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Greatly extended storage stability of electrochemical DNA biosensors using ternary thiolated self-assembled monolayers

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ABSTRACT

While high storage stability of sequence-selective DNA biosensors is crucial towards their routine applications, commonly used electrochemical hybridization biosensors are characterized with limited storage stability. In this article we demonstrate that recently developed ternary thiolated monolayers impart dramatic improvement in the storage stability of DNA electrochemical biosensors. In particular, highly stable multicomponent interfaces are prepared by co-immobilizing the thiolated capture probe (SHCP) with 1,6-hexanedithiol (HDT) on gold substrates, followed by the incorporation of 6-mercapto-1-hexanol (MCH) diluent. The resulting (SHCP/HDT+MCH) DNA hybridization recognition platform offers substantially higher storage stability compared to conventional binary (SHCP+MCH) monolayers. The (SHCP/HDT+MCH) ternary monolayers maintain their initial signal (S)-to-noise (N) ratio (S/N) over a prolonged 3 months period upon storage at 4 °C, compared to the rapid sensitivity loss observed using the common binary interfaces. This attractive stability performance promises the convenient usage of pre-prepared electrodes after prolonged time storage without any treatment. Such dramatic improvements in the storage stability have been achieved through a rational optimization of the concentration ratio of the SHCP and the other components of the ternary SAM. The improved storage stability of SHCP/HDT+MCH interfaces observed at higher concentrations of SHCP is attributed to a hindered displacement of SHCP by MCH in the resulting compact layers. The ability to design highly stable nucleic acid interfaces using common chemicals obviates the need of using specialized expensive reagents.

1. Introduction

Sequence-specific electrochemical DNA biosensors have received considerable interest and offer advantages over conventional slow/laborious DNA hybridization techniques, including high sensitivity, good selectivity, low cost, miniaturization, portability, lower power requirements and independence from sample turbidity [1–4]. The overall performance of electrochemical DNA hybridization biosensors is strongly dependent upon the surface chemistry used for interfacing the DNA probe and the electrode transducer. In particular, control of the surface chemistry and coverage of the electrode substrate are essential for maximizing the hybridization efficiency and minimizing of non-specific adsorption events. Particular attention has been given to self-assembled monolayers (SAMs) which provide well-ordered assemblies on electrode substrates. Binary alkanethiol SAMs with different chain lengths offer controllable surfaces with different chemical functionalities for DNA biosensing [5,6]. Most of the common methods are based on the adsorption of thiolated species on gold substrates due to their high affinities to gold [7]. Although such SAM-based DNA biosensor interfaces have been widely investigated with regard to the sensitivity and selectivity of these devices, little attention has been given to their long-term storage stability (which is crucial for diverse practical applications). For example, SAMs stability is of the utmost importance, if they need to be applied as a biosensor interface in real diagnostic applications [8]. Some researchers have tried to circumvent this prolonged storage stability problem through the use of new alkanethiols which increase the forces of attraction between the alkyl chains or through self-assembly systems which form more robust bonds with the underlying substrate using more attachment points or different surface chemistry [9].

In most of the studies, a thiol-derivatized specific ss-oligonucleotide capture probe (SHCP) is immobilized on the electrode, following a common spacer thiol treatment. The preparation of these binary SHCP+MCH SAMs has demonstrated to solve the main drawbacks faced by the unary SAMs fabricated by assembling the SHCP alone which lead to a very low hybridization efficiency (0%–5%) with the target nucleic acid [10,11]. The MCH displaces the non-specific adsorptive contacts between nucleotides and gold, occupies the vacant places within the monolayer

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(minimizing non-specific adsorptions), serves as a spacer between the DNA probe strands by reducing steric hindrance and forces them to an extended conformation amenable for hybridization [10–13]. However, it has been reported that these HS-anchored probes are largely displaced with time from the gold surface by these short-chain thiol compounds, such as MCH, dithiothreitol (DTT) or mercaptoethanol, most probably due to removal of unspecific and/or replacement of weekly bound probes [14–16]. As a result, such binary SHCP+MCH interfaces suffer from poor storage stability (with a 50% loss of the initial signal after 3 weeks of storage) [15]. The removal of the SHCP from the surface leads to a rapid and sharp decrease in the hybridization efficiency and hence in the sensitivity of the sensor. As a result, the MCH assembly is commonly carried out during the day of the measurement, just before the actual DNA hybridization sensing. MCH adsorption could be accomplished by a mechanism of displacement of nucleotide–gold contacts, which leaves empty places within the monolayer. The possibility of a thiol–thiol exchange mechanism has also been proposed [5]. This means that the sulfur–gold bond between the DNA linked probe and the substrate could be exchanged for one between MCH and the substrate [16]. In addition, the biologically relevant monolayers assembled through the thiol–gold bond have limited long term stability [17].

High storage stability of electrochemical DNA biosensors is crucial for many practical/routine applications in diverse fields, including decentralized field testing, genetic screening and detection and rapid clinical diagnosis (avoiding the time consuming preparation of a fresh biosensor). There have been a few attempts to prepare electrochemical DNA hybridization biosensors with high storage stability, using specialized reagents and surface chemistries [17]. Instead of using monothiol modifications, DNAs modified with two and even three thiol groups, such as dithiol phosphoramidite (DTPA)-modified capture probes, were shown recently to improve the DNA–gold linkage stability [18,19]. The thiol SS dipod DTPA is a disulfide-containing modifier designed to functionalize synthetic nucleic acids with multiple thiol groups and can be incorporated at any position of the oligonucleotide. Each DTPA addition leads to two thiol groups and a single ss-DNA can be modified with up to three DTPA moieties. Johnson et al., developed a robust attachment strategy by using a thin layer of poly(mercaptopropyl)methylsiloxane that was used to link maleimide-functionalized DNA to the surface through a network of thiol bonds [20,21]. Liepold et al. demonstrated that a monolayer prepared by assembling of capture probes modified with three DTPA groups on gold nanoparticles (AuNPs) were not displaced even by millimolar concentrations of HDT [14]. Li et al. demonstrated also the use of new trithiols terminated oligonucleotides to prepare extremely stable DNA–nanoparticle conjugates [22]. The issue of monothiolated-DNAs stability on gold has tried to be addressed also by Sakata et al. [23] using a novel tripod binder and by Plaxco and co-workers [24] using the flexible Letsinger-type trihexylthiol anchor. Day has recently described the synthesis of multithiolated DNA molecules to produce monolayers of ss-DNAs on gold substrates [17]. However, such multidentate thiol DNAs are more expensive to synthesize or not commercially available, and monothiol DNA is still the most widely used reagent leading also to the higher monolayer coverage [25] but with limited storage stability and hence further improvements are desired for diverse practical applications.

This article demonstrates that a dramatic improvement in the storage stability of DNA electrochemical biosensors can be achieved through the use of a dithiol-based ternary alkanethiol monolayer on gold substrates prepared by co-immobilization of SHCP with HDT, followed by the incorporation of MCH as diluent (SHCP/HDT+MCH). In order to address the issue of moderate stability of monothiolated DNA monolayers, we have systematically compared the prolonged stability of different binary and multicomponent ternary layers prepared with different backfillers. The role of the concentration and the assembling time of some components have been evaluated. Our results demonstrate that photolithography 16-sensor Au electrode arrays modified with the SHCP/HDT+MCH monolayer retain their attractive signal-to-noise (S/N) characteristics over a prolonged 3 months of dry storage at 4 °C, and that the exact composition of the monolayer (mainly the molar ratio between SHCP and MCH) plays an important role in this prolonged dry storage stability.

2. Materials and methods

2.1. Reagents and solutions

6-mercapto-1-hexanol (MCH, 97%), 1,6-hexanedithiol (HDT, 96%), DL-dithiothreitol (DTT), 11-mercaptoundodecanoic acid (MUA), Trizma hydrochloride (Tris–HCl), ethylenediaminetetraacetic acid (EDTA), polyethylene glycol (PEG), ethanolamine, *N*-hydroxysuccinimide (NHS), 1-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. The blocking agent casein was obtained from Pierce (Rockford, IL). The enzyme substrate 3,3′,5,5′-tetramethylbenzidine (TMB, Neogen K-blue enhanced activity substrate, containing H_2O_2) was obtained from Neogen (Lexington, KY). The conjugated anti-fluorescein isothiocyanate-horseradish peroxidase (anti-FITC-HRP, Fab fragments) was purchased from Roche Diagnostics (Mannheim, Germany).

The buffers used in the different experimental steps were as follows: the immobilization buffer (IB) contained 10 mM Tris–HCl, 1 mM EDTA, and 0.3 M NaCl (pH 8.0), the hybridization buffer (HB) was a 1.0 M phosphate solution containing 2.5% BSA (pH 7.2), and the binding buffer (BB), for the incorporation of the conjugated anti-FITC-HRP, consisted of PBS (1 ×) containing 0.5% casein (pH 7.2).

All synthetic oligonucleotides used, designed for detecting a characteristic region of *E. coli* 16S rRNA [26], were purchased from Integrated DNA Technologies, Inc. (CA, USA) and are listed in Table S-1 (in the supporting information).

2.2. Apparatus and electrodes

Chronoamperometric measurements were performed on disposable 16-sensor Au electrode arrays prepared by photolithography (GeneFluidics Inc. Irwindale, CA, USA). Each sensor consisted of a 2.5 mm diameter central gold working electrode, surrounded by a gold counter electrode and a gold pseudo-reference electrode. The sensor chip was driven by a computer-controlled PalmSens handheld potentiostat with an eight-channel PalmSens Multiplexer (Palm Instruments BV, Houten, The Netherlands).

2.3. Procedures

2.3.1. Preparation of the sensing interfaces on the working electrodes

For the preparation of SHCP/3rd component+MCH ternary monolayers, a mixture of SHCP and the freshly prepared 3rd component (HDT or DTT), with appropriate concentrations, was prepared in IB buffer and allowed to stand for 10 min. Aliquots of 6 μL of this mixture were cast over each Au working electrode in the 16-sensor array and incubated overnight at 4 °C in a humidified chamber. After washing with water and drying with nitrogen, the mixed monolayer-modified Au sensors were subsequently treated with 6 μL of a MCH aqueous solution (0.1–1.0 mM, in IB

buffer) for 50 min to obtain the ternary SAM interfaces. Finally, the sensors were thoroughly rinsed with water and dried under nitrogen.

For the preparation of SHCP+MCH binary monolayers, a SHCP solution with appropriate concentration was prepared in IB buffer and the same procedure and MCH post-treatment given above was performed.

For the preparation of SHCP+MUA+MCH monolayers, a SHCP solution with appropriate concentration was prepared in IB buffer and same procedure given above was performed. SHCP-modified Au sensors were subsequently treated with 6 μL of a MUA aqueous solution (1.0 mM, in PBS (1 ×) pH 7.2) for 1 h with the same MCH treatment mentioned above.

For the preparation of NH_2-CP monolayers, the 16-sensor Au electrode array was modified by immersion overnight in an ethanolic solution containing MUA/MCH (2.5/7.5 mM) or HDT/MUA/MCH (0.3/2.5/7.5 mM). The resulting modified arrays were thoroughly rinsed with water and dried under nitrogen. To convert the carboxylic terminal groups of the binary SAM to amine-reactive esters a 4 μL drop of a 200 mM EDC/50 mM NHS solution (prepared in deionized water), was applied to each working electrode and allowed to incubate for 10 min. After washing with water and drying with nitrogen, the activated monolayer-modified working Au sensors were subsequently treated with 4 μL of 2 μM NH_2-CP solution (prepared in PBS (1 ×) pH 7.2) and incubated at room temperature for 45 min. After thoroughly rinsing the array with water and drying under nitrogen a 30 μL drop of 1 M ethanolamine was cast to cover all three electrodes and incubate at room temperature for 10 min in order to block the remaining reactive groups of the activated monolayer [27]. All these incubation steps were performed at room temperature in a humidified chamber. Finally, the sensors were thoroughly rinsed with water and dried under nitrogen.

2.3.2. DNA hybridization assay in HB

The sensor response was evaluated with a sandwich-type hybridization assay, using a FITC-labeled detector probe (FITC-DP) and an anti-FITC-HRP as the reporter molecule. TMB was the selected co-substrate for the electrochemical measurement of the activity of the captured HRP reporter. 1 nM target DNA was mixed with 0.25 μM FITC-DP in HB and allowed to react for 15 min for homogeneous hybridization. Aliquots of 4 μL of the preformed hybrid solution were cast on each of the SAM-modified Au sensors and incubated for 15 min. Before casting the hybridization solution a previous treatment with PEG was carried out over the NH_2-CP modified Au electrode arrays by dropping a 4 μL drop of a 0.05% (w/v) polyethylene glycol 3350 (PEG) solution, in PBS (1 ×) pH 7.2, onto each working electrode and incubating 10 min at room temperature in a humidified chamber [2]. After hybridization, the array was washed and dried and each working electrode was incubated with 4 μL of a 0.5 U mL^{-1} anti-FITC-HRP solution in BB for 15 min. Subsequently, the array was washed and dried, and a prefabricated plastic 16-well manifold (GeneFluidics, Irwindale, CA, USA) was bonded to the sensor array. To perform the chronoamperometric detection, 50 μL of the TMB–H_2O_2 K-Blue reagent solution were placed sequentially on each of the sensors in the array, covering the area of three electrodes. After 30 s, the potential was stepped to −200 mV (vs. the gold pseudo-reference electrode) and the current was sampled during 60 s.

2.3.3. Dry storage stability studies

In order to evaluate the dry storage stability of the different interfaces, the 16-sensor Au electrode arrays, pre-modified with the SAM under study and dried, were stored at 4 °C in a humidified chamber. The long-term stability of coated Au sensors was tested periodically by evaluating the response for 1 nM target DNA, the noise level (response for 0 nM target DNA), and the corresponding S/N provided after hybridization in HB.

3. Results and discussion

3.1. Comparison of the dry storage stability of different monolayers

The prolonged storage stability of the ternary (SHCP/HDT+MCH) DNA recognition platform was compared with that of conventional binary (SHCP+MCH) monolayer. An extended period of 3 months was employed to examine and compare the long-term stability of both interfaces using storage at 4 °C in dry conditions. Fig. 1 compares the chronoamperometric responses obtained for 1 nM (solid lines) and 0 nM (dotted lines) of target DNA at the common binary SHCP+MCH interface (Right) with those observed at the new HDT-based ternary layer (Left) at the preparation day and after 30 and 90 days of dry storage time, respectively. The binary layer displays a complete loss of its DNA hybridization and background signals within one month of storage. In contrast, the ternary interface retains over 70% of its initial response after the entire 90-day period. Notice also that the greatly improved signal-to-noise characteristics of the ternary layer are retained during this prolonged 3-month storage study.

Fig. 2 compares the stability of the binary and ternary SAM-DNA interfaces over these 30-and 90-day storage periods, respectively. The binary interface displays a rapid loss of its DNA hybridization signals, with 62%, 89% and 96% current diminutions after 10, 20 and 30 days, respectively. In contrast, no change in the sensitivity of the ternary monolayer is observed during the initial (30-day) storage period, with only 20% and 29% decreases

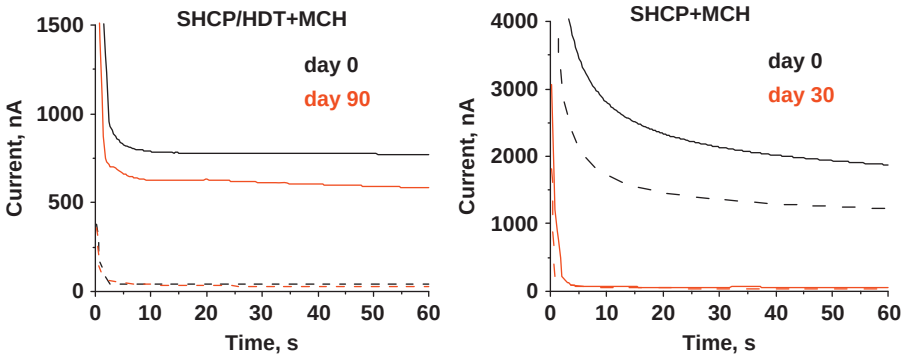

Fig. 1. Stability of binary and ternary DNA interfaces. Chronoamperometric responses obtained for 1 nM (solid lines) and 0 nM of target DNA (dashed lines) after the indicated storage times with SHCP/HDT+MCH or SHCP+MCH modified Au electrode arrays. Potential stepped to −200 mV. SAMs composition: [SHCP]=1 μM, [MCH]=1 mM and [HDT]=300 μM when used.

after 60 and 90 days, respectively and no apparent loss of the overall S/N characteristics over the entire 3 months period.

This dramatic improvement of the long-term stability of this layer can be attributed to minimal displacement of SHCP by MCH in the presence of HDT. Apart from its attractive prolonged stability (Figs. 1 and 2), greatly improved S/N characteristics were offered by the SHCP/HDT+MCH ternary monolayer, in comparison with the SHCP+MCH, reflecting the effective minimization of non-specific DNA adsorption effects and hence of background current contributions [28–30]. Ricci et al. also observed a substantial (50%) loss of the initial signal after a 3-weeks storage working of an E-DNA sensor based on SHCP+MCH SAMs [15].

The storage stability of another recently reported SHCP+MUA+MCH ternary monolayer [31] was also examined and compared with that of the present SHCP/HDT+MCH monolayer. Yet, the SHCP+MUA+MCH displayed a 62% loss of its initial S/N ratio within 45 days (Fig. S 1 in the supporting information), i.e., a substantially lower storage stability compared to the HDT-based ternary layer.

Alkanethiol SAMs has a much higher stability than thiolated-DNAs on gold [25]. Unlike the ability of ds-DNAs to form well-ordered SAMs [32], thiol-modified ss-DNAs do not form a compact monolayer due to a lack of lateral hydrophobic forces and thus can be displaced more easily. Therefore, we also compared the prolonged storage stability of multicomponent SAMs prepared by covalent immobilization of NH_2-CPs on MUA-based alkanethiol SAMs. These results demonstrated that NH_2-CP+HDT/MUA/MCH and NH_2-CP+MUA/MCH monolayers Au electrode arrays exhibited 80% and 39% losses of their initial S/N after 21 days of dry storage, respectively (see Fig. 3). It should be noted that despite its inferior stability the NH_2-CP+HDT/MUA/MCH monolayer provides higher S/N characteristics than the NH_2-CP+MUA/MCH interface, demonstrating (again) the attractive features offered by using HDT as a 3rd component of the underlying interface.

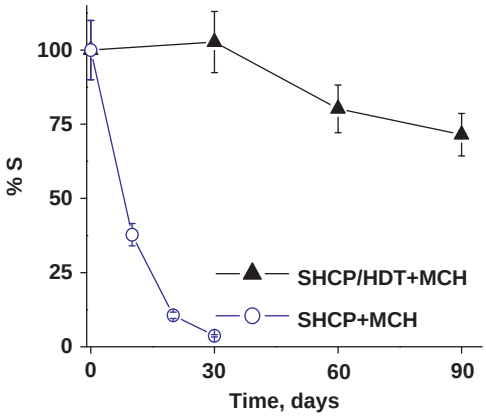

Fig. 2. Stability of binary and ternary DNA interfaces. Dependence of the % of the remaining S for 1 nM target DNA with Au electrode arrays modified with the SHCP+MCH and SHCP/HDT+MCH monolayers in HB over prolonged storage time. SAMs composition, as in Fig. 1. Error bars were estimated from five parallel experiments.

3.2. Comparison of the behavior of the ternary monolayer putting the MCH from the beginning or the day of measurement

Storage stability studies have been carried out by preparing the SHCP/HDT overnight interfaces and casting the MCH the next day (day 0 of the study) or storing the SHCP/HDT-modified Au electrode arrays and making the post-treatment with MCH in the control day (just before the measurements). The obtained results (see Fig. 4A,B) demonstrate a gradual decrease in the S/N with time only in the case of ternary monolayers containing the MCH component from the beginning. The release of the SHCP from the surface leads to a drastic decrease in the hybridization efficiency, and hence in the resulting signal and the S/N characteristics. These results seem to indicate that the most likely origin of the monolayer loss is due to the displacement of SHCP after prolonged time by MCH and not to the oxidation of the thiolated headgroup and its subsequent desorption from the gold surface reported by other authors [33].

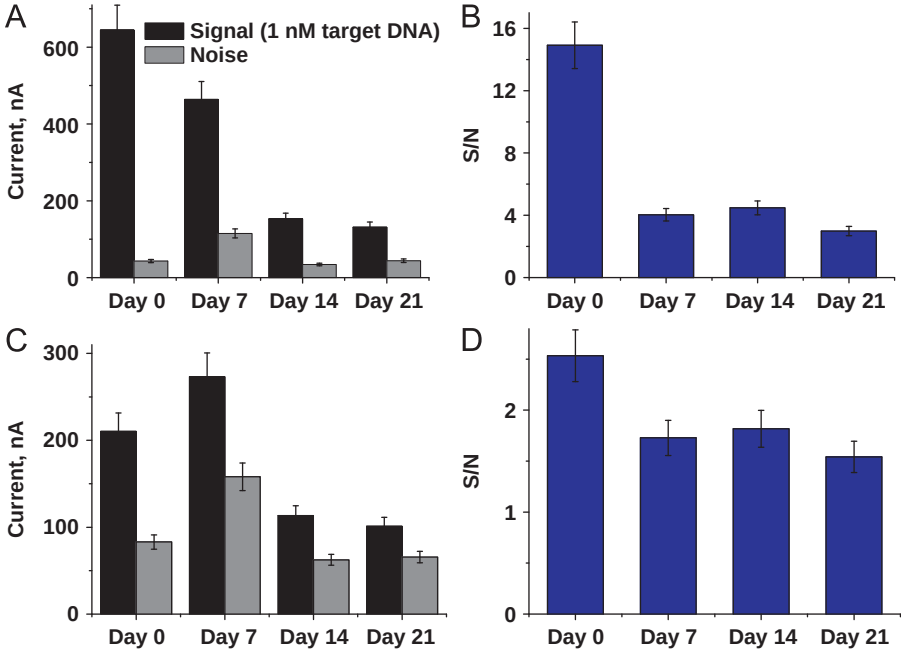

Fig. 3. Stability of Au electrode arrays modified with NH_2-CP+HDT/MUA/MCH (A,B) and NH_2-CP+MUA/MCH (C,D) monolayers: (A,C) Signal (S) and noise (N) values obtained for 1 nM (black columns) and 0 nM (gray columns) target DNA in HB, and (B,D) the resulting S/N ratios obtained. SAMs composition: [NH_2-CP]=2 μM, [HDT]=300 μM, [MUA]=2.5 mM, [MCH]=7.5 mM. Error bars were estimated from five parallel experiments.

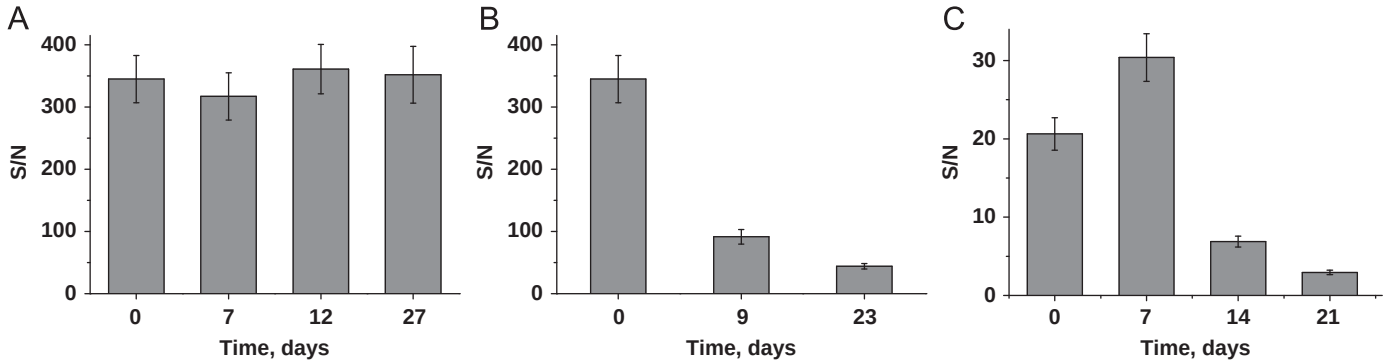

Fig. 4. Factors affecting the stability: A), B) Long-term stability of Au electrode arrays modified with the SHCP/HDT+MCH and storage at 4 °C over indicated intervals. S/N ratios obtained 1 nM target DNA by casting MCH A) the day of testing B) from the beginning (day 0 of the stability study). C) S/N ratio obtained for 1 nM target DNA with Au electrode arrays modified with the ternary SHCP/HDT+MCH monolayers in HB over prolonged storage time. SAMs composition: [SHCP]=0.05 μM, [HDT]=300 μM, [MCH]=1 mM (A,B) and 0.1 mM (C). Error bars were estimated from five parallel experiments.

These results, which evidence the MCH-induced desorption of SHCP, are in agreement with the one obtained by Arinaga et al. [34]. Their results demonstrate the development of vacancies/holes in the SHCP+MCH layer during long storage attributable to desorption of SHCP by MCH. Indeed, DNA surface coverage measurements reveal desorption of a 10% of the layer overnight.

These insights into the mechanism of desorption is essential both to evaluate the viability of this surface modification method and to point to the best surface chemistries that could resist this mechanism and may exhibit higher long-term stability.

3.3. Influence of the composition and density of the monolayer on the prolonged storage stability of the SHCP/HDT+MCH interface

It is reasonable to expect that DNA monolayers that display lower surface densities (by 1-2 orders of magnitude) could be more susceptible to thiol–thiol exchange [25]. Initially, the vacant surface sites around each probe leave available places once the nucleotide–gold contacts are removed; incoming MCH molecules can attack and displace a thiol–gold bond between a probe and the substrate [16].

In order to evaluate if the composition of the monolayer (and hence its coverage) plays an important role in its dry storage stability, comparison studies have been carried out with SHCP/HDT+MCH SAMs prepared using 0.05 or 1 μM of SHCP and fixed HDT and MCH concentrations (300 μM and 1 mM, respectively). As illustrated in Fig. S 2 (in the supporting information) the sensor prepared with 0.05 μM SHCP maintained only the 14% of the original S/N after 23 days of storage in dry conditions while the SHCP/DTT (a cyclic dithiol)+MCH monolayer kept only the 6% of the original S/N under the same working conditions (Fig. S 3 in the supporting information). In contrast, SHCP/HDT+MCH-modified Au electrode arrays (using 1 μM of SHCP) showed no decrease in S/N even after 3 months (Fig. 5). These results suggest that increasing the level of SHCP of the SHCP/HDT+MCH to the micromolar concentration, commonly used with the binary (SHCP+MCH) SAM leads to an enhanced storage stability of the resulting ternary SAM. The inferior prolonged stability of the SAMs prepared with the lower SHCP concentration (both using linear or cyclic dithiols) can be attributed to their lower surface density, which leaves a higher number of vacant places giving more opportunities to MCH for displacement [16].

Despite this better stability a 17 times lower S/N is observed when the DNA recognition interface is prepared using 1 μM of SHCP. This can be due to the drastic effect reported about the coverage of the ss-DNA probe on the hybridization efficiency of the biosensor. For an optimum performance there should be a balance between the increase in sensitivity achieved with the number of DNA probes immobilized on the surface and the space needed between them to limit repulsion of the incoming targets and steric effects between probe strands [10,35].

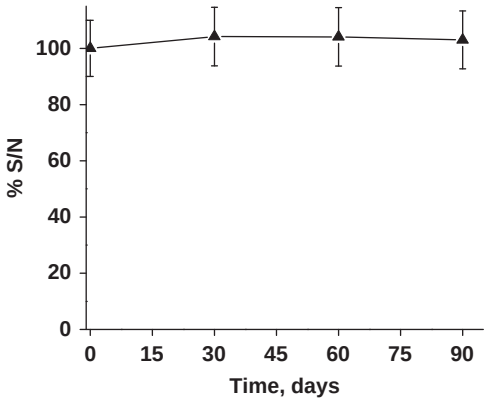

Fig. 5. % S/N obtained for 1 nM target DNA with Au electrode arrays modified with the ternary SHCP/HDT+MCH monolayer in HB over prolonged storage time. SAMs composition: [SHCP]=0.05 μM, [HDT]=300 μM, [MCH]=1 mM. Error bars were estimated from five parallel experiments.

We evaluated also the effect of MCH concentration on the storage stability of SHCP/HDT+MCH layers. Our results demonstrate that while lowering the concentration of MCH (until 0.1 mM level) had a significant effect on the final performance of the ternary layer (by giving a S/N ~10 times lower) did not improve its long-term stability (Fig. 4C). In this sense, it should be mentioned that Park et al. claimed that 0.1 mM is the threshold value for DNA to be removed by MCH [13].

3.4. The role of HDT on the storage stability of monolayers

To examine the role of the linear dithiol compound, HDT, we also assessed the performance and prolonged stability of the SHCP/HDT monolayer. Without MCH the S/N was relatively low, supporting the MCH role in these monolayers to provide a standing-up configuration of the SHCP, with improved orientation and hybridization efficiency [29]. A decrease of 39% in S/N was obtained with this binary monolayer after 30 days of storage in dry conditions (Fig. 6).

Taking into account the dramatic effect of the SHCP concentration on the stability of these ternary interfaces, future efforts will aim at guiding and tailoring the surface density of the monothiolated DNA layer. Particularly in comparing the stability

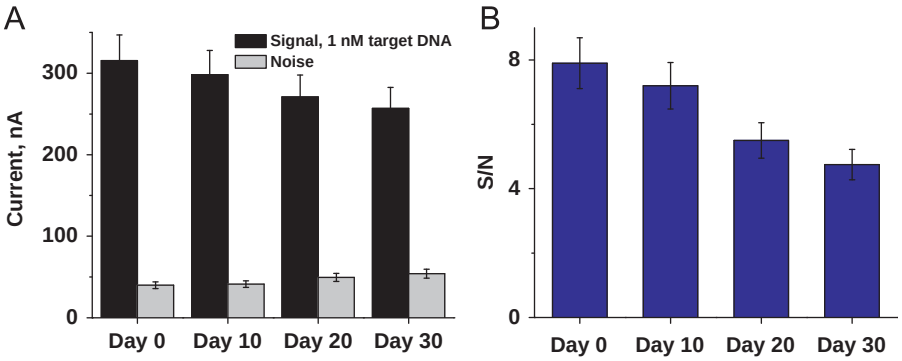

Fig. 6. Stability of Au electrode arrays modified with the binary SHCP/HDT monolayer: (A) signal (S) and noise (N) values obtained for 1 nM (black columns) and 0 nM (gray columns) target DNA in HB, and (B) the resulting S/N ratios obtained. SAMs composition: [SHCP]=1 μM, [HDT]=300 μM. Error bars were estimated from five parallel experiments.

of these SAMs (based on a competitive alkanethiol replacement procedure) with those prepared by the selective desorption of mixed alkanethiols SAMs and subsequent assembling of the SHCP. In this later protocol, a careful control of the gold domains sizes created allows tailoring the SHCP surface density and hence the performance and stability of the resulting interface [6].

4. Conclusions

We have shown that a judicious design of thiolated self-assembled ternary monolayer can lead to a dramatic improvement of the storage stability of electrochemical DNA hybridization biosensors. The results underscore the importance of a detailed optimization of the composition of the ternary SAM for enhancing substantially the stability and storage lifetime of the resulting biosensors. The greatly extended stability demonstrated by the Au electrode arrays modified with the SHCP/HDT+MCH enables advanced preparation and storage, as desired for practical real-life applications of these sensing devices.

In particular, ternary SAM interfaces, based on the use of a linear dithiol, a monothiolated capture probe and the common MCH spacer, impart a dramatic storage stability to DNA hybridization biosensors compared to the commonly used binary SAM. The enhanced stability may lead also to improved stability of DNA biosensors based on other transduction modes (e.g., QCM, SPR) that rely on SAMs on gold surfaces, thus expanding the scope of these highly stable interfaces. Such improvements would thus facilitate and simplify diverse practical applications of DNA hybridization biosensors.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.033.

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