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Strong Resistance of Phosphorylcholine Self-Assembled Monolayers to Protein Adsorption: Insights into Nonfouling Properties of Zwitterionic Materials

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Abstract: In this work, we show the strong resistance of zwitterionic phosphorylcholine (PC) self-assembled monolayers (SAMs) to protein adsorption and examine key factors leading to their nonfouling behavior using both experimental and molecular simulation techniques. Zwitterions with a balanced charge and minimized dipole are excellent candidates as nonfouling materials due to their strong hydration capacity via electrostatic interactions.

Introduction

Poly(ethylene glycol) (PEG) is one of the best synthetic nonfouling materials.[1] However, it is now recognized that PEG decomposes in the presence of oxygen and transition metal ions found in most biochemically relevant solutions.[1−3] It was also shown that grafted PEG brushes exhibit protein resistance at room temperature, but lose their protein repulsive properties above 35 °C.[4] It is of great interest to search for alternative nonfouling materials other than PEG.[1,3] It is generally believed that water plays an important role in surface resistance to protein adsorption.[5−7] While hydrophilic and neutral PEG forms a hydration layer via hydrogen bonds, zwitterions form a hydration layer via electrostatic interactions. It is expected that zwitterions are capable of binding a significant amount of water molecules and therefore are potentially excellent candidates for super-low fouling materials.

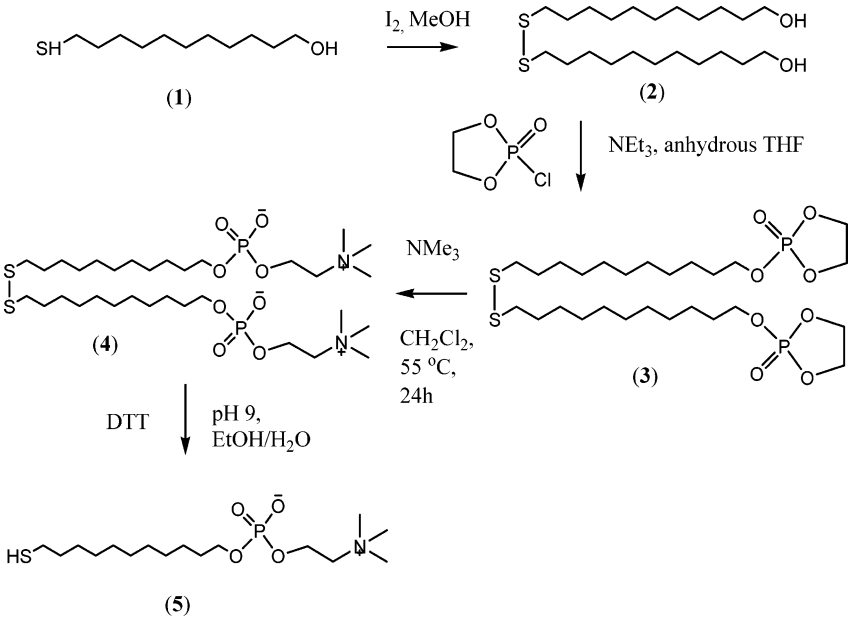
The lipid components that constitute the outside surface of a cell membrane are mainly zwitterionic phospholipids and are believed to be nonthrombogenic.[8,9] 2-Methacryloyloxyethacrylate (MPC)-based polymers have been shown to significantly reduce protein adsorption compared to relevant controls and have been widely used for various applications.[10−12] Langmuir−Blodgett and self-assembly are two excellent methods to create thin films with a high degree of structural control. Langmuir−Blodgett deposition, spin-coating, and vesicle fusion are often used to prepare planar supported lipid bilayers (PSLB).[13] Polymerizable PC lipids were introduced to stabilize the fluid PSLB.[14] It was shown that the redox(poly) bis-SorbPC surface[15] adsorbed only 6% of a monolayer (ML) of bovine serum albumin (BSA) with respect to that on the arachidic acid monolayer.

An alternative approach is to form PC-terminated SAMs on gold as shown in the pioneering work by Whitesides'[16] and Cooper's[17] groups. However, fibrinogen (Fg) adsorption was ∼23% and ∼35% of a ML with respect to that on methyl-terminated SAMs as measured in their own studies, respectively. Recently, Chung et al.[18] reported another study of PC SAMs. Fibrinogen adsorption was 18% of a ML with respect to that on methyl-terminated SAMs also measured in their experiments. It is speculated[15] that relatively high protein adsorption on PC SAMs may be due in part to the packing arrangement of a PC SAM, which is not likely to assemble with the same surface area per PC moiety as that found in a fluidic PC lipid bilayer. Consequently, the dipole orientation of the PC lipid bilayer and associated water molecules may be altered in a PC SAM relative to a PC lipid bilayer.[15] However, it was observed that even mixed $N^+(CH_3)_3$ and SO_3^- zwitterionic SAMs adsorbed less than 1% of a ML of Fg from a 1 mg/mL solution in a study by Holmlin et al.[16]

In this work, we synthesized PC thiols from a modified synthetic route, characterized PC SAMs on Au(111) using X-ray photoelectron spectroscopy (XPS) and atomic force microscopy

(1) Ostuni, E.; Chapman, R. G.; Holmlin, R. K.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605.
(2) Shen, M. C.; Martinson, L.; Wagner, M. S.; Castner, D. G.; Ratner, B. D.; Horbett, T. A. *J. Biomater. Sci., Polym. Ed.* **2002**, *13*, 367.
(3) Luk, Y. Y.; Kato, M.; Mrksich, M. *Langmuir* **2000**, *16*, 9604.
(4) Leckband, D.; Sheth, S.; Halperin, A. *J. Biomater. Sci., Polym. Ed.* **1999**, *10*, 1125.
(5) Harder, P.; Grunze, M.; Dahint, R.; Whitesides, G. M.; Laibinis, P. E. *J. Phys. Chem. B* **1998**, *102*, 426.
(6) (a) Zheng, J.; Li, L.; Chen, S.; Jiang, S. *Langmuir* **2004**, *20*, 8931. (b) Zheng, J.; Li, L.; Tsao, H.; Sheng, Y.; Chen, S.; Jiang, S. *Biophys. J.* **2005**, *89*, 158.
(7) Li, L. Y.; Chen, S. F.; Zheng, J.; Ratner, B. D.; Jiang, S. Y. *J. Phys. Chem. B* **2005**, *109*, 2934.
(8) Lewis, A. L. *Colloids Surf., B* **2000**, *18*, 261.
(9) Vermette, P.; Meagher, L. *Colloids Surf., B* **2003**, *28*, 153.
(10) Ishihara, K.; Fukumoto, K.; Iwasaki, Y.; Nakabayashi, N. *Biomaterials* **1999**, *20*, 1545.
(11) Durrani, A. A.; Hayward, J.; Chapman, D. *Biomaterials* **1986**, *7*, 121.
(12) Nakaya, T.; Li, Y. J. *Prog. Polym. Sci.* **1999**, 143.
(13) Malmsten, M. *J. Colloid Interface Sci.* **1995**, *4*, 106.
(14) Hayward, J.; Chapman, D. *Biomaterials* **1984**, *5*, 135.
(15) Ross, E. E.; Spratt, T.; Liu, S.; Rozanski, L. J.; O'Brien, D. F.; Saavedra, S. S. *Langmuir* **2003**, *19*, 1766.
(16) Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841.
(17) Tegoulia, V. A.; Rao, W.; Kalambur, A. T.; Rabolt, J. F.; Cooper, S. L. *Langmuir* **2001**, *17*, 4396.
(18) Chung, Y. C.; Chiu, Y. H.; Wu, Y. W.; Tao, Y. T. *Biomaterials* **2005**, *26*, 2313.

Scheme 1. Synthesis route of 11-mercaptoundecylphosphorylcholine



(AFM), and evaluated the adsorption of Fg and BSA using a surface plasmon resonance (SPR) sensor. In addition, we also performed molecular simulation studies of the packing structure and density of PC SAMs on Au(111) to provide molecular-level information for the further interpretation of our experimental results. The objectives of this work are to study the nonfouling properties of PC SAMs and to provide insights into the molecular-level nonfouling mechanism of zwitterionic materials.

Materials and Methods

Chemicals. 11-Mercaptoundecanol, 1-octanethiol, 1-hexadecanethiol, iodine, triethylamine, trimethylamine, tetrahydrofuran (anhydrous, 99.9%), 1,4-dithio-D,L-threitol, and sodium bisulfite were purchased from Sigma-Aldrich and used as received. 2-Chloro-1,3,2-dioxaphospholane-2-oxide, and *N*,*N*-dimethanolamine were purchased from Acros Organics. Human Fg and BSA were also purchased from Sigma-Aldrich.

Synthesis of 11-Mercaptoundecylphosphorylcholine. The reaction route for the synthesis of 11-mercaptoundecylphosphorylcholine (PC thiol) is presented in Scheme 1. A methanol solution (30 mL) containing 11-mercapto-1-undecanol (2.05 g) (**1**) was titrated by 1 M iodine methanol solution until the solution turned light yellow, and then the reaction was quenched with sodium bisulfite. 11-Hydroxyundecyl disulfide (**2**) was precipitated from the methanol solution at 0 °C and recrystallized from ethanol with a yield of 1.85 g (90%). **2** (0.407 g, 1 mmol) and triethylamine (0.35 mL, 2.5 mmol) were dissolved in 15 mL of chloroform ($CHCl_3$). This solution was slowly added into 2-chloro-1,3,2,-dioxaphospholane-2-oxide (0.21 mL, ~2.4 mmol) anhydrous THF (40 mL) solution at −18 °C. The mixture was allowed to warm to ambient temperature over 2 h. The reaction mixture was then cooled to 0 °C and filtered. The filtrate was concentrated in vacuo at room temperature without further purification, and the product (**3**) is a colorless to light-yellow oil. **3** and trimethylamine (0.5 mL, 5.5 mol) in anhydrous methane chloride (35 mL) at −15 °C were sealed in a bomb, which was heated to 55 °C and stirred for 24 h. The reaction mixture was cooled and filtered to give a white solid powder (**4**, 0.4 g). The white solid powder (0.4 g) was dissolved in ethanol (3 mL), and 1,4-dithio-D,L-threitol (DTT, 0.4 g) was added. The pH of the solution was adjusted to 9 with concentrated $NH_3 \cdot H_2O$. The solution was stirred for half an hour and purified by flash chromatography on silica using a mixed eluent (i.e., chloroform:ethanol:water, 4:8:4). The fraction of 11-mercaptoundecylphosphorylcholine was concentrated in vacuo by a rotary evaporator at room temperature. Anhydrous ethanol was added to help remove water. The final product (**5**) is a white solid powder (0.16 g, total yield 21%). ^{1}H NMR (MeOD, 300 MHz): δ 1.27−1.48 14H, 1.55−1.75 4H, 2.51 2H, 3.24 9H, 3.65 2H, 3.88 2H, 4.26 2H.

All intermediate compounds and the final product were characterized by a Bruker Esquire Ion Trap LC−Mass Spectrometer. All molecular weight peaks match well with their theoretical values. **2** is closer to 100% pure from thin-layer chromatography (TLC). In the conversion of **2** to **3**, there were some unreacted hydroxyl groups although extra 2-chloro-1,3,2,-dioxaphospholane-2-oxide was added. However, these unreacted hydroxyl groups do not affect the conversion of **3** to **4**. **3** and **4** were obtained without further purification. Due to strong electrostatic interactions between phosphorylcholine groups and silica, the retention factor (R_f) value of **4** is only less than 0.2 even in the eluent with high water content (the volume ratio of ethanol to water is 8:3). The R_f value of **5** is 0.35 when eluted by 8:3 of ethanol:water and 0.55 when eluted by 8:3:0.1 of ethanol:water:HCl in volume. In the conversion of **3** to **4**, an asymmetrical disulfide containing one PC group and one phosphate [$(CH_2)_{11}OPO_2H(OCH_2CH_2OH)$] group could be produced. This negatively charged thiol could affect the quality of PC SAMs and protein adsorption on PC SAMs. To check for the existence of this side product in **5**, a small amount of concentrated HCl was added to the eluent to increase the difference in R_f value between PC thiol and $HS(CH_2)_{11}OPO_2H(OCH_2CH_2OH)$ thiol.

SAM Preparation and Characterization by X-ray Photoelectron Spectroscopy. Gold-coated chips were washed with ethanol and cleaned in a UV cleaner for 15 min. They were dipped into either (acidic) ethanolic or (basic) aqueous solution of 0.1 mM PC thiol overnight. The chips covered with alkanethiolate SAMs and PC SAMs were washed with ethanol and Millipore water, respectively, and then dried with filtered, compressed air. The procedure was repeated three times. PC samples analyzed by XPS were put in a vacuum chamber within 30 min of preparation or stored in N_2 before they were put in the vacuum chamber. XPS measurements were conducted using a Surface Science Instrument X-Probe spectrometer (Mountain View, CA) equipped with a monochromatic Al Kα source (KE = 1486.6 eV), a Hemispherical analyzer, and a multichannel detector. All XPS data were

acquired at a nominal photoelectron takeoff angle of 55°. SSI data analysis software was used to calculate elemental compositions from peak areas.

SAM Characterization by Ellipsometry. Ellipsometry was performed using a spectroscopic ellipsometer (Sentech SE-850, GmbH). Sample preparation is the same as in XPS experiments. Five separate spots were measured at three different angles of incidence (50°, 60°, and 70°) in the vis region. The same batch of gold-coated chips was cleaned by UV−ozone cleaner for 20 min, washed with ethanol and Millipore water, and dried with filtered air. These bare gold-coated chips were used as reference. The thicknesses of films under study were determined using the Cauchy layer model with an assumed refractive index of 1.45 [16] and 1.50.[19]

Protein Adsorption by Surface Plasmon Resonance Sensor. A custom-built highly sensitive SPR biosensor was used. Unlike many SPR sensors based on angular measurements,[20,21] this SPR biosensor is based on wavelength interrogation. It was reported in our previous studies that 5 ng/mL of staphylococcal enterotoxin B (SEB) was detectable by a direct method and 0.5 ng/mL SEB with a sandwich method using this sensor.[22] The chips were coated with adhesion-promoting chromium layer (thickness $\approx$ 2 nm) and surface plasmon active gold layer (thickness = 50 nm) by electron beam evaporation in a vacuum. The chip modified with a sensing layer was attached to the base of the prism, and optical contact was established using refractive index matching fluid (Cargille). A dual-channel Teflon flow cell containing two independent parallel flow channels with small chambers was used to contain a liquid sample during experiments. A peristaltic pump (Ismatec) was utilized to deliver liquid sample to the two chambers of the flow cell. The flow rate of 0.05 mL/min was used throughout experiments.

All protein adsorption measurements were performed in physiological PBS solution (10 mM sodium phosphate, 138 mM NaCl, and 2.7 mM KCl at pH 7.4). SPR was first stabilized with PBS solution for 20 min, then the protein solution was flowed into the system at 1 mg/ml for 10 min, and the SPR was then flushed with PBS solution for 5 min.

Nanografting. A Nanoscope IV AFM (Veeco, CA) equipped with E scanner was used for nanografting. A sample was mounted inside a quartz fluid cell, which allowed the injection and removal of solution from the flow cell for AFM lithography and imaging in a liquid environment. Si_3N_4 cantilevers with a force constant of 0.32 N/m were used. The AFM tip was used to remove C16 SAM chains within an area of 100 nm × 80 nm in an aqueous solution (pH 10) containing PC thiol (0.4 mM) under a minimal force of 5 nN. When C16 chains were removed, PC thiols refilled the empty space. Both topographic and frictional AFM images were acquired under 0.2 nN.

Results and Discussion

Synthesis of PC Thiol. In this work, PC thiol was synthesized following a modified scheme (Scheme 1) based on the methods reported by Holmlin et al. [16] and Chung et al.[18] with two considerations. For synthesis of a thiol from a thioester in the conventional method, such as the one by Holmlin et al.,[16] the thioester group is converted to the thiol group by hydrolysis, which could also hydrolyze the phosphorylcholine group. When starting with a disulfide, one can avoid the undesirable hydrolysis step, particularly after the formation of the PC group. For synthesis of PC thiols starting from a disulfide by Chung et al., it is hard to purify the asymmetrical disulfide containing one PC group and one phosphate group, which will later affect the quality of PC SAMs and protein adsorption. Thus, by reducing the disulfide to a thiol under mild conditions, pure PC thiol can be obtained with simpler purification and higher yield (21% after purified by flash chromatography).

Table 1. Elemental Compositions of PC SAMs on Au(111) Determined from XPS[a]

elements	theory	pH 10	acidic ethanol solutions
C	69.6	63.2	62.1
N	4.4	4.0	3.9
P	4.4	3.8	4.5
O	17.7	26.6	27.3
S	4.4	2.3	2.2

[a] The gold ratio is 23 ± 0.7% at pH 10 and 22.8 ± 0.9% from acidic ethanol solution if gold is counted.

Preparation and Characterization of PC SAMs. The quality of a PC SAM is critical to its nonfouling property and will depend on its preparation conditions. As discussed above, a trace amount of $OPO_2H(OCH_2CH_2OH)$-terminated thiol from impurity in the final product could alter the properties of PC SAM from neutral to negatively charged, leading to protein adsorption. In this work, we prepared PC SAMs from an acidic ethanol solution and from an aqueous solution under the basic condition at pH 10. An ethanol solution of PC thiol prepared from PC thiol dried under vacuum is slightly acidic. It was found that the N/P ratio of ~1:1 was achieved for PC SAMs formed from a basic assembly solution, while a ~18% lower N/P ratio was observed under an acidic assembly solution (Table 1). From Table 1, under both basic and acidic conditions, bound sulfur species and the relative film thickness based on gold elemental percentages or C/Au ratios do not show obvious changes. Although only a trace amount of phosphate-terminated thiol occurs in our TLC results, it could preferentially absorb in acidic conditions. PC SAMs prepared in a basic aqueous solution of PC thiol will suppress the assembly of phosphate-terminated thiol. The composition of PC SAMs formed under basic conditions agrees well with the theoretical value except for slightly lower elemental percentages of sulfur and higher percentages of oxygen (Table 1). The slightly lower amount of sulfur is due to the signal attenuation of bound sulfur species on the gold surface by the top organic layer. The high-resolution spectra of the S_{2p} region from XPS shows that most of the sulfur species are bound to the surface, with very low amounts of unbound or oxidized sulfur species (Figure 1).[23,24] XPS results show that high-quality PC SAMs are formed. It can be seen from Table 1 that the elemental composition of oxygen is higher than the theoretical value, which was also observed previously in other work.[17,18] Since few oxidized sulfur species are observed, oxidized sulfur species do not account for the large increase in oxygen content. In the previous work on MPC polymers, it was found that each MPC monomer contained two hydrated water molecules.[25] Some of these tightly associated water molecules would remain even under high vacuum in XPS experiments and contribute to the higher oxygen content. The similar phenomenon of tightly associated water on cadmium carboxylate layers was observed in earlier XPS experiments.[26]

(19) Fabianowski, W.; Coyle, L. C.; Weber, B. A.; Granata, R. D.; Castner, D. G.; Sadownik, A.; Regen, S. L. *Langmuir* **1989**, *5*, 35.
(20) Liedberg, B.; Lundstrom, I.; Stenberg, E. *Sens. Actuators, B* **1993**, *11*, 63.
(21) Melendez, J.; Carr, R.; Bartholomew, D. U.; Kukaskis, K.; Elkind, J.; Yee, S. S.; Furlong, C.; Woodbury, R. *Sens. Actuators, B* **1996**, *35*−*36*, 212.
(22) Homola, J.; Dostálek, J.; Chen, S. F.; Rasooly, A.; Jiang, S. Y.; Yee, S. S. *Int. J. Food Microbiol* **2002**, *75*, 61.
(23) Sun, F.; Grainger, D.; Castner, D.; Leachscampavia, D. *Macromolecules* **1994**, *27*, 3053.
(24) Castner, D.; Hinds, K.; Grainger, D. *Langmuir* **1996**, *12*, 5083.
(25) Umeda, T.; Nakaya, T.; Imoto, M. *Makromol. Chem.* **1982**, *3*, 457.
(26) Roberts, G. G. *Adv. Phys.* **1985**, *34*, 475.

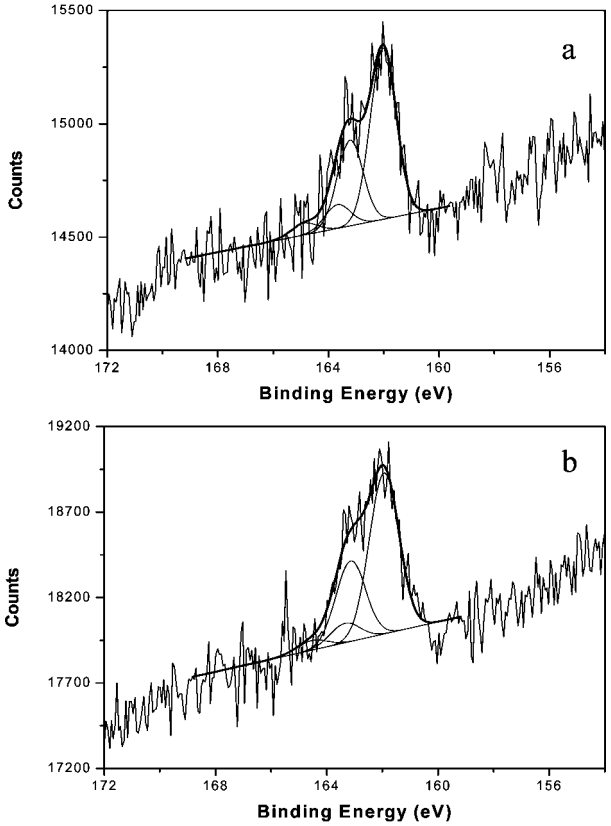


Figure 1. High-resolution spectra of the S_{2p} region from XPS for 11-mercaptoundecylphosphorylcholine SAMs prepared from a solution at (a) acidic and (b) pH 10. The peaks were fit using two S_{2p} doublets with 2:1 area ratio and splitting of 1.2 eV. The high-resolution sulfur data show up to 89% sulfur species at 162 eV, indicating that the majority of sulfur species is bound to the gold surface.

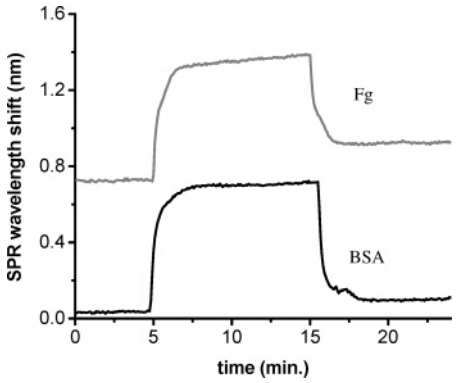


Figure 2. Adsorption of 1 mg/ml Fg and BSA in PBS buffer (0.13 M and pH 7.4) on PC SAMs from SPR. The typical SPR wavelength shifts for Fg and BSA adsorption on methyl-terminated SAMs are 16 nm[7] and 7 nm,[33] respectively; 1 nm wavelength shift in SPR is equivalent to 0.15 mg/m^2 adsorbed proteins.

In addition, the contact angle of PC SAMs measured with Millipore water under ambient condition is 17 ± 2°.

Protein Adsorption on PC SAMs. The adsorption of Fg and BSA on PC-terminated SAMs prepared from the aqueous solution of PC thiol at pH 10 was investigated by an SPR sensor. It is shown in Figure 2 that the adsorbed amounts of Fg and BSA on PC SAMs are 0.03 mg/m^2 (or 1% of a ML) and 0.01 mg/m^2 (or 0.7% of a ML) from PBS buffer containing 1 mg/mL Fg and BSA, respectively. Protein adsorption is much lower on our PC SAMs than on those PC SAMs reported previously and is even lower than on the redox(poly) bis-SorbPC surface.[15]

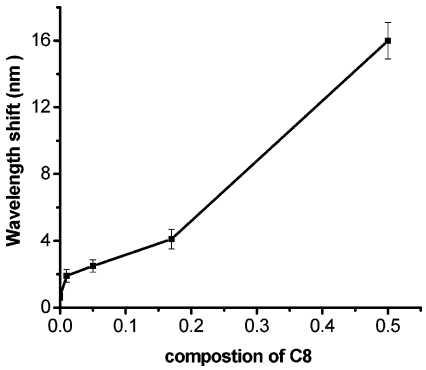


Figure 3. Fg adsorption on mixed C8 and PC SAMs from PBS buffer. With the increase of C8 composition, Fg adsorption increases.

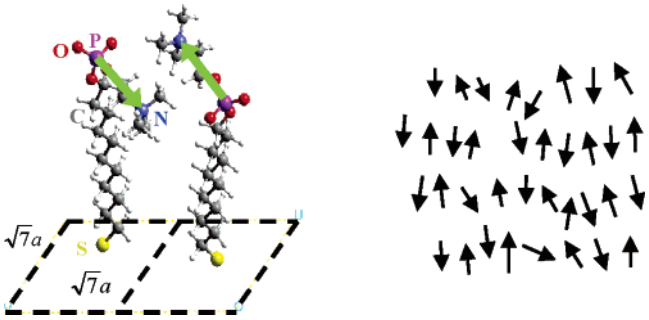


Figure 4. (a) Molecular configuration of PC SAMs from molecular simulations and (b) antiparallel orientation of PC headgroups for dipole minimization.

Thus, the PC headgroups are intrinsically nonfouling. It should be emphasized that the N/P ratio is critical to the nonfouling behavior of a zwitterionic surface for charge balance. Comparing our XPS results in Table 1 with those published previously,[17,18] it can be seen that the N/P ratio from our PC thiol SAM formed at pH 10 is 1.05:1, which is very close to the theoretical value of 1:1 and which has 1% of a ML of Fg adsorption. The N/P ratio of our PC thiol SAM formed at acidic ethanol solution was 0.87:1, while Fg adsorption on the PC SAM is 3.5% of a ML. This may be the key reason our PC surface resists protein adsorption. However, in the work by Tegoulia et al.[17] and Chung et al.,[18] the N/P ratio is 0.59:1, and protein adsorption is relatively high. It should be pointed out that different methods were used to measure protein adsorption on PC SAMs, such as angular measurement-based SPR sensors,[16] wavelength interrogation-based SPR sensor in this work, radio-label,[17] and QCM.[18] Each method has its own assumption to convert its response to the amount of adsorbed proteins. Thus, the absolute amount of adsorbed protein is often hard to obtain. For fair comparison, the percentage of protein (Fg or BSA) adsorption presented in this work is normalized with respect to that on methyl-terminated SAMs or hydrophobic surfaces as measured with the same method. Furthermore, since protein adsorption depends on the ionic strength and the pH value of a protein solution, protein adsorption is compared in physiological PBS buffer. Therefore, the N/P ratio from XPS could serve as an indicator for the quality of the PC SAMs.

Protein Adsorption on Mixed SAMs. Protein adsorption on mixed PC and CH_3 SAMs was also investigated. It was shown in our previous work that OEG SAMs of moderate surface density were more resistant to protein adsorption than the compact OEG SAM due to a large number of hydration water

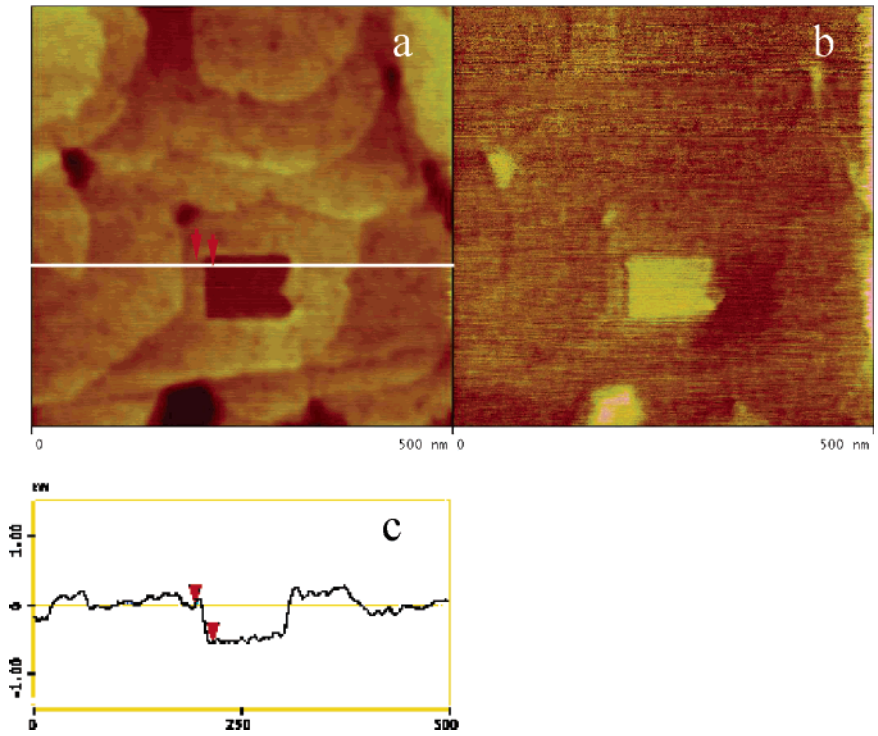


Figure 5. In situ AFM lithography for determining the height of PC SAMs on Au(111). The background is $HS(CH_2)_{15}CH_3$ (C16) SAMs and the dark 100 nm × 80 nm area in the topographic image (a) is back-filled with PC thiol. The corresponding white square in frictional image (b) shows higher friction on PC SAMs than on C16 SAMs. The cross-sectional analysis (c) shows ~5.6 Å height difference between C16 SAM and PC SAM.

molecules around the OEG chains and the more flexible structure of the OEG chains.[6,7] Results in Figure 3 show that protein adsorption increases on mixed SAM surfaces coadsorbed from an (acidic) ethanol solution of PC and $HS(CH_2)_7CH_3$ (C8) thiols. The amount of adsorbed proteins on mixed SAMs coadsorbed from PC and C8 thiols even containing only 1% C8 is 3 times higher than that on the PC SAM formed in an (acidic) ethanol solution and 10 times higher than that on the PC SAM formed in an aqueous solution at pH 10 (Figure 2). In fact, our simulation results show that pure PC SAMs have packing densities similar to those of PC lipids and the charge interaction of PC groups is the dominant factor for the packing density. Thus, it is not surprising that pure PC SAMs are more resistant to protein adsorption than mixed PC SAMs. Furthermore, AFM images of mixed-SAM surfaces show strong phase segregation. This is also a part of the reason for higher protein adsorption even with a small amount of C8 thiol in the coadsorption solution. This result also indicates that the purity of PC thiols is important to the formation of protein-resistant PC SAMs.

Molecular Packing Structure of PC SAMs. To provide molecular-level information regarding the packing structures and properties of PC SAMs, we performed molecular simulation studies of PC SAMs on Au(111). All simulations were performed using the BIO_SURFACE[6] program developed in our group, capable of simulating biomolecular interactions with various material surfaces. Molecular mechanics was performed first in continuous medium followed by molecular dynamics simulations at 300 K in both implicit and explicit solvents. The all-atom CHARMM27 force field was used. To obtain the lowest-energy structure of PC SAMs on Au(111), we need to consider two important parameters in our molecular simulations: (a) the lattice structure of PC SAMs ranging from three to eight gold atoms per unit cell and (b) the packing pattern of a pair of PC chains, which could have the parallel, antiparallel, or random orientation of their dipoles (the vector pointing from P to N). A simulation box containing 36 PC chains was used in this work, and all possible lattice structures and packing patterns were considered. Our simulation results show that the lowest-energy configuration of PC SAMs has a $(\sqrt{7}\times\sqrt{7})R60°$ lattice structure with a thickness of 14.4 Å. This corresponds to a chain-chain spacing of ~7.63 Å or an area of 50.42 $Å^2$ per chain, which is very close to that of membrane lipids obtained from X-ray deflection experiments.[27] Unlike lipids for which each PC headgroup has two alkyl chains, each of the PC headgroups in our PC SAMs has only one alkyl chain. In our AFM studies, it was found that PC SAMs were more easily penetrated by an AFM tip, indicating that PC SAMs have less dense alkyl chains than alkanethiolate SAMs or lipid layers. Our simulation results also show the angle between the vector pointing from P to N and the normal gold surface is about 80°, indicating that the orientation of PC headgroups lies nearly parallel to the gold surface as shown in Figure 4a. This was also observed previously for lipids.[28] Furthermore, our simulation results show that PC headgroups prefer to have an antiparallel orientation with respect to each other as shown in Figure 4b. This was also observed for zwitterionic peptide amphiphiles.[29] With the antiparallel configuration, the electrostatic energy and net dipole moment of PC SAMs are minimized. Large dipole accumulation on the surfaces will lead to protein adsorption. Detailed simulation results will be published elsewhere.

(27) Hauser, H.; Pascher, I.; Pearson, R. H.; Sundell, S. *Biochim. Biophys. Acta* **1981**, *650*, 21.
(28) Sheng, Q.; Schulten, K.; Pidgeon, C. *J. Phys. Chem. B* **1995**, *99*, 11018.
(29) Tsonchev, S.; Troisi, A.; Schatz, G. C.; Ratner, M. A. *J. Phys. Chem. B* **2004**, *108*, 15278.

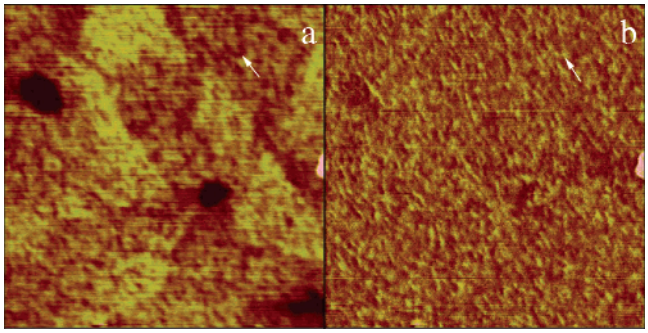


Figure 6. Phase-segregated C16- and PC-mixed SAM for determining the height of PC SAMs on Au (111) by AFM. The features (e.g., the one marked by an arrow) having lighter color in the topographic image (a) are darker in the corresponding frictional image (b).

The Thickness of PC SAMs. The thickness of PC SAMs would be 21.2 Å (equivalent to that of C18 SAMs) if the PC chains are all-trans and tile 30° from the normal gold surface as do CH_3- and OH-terminated alkanethiols on gold surfaces.[5,30] Our molecular simulation results show that the angle between the vector pointing from P to N and the normal gold surface is about 80°, and the thickness of PC SAMs is 14.4 Å. In this work, two AFM experiments were used to estimate the thickness of PC SAMs and to verify the simulation results. One is the in situ nanografting of PC thiols on $HS(CH_2)_{15}CH_3$ (C16) SAMs using AFM,[31] in which the AFM tip scans over C16 SAMs to remove C16 chains within a certain area, and PC thiols in the solution then fill the empty space. The AFM tip scans again with low force to obtain an image. It is found in the nanografting experiment that C16 SAMs are higher than PC SAMs by ~5.6 Å (Figure 5). Thus, the thickness of PC SAMs is 13.7 Å with the thickness of C16 equal to 19.3 Å.[5,30] To exclude the possibility of gold layer plastic deformation by extremely high forces in AFM,[32] a minimal force (~5 nN) was applied to remove the C16 thiol. Since PC SAMs in the nanografting area were formed in a short time before being imaged, these (immature) SAMs could be lower in height than those (mature) PC SAMs formed overnight. In the second method, mixed SAMs were formed in two steps. First, cleaned Au(111) chips were immersed in a solution of 1 mM C16 thiol for 10 min, rinsed thoroughly, and immersed in a solution of 0.1 mM PC thiol overnight. The topographic image of mixed PC and C16 SAMs in Figure 6a shows phase segregation with 2−4 Å height difference. From the frictional image in Figure 6b, it can be seen that the spot having lower friction is higher in the topographic image in Figure 6a. As can be seen from Figure 5, C16 has lower friction. Thus, C16 chains are higher than PC chains in the mixed SAMs.

The thickness of the PC SAMs was also measured by ellipsometry to be 9.5 ± 0.2 or 8.9 ± 0.2 Å if a refractive index of 1.45[16] or 1.50[19] is used. It should be pointed out that 1.45 and 1.50 are obtained for C16 thiol and phosphorylcholine lipid, respectively. They are also used to describe the compact structure of C16 SAMs and lipid bilayers. However, PC SAMs have loose structures with $(\sqrt{7}\times\sqrt{7})R60°$ (~50% less dens than CH_3 and OH SAMs). Thus, it is expected that the thickness of PC SAMs obtained from ellipsometric measurements is significantly lower than their actual height if the refractive index of 1.45 or 1.50 is used. Therefore, AFM results confirm those from simulations that the orientation of PC headgroups lies nearly parallel to the gold surface. Further ellipsometric measurements suggest that the packing density of PC SAMs should be lower than that of CH_3 SAMs as also predicted in molecular simulations.

Conclusions

It is demonstrated in this work that zwitterionic PC SAMs are highly resistant to protein adsorption. Our experimental results show that PC SAMs have very low protein adsorption when the N/P ratio is close to 1:1 and the charges are balanced. The N/P ratio from XPS could be a good indicator of charge balance. Due to the strong dipole of zwitterions, appropriate packing is needed to minimize their net dipole. Our simulation results show that PC head groups have similar packing densities to membrane lipids and prefer to have an antiparallel orientation for dipole minimization. Although zwitterions have a strong hydration layer via electrostatic interactions and should highly resist protein adsorption, balanced charge and minimized dipole are two key factors for their nonfouling behavior.

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(30) Bain, C. D.; Whitesides, G. M. *J. Phys. Chem. B* **1989**, *93*, 1670.
(31) Liu, G. Y.; Xu, S.; Qian, Y. L. *ACC. Chem. Res.* **2000**, *33*, 457.
(32) Salmeron, M.; Folch, A.; Neubauer, G.; Tomitori, M.; Ogletree, D. F.; Kolbe, W. *Langmuir* **1992**, *8*, 2832.
(33) Li, L. Y.; Chen, S. F.; Jiang, S. *Langmuir* **2003**, *19*, 2974.