Supporting Information

Nanoprobes to interrogate nonspecific interactions in lipid bilayers: from defect-mediated adhesion to membrane disruption

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1. Synthesis and characterization of lipid adhesive/non-adhesive nanoprobes

1.1 Experimental details

1.1.1 Materials

Phosphotungstic acid hydrate (Reagent grade solubilized at 2 wt% in deionized water), Ammonium hydroxide (NH4OH, ACS reagent, 28–30% solution), Ethanol abs. (EtOH, VWR, 99.9%), Methanol (MeOH, HPLC grade), Tetraethylorthosilicate (TEOS, 99%), Toluene (99.8%), Acetonitrile (99.5%), Dichloromethane (DCM, 99.8%), Acetone (ACS reagent), N,N-Dimethylformamideme (DMF, anhydrous 99.8%), Hydrochloric acid (HCl, ACS reagent 37%), Potassium hydroxide (KOH, pellets), Sodium phosphate monobasic, Disodium hydrogen phosphate, Potassium chloride (KCl),2-(Dimethylamino) ethyl methacrylate (DMAEMA, contains 700-1000 ppm monomethyl ether hydroquinone as inhibitor, 98%), Propargyl acrylate (PA, 98%), Poly(ethylene glycol) methyl ether thiol (PEG-T, average Mn 5000), 1-Hexadecanethiol (HD-T, ≥ 95%), were purchased from Sigma Aldrich. Silicon dioxide microparticles (1.0 micron, 99.9%) were purchased from Alfa Aesar. Deionized water was obtained from a Milli-Q purification system (Millipore). 3-(trimethoxysilyl) propyl 3-[bis(2,4,6-trimethyl-benzoyl) phosphinyl]-2-methyl-propionate (TMESI2-BAPO) was synthesized via a stable bis(mesityl)phosphane intermediate as reported elsewhere.\(^1\)

1.1.2 Synthetic protocols

The synthetic protocol for the synthesis of the lipid adhesive/non-adhesive nanoprobes was inspired by a former publication in our group.\(^2\) It involves three steps of functionalization from photoactive nanoparticles, as follows. (Step 1): A photografting-from process involving the copolymerization of 2-(dimethylamino) ethyl methacrylate (DMAEMA) and propargyl acrylate (PA) from photoactive silica nanoparticles (functionalized with TMESI2-BAPO); this step leads to the formation of a product called clickable-NPs. (Step 2): A toposelective post-polymerization modification of P(DMAEMA-ran-PA) hairy surface with thiol-terminated poly(ethylene glycol) chains (PEG-T) via thiol-yne click reactions performed with the clickable-NPs immobilized on negatively charged microscaffolds; this step leads to the formation of a product called clickable/nonadhesive-NPs (Step 3): Final post-polymerization modification of available ‘clickable’ domains of the clickable/nonadhesive-NPs via thiol-yne (and -ene) by using 1-hexadecanethiol (HD-T); this step leads to the formation of a product called adhesive/nonadhesive-NPs with compartmentalized surface heterogeneity.

\(^1\) Sangermano, M., Periolatto, M., Castellino, M., Wang, J., Dietliker, K., Grutzmacher, J. L., & Grutzmacher, H. (2016). A Simple Preparation of Photoactive Glass Surfaces Allowing Coatings via the “Grafting-from” Method. ACS applied materials & interfaces, 8(30), 19764-19771.

\(^2\) Razza, N., Rizza, G., Coulon, P. E., Didier, L., Fadda, G. C., Voit, B., ... & Sangermano, M. (2018). Enabling the synthesis of homogeneous or Janus hairy nanoparticles through surface photoactivation. Nanoscale, 10(30), 14492-14498.
Silica nanoparticle synthesis:

Monodisperse silica nanoparticles were synthesized using a one-pot hydrolysis-condensation procedure of TEOS in ethanol with ammonia hydroxide as catalyst based on a modified Stöber approach. In a typical procedure, 145 ml of absolute ethanol and 10 ml of ammonia solution are added in a 250 ml round-bottom flask. The flask is sealed with a silicone septum and heated to 60 °C. When the temperature is stable 5 ml of TEOS are added and the solution is kept at 60 °C under continuous stirring at 700 rpm overnight. After hydrolysis-condensation, the obtained nanoparticles are washed 5 times with absolute ethanol by centrifugation/redispersion cycles. For each step, the particles were collected after 30 minutes of centrifugation at a relative centrifugal force of 15400 g. At each vial, containing about 60 mg of nanoparticles, 30 ml of washing solvent were added to redisperse the particles. Finally, the purified particles can be dried in a vacuum oven under reduced pressure at 60 °C.

Preparation of negatively charged silica microscaffolds: OH-SMSs:

Commercially available silica microparticles (1 µm in diameter) were cleaned with wet chemical treatment (RCA-SC1) to remove contaminants and activate the silanols on the silica surface. Silica microparticles (1 g) were added to 100 ml hydrogen peroxide (30%), 100 ml ammonia hydroxide (28-30%), and 100 ml deionized water. The mixture was sonicated and stirred for 1 hour at 70 °C in an open round-bottom flask. The particles were then collected by centrifugation and washed 5 times with absolute ethanol by centrifugation/redispersion cycles.

Functionalization of silica nanoparticle with TMESI²-BAPO: photoactive-NPs (I):

Purified and dried silica nanoparticles were dispersed in 125 ml of toluene by sonication (final concentration 17.5 g/l). Then, 0.2 ml of TMESI²- BAPO were added to the reaction mixture. The colloidal suspension was transferred in a round-bottom flask and sealed. After 48 hours of stirring at room temperature, the particles were collected by centrifugation and washed four times in toluene, two times in acetone by centrifugation/redispersion cycles. For each step, the particles were collected after 30 minutes of centrifugation at a relative centrifugal force of 15400 g. At each vial, containing about 60 mg of nanoparticles, 30 ml of washing solvent were added to redisperse the particles. After purification, the particles were dried under reduced pressure. Finally, the functionalized photoactive nanoparticles were dispersed and stored in acetonitrile with a concentration of 15 g/l.

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Berger, S., Ionov, L., & Synytska, A. (2011). Engineering of Ultra-Hydrophobic Functional Coatings Using Controlled Aggregation of Bicomponent Core/Shell Janus Particles. Advanced Functional Materials, 21(12), 2338-2344.
Synthesis of clickable-NPs (II):

In a typical procedure, 900 mg of BAPO functionalized silica nanoparticles are added to 210 ml of DMF and sonicated for 30-45 min until the particles are properly dispersed. Then, 12.04 mg of DMAEMA and 1.96 of PA are added to the colloid under stirring (9:1 molar ratio). The whole system is then transferred to a lab-made UV-reactor. It consists of a round-bottom flask with three necks for argon inlet and outlet and optical fiber connection through the main neck. The optical fiber is adjusted at 5 cm from the level of the liquid and connected to a light source (LC8 Lightning cure, Hamamatsu Photonics, Hamamatsu, Japan equipped with a Mercury-Xenon lamp with a spectral range distribution from a wavelength of 250 nm to visible light). The flask was covered with aluminum foil and immersed in a bath of water at room temperature to prevent any overheating. The mixture was purged with argon for 20 min under gentle stirring before light exposure. Then, the light source was activated with an intensity of 60 mWcm\(^2\) (UVA) for 1 hour to induce photopolymerization. Finally, the polymer-grafted nanoparticles were collected by centrifugation and washed eight times with DMF (x2), acetone (x2), ethanol (x2), and finally acid water (x2). For each step, the particles were collected after 30 minutes of centrifugation at a relative centrifugal force of 15400 g. At each vial, containing about 60 mg of nanoparticles, 30 ml of washing solvent were added to redisperse the particles. After the washing steps, the particles were dialyzed against ultrapure water for 96 hours and changing the dialysate every 4 hours (dialysis tubing of regenerated cellulose M.W. > 12,000 Da, from Spectrum Labs). The synthetic route is schematically reported in step 1 of Figure S1

![Figure S1](image-url): Schematic representation of the fabrication process via photografting-from and light-induced thiol-yne click reactions. (Step 1) Synthesis of clickable nanoparticles from photoactive nanoparticles via photogeneration of P(DMAEMA-ran-PA) pH-responsive and clickable surfaces
through the photografting-from method. The tabs below each product report the schematic structure of the surface in each specific step.

**Synthesis of clickable/nonadhesive-NPs (III) and fully nonadhesive-NPs (V):**

The designed procedure for the synthesis of the intermediate anisotropic nanoparticles is based on electrostatic immobilization of clickable nanoparticles (II) onto negatively-charged silica microscaffolds (OH-SMPs). The immobilization is followed by the toposelective click reactions. In a typical procedure, 50 ml of clickable-NPs at a concentration of 2.5 mg ml-1 were added dropwise to 20 ml of (OH-SMSs) at a concentration of 200 mg ml-1. The colloids were left interact at a pH of 5.5 for 1 hour under gentle stirring. These quantities were calculated to have an excess of microscaffolds and, thus, to ensure the immobilization of all the clickable-NPs. After the incubation time, the colloids were diluted to reach a total volume of 120 ml of water at a pH of 5. At this point, 4550 µl of polymer solution (PEG-T in water, 40 mg ml-1), and photoinitiator solution (I2959 in 2:1 MeOH : Water solution, 160 mg ml-1). After 30 min of incubation, the whole colloid was transferred in the above-mentioned UV-reactor and irradiated for 50 minutes with an intensity of 60 mWcm-2 (UVA). After irradiation, 1 ml of H2O2 solution was added to oxidize the unreacted thiol end-groups of PEG-T and avoid click reaction after the disassembly of nanoparticles. After 10 min of extra irradiation the presence of H2O2, the pH of the colloid was adjusted to 2 by adding few drops of HCl, 4 ml of KCl solution (3 M) were added to help to increase the ionic strength of the solution and the whole system was sonicated for 30 minutes to promote the disassembly. After sonication, the nanoparticles were collected by vacuum filtration through track-etched polycarbonate membranes (1 µm pore size, Whatman® Nuclepore). The vacuum filtration was repeated at least three times. Finally, the partially PEGylated clickable nanoparticles were purified via centrifugation and redispersion cycles repeated 5 times with acetone. Finally, the partially PEGylated clickable nanoparticles intermediate was dried by using a rotary evaporator at 45 °C for 4 hours. The synthetic route is schematically reported in step 2 of Figure S3. Similarly, homogeneous hydrophilic particles were obtained by using the above-mentioned strategy but without the immobilization of the clickable-NPs onto negatively-charged silica microscaffolds (OH-SMPs). In this case, the clickable-NPs were homogeneously modified with PEG-T leading to the formation of so-called fully nonadhesive-NPs. The synthetic route is schematically reported in step 2’ of Figure S2.
Figure S2: Schematic representation of the fabrication process of uniformly PEGylated nonadhesive nanoprobe via light-induced post-polymerization modification with PEG-T. (Step 2'). The tabs below each product report the schematic structure of the hairy surface photogenerated in each specific step.

Synthesis of adhesive/nonadhesive anisotropic NPs (IV):

In a typical procedure, 70 mg of partially PEGylated nanoparticles adhesive/nonadhesive-NPs were added to 30 ml of DCM and sonicated in an ice bath until a suitable dispersion was obtained. Then, 132 µl HD-T solution (2.3 mg ml-1 in DCM) and 236 mg of I-2959 were added to the colloid and sonicated for a few minutes. The whole system was then transferred to the above-mentioned UV-reactor and immersed in an ice bath. After 50 minutes of irradiation with an intensity of 60 mWcm-2 (UVA), the DCM was evaporated by using a rotary evaporator. Finally, the so produced amphiphilic adhesive/nonadhesive-NPs were purified via centrifugation and redispersion cycles repeated 5 times with acetone. The synthetic route is schematically reported in step 3 of Figure 6.4.
Figure S3: Schematic representation of the fabrication process of clickable/nonadhesive-NPs via toposselective light-induced post-polymerization modification with PEG-T. (Step 2) Synthesis of amphiphilic adhesive/nonshedesive-NPs via light-induced post-polymerization modification with HD-T (step 3). The tabs below each product report the schematic structure of the hairy surface photogenerated in each specific step.

1.1.3 Characterization techniques

Thermogravimetric analyses, TGA:

All the experiments were conducted on a thermal analyzer STA 449 F1 Jupiter (NETZSCH GmbH & Co) with a few mg of sample loaded in an alumina crucible and heated from room temperature to 800 °C with a heating rate of 10 K min⁻¹ under inert helium atmosphere. To promote the oxidation and, thus, the volatilization of carbon residues, at 700 °C the atmosphere was switched from helium to oxygen. However, no weight change was detected after in oxidative condition. The polymer fractions were evaluated by subtracting the total mass loss of BAPO functionalized nanoparticles to the total mass loss of the grafted particles (mass losses were evaluated between 140 and 800 °C).

Infrared spectroscopy, FTIR:

in transmission mode was to check the presence of “clickable” functionalities after the light-induced surface-initiated co-polymerization of DMAEMA and PA. To do so, we compared the FTIR spectra for native silica nanoparticles and the clickable-NPs. In typical sample preparation, 3 mg of dried sample was mixed with 97 mg of KBr. The two components were mixed by using an agate laboratory mortar and pestle. The mixed powder was transferred into a 70 mm pellet die and pellets were fabricated by using a laboratory benchtop hydraulic press while connecting the die to a vacuum pump. The procedure was repeated 4-5 times till pellet
optical clarity was obtained. Spectra were collected by using a Nicolet iS50 FTIR spectrometer. Spectra were recorded in absorbance mode with a resolution of 4 cm⁻¹ and a wavelength ranging from 1400 to 4000 cm⁻¹, averaging 10 scans for each spectrum.

**X-ray photoelectron spectroscopy, XPS:**

Spectra were recorded using a Thermo Scientific instrument equipped with a monochromatic Al Ka X-ray source (1486.6 eV). All samples were analyzed with a combined electron and Argon ion gun neutralizer system to reduce the charging effect during the measurements. All core-level peak energies were referred to C1s peak at 284.5 eV, and the background contribution in high-resolution scans was subtracted employing a Shirley function. The average chemical composition was calculated from wide scan spectra in two different locations on each surface. The peaks were fitted using a Gaussian/Lorentzian mixed-function employing Shirley background correction (Software Thermo Avantage v5.906). All analyses were performed at room temperature.

**Conventional transmission electron microscopy, TEM:**

All transmission electron microscopy investigations were conducted on JEOL 2010 FEG (JEOL Ltd.) with an acceleration voltage of 200 kV. The images were acquired with an ORIUS SC 200 (2k x 2k) camera. Samples of clickable-NPS for positive stain TEM were prepared by depositing a drop of colloid (2.5 mg ml⁻¹) on carbon-coated TEM grids (glow discharged for 30 sec with a current of 25 mA). After 1 minute, the liquid in excess was blotted with filter paper. Immediately after, the grid was washed twice with deionized water by rapidly blotting the water drop from the grid. Then the sample was stained with a drop of phosphotungstic acid solution (PTA, 2 wt% in ultrapure water adjusted by using sodium hydroxide to reach the pH of interest). After 30 seconds, the liquid in excess was blotted away with filter paper and washed twice with deionized water as above. Finally, another drop of phosphotungstic acid solution was deposited on the grid. After 30 seconds the liquid was blotted and the grid was dried under a flux of nitrogen. Since the PTA staining agent does not allow a clear identification of the compartmentalized morphology after the post-polymerization modification, the anisotropic functionalization was highlighted by using gold nanoparticles. To do so, gold nanoparticles (5 nm, citrate-capped) were incubated with clickable-NPs and clickable/nonadhesive-NPs to highlight the different gold stain after partial PEGylation via post-polymerization modification. In a typical procedure, 200 µl Au NPs (at a concentration of 5.5 x 10¹⁵ particles/ml) were added to 100 µl of hairy colloid (either with clickable-NPs and clickable/nonadhesive-NPs at a concentration of 2.5 mg ml⁻¹) and let interact for 30 min. Then, the with clickable-NPs and the clickable/nonadhesive-NPs were purified from unbound Au NPs by centrifugation. Subsequently, a drop of hairy nanoparticles stained with Au NPs was deposited on carbon-coated TEM grids (glow discharged for 30 sec with a current of 25 mA). After 1 minute, the liquid in excess was blotted with filter paper. Finally, the grid was dried under a flux of nitrogen and analyzed under the conditions reported above.
Zeta potential measurement:

Electrokinetic measurements via electrophoresis of clickable-NPs were carried out with a Zetasizer Nano ZS from Malvern Instruments Ltd. and an MPT-2 auto titrator. For the measurements, the particles were suspended (0.4 mg ml\(^{-1}\)) in a solution of 1mM KCl in water. The pH of the prepared suspensions was controlled by adding either 0.1 M KOH or HCl aqueous solutions. Three measurements were recorded for each sample at each pH value.

Neutron reflectivity of planar brush:

Measurements of the structural properties of the pH-responsive layer with adhesive functionalities P(DMAMEMA-r-PA)-g-HD were carried out using specular neutron reflectivity. The polymer brushes were grown by grafting-from technique on silicon wafers. The estimated molar structure ratio was 90% DMAEMA and 10% PA assumed to be equal to the polymer feeding ratio. The polymer density was 1.3g/cm\(^3\). Measurements were performed at the HERMES reflectometer at the ORPHEE reactor (Laboratoire Leon Brillouin, CEA-Saclay). The incident grazing angle was set to θ=1.5° and the neutron beam wavelength range was λ= 1-30 Å, giving a corresponding wave vector range Q = 0.01-0.2 Å\(^{-1}\). A liquid cell was mounted on the top of the wafer and filled with a solution at different pD values. The pD values were 2, 3, 4, 5, 6, 7. Samples were not analyzed for pD > 7 because preliminary screening showed no conformational changes for higher pD values. All the solutions were prepared in D\(_2\)O. The buffer was prepared in 5 mM citrate buffer, 100mM KCl, and the final pD value was adjusted with DCl or NaOD. Typical counting times, including background measurements, were 4 h. For each sample, specular reflectivity was normalized to the direct beam incident intensity. The total reflection plateau was used for the normalization of the reflectivity curves. The data were fitted with a multilayer model and convoluted with the resolution function in the transfer vector of the spectrometer. Data reduction and fit were done with PASINET (http://www-llb.cea.fr/Phocea/Page/index.php?id=84), an in-house program developed by D. Lairez at LLB. We used a multilayer-based fitting program; it uses a least-squares algorithm to fit 3 fixed and 5 variable parameters for each reflectivity curve. The neutron beam enters through one side of the silicon wafer, is reflected at the silicon-solvent interface, and exits from the other side of the wafer. Both the substrate and bulk solvent were treated as semi-infinite layers of indefinite thickness. In the fit procedure, we fixed R/RF=1, the scattering length density of the silicon \(\rho_{Si}= 2.07 \times 10^6 \text{ Å}^2\), and silicon roughness \(\sigma_{Si}=0\). The 5 variable parameters were the SLD of D\(_2\)O \(\rho_{D2O}\), that was allowed to vary from its ideal value of \(\rho_{D2O}= 6.35 \times 10^6 \text{ Å}^2\), the SLD \(\rho_{pD}\) of the polymer layer, its thickness \(h_{pD}\) and roughness \(\sigma_{h}\), and the background.

1.2 Results and discussion

After having functionalized the surface of nanoparticles with TMESI\(^2\)-BAPO, the size of photoactive-NP was evaluated by conventional TEM. From the analysis, an average diameter of 39 ± 5 nm. Upon light-exposure, two phosphoryl radicals are generated for each initiator site which can initiate the polymerization of the available monomer. For this study, surface-anchored copolymers were photogenerated containing both tertiary amines (from DMAEMA) to introduce the pH-adjustable positive charge upon amine protonation, and acetylene groups.
(from the PA) to introduce ‘clickable’ moieties. In most cases, it is necessary to protect and then deprotect the acetylene of propargyl acrylate with an alkylsilyl, before and after the polymerization, respectively.⁴⁻⁵ This is needed to avoid the formation of a cross-linked system, which would result in large agglomerates during grafting.

Nevertheless, by light-initiating the polymerization with phosphoryl radicals, PA can be copolymerized with other monomers without the use of any protection for the acetylene groups. In doing so, previous studies demonstrated that most of the acetylene groups are maintained and only a small degree of branching may be observed.⁶ The reasons for that can be found in the relative reactivity of phosphoryl radicals toward various π-bonds, such as triple bond (acetylene) and the double bound (acrylic) of PA and double-bond (methacrylic) of the copolymerizing monomer. Electron paramagnetic resonance studies, on phosphoryl radicals, have shown that the quenching rate constant toward triple bond of the acetylene is significantly lower (more than an order of magnitude) than toward the acrylic double bond of PA⁶ or to a methacrylic double bond.⁷ In this study, the surface-anchored phosphoryl radicals lead to the formation of brushes with pH-responsive and clickable character. The thickness of such polymer corona on the NP surface was evaluated via TEM after positive stain with PTA (Figure S4 a-b). A thickness of 4.3 ± 0.7 nm, in a dry state, was measured by analyzing the transmitted electron intensity profiles (Figure S4 c) of several hundreds of nanoparticles. The anisotropic features of the partial PEGylation was unveiled by TEM after incubating clickable/nonadhesive-NPs with gold nanoparticles. As reported in Figure S4 e, due to the presence of long PEG chains on a hemisphere of the clickable/nonadhesive-NPs, the gold nanoparticles cannot reach the buried amines, and therefore no stain is observed. On the other hand, clickable-NPs before the toposelective PEGylation were homogeneously stained with gold nanoparticles, confirming homogeneous surface chemistry.

The thermal stability of adhesive/nonadhesive-NPs and its intermediates was evaluated through TGA. Within the evaluation window in inert conditions (100 - 700 °C), we observe a two-step decomposition profile as reported Figure S5 and Table 1. Additionally, TGA allowed us to estimate the polymer grafting yield by looking at the total mass loss in comparison with the loss of TMESI²-BAPO functionalized nanoparticles. The total mass loss was estimated after switching the atmosphere from helium to oxygen at 700 °C. This was done because of the tendency of DMAEMA-based copolymers to form solid carbonization products which can be removed by using an oxidizing atmosphere (e.g. containing oxygen). We found that the grafted polymer accounts for 30.5% of the total nanoparticle weight. Additionally, by

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⁴ Wei, X., Chen, W., Chen, X., & Russell, T. P. (2010). Disorder-to-order transition of diblock copolymers induced by alkyne/azide click chemistry. Macromolecules, 43(14), 6234-6236.
⁵ Kitaura, T., Tomioka, H., Fukatani, N., & Kitayama, T. (2013). Anchimeric assistance on sequence regulation in partial modification of isotactic poly (propargyl methacrylate) by click reaction. Polymer Chemistry, 4(4), 887-890.
⁶ Ciftci, M., Kahveci, M. U., Yagci, Y., Alonos, X., Ley, C., & Tar, H. (2012). A simple route to synthesis of branched and cross-linked polymers with clickable moieties by photopolymerization. Chemical Communications, 48(82), 10252-10254.
⁷ Sluggett, G. W., McGarry, P. F., Koptyug, I. V., & Turro, N. J. (1996). Laser flash photolysis and time-resolved ESR study of phosphinoyl radical structure and reactivity. Journal of the American Chemical Society, 118(31), 7367-7372.
considering the size of the nanoparticles, we calculated an average polymer per unit area of $13.5 \pm 2.5 \text{ mg m}^{-2}$. As summarized in Table 1, we observed an increase in weight loss after one (PEG-T only) and two steps (PEG-T and HD-T) photo-click reactions.

Figure S4: (a) TEM micrograph of clickable-NPs after positive stain with PTA along with (b) a magnification of single stained nanoparticle. (c) Example of the intensity profile of a positive-stained clickable-NPs used to estimate the silica core size and poly(DMAEMA-ran-PA) polymer shell thickness. (d) clickable-NPs and (e) clickable/nonadhesive-NPs after incubation with gold nanoparticles to highlight the toposelective modification with PEG-T.

Table 1. Summary of weight loss percentage from thermogravimetric analysis for the final anisotropic amphiphilic nanoparticles adhesive/nonadhesive-NPs and any other intermediate particle system. Weight loss is evaluated between 140 and 800 °C.

| Particle system                  | Total | Polymer | P(DMAEMA-r-PA) | PEG-T | HD-T |
|---------------------------------|-------|---------|----------------|-------|------|
| Photoactive NPs (I)             | 12.5  | n/a     | n/a            | n/a   | n/a  |
| Homogeneous yne(+HNPs) (II)     | 43.0  | 30.5    | 30.5           | n/a   | n/a  |
| Hydrophilic yne/hydro(+HNPs) (III)| 49.2  | 36.7    | 30.5           | 6.2   | n/a  |
| Amphiphilic lipo/hydro(+HNPs) (IV)| 53.4  | 40.9    | 30.5           | 6.2   | 4.2  |
From a chemical viewpoint, the photografting of P(DMAEMA-ran-PA) from the photoactivated nanoparticles was evaluated by FTIR. As reported in Figure S6, a broad and intense peak is located at 3420 cm\(^{-1}\) which corresponds to the OH stretching vibration of the silica surface. Additionally, a small peak located at 2950 cm\(^{-1}\) indicated the presence of C-H stretching from CH\(_2\), largely present in the P(DMAEMA-ran-PA) shell. This is furtherly confirmed by another peak located at 1470 cm\(^{-1}\) and due to C-H bending from CH\(_2\). A clear confirmation of the presence of PA blocks in the copolymer structure is indicated by the small peak located at 2125 cm\(^{-1}\) (better visible in the inset of Figure S6). This peak of the alkyne is due to acetylene-hydrogen stretching C≡C-H and clearly shows the presence of ‘clickable’ functionalities in the system. Moving toward smaller wavenumbers, two sharp peaks located at 1730 and 1660 cm\(^{-1}\) can be identified. These are ascribed to C=O stretching vibration and C=C alkenyl stretching, respectively. The C=O stretching vibration is typical of polymers originating from acrylic and methacrylic monomers, whereas the C=C alkenyl stretching is likely indicating the presence of some degree of branching due to the acetylene functionalities of PA.

Furthermore, XPS analysis conducted on grafted clickable-NPs, clickable/nonadhesive-NPs intermediate, and final adhesive/nonadhesive-NPs showed the evolution of the chemical composition for each step of thiol-yne photo-click modification (Figure S7). The surveys (left Figure S7) show all the elements present and their chemical states. In the dotted frames the position of C1s and S2s peaks is reported (the latter is not visible due to its lower intensity). For C1s and S2s, high-resolution spectra were collected as reported in the middle and right spectra of Figure S7. When going from clickable-NPs to clickable/nonadhesive-NPs intermediate, PEG-T is introduced in the brush architecture. This chemical modification introduces thiol (i.e. thiol-yne click chemistry) and ether bonds C-O-C (from the PEG
backbone). Although the contribution to the C1s from adventitious carbon cannot be excluded, it is clear from the high-resolution C1s peak of clickable-NPs and clickable/nonadhesive-NPs that the introduction of PEG increases the C-O content assigned to the peak located at about 286 eV. Indeed, the C-O contribution to the C1s goes from 19.8% - before the modification with PEG-T – to 30.7% after the modification with PEG-T. The introduction of PEG-T is further confirmed by the appearance of S 2s peak located at about 232 eV. In the second modification step, the introduction of HD-T led to a further increase in sulfur content while the C-C contribution to the C1s goes from 61.9% - before the modification with HD-T – to 71.0% after the modification with HD-T.

Brush swelling as a function of DMA protonation for the adhesive domain of nanoprobes was studied by conducting neutron reflectivity experiments on planar adhesive brushes (P(DMAEMA-ran-PA) clicked with HD-T). A representative fit for neutron reflectivity data is reported in Figure S8 and a summary of fit results is reported in Table 2. Please note that the compact conformation of planar brushed may induced shifts in the pKa of DMA pendant of the chains, which in turn is translated in a conformational change happening at lower pH. This has already been reported for both polymer brushes and aminoacid residues buried in proteins.

![Figure S6](image)

**Figure S6**: FTIR spectra of polymer grafted clickable-NPs. The presence of clickable functionalities is highlighted by the magnification between 2100 and 2180 cm⁻¹ on the inset.

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8 Schuwer, N. B. (2011). Synthesis of Responsive Polymer Brushes for Sensing Applications (Thesis No. 5077); EPFL.

9 Isom, D. G., Castañeda, C. A., & Cannon, B. R. (2011). Large shifts in pKa values of lysine residues buried inside a protein. Proceedings of the National Academy of Sciences, 108(13), 5260-5265.
Figure S7: XPS spectra of polymer grafted clickable-NPs (a) clickable/nonadhesive-NPs intermediate (b) and final adhesive/nonadhesive-NPs (c). For each particle system survey spectrum and high-resolution spectra for C1s and S2s are included.

Table 2. Neutron reflectivity fit results for planar adhesive polymer brushes on planar silicon substrates.

| pD | Polymer Thickness (Å) | SLD polymer (x10^{-6} Å^2) | roughness (Å) | SLD solvent (x10^{-6} Å^2) | Background |
|----|-----------------------|----------------------------|---------------|-----------------------------|------------|
| 2  | 126                   | 5.1                        | 62            | 6.31                        | 1.6983e-06 |
| 3  | 86.1                  | 4.7                        | 60.5          | 6.31                        | 4.0e-06    |
| 4  | 82.2                  | 4.60                       | 53            | 6.32                        | 4.0e-06    |
| 5  | 77.7                  | 4.5                        | 58.7          | 6.32                        | 5.2e-6     |
| 6  | 65.8                  | 4.4                        | 55.5          | 6.33                        | 4.0e-06    |
| 7  | 64                    | 4.4                        | 54            | 6.32                        | 1.6983e-06 |
2. **Nanoprobe-lipid membrane interactions**

2.1. **Experimental details**

**Preparation of large unilamellar vesicles (LUVs):**

LUVs (a.k.a. large liposomes) were prepared by extrusion. In a typical procedure, 4 mg of lyophilized DPhPC lipids were dissolved in 250 µl CH₂Cl₂ and transferred in a 2 ml round-bottom flask. The CH₂Cl₂ was evaporated by gentle argon flux while rotating the flask. After solvent evaporation, the flask was placed for 2 hours in a high vacuum chamber (0.5 mbar) to further boost the residual solvent removal. The so obtained lipid cake was then hydrated in 1 ml phosphate buffer with a pH of interest. The hydration was carried out for 2 hours alternated each 30 min by short mixing with a vortex mixer. Then, the hydrated lipid suspension was subjected to 5 freeze-thaw cycles by alternatively placing the vial first in cooling acetone - liquid nitrogen bath and then in a warm water bath. The lipid suspension was den extruded by using an extrude set equipped with two 1 ml syringes separated by an extrusion block containing two single-use polycarbonate membranes (Mini-Extruder from Avanti® Polar Lipids, Inc.). Before extrusion, the syringes were wetted with the buffer of interest to limit the dead volumes and facilitate extrusion. The lipid suspension was first passed 11 times through 0.4 µm polycarbonate membranes and finally collecting the liquid in the initially-empty syringe. This allows for avoiding contamination from foreign materials. The extruded suspension was then extruded additional 11 times through 0.1 µm polycarbonate membranes to finally obtain large unilamellar vesicles. The liposome suspension was stored at 4 °C for a maximum of 3 days. Measurements of the structural properties of the pH-responsive layer with adhesive functionalities.

![Graph showing neutron reflectivity data](image-url)

**Figure S8:** Representative fit of neutron reflectivity data based on the best-fit parameters.
Conventional transmission electron microscopy (TEM) for liposome/nanoprobe assembly:

The interactions with lipid membranes were investigated for adhesive/nonadhesive-NPs. In a typical procedure, 10 µl of adhesive/nonadhesive-NPs dispersion was let interact with 100 µl of LUVs suspension for 30 min and then analyzed by using negative stain TEM. Uranyl acetate was chosen as a staining agent following the same staining protocol described in the previous section. It is important to note that although the nanoparticles were incubated in a specific buffer condition, before staining the deposited samples were thoughtfully washed with ultrapure water. This step is necessary for two reasons: (i) phosphate residues can crystallize during the drying process, contaminating the sample; (ii) phosphate ions can interact with uranyl ions to produce a fine crystalline precipitate that obscures the sample. Despite its rapidity, the washing step changes pH and ionic strength and subsequently the interacting conditions. Therefore, it is not possible to rely on this technique to distinguish different behaviors depending on the pH value. However, this rapid technique allows us to visualize whether interactions between nanoparticles and lipid membranes occurred.

Liquid phase transmission electron microscopy (LP-TEM):

A better understanding was gained by using LP-TEM analysis, which was used to directly visualize, in situ, the interaction between nanoprobes and liposomes. LP-TEM allows access to sample behavior in water by direct visualization of liquid water. This is possible by embedding the liquid sample in a microfabricated cell composed of two silicon chips with electron transparent Si₃N₄ windows separated by 50 nm gold spacers. The Si₃N₄ windows are surrounded by a liquid reservoir which connected to a flow system embedded in a dedicated sample holder. The whole microfabricated device can be inserted inside the microscope thanks to vacuum isolation using O-ring seals. LP-TEM experiments were performed in a Poseidon 210 liquid flow sample holder (Protochips Inc.) loaded with microfabricated E-chips with thin silicon nitride amorphous windows. The E-chips were rinsed in acetone first and then ethanol (2 min each) to remove the protective coating layers. After being dried with a gas duster, the E-chips were plasma cleaned for 1 minute to enhance the silicon nitride membrane hydrophilicity and to remove any organic contaminants. During the LP-TEM analysis, low electron dose conditions were used utilizing a programmable syringe pump (Harvard Apparatus, USA). The image processing was carried out using Gatan Microscopy Suite (GMS3) and ImageJ software. In a typical procedure, after having loaded the bottom E-chip inside the sample holder, a drop of the liposome suspension was deposited on it. Then, the system was sealed with the top E-Chip with the silicon nitride windows facing the liquid. Then, the system was sealed with the top E-Chip with the silicon nitride windows facing the colloid. After the assembly, the vacuum seal was tested with a turbo pumping station (Pfeiffer Vacuum Technology AG, Germany). Once a liposome is identified, the flux is turned on (100 µl h⁻¹ of buffer at a pH of 6.6) utilizing a programmable syringe pump (Harvard Apparatus, USA). The flux contains nanoprobes in a 6.6 pH buffer. The view is kept on the liposomes, which are visualized waiting for the nanoparticles to arrive. When the nanoparticles reach the liposomes, the flux is turned off and the interactions start to be recorded. It is important to note that an acidic buffer was used to adjust pH because the pH difference between the inner volume
of the liposome and the external media may induce instability to the self-assembled structure. Since the beam induces acidification of the irradiated liquid environment, we decided to opt for this strategy so that the pH can be adjusted homogeneously inside and outside the liposomes. Although working with low electron doses, this effect is still important since, in this case, there is no water flux to remove the radiolysis products. To have an idea of this effect, studies have demonstrated that under beam irradiation the entire liquid cell will have an increased $\text{H}_2\text{O}^+$ concentration and thus a reduced pH. For instance, starting from pure water initially at pH 7, after being exposed to a 1 μm radius beam of 1 nA at 300 kV its pH will eventually drop to ~4.9 within the irradiated region and ~6.1 outside.\footnote{Schneider, N. M. (2016). Electron beam effects in liquid cell TEM and STEM. liquid cell electron microscopy, 140-163.} This effect cannot even be compensated by increasing the buffer concentration because such an increase in concentration could only introduce additional artifacts due to salt precipitation within the liquid cell. To achieve better contrast, energy filter TEM (EFTEM) was used by collecting only zero-loss electrons with a GIF Tridiem (Gatan GmbH) post-column energy filter used in image mode.

**Lipid membrane electrical measurements:**

Interactions between the nanoparticle and cell membranes were investigated by using free-standing planar unsupported bilayer lipid membranes (hereafter p-BLMs) as a cell membrane model. In a typical electrical measurement on p-BLMs, a membrane-forming solution was prepared by adding DPhPC phospholipids in n-decane at a concentration of 10 mg ml$^{-1}$ and stored at -20 °C when not in use. The adopted experiment configuration is schematically reported in Figure S9. The cell is a two-part system consisting of a black Delrin chamber (trans) and a removable cuvette (cis) with a 150 μm in diameter hole, both from Warner Instruments. The p-BLM is formed over a hole separating the two cis/trans chambers. To do so, 0.5 μl of the membrane-forming solution is painted on and around the hole by using a long gel-loading pipet tip and let it dry in air for at least 30 min. This step allows acts as surface pre-treatment which will help the actual membrane formation in the following steps. Once the lipid primer-coating is applied, the two chambers are filled with 750 μl buffer solution at a specific pH. At this point, the two Ag/AgCl electrodes are inserted in the solution and connected to a waveform generator (33500B Series, Keysight Technologies). A voltage of 50 mV is applied with a sinusoidal waveform and a frequency of 10 Hz. During the experiments, the ionic current (values in the order of pA) was measured. To do so, the resulting ionic current was amplified by using the Axopatch 200B amplifier. The amplified current was digitized with a 16 bits analog-digital oscilloscope (PicoScope®, Pico Technologies). To reduce the noise, the whole-cell system was closed inside a Faraday copper cage and placed on an anti-vibration marble table. The data acquisition was handled by using dedicated Matlab macros. From the ionic current and voltage, the complex impedance $Z_{m^*}$ of the system could be estimated in terms of real (Rm, resistance) and imaginary (Xm, capacitive reactance) parts.
Figure S9. Electrical measurements of p-BLM. Simplified measurement design with two compartments (cis and trans) filled with an electrolyte solution (phosphate buffer) and connected to an AC power generator via two Ag/AgCl electrodes. The two compartments are separated by a small hole with a circular shape and with a diameter of 150 µm. The p-BLM is painted on that hole and afterward, current and voltage are monitored to calculate the membrane complex impedance overtime. To interrogate nanoprobe-membrane interactions, nanopores are added in the cis compartment and the complex impedance is monitored over time. Nanopores that anchor at the lipid bilayer are responsible for decreasing the imaginary component of the complex impedance.

The p-BLM is formed by painting the immersed hole with an additional 0.5 μl of the membrane-forming solution with a long gel-loading pipet. Its formation could be detected electrically by the change in the measured ionic current and, consequently, by the change in complex impedance. In terms of impedance, once the membrane is formed, the mainly-resistive system becomes mainly-capacitive because of membrane formation. The formation of a p-BLM could be identified and distinguished from an amorphous lipid film by looking at the capacitance values. Only p-BLMs with a capacitance (calculated from $X_m$) lying between 50 and 70 pF were accepted for the actual measurements. The quality of the formed membranes was also evaluated optically using a 2000x digital camera. Good quality p-BLMs showed a dark central spot (the actual lipid bilayer) surrounded by a solvent annulus with a toroidal shape. After the membrane formation, the power supply was turned off for 10 minutes, allowing the n-decane to evaporate. Then, after a further optical inspection, the impedance of the p-BLM started being measured overtime with averaged acquisition every 10 periods for a total acquisition time of 10 minutes. Depending on their quality, the membrane may evolve, more or less rapidly over time. This innate evolution primarily affects the imaginary component of the complex impedance. In this study, if the imaginary component $X_m$ of the complex impedance varied overtime less than 0.5 % min⁻¹, the membrane was used for the actual experiment, and discarded otherwise. This criterion is fundamental to achieve a good signal to noise ratio, otherwise, we might encounter misleading interpretation of the electrical properties of the membrane after nanoprobe additions. In all experiments, the complex impedance was measured at room temperature. After about 3 minutes, a few μl of nanoparticle were added to the cis chamber, whereas an equivalent volume of buffer solution was added to the trans chamber to compensate for the
volume additions. The nanoprobe addition was so that an average concentration of 230 pM was reached in the cis chamber (concentration calculated assuming a nanoparticle radius equal to its average from TEM). Note that just before the NP addition, the measurement was paused to freely open the Faraday cage and close it afterward. The whole NP addition step required about 10-15 seconds. The electrical measurement was carried out for 7 minutes in total. No reliable information could be obtained from longer measurement times because membrane instability developed overtime. Control tests were performed by using (i) only buffer additions in both chambers (same volume as for nanoparticle experiments) to check the presence of artifacts from the volume addition, and (ii) homogeneous nonadhesive nanopores to confirm that the adhesion to lipid membranes is coming from adhesive the domain of the nanopores. After each experiment, the cell was thoughtfully washed with a dilute solution of sodium hydroxide, ultrapure water, and ethanol in the reported order. Then, it was let dry completely in an oven at 50 °C. When not in use the Ag/AgCl electrodes were stored in dilute sodium hypochlorite solution and thoughtfully washed with ultrapure water before their use.

2.2. Supplementary results

![Figure S10](image_url)

Figure S10. Temporal evaluation of transmembrane complex impedance before and after homogeneous nonadhesive nanoprobe addition via membrane electrical measurements. Averaged results normalized capacitive reactance ($X_m / X_m^0$)*100 (A) resistance ($R_m / R_m^0$)*100 (B) and impedance phase shift (C). No statistically significant differences were identified within the investigated pH range. A final average concentration of 230 pM was used in all the experiments.

3 Molecular dynamics simulations

Molecular dynamics (MD) simulations are performed using the g GROMACS 4.5.6 MD package. Graphical images of the molecular system are represented with the VMD software. The MARTINI 2.0 coarse-grained (CG) force field is used in the molecular modeling of the system. In line with the

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11 Hess, B., Kutzner, C., Van Der Spoel, D., & Lindahl, E. (2008). GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. Journal of chemical theory and computation, 4(3), 435-447.
12 Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: visual molecular dynamics. Journal of molecular graphics, 14(1), 33-38.
13 Marrink, S. J., Risselada, H. J., Yefimov, S., Tieleman, D. P., & De Vries, A. H. (2007). The MARTINI force field: coarse grained model for biomolecular simulations. The journal of physical chemistry B, 111(27), 7812-7824.
MARTINI approach, the atomistic system is mapped in a 4:1 average scheme, namely four heavy atoms, on average, are grouped in a unique molecular group or entity denominated “bead”. Each bead type reproduces different behavior in terms of the properties of the atomic group it represents. More specifically, MARTINI bead types are divided into four categories: polar (P), nonpolar (N), apolar (C), and charged (Q). Each particle, in turn, is identified by subtypes, which represent more accurately the corresponding chemical properties: i. hydrogen-bonding as donor d, acceptor a, none 0, or both of them, da, of the corresponding atomic structure; or ii. degree of polarity, from 1 (lowest) to 5 (highest). It is worth noticing that, sometimes, the CG mapping can be 3:1 or 5:1 (i.e., grouping 3 or 5 heavy atoms in a bead), to better reproduce the atomistic system in terms of chemical and physical properties. In this work, the polymer chain and the lipid membrane are mapped and their bonded potentials parameters (bonds, angles, dihedrals) are obtained by matching the atomistic system distributions, in line with the corresponding original works.14,15,16,17 Concerning the polymer modeling, the main backbone chain is described as a sequence of SC1 bead types (3:1 mapping, shared atomistic CH2), in line with Panizon, et al. (2015).16 Bending and torsional potentials are modeled following the method proposed by Bulacu et al. (2013)14, in which restricted bending (ReB) functions are used for angle potentials, increasing the numerical stability of the simulation. The advantage of such an approach is that the main backbone can never reach the 180° angle collapsing on itself. ReB potential is therefore employed for angles, whereas standard proper dihedrals function with multiplicity = 1,2 is considered to model torsions of the main backbone. In line with the MARTINI force field and with the mapping proposed by Lee & Larson (2008)15, chains of P(DMAEMA-ran-PA) clicked with HD-T are modeled in the following way: the ester part corresponds to a Na bead type, whereas the dimethyl amine part is mapped with an N0 bead type. Bonding potentials parameters of these structures are chosen to reproduce the corresponding atomistic part, in line with Lee & Larson (2008)15. The sulfide groups are mapped as a C5 bead type, corresponding to the atomistic CH2-S-CH2-CH2 group and the alkyl tail as a sequence of three C1 beads (Wassenar, et al., 2015).17 Lipid DPPC membrane topology is taken from literature.13,17,18 CG beads non-bonded interactions are modeled through pairwise Lennard-Jones (LJ) 12-6 potential:

\[ U_{\text{LJ}}(r_{ij}) = 4\varepsilon_{ij} \left[ \left( \frac{a_{ij}}{r_{ij}} \right)^{12} - \left( \frac{a_{ij}}{r_{ij}} \right)^{6} \right], \]  

(1)

14 Bulacu, M., Goga, N., Zhao, W., Rossi, G., Monticelli, L., Periole, X., ... & Marrink, S. J. (2013). Improved angle potentials for coarse-grained molecular dynamics simulations. Journal of chemical theory and computation, 9(8), 3282-3292.
15 Lee, H., & Larson, R. G. (2008). Coarse-grained molecular dynamics studies of the concentration and size dependence of fifth- and seventh-generation PAMAM dendrimers on pore formation in DMPC bilayer. The journal of physical chemistry B, 112(26), 7778-7784.
16 Panizon, E., Bochicchio, D., Monticelli, L., & Rossi, G. (2015). MARTINI coarse-grained models of polyethylene and polypropylene. The Journal of Physical Chemistry B, 119(25), 8209-8216.
17 Wassenar, T.A.; Ingolfsson, H.I.; Bockmann, R.A.; Tieleman, D.P.; Marrink, S.J. Computational lipidomics with INSANE: a versatile tool for generating custom membranes for molecular simulations. J. Chem. Theory Comput. 2015, 11(5), 2144-2155.
18 Marrink, S. J., De Vries, A. H., & Mark, A. E. (2004). Coarse grained model for semiquantitative lipid simulations. The Journal of Physical Chemistry B, 108(2), 750-760.
where $\varepsilon_{ij}$ is the strength of the energy interaction, $\sigma_{ij}$ is the distance at which the inter-particle potential is null, between two generic beads $i$ and $j$ that interact at a distance $r_{ij}$. For charged particles (bead type Q), also the Coulombic potential is implemented:

$$U_{el}(r_{ij}) = \frac{q_i q_j}{4\pi \varepsilon_0 \varepsilon_r r_{ij}}$$  \hspace{1cm} (2)

where, charged particles that carry a charge $q_i$ and $q_j$ interacts in a medium with relative dielectric constant $\varepsilon_r = 15$ for explicit screening, at a distance $r_{ij}$.

**Coarse-Grained Lipid bilayer formation:**

The lipid bilayer is formed through spontaneous self-assembly of about 400 lipid molecules in a water solution. A cubic simulation box is chosen with a box length equal to 11 nm and 8000 water molecules. In line with the MARTINI 2.0 force field, a four-to-one mapping is chosen and the DPhPC is mapped as a DPPC, as an initial assumption, being the two lipids structure very similar to each other from a coarse-grained (CG) representation point of view. The DPPC MARTINI topology has the advantage of having already been investigated and, consequently, been available in the literature. The choline and phosphate groups are both modeled via a hydrophilic CG particle bearing a positive and negative charge, respectively Q0 (blue) and Qa (dark yellow) bead types from the MARTINI 2.0 table. The phytanoyl tails are represented by four hydrophobic CG particles, C1 bead types (light blue). The glycerol ester backbone is modeled by two particles of intermediate hydrophilicity, represented with the Na bead type. The water CG particle is hydrophilic, labeled as P4, representing four real atomistic water molecules. Lipid mapping is shown in Figure S11.

![Figure S11. Mapping of the lipid bilayer (left). The CG representation corresponds to: Q0 bead type for the choline head of the lipid (blue), bead type Qa for the phosphate groups (dark yellow), Na bead type to represent glycerol ester groups, and C1 bead types for the alkyl tails. On the right, its stylized bond-style representation.](image-url)
The initial assumptions about the lipid molecular modeling stated above are furthermore justified by previous works,\textsuperscript{19,20} under suitable operating conditions. One of them is represented by the temperature at which the simulations are carried out. Indeed, despite the experimental gel to the liquid-crystalline phase transition of DPPC is detected to be at 315 K,\textsuperscript{21} in full-atom (AA) and coarse-grained molecular dynamics (CGMD) simulations it takes place at 305 K.\textsuperscript{22,23} Being the physiological temperature of 310 K the value at which biological experiments are usually performed, operating at 315 K represents, therefore, a good initial assumption, assuring the system to always be above its gel-to-liquid-crystalline phase transition throughout all the MD simulations conducted in this work. Another advantage that comes out from operating at 315 K consists of having a lateral diffusion coefficient of the DPPC membrane closer to the DPhPC one than at it is at higher temperatures.\textsuperscript{24}

Lipid self-assembly simulations are performed in NPT ensembles, with 8000 water and 398 lipids molecules, at 1 bar and 315 K. The system is minimized employing the steepest descent algorithm and equilibrated for 30 ns (with a leap-frog integrator), to let the disordered molecules in the box self-assemble, forming the bilayer. During the equilibration, the pressure coupling is isotropic and carried out through the Berendsen barostat and pressure time constant equal to 3.0 ps, while the simulation run is made for 30 ns more, with a semi-isotropic pressure coupling to reach the zero surface tension on the bilayer. Concerning the simulation run, the Parrinello-Rahaman pressure coupling is implemented with a time constant that spans in the range 4-8 ps. In both equilibration and run, the temperature of 315 K is imposed thanks to the velocity-rescale algorithm and a time constant equal to 1.0 ps. Intermolecular and electrostatic non-bonded interactions are considered with shifted (in the range 0.9-1.2 nm) Lennard-Jones (L J) and Coulombic potentials. The relative dielectric constant is set equal to 15 since non-polarizable water is used. The cut-off distance for the short-range neighbor list is 1.4 nm and periodic boundary conditions along x, y, and z directions are taken into account. At the end of this procedure, the bilayer is formed and stabilized, as shown in Figure S12.

As clearly visible in Figure S12, the lipid bilayer has overcome its gel-to-liquid phase transition, due to the disordered structure it forms. The single light green beads on the top and bottom of the snapshot corresponding to the CG water.

\textsuperscript{19} Hoiles, W., Krishnamurthy, V., & Cornell, B. (2018). Dynamics of Engineered Artificial Membranes and Biosensors. Cambridge University Press.
\textsuperscript{20} Stoddart, D., Ayub, M., Höfler, L., Raychaudhuri, P., Klingelhofer, J. W., Maglia, G., ... & Bayley, H. (2014). Functional truncated membrane pores. Proceedings of the National Academy of Sciences, 111(7), 2425-2430.
\textsuperscript{21} Janiak, M. J., Small, D. M., & Shipley, G. G. (1976). Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl and dipalmitoyllecithin. Biochemistry, 15(21), 4575-4580.
\textsuperscript{22} Leekumjorn, S., & Sum, A. K. (2007). Molecular studies of the gel to liquid-crystalline phase transition for fully hydrated DPPC and DPPE bilayers. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1768(2), 354-365.
\textsuperscript{23} Marrink, S. J., Risselada, J., & Mark, A. E. (2005). Simulation of gel phase formation and melting in lipid bilayers using a coarse grained model. Chemistry and physics of lipids, 135(2), 223-244.
\textsuperscript{24} Wang, S., & Larson, R. G. (2014). Water channel formation and ion transport in linear and branched lipid bilayers. Physical Chemistry Chemical Physics, 16(16), 7251-7262.
Lipid membrane – Polymer brush coupling:

Once the CG DPPC lipid bilayer is created and stabilized (as discussed above), then it is coupled with the CG polymer brush. The single polymer chain consists of P(DMAEMA-ran-PA) clicked with HD-T made of 60 monomeric units with a DMAEMA : PA molar ratio of 9:1 (assumed to be equal to the feed ratio). This guarantees a full stretched polymer chain length approximatively equal to 12 nm, very close to the swollen brush thickness used in the experimental set-up. A graphical representation of the single CG polymer chain is shown in Figure S13. The coupled system is depicted in Figure S14.

After creating a single polymer chain, its structure is replicated in x- and y-direction of the simulation box and the intermolecular spacing is chosen in line with the experimental data referred to as the grafting density. More specifically, a probe core diameter and bush length are taken from experimental measurements were coupled with an average grafting density assumed to be equal to 0.5 chain/nm². This corresponds to about 1256 polymer chains in half
nanoprobe surface area. Considering the brush length and the number of polymer chains (1256, constant), an outer grafting density can be calculated as the number of polymer chains divided by the external surface area of a sphere with a diameter equal to the summation of the inner core diameter plus the brush length. The outcome is an external grafting density equal to 0.125 chains/nm². Assuming to investigate only a small portion of the brush-membrane contact (sufficiently small to assume curvature effects being negligible), a contact surface area equal to 11 nm x 11 nm (in x and y directions) is chosen for this work, in line with the lipid membrane set-up described above. This corresponds to an external surface area of the grafted nanoprobe of about 121 nm². At this point, it is trivial to find the number of polymer chains exposed at the outer surface area of the grafted nanoparticle, using the external grafting density: the number of chains = external grafting density × the available area = 0.125 chains/nm² × 121 nm² = 16 chains ca. The polymer brush is therefore made of 16 chains spatially equally distant from each other in a square 4 by 4 configuration. The heads of each polymer chain are fixed at a certain z-axis height thanks to a suitable positional restrain, the numerical details thereof will be provided in the corresponding section below. Figure S5 shows the CG polymer brush, with particular attention to the positional restrained heads (large blue spheres).

Figure S14. CG coupled system, in the MARTINI representation.

To investigate the effects of the protonation degree α, a given amount of DMA units is suitably protonated, following a random but decreasing order from the outer to the inner part of the brush, in line with an experimental setup. The system is electrically neutralized with the proper amount of Na⁺ (sodium) and Cl⁻ (chloride) CG counterions in solution (one ion CG bead type accounts for the atomistic ion plus the first hydration shell around it), in line with the
classical MD framework. The protonated DMA units correspond to a CG bead type Q0 of the MARTINI force field (dark blue in Figure S13). The simulation box is kept sufficiently high (initial z-direction length equal to 30 nm) to prevent undesired effects due to the periodic boundary conditions along z. The initial configuration is made in such a way that the polymer brush–to–lipid membrane distance is always greater than the cut-off distance, i.e. the two systems do not see each other (no nonbonded interactions) at the beginning.

Figure S15. CG polymer brush in a relaxed conformation, in the MARTINI representation. The large blue spheres represent the positional restrained heads.

Umbrella sampling calculation:

Free energy calculations are performed in the molecular simulations conducted in this work. The polymer brush movement towards the lipid membrane is investigated through progressive advancement steps (reaction coordinates) of Umbrella Sampling (US) calculations.\(^\text{25}\) US represents one of the most used free energy methods in analyzing macromolecular interactions in the MD field.\(^\text{26,27}\) The US is here employed to obtain the free energy profile, or potential of mean force (PMF), referred to the lipid-polymer interaction in terms of relative approaching, grafting, adhesion, and eventual disruption of the lipid bilayer at different protonation degrees of the polymer brush.

In line with the US method, a series of configurations are generated along a distance or reaction coordinate, say \(\xi\), and a harmonic biasing potential \(w_i(\xi)\) is applied to guarantee the system to uniformly sample all the configurations at every chosen reaction coordinate, \(\xi_i\):

\(^{25}\) Kästner, J. (2011). Umbrella sampling. Wiley Interdisciplinary Reviews: Computational Molecular Science, 1(6), 932-942.

\(^{26}\) Patey, G. N., & Valleau, J. P. (1973). The free energy of spheres with dipoles: Monte Carlo with multistage sampling. *Chemical Physics Letters*, 21(2), 297-300.

\(^{27}\) Torrie, G. M.; Valleau, J. P. Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling. *J. Comput. Phys.* **1977**, **23**, 187-199.
\[ w_i(\xi) = \frac{K_i}{2} (\xi - \xi_i)^2, \]

where \( K_i \) is the spring constant of the harmonic potential. More specifically, the system is brought to evolve for a given time (simulation time), in every reaction coordinate interval \( i \) between \( \xi \) and \( \xi + \Delta \xi \), also labeled as “sampling window”. Every distance, \( \xi_i \) corresponds to a sampling window investigation. The number of reaction coordinate intervals (or, equally, sampling windows) must be high enough (i.e., small \( \Delta \xi \)) to guarantee phase space overlap of the thermodynamic transformation and, at the same time, not too large for computational costs’ reasons. The usual approach consists of a trial and error strategy from the coarser to the finer interval \( \Delta \xi \) (namely from smaller to a larger number of intervals/windows) at which a continuous PMF is obtained, that is, a good overlap of the sampling systems distributions (histograms) is reached. In this US framework, the lipid bilayer represents the reference group, whereas the polymer brush is the pulled one; the reaction coordinate \( \xi \) for the thermodynamic pathway refers to the spatial distance between the center of mass (COM) of these two groups, as schematically shown in Figure S16. Independent simulations are carried out for each sampling window, as it will be reported below. The effect of the bias is ultimately removed through the weighted histogram analysis method (WHAM). The error analysis on the US method is conducted utilizing the bootstrap method, a very common routine for the free energy calculations presented here.

**Figure S16.** Reaction coordinate \( \xi \) along which (z-directions) the thermodynamic calculations of umbrella sampling (US) are carried out. For each sampling interval \( \Delta \xi \), a set of independent simulations is conducted.

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28 Kumar, S., Rosenberg, J. M., Bouzida, D., Swendsen, R. H., & Kollman, P. A. (1992). The weighted histogram analysis method for free‐energy calculations on biomolecules. I. The method. Journal of computational chemistry, 13(8), 1011-1021.

29 Hub, J. S., & de Groot, B. L. (2006). Does CO2 permeate through aquaporin-1?. Biophysical Journal, 91(3), 842-848.
This modeling and simulation approach brings a twofold advantage: on one side, it is possible to study the dynamic evolution (over simulation time) and the behavior of the system at each US interval/window of the progressive polymer brush advancement towards the lipid membrane, at the molecular scale; on the other side, by looking at the PMF profiles, it is possible to draw out a solid and robust comparison among systems at different protonation degrees. The PMF profiles constitute a footprint of the thermodynamic pathway related to the adhesion and the eventual membrane disruption at the considered protonation degrees and can be used as a powerful tool to calculate the free energy of adhesion-related to the local polymer brush-lipid membrane interactions. We recommend the cited references\textsuperscript{25,30} for a complete understanding of the statistical thermodynamics background related to the PMF calculations conducted in the current work.

**Numerical details of the US simulations**

The whole system described above (membrane, brush, water, and ions) is simulated in an orthogonal simulation box with size 11 nm x 11 nm x 30 nm, respectively in x-, y-, and z-directions. The US reaction coordinate (i.e., the polymer brush translational movement) is oriented along the z-axis. Therefore, the lipid membrane lies on the x-y plane. The simulation box size in the z-direction is sufficiently high to avoid any numerical instabilities during the simulation run. The energy minimization of the system is conducted using the steepest descent algorithm with tolerance set to 100 kJ/(mol∙nm) and an initial step size equal to 0.01 nm. For each configuration (sampling window) of the US calculation, an independent equilibration and a simulation run are carried out. All simulations are performed in NPT ensembles. Equilibration is run for 1 ns with a 0.01 ps timestep. The temperature is fixed at 315 K with a velocity-rescale thermostat and a time constant equal to 1 ps. A semi-isotropic pressure coupling (necessary to avoid any longitudinal tension across the membrane surface) fixes the system at 1 bar during equilibration with a Berendsen barostat, with a time constant and compressibility respectively equal to 1 ps and 4.5⋅10\textsuperscript{-5} bar\textsuperscript{-1} along both the x-y plane and the z-axis. The main simulation run is done for 10 ns with a 0.01 ps timestep. Temperature and pressure have the same values as for the equilibration phase, but the coupling is imposed employing a Nose-Hoover thermostat with a time constant equal to 2 ps and a Parrinello-Rahman barostat (semi-isotropic) with a time constant equal to 8 ps. Intermolecular forces are modeled via the Lennard-Jones (LJ) and Coulombic potentials reported in Eq. (1) and (2). Electrostatic interactions are governed using the Particle-Mesh Ewald (PME) summation with a Coulomb cut-off radius equal to 1.4 nm and a PME interpolation order set to 4. The relative dielectric constant is equal to 15. The grid Fourier-spacing is set to 0.21 nm. Concerning the non-bonded LJ interactions, the Verlet cut-off scheme is applied, with the cut-off radius equal to 1.4 nm. The cut-off distance for the short-range neighbor list is 1.4 nm and periodic boundary conditions are considered along x-, y- and z-directions. The integration of Newton’s equations of motions is conducted using a leap-frog algorithm for all the simulations. A harmonic biasing potential is used in the pull code; its mathematical expression is reported in

\textsuperscript{30} Hub, J. S., De Groot, B. L., & Van Der Spoel, D. (2010). g_wham - A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. Journal of chemical theory and computation, 6(12), 3713-3720.
Eq. (3), where $K_i = 1000$ kJ/(mol·nm$^2$). The pulling rate is kept null, overcoming the relative difficulty in determining the best parameter for such a complex system; therefore, the configurations are generated by purely translating the first equilibrated system along the reaction coordinate $\xi$ (z-direction). The whole spatial translational movement (polymer brush advancement towards the lipid membrane) is about 10 nm. The chosen sampling window/interval $\Delta \xi = 0.2$ nm for a total of about 50 configurations generated. As stated before, a set of independent simulations is performed for each of them. The heads of the 16 polymer chains composing the brush are position-restrained with a spring force with a relative spring constant set equal to 5000 kJ/(mol·nm$^2$). This allows the user to mimic the radial non-uniform behavior due to the grafting onto the metallic nanoparticle core. The investigated protonation degrees $\alpha = 0$ (not charged), 0.25, 0.50, 0.75 and 1.0 (fully charged).

**Error analysis with the bootstrap method**

Statistical errors on the US calculations are performed using the bootstrap analysis.$^{30}$ Average profile and standard deviations are reported for just one case, for a sake of brevity, for the case of $\alpha = 0.50$ (Figure S17) together with the corresponding umbrella histograms. A good overlap among the distributions is reached, which turns out to be sufficient to obtain a continuous PMF profile. All the other investigated protonation degrees $\alpha$ show similar profiles and the same order of magnitude of the error on the US calculation. The maximum error estimated is about $\pm 35$ kJ/mol.

![Average and standard deviation from bootstrapping](image)

**Figure S17.** Error analysis from bootstrapping method (top, average, and error bars) and umbrella histograms (bottom) as function of the reaction coordinate $\xi$, for the case of $\alpha = 0.50$. 