for four hours in order to quench the polymerization reaction. The solution was subsequently filtered and the solvents were evaporated. The obtained PDMS$_{25}$-H was dissolved in dried toluene along with 2-allyloxyethanol and Karstedt’s catalyst (platinum (0)-1,3-divinyl-1,1,3,3-tetramethyl-disiloxane) and the reaction proceeded overnight at 110 °C to obtain the hydroxyl-terminated PDMS$_{25}$-OH. PDMS$_{25}$-OH was then dissolved in dichloromethane for purification and a colorless oil was obtained.

In the second step, the PMOXA block was attached to the PDMS$_{25}$-OH block via cationic ring opening polymerization of 2-methyl-2-oxazoline (MOXA). PDMS$_{25}$-OH was dried under vacuum overnight at 100 °C. The compound was then dissolved in hexane and trimethylamine was added in a flask while being cooled. Trifluoromethanesulfonic anhydride in hexane was added dropwise in the flask and the solution was then filtered through a glass frit and the triflate-activated PDMS-OTf was obtained. Dried ethyl acetate and MOXA were added and the reaction proceeded at 40 °C for 63 h. The reaction was subsequently quenched by the addition of water and triethylamine. The obtained copolymer was then dissolved in methanol and centrifuged for purification, affording PMOXA$_{10}$-b-PDMS$_{25}$. The solvent from the bottom methanol-enriched phase was evaporated and the purified PDMS$_{25}$-b-PMOXA$_{10}$ was obtained.
as a slightly yellow gel (10.74 g, M_n (GPC) = 2850 g mol⁻¹, 3.77 mmol). PDMS_{25}-OTf and PDMS_{25}-b-PMOXA_{10} where characterized by ¹H-NMR: PDMS_{25}-OTf: ¹H-NMR (500 MHz, CDCl₃, 295 K, δ, ppm): 0.07 (m, 157H, -Si(CH₃)₂-), 0.54 (m, 4H, -Si-CH₂-), 0.88 (t, 3H, -CH₂-CH₃), 1.32 (m, 4H, -Si-CH₂-CH₂-CH₃), 1.62 (m, 2H, -SiCH₂-CH₂-CH₂-O-), 3.45 (m, 2H, -SiCH₂-CH₂-CH₂-O-), 3.74 (m, 2H, -O-CH₂-CH₂-OH), 4.61 (m, 2H, -O-CH₂-CH₂-OTf).
PDMS_{25}-b-PMOXA_{10}: ¹H-NMR (500 MHz, CDCl₃, 295 K, δ, ppm): 0.07 (m, 152H, -Si(CH₃)₂-), 0.51 (m, 4H, -Si-CH₂-), 0.87 (t, 3H, -CH₂-CH₃), 1.31 (m, 4H, -Si-CH₂-CH₂-CH₃), 1.57 (m, 2H, -Si-CH₂-CH₂-CH₂-O-), 2.14 (m, 30H, -N-CO-CH₃), 3.46 (m, 43H, -O-CH₂-CH₂-N-CH₂-CH₂-N-), 3.80 (m, 2H, -N-CH₂-CH₂-OH).

For the assembly of the membranes, PMOXA_{10}-b-PDMS_{25} and PEO_{45}-b-PEHOx_{20} copolymers of similar lengths were used. Their molecular weight and the dispersity were extracted from ¹H-NMR and GPC data, respectively.

2. Methods

Preparation of the solid supports. The surface of silicon (Si) wafers (Si-Mat Silicon Materials, Germany, composition N/Phos, type of orientation <100>) were activated and modified, rendering them either hydrophilic or hydrophobic. The substrates were washed with ethanol and water, dried using nitrogen and the surface was treated with UV-ozone (Jelight Company Inc., Irvine, USA) for 20 minutes to remove any impurities and to render the surface hydrophilic. To obtain the hydrophobic functionalization, a silanization of the native hydrophilic silicon oxide layer³ according to a previously described procedure⁴ has been performed. In brief, substrates rendered hydrophilic by the previously described protocol were immersed in 40 mL toluene mixed with 250 µL trichloro(octadecyl)silane in a glovebox under inert gas atmosphere and left to react for 2 h, resulting in –OH groups on the activated wafers being capped by the silane group to form a covalent Si-O-Si bonds. Substrates were subsequently rinsed with toluene, ethanol and water under ambient atmosphere, thus leading
to clean surfaces with a surface-located hydrocarbon chain. A contact angle of 110 ° was measured after the silanization, confirming the hydrophobic character of the surface obtained.

**Polymer monolayer formation.** Monolayers of the amphiphilic diblock copolymers were obtained in a Kibron Microtrough G2 made of Teflon™ (Kibron Inc, Sweden) of area 16480 mm², equipped with two hydrophilic Delrin™ barriers providing symmetrical film compression and preventing monolayer leakage. The same setup equipped with a dipping module for LB and LS deposition was employed for the transfer of the formed monolayers onto the Si substrates. A platinum Wilhelmy plate (perimeter 8 mm) was used to monitor the surface pressure with an accuracy of 0.1 mN m⁻¹. The solutions of diblock copolymers were freshly prepared at 1 mg mL⁻¹ in chloroform, sonicated for 15 minutes prior to use, then spread dropwise onto the water surface. Before compression, the polymer monolayers at the air/water interface were allowed to stabilize for 15 min to adapt their most favorable conformation and then they were compressed at a rate of 1 mm min⁻¹ while simultaneously recording the surface-pressure isotherms. Before each isotherm recording, the surface pressure was set to 0 mN m⁻¹, the solutions were spread and compressed at a rate of 31 mm min⁻¹ according to the protocol described above.

The air/water interface was monitored using a Brewster angle microscope coupled to a symmetric goniometer (EP³SW system Nanofilm Technologie, Göttingen, Germany) equipped with an Nd YAG laser light source emitting at a wavelength of 532 nm. The Brewster angle for water was set at 53° with polarizer, condenser and analyzer set at 6°, 0° and 4° respectively. A Nikon 10x objective was used to form the image and was collected with a monochrome CCD camera giving an image size of 220 x 250 µm². All experiments were carried out at 20 °C and under dust-free conditions.

**Leakage detection.** To ensure that no significant leakage from the barriers was occurring during the LB and LS transfers, weights were placed on the barriers and a comparison in the
evolution of the trough area was made by spreading a polymer mixture after stabilization of the monolayers at the air/water interface.

Up until 100 seconds the barriers were closing to reach the desired surface pressure. Then the stabilization of the monolayer took place while a linear decrease of the trough area occurred. The presence of weights influences the slope of the decrease by rendering the curve flatter. In both cases, negligible leakage of the monolayer was observed, slowed down by the presence of weights on the barriers. Either way, the desired surface pressure value for the deposition could be reached, therefore the polymer film transfers were made without the presence of weights on the barriers.

**Transfer onto hydrophilic substrates.** Polymer monolayers at different molar ratios of the respective diblocks were transferred onto Si wafers previously immersed into the subphase using a dipper. For each polymer mixture, a target surface pressure of approximately 85% of the surface pressure value at the monolayer breaking point was set for the transfer, as determined from Langmuir isotherms recorded for the respective mixtures. The monolayers were transferred at a constant rate of 0.5 mm min\(^{-1}\) after a stabilization time of 15 min for the polymer chains to adopt their most favorable orientation. Deposition of a bilayer onto hydrophilic substrates involved Langmuir-Blodgett (LB) transfer as the first step, followed by Langmuir-Schaefer (LS) horizontal transfer by bringing the surface of the substrate to the monolayer formed at the air/water interface at 10 mm min\(^{-1}\) and pulling it out from the surface after a few seconds at 0.5 mm min\(^{-1}\).

**Transfer onto hydrophobic substrates.** The monolayers were deposited onto hydrophobic substrates Langmuir-Schaefer horizontal transfer at 10 mm min\(^{-1}\) for the downward motion followed by an upward motion at 0.3 mm min\(^{-1}\). Bilayers were obtained using Langmuir-Blodgett transfer, with the first layer via a downward motion and the second one in upward motion, both at 0.5 mm min\(^{-1}\). This strategy ensured the adhesion to the substrate of the
hydrophobic blocks during the downward pass while avoiding any unnecessary sample manipulation and subphase cleaning before the deposition of the second layer using LB in an upwards motion.

All samples were stored either in water or in a dry state, depending on the further characterization steps.

3. Characterization of the films

Ellipsometry measurements. The thickness of the films was measured in air, using an imaging ellipsometer coupled with a Brewster angle microscope equipped with a Nd-YAG laser (532 nm). The refractive index was set to 1.5 and the angle of incidence (AOI) variation mode ranged from 50° to 55°. Each measurement was repeated on five different spots of the sample directly after the transfer onto the solid support. The transfer ratio (TR) was calculated to assess the transfer efficiency of amphiphilic block copolymers from the air/water interface onto a solid support, expressed as the ratio between the area diminution (Δ) of the trough during the transfer divided by the solid support area:

Equation S1:

\[
TR \equiv \frac{\Delta \text{monolayer area during deposition}}{\text{Total surface area of the substrate}}
\]

All measurements have been performed at an ambient temperature of 20 ºC at least three times unless specified otherwise.

Surface wettability. The wetting properties films were studied by sessile drop goniometer using a Drop Shape Analyzer DSA25 (KRÜSS, NC, USA) coupled with a CDD camera (IDS, Germany). Water droplets of constant volume (2 µL) were dropped onto the sample surface using a microsyringe with simultaneous video recording. The average static contact angle was then calculated based on the video using the instrument software (Krüss ADVANCE v.1.7.2.1) and fitted using the Young-Laplace equation.¹
**Surface morphology.** The polymer films morphology, both in air and in water, were investigated using atomic force microscopy (AFM) (NanoWizard Ultra, JPK Instruments, Bruker, USA) in intermittent contact mode. Commercially available aluminum coated cantilever DNP-S A (resonant frequency 65 kHz, spring constant 0.35 Nm\(^{-1}\)) was used for imaging all the samples in water. Tap300 Al-G (resonant frequency 300 kHz, spring constant 40 Nm\(^{-1}\)) and Tap150 Al-G (resonant frequency 150 kHz, spring constant 5 Nm\(^{-1}\)) were used for imaging in air bare silicon wafer and silicon substrates covered with polymer films respectively.

**Fluorescence labelling of the polymer films.** PMOXA\(_{10-b}\)-PDMS\(_{25}\) was covalently labelled using lissamine rhodamine B sulfonyl chloride (LRB-SC) though the -OH group of the hydrophilic PMOXA moiety. Bilayers of PEO-\(b\)-PEHOx, 90:10, 50:50 and PMOXA-\(b\)-PDMS films were deposited on glass cover slips (22x32 mm, thickness #1, Menzel-Gläser) using LB and LS deposition techniques. Subsequently, the polymer film covered glass slide was put in contact with a solution of LRB-SC at a concentration of 4 mg mL\(^{-1}\) at pH 9 on the side where the film was deposited. Following, the surfaces were rinsed and stored in water upon further characterization.

**Confocal laser scanning microscopy (CLSM)**

Confocal laser scanning microscopy (CLSM) was used to observe the labelled polymer films at room temperature using the Zen Black software. The measurements were obtained with a Zeiss LSM 510-META/Confcor2 laser-scanning microscope equipped with a DPSS 561-10 laser (561 nm) and a 40x water immersion objective (C-Apochromate 40×/1.2 W korr FCS M27), with the pinhole kept to 1 AU. The laser intensity, as well as the detector and amplifier gains were adjusted to optimize the quality of the images, which were recorded at a resolution of 512 x 512 pixels with a scan time of 5.03 seconds.
Differential scanning calorimetry (DSC)

Thermal transitions were measured using 10 mg of polymer or polymer mixtures for each measurement on a DSC 214 Polyma (Netzsch GmbH, Austria) under a nitrogen atmosphere from -150 °C to 120 °C with a cooling rate of 40 K min\(^{-1}\) and a heating rate of 10 K min\(^{-1}\). The DSC curves shown correspond to the second heating curve.

![DSC curves](image)

*Figure S1. Differential scanning calorimetric curves (second heating) of PEO\(_{45}\)-b-PEHOx\(_{20}\) and PMOXA\(_{10}\)-b-PDMS\(_{25}\) at 100:0; 90:10; 70:30; 50:50; 30:70; 10:90 and 0:100 molar ratios.*

Supporting Data

**Table S1**: Maximum surface pressures (SP°) and SP° for deposition for various molar ratios of PEO\(_{45}\)-b-PEHOx\(_{20}\) and PMOXA\(_{10}\)-b-PDMS\(_{25}\) based layers onto hydrophilic silicon substrates.

| Molar ratio | Max SP° (mN m\(^{-1}\)) | SP° for deposition (mN m\(^{-1}\)) | TR of monolayers | TR of bilayers |
|-------------|--------------------------|-----------------------------------|-----------------|----------------|
| 100:0       | 46                       | 39                                | 1.12            | 1.5            |
| 90:10       | 45                       | 39                                | 0.99            | 1.01           |
| 70:30       | 50                       | 41                                | 0.89            | 1.0            |
| 50:50       | 51                       | 42                                | 1.2             | 1.15           |
Table S2: Maximum SP° at breaking point, and SP° for deposition for various molar ratios of PEO<sub>45</sub>-b-PEOH<sub>20</sub>: PMOX<sub>10</sub>-b-PDMS<sub>25</sub> onto hydrophobic substrates.

| Molar ratio | Max SP° (mN m<sup>-1</sup>) | SP° for deposition (mN m<sup>-1</sup>) | TR of monolayers | TR of bilayers |
|-------------|----------------------------|--------------------------------------|-----------------|---------------|
| 100:0       | 46                         | 39                                   | 1.28            | 1.14          |
| 90:10       | 45                         | 39                                   | 0.91            | 1.31          |
| 70:30       | 50                         | 41                                   | 0.91            | 0.44          |
| 50:50       | 51                         | 42                                   | 1.31            | 0.57          |
| 30:70       | 50                         | 43                                   | 0.99            | 0.74          |
| 10:90       | 50                         | 44                                   | 0.95            | 0.57          |
| 0:100       | 51                         | 45                                   | 0.18            | < 0           |

Table S3: Polymer film thickness values varying with PEO<sub>45</sub>-b-PEOH<sub>20</sub>: PMOX<sub>10</sub>-b-PDMS<sub>25</sub> molar ratio.

| Sample     | Thickness of monolayers (nm) | Thickness of bilayers (nm) |
|------------|------------------------------|----------------------------|
|            | On hydrophilic substrates | On hydrophobic substrates | On hydrophilic substrates | On hydrophobic substrates |
| 100:0      | 0.7 ± 0.0          | 1.8 ± 0.1            | 2.5 ± 0.2         | 4.2 ± 0.1            |
| 90:10      | 0.9 ± 0.1          | 2.1 ± 0.2            | 3.5 ± 0.3         | 3.6 ± 0.2            |
| 70:30      | 1.1 ± 0.0          | 2.2 ± 0.1            | 3.1 ± 0.1         | 4.5 ± 0.1            |
| 50:50      | 1.2 ± 0.1          | 2.3 ± 0.3            | 3.5 ± 0.5         | 4.2 ± 0.1            |
| 30:70      | 1.2 ± 0.0          | 1.4 ± 0.0            | 3.7 ± 0.8         | 0.8 ± 0.0            |
| 10:90      | 1.7 ± 0.1          | 1.5 ± 0.0            | 3.9 ± 0.1         | 1.4 ± 0.0            |
| 0:100      | 2.8 ± 0.1          | 0.5 ± 0.1            | 4.8 ± 0.2         | 0.9 ± 0.1            |
Figure S2. Surface modification of (A) native silicon wafers to obtain (B) hydrophilic “SiOH” and (C) hydrophobic “SiCH₃” silicon substrates.

Figure S3. Shift in phase (upper row, blue cyan) and height type (lower row, red orange) images of monolayers and bilayers deposited onto hydrophilic Si substrates from PEO₄₅-b-PEOHₓ₂₀ and PMOXA₁₀-b-PDMS₂₅ solutions mixed at 70:30, 50:50, 30:70 and 10:90 molar ratios. Imaged in air. Scale bar 1 µm.
**Figure S4.** Shift in phase (blue cyan) and height type (red orange) images of mixtures films of PEO$_{45}$-b-PEHO$_{20}$ : PMOXA$_{10}$-b-PDMS$_{25}$ 70:30 and 50:50 transferred onto hydrophilic silicon wafers in the form of monolayers and bilayers. Imaged in water. Scalebar 1 µm.

**Figure S5.** Shift in phase (upper row, blue cyan) and height type (lower row, red orange) images of silanized silicon substrates imaged in A. air and B. water. Scale bar 1 µm.
Figure S6. A. Shift in phase (upper row, blue cyan) and height type (lower row, red orange) images of one component films of PEO\textsubscript{45}-b-PEHO\textsubscript{20} and PMOXA\textsubscript{10}-b-PDMS\textsubscript{25}, and of mixtures films 90:10 transferred onto hydrophilic silicon wafers in the form of monolayers and bilayers. Imaged in air. Scalebar 1 µm. B. Height profiles of the corresponding copolymer membranes displaying height (nm) vs. offset (µm).

PMOXA\textsubscript{10}-b-PDMS\textsubscript{25} in the monolayer form exhibited a carpet-like texture covered with round nanostructures of ca. 50-100 nm in diameter, presumably due to the formation of vesicles while drying due to the self-assembly properties of the chosen copolymers (Figure S6A, monolayers). Structures of a comparable size have been observed in BAM micrographs (Figure
which confirms the formation of vesicles while at the air/water interface, which the drying process can be analogous to. The formation of such structures occurred in case of the 90:10 molar ratio, where the addition of a small amount of PEO\textsubscript{45}-b-PEHO\textsubscript{20} results in the decrease in number of structures and shrinkage in their size. For one-component membranes, no notable difference in phase shift between the structures and the continuous phase for the monolayers was observed but for the 90:10 molar ratio composition, a domain separation phenomenon occurred and significant for the PEO\textsubscript{45}-b-PEHO\textsubscript{20} one-component monolayer, where structures appear to be grouped within domains. This is presumably the effect of the hydrocarbon side chain present in the PEHO\textsubscript{20} block. The roughness of the monolayers ranged from 1.6 nm to 2.8 nm with the PEO\textsubscript{45}-b-PEHO\textsubscript{20} and the 90:10 films exhibiting similar surface architecture (Figure S6A, bilayers). Comparably, bilayers of the same compositions display generally an increased roughness as opposed to their monolayer analogues and higher differences between highest and lowest point ranging from 10 to 30 nm, showing the presence of the second layer and the increased thickness as compared to the monolayers (Table S3). However, no pattern is visible on the phase images, suggesting that either the domain separation does not occur or takes place on a subnanoscale level.

![Figure S7. Shift in phase (blue cyan) and height type (red orange) images of mixtures films 90:10 and 50:50 transferred onto hydrophilic silicon wafers in the form of monolayers and bilayers. Imaged in water. Scalebar 2 µm.](image-url)
Figure S8. CLSM micrographs of one-component films of A. PEO_{45}-b-PEHO_{20} B. 90:10 ; C. 50:50 and D. PMOXA_{10}-b-PDMS_{25} transferred onto hydrophilic glass cover slips in the form of bilayers. Measured in water. Scalebar 1 µm.
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