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Electrochemical Release of Amine Molecules from Carbamate-Based, Electroactive Self-Assembled Monolayers

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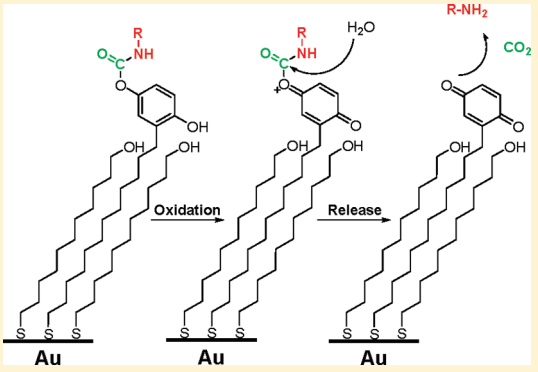
Supporting Information

ABSTRACT: In this paper, carbamate-based self-assembled monolayers (SAMs) of alkanethiolates on gold were suggested as a versatile platform for release of amine-bearing molecules in response to the electrical signal. The designed SAMs underwent the electrochemical oxidation on the gold surface with simultaneous release of the amine molecules. The synthesis of the thiol compounds was achieved by coupling isocyanate-containing compounds with hydroquinone. The electroactive thiol was mixed with 11-mercaptoundecanol [$HS(CH_2)_{11}OH$] to form a mixed monolayer, and cyclic votammetry was used for the characterization of the release behaviors. The mixed SAMs showed a first oxidation peak at +540 mV (versus Ag/AgCl reference electrode), indicating the irreversible conversion from carbamate to hydroquinone groups with simultaneous release of the amine molecules. The analysis of ToF-SIMS further indicated that the electrochemical reaction on the gold surface successfully released amine molecules.



INTRODUCTION

Controlled release of (bio)functional molecules from solid surfaces is an indispensable and important step in many technologically important areas, including stimuli-responsive surfaces, biochips and sensors, and microfluidics, as well as fundamental studies in surfaces and interfaces.[1−3] For example, the *in vitro* developmental behaviors of neurons, including neurite outgrowth and synaptogenesis, on an artificial substrate, could be investigated and controlled, along with nanotopographical effects, by spatio-selective release of neurotransmitters and other neuron-active ligands at a predetermined time.[4−6] The electrochemical strategies for surface reactions-on-demand[7] are favored over other methods, because they are seamlessly coupled with the existing electronic devices and are controllable spatiotemporally in real time with ease.[8,9] The self-assembled monolayers (SAMs) of thiolates on gold prove to be a simple but versatile platform for the electrochemical strategies, because they are not only chemically robust, but flexible in a sense that their physicochemical properties can be readily tailored by terminal moieties and postreactions on the SAMs.[10−14] Since Mrksich's pioneering work of the electroactive hydroquinone (HQ)-terminated SAMs on gold,[15−18] several methods, mainly HQ-based ones, have been developed for the electrochemical release of molecules from surfaces.[19−24] For example, the quinone propionic ester moiety underwent lactonization by its reduction to HQ, concomitant with release of biotin, RGD peptide, or γ-aminobutyric acid.[19−22] In addition, the *O*-silyl-HQ ether group was sophisticatedly designed to release a silanol ligand by oxidation to quinone (Q) and subsequent hydrolysis of the silyl ether.[22,23] The HQ-terminated SAMs were also deliberately coupled with oxime chemistry to release hydroxyl groups by combination of redox reactions and pH changes.[25] In addition, the oxime chemistry has been utilized to investigate cell adhesion on surfaces with HQ-terminated SAMs in real time.[26,27] However, the previous reports have mainly been limited to the release of alcohol molecules, and the demonstrated applications to the detachment of cells through the release of the RGD peptide, although the intensive and cumbersome synthetic procedures were employed to prepare the required HQ thiol compounds. Moreover, there have been few reports on the controlled release of functional molecules that are biologically operational after release. We envisioned that biological events could be monitored and/or regulated on artificial, dynamic surfaces with electrochemical release of biologically active small molecules, such as dopamine, γ-aminobutyric acid, and amino acids, which contain the amine functional group. In this work, we developed carbamate-based chemistry for releasing target amine molecules in the electrochemical way. The electrochemical reactions on the SAMs were

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monitored by cyclic voltammetry, and confirmed by time-of-flight secondary ion mass spectroscopy (ToF-SIMS).

EXPERIMENTAL SECTION

Formation of Self-Assembled Monolayers (SAMs). Gold substrates were prepared by thermal evaporation of 5 nm of titanium and 100 nm of gold onto silicon wafers. The gold substrates were cleaned for 1 min in piranha solution (3:7 by volume of H_2O_2 and H_2SO_4) before formation of SAMs. Mixed monolayers were formed by immersing the gold-coated substrates in the ethanolic solution of the synthesized hydroquinone derivative (HQ-SH, HQ-PhBr, HQ-EtCl, HQ-Et, or HQ-Dopa; 0.4 mM) and 11-mercaptoundecanol (0.6 mM) for 18 h. The substrates were removed from the solution, washed with ethanol, and dried with a stream of Ar gas.

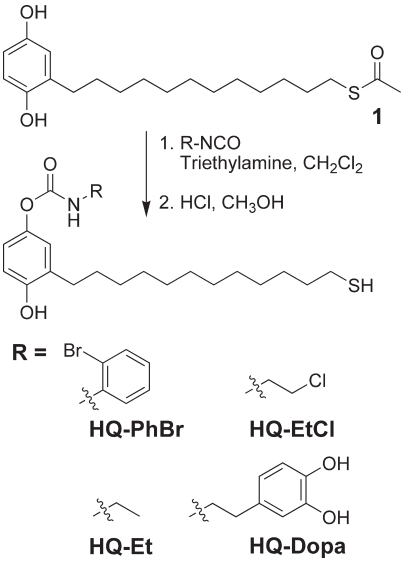

Figure 1. Synthesis of the carbamate-containing thiol compounds.

Characterizations. Cyclic voltammetry was performed by using PGSTAT302N potentiostats (Autolab, The Netherlands). Electrochemical measurments on the SAMs were performed in a PBS buffer, with the platinum wire as a counter electrode, Ag/AgCl as a reference, and the monolayer as a working electrode. ToF-SIMS data were obtained with a ToF-SIMS V instrument (ION-TOF GmbH, Germany) equipped with a bismuth liquid metal ion gun (LMIG). SIMS spectra of negative ions were obtained by using Bi^+ primary ions operated at 25 keV with a pulse width of 16.8 ns (0.58 ns after bunching) and a repetition rate of 5 kHz in the high-current bunched mode. The analysis area ($300 \times 300\ \mu m^2$) was randomly rastered by the primary ions, and the primary ion dose was maintained below 10^{12} ions/cm^2 to ensure static SIMS conditions. The mass resolution ($M/\Delta M$) at m/z 500 was usually more than 7000, and the mass calibration of negative ion spectra was internally performed by using CH^- and CH_3^-. To enhance the mass accuracy at high mass values, recalibration was performed by using $C_{11}H_{23}OSAu_2^-$ and $C_{18}H_{29}O_2SAu_2^-$ (mass accuracy ~19.225 ppm at m/z 597.08).

RESULTS AND DISCUSSION

The carbamate linkage of our system was formed by coupling the hydroxyl group of HQ-derivative (**1**) with isocyanate, which would be released in the form of amine after electrochemical oxidation (Figure 1). After formation of the carbamate linkage, the thioacetyl group was converted to the thiol group. Synthesis of HQ-Dopa required additional steps for the protection and

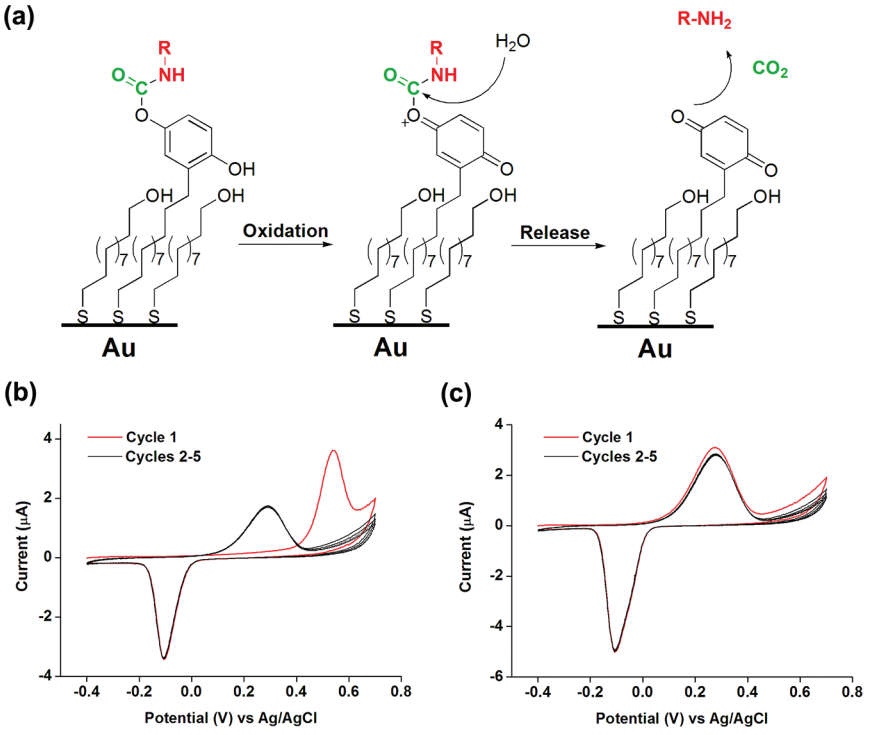

Figure 2. (a) Carbamate-based, electrochemical method for releasing amine from the gold surface. Hydroquinone (HQ) is electrochemically oxidized to the benzoquinonium cation, which is decomposed by the attack of water, releasing the amine ligand and CO_2. Cyclic voltammograms of (b) mixed HQ-PhBr and (c) mixed HQ-SH SAMs. The CV was recorded in PBS (pH 7.4) at a scan rate of 25 mV/s. The CV in red is for the first scan, and the ones in black for the next scans.

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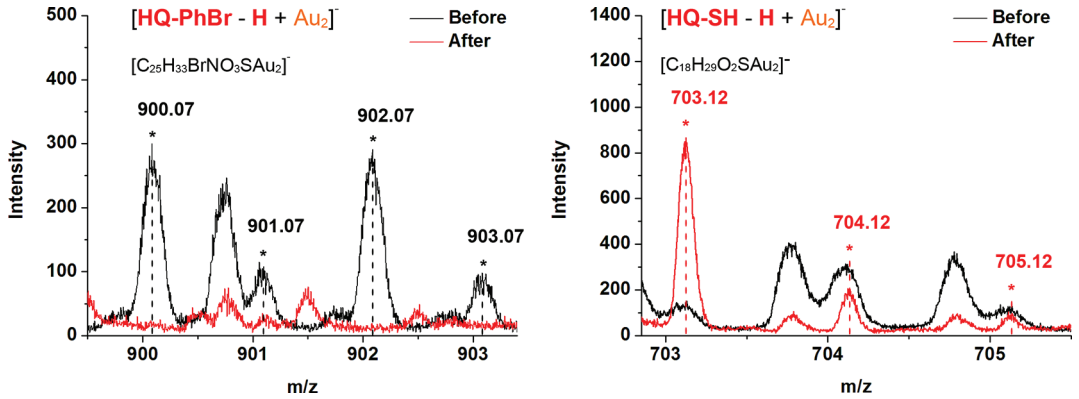

Figure 3. ToF-SIMS spectra of mixed HQ-PhBr SAMs before (black line) and after (red line) electrochemical reactions (10 scans; the isotope peaks are marked with *).

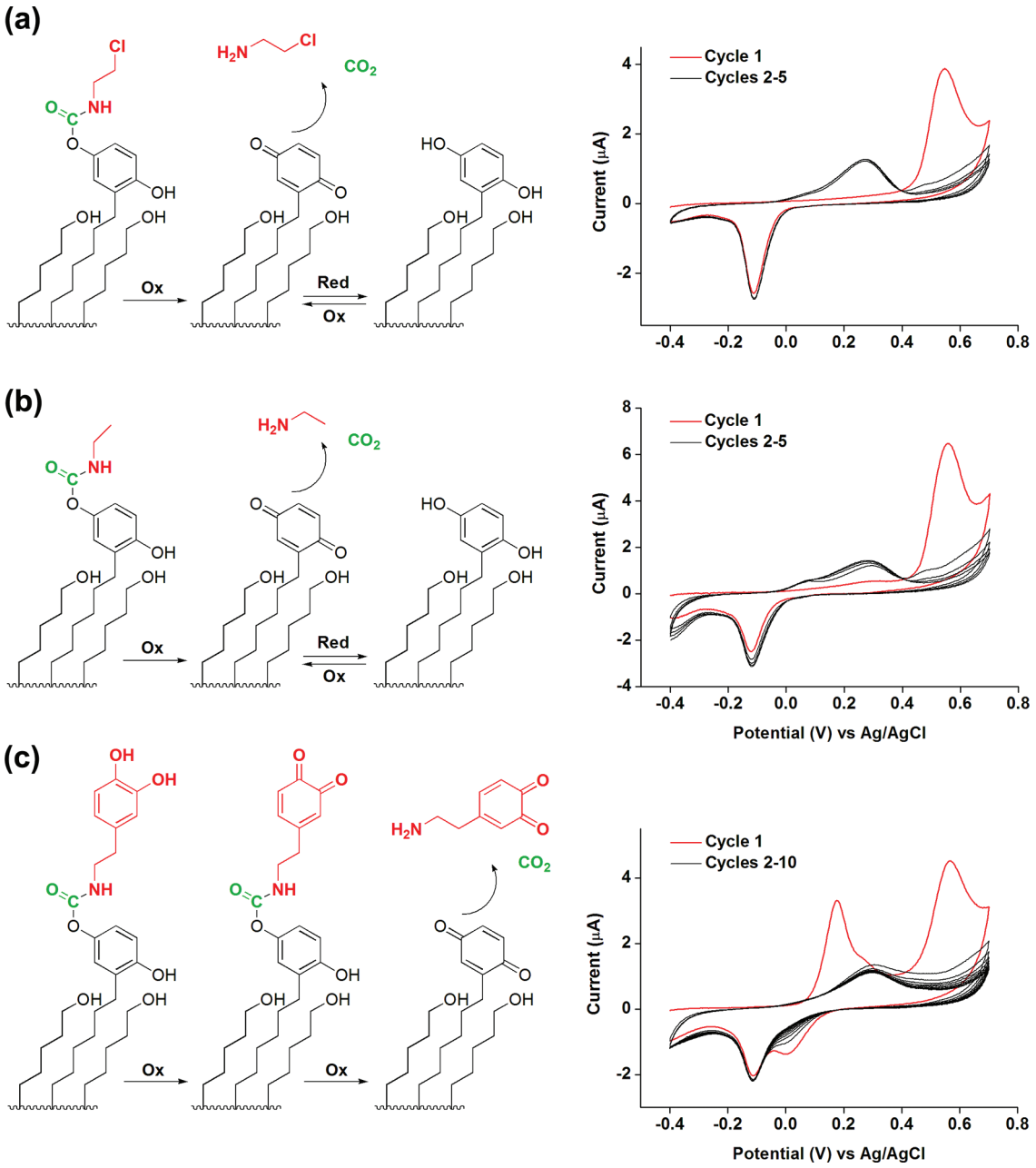

Figure 4. Oxidative release of amine molecules: (a) HQ-EtCl, (b) HQ-Et, and (c) HQ-Dopa.

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deprotection of the reactive catechol groups (see the Supporting Information for experimental details). A carbamate linkage with different bond connectivity has previously been used by Kwak for protecting biotin with HQ on gold, where the oxidation to Q led to the release of Q and CO_2 and the controlled exposure of biologically active biotin.[28] In contrast, our design ensured that the carbamate group would be attacked by water, upon the electrochemical oxidation of HQ to Q, and the amine ligand would be released from the gold surface (Figure 2a). Prior to the SAM formation, we investigated the electrochemical behaviors of the compounds in solution (acetonitrile:water = 9:1 containing 100 mM tetrabutylammonium hexafluorophosphate) by cyclic voltammetry (see the Supporting Information for the cyclic voltammograms).[24] The cyclic voltammograms (CVs) of all the compounds showed the oxidation peak of the HQ-carbamate groups in the range of 0.99−1.09 V in addition to that of the thiol group about 1.26−1.36 V. We also observed the oxidation peak of the 1,2-dihydroxybenzyl group at 0.75 V before the oxidation of the HQ-carbamate and thiol groups. The results indicated that the oxidation potential of the HQ-carbamate groups was not affected by the ligands attached to HQ.

We first formed the mixed SAMs of HQ-PhBr and 11-mercaptoundecanol (2:3) on gold. The electrochemically inert hydroxyl background of 11-mercaptoundecanol was used to isolate the electroactive components of the SAMs from one another, and its hydrophilicity was also well-suited for close contact with aqueous media. We characterized the SAMs by IR spectroscopy and cyclic voltammetry (see the Supporting Information for the IR spectrum). In the IR spectrum, we observed the characteristic peak of HQ-PhBr at 1436 cm^{-1} (C−N stretching), in addition to the peaks at 748 (C−H bending) and 1092 cm^{-1} (C−O−C stretching), indicating the successful formation of the mixed SAMs. The release behavior of 2-bromoaniline from the mixed SAMs of HQ-PhBr was monitored by CV (PBS buffer; scan rate: 25 mV/s) (Figure 2b). On the forward scan of the first cycle, the oxidation of HQ-PhBr occurred at 540 mV, with release of 2-bromoaniline. Integration under the first oxidation peak area revealed that the surface coverage of HQ-PhBr was 5.40×10^{13} molecules/cm^2 (see the Supporting Information for the surface coverage of the other carbamate-based compounds in the mixed SAMs). On the return scan, the Q-terminated SAMs were reduced back to HQ-terminated ones at −110 mV. In the following scans, we did not have the previously observed oxidation peak (at 540 mV), but did see a new oxidation peak at 290 mV with the reduction peak at −110 mV. The peak positions were not changed during the next 4 cycles. We compared these stationary redox peaks with those for the mixed SAMs of 2-(12-mercaptododecyl)hydroquinone (HQ-SH) and 11-mercaptoundecanol (the control; Figure 2c). The redox peak positions of the mixed HQ-SH SAMs were found to be identical to those for the HQ-PhBr SAMs after the first cycle. In addition, the calculated surface coverage of HQ-SH after 5 scans (5.36×10^{13} molecules/cm^2) was nearly the same as that of HQ-PhBr (5.40×10^{13} molecules/cm^2), implying that the release of 2-bromoaniline was nearly quantitative, and the desorption of the thiol compounds was negligible for HQ-PhBr. The results clearly indicated that the electrochemical release of 2-bromoaniline was achieved during the first oxidative potential, followed by the typical redox activity of the HQ moiety. We further characterized the SAMs on gold before and after the electrochemical reactions by ToF-SIMS (Figure 3). Before the reaction, we observed a mass peak at 900.07, which corresponded to the gold-adduct

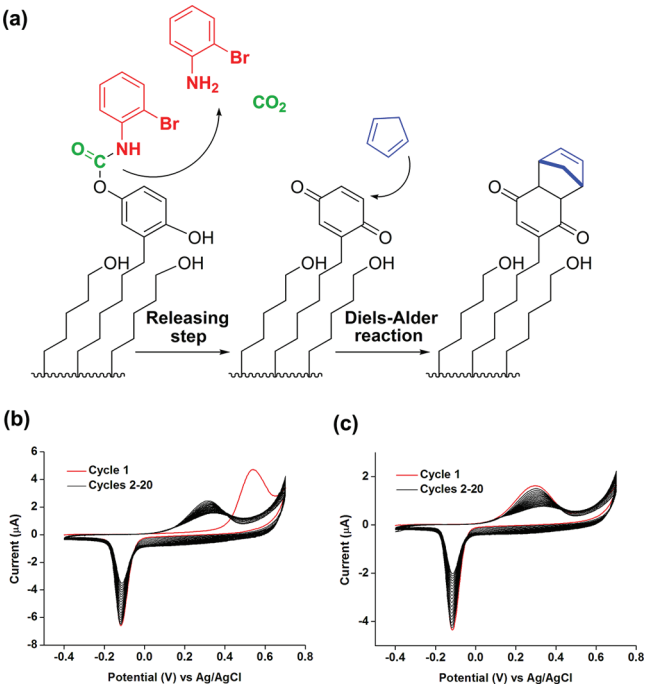

Figure 5. (a) Oxidative release of 2-bromoaniline and subsequent Diels−Alder reaction with cyclopentadiene. Consecutive cyclic voltammograms of (b) mixed HQ-PhBr SAMs and (c) mixed HQ-SH SAMs (THF:PBS buffer = 1:1 containing 5 mM cyclopentadiene; scan rate: 25 mV/s).

HQ-PhBr ($[HQ\text{-}PhBr - H + Au_2]^- = [C_{25}H_{33}BrNO_3Au_2]^-$) with 3 additional types of carbon- and bromine-related isotope patterns (m/z 901.07, 902.07, and 903.07). After electrochemical reaction, these isotope patterns disappeared, and a new mass peak appeared at 703.12, which corresponded to the gold-adduct HQ-SH ($[HQ\text{-}SH - H + Au_2]^- = [C_{18}H_{29}O_2SAu_2]^-$). Compared with the gold-adduct HQ-SH, the gold-adduct HQ-PhBr showed more apparent and intense isotope patterns, probably due to the existence of bromine, which has two stable isotopes (^{79}Br, ^{81}Br). In the case of the gold-adduct HQ-SH, the isotope peaks (m/z 704.12 and 705.12) were relatively weak and found to overlap with other peaks. The ToF-SIMS data clearly confirmed that the carbamate-based, electroactive SAMs released the target amine molecules via electrochemical reactions.

For the generalization of our system, two aliphatic amines, 2-chloroethylamine and ethylamine, were tested for the electrochemical release after formation of the mixed SAMs with 11-mercaptoundecanol. Both SAMs were observed to have the oxidation peak in the range of 540−550 mV during the first scan, indicative of the general applicability of the carbamate-based release system (Figure 4a and b). Similar to HQ-PhBr, the typical redox peaks of HQ were observed after the release of the amine molecules for both SAMs. HQ-Dopa, which contains dopamine, a well-known neurotransmitter, was also employed as a representative model for the electrochemical release of neurotransmitters (Figure 4c). Consistent with the solution-based electrochemical behavior, the oxidation of the catechol (1,2-dihydroxybenzene) group in dopamine was observed first at 180 mV, followed by the oxidation of HQ at 560 mV and the subsequent release of dopamine, probably implying that dopamine would be released as an oxidized form. Taken together, the results showed that various amine molecules, especially

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biologically functional amine molecules, could be released in a controlled manner by using the carbamate-based strategy.

The generation of the electroactive HQ−Q pair after electrochemical release is one of the advantages of the carbamate-based system over other methods, because postfunctionalizations, such as attachment of ligands, are possible via well-established reactions of HQ and Q.[23,25] As proof-by-demonstration, we performed the Diels−Alder reaction of cyclopentadiene on the mixed HQ-PhBr SAMs (Figure 5a). As expected, 2-bromoaniline was detached from the surface in the presence of cyclopentadiene (5 mM in THF:PBS (1:1 v/v)) during the first scan, confirmed by CV (Figure 5b). The current of the HQ redox peaks, however, decreased gradually in the following scans because of the Diels−Alder reaction between Q at the surface and cyclopentadiene in solution;[17,18] in contrast, the stationary redox-peak current was observed in the absence of cyclopentadiene (Figure 2b). As another control experiment, the Diels−Alder reaction on the mixed HQ-SH SAMs was performed (Figure 5c). In this control, we observed the same CV except for the oxidation peak corresponding to the release of 2-bromoaniline. The results indicated that the electroactive surfaces functioned properly after release of amine molecules.

CONCLUSIONS

In summary, we developed a carbamate-based surface chemistry for electrochemical release of amine molecules from the gold surface. Considering that many biologically active small molecules contain the amine functional group, we believe that our method could be utilized for investigating cellular metabolism via spatiotemporally controlled, intimate release of the molecules of interest,[29] including neurotransmitters. In addition, the reusability of the surfaces for postattachment also could be beneficial in the design of biologically functional surfaces that interact with living cells.

ASSOCIATED CONTENT

Supporting Information. Experimental details and characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‡These authors contributed equally to this work.

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