Stimuli-responsive functionalization strategies to spatially and temporally control of surface properties: Michael vs. Diels Alder type additions

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ABSTRACT

Stimuli-responsive self-assembled monolayers (SAMs) are used to confer switchable physical, chemical, or biological properties to surfaces through the application of external stimuli. To obtain spatially and temporally tunable surfaces, we present microcontact printed SAMs of an hydroquinone molecules that are used as a dynamic interface to immobilize different functional molecules either via Diels Alder or Michael thiol addition reactions upon the application of a low potential. In spite of the common use of such reactions and the potential applicability of the resulting surfaces in different fields ranging from sensing to biomedicine through data storage or clean up, a direct comparison of the two functionalization strategies on a surface has not yet been performed. Although the Michael thiol addition requires molecules that are commercial or easy to synthesize in comparison with the cyclopentadiene derivatives needed for the Diels Alder reaction, the latter reaction produces more homogeneous coverages in comparable experimental conditions.

INTRODUCTION

Self-assembled monolayers (SAMs) are used to modify substrates in order to confer to their surfaces a specific function, such as to enhance/decrease the wettability, friction, adhesion, or biocompatibility, among others.^{1,2} One of the most studied SAMs are formed by alkanethiolates on gold, since long chain alkanethiolates form densely packed, highly ordered trans-extended monolayers in a rapid and spontaneous manner on gold.^{3,4} One way to use such SAMs for modifying the surface properties of a substrate is to replace the terminal CH₃ units of alkanethiols by a chemical group that will introduce the desired functionality to the surface. This can be done either using directly ω -functionalized alkanethiols to form the SAM or modifying the SAM after its formation. If the terminal functional group responds to a given external stimuli both strategies permit to generate stimuli-responsive surfaces exhibiting switchable physical, chemical, or biological properties, which can be tuned in an accurate and predictable way at any given time. Such smart surfaces can find numerous applications in science and technology ranging from environmental, sensing, or biomedicine to cleanup and data storage. In particular, methods to modify SAMs after their formation are critical to develop such complex smart surfaces.⁵ For example, a composition change can be caused by the isomerization of an immobilized ligand between a structure that is active and another that is inactive.⁶ Alternatively, the chemical change can be triggered by activating the substrate, thus allowing the immobilization of ligands present in solution. In some cases, the reaction can even be reversed leading to the release of the ligand from the substrate.⁷⁻¹⁰ These strategies rely on interfacial chemical reactions initiated by an external non-invasive stimuli, such as light or an electrical potential,^{3,11,12} enabling a spatiotemporal control over the surface environment.^{6,13} Dynamic control over the properties of surfaces can be performed by applying an electrical potential to

generate oxidation or reduction reactions to attached molecules leading to two different redox states. For example, a switch in surface wettability could be obtained by altering the electronic states of single-layered molecules between hydrophilic and hydrophobic states,¹⁴ yielding interesting substrates for microfluidic,¹⁵ self-cleaning,¹⁶ or sensor devices.¹⁷ Similarly, surfaces that can dynamically regulate biological functions in response to applied stimuli, can be useful for biological and medical applications, such as to control cell guidance.^{18,19}

SAMs functionalized with hydroquinone (HQ) and its oxidized quinone (Q), as terminal groups, can be used as a dynamic interfaces to immobilize different functional molecules on surfaces via stimuli activated Diels Alder (DA) reactions triggered by the application of a low electrical potential.²⁰ The DA reaction is a reversible cycloaddition occurring between a conjugated diene (in the *cis* configuration) and an electron-deficient dienophile. Advantageously this reaction is orthogonal, efficient, atom conservative, and does not require a catalyst. It is also insensitive to the reaction conditions, such as air and humidity, allowing its use under ambient conditions.²¹ One of the pairs of diene and electron-deficient dienophile most used for DA reactions is cyclopentadiene (Cp) and HQ derivatives, respectively. They react in mild conditions but show some important drawbacks. On one side the synthesis of the needed HQ-tagged molecules is complex and not standardized.²² On the other side, it is also very challenging to synthesize the Cp-terminated functional derivatives for the DA reactions, due to the low functionalization reproducibility caused by the variability of Cp derivatives on the chemisorption process previous to the DA reaction.²³

In order to avoid the use of Cp or other less favorable dienes, Michael thiol addition (MA) reactions can be considered as an alternative to HQs for SAM derivatization in spite of the differences in their reaction mechanisms. MA reactions involve the addition of a nucleophile,

which is referred to as the ‘Michael donor,’ to an activated electrophilic alkene, named the ‘Michael acceptor’, resulting in a ‘Michael adduct’. Even though, this type of reaction is usually performed with enolate nucleophiles and activated alkenes there are many other functional groups that possess enough nucleophilicity to undergo this reaction. Indeed, several non-enolate nucleophiles were previously used, such as amines,²⁴⁻²⁶ thiols,²⁷ azides,²⁸ and phosphines.²⁹ MA rates of thiols reacting with acrylates or vinyl sulfones are dependent on the pH of the reaction media and they are reliant on the particular pKa of each thiol. This is explained by the fact that the reacting species are the thiolates rather than the pristine thiols.³⁰ Interestingly, MA reactions readily occur at physiological conditions and are selective to thiol containing proteins over amine nucleophiles, for example.²⁷ Thus, reactions with lysine residues required a higher pH of 9.3 and their rates are still dramatically slower than the reaction with cysteine at a comparable pH. Another important advantage of MA reactions for SAM derivatization is the fact that the synthesis of functional molecules with thiol terminal groups are easier to perform than the Cp derivatives and the resulting compounds are more stable. Nevertheless, there is no comparative study between the interfacial DA and MA reactivity of Cp- and thiol-terminated functional molecules, respectively, onto an electrochemically activated quinone (Q) SAM. Thus, the differences on the spatial and temporal control given by these two different dynamic surfaces have not yet been determined.

Here we will report on the differential interfacial reactivities of SAMs with Cp- and thiol-terminated functional molecules and their implications on the modification of properties of gold substrates. To compare the performances of these two surface reactions two fluorescein derivatives were synthesized, one with a Cp (**1**) and another with a thiol (**2**) termination, which were used as fluorescent probes to interrogate the surface functionalization. Both molecules were

linked to a readily prepared hydroquinone terminated SAM, **S1**, which offers temporal control through its reversible conversion to a benzoquinone terminated SAM, **S2**, in a redox process that involves two electrons and two protons (Scheme 1a). SAM **S1** consisted of electroactive HQ terminal group that was anchored to gold surfaces through thiols, as previously described by Lee et al,^{31,32} who pioneered the use of the DA reaction for surface functionalization with biological ligands.^{33,34} Moreover, the SAM strategy offered us the possibility to precisely control the density of electroactive ligands on the surface by mixing two different pegylated alkanethiol molecules, like the electroactive HQ (**3**) and the hydroxyl-terminated analogue (**4**). The alkyl chains of these molecules confer structural stability to the monolayer, through the formation of a compact 2D packing mediated by van der Waals $\text{CH}_2\cdots\text{CH}_2$ interactions, whereas the ethylene glycol linker units give hydrophilicity to the surface and prevent unspecific binding of proteins and cells, if desired.^{35,36}

Scheme 1. (a) Schematic representation of the electroactivated interfacial DA and MA reactions. SAM **S1** was electrochemically oxidized to SAM **S2**, which reacted to molecules functionalized either with ω -terminated Cp or thiol groups to produce SAMs **S3** and **S4**, respectively. **(b)** Molecular structures of fluorescein molecules functionalized with Cp, **1**, and thiol, **2**, terminating

groups as well as the thiolated HQ derivative, **3**, and its analogous hydroxyl derivative **4**. All these molecules contain a polyethylene chain bridging the ω -terminated group and the anchoring unit. In such molecules the alkyl chain provides structural order to get compact molecular monolayers, whereas the oligo(ethylene glycol) chain prevents unspecific protein and cell adsorption.

As mentioned above, one of the main disadvantages of the DA reaction when using HQ as dienophile has been attributed to synthetic difficulties^{22,23} to achieve the desired molecules, which hinder its extended use for a wide range of applications. In the present study we firstly optimized the synthesis of the HQ derivative **3** to form the electroactive SAM **S1** providing a complete explanation of the synthetic route. Moreover, a multitechnique approach was used for the surface characterization, which includes X-ray photoelectron spectroscopy (XPS), cyclic voltammetry (CV), and contact angle measurements for both the reduced **S1** and oxidized **S2** SAMs. Then, we analyzed the performance of the DA and MA reactions as surface functionalization strategies to obtain spatial and temporal stimuli-responsive SAMs. Specifically, electroactivated SAM **S2** with Q terminal group was conjugated with the newly synthesized fluorescein terminated molecules **1** and **2**, with Cp and thiol anchoring groups respectively, to demonstrate temporal control over the surfaces functionalization. To achieve a spatial control, the microcontact printing technique was used,³⁷ thereby obtaining a pattern with inert areas consisting of hydrophilic monolayers of hydroxyl molecules **4**, resistant to non-specific adsorption of proteins and cells, surrounded by areas functionalized with SAMs of HQ derivative **3**. Due to the different lengths of molecules **3** and **4** the HQ groups jut from the OH groups on these patterned surfaces allowing the specific interactions of Q groups on the surface of triggered SAMs.

EXPERIMENTAL

Synthesis.

All chemicals involved in the synthesis were purchased from Sigma Aldrich (US) and used without further purification.

Compound **4** consisting of an alkanethiol functionalized with a tetra(ethylene glycol) group, the 1-mercaptoundec-11-yl-tetra(ethylene glycol), was synthesized through a three step procedure (Scheme 2a). The synthesis of the hydroquinone-tetra(ethylene glycol)alkanethiol **3** consisted of the subsequent modification of the alkanethiol **4** with the hydroquinone moiety **8** (Scheme 2b) resulting in a six step procedure (Scheme 2a). Treatment of tetra(ethylene glycol) with 11-bromo-1-undecene in a saturated aqueous NaOH solution afforded the alkene **5**. Subsequent addition of thiolacetic acid in the presence of a radical initiator gave the thioacetate **6**, and the final hydrolysis afforded thiol **4** (OH-SH). Compound **8** was prepared in two synthetic steps from commercially available materials (Scheme 2b). The first reaction is based on the addition of dihydropyran to hydroquinone to provide the protected *di*-tetrahydropyrane-hydroquinone **7**, which was purified via recrystallization in hexane after completion of the reaction (monitored with TLC). Deprotonation of **7** with *t*-butyllithium followed by addition of dibromobutane provided the *di*-tetrahydropyran-hydroquinone-butylbromide **8**. We successfully optimized the purification step taking into consideration the solvent used to elute the product in the column chromatography, which presents an *R_f* value very similar to the starting material. Specifically, we used a mixture of chloroform and dichloromethane in comparison with the previously described hexane-ethyl acetate mixture.⁹ Precursor **8** was then used to convert thiol **4**

into the hydroquinone derivative **3** (HQ-SH). First, thiol **4** was protected with triphenylmethyl chloride, and the subsequent addition of the masked hydroquinone **8**, gave the intermediate **10**, which after treatment with TFA afforded **3** (HQ-SH). This second synthetic route may be shortened, since the hydrolysis of the thioacetate **6** to obtain **4**, and subsequent protection with triphenylmethyl chloride to obtain **9**, can be carried out in a one-pot sequence (for more details see SI).

Scheme 2. (a) Synthesis of thiol **4** (OH-SH) and the hydroquinone derivative **3** (HQ-SH). (b)

Synthesis of **8** from hydroquinone and dihydro-2H-pyran.

Fluorescent molecules **1** and **2** were prepared from the common precursor **14** (CF-OEG-NH₃Cl) which were synthesized in a two-step procedure starting from commercially available 5(6)-carboxyfluorescein (CF), see Scheme 3. CF was activated as the succinimidyl ester and reacted with 1-(*tert*-butoxycarbonylamino)-4,7,10-trioxa-13-tridecanamine to prepare compound **13** (CF-OEG-Boc). Removal of Boc protecting group afforded derivative **14**. The cyclopentadienyl derivative **12** was prepared in a three-step synthesis starting from sodium cyclopentadienylide (NaCp). First, NaCp was alkylated with methyl bromoacetate and the resulting methyl ester was hydrolyzed to obtain **11** (Cp-COOH), which was then converted to the corresponding activated succinimidyl ester **12** (Cp-COOSu). The coupling between **12** and **14** afforded the carboxyfluorescein derivative **1** (CF-OEG-Cp). On the other hand, 3-(tritylthio)propanoic acid was converted to the corresponding succinimidyl ester and reacted with **14** to obtain the thiol-protected derivative **15** (CF-OEG-STrt). Finally, the trityl protecting group was removed to obtain compound **2** (CF-OEG-SH) (for more details see SI).

Scheme 3. Synthesis of the fluorescein-containing molecules **1** (with Cp) and **2** (with thiol).

These compounds were purified and characterized using ^1H -NMR and Electrospray (ESI-MS). ^1H -NMR experiments were carried out on a Bruker Advance 400 MHz spectrometer.

Self Assembled Monolayer Preparation. Substrates were prepared by evaporation of titanium (50 nm) as adhesion layer and gold (100 nm) onto silicon wafers and cut into substrates of 1x2 cm. These gold substrates were cleaned by sonication in isopropanol, acetone, and ethanol (HPLC grade), exposed to ozone for 20 minutes and immersed in absolute ethanol (HPLC grade) during 30 minutes. Finally, substrates were dried with a stream of nitrogen and immersed in a solution containing molecules **3** and **4** in a 1:1 ratio and a 1 mM total concentration in absolute ethanol to obtain SAM **S1**. Finally, they were left immersed in the solution and under an inert atmosphere overnight. Before use, SAMs **S1** were rinsed with absolute ethanol and dried with a stream of nitrogen.

XPS Measurements. X-ray photoelectron spectroscopy measurements on SAMs **S1**, **S2** and **S3**, were performed with a Phoibos 150 analyzer (SPECS GmbH) in ultra-high vacuum conditions (base pressure $5 \cdot 10^{-10}$ mbar) with a monochromatic aluminium K alpha X-ray source (1486.74 eV). The energy resolution measured by the FWHM of the Ag 3d5/2 peak for a sputtered silver foil was 0.6 eV. The spot size was 3.5 mm by 0.5 mm.

Contact Angle Measurements. Contact angle measurements on SAMs **S1** and **S2** were performed with Milli-Q (MQ) water under ambient temperature and humidity with a Contact Angle Measuring System DSA 100 from KRÜSS.

Electrochemical Measurements. Before performing electrochemical measurements, substrates with SAM **S1** were rinsed with absolute ethanol and dried with a stream of nitrogen. Later on SAMs were electrochemically characterized using CV with an AUTOLAB 204 and the NOVA

1.9 software. A custom built electrochemical cell with a Pt-wire as counter electrode, a Ag/AgCl electrode as reference electrode, and the SAM **S1** on gold as working electrode were used. The electrolyte was a 1 M HClO₄ aqueous solution, which was purged with argon before its use. The area exposed to the electrolyte was approximately 1 cm².

Microcontact Printing.

Preparation of stamps. Stamps of poly(dimethylsiloxane) (PDMS; Dow Corning Sylgard 184) were prepared by casting the prepolymer onto photolithographically fabricated master patterns on silicon, which were previously silanized to increase their hydrophobicity.^{4,38}

Preparation of dynamic surfaces with spatial control. Micropatterned SAMs were prepared onto gold substrates produced and cleaned as explained above. A PDMS stamp with a pattern of 20 μm wide lines was used to microcontact print octadecanethiol (1 mM) onto the gold substrate. The stamp was firstly cleaned with ethanol and dried with a nitrogen stream, thereafter it was immersed during 10 seconds in the octadecanethiol solution, dried under a stream of nitrogen, and put into contact with the clean gold surface during 1 minute. The stamp was then peeled off cautiously from the surface and the substrate was transferred into the backfilling solution, a 1 mM ethanolic solution of the synthesized molecules **3** and **4**. Ratios of 1:1 and 1:99 of molecules **3:4** were used for the CV characterization and the fluorescein molecule immobilization studies, respectively. The substrates were left immersed in the solution and under an inert atmosphere overnight. Before further use of these SAMs, they were rinsed in absolute ethanol and dried with a stream of nitrogen.

Figure 1. Scheme of the preparation of a dynamic substrate with spatial (lines of 20 μm width) and temporal control of the functionalization. First, microcontact printing was used to pattern hydrophobic areas of octadecanethiol on bare gold. Then, the non-functionalized areas were filled with a mixed SAM of **4** and the electroactive hydroquinone terminated **3**.

Stimuli activated surface functionalization.

For the DA surface confined reaction, first a voltage of +800 mV for 60 seconds was applied to SAM **S1** in order to oxidize all the hydroquinone groups of the SAM. The resulting SAM **S2** was then rinsed with MQ-water and immersed in a 20 mM solution of fluorescent Cp-terminated derivative **1** in THF:water (1:1) during 30 minutes to give the SAM **S3** after proper rinsing with MQ-water and characterized by fluorescence microscopy.

For the MA surface confined reaction, the SAM **S2** was obtained as explained above. Thereafter, it was immersed in a solution of fluorescent thiolated molecule **2** in a mixture of EtOH:water (9:1) to give the SAM **S4**. To optimize the homogeneity of the pattern, different experimental conditions were tested. Specifically, the concentration of **2** was varied from 10 μM to 20 mM and the immersion times were ranged from 30 minutes to overnight (SI).²⁸

After functionalization, the substrates were rinsed with MQ-water and characterized by fluorescence microscopy .

Fluorescence Microscopy. Fluorescein functionalized substrates were observed with an Olympus BX51 microscope equipped with a CCD camera Olympus DP20

RESULTS AND DISCUSSION

Characterization of the electroactive hydroquinone SAM S1.

CVs using the prepared mixed SAM S1, prepared with a 1:1 molar ratio of **3** and **4**, as the working electrode at different scan rates are depicted in Figure 2. Current peaks are found between 0.43-0.47 V for the oxidation and 0.33-0.26 V for the reduction, in agreement with a previous report.³¹ The linear relationship between the current intensity and the scan rate (linear regression gave $R^2 = 1.000$ and $R^2 = 0.996$ for anodic and cathodic peaks, respectively) confirmed that the molecules were surface confined. This relationship is usually obtained in rapid reversible redox processes of immobilized redox couples on a surface.^{20,39} The surface coverage was estimated by integrating the oxidation peak area observed from the CV in 1 M HClO₄, taking a 2e⁻, 2H⁺ reaction mechanism, and applying the equation $Q = nFA\Gamma$,. In this equation n is the number of electrons involved in the redox couple (n=2 for hydroquinone/quinone), F is Faraday's constant, A is the surface area of the electrode that is in contact with the electrolyte, and Q is the total charge. By integrating the area under the reductive wave, Q was obtained giving place to a density of $\Gamma = 6.21 \times 10^{-12}$ moles/cm² for the 50% quinone monolayer and 1.24×10^{-11} moles/cm² for the completely covered one, similarly to what it was previously reported.^{20,24}

Figure 2. CVs of the SAM **S1** with a 1:1 molar ratio between **3** and **4**. Inset: relation between scanning rate and the reduction and oxidation peak intensity (current) of the SAMs.

Contact angle measurements were performed for bare gold and substrates functionalized with SAM **S1** using **3** and **4** (1:1) before and after the hydroquinone oxidation (see experimental part) to the benzoquinone derivative. The values obtained were 66.1, 56.1, and 80.8°, respectively (Figure 3).

Figure 3. Contact angle images of (a) bare gold, (b) SAM S1, and (c) SAM S2.

The bare gold surface showed larger contact angle than the SAM S1 due to hydrophilicity provided by the hydroxyl groups on the surface. On the other hand, SAM S2 presents the highest contact angle, being the most hydrophobic surface, due to the presence of the carbonyl groups.

Stimuli activated SAM reactivity for spatial and time controlled surface functionalization.

The activation of the SAMs towards the DA and MA interfacial reactions was studied by CV. SAMs with a 1:1 molar ratio of **3** and **4** were prepared as previously described and solutions of either 15 mM of hexyl-Cp and hexanethiol in THF:PBS (1:1) were used as non-electroactive target ligands to react with the electroactivated surface. Thus, SAM S1 was oxidized to benzoquinone by applying a potential of +650 mV to obtain the SAM S2. In order to see how time is needed to apply the potential for a complete oxidation of SAM S1 to S2, the potential was applied during 20 seconds three consecutive times. After every 20 seconds of oxidation, **the SAM** was immersed in the target ligand solution during 30min. After every oxidation and

immersion (reaction) step, a CV was performed in order to check the different evolution of the DA or MA interfacial reactions. As seen in Figure 4, the redox peak during the DA reaction with the hexanethiol target ligand disappears after a total 60 seconds of oxidation, as expected, since the cycloadduct formed is not electroactive anymore. On the contrary, the redox peaks of the MA reaction with the hexyl-Cp were only slightly decreased and shifted since in this case, the product of the reaction is still electroactive and thus the redox signal is not lost.²⁴

Figure 4. DA and MA interfacial reactions using 1M HClO₄ as electrolyte for the oxidation and ligand solutions (hexyl-Cp and hexanethiol) of 15 mM in THF:PBS (1:1) for the interfacial

reactions. Cyclic voltammograms monitoring the three oxidation steps of 20 seconds followed by 30min immersion in the ligand solution.

In order to include not only a temporal control, but also a selective spatial functionalization upon a certain external stimulus (applied potential), the microcontact printing technique was used to anchor the electroactive molecule **3** only on specific areas of the surface, e.g. in stripes of 20 μm in width.

To compare the differences between the DA and the MA surface confined reactions, fluorescence microscopy images were taken after immersion of the resulting SAM **S2** in a 20 mM solution of fluorescent ligands **1** and **2** for 30min. Oxidation of **S1** was performed by applying a voltage of +800 mV during 60 seconds to ensure the presence of benzoquinone groups on the surface, yielding **S2**. For the DA interfacial reaction a ligand solution of **1** in THF:water 1:1 was used, whereas for the MA interfacial reaction a ligand solution of **2** in EtOH:water 9:1 was employed. The luminescent micropattern obtained after the surface confined reactions is shown in Figure 5.

In spite of the broad experimental conditions that were tested for the MA reaction (see experimental section) varying the concentration of the fluorescent molecule **2** and the immersion times, as it can be observed in Figure 5b, the MA does not give place to a homogeneous functionalization, but fluorescent patchy areas. The DA reaction has been described to show a rate enhancement in polar solvents and in ethylene glycol (EG) environments due to the stabilization of its transition state.⁴⁰ On the other hand, the MA, in spite of being considered a powerful click reaction,²⁷ needs mild catalysis (i.e. amounts of a base or a nucleophile in the

media) to increase its reaction kinetics and yield, which explains the formation of patchy areas in its absence.

In the present conditions, as we compared the two reactions under similar conditions, we did not add any catalyst. Given the considerable variability of the catalytic reaction, which depends on the pKa of the used thiol (ranging from 7 to 11), the strength and concentration of the catalyst as well as the steric hindrance between the reactants,²⁷ an optimization of the reaction conditions should be considered for each system.

Figure 5. Fluorescence images of molecules **1** and **2** (20 mM) covalently attached (after 30 minutes immersion time) on micropatterns consisting of 20 μm wide stripes of electroactivated hydroquinone surface of SAM **S1** to **S2** (during 60 seconds) that reacted through (a) DA reaction and (b) MA reaction, respectively (Scheme 1). The stripe width of the pattern in the left image is narrower than the 20 μm of the PDMS stripes, which can be attributed to the incomplete contact printing of the SAM **S1** due to the fact that this process is very pressure dependent.

CONCLUSIONS

A direct comparison of the electroactivated DA and MA functionalization strategies of surface has been performed. To produce spatially and temporally switchable surfaces, we prepared microcontact printed SAMs of HQ that were used as a dynamic interface to immobilize different functional molecules via DA or MA reactions upon the application of a low electrical potential. The synthesis of a thiolated HQ derivative containing a bridge with a pegylated chain followed by an alkylated chain is thoroughly described as well as two novel fluorescein pegylated derivatives tagged with either Cp or thiol groups to compare the interfacial DA and MA reactions. In spite of the different experimental condition used, the MA does not give place to a homogeneous functionalization, but patchy areas compared to the fully covered surfaces obtained through the DA interfacial reaction. Given that the synthesis of the Cp derivatives is more complex and the compounds obtained less stable than the thiol analogues, we can conclude that there is not a clearly advantageous reaction over the other and therefore their use should be decided according to the singularities of each system such as the difficulty of synthesizing specific Cp-derivatives, the need for fully coated surfaces, or the possibility of adding a catalyst.

ASSOCIATED CONTENT

Supporting Information. The following files are available free of charge.

Synthetic procedures and ^1H -NMR spectra of compounds, XPS (C, S, O) of SAMs.

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ABBREVIATIONS

SAM, self assembled monolayer; DA, Diels Alder reaction; MA, Michael thiol addition reaction; Cp, cyclopentadiene; HQ, hydroquinone; EG, ethylene glycol; CF, 5(6)-carboxyfluorescein; MQ, Milli-Q; PDMS, poly(dimethylsiloxane); XPS, X-ray photoelectron spectroscopy; CV, cyclic voltammetry; TLC, thin-layer chromatography; HPLC, high performance liquid chromatography.

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