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Lara Tauk, Thierry Thami, Lynda Ferez, Armagan Kocer, Jean-Marc Janot,  
Philippe Dejardin

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1 Thin phosphatidylcholine films as background  
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3 functionalization for biomedical applications.

4

5 *Lara Tauk<sup>1</sup>, Thierry Thami<sup>1</sup>, Lynda Ferez<sup>1</sup>, A. Kocer<sup>2,3</sup>, Jean-Marc Janot<sup>1</sup>, Philippe*  
6 *Déjardin<sup>1\*</sup>*

7

8 <sup>1</sup>Institut Européen des Membranes, Université Montpellier 2 (ENSCM, UM2, CNRS),  
9 CC047, 2 Place Eugène Bataillon, F-34095 Montpellier Cedex 5 (France)

10 <sup>2</sup>Biomade Technology Foundation, Nijenborgh 4, 9747AG, Groningen, The Netherlands.

11 <sup>3</sup>Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute,  
12 University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.

13

14 \*Corresponding author, [philippe.dejardin@iemm.univ-montp2.fr](mailto:philippe.dejardin@iemm.univ-montp2.fr), Tel +33 467149121, Fax  
15 +33 467149119.

16

1

2 ABSTRACT Non-specific adsorption is a crucial problem in the biomedical field. To  
3 produce surfaces avoiding this phenomenon, we functionalized thin (7 - 180 nm)  
4 poly(methylhydrosiloxane) (PMHS) network films at room temperature ( $\approx 20^{\circ}\text{C}$ ) with  
5 phospholipids (PL) bearing a phosphorylcholine head. Regardless of their mode of  
6 preparation (casting or immersion), all surfaces appeared to be very hydrophilic with a captive  
7 air-bubble contact angle stabilized around  $40^{\circ}$ . The thin films were protein-repellent in  
8 phosphate saline buffer pH 7.4 according to analysis by normal scanning confocal  
9 fluorescence. Neither was any adsorption or spreading of L- $\alpha$ -Phosphatidylcholine liposomes  
10 on such films observed. In addition, amino functional groups could be easily attached to the  
11 surface remaining available for further functionalization.

12

13 Keywords: biocompatibility; phosphorylcholine; phosphatidylcholine; biomaterials; PMHS;  
14 anti-fouling

15

16

1

2 **1. Introduction**

3

4 The efficient functioning of sensors for biomedical analysis is dependent on the  
5 integrity and accessibility of the surface-fixed molecule designed for molecular recognition,  
6 on the one hand, and on the surroundings designed for neutral behavior, especially to avoid  
7 nonspecific adsorption, on the other hand. Thus blocking buffers are proposed in diagnostic  
8 kits with for instance albumin[1, 2]. In addition, fabrication of polymer films on solid  
9 supports with defined structures and properties such as uniformity, stability, and  
10 reproducibility is crucial in the development of thin-film chemical sensors. It is still difficult  
11 to fabricate ultrathin polymer films that are uniform, continuously defect free, and stable.  
12 Moreover, controlled growth of stable polymer films at the nanoscale level, which is  
13 important in many applications, remains a challenge. Some control of film thickness,  
14 uniformity, stability is needed to achieve reproducibility[3].

15 A crucial aspect in the design of biomaterials surfaces is to achieve control also over  
16 the interfacial interactions between the synthetic material/device and the biological medium[4,  
17 5]. Protein-resistant ("non-fouling") surfaces are particularly important in the context of  
18 blood-contacting biomedical devices, and as non-interactive background for bio-diagnostic  
19 surfaces. There is a very limited number of effective non-fouling surfaces available to meet  
20 the challenges of practical applications [6]. Poly(ethylene glycol) (PEG) and oligo(ethylene  
21 glycol) (OEG) have been widely used to resist nonspecific protein adsorption [7, 8] owing to  
22 their ability to form a hydration layer via hydrogen bonds but the strength of these bonds  
23 decreases as the temperature increases[9]. Moreover, PEG or OEG can decompose in the  
24 presence of oxygen and transition metal ions[10]. Recently zwitterionic (sulfobetaine;  
25 carboxybetaine) materials have been found to exhibit ultralow protein adsorption [11, 12]

1 (fibrinogen adsorption  $< 5 \text{ ng cm}^{-2}$ ). This is due to the zwitterion structure which retains a  
2 large amount of tenaciously held water independent of temperature because of hydration via  
3 ionic solvation[13]. The interface is expected to be electrically neutral over a wide pH range  
4 (5-9) with phosphate and sulfonate zwitterions while variations can be exploited with carboxy  
5 groups. Coated surfaces with zwitterionic phospholipids have been shown to confer high  
6 resistance to protein adsorption[14]. The mechanism of protein adsorption resistivity on the  
7 phosphorylcholine modified surface is thought to be based on the interaction between water  
8 and phosphorylcholine groups. It is reported that when the water fraction on the polymer  
9 surface is maintained at a higher level, the proteins can contact the surface reversibly without  
10 significant conformational changes[15]. The large amount of water around the  
11 phosphorylcholine groups is thought to repel proteins and even prevent conformational  
12 changes of the few adsorbed proteins[16-18].

13 Recently a zwitterionic trimethoxysilane was synthesized for direct grafting on  
14 oxidized silicon wafers[19]. In the present work we applied the strategy of surface anchoring  
15 a reactive poly(methylhydrosiloxane) (PMHS) network, as in the development of a sensor for  
16 nitroaromatics[20], followed by covalent grafting of phospholipids bearing the zwitterionic  
17 phosphorylcholine head on the polymer active sites (SiH) via the hydrosilylation reaction  
18 (Fig. 1a). X-ray photoelectron analysis was used to follow the reaction. The captive air bubble  
19 contact angle in water was measured to characterize the samples surface hydrophilicity.

20 We considered especially thin PMHS films (10-200 nm), extending the previous study  
21 on films of thickness in the micrometer range[21], with the aim of creating low roughness  
22 surfaces not exhibiting the grooves shown in that previous work but conserving their protein-  
23 repellent character. Protein adsorption was analyzed by the normal scanning confocal  
24 fluorescence technique.

25

## 2. Materials and Methods

### 2.1 Chemicals

Both the precursors methyldiethoxysilane  $\text{HSi}(\text{CH}_3)(\text{OCH}_2\text{CH}_3)_2$  (DH) and triethoxysilane  $\text{HSi}(\text{OCH}_2\text{CH}_3)_3$  (TH) were purchased from ABCR (Karlsruhe, Germany) and used as received. Water for substrate cleaning was obtained from a Milli-Q water purification apparatus (Millipore). Absolute ethanol for sol-gel synthesis was of synthesis grade purity. Trifluoromethanesulfonic acid  $\text{CF}_3\text{SO}_3\text{H}$  was purchased from Aldrich. Toluene was distilled before use for thin film hydrosilylation. The platinum-divinyltetramethyldisiloxane complex in xylene (PC072) (platinum concentration of about 0.1 M assuming 2.4% (w) Pt in xylene), also known as Karstedt's catalyst, was purchased from ABCR. 1,2-dilinoleoyl-sn-glycero-3-phosphorylcholine (18:2 Cis) (PL) was purchased from AvantiPolarLipids. The molecule bears the zwitterionic phosphorylcholine head and two unsaturated cis carbon-carbon bonds on each of the two fatty chains. 3-(ethoxydimethylsilyl)-propyl amine (Aldrich, 588857) and hexamethyldisilazane were used as received.

### 2.2 Proteins and labeling

Bovine serum albumin (A-7638), cytochrome C (C-2506),  $\alpha$ -chymotrypsin (C-4129), avidin (A9275) were purchased from Sigma-Aldrich; some avidin from Fluka (No. 11368). Given their isoelectric point, at physiological pH 7.4, BSA ( $67\,000\text{ g mol}^{-1}$ , pI 5.6) is negative,  $\alpha$ -chymotrypsin ( $25\,300\text{ g mol}^{-1}$ , pI 8.1) slightly positive, avidin ( $66\,000\text{ g mol}^{-1}$ , pI 10) and cytochrome C ( $12\,400\text{ g mol}^{-1}$ , pI 10.2) strongly positive.

1 Labeling of proteins was performed with Alexa-fluor-594 succinimidyl ester (InvitroGen,  
2 A30008). Typically 500  $\mu$ L of protein solution were added to dry fluorophore Alexa-594 in  
3 molar ratio 1:1 and allowed to react for 0.5 h at 20°C. Avidin was also labelled via previous  
4 biotin-ethylenediamine hydrobromide (Sigma B9181) reaction with alexa succinimidyl ester  
5 as described elsewhere [22]. The mixture was then put in an *ad hoc* microtube with filter  
6 (Biospin P6) and centrifuged at 16 000 g for 1 min according to the supplier of the kit  
7 (Labelling kit A10239, InvitroGen). The labeling ratio [Alexa] / [protein] (0.3-0.5) was  
8 determined from the UV absorbances at 280 nm where both the label ( $\epsilon_{280-Alexa} = 50\ 400\ M^{-1}$   
9  $cm^{-1}$ ) and protein absorb and at 590 nm where only the label ( $\epsilon_{590} = 90\ 000\ M^{-1}\ cm^{-1}$ )[23]  
10 absorbs. Protein solutions were prepared in 10mM sodium phosphate buffer pH 7.4 with  
11 0.15M NaCl in de-ionized water (MilliQ system, Millipore) and stored at 4°C.

12

### 13 *2.3 Liposome preparation and labeling*

14

15 One hundred milligrams of L- $\alpha$ -Phosphatidylcholine (Soy-20%) (Avanti Polar Lipids  
16 Inc. 541601) was dissolved in 5ml of chloroform containing 100 $\mu$ L of a 1,1'-dioctadecyl-  
17 3,3,3',3'-tetramethylindodicarbocyanine perchlorate solution (Invitrogen Inc.) (1mg/ml in  
18 methanol). After chloroform had been thoroughly removed under vacuum, phosphate buffered  
19 saline (PBS, 10mM, pH 8.1, 150mM NaCl) was added and mixed in. Aggregated particles  
20 were then sonicated until complete dissolution. The mixture was then taken through a freeze-  
21 thaw procedure five times. This procedure involved freezing the solution by immersion in  
22 liquid nitrogen, followed by thawing by immersion in 60 °C water. After the freeze-thaw  
23 process, the vesicle suspension was divided into 10 samples of 500 $\mu$ L, frozen once again by  
24 immersion in liquid nitrogen and stored at -80°C. Before use, the vesicle suspension was  
25 heated by immersion in 60°C water and regularized by extrusion through polycarbonate

1 membrane filters (Avestin Inc.) of pore diameter 400 nm mounted in a mini-extruder (Avestin  
2 Inc.). We subjected the samples to 11 passes through the membrane.

3 The same procedure without the label DiD was applied to obtain unlabeled liposomes  
4 whose size distribution was measured by photon cross-correlation spectroscopy (Nanophox,  
5 Sympatec - France).

6

## 7 *2.4 Methods of preparation of the support*

8

### 9 *2.4.1 Substrate cleaning and activation*

10

11 Silicon wafers Si(100) (ACM, France) square strips of  $2 \times 2 \text{ cm}^2$  were used as  
12 substrates for spin-coating deposition. To bond covalently the PMHS thin films to native  
13 oxide silica (thickness ca 2 nm) and to glass surfaces, they were cleaned and activated with  
14 oxygen plasma for 60 seconds.

15

### 16 *2.4.2 PMHS film*

17

18 PMHS thin films were prepared at  $22 \pm 1^\circ\text{C}$  by sol-gel polymerisation of DH and TH  
19 as crosslinker, as described elsewhere[24]. This procedure gave layers of reproducible  
20 homogeneity and thickness as controlled by ellipsometry (Plasmos SD 2300 ellipsometer  
21 (München Germany)) [25]. We used refractive index  $n = 1.4$  for PMHS.

22

### 23 *2.4.3 Grafting PMHS with phospholipids*

24

25 *a-by casting*: the phospholipid solution was prepared under argon. 20 mg of PL were  
26 dissolved in 8 mL of dry toluene (2.5 mg / mL), with additional 7  $\mu\text{L}$  of the Karstedt catalyst



1 (xylene solution of platinum divinyltetramethyldisiloxane complex). The molar ratio of  
2 phospholipid over hydrogenosilane group SiH was 1.5. PMHS coated silicon wafers ( $2 \times 2$   
3  $\text{cm}^2$ ) and glass surfaces ( $1.5 \text{ cm}^2$ ) were covered by 200  $\mu\text{L}$  and 80  $\mu\text{L}$  of phospholipid solution  
4 respectively. The samples were kept for one hour under a cap to slowly evaporate the solvent  
5 and then left in open air for quick evaporation.

6 *b-by immersion*: silicon wafers were immersed in the phospholipid solution (2.5 mg/mL)  
7 for one hour.

8 For both methods, the samples were then rinsed successively in different  
9 toluene/chloroform mixtures from 100/0 to 0/100 (% vol.) with a gap of 20% and dried under  
10 a stream of Argon. We will refer to the PMHS layer having reacted with PL as PL-PMHS( $h$   
11 nm) where  $h$  is the PMHS initial thickness measured by ellipsometry.

## 12

### 13 2.5 Techniques of characterization of the support

#### 14

#### 15 2.5.1 X-ray photoelectron spectroscopy

16

17 The surface elemental compositions of unmodified and modified PMHS surfaces were  
18 analysed by X-ray photoelectron spectroscopy (XPS). The spectrophotometer (ESCALAB  
19 250, Thermo Electron, UK) was equipped with a monochromatic Al K $\alpha$  (1486.6 eV) radiation  
20 source. The acceleration tension and power of X-ray source were 15 kV and 100 W,  
21 respectively. The samples were analysed at a pressure in the  $10^{-9}$  mbar range. The electron  
22 take-off angle with respect to the sample surface was  $90^\circ$ . The analysed spot size was  
23 approximately  $400 \mu\text{m}^2$ . The XPS composition corresponds to depths of 5-10 nm. Survey  
24 scans (0-1350 eV) at low resolution were performed to identify constitutive elements. High  
25 resolution C $_{1s}$ , Si $_{2p}$ , O $_{1s}$ , N $_{1s}$  and P $_{2p}$  spectra were recorded to obtain more detailed information

1 about the nature of the surface. The peaks were fitted with Gauss–Lorentz curves. They  
2 provided the various surface atomic ratios from the corresponding peak areas, by assuming  
3 the total area corresponded to 100%, after correction with the theoretical sensitivity factors  
4 [26]. The spectra of both pristine PMHS and PL-PMHS were calibrated using the  
5 hydrocarbon contaminant or alkane C<sub>1s</sub> peak set at 284.8 eV. In both cases, the peak of Si-  
6 CH<sub>3</sub> was found at a binding energy (BE) of 284.0 eV in agreement with PDMS polymer at  
7 284.4 eV [27].

8

### 9 *2.5.2 Captive air bubble contact angle in water*

10

11 The samples were characterized by captive air-bubble (7.6 μL) contact angle (GBX -  
12 Digidrop, Romans, France). The contact angle was calculated after 1 min using computerized  
13 image analysis. The data are relative to the angle through the liquid as generally defined[28],  
14 in opposition to its complement angle to 180° through air as used in a previous work[21]

15

### 16 *2.5.3 AFM*

17

18 Atomic Force Microscopy (AFM) experiments were performed using a Dimension  
19 3100 microscope equipped with a Nanoscope IIIa controller system (Digital Instruments,  
20 Veeco Metrology Group). AFM images were obtained by scanning in tapping mode in water  
21 or under ambient conditions in air using silicon SPM probes (stiffness  $k \approx 2$  N/m, resonance  
22 frequency of 67 kHz, pointeprobeplus, Nanosensors). The root mean square average  
23 roughness ( $R_q$ ) was analyzed with the Nanoscope software (version 5.31r1).

24

### 25 *2.6 Adsorption: Flow cell and Determination of interfacial concentration*

1

2           The experiments were performed at  $T = 19^{\circ}\text{C}$  in a slit flow cell of thickness  $\approx 100 \mu\text{m}$   
3 and flow rate corresponding to wall shear rate  $1000 \text{ s}^{-1}$ . Confocal measurements[29, 30] with  
4 inverted microscope configuration were performed at 3 cm from the slit entrance. Interfacial  
5 concentration was evaluated as follows: the fluorescence signal  $F_{\text{sol}}$  from solution at  
6 concentration  $C$  is relative to an effective volume  $V$  while the signal  $F_{\text{surf}}$  from surface  
7 concerns interfacial concentration  $\Gamma$  over area  $A$ .  $F_{\text{surf}} \propto \Gamma A$  and  $F_{\text{sol}} \propto C V$  therefore  $\Gamma =$   
8  $(V/A) (F_{\text{surf}}/F_{\text{sol}}) C$ . In a previous paper[29] the order of magnitude of  $V/A$  was estimated from  
9 the focus radius for  $A$  and  $1 \mu\text{m}^3$  taken as the confocal volume  $V$ . A more precise  
10 determination is obtained considering the adsorbed layer as a Dirac function of  
11 fluorescence[21]. Then  $V/A = w_D$  is the area under the normalized fluorescence peak  $f_i(y) =$   
12  $F_i(y) / F_i(0)$  resulting from the convolution of the fluorescent interface positioned at  $y = 0$  with  
13 the laser beam.

14

### 15 **3. Results and Discussion**

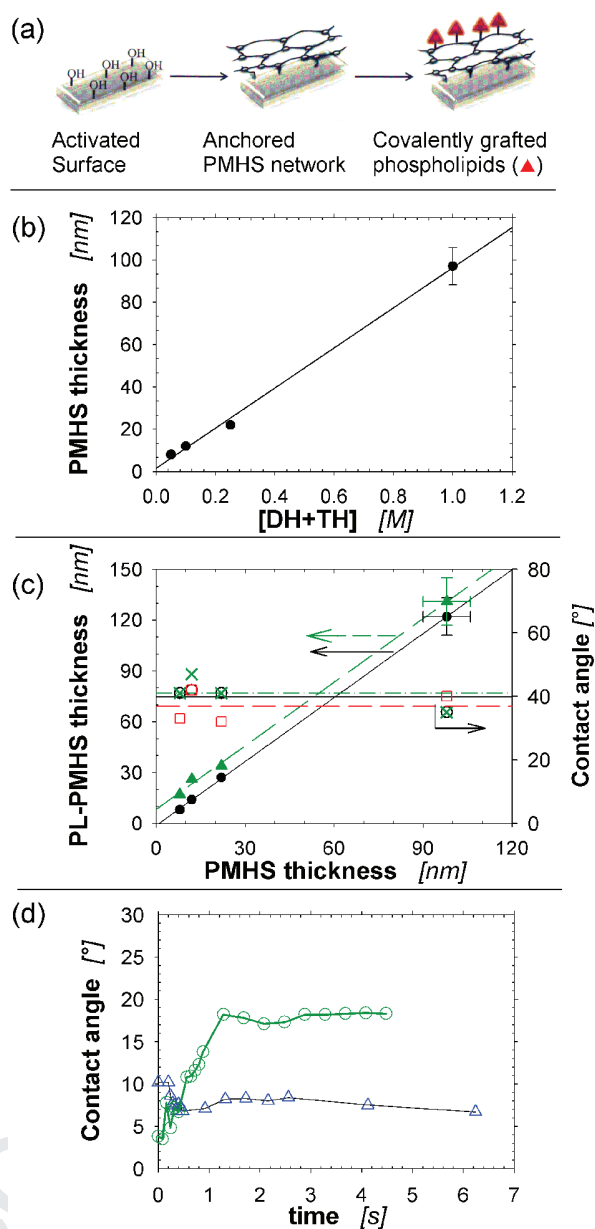
16

#### 17 *3.1 Physico-chemical characterization of PMHS and PL-PMHS films*

18

19           The ellipsometric thickness of PMHS film on oxidized wafers as a function of initial  
20 concentration of monomers (DH and TH) is provided in Fig. 1b. The variation was linear with  
21 a slope of  $95 \pm 4 \text{ nm M}^{-1}$ . After hydrosilylation reaction had proceeded over one hour by  
22 means of casting or immersion the thickness of the PL-PMHS layer was measured and the  
23 hydrophilicity characterized by captive air bubble contact angle (Fig. 1c). Both techniques  
24 gave the same results suggesting the occurrence of a quick interfacial reaction over less than  
25 one hour and efficient removal of excess phospholipids in the casting method. An average

1 angle of  $40^\circ$  was observed whatever the initial PMHS thickness, much below the value of  $90^\circ$   
2 observed with hydrophobic PMHS. The ellipsometric thickness increased as expected after  
3 reaction of the PMHS layer with phospholipids. Conditioning the samples overnight in Milli-  
4 Q water gave rise to an additional increase of thickness. Over the range examined we can  
5 attribute a mean 26% increase of thickness from the reaction with phospholipids and an 8 nm  
6 increase from conditioning the material in water. Conditioning the PL-PMHS for several days  
7 in water led to rolling bubble ( $0^\circ$ ) possibly stabilized at a slightly higher contact angle (Fig.  
8 1d).  
9



1

2 **Figure 1** Method of surface treatment and physico-chemical characteristics of the films. (a)

3 Illustration of surface silanols of oxidized silicon wafer as anchoring points of PMHS network

4 for subsequent phospholipid grafting. (b) Thickness of PMHS film on oxidized silicon wafers

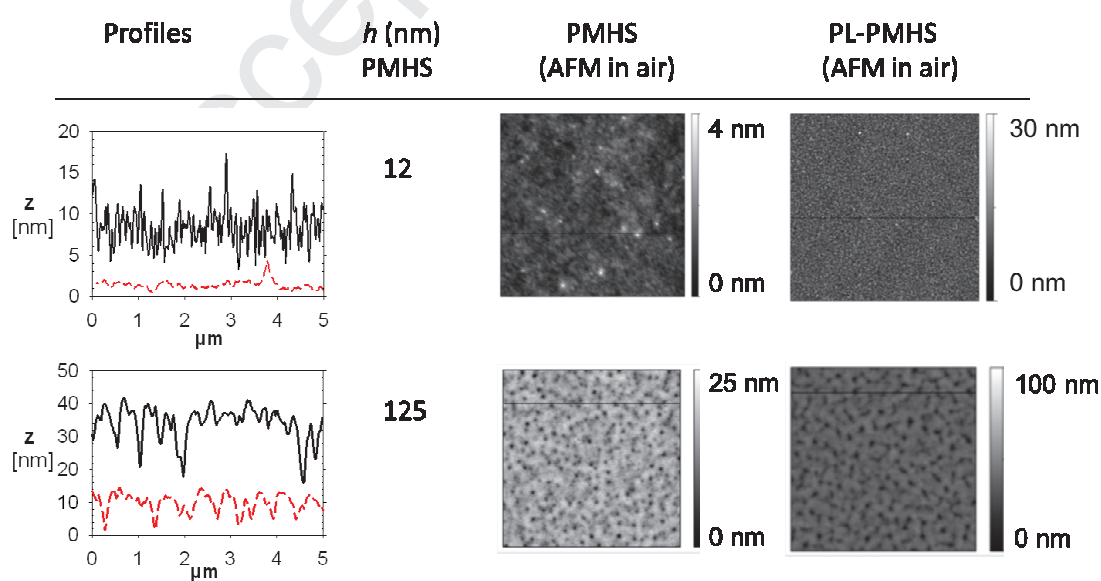
5 as a function of initial concentration of monomers. Slope  $95 \pm 4 \text{ nm M}^{-1}$ . Dispersion of data at6 low concentration is of the order of the symbol size. (c *left scale*) PL-PMHS (reaction with PL7 solution 2.5 mg/mL) thickness after toluene/ chloroform rinsing (●; full line, slope  $1.26 \pm$ 8  $0.01$ ) and additional overnight immersion in water (▲; dashed line, slope  $1.25 \pm 0.03$ ,

1 ordinate at the origin  $8 \pm 2$  nm) as a function of original PMHS thickness. (*c right scale*)  
 2 Captive air bubble contact angle on PL-PMHS, created via casting (o) or immersion (x,  
 3 wafer; □, glass), as a function of initial PMHS thickness. (*d*) Examples of variation of contact  
 4 angle with time (rolling bubble over  $\sim 1$  cm) for samples conditioned for several days in  
 5 water.

6

7 The structures of the PMHS and PL-PMHS films were examined by AFM (Fig. 2).  
 8 PMHS roughness was lower than 1 nm for a very thin layer (12-20 nm) while structures  
 9 appeared for 125nm. Reaction of PL induced always an increase in roughness (Table 1). The  
 10 12 nm thin PMHS sample showed a roughness  $R_q$  of 0.4 nm with a few defects. PL grafting  
 11 induced an increase of roughness to 2.03 nm as measured in air. Immersion in water  
 12 decreased the roughness to 1.28 nm. This may be partly due to the reorganisation of  
 13 phospholipids chains in the presence of an aqueous medium in order to minimise the free  
 14 energy of the system. The minimum 0.9 nm for the PL-PMHS roughness was found for *ca.* 20  
 15 nm thick PMHS.

16



17

1

2 **Figure 2** Characterization of the films by atomic force microscopy. AFM pictures ( $5\ \mu\text{m} \times 5$   
3  $\mu\text{m}$ ) of PMHS layers (thickness  $h$ ) and of their derived PL-PMHS layers. Corresponding  
4 profile samples of PMHS (dashed line) and PL-PMHS (full line).

5

$h$ (PMHS)	$R_q$ (PMHS)	$R_q$ (PL-PMHS)
(nm)	(nm)	(nm)
12	0.40	2.03
20	0.60	0.90
125	2.5	5.6

6 **Table 1** Roughness  $R_q$  over  $10\ \mu\text{m} \times 10\ \mu\text{m}$  samples of PMHS and derived PL-PMHS for  
7 different initial PMHS thickness  $h$ .

8

### 9 3.2 Chemical characterization: XPS analysis of the interface

10

11 The surface elemental compositions of pristine PMHS and PL-PMHS were determined  
12 from high resolution XPS spectra. To summarize the characterization of PL-PMHS layers,  
13 they are very hydrophilic due to the fast reaction of PL at the top of the layer. There was  
14 however competition with the side reaction of SiH with traces of water giving SiOH groups  
15 which may recombine to produce siloxane bridges. The formation of new SiOH or SiOSi  
16 bonds in the PL-PMHS layer was shown by the evolution of the two distinct doublets ( $\text{Si}_{2p^{3/2}}$ )  
17 of the  $\text{Si}_{2p}$  PMHS spectrum by reaction with PL at binding energies 101.2 (21%  $\rightarrow$  86%) and  
18 102.3 eV (79%  $\rightarrow$  14%) with small shifts in energy. We will see below how the density of  
19 available SiOH can be determined and used for functionalization.

20

1 *3.3 Protein adsorption*

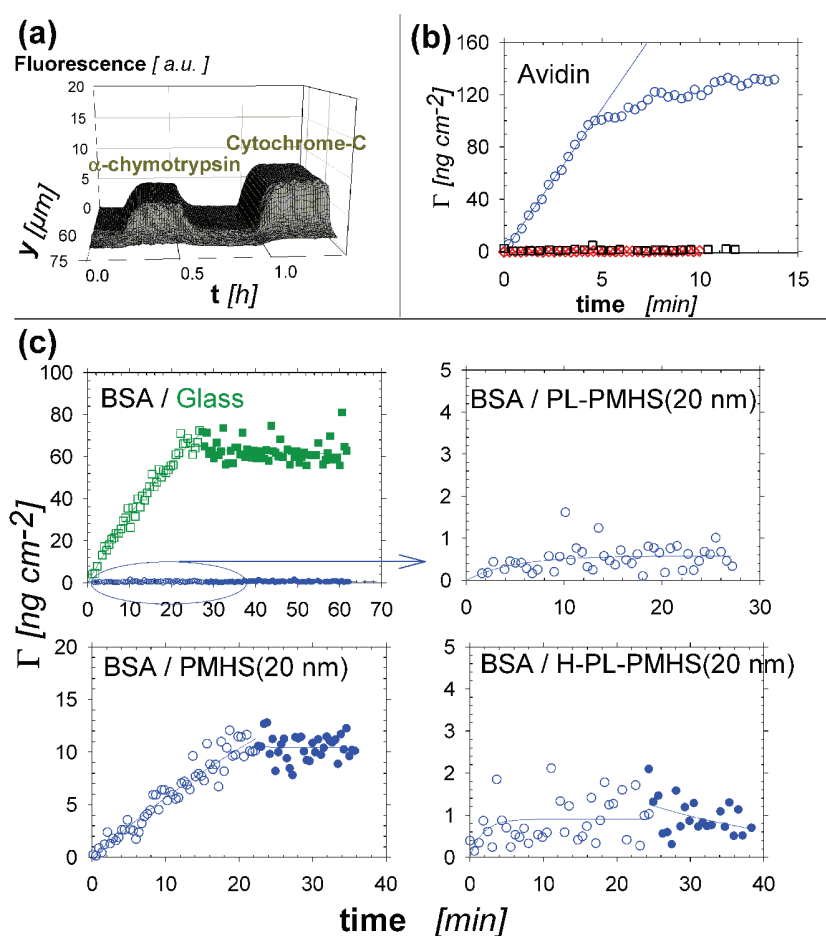
2

3 The wettability behaviour of the PL-PMHS surfaces (Fig. 1c-d) was indicative of a  
4 probable protein-repellent character. We investigated protein adsorption from single protein  
5 solution in PBS buffer pH 7.4 (avidin, bovine serum albumin (BSA),  $\alpha$ -chymotrypsin and  
6 cytochrome-C). Avidin is a model of a very stable hard protein. As most surfaces are  
7 negative, we considered preferably positive proteins at neutral pH, to check the surface  
8 passivation with respect to protein adsorption. It should be borne in mind however that the  
9 ionic strength of PBS buffer is not small with 0.15 M NaCl. Conversely the negative BSA can  
10 be viewed as a soft protein model, able to be adsorbed and denatured on many materials  
11 whatever their charge, thus a good candidate to check for possible hydrophobic interactions  
12 with surfaces. Indeed, this protein is often used in blocking buffers to prevent subsequent  
13 nonspecific adsorption.

14 The protein-repellent character of the PL-PMHS(20 nm) surface was checked in the  
15 configuration of flowing solutions in a slit. No adsorption of  $\alpha$ -chymotrypsin and  
16 cytochrome-C was observed (Fig. 3a).

17





1

2 **Figure 3** Adsorption under laminar flow conditions with wall shear rate  $\gamma = 1000 \text{ s}^{-1}$ . Closed  
 3 symbols for the buffer rinsing step. (a) 3D graphs of successive flows of  $\alpha$ -chymotrypsin and  
 4 cytochrome C solutions (150 nM) with intermediate flow of PBS buffer showing no  
 5 adsorption on the wall of PL-PMHS covered oxidized silicon wafer (foreground,  $y \approx 67 \text{ }\mu\text{m}$ ).  
 6 (b) avidin on (●) sulfochromic acid treated glass,  $C_b = 5.0 \text{ }\mu\text{g/mL}$ ; (■) PL-PMHS(7 nm),  $C_b$   
 7  $= 10 \text{ }\mu\text{g/mL}$ ,  $\Gamma = 1.2 \pm 0.9 \text{ (SD) ng cm}^{-2}$ ; (◆) PL-PMHS(180 nm),  $C_b = 5.0 \text{ }\mu\text{g/mL}$ ,  $\Gamma = 0.1$   
 8  $\pm 0.9 \text{ ng cm}^{-2}$ ; (c) BSA adsorption kinetics on different surfaces,  $C_b = 10 \text{ }\mu\text{g/mL}$ : (top left) (□)  
 9 sulfochromic acid treated glass, (○) PL-PMHS(20 nm), shown at a different scale on (top  
 10 right)  $\Gamma = 0.5 \pm 0.3 \text{ ng cm}^{-2}$ ; (bottom left) PMHS(20 nm); (bottom right) PL-PMHS(20 nm)  
 11 treated with HMDS.

12

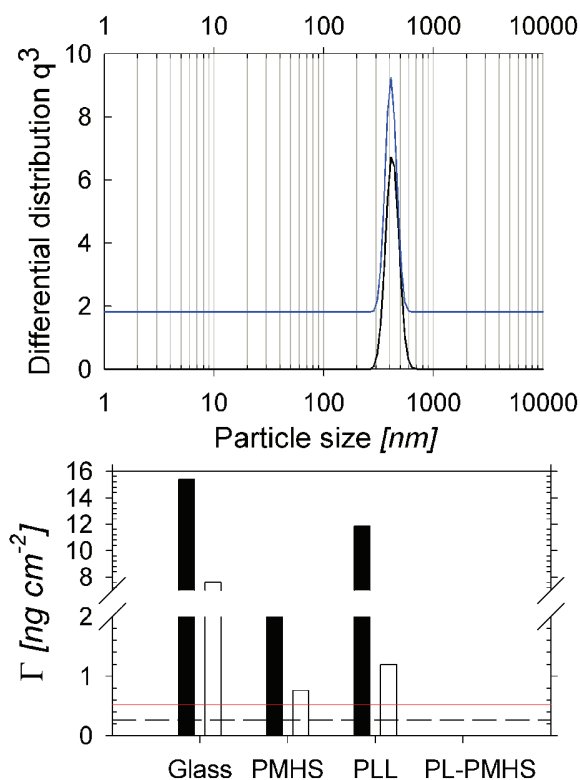
1           The adsorption of avidin was examined on different PL-PMHS substrates derived  
2 from the PMHS initial thicknesses of 7 nm and 180 nm (Fig. 3b). The interfacial  
3 concentration was 1.2 and 0.1 ng cm<sup>-2</sup> respectively, with dispersion around 1 ng cm<sup>-2</sup>. Let us  
4 recall that there was a slight displacement in the plane of substrate between the normal  
5 scanings to avoid repetitive exposure to the laser beam which could induce a photobleaching  
6 effect. This procedure constituted then also an examination of the interface homogeneity.  
7 Conversely, adsorption on sulfochromic acid treated glass was large with an initial linear  
8 variation up to *ca.* 0.1 µg cm<sup>-2</sup>. The initial kinetic constant *k* was 7.4 × 10<sup>-5</sup> cm s<sup>-1</sup>. With *D* ≈ 6  
9 10<sup>-7</sup> cm<sup>2</sup> s<sup>-1</sup>, the transport limited constant *k<sub>Lev</sub>* is 2.65 10<sup>-4</sup> cm s<sup>-1</sup>. Applying the expression  
10 [31, 32]  $k_a = k (b_1 u + 1) (b_2 u + 1) / ((u-1) (a_1 u - 1))$ , where  $u = k/k_{Lev}$  and numerical coefficients  
11  $a_1 = 0.556$ ,  $b_1 = -0.681$ ,  $b_2 = -0.0484$ , led to  $k_a \approx 1.0 \times 10^{-4}$  cm s<sup>-1</sup>. In any case, the  
12 phospholipid treatment was very efficient in preventing avidin adsorption, even at very small  
13 thickness of PMHS (7 nm), exhibiting the good quality of the coverage and the corresponding  
14 screening of the substrate.

15           The adsorption of Alexa-BSA was studied on PMHS(20 nm), PL-PMHS(20 nm) and  
16 PL-PMHS followed by HMDS capping, denoted H-PL-PMHS(20 nm) (Fig. 3c). All surfaces  
17 were immersed overnight in water and exposed to flowing buffer and solution with  
18 sulfochromic acid treated glass as the other face. Whatever the surface, when adsorption  
19 occurred no rapid desorption was observed by flowing buffer. The PMHS surface exhibited  
20 some adsorption, 10 ng/cm<sup>2</sup> over 20 min without a plateau ( $k = 1.0 \cdot 10^{-6}$  cm s<sup>-1</sup>). The H-PL-  
21 PMHS showed a very small interfacial concentration ( $\Gamma = 0.8 \pm 0.5$  ng/cm<sup>2</sup>) comparable to  
22  $0.5 \pm 0.3$  ng/cm<sup>2</sup> observed on PL-PMHS. It might reflect the burying of the trimethyl groups  
23 in the hydrophobic domains of lipid chains avoiding their direct exposure to the aqueous  
24 medium thus preventing hydrophobic interactions with the protein.

1 Our studies showed that phosphorylcholine modified PMHS layers experienced almost  
 2 “zero” protein adsorption. The decrease due to phosphorylcholine groups agrees also with  
 3 previous studies to improve the biocompatibility of poly(dimethylsiloxane) (PDMS)[33]. The  
 4 polymer of 2-methacryloyloxyethyl phosphorylcholine (MPC) was grafted by surface-  
 5 initiated photo-induced radical polymerization. The *in vitro* single protein adsorption on the  
 6 poly(MPC)-grafted PDMS decreased 50-75% compared to the unmodified PDMS. On  
 7 poly(ether-ether-ketone) (PEEK) treated with MPC, BSA adsorption studies[34] showed a  
 8 significant effect of MPC till 90% adsorption reduction with respect to PEEK.

9 Adsorption of labeled (DiD) liposomes of L- $\alpha$ -Phosphatidylcholine (diameter 400 nm)  
 10 was measured by scanning confocal fluorescence as for the proteins. Based on an average area  
 11 per head of 0.5 nm<sup>2</sup> or diameter per head of 0.8 nm, the adsorption at different interfaces is  
 12 shown in Fig. 4.

13



14

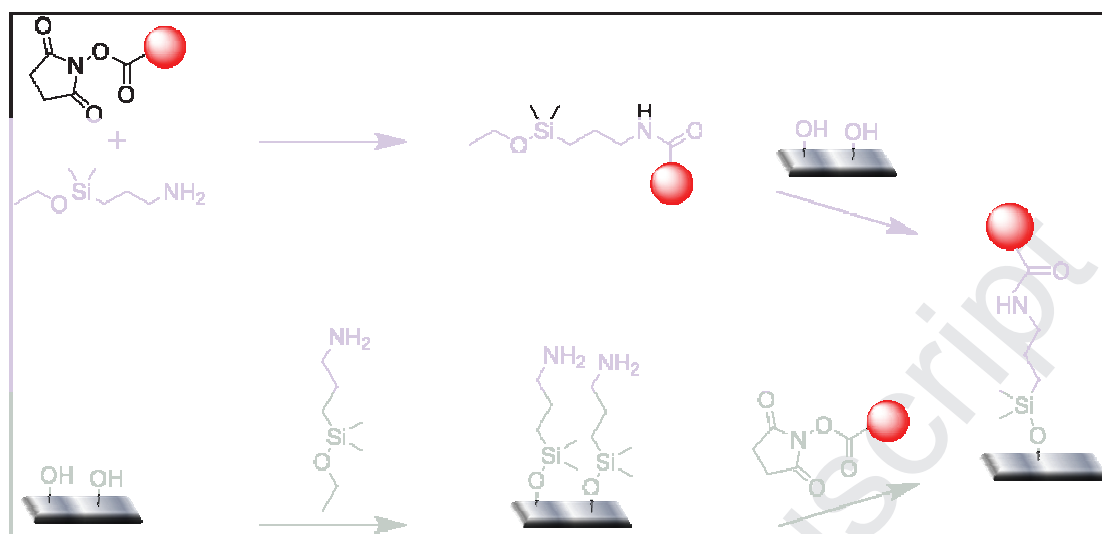
1 **Figure 4** Characterization of liposomes and of their interaction with surfaces. (top) Photon  
2 cross-correlation spectroscopy of unlabeled L- $\alpha$ -Phosphatidylcholine liposomes showing the  
3 reproducibility of the size 400 nm in the preparation. (bottom) Adsorption-spreading of  
4 labelled liposomes on piranha treated glass, PMHS, poly(L-Lysine) (PLL) and PL-PMHS,  
5 after one (full bar) and a few rinsings (empty bar). Horizontal lines correspond to estimated  
6 monolayer (dashed line) and bilayer coverages (full line).

7  
8 It can be seen that PL-PMHS constitutes a repellent surface with respect to L- $\alpha$ -  
9 Phosphatidylcholine liposomes, despite this lipid being not just a pure simple lipid but a  
10 mixture of lipids (24% PC, 18% Phosphatidylethanolamine, 11.5% Phosphatidylinositol, 4.3  
11 % Phosphatidic acid, 4.6% LysoPC and some other unknown lipids).

### 12 13 *3.4 Functionalization of PL-PMHS Surface*

14  
15 The XPS analysis after PMHS hydrosilylation with PL exhibited the side reaction with  
16 water giving silanol groups, which may evolve to siloxane bridges too. Such interfacial  
17 silanols could be available for subsequent reaction. Their presence was checked by reaction of  
18 a monoethoxy silane coupling agent bearing alexa as fluorescent probe (Scheme 1). Indeed,  
19 we observed a high fluorescence signal thus confirming the possibilities of chemical  
20 functionalization at such surfaces having an inert background of C18-phospholipids bearing a  
21 phosphorylcholine head. No coupling reaction occurred after pretreatment of PL-PMHS with  
22 hexamethyldisilazane (HMDS) as the silanols were neutralized by bulky trimethylsilyl  
23 groups.

24



1

2 **Scheme 1** Grafting reaction of Alexa, sketched as a big sphere, to surface silanols. (top) via  
 3 previous formation of amide link between alexa and monoethoxy silane coupling agent and  
 4 (bottom) via previous reaction of surface silanols with aminosilane.

5

6 PMHS did not exhibit any coupling reaction with alexa monoethoxy silane in toluene /  
 7 ethanol (70/30), thus suggesting the relative stability of the interfacial hydrogenosilane (SiH)  
 8 functional groups in the absence of water and catalyst. Moreover, this demonstrates that the  
 9 Karstedt' catalyzed side reaction of SiH with water plays a major role in the formation of  
 10 interfacial silanol on such PL-PMHS surface. After coupling the alexa probe, the silanol  
 11 concentration can be thus estimated by assuming that the coupling reaction is total. According  
 12 to the method developed above for determination of interfacial concentration, the mean  
 13 degree of functionalization of the PL-PMHS layer was measured to be  $3.0 \times 10^{-2} \text{ nm}^{-2}$ .

14 Assuming that all molecules were grafted at the interface the mean distance between the sites  
 15 was 5.7 nm. This order of magnitude is acceptable for a quite dense packing of proteins on a  
 16 neutral background and can be probably modified by changing the experimental conditions. In  
 17 addition, we showed that the silanols of the interface could react also with  
 18 aminomonoethoxysilane to provide a phosphorylcholine background surface with amino

1 groups available for amide junctions (Scheme 1). This was carried out with the alexa ester  
2 leading to the same interface as before with the same site density.

3

#### 4 **4. Conclusion**

5

6 Polymethylhydrosiloxane anchored on an activated oxidized silicon wafer or glass can  
7 be deposited as a very thin film of low roughness and functionalized with phospholipids  
8 bearing a phosphorylcholine head. The functionality of the surface was analyzed by XPS and  
9 the correlated wettability change determined by contact angle measurements with captive air  
10 bubble. The new interface was observed to be very hydrophilic and protein-repellent at  
11 neutral pH in phosphate saline buffer. L- $\alpha$ -Phosphatidylcholine liposomes containing different  
12 phospholipids could not bind to such interfaces. Thus tailored surfaces can be produced to fix  
13 specific cells with appropriate functionalization. As the stiffness is a pertinent parameter in  
14 cell spreading[35], one perspective would be to vary the DH/TH ratio in the synthesis of the  
15 PMHS films with better control of water in subsequent grafting. We have shown that the  
16 silanol groups originating from the side-reaction with traces of water provided possibilities for  
17 further functionalization. The prevention of protein adsorption is attributed to the zwitterionic  
18 character of the phosphorylcholine head which maintains a water-rich layer at the interface.  
19 We found that a 20 nm thick PMHS layer led to very flat surfaces with roughness less than 1  
20 nm. We have shown finally that the protein-repellent interface can be easily provided with  
21 amine functional groups. Therefore such protein-repellent interfaces can be useful in the  
22 biomedical domain, e.g. for biosensors, biomaterials and cell culture, by reducing strongly the  
23 non-specific adsorption, on the one hand, and offering in addition possibilities for grafting  
24 desired functions, on the other hand. Moreover, the covalent coverage with phospholipids and  
25 the sites for further functionalization are generated in a one step process.

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19  
20  
21



1 **Captions to illustrations**

2

3 **Figure 1** Method of surface treatment and physico-chemical characteristics of the films. (a)  
 4 Illustration of surface silanols of oxidized silicon wafer as anchoring points of PMHS network  
 5 for subsequent phospholipid grafting. (b) Thickness of PMHS film on oxidized silicon wafers  
 6 as a function of initial concentration of monomers. Slope  $95 \pm 4 \text{ nm M}^{-1}$ . Dispersion of data at  
 7 low concentration is of the order of the symbol size. (c *left scale*) PL-PMHS (reaction with PL  
 8 solution 2.5 mg/mL) thickness after toluene/ chloroform rinsing ( $\bullet$ ; full line, slope  $1.26 \pm$   
 9  $0.01$ ) and additional overnight immersion in water ( $\blacktriangle$ ; dashed line, slope  $1.25 \pm 0.03$ ,  
 10 ordinate at the origin  $8 \pm 2 \text{ nm}$ ) as a function of original PMHS thickness. (c *right scale*)  
 11 Captive air bubble contact angle on PL-PMHS, created via casting (o) or immersion ( $\times$ ,  
 12 wafer;  $\square$ , glass), as a function of initial PMHS thickness. (d) Examples of variation of contact  
 13 angle with time (rolling bubble over  $\sim 1 \text{ cm}$ ) for samples conditioned for several days in  
 14 water.

15 **Figure 2** Characterization of the films by atomic force microscopy. AFM pictures ( $5 \mu\text{m} \times 5$   
 16  $\mu\text{m}$ ) of PMHS layers (thickness  $h$ ) and of their derived PL-PMHS layers. Corresponding  
 17 profile samples of PMHS (dashed line) and PL-PMHS (full line).

18

19 **Figure 3** Adsorption in laminar flow conditions with wall shear rate  $\gamma = 1000 \text{ s}^{-1}$ . Closed  
 20 symbols for the buffer rinsing step. (a) 3D graphs of successive flows of  $\alpha$ -chymotrypsin and  
 21 cytochrome C solutions (150 nM) with intermediate flow of PBS buffer showing no  
 22 adsorption on the wall of PL-PMHS covered oxidized silicon wafer (foreground,  $y \approx 67 \mu\text{m}$ ).  
 23 (b) avidin on (o  $\bullet$ ) sulfochromic acid treated glass,  $C_b = 5.0 \mu\text{g/mL}$ ; ( $\square$ ) PL-PMHS(7 nm),  $C_b$   
 24  $= 10 \mu\text{g/mL}$ ,  $\Gamma = 1.2 \pm 0.9 \text{ (SD) ng cm}^{-2}$ ; ( $\diamond$ ) PL-PMHS(180 nm),  $C_b = 5.0 \mu\text{g/mL}$ ,  $\Gamma = 0.1$   
 25  $\pm 0.9 \text{ ng cm}^{-2}$ ; (c) BSA adsorption kinetics on different surfaces,  $C_b = 10 \mu\text{g/mL}$ : (top left) ( $\square$ )

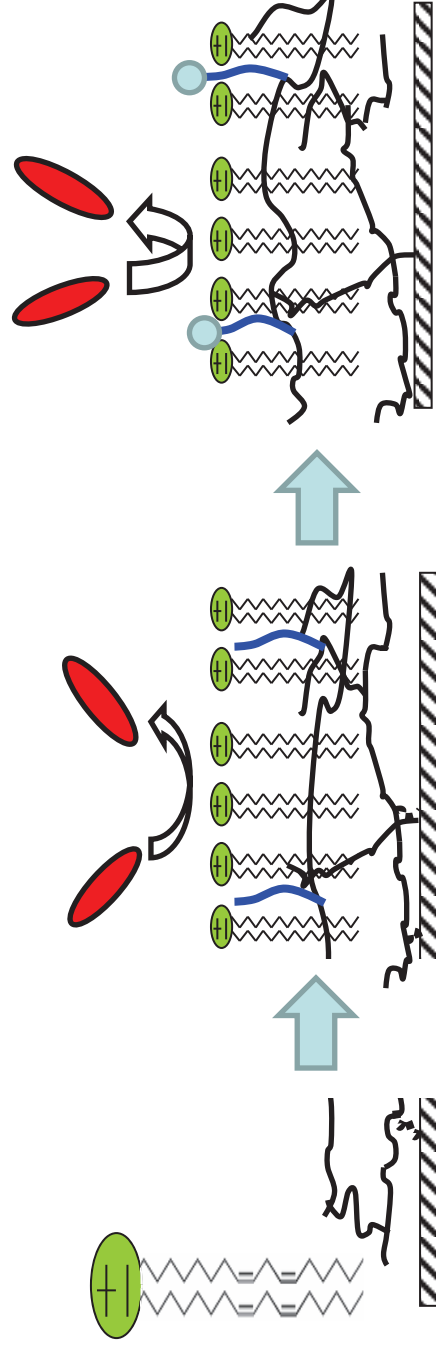
1 sulfochromic acid treated glass, (o) PL-PMHS(20 nm), shown at a different scale on (*top*  
2 *right*)  $\Gamma = 0.5 \pm 0.3 \text{ ng cm}^{-2}$ ; (*bottom left*) PMHS(20 nm); (*bottom right*) PL-PMHS(20 nm)  
3 treated with HMDS.

4  
5 **Figure 4** Characterization of liposomes and of their interaction with surfaces. (top) Photon  
6 cross-correlation spectroscopy of unlabeled L- $\alpha$ -Phosphatidylcholine liposomes showing the  
7 reproducibility of the size 400 nm in the preparation. (bottom) Adsorption-spreading of  
8 labelled liposomes on piranha treated glass, PMHS, poly(L-Lysine) (PLL) and PL-PMHS.  
9 After (full bar) one and (empty bar) a few rinsings. Horizontal lines correspond to estimated  
10 (dashed line) monolayer and (full line) bilayer coverages.

11  
12 **Scheme 1** Grafting reaction of Alexa, sketched as a big sphere, to surface silanols. (top) via  
13 previous formation of amide link between alexa and monoethoxy silane coupling agent and  
14 (bottom) via previous reaction of surface silanols with aminosilane.

- 15  
16 - Simple method to build a thin phospholipid layer covalently linked to a polymer network  
17 - From phospholipids, building of a stable background dense layer of phosphorylcholine  
18 heads.  
19 - Possibilities of additional routine chemistry for fonctionnalization over the  
20 phosphorylcholine background.

21



Functionalizable protein-repellent interface