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## Design of tethered bilayer lipid membranes, using wet chemistry via aryldiazonium sulfonic acid spontaneous grafting on silicon and chrome

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Ophélie Squillace<sup>1</sup>, Thomas Perrault<sup>1</sup>, Magdalena Gorczynska<sup>1,2</sup>, Andrew Caruana<sup>3</sup>, Anna Bajorek<sup>2</sup>, and Guillaume Brotons<sup>1\*</sup>.

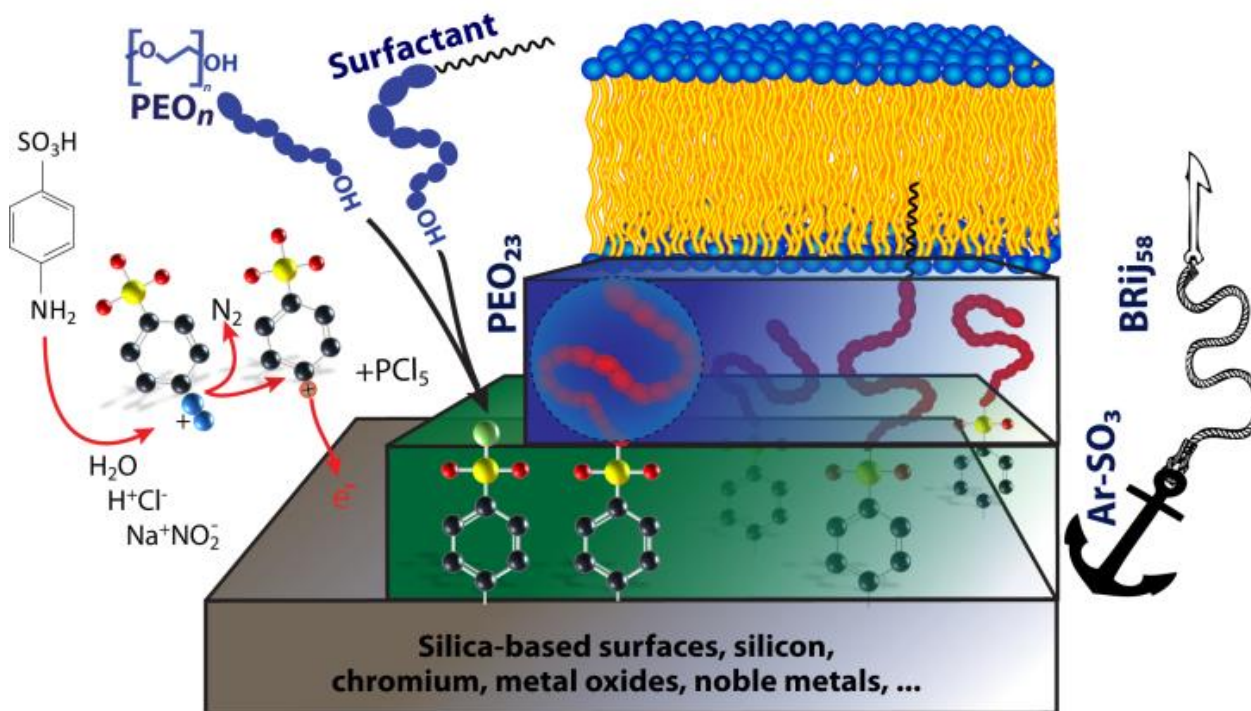
<sup>1</sup>IMMM, Institut des Molécules et Matériaux du Mans UMR CNRS 6283, Université du Mans - UFR Sciences et Techniques, Avenue Olivier Messiaen, 72085 Le Mans, France.

<sup>2</sup> A. Chełkowski Institute of Physics, University of Silesia in Katowice, 75 Pułku Piechoty 1A, Chorzów, Poland

<sup>3</sup> STFC ISIS Facility, Rutherford Appleton Laboratories, Chilton, Didcot OX11 0QX, UK.

Solid Supported Tethered Single Lipid Bilayer; Planar Membrane Sensors; Anchor-Harpoon hybrid coatings to stabilize hydrated and Fluid Membranes; Surface Functionalization by Covalent Wet Chemistry and Spontaneous Grafting of Aryldiazonium Sulfonic Acid; Covalent Polymer Ultra-Thin Films and Surfactants Molecular Coatings.

Graphical abstract



### Highlights :

- Bilayer lipid membranes are tethered to wafers via simple surfactants wet chemistry
- Solid supported single biomembrane sensors obtained from wet chemistry on Si and Cr
- Grafted commercial surfactants are anchor-harpoon molecules for biomembranes on solid
- Aryldiazonium salt wet chemistry on solids to form Brij and PEG coatings for bilayers
- XPS and neutrons probe the structure of a hydrogel cushion that supports biomembranes

**ABSTRACT** We describe a bottom-up surface functionalization to design hybrid molecular coatings that tether biomembranes using wet chemistry. First, a monolayer was formed by immersion in a  $\text{NH}_2\text{-Ar-SO}_3\text{H}$  solution, allowing aryldiazonium salt radicals to spontaneously bind to it via strong C bonding. After formation of the air-stable and dense molecular monolayer ( $-\text{Ar-SO}_3\text{H}$ ), a subsequent activation was used to form highly reactive  $-\text{Ar-SO}_2\text{Cl}$  groups nearly perpendicular to the monolayer. These can bind commercial surfactants, PEGylated oligomers and other inexpensive molecules via their  $-\text{OH}$ ,  $-\text{COOH}$ , or  $-\text{NH}_2$  chain end-moieties, to build hybrid coatings. Metal and oxidized chromium, semi-conductor n-doped silicon (111), are the substrates tested for this protocol and the aromatic organic monolayer formed at their surface was characterized by X-ray photoelectron spectroscopy (XPS). XPS reveals unambiguously the presence of C-Cr and C-Si bonds, ensuring robustness of the coatings. Functional sulfur groups ( $-\text{SO}_3\text{H}$ ) cover up to  $6.5 \times 10^{-10}$  mol  $\text{cm}^{-2}$  of the silicon interface and  $4.7 \times 10^{-10}$  mol  $\text{cm}^{-2}$  of the oxidized chromium interface. These surface concentrations are comparable to the classic values obtained when the prefunctionalisation is driven by electrochemistry on conductors. Tethered lipid membranes formed on these coatings were analyzed by neutron reflectivity at the interface of functionalized n-doped silicon substrates after immersion in a solution of lipid vesicles and subsequent fusion. Results indicate a rather compact hybrid coating of Brij anchor-harpoon molecules that maintain a single lipid bilayer above the substrate, on top of a hydrated PEO cushion.

## INTRODUCTION

Large-scale production of membrane sensors and other analytical platforms using biomimetic membranes is gaining attention since such platforms are great tools for biological studies that are at the core of societal and environmental issues. In the present case, we build model lipid bilayer sensors to study biomembrane interactions with microplastic and nanoplastic particles, whose possible translocation, or subsequent transfer along the trophic chain, are growing concerns. Even though it is the object of multiple studies in recent years, observation of micro/nanoplastic translocation and interactions is very challenging, and as such the route of their entry into organisms is not yet identified[1]. Conclusive dedicated techniques such as an *in vitro* approach, applying Ussing chambers or any other electrochemical or optical sensors, should be considered to accurately show the transport of microplastics/nanoplastics and potential associated effects on passive or active flux across membranes[2]. Moreover, such membrane sensors built at the solid/liquid interface, offer a relevant environment for gaining a better fundamental understanding of the molecular structure, interactions and dynamics of proteins [3] and lipids[4]. In this context, the surface functionalization of sensor platform metals and semi-conductors to tune interfacial properties and to introduce new surface chemistries is central[5, 6], and great interest is borne to hybrid coatings that can stabilize a partially tethered bilayer lipid membrane (tBLM) at the sensor interface[7-9]. The main difficulty in such systems is maintaining membrane fluidity, hydration and biological function in close proximity to a solid surface. To meet these requirements, an optimal environment can be provided by diluting “anchor-harpoon” molecules (whose alkyl chains insert into, and tether the membrane) in a hydrogel layer that offers a soft cushion of hydration to the membrane. PEGylated chains (PolyEthylene Glycols or PolyEthelene Oxide) form a good cushion, and also introduce anti-fouling properties against proteins[10].

To graft such hybrid coatings to the substrate, several methods are now standard: grafting self-assembled monolayers (SAM) like alkanethiols on noble metals[11]; silane coupling on oxidable metals[12] and silica-based material[13-15]; alkenes on doped silicon[16, 17]. For bulk or thin film electrode coatings, assisted electrochemical reactions are even more versatile and some offer robust carbon covalent bonding which are resistant to ultrasonic stress in solvents. Among those, the reduction of aryldiazonium salts[18] gives covalently bonded poly(phenylene)-like molecular coatings with near-perpendicular  $\pi$ -conjugated molecules that maintain excellent electron transport across the interface. First developed for glassy carbon, the reaction has been extended to a large variety of electrode materials: metals[19]; a large variety of industrial, coinage and noble metals (Co, Ni, Cu, Zn, Pt, Au)[20]; and semiconductors[21] including silicon[22, 23]. The process generally involves a highly reactive aryl radical or cation, formed by splitting the C-N bond, and the acceptance of an electron from the substrate accompanied by nitrogen release. Since different aryldiazonium salts are air-stable and exist with additional side groups on the grafted non-polar aromatic cycle, this reduction has been used to introduce different surface chemistries to electrodes ( $-\text{OH}$ ,  $-\text{COOH}$ ,  $-\text{NH}_2$ ...).

Recently, a two-step process was proposed to build hybrid molecular coatings from commercial unreactive molecules[24]: (1) using a prefunctionalization obtained by the reduction of aryldiazonium salts with a sulfanilic acid group to form a dense monolayer ( $-\text{Ar-SO}_3\text{H}$ ); and (2) activating the layer to present a para-benzene sulfonyl chloride group ( $-\text{Ar-SO}_2\text{Cl}$ ), known for its high reactivity toward weak nucleophiles. This process enabled the grafting of surfactants often considered as non-reactive via their hydroxyl ( $-\text{OH}$ ) end-moieties. Several other common end-moieties react as well, such as  $-\text{COOH}$  or  $-\text{NH}_2$ , so a huge catalogue of inexpensive molecules is available for the preparation of complex analytical platforms. While preparing hybrid coatings often requires engaging in cumbersome chemistry, for both the synthesis of the tether and the PEGylated molecules that can bind to the specific sensor support, we avoided this difficulty by grafting non-ionic diblock

oligomers for the “anchor-harpoon” molecule (Brij58:  $C_xH_{2x+1}(OCH_2CH_2)_nOH$  with  $x=16$  and  $n=23$ ) and short PEO chains for the cushion ( $(OCH_2CH_2)_nOH$  with  $n=9$  and  $n=23$ )[24, 25].

Since aryldiazonium salts can spontaneously bind to various substrates from a simple immersion in solution[26-29], here we explored the functionalization without electrochemistry and extended our previous protocol for the preparation of tBLM on electrodes to very poor or non-conducting substrates. Tethered on such substrates via simple chemistry, membranes become a platform of study adapted for techniques such as neutron reflectivity (NR)[8] without the necessity of an extra layer of metal on silicon that could increase the roughness of the surface and result in a loss of resolution or complexification of the interface profile. First, the present study demonstrates that analogous benzenesulfonic acid gives secondary products harvesting free electrons at the silicon interface that form covalent organic monolayers at room temperature, in absence of externally applied potential or exogenous reductive species. Second, it shows that this protocol of functionalization is suitable for keeping a single lipid bilayer several angstroms away from the surface above a highly hydrated cushion. The covalent bonding, based on spontaneous electron transfer at the open circuit potential (OCP) of the substrate material in solution, is demonstrated by X-ray photoelectron spectroscopy (XPS) investigations on conductors and a semi-conductor: thin metal chromium films (denoted CrA) thermally evaporated on glass and n-doped silicon (111) wafers (denoted Sil). The functionalization on semi-transparent chromium electrodes was previously studied[25] to build tBLM in an environment appropriate for checking the membrane fluidity and lipid dynamics via fluorescence techniques, and for investigating structure at high-resolution via x-ray, neutron and light scattering methods. We validate here by XPS the grafting of aryldiazonium salts by electro-reduction using cyclic voltammetry (CV) as previously described[24, 25] and use it as a control. The values of surface concentration of the grafted groups obtained by XPS and NR are comparable and similar to the values of reference[18, 24, 30, 31].

## MATERIALS AND METHODS

**Chemicals and Materials** Ethanol (EtOH, 99.8%), acetone (99.5%), dichloromethane (99.9%), acetonitril (99.8%), methanol (99.7%), sulfanilic acid ( $NH_2-Ar-SO_3H$ ), hydrochloric acid (HCl, 37%), sodium nitrite ( $NaNO_2$ ), Brij58: ( $C_{16}H_{33}(OCH_2CH_2)_{20}OH$ ),  $PEO_9-CH_3$  ( $H(OCH_2)_9CH_3$ ),  $PEO_{23}-OH$  ( $H(OCH_2)_{23}OH$ ), 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) were purchased from Sigma-Aldrich (St Quentin Fallavier, France) and used without further purification. Milli-Q water (Versol, Aguetant, Lyon, France) was used. N-doped Silicon (111) wafers (250  $\mu m$  and 5 mm thick) were purchased from Siltronix, France. Chromium films of 12-30 nm thickness were prepared in our lab by the thermal evaporation[24] of chromium from pellets and condensation onto microscopy cover glasses (thickness  $\sim 170 \mu m$  and optical index  $\sim 1.5$  from Cole-Palmer<sup>TM</sup>) that were cleaned in a mixture of methanol and hydrochloric acid (1:1), rinsed with purified water, dried and stored in a dry atmosphere.

**Preparation of SUVs** DOPC lipids, dispersed in chloroform were first evaporated, dried in high vacuum chamber for 2 hours, and rehydrated overnight in solutions of  $D_2O/NaCl$  at 200 mM to make solutions of 1 mg/mL lipid concentration. The solutions were then sonicated at 60 °C for 30 min and filtered through a membrane with pore size of 200 nm (PALL LIFE SCIENCES GHP Acrodisc GF 13 mm syringe filter).

**Surface preparation** All vials and laboratory containers were pre-cleaned twice before use with 1 mol/L KOH and 5% (v/v) 2-propanol solution and then 1 mol/L HCl solution. Extensive Milli-Q water rinsing was used between each step. The surfaces were first cleaned with fresh water piranha solutions (70 °C, 10 min) and then with fresh HF 1% immersions of 2 min. Surfaces for XPS measurements (sample CrA and Sil) were functionalized by long dips of 72 h in a solution of sulfanilic acid (0.2 mM) prior dissolution in a 1 M HCl solution at room temperature and stirred with 0.2 mM of  $NaNO_2$  for 1 h at 0 °C. Samples were then rinsed with pure water and ethanol to remove all residual chemicals. The n-doped Silicon (111) wafer for NR was functionalized with Brij58 molecules after long dips of 24 h in solutions of sulfanilic acid (5 mM) prior dissolution in 1 equivalence medium of  $NaNO_2/HCl$  (1 M) and 18 h in an organic solution of Brij58 (solubilized in acetonitrile at a concentration of  $0.8 \times 10^{-5}$  M).

**Contact Angle (CA) Measurements** Water contact angles were measured using a homemade set-up with a halogen source and a telecentric lens on the source and camera pathways. Contact angles were determined with a precision of 0.1°. At least 5 drops of 1  $\mu L$  were deposited on separated regions over the surface to obtain an average value for each sample.

**X-ray photoelectron spectrometry (XPS)** X-ray electron spectroscopy (XPS) measurements were carried out using a Physical Electronics (PHI 5700) XPS/ESCA spectrometer under ultra-high vacuum conditions of about  $10^{-9}$  Torr. All XPS data were obtained at room temperature by using a monochromatized X-ray source Al  $K\alpha$  (1486.6 eV) operating at 150 W power (15 kV  $\times$  10 mA). The survey spectra were acquired with the pass energy of 187.85 eV and high resolution core level lines with the pass energy of 11.75 eV. All performed measurements were done in two consecutive modes. The first one is normal-mode with a 45° take off angle. The second so-called grazing mode was performed tilting the sample by 30° to take off angle 15° in order to analyze in detail chemical states on the surface. All the obtained XPS spectra were calibrated using the C1s peak (BE=284.8 eV) as this carbon peak usually originates from the carbon adsorbed on the surface of the sample

and is used as reference for charge correction. All measured spectra were processed with the use of MultiPak 9.4 software. The core level lines O1s, C1s, S2p and Si2p were fitted with the use of a combination Gauss-Lorentz shape of photoemission line and Shirley background. Additionally, the Cr2p<sub>3/2</sub> line was fitted with the asymmetric Doniach – Sunjić (DS) line shape typically used for analyzing photoemission spectra from metals[32]. More information can be found in the SI.

**Neutron Reflectivity (NR)** Measurements were carried out at the spallation neutron source at the ISIS facility (Didcot, UK), on POLREF in the non-polarized mode (this instrument is usually used for magnetic samples and has a wavelength range 1-15 Å). The functionalized silicon (111) wafer was mounted in a fluidic cell that was connected to a HPLC pump via several channels to achieve solvent switch at a rate of 1 mL/min. Measurements were performed in three different solvents to change the contrast at interfaces: D<sub>2</sub>O, H<sub>2</sub>O (the highest background from hydrogen incoherent neutron scattering) and a so-called “silicon match water” (SMW) mixture corresponding to 62% in volume of H<sub>2</sub>O and 38% of D<sub>2</sub>O (with nearly the same neutron scattering length density, NSLD, as the silicon substrate). The NR measurements were always carried out at rest, after solvent exchange involving at least 30 mL injections for a sample cell volume of 3 mL of solvent. The sample was set up in a horizontal geometry with the incident beam being reflected at the solid/liquid interface on the silicon side. Three angles of incidence were used to establish the full Q-range NR curve analyzed for each sample (0.5°, 1.2° and 2.5°). NR curves were analyzed using the ReFlid software and the DREAM method in the Python language [33-35]. The NR curve fit is based on the calculation of the neutron reflectivity from a one-dimension NSLD slab profile depending on few parameters per layer: a NSLD, a thickness, and each interface is described with an error function profile based on a roughness parameter. For each sample, we simultaneously fitted the three solvent curves using the same fit parameters that define all thicknesses and interface roughnesses for the three NSLD profiles. For the neutron reflectivity fits, the three different NR curves are fitted simultaneously. The NSLD of hydrated layers that includes solvent molecules (H<sub>2</sub>O, D<sub>2</sub>O or SMW) are the only contrast-dependent fit parameters. Otherwise the NSLD of non-hydrated layers are fitted with a single parameter and considered as identical for all contrast measurements. More information can be found in the corresponding SI section.

## RESULTS AND DISCUSSION

As a simple method to quickly check and validate the grafting of the Ar-SO<sub>3</sub>H prefunctionalization, contact angles (CA) of sessile drops on the different surfaces were measured. The noticeable change in wettability before and after functionalization was observed for each chromium sample (from  $\theta_i=52.1^\circ$  to  $\theta_i=16.4^\circ$ ) and differed only slightly from the silicon substrate, whose bare surface was more hydrophilic than chromium (from  $\theta_i=20.3^\circ$  to  $\theta_i=19.1^\circ$ ). This comes in additional support to the results obtained from XPS and Neutron.

### Investigation of the functionalization by XPS

XPS measurements were carried out at two take-off angles, 45° and 15°, to discriminate the surface elements from those of the substrate. All expected elements were found: carbon C, oxygen O, sulfur S, and on the substrates, chromium Cr or silicon Si (and phosphorus P for n-doped wafers). Survey XPS spectra from CrA sample and Si sample are shown in **Figure 1** and corresponding element quantities by percentage are shown in **Table 1**.

CrA			Si1		
Element	45°	15°	Element	45°	15°
C	43.7%	49.0%	O	44.8%	48.8%
O	34.4%	32.6%	Si	34.4%	33.8%
Cr	15.5%	12.7%	C	16.1%	15.5%
S	2.4%	2.9%	P	3.2%	1.5%
N	2.4%	1.8%	N	0.4%	<0.5%
Si	1.8%	1.0%	S	<0.1%	<0.1%

**TABLE 1. Percentage of elements probed on functionalized chromium films (CrA) and silicon (Si1) substrates.**

The presence of each element, C1s, O1s, S2p, Cr2p or Si2p confirms the grafting process with Ar-SO<sub>3</sub>H after extensive ultrasonic rinsing. The first three characteristic peaks respectively appear in the ranges of 282.8-290.5 eV, 530.4-534.1 eV, 167.99-169.15 eV on both chromium and silicon, and the last two in the range of 573.9-578.4eV for chromium and 99.0-103.3eV for silicon as expected[36-38]. As the take-off angle is decreased the detection is progressively more sensitive to the top layer of the chromium coated glass, that is the substrate/monolayer interface, and this is consistent with the observed increase of the carbon peak and decrease of the silicon peak. Likewise, for the functionalized silicon, the oxygen peak is increased while probing upward the substrate/monolayer interface while the phosphorus peak, which is related to the doping of the silicon, decreases. The increase of detected sulfur is also noticeable on chromium in the challenging-to-measure S2p peak (only 1 atom per molecule with a relatively small S photoionization cross-section in comparison to the other elements considered). This confirms that the Ar-SO<sub>3</sub>H groups are on top of the substrates. The presence of nitrogen

in the range of 400-405 eV is attributed to traces of dinitrogen and nitro groups[39, 40]. Its origins may lie in: the incomplete yield of the reduction leading to the radical formation, and subsequent covalent binding of the Ar-SO<sub>3</sub>H group, due to mild conditions[40] and reaction of aryldiazonium salt solution with the already-grafted species[39]. In spite of the detection of nitrogen traces that could underpin a lack of total grafting efficiency of the groups to the surface, the values obtained from the calculation of the surface concentration  $\Gamma$  of the grafted groups are close to those expected for monolayer coverage[18, 30, 31] and to the coverage of bound PEO onto Ar-SO<sub>3</sub>H grafted under electric field[24]:  $\Gamma_{Cr}=4.7 \times 10^{-10}$  mol cm<sup>-2</sup> on chromium and  $\Gamma_{Si}=6.5 \times 10^{-10}$  mol cm<sup>-2</sup> on silicon. The expressions of  $\Gamma$  for chromium[41, 42] and for silicon are respectively given as follow:

$$\Gamma = \frac{S_{2p}}{Cr_{2p_{3/2}}} \times 2.3 \times 10^{-9}; \quad \Gamma = \frac{S_{2p}}{Si_{2p}} \times 1.3 \times 10^{-9}.$$

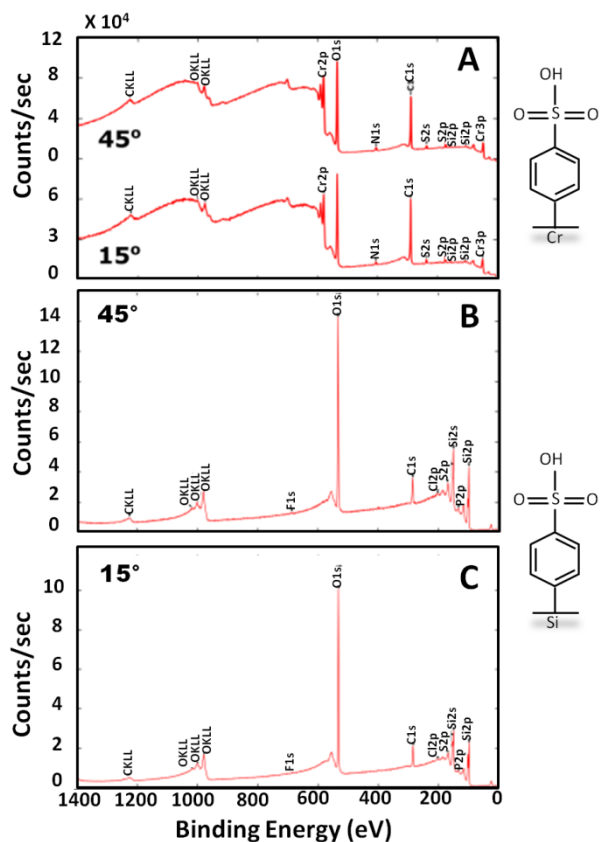
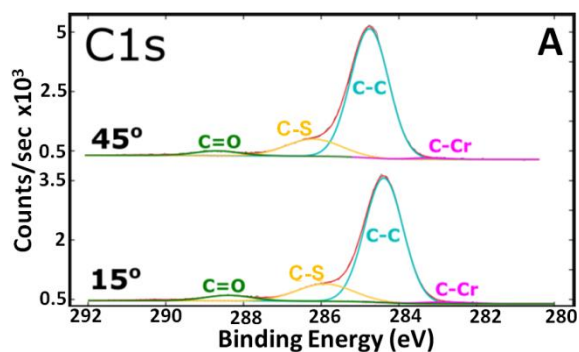


FIGURE 1. Survey spectra of A) functionalized chromium and B) silicon, collected at 45 ° and 15 ° of irradiating angle.

For each type of substrate, the peaks in **Figure 2** related to C1s, O1s and S2p were fitted and analyzed, along with Cr2p<sub>3/2</sub> for functionalized chromium and Si2p for functionalized n-doped silicon. More details about the XPS set-up, fits and bands attribution can be found in the SI along with a summarized table of the elements' positions on the binding energy scale.



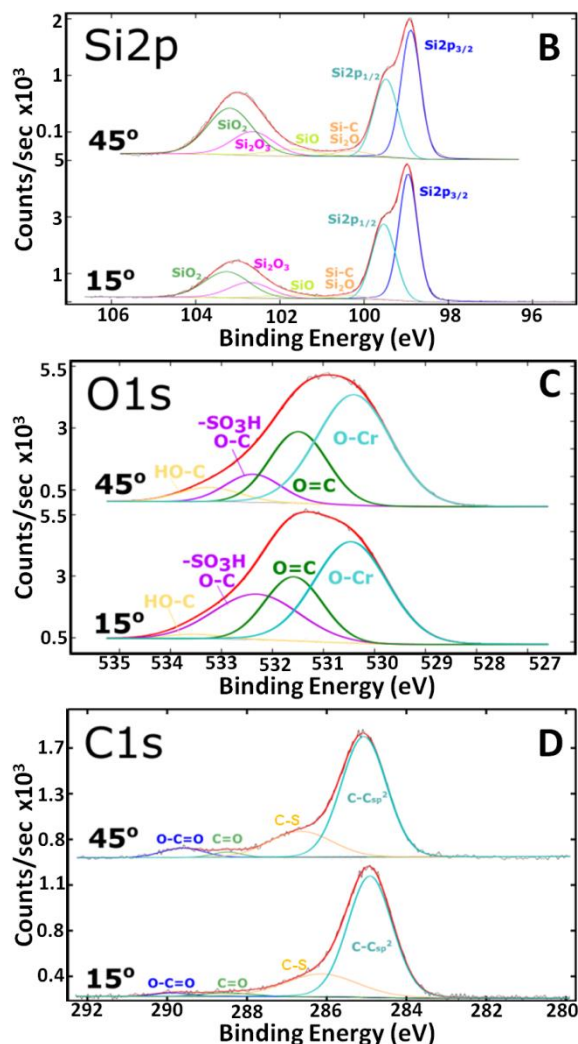


FIGURE 2. Fits of the XPS peaks A) C1s on Cr, B) Si2p on Si, C) O1s on Cr, D) C1s on Si of data collected at 45° and 15° of irradiation.

The presence of specific bonds are expected as a fingerprint of the grafted molecules such as O-SO<sub>2</sub>H, C-S, C-Cr or C-Si; of surface oxidation such as Cr-O or SiO, Si<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>; and O-C=O, or C-OH partly due to the sensitivity of the technique to possible contaminants of the surface[43].

The fingerprint of each substrate, chromium and silicon, is clearly displayed in the XPS data. For silicon, the band at 99.0 eV is characteristic of the silicon surface, and the additional peak at ~102.0 eV is assigned to the oxidation states. For chromium, only the Cr2p<sub>3/2</sub> region was analyzed, the Cr2p peak being separated into a spin-orbit doublet with no overlap. The band at 474.0 eV is characteristic of the chromium surface, and an additional broad peak at ~576.8 eV is assigned to the oxidation states and bonds to organic molecules. The features of the grafted molecules are identified in the C1s signal by the bonds C-C(aromatic) at 284.5 eV on Cr and 284.9 eV on Si and C-S at 286.0 eV on both substrates[44, 45]. It is possible that the peak attributed to C-S includes a contribution of C-O bonds, due to the grafting on oxides, since those binding energies overlap. The -SO<sub>3</sub>H (overlapping with C-O) is also seen in the O1s signal at 532.3 eV on Cr and 532.4 eV on Si[46, 47]. The traces of pollutants common to air exposed samples are detected in the C1s and O1s peaks and do not exceed 1.7 in % area.

#### Investigation of the tBLM by NR

In addition to XPS measurements, we also performed neutron reflectivity experiments on doped silicon (111) thick wafers. Data obtained in three different contrasts (100% H<sub>2</sub>O, 100% D<sub>2</sub>O, 62% H<sub>2</sub>O/38% D<sub>2</sub>O) before and after the SUV solution injection were analyzed using the ReFlid software[33-35] based on the fit of the NR curve from a neutron scattering length density (NSLD) profile ( $\rho_{bNR}(z)$ ). It is based on a box model where each layer is depicted from few fitting parameters: its thickness  $e$ , top roughness  $\sigma$  and NSLD (Table S-4,6). Best fits to the data profiles are shown in the form of a sum of errors function as follows[48, 49] (Figure 3):

$$NSLD(z) = \sum_{i=1} (\rho_i - \rho_{i+1}) * \frac{1}{2} \left[ \operatorname{erf} \left( \frac{z - z_i}{\sqrt{2}\sigma_i} \right) + 1 \right]$$

In this way, the NSLD of a layer corresponds to the sum of the NSLD of each material that composes a layer, weighted by its volume fraction in the layer. Thus, the fit is based on the determination of the volume fractions  $\varphi_l$  of each component in each layer  $l$  (**Figure S-4,7**). In our case, we don't expect that the volume fraction of each molecule is dependent on the solvent used for a stable membrane, but since during the fit the total NSLD of hydrated layers are contrast-dependent parameters, as mentioned before, the volume fractions calculated after the fit may differ between the three contrasts. Thus, we present their averaged values obtained from the three solvent contrasts NSLD profiles (**Figure S-5,8 and Table S-5,7**). Then we can estimate the density of molecules in each layer  $l$  of thickness  $e_l$  with  $l$  ranging from 1 to 5 for the 5 maximum layers created to describe the sample, using the following equation:

$$N_{l,molecules} = \varphi_l \frac{e_l}{V_{molecule}}$$

The NSLD value for the layer that contains the aryldiazonium molecules ( $\rho=1.6 \times 10^{-6} \text{ \AA}^{-2}$ ) corresponds thus to a grafting density  $\Gamma \sim 3.6 \times 10^{-10} \text{ mol.cm}^{-2}$  which is smaller than but comparable to the one found in the XPS experiments, carried out in more favorable conditions. For an ideal close-packed monolayer on Si(111), the highest density reached is  $\Gamma_{\max} \sim 13 \times 10^{-10} \text{ mol.cm}^{-2}$ [50]. From the grafting density of aryldiazonium, we can also estimate the mean in-plane distance between two grafting points ( $D=2/\sqrt{\pi\Gamma}$ ), which corresponds to roughly  $8.0 \text{ \AA}$ .

After the pure Brij58 functionalization and before SUV injection, the most favorable molecular configurations correspond to alkyl chains (hydrophobic and with  $\rho=0.35 \times 10^{-6} \text{ \AA}^{-2}$ ) lying close to the substrate interface and avoiding water, whereas the PEGylated chains (hydrophilic,  $\rho=0.68 \times 10^{-6} \text{ \AA}^{-2}$ ) are expected to be well hydrated. This is confirmed by the volume fractions obtained for layers 3 and 4, which correspond respectively to a mixture of solvent/PEG/Alkyl chains and a mixture of solvent/PEG chains. Alkyl chains are thus mainly located close to the silicone interface from hydrophobic interactions (the surface density of alkyl chain is more important than PEG chains in layer 3). Layer 4 can be pictured as loops of hydrated PEG chains. If we consider a much simpler model, with PEGylated chains made of  $N \sim 20$  monomers and with a free end, we can estimate their extension using a Kuhn length of  $b=11 \text{ \AA}$ [50], which corresponds to the chain segment length in a freely jointed model. Then considering the flexibility Flory characteristic ratio  $C_{\infty}=(b/a)^2=6.7$ , we can determine the real monomer length  $a=4.25 \text{ \AA}$ . Following the Alexander-de Gennes model of graft polymer chains, the non-dimensional grafting density  $(a/D)^2$  is here higher than  $N^{-6/5}$ , so each grafted PEGylated chain should adopt a semi-diluted configuration. In that configuration, the vertical extension of these chains should be equal to  $L_{PEG}=aN(a/D)^2=55.6 \text{ \AA}$ . For the alkane chain, we can estimate its length by the following equation:  $L_{C_{16}H_{33}} \approx 7.5 * (2d_{C-C} * \sin\theta) = 21.8 \text{ \AA}$ , with  $d_{C-C}=1.54 \text{ \AA}$  the bond length between two carbon atoms and  $\theta \approx 109.47^\circ$  corresponding to the angle between carbon bonds. This justifies the thicknesses found for layer 3 ( $C_{16}H_{33}$ +Part of PEGylated chains) and layer 4 (Extended PEGylated chains) at  $23.3 \text{ \AA}$  and  $26.7 \text{ \AA}$  respectively.

After contact with injected DOPC SUVs, a clear single bilayer lipid membrane is formed from vesicle fusion and a hydration cushion of roughly  $7.5 \text{ \AA}$  exists between the silicone substrate and the inner lipid head groups, with a highly hydrated PEG sublayer ( $\sim 70\%$  of water for  $\sim 30\%$  of PEG blocks). The averaged volume fraction profile of each molecular component in the layer is presented in **Figure 4** after simultaneous analysis of the three solvent contrasts used for this sample in the neutron reflectivity experiment (see **SI Table S-7** for precise values). The presence of water in the NSLD of layer 5, which represents the bilayer hydrocarbon tail region, is attributed to pores and bilayer defects that were identified previously from the transmembrane ion currents detected by electrochemical impedance spectroscopy[24]. The hydration of head group layers ( $39.53\%$ ) obtained is much larger than the tail one ( $13.89\%$ ), but not as much as reported in the literature with head hydrations reaching  $80\%$ . This issue comes in our case from the high background level we measured for the  $H_2O$  contrast sample. The bilayer's characteristic dimensions obtained from the NR fits are of  $12 \text{ \AA}$  for the head group layers ( $\rho=1.78 \times 10^{-6} \text{ \AA}^{-2}$ ) and  $23 \text{ \AA}$  for the inner tail region ( $\rho=0.21 \times 10^{-6} \text{ \AA}^{-2}$ ). This leads to a total bilayer thickness of  $45 \text{ \AA}$  which is perfectly consistent with the literature data for hydrated DOPC membranes[51]. Unfortunately, it was not possible to distinguish unambiguously the inserted alkyl chains of grafted Brij58 molecules (harpoons) from the lipid chains since both have the same NSLD ( $\rho=0.21 \times 10^{-6} \text{ \AA}^{-2}$  for lipid chains and  $\rho=0.35 \times 10^{-6} \text{ \AA}^{-2}$  for alkyl chains from our calculations based on the pure product densities).



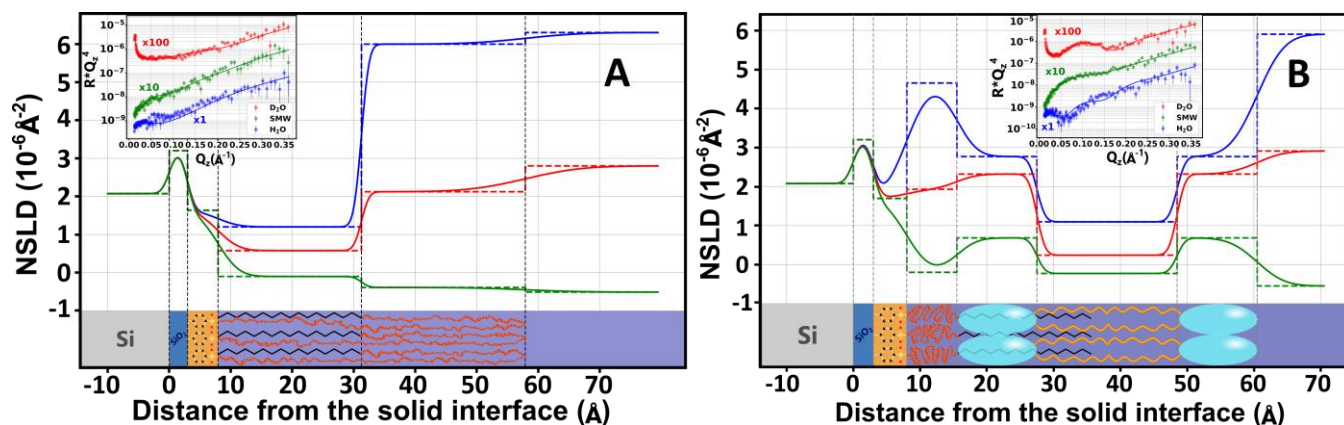


FIGURE 3. NSLD profiles obtained from fits of NR measurements A) silicon substrate functionalized with Brij58, B) after SUV deposition and the spontaneous formation of a Single Tethered Bilayer Lipid Membrane. Red line represents the contrasts for  $\text{D}_2\text{O}$ , green line for SMW and blue for  $\text{H}_2\text{O}$ .

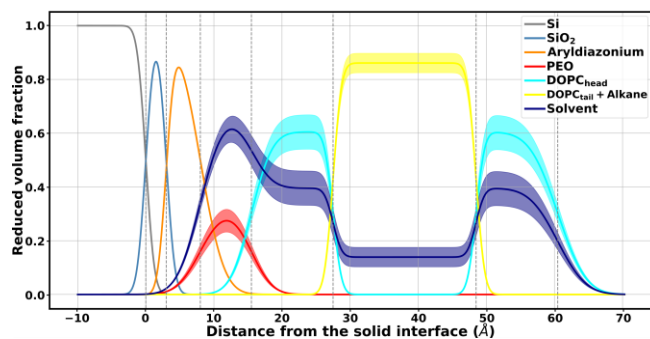


FIGURE 4. Averaged volume fraction profile for each molecular component in the layers. The sample is silicon substrate functionalized with Brij58 after SUV deposition and the formation of a single bilayer membrane

## CONCLUSION

In conclusion, spontaneous covalent grafting of aryldiazonium salt radicals to a material surface, by simple immersion at room temperature, made our protocol more straightforward by greatly simplifying the prefunctionalization step using wet chemistry[25], and still preserved the formation of  $-\text{Ar}-\text{SO}_3\text{H}$  monolayers via strong bonds between carbon and surface atoms. The second step was unchanged and also consisted in simple immersions to activate the  $-\text{Ar}-\text{SO}_3\text{H}$  groups into  $-\text{Ar}-\text{SO}_2\text{Cl}$ , and to create irreversible bonding of molecules from their  $-\text{OH}$ ,  $-\text{COOH}$ ,  $-\text{NH}_2$ , etc groups. We succeeded with simple immersions to reproduce the functionalization previously obtained by electro-grafting (assisted with cyclic voltamperometry, CV[24, 48]) on chromium thin films and n-doped silicon ( $\text{III}$ ) wafers. For all samples, atomic force microscopy gave surface roughness within the expected range of 0.3-0.5 nm[45]. XPS analysis revealed all expected bonds ( $-\text{SO}_3\text{H}$ , then C-Cr, C-Si and the challenging-to-measure S/2p peaks), and supported a rather compact layer with a surface coverage density similar to the one previously obtained by electrochemical analysis. Multiple oxidation states of both chromium and silicon did not impede the functionalization. Insightful investigation of  $\text{C}1s$ ,  $\text{O}1s$ ,  $\text{S}2p$ ,  $\text{Cr}2p_{3/2}$  and  $\text{Si}2p$  emission peaks also confirmed the expected molecule ratios at each step. By using wet chemistry in the absence of externally applied potential or additional reductive species, instead of electrochemistry, we greatly simplified this protocol for large scale production of smart coatings for sensors, envisioned for almost-isolating semi-conducting or metallic surfaces.

## ASSOCIATED CONTENT

### Supporting Information

I) XPS instrumentation and measurements; XPS fits for Chromium substrates; XPS fits for Silicon substrate. II) Neutron Reflectivity. NR instrumentation, measurements and fitting The Supporting Information is available free of charge on the BBA Biomembranes publications website.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: guillaume.brotons@univ-lemans.fr.

### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript and declare no competing interests.

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