# Multiple Surface Functionalities through Step-by-Step Hydrolysis of Self-Assembled Monolayers

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Self-assembled monolayers (SAMs) of *N*-(3-diethylphosphatoxy)propyl-11-mercaptoundecanamide (PPMA) were prepared on gold surfaces. The SAMs, consisting originally of a phosphonate surface functionality, were subjected to step-by-step acidic hydrolysis to reveal two different functional groups: (a) a hydroxyl and (b) a carboxylic group. At each stage these SAMs were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray photoelectron spectroscopy (XPS), and water contact angle measurements. To our best knowledge, for the first time a methodical approach was used to obtain three different surface functionalities from one type of molecule on the surface of gold. These SAMs could provide a novel approach for conducting high-throughput investigations, for instance for cell adhesion. The present study contributes toward the understanding of reactions at interfaces as it demonstrates the influence of step-by-step hydrolysis on SAMs.

## Introduction

Interface reactions have provided a crucial gateway for surface engineering for specific applications, ranging from biomaterials,[1−3] tissue engineering,[4−7] biosensors,[8,9] chemical sensors, [10,11] and electronics[12] to surface catalysis,[13] among others.[14,15] With the aid of sophisticated surface analytical techniques, it has become possible to analyze one-molecule-thick designed surfaces.[16,17]

As such, self-assembled monolayers (SAMs) have provided an opportune method of studying surface reactions on a variety of substrate surfaces.[18−20] SAMs are referred to as the spontaneous organization of molecules on surfaces.[21] The organization of these molecules on a given surface is a thermodynamically driven process, controlled by a number of different properties imparted by the molecule: a "head group" reactive toward a substrate, a spacer chain that controls the packing ability and self-organization of the molecules, and a "tail group" that defines the surface property of the SAM on the given substrate.

Since the research undertaken by Kumar and co-workers[22] on alkanethiols on gold, a whole array of head groups has been identified, containing select affinities for given substrates, thus making it relatively easy to prepare a whole host of SAMs conveying different tail groups.[23−27] The most studied SAMs are, notably, thiols on gold, as sulfur−gold interactions are known to be very strong.[28] However, the thiol moieties are not limited only to gold but also assemble

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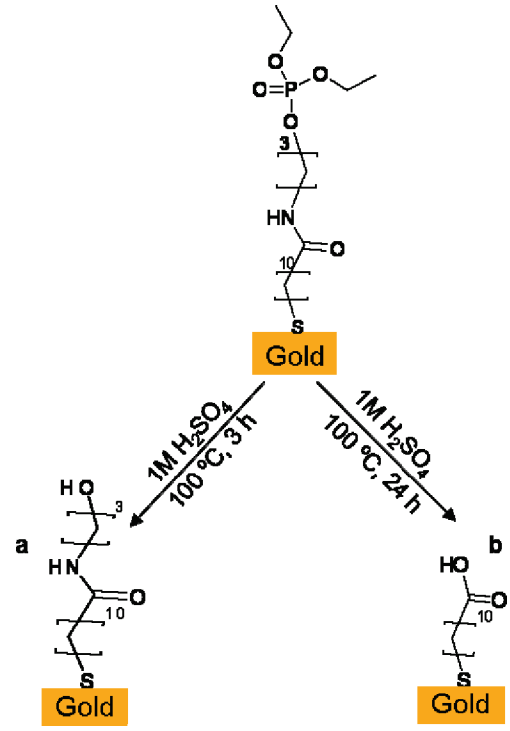
on Cu,[29] Ag,[29] Ni,[30] Pd,[31] and many more.[32] Nogues and Lang[33] investigated the effects of alkylthiols on zinc surfaces as an anticorrosion barrier. SAMs have also made important contributions toward the influence of chemical functional groups on cell and protein interactions, a factor that is essential for the understanding and optimization of implants within the human body.[34−37]

The above examples provide a brief overview of some of the recent investigations and applications of self-assembled monolayers. Transformations of the surface properties of SAMs on substrate surfaces provide an informed insight into the stability and the kinetics of reactions at interfaces.

The chemical modifications of the surface groups on SAMs can be achieved in a number of ways: via photochemistry,[38] electrochemistry,[39] polymerization,[40−42] reduction and oxidation,[43] and thus allowing for the opportunity to further derivatize the surface for selected applications. Rutenberg et al.[44] conducted surface ring opening metathesis polymerization reactions on gold surfaces to develop polymer brushes.

Vaidya et al.[45] investigated the base-catalyzed hydrolysis of two differing nitrophenyl disulfide esters, where it was observed that both the availability of defect sites and packing density have an influence on the hydrolysis rate. Surface modifications concerning hydrolysis have largely been studied on SAMs containing facile leaving groups, such as *N*-hydroxysuccinimide- (NHS-) derivatized SAMs.[46−49] However, to our best knowledge, no research has been undertaken concerning step-by-step hydrolysis of SAMs followed by further derivatization. Step-by-step hydrolysis of SAMs may provide a novel path to preparing multiple functionalities on the surface, a methodology that would be beneficial to ascertain the influence of a multitude of surface chemical groups in cell studies, corrosion investigations, wettability, and friction via simple multifunction chemistry.[47]

**Scheme 1. Illustration of Controlled Hydrolysis of PPMA Molecules as SAMs on Gold**[a]




[a] PPMA SAMs were controllably hydrolyzed (by use of 1 M $H_2SO_4$) for selected time periods to hydrolyze (a) phosphonate groups and (b) amides.

SAMs can be prepared by the conventional method of immersing substrates in solution for varying time lengths or by the more acclaimed route of using microcontact printing. Microcontact printing uses an elastomeric stamp, polydimethylsiloxane (PDMS), to transfer molecules (termed as "ink") to any given substrate.[50,51]

Thus, the aim of the present study was to demonstrate that, for the first time, step-by-step hydrolysis of *N*-(3-diethylphosphatoxy)propyl-11-mercaptoundecanamide (PPMA) SAMs on gold can be used to expose two different chemical functional groups (Scheme 1). Use of a single molecule (PPMA) for creating different functional groups on gold, we believe, can open up a novel gateway for the preparation of substrates with differing functionalities. In this instance we began with a phosphonate surface group and through time-dependent hydrolysis are able to produce a hydroxyl and a carboxyl surface functionality. This method circumvents the need to synthesize[47] or purchase a variety of different molecules containing the desired chemical groups, as through step-by-step hydrolysis different groups can be achieved. In addition, it also provides a new insight into the stability of this SAM when subjected to step-by-step hydrolysis as opposed to the hydrolysis of facile leaving groups.

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## Experimental Section

**General Methods.** All chemicals for the synthesis of the specified ink were purchased from Sigma−Aldrich Logistik GmbH (Schnelldorf, Germany) and used as received. *N*-(3-Diethylphosphatoxy)propyl-11-mercaptoundecanamide was synthesized in accordance with Borgh et al.[52] and Svedhem et al.[53] The synthesis consisted of four steps, where the characterization and experimental description of the main molecule product is given below.

***N*-(3-Diethylphosphatoxy)propyl-11-mercaptoundecanamide [$HS(CH_2)_{10}CONH(CH_2)_3OP(O)(OEt)_2$].** A solution of *N*-(3-diethylphosphatoxy)propyl-11-(acetylthio)undecanamide (0.42 g, 0.92 mmol) in methanol (15 mL) was purged with argon, after which NaOMe (23.7 mg, 0.44 mmol) was added to the reaction mixture, and stirred for 12 h. The reaction mixture was then cooled to 0 °C, and zinc powder and acetic acid (7.4 mL, 123.33 mmol) (1.13 g, 17.34 mmol) were slowly added. The reaction mixture was warmed up to room temperature and stirred for 30 min. After that the zinc was removed by filtration, and the filtered organic solution was diluted with ethyl acetate (10 mL). The organic phase was washed once with distilled water, brine, distilled water, and dried over $Na_2SO_4$. The solvent was evaporated, and the crude product was purified by column chromatography (ethyl acetate/methanol 95/5). Yield: 0.25 g (66%) *N*-(3-diethylphosphatoxy)propyl-11-mercaptoundecanamide as a white-yellow solid wax. TLC (ethyl acetate/methanol 95/5) $R_f = 0.5$; $^1$H NMR ($CDCl_3$) $\delta$ 6.41 (1H, br s); 4.15−4.03 (6H, m); 3.38−3.31 (2H, q); 2.53−2.42 (2H, m); 2.17−2.02 (3H, m); 1.89−1.79 (2H, m); 1.57−1.51 (4H, m); 1.35−1.20 (18H, m); $^{13}$C NMR ($CDCl_3$) $\delta$ 173.39; 65.06; 64.97; 64.01; 63.91; 36.71; 35.48; 33.97; 29.82; 29.73; 29.63; 29.38; 28.97; 28.29; 25.70; 24.57; 16.13; 16.03. Micro-ESI-MS *m*/*z* ($CHCl_3$/MeOH): calcd for $C_{18}H_{38}NO_5PS$ $[M]^+ = 411.6$; found $[M + Na]^+ = 434.2$. Elemental analysis: calcd, C 52.53, H 9.31, N 3.40, S 7.79, P 7.53, O 19.44; found, C 52.28, H 9.11, N 3.38, S 7.76.

**Preparation of *N*-(3-Diethylphosphatoxy)propyl-11-mercaptoundecanamide (PPMA) SAMs.** First, gold samples were prepared by physical vapor deposition (Leybold Vakuum GmbH): 20-nm-thick titanium films were deposited onto borofloat glass (Φ 15 mm, Borofloat, Jena Engineering, Jena, Germany) substrates via electron-beam deposition, at a rate of 0.26 nm/s. This was then followed by thermal deposition of gold with a thickness of 200 nm onto the titanium-coated surface at a rate of 0.4 nm/s. The gold substrates were sonicated for 10 min in HPLC-grade ethanol (VWR, Darmstadt, Germany) and then cleaned in argon plasma for 5 min. After this, SAMs of PPMA were prepared by immersing the gold substrate into a 1 mM ethanolic solution of PPMA for 24 h at room temperature, followed by rinsing with ethanol and drying under a stream of nitrogen.

**Step-by-Step Hydrolysis of PPMA SAMs.** SAMs were subjected to hydrolysis at 100 °C in 1 M $H_2SO_4$ for 3, 6, and 24 h time lengths (see Scheme 1). Following hydrolysis, these were rinsed with 1 M NaOH and distilled water. The surfaces hydrolyzed for 24 h revealed a carboxylic moiety that was activated using a 1:2 ratio of 1-(3-*N*,*N*′-dimethylaminopropyl)ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) aqueous solutions at room temperature for 4 h. This was then reacted with a 0.1 M ethanolic solution of 1,3-propyldiamine overnight at room temperature, to form an amine-terminated group.

## Materials Characterization

**NMR, Elemental Analysis, and Mass Spectrometry.** $^1$H and $^{13}$C NMR of the synthesized compound were characterized by use of deuterated chloroform on a Bruker DRX 400 and a Bruker AC 250 with tetramethylsilane as the internal standard. Elemental analysis was performed on a CHNS-932 Automat Leco. Electrospray ionization (ESI) mass spectrometry was measured in methanol solution on a MAT 95 XL (Finigan) with a sector field analyzer.

**Fourier Transform Infrared Spectroscopy.** The IR spectra of the PPMA SAMs were recorded on a Bio-Rad FT 25−ATR cuvette (Diamond) system. The number of scans taken was 64, with a resolution of 8 $cm^{-1}$.

**X-ray Photoelectron Spectroscopy.** Analysis of SAMs was performed on a Quantum 2000 (PHI Co., Chanhassen, MN) instrument, with a focused monochromatic Al Kα source (1486.6 eV) for excitation. The power of the X-ray source was kept at 25.7 W. Multipak software provided by the manufacturer was used for curve-fitting and data analysis. The binding energy scale of the spectra was aligned via the C 1s spectra at 284.8 eV. Measurements were made at takeoff angles of 45°.

**Contact Angle Measurements.** A DSA10 drop shape analysis system (Krüss GmbH, Hamburg, Germany) was used to measure the advancing contact angles of the PPMA SAMs on gold substrates with Millipore water. The initial drop volume was 10 μL and the dosing rate was set at 10 μL/min for every measurement. All measurements were performed at room temperature.

**Ellipsometry.** Ellipsometry of the samples was taken on an $EP^3SE$ ellipsometer having a xenon arc lamp with interference filters. The wavelength was set to 630.1 nm at an angle of 70°. The thickness of the SAMs was measured before and after hydrolysis. For each sample, five different points were measured.

## Results and Discussion

FT-IR spectra were taken of the native PPMA SAM before and after hydrolysis at the selected time lengths. Prior to hydrolysis, the P═O, C−N, and C═O vibrations were evident at 1261, 1645, and 1727 $cm^{-1}$, respectively (Figure 1a).[46,52] It is known that SAMs consisting of highly ordered, densely packed monolayers have $CH_2$ asymmetric and symmetric stretching modes near 2920 and 2851 $cm^{-1}$.[54]

In accordance with the $CH_2$ asymmetric and symmetric stretching modes at 2927 and 2855 $cm^{-1}$ (Figure 1), respectively, it is possible that the SAMs form a gauche like assembly and are not densely packed on the surface, as the stretching modes differ from those expected for densely packed SAMs.[54] This is very similar to the packing density achieved by Borgh et al.[52] and is caused by the bulky phosphonate ester groups on the SAM surface. Following hydrolysis (Figure 1b), the peaks have only been shifted by very small amounts around 2928 and 2857 $cm^{-1}$ for the $CH_2$ asymmetric and symmetric stretching modes, respectively. Upon hydrolysis for 3 h (Figure 1b), the vibration stretches corresponding to amide I and II (small shoulder peak around 1560 $cm^{-1}$; this is also confirmed in the XPS data as the N 1s peak is still present) remain; however, the vibration stretches for P═O and P−O−C are not visible and a broad OH vibration stretch is seen around 3200−3600 $cm^{-1}$. Thus, it is confirmed that the phosphate group has been hydrolyzed (see Scheme 1a).

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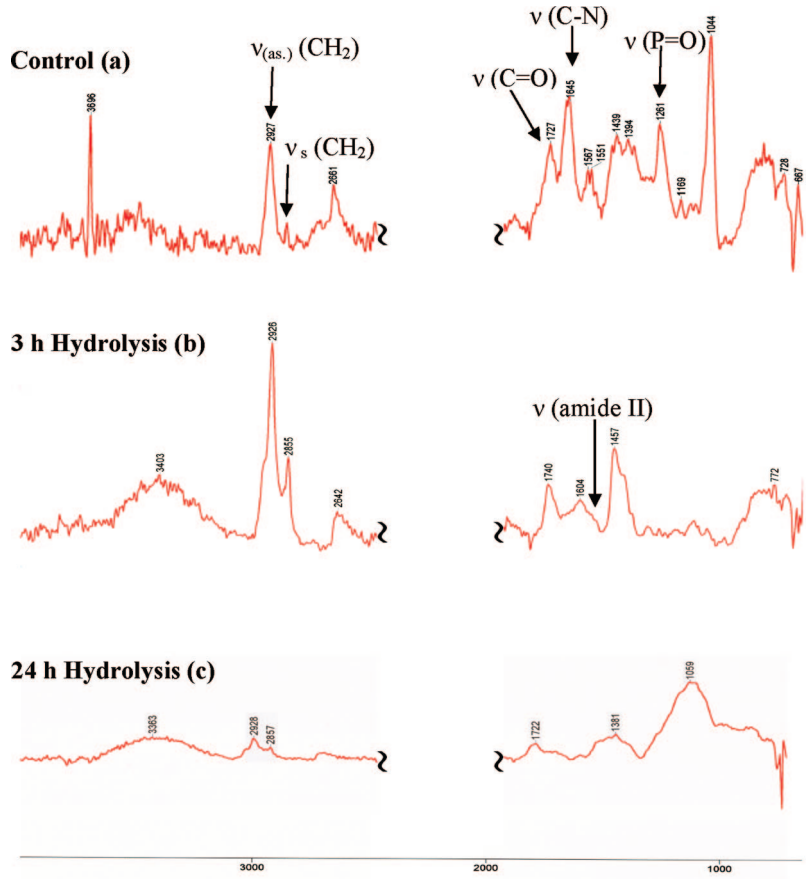


**Figure 1.** FT-IR spectra of the step-by-step hydrolysis of PPMA on gold, before and at 3 and 24 h time lengths. The control (a), native PPMA, shows the peaks corresponding to the phosphonate (P═O) groups and amides. In the 3 h hydrolysis (b) period, there are far fewer peaks observed between 1000 and 1400 $cm^{-1}$. In the 24 h hydrolysis (c) period, the only remaining peaks visible belong to the carboxyl group and a broad OH peak is clearly seen with the lack of a vibration peak for the amide moiety, due to near-complete hydrolysis of the bond.

After 24 h of hydrolysis (Figure 1c), the amide stretch has disappeared and a vibration band around 3343 $cm^{-1}$, corresponding to the -COOH stretch, is observed. It should be noted that the intensities of the $CH_2$ asymmetric and symmetric stretching modes are significantly reduced, but this phenomenon is attributed more to the sensitivity of the IR instrument.

XPS measurements were taken before and after hydrolysis. The C 1s high-resolution XPS data of the assembled monolayer, together with N 1s and P 2p and their hydrolysis, is shown in Figure 2. The native (control) SAM C 1s binding energy consists of two specific binding energies at 287.58 and 288.60 eV (together in the shoulder peak) indicative of the C═O and C−N peaks being present; upon hydrolysis from 3 to 24 h, the shape of the peak changes markedly (see Figure 2). First, from the XPS data obtained for SAMs hydrolyzed for 3 h, the C 1s peak has changed, where at a binding energy of 286.52 eV a broadening of the main carbon peak is visible.

The peak observed at 286.52 eV in the C 1s XPS data corresponds to the C−O bond as there is a lack of a P 2p peak at 134.37 eV (Figure 2), indicating that the phosphate group has been hydrolyzed. This is also confirmed by the decrease in the contact angle of the control from 65.1° ± 0.3° to 41.6° ± 1.64° on the 3 h hydrolyzed SAM. After 24 h of hydrolysis, the peak at 288.60 eV was somewhat diminished; this, coupled with a small N 1s peak at 400.31 eV, confirmed the loss of the vast majority (88%) of the amide bonds from the surface, as also confirmed by a contact angle measurement of 38° ± 1.26°. The phenomena observed from the XPS data clearly depict that the first bonds to be hydrolyzed were the phosphate ester bonds: within 3 h the P 2p peak at 134.37 eV disappeared, revealing a hydrophilic OH moiety at the surface as shown in Scheme 1 and as supported by both FT-IR, XPS, and contact angle measurements. Phosphate esters are analogous to carbon esters and thus are far easier to hydrolyze, as it is known that amide bonds tend to be very stable, requiring longer acidic conditions to achieve successful hydrolysis. To verify that there was no loss of the sulfur bond to the gold, XPS angle-dependent measurements were conducted on the 24 h hydrolysis samples.

As shown in Figure 3, after 24 h of hydrolysis at 100 °C, the thiol SAMs on the gold surface remain unaffected and have not been removed from the surface. Amide-containing SAMs form a packing order hierarchical to that of alkanethiolates on gold,[55] an effect caused by hydrogen-bond interaction between the carbonyl and amine moieties within the chains, strengthening the intermolecular interactions and forming linear networks on the surface.[56]

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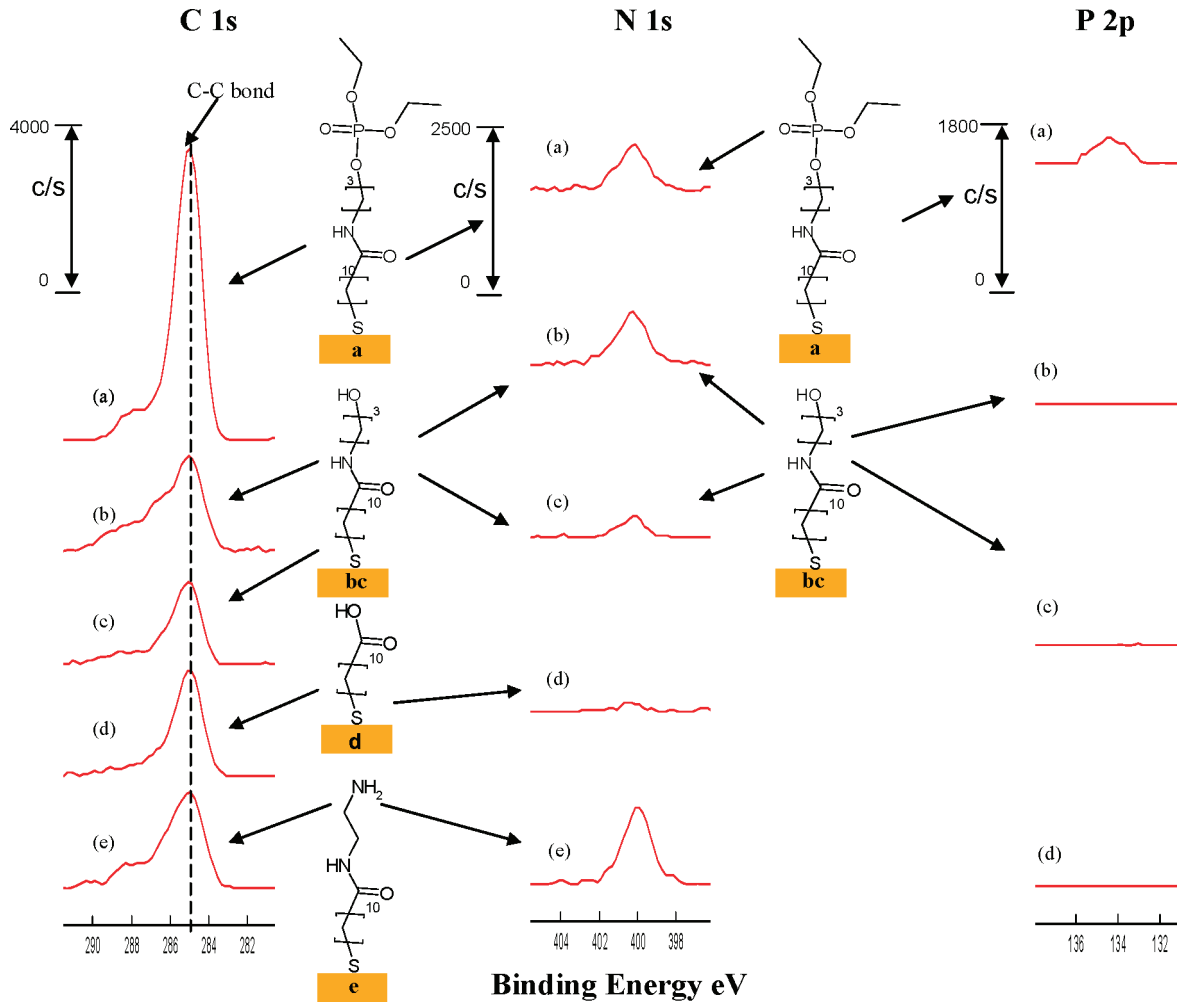


**Figure 2.** XPS spectra of C 1s, N 1s, and P 2p data of *N*-(3-diethylphosphatoxy)propyl-11-mercaptoundecanamide on gold, hydrolyzed at 100 °C in 1 M $H_2SO_4$. (a) Control; (b) 3 h hydrolysis; (c) 6 h hydrolysis; (d) 24 h hydrolysis; (e) amidation.

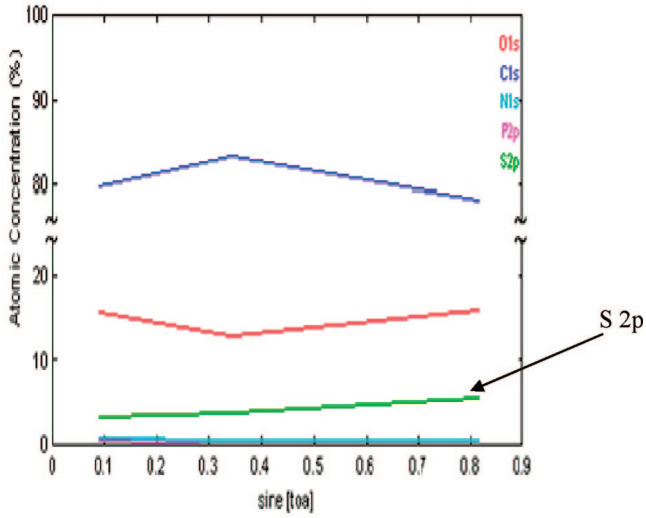


**Figure 3.** XPS angle-dependent measurements of the SAMs after 24 h of hydrolysis at 100 °C in 1 M $H_2SO_4$. This graph shows that even after 24 h of hydrolysis the thiol group is still on the gold surface.

The confirmation of the thiol moiety on the gold surface even after being subjected to temperatures of 100 °C under acidic conditions exemplifies the stability of the sulfur−gold bond. This aspect has been confirmed in a previously reported research investigation conducted by Nuzzo et al.[57] They have shown that thiol SAMs are stable up to temperatures of 170 °C; however, Schlenoff et al.[58] observed that at 100 °C there was some loss of the hexadecanethiol on the gold surface. The thermal effects on amide-modified thiols on gold have not yet been studied and could be an investigation carried out in future studies. The slow impeding surface hydrolysis of the amides within the SAMs may be explained by the following factors: solvent and "steric" effects.[14]

When the reaction conducted herein is compared to those of the ester hydrolysis conducted by Stirling and co-workers,[59] it can be hypothesized that the rate of hydrolysis is also influenced by the position of the amide group within the chain. In this instance, the amide moiety is largely on the surface, 12 carbons away from the gold surface, and thus is readily accessible for acidic attack. It is proposed that the initial hydrolysis begins at the SAM surface defect sites and

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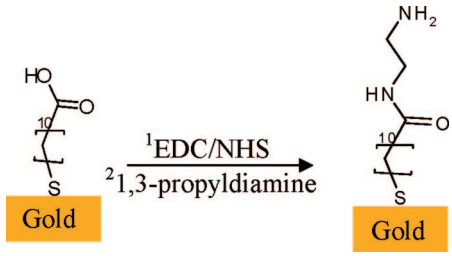
**Scheme 2. Amidation of 24-h Hydrolyzed PPMA SAM with 1,3-Propyldiamine**




surface boundaries where it is much more facile to solvate the region; thus it could be seen as the rate-determining step, after which the rate of hydrolysis is expected to increase as accessibility to neighboring amide moieties by the $OH^-$ becomes readily available.

Following successful hydrolysis of the amide moiety within the SAMs, the resulting carboxylic-terminated groups were activated via the common method of EDC/NHS followed by amidation with propyldiamine (Scheme 2).

After activation of the SAM with NHS, it was treated with propyldiamine to re-form the amide functionality, as is clearly shown in the XPS C 1s and N 1s spectra (Figure 2): the C 1s peak develops a shoulder having a binding energy of 288.95 eV and a N 1s peak is once again visible at 400.31 eV, corresponding to the newly formed C−N bond.

In this instance we have shown the creation of an alcohol and a carboxylic group from a phosphate-terminated functionality. The creation of different chemical functionalities creates the potential for conducting further surface reactions such as demonstrated here by amidation of the carboxylic functional group. These newly formed functionalities could be utilized for cell studies,[60] ranging from hydroxyapatite induction by using the native phosphate groups, as phosphonic acid groups,[61] through to cell repulsion by using the exposed alcohol groups.[36]

Ellipsometry was used to deduce the thickness of the PPMA SAMs before and after hydrolysis. The thickness of the original PPMA SAM was shown to be 1.6 ± 0.8 nm. This thickness suggests that the SAMs are not densely packed, and combining the thickness with the IR stretch bands of the $CH_2$ bonds, it can be stated that the monolayers form a gauche like assembly. The length of the PPMA molecule is 2.3 nm (as calculated by the ChemDraw software 8.0) and in an all-trans conformation this length would be shown; as such, this suggests that the molecules are in fact sitting at an angle close to 45° on the substrate surface, resulting in a reduced thickness. Following hydrolysis after 3 h, the thickness of the SAM was reduced to 0.7 ± 0.4 nm; however, the length of the molecule is 1.9 nm. This suggests that the SAMs may contain some degree of porosity.[62] After 24 h of hydrolysis the thickness of the film remains very similar, as it is 0.7 nm ± 0.98 nm. This similarity can be due to the removal of only a very short chain (see Figure 2b−d).

## Conclusions

Herein we have demonstrated that step-by-step hydrolysis can be used for the exposure of different chemical functional groups, using *N*-(3-diethylphosphatoxy)propyl-11-mercaptoundecanamide. From XPS and FT-IR measurements it was shown that these SAMs still remained stable even after 24 h of hydrolysis at 100 °C in a 1 M $H_2SO_4$ solution; this was further emphasized by continuing on with an amidation on these newly derivatized surfaces. It is suggested that these surface reactions could be put to use in areas investigating the influence of multiple surface functionalities for varying applications where one molecule can be potentially used to produce two reactive surface groups as preliminary steps. It also circumvents the need to conduct exhaustive chemical synthesis, whereby here this is simplified by simple surface hydrolysis. It further provides a key contribution toward scientific research on the effects of step-by-step hydrolysis of SAMs, an area that has not been fully investigated. In addition the SAMs will be used for the preparation of varying functional groups for cell adhesion studies.

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