

Supporting Information

Visualizing the Solid-Liquid Interface of Conjugated Copolymer Films Using Fluorescent Liposomes

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Experimental methods

Materials

Polystyrene-block-polyethylene glycol (PS-b-PEG), phosphate buffered saline (PBS) and chloroform were purchased from Sigma-Aldrich. The lipids, i.e., 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) and 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPhPE), were purchased from Avanti Polar Lipids (Alabaster, USA). N-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY-DHPE) was purchased from Thermo Fisher Scientific. The series of NDI-T2 copolymers with 0, 10, 50, 90 and 100% glycol chain density was synthesized according to a previously reported protocol based on Stille polymerization.¹

Film preparation

Microscope glass slides and cover glasses (24 × 32 mm; No. 1.5; VWR) were cleaned in NH₄OH:H₂O₂:H₂O (1:1:5) solution for 10 min at 80 °C, followed by O₂ plasma cleaning for 2 min. NDI-T2 copolymers were dissolved in chloroform at a concentration of 4mg/mL. A thin layer of Parylene C was deposited on the clean glass slides before the polymers were spin-cast at 1000 rpm for 30 seconds. After spin-casting, the films were annealed at 40 °C for 1 hour. For comparison, we also drop-casted the same polymer solutions on these substrates, left to dry in ambient atmosphere, followed by the same annealing protocol as used for the spin-cast films.

Vesicle preparation

DPhPC and DPhPE were mixed in chloroform (molar ratio 7:3) with 0.5 mol% BODIPY-DHPE. Bulk chloroform was evaporated under a stream of N₂ and any remaining solvent was removed under vacuum for at least 3 hours. PBS was then added to the dried lipid film which was re-

suspended in a sonication bath for 30 minutes. The resulting multi-lamellar vesicles were extruded through a polycarbonate membrane (Whatman Nucleopore) with a pore size of 50 nm using a mini-extruder (Avanti Polar Lipids).

AFM measurements

The morphology of the polymer films was characterized with an Agilent 5500 SPM system (Agilent Technologies, USA). Measurements were carried out in tapping mode in air or when the films were immersed in PBS.

TEM measurements

Thin films were formed by spin-casting NDI-T2 copolymer solutions on clean glass substrates. The films were delaminated onto an air-water interface after immersing the samples in DI water and transferred to TEM grids (Electron Microscopy Sciences LC305-Cu). A Titan CT transmission electron microscope (FEI) was operated at 300kV and a Gatan ultra-scan CCD camera was used for high-resolution TEM imaging. Images were analyzed using Gatan Microscopy Suite software (GMS 3). Using these images, we estimated the width of the fibrils by drawing a line across a group of fibrils which were in parallel to one another and then by measuring the average distance value over the number of fibrils within this line.

FRAP measurements

Fluorescent images were taken with a Zeiss LSM 880 inverted microscope (Zeiss Germany). The NDI-T2 copolymer films were incubated with 0.5 mol% BODIPY labeled DPhPC/DPhPE vesicles overnight at room temperature. The samples were gently washed with PBS to remove excess vesicles before imaging. A laser irradiation was used to bleach a circular spot in the lipid membrane. The recovery of fluorescence intensity of the bleached spot was recorded to determine

the mobility of the lipid bilayer using the FRAP module in ZEN software. The time $t_{1/2}$ required to achieve half of the maximum recovery intensity was obtained after fitting the data with the FRAP module. The diffusion coefficient (D) was calculated using the following equation:

$$D = \frac{w^2}{4t_{1/2}}$$

where w is the radius of the photobleached spot.

Surface free energy measurements

The surface free energy of copolymer films was determined from static contact angle measurements using a KRUSS DSA100E drop shape analyzer and Advance software (Germany). To calculate the surface free energy of each copolymer film, the contact angles of 3 different liquids, that are water, 1,2-diiodomethane and ethylene glycol, were measured using the sessile drop method.² Each contact angle value was used in the Owens, Wendt, Rabel and Kaelble (OWRK) model to calculate the total surface free energy as well as its polar and dispersive components. The work of adhesion was calculated according to the Young and Dupree equations. The surface tension of the lipid vesicle solution was calculated as 71 mN/m via the pendant drop method and the contact angle of the lipid vesicle solution on top of each copolymer film was calculated using the sessile drop method.

XPS measurements and the analysis of the spectra

XPS experiments were performed using a Kratos Axis Supra instrument equipped with a monochromatic Al K α x-ray source ($h\nu = 1486.6$ eV) which was operated at a power of 150 W and under UHV (in the range of $\sim 10^{-9}$ mbar). All spectra were recorded in hybrid mode using electrostatic and magnetic lenses. The survey and high-resolution spectra were acquired at fixed

analyzer pass energies of 80 eV and 20 eV, respectively. In order to avoid differential charging, we mounted the samples in floating mode and the spectra were thus acquired using charge neutralization. We carried out deconvolution of high-resolution spectra of C 1s as shown in Figure S6. The C 1s spectra were deconvoluted into four or five peaks attributed to seven different binding environments. The peaks at 284.4 eV and 284.8 eV are attributed to $\underline{\text{C}}=\text{C}$ and $\underline{\text{C}}-\text{C}$ bonds, respectively, while the peak at 285.2 eV corresponds to $\underline{\text{C}}-\text{N}$ and $\underline{\text{C}}-\text{S}$ bonds. The peak at 286.2 eV is related to $\underline{\text{C}}-\text{O}-\text{C}$ bond which is a characteristic of ethylene glycol.³ The peak at 288.4 eV is assigned to the carbonyl moiety, that is $\underline{\text{C}}=\text{O}$ and $\text{N}-\underline{\text{C}}=\text{O}$. By integrating the area under each peak, we estimated the percentage of each bond relative to the total carbon atoms at the surface.

QCM-D measurements

QCM-D measurements were performed on the QSense Analyzer system (Biolin Scientific AB, Sweden) using NDI-T2 polymer films coated on Au sensors. The measurements were carried out at 24°C with a flow rate of 100 $\mu\text{L}/\text{min}$ controlled by a peristaltic pump. After stabilization of the baseline in PBS, vesicles in PBS (0.5 mg/mL) were injected into the system. The adsorption of vesicles on top of the films was monitored through the changes in resonance frequency (Δf) and energy dissipation (ΔD) as a function of time using several overtones. The data presented in Figure S7 were measured at the 5th overtone.

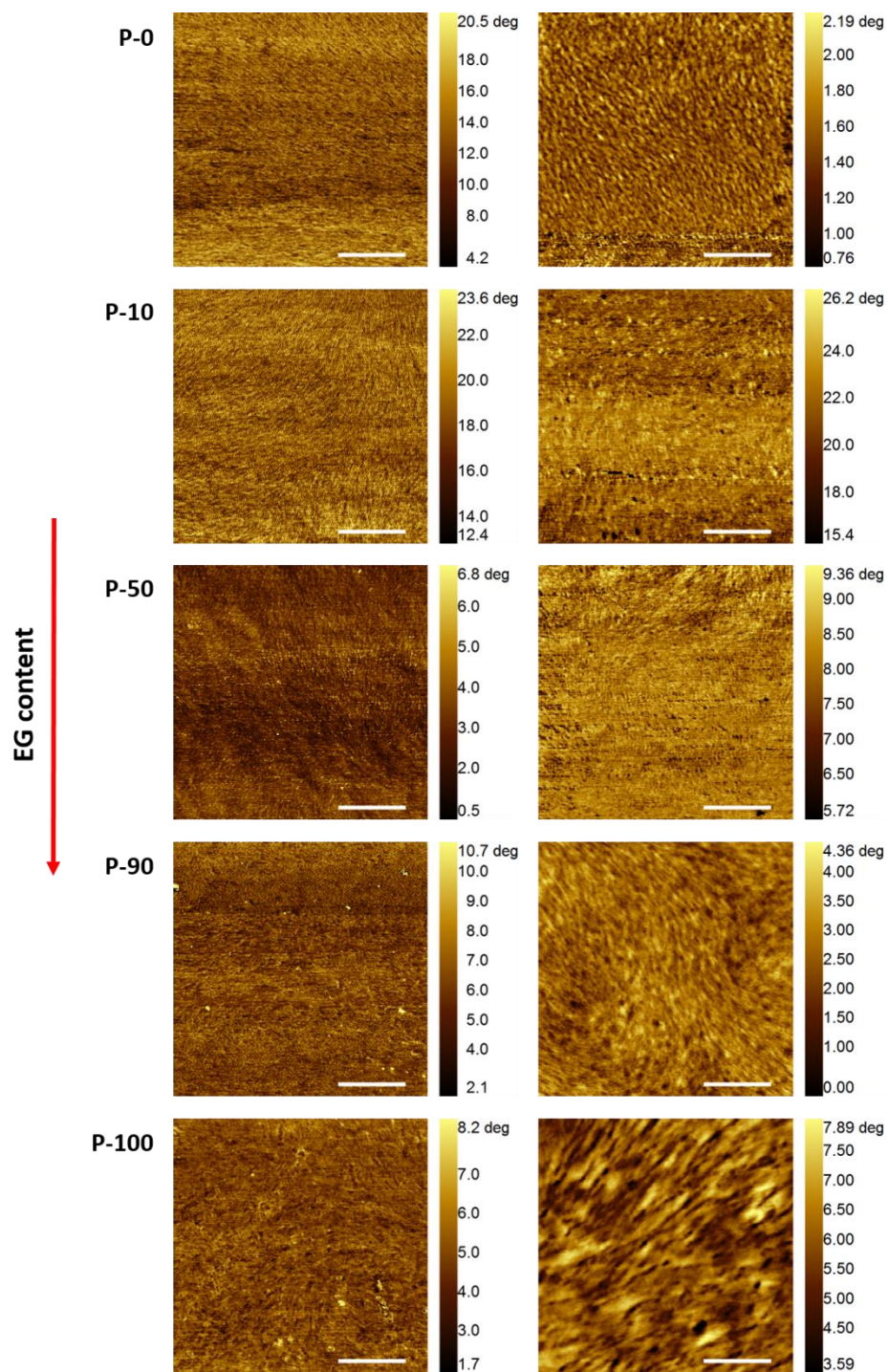


Figure S1. AFM phase images of the polymer films characterized in air (left panel) and in PBS (right panel). The arrow shows the increase in EG percentage in the structure, from 0 to 100%.

Scale bar = 500 nm.

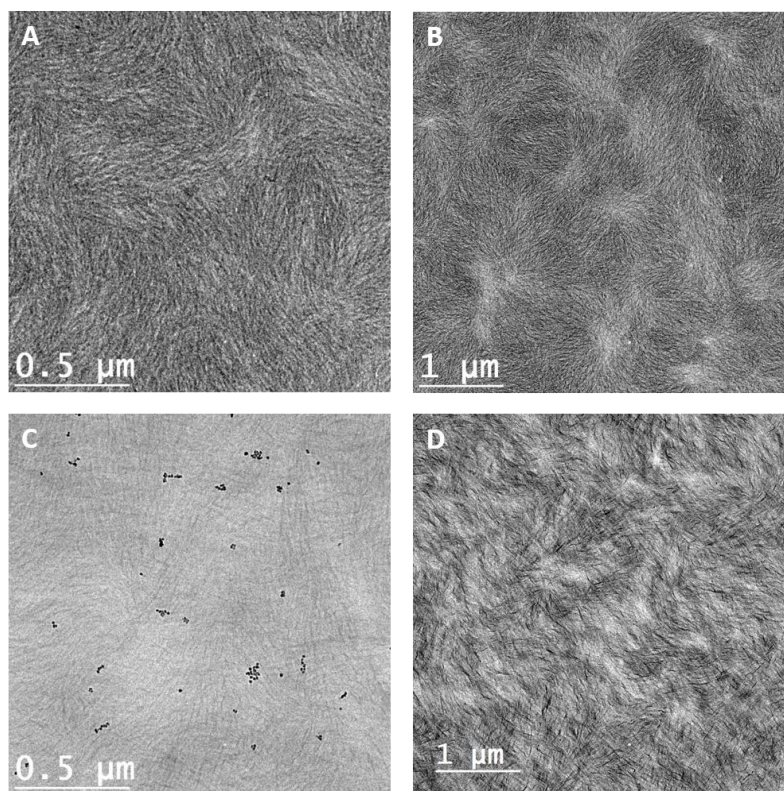


Figure S2. TEM images of polymer films containing (A) 10% (B) 50% (C) 90% (D) 100% EG in their structure.

Table S1. Contribution of the bright and dark regions in fluorescent images for P-10, P-50 and P-90. Also shown is the values for the PS-b-PEG film.

	P-10	P-50	P-90	PS-b-PEG
Bright region (%)	6.7	11.7	13.5	12.0
Dark region (%)	93.3	88.3	86.5	88.0

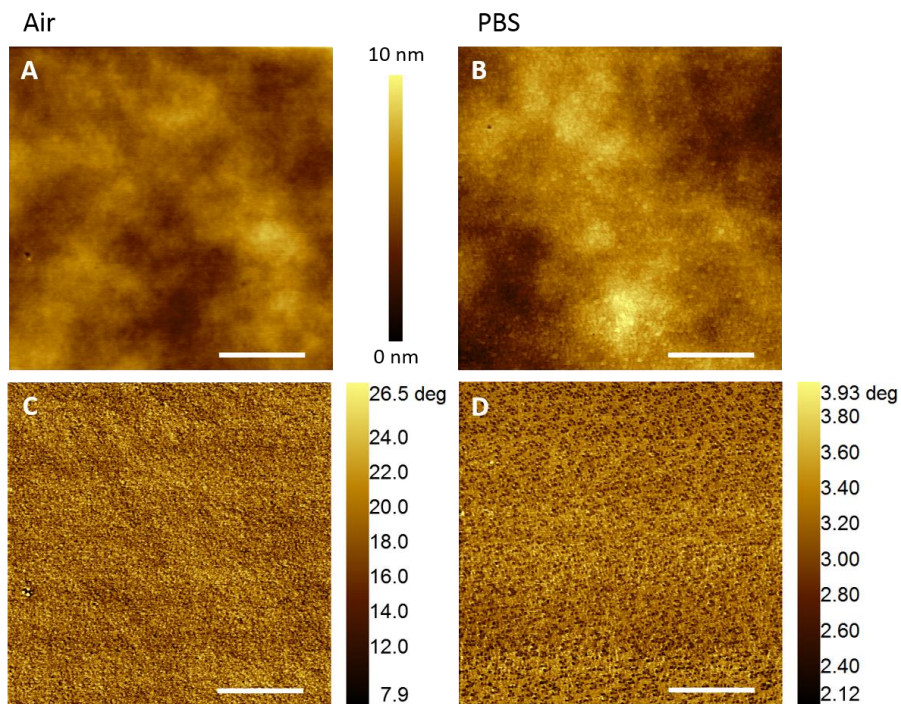


Figure S3. AFM topography (top) and phase (bottom) images of PS-b-PEG film in air (A, C) and in PBS (B, D). Scale bar = 500 nm.

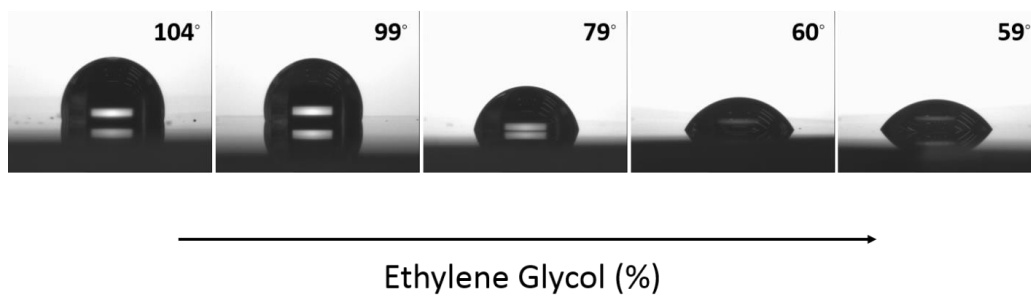


Figure S4. Digital pictures of the drops of the lipid vesicle solution on top of NDI-T2 films with EG content varying from 0% to 100% (left to right).

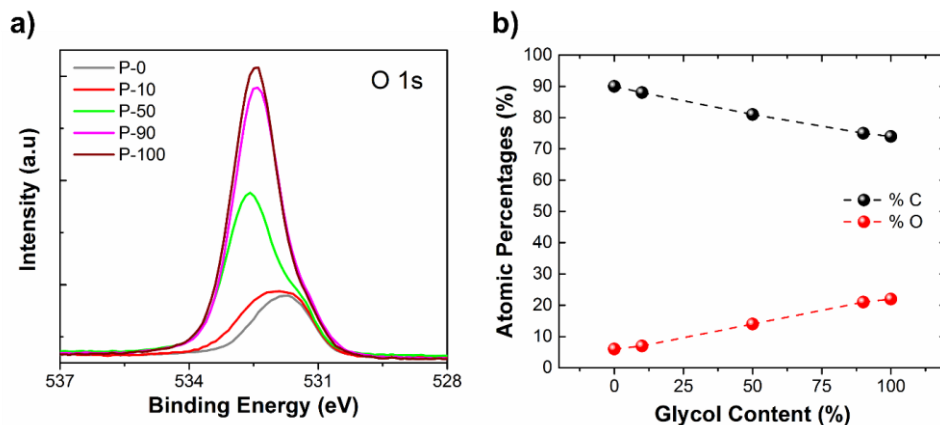


Figure S5. (a) High resolution XPS O 1s spectra and (b) the atomic percentage of carbon (black dots) and oxygen (red dots) atoms relative to the total amount of elements found at the surface of polymer films. The spectra were acquired using charge neutralization and referenced to C 1s at 284.8 eV.

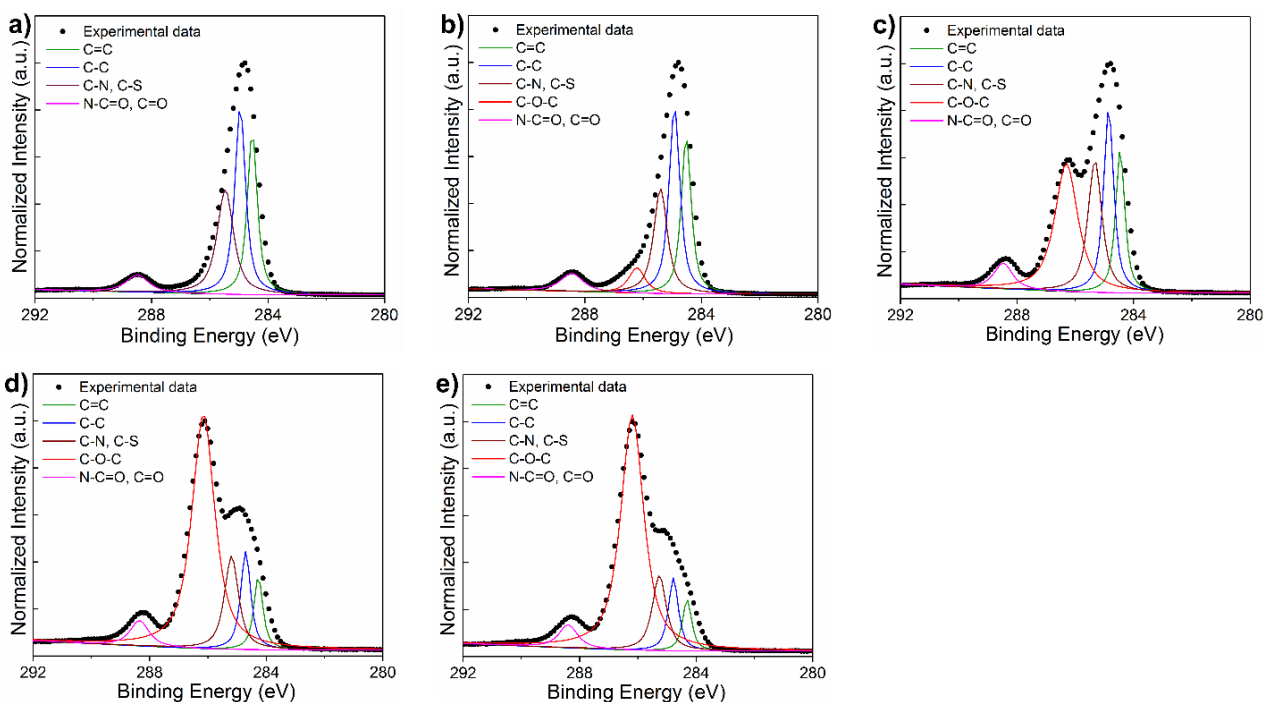


Figure S6. XPS C 1s spectra of NDI-T2 films with different glycol contents (a: P-0, b: P-10, c: P-50, d: P-90 and e: P-100). The spectra were normalized to the maximum intensity and the

background subtraction was carried out using Tougaard method. The C 1s spectra were deconvoluted and fit into several distinct peaks with Gaussian and Lorentzian method, representing different types of carbon bonds.

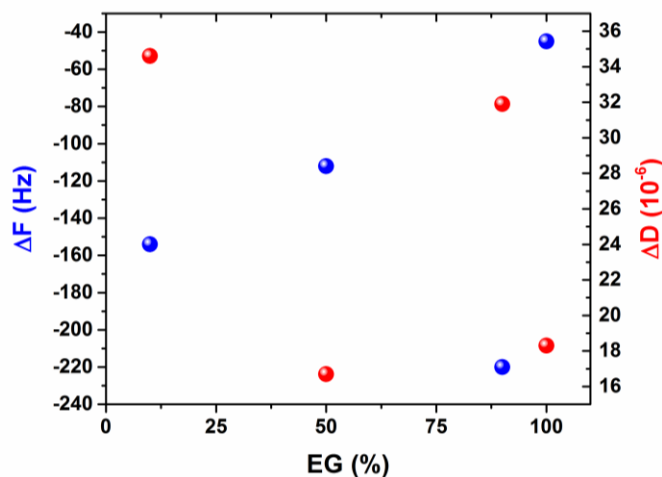


Figure S7. QCM-D data showing frequency and dissipation shifts (Δf and ΔD , respectively) upon the adsorption of vesicles onto the polymer films with various EG concentrations. The higher the Δf , more mass (vesicles) is accumulated on top of the film.

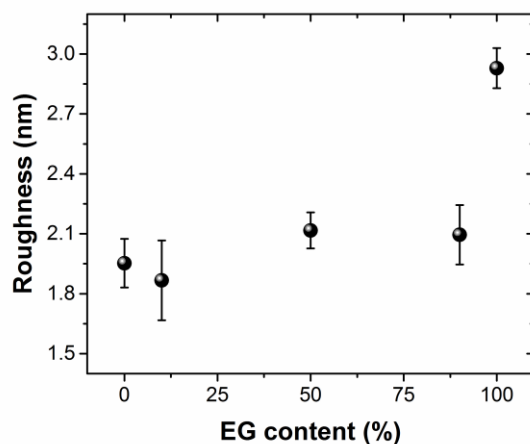


Figure S8. The roughness of polymer films characterized in PBS. The films have a thickness of ca. 60 ± 10 nm.

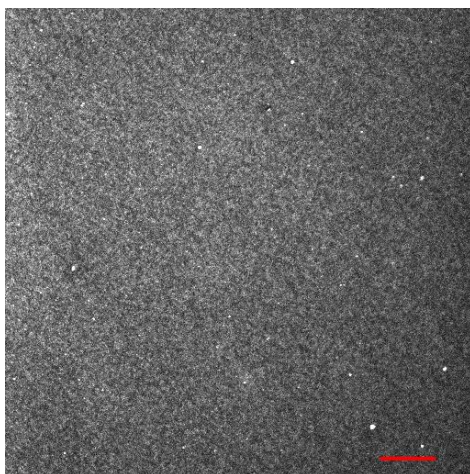


Figure S9. Fluorescence image of a drop-cast P-50 film after incubation with 0.5 mg/mL of the vesicle suspension in PBS. Scale bar is 50 μm .

Table S2. Comparison of various carbon bonds observed for P-50 films prepared via spin-casting and drop-casting.

deposition	% C=C	% C-C	% C-N and C-S	% C-O-C	% N-C=O
Spin-cast	15.5	21.8	22.0	34.9	5.8
Drop-cast	21.9	27.7	23.9	16.7	9.8

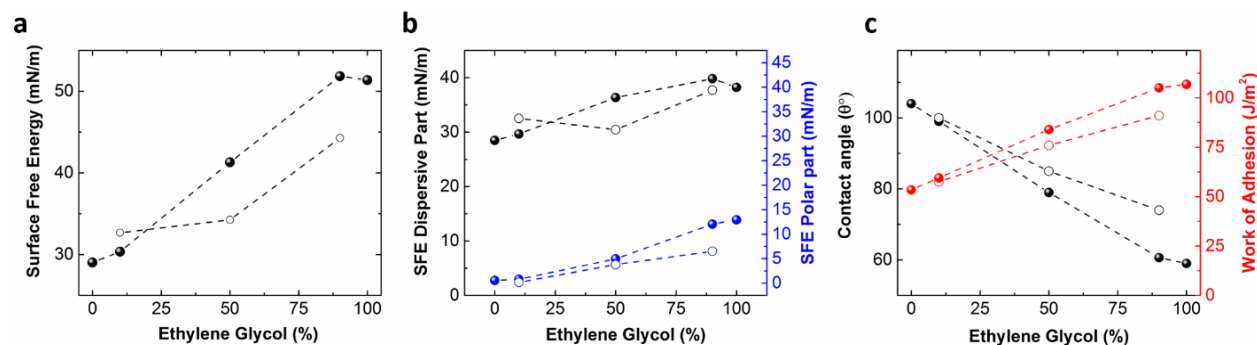


Figure S10. (a) Surface free energy (SFE) of the polymer films and (b) SFE components deconvoluted into dispersive (left axis) and polar parts (right axis) as a function of the EG content in the polymer composition. (c) Contact angle (left axis) and the work of adhesion (right axis) of the lipid vesicle solution on top of each film. For all the plots, solid and empty circles correspond to spin-cast and drop-cast samples, respectively.

References

- (1) Giovannitti, A.; Maria, I. P.; Hanifi, D.; Donahue, M. J.; Bryant, D.; Barth, K. J.; Makdah, B. E.; Savva, A.; Moia, D.; Zetek, M.; Barnes, P. R. F.; Reid, O. G.; Inal, S.; Rumbles, G.; Malliaras, G. G.; Nelson, J.; Rivnay, J.; McCulloch, I. The Role of the Side Chain on the Performance of N-type Conjugated Polymers in Aqueous Electrolytes. *Chem. Mater.* **2018**, *30* (9), 2945–2953
- (2) Kwok, D. Y.; Neumann, A. W. Contact Angle Measurement and Contact Angle interpretation. *Adv. Colloid Inter. Sc.* **1999**, *81*, 167-249.
- (3) Popat, K. C.; Sharma, S.; Desai, T. A. Quantitative XPS Analysis of PEG-Modified Silicon Surfaces. *J. Phys. Chem. B* **2004**, *108*, 5185-5188.