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On-Demand Electrochemical Activation of the Click Reaction on Self-Assembled Monolayers on Gold Presenting Masked Acetylene Groups

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Supporting Information

ABSTRACT: We report on a new surface modification method for grafting a "dynamic" property for on-demand activation of the click reaction. Our approach utilizes the acetylene group masked with dicobalt hexacarbonyl, $Co_2(CO)_6$, which is not reactive toward the click reaction. Electrochemical treatment reveals the acetylene group on the selected region, which is then used as a chemical handle for surface functionalization via the click reaction with an azide-containing molecule. Electrochemical and chemical conversions on the surface were verified by cyclic voltammetry, X-ray photoelectron spectroscopy, and fluorescence spectroscopy. We have demonstrated immobilization of an azide-modified RGD peptide and promotion of cell adhesion/migration to the region of electrochemical induction.

Since the discovery of the Cu(I)-catalyzed Huisgen cycloaddition ("click") reaction between azides and alkynes in 2002, it has been widely used because of its simplicity, mild reaction conditions, high chemoselectivity, bioorthogonality, and tolerance toward many organic functional groups.[1−3] As such, the click reaction has been actively harnessed for immobilization of biomolecules, including oligonucleotides,[4] peptides,[5−7] and proteins,[8−10] to the surface of various materials, such as self-assembled monolayers (SAMs) on gold. SAMs on gold provide convenient, flexible, and biocompatible systems; therefore, they have been applied to various biological studies, such as cell adhesion, biochips, sensors, and arrays of biomolecules.[11−16] For most applications, SAM-based biointerfaces rely on the "static" property of monolayers, which carry out their predetermined roles once they are formed. Recently, "dynamic" SAMs have been developed, where surface properties can be modulated by an external stimulus, allowing the activities of the monolayers to be "turned on" from a dormant state to an active state for initiation of ligand immobilization, cell adhesion, and actuation for biochemical sensors.[17,18]

Here we report on a new strategy for on-demand electrochemical activation of the click reaction on SAMs on gold. With this strategy, the combination of the click reaction and an electrochemical reaction provides a new method of selective surface modification, enabling the "dynamic" property of SAMs. The alkyne groups presented on the surface are masked with dicobalt hexacarbonyl [$Co_2(CO)_6$] groups,[19,20] making them inert toward the click reaction. Upon electrochemical treatment, $Co_2(CO)_6$ is oxidatively degraded to reveal the alkyne groups, which are further used as chemical handles for incorporation of azide-containing molecules onto the surface via the click reaction (Figure 1).

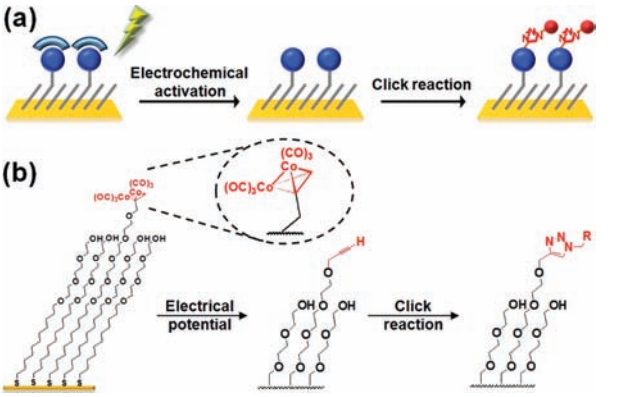

Figure 1. Schematic representations of (a) on-demand activation of click chemistry and (b) the chemical structure of the monolayer.

We have demonstrated our approach using SAMs prepared with a mixture of acetylenic $Co_2(CO)_6$-terminated disulfide and tri(ethylene glycol)-terminated disulfide. Acetylenic $Co_2(CO)_6$-terminated disulfide was synthesized in six steps from commercially available reagents [for the synthetic scheme and detailed experimental procedures, see the Supporting Information (SI)]. The tri(ethylene glycol) group ensures that the modified surfaces are inert to nonspecific protein adsorption, thereby reducing false-positive results.[21]

First, using cyclic voltammetry (CV), we characterized the electrochemical conversion of an acetylenic $Co_2(CO)_6$ complex and investigated the yield of click chemistry to tether azide-containing molecules to the electrochemically revealed acetylene groups. An acetylenic $Co_2(CO)_6$ complex-presenting monolayer was prepared with a density of 10% among the tri

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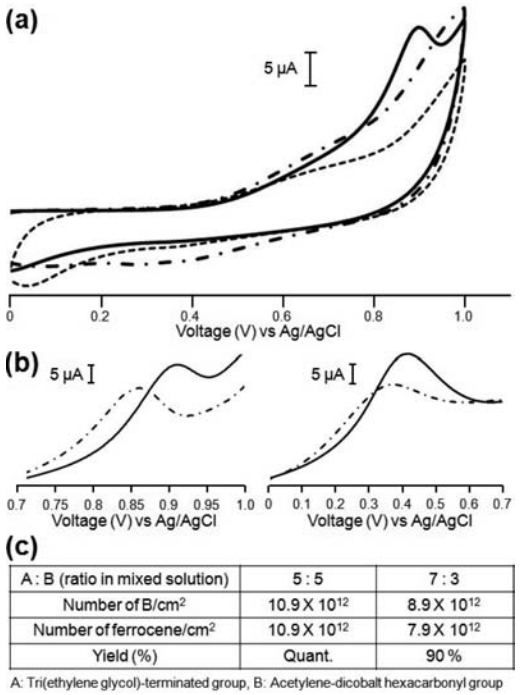

(c)

A : B (ratio in mixed solution)	5 : 5	7 : 3
Number of B/cm^2	10.9 X 10^{12}	8.9 X 10^{12}
Number of ferrocene/cm^2	10.9 X 10^{12}	7.9 X 10^{12}
Yield (%)	Quant.	90 %

A: Tri(ethylene glycol)-terminated group, B: Acetylene-dicobalt hexacarbonyl group

Figure 2. (a) Cyclic voltammetry of the acetylenic $Co_2(CO)_6$ complex-presenting monolayer: first scan (solid line); second scan (dashed line). The free acetylene-presenting monolayer gave no apparent anodic peak (dot-dashed line). (b) Cyclic voltammograms recorded for (left) acetylenic $Co_2(CO)_6$ complex-presenting monolayers (scan rate 0.1 V/s) and (right) the monolayers after azidoferrocene was incorporated by the click reaction (scan rate 0.3 V/s). The monolayers were prepared with tri(ethylene glycol)-terminated disulfide and acetylenic $Co_2(CO)_6$ complex-terminated disulfide in a solution ratio of 50:50 (solid line) and 70:30 (dot-dashed line). (c) Numbers of acetylene dicobalt hexacarbonyl groups and ferrocene groups on the monolayers as calculated by integration of the anodic peaks.

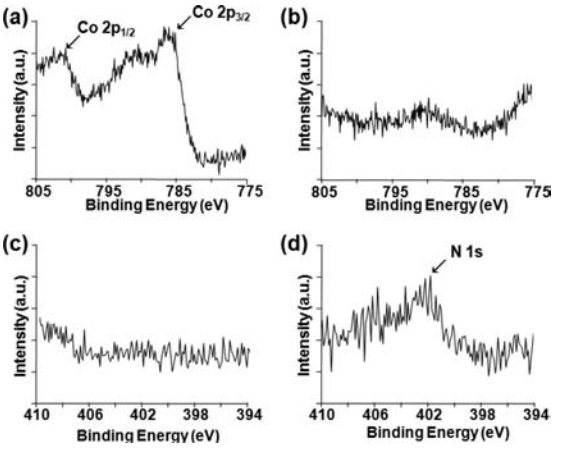

Figure 3. High-resolution XPS spectra of the acetylenic $Co_2(CO)_6$ complex-presenting monolayer. (a, b) Scans of the Co 2p region (a) before and (b) after electrochemical treatment, showing electrochemical oxidative degradation of the $Co_2(CO)_6$ complex to reveal an alkyne group. (c, d) Investigation of the N 1s region of the electrochemically treated monolayer (c) before the click reaction, which gave no apparent peak, and (d) after the click reaction, which gave an N 1s peak, demonstrating attachment of 2-azidoacetic acid.

(ethylene glycol) group background. Figure 2a shows the cyclic voltammograms recorded for the monolayer in a degassed phosphate-buffered saline (PBS) solution at pH 7.4 with a scan range between 0 and +1.0 V vs Ag/AgCl at a scan rate of 0.2 V/s. An anodic peak at 850 mV, which was clearly observed on the first scan (solid line), showed a dramatic decrease on the second scan (dashed line), indicating completion of electrochemical oxidative degradation of the $Co_2(CO)_6$ group within one scan. As a control, we conducted the same experiment using a free acetylene-presenting monolayer. CV gave only a nonfaradaic current (dot-dashed line) that was similar to the second scan of the acetylenic $Co_2(CO)_6$ complex-presenting monolayer. As an additional control, a monolayer presenting the acetylenic $Co_2(CO)_6$ complex was treated with an electric potential at 850 mV followed by analysis with CV, which showed only a nonfaradaic current (data not shown). These results verified that there were no electroactive molecules on these control monolayers and that the anodic peak at 850 mV arose from the electroactive acetylenic $Co_2(CO)_6$ complex. Next, we quantified the dicobalt hexacarbonyl groups on the monolayers and the azide-containing molecules that were incorporated onto the surface via the click reaction. Cyclic voltammograms were recorded for acetylenic $Co_2(CO)_6$ complex-presenting monolayers (Figure 2b, left) as well as for the monolayers after electric potential treatment and incorporation of azidoferrocene by the click reaction (azidoferrocene, 10 mM; copper(II) sulfate, 60 mol %; sodium ascorbate, 60 mol %; 37 °C for 1 h) (Figure 2b, right). The monolayers were prepared with tri(ethylene glycol)-terminated disulfide and acetylenic $Co_2(CO)_6$ complex-terminated disulfide in solution ratios of 50:50 (solid line) and 70:30 (dot-dashed line). Acetylene dicobalt hexacarbonyl groups and ferrocene groups on the monolayers were then quantified by integration of the anodic peaks. CV analysis showed that the number of ferrocene groups almost reached that of the acetylene dicobalt hexacarbonyl groups, indicating that the azide-containing molecule was incorporated onto the activated surface via the click reaction with a yield of >90% (Figure 2c). Taken together, the electrochemical characterizations clearly show that electrochemical/chemical modifications on monolayers (i.e., the deprotection and click reactions) proceeded as shown in Figure 1b in very high yield.

Next, X-ray photoelectron spectroscopy (XPS) was used to examine all of the electrochemical and chemical conversions on the monolayer. The acetylenic $Co_2(CO)_6$ complex-presenting monolayer gave a Co $2p_{3/2}$ peak at 786 eV and a Co $2p_{1/2}$ peak at 801 eV (Figure 3a). The monolayer was then treated with an electric potential; washed with deionized water, EtOH, and EDTA solution (50 mM, pH 8.0); and analyzed using XPS. The two peaks at 786 and 801 eV corresponding to Co were absent (Figure 3b), illustrating electrochemical oxidative degradation of the $Co_2(CO)_6$ complex to reveal an alkyne group, as depicted in Figure 1b. Investigation of the N 1s region (394–410 eV) for this monolayer gave no apparent peak, suggesting that there were no nitrogen-containing moieties on the surface (Figure 3c). An identical monolayer was subjected to the click reaction with 2-azidoacetic acid. XPS of this monolayer clearly showed a N 1s peak at 402 eV, implying the presence of a triazole ring moiety and therefore attachment of 2-azidoacetic acid via click chemistry (Figure 3d). The control experiment using a free acetylene-presenting monolayer gave the N 1s peak only after the click reaction (Figure S1a,b in the SI), while no N 1s peak was observed for the acetylenic $Co_2(CO)_6$ complex-presenting

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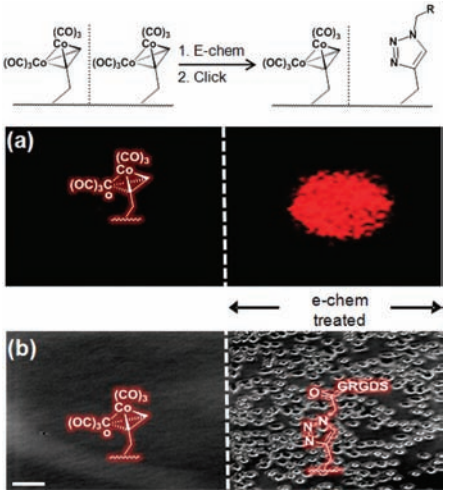

Figure 4. Selective activation of the click reaction and attachment of (a) azide-linked rhodamine and (b) N_3-GRGDS followed by CHO cell adhesion to the activated region of the monolayer. Scale bar = 100 μm.

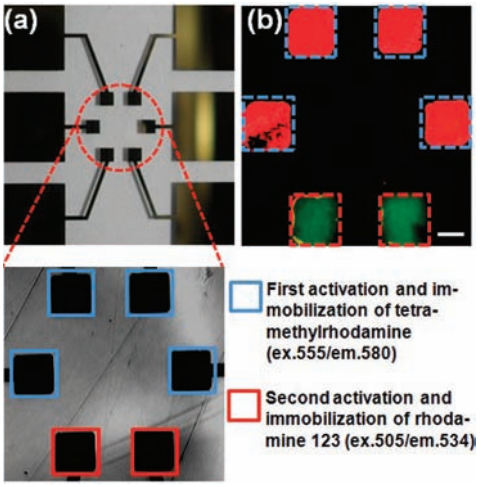

Figure 5. Selective and sequential activation of the click reaction and immobilization of fluorescent dyes to activated gold electrodes. (a) Optical image of a micropatterned substrate. (b) Fluorescence images of the electrodes after activation/immobilization of four electrodes and successive activation/immobilization of additional two electrodes. Scale bar = 25 μm.

monolayer after treatment with click reagents (Figure S1c,d). Taken together, the XPS analyses confirmed that the alkyne group was electrochemically regenerated and that subsequent click chemistry resulted in the introduction of an azide-containing molecule to the monolayer.

To demonstrate the validity of our strategy of electrochemical activation of the click reaction, we examined the selective modification of a surface. Two acetylenic $Co_2(CO)_6$ complex-presenting monolayers at densites of 50 and 5% were prepared, and half of the monolayers were treated with an electric potential. The monolayers were then subjected to the click reaction with tetramethylrhodamine azide and N_3-GRGDS peptide (for the synthesis of this peptide, see the SI), respectively. The Arg-Gly-Asp (RGD) peptide, which is found in many extracellular matrix proteins, is a ligand for cell-surface integrin receptors and

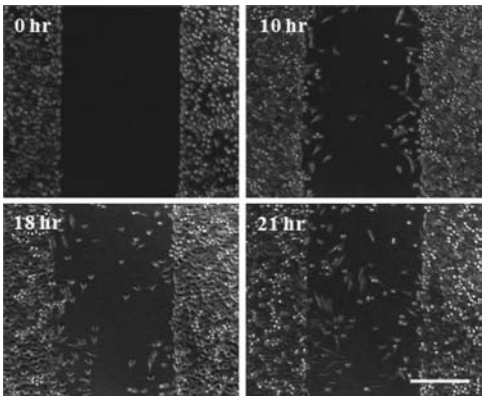

Figure 6. Application of a dynamic substrate to the study of cell migration. Stripe features on a monolayer that were surrounded by acetylenic $Co_2(CO)_6$ groups presented fibronectin. HeLa cells adhered to and spread on the stripe regions. Treatment of the monolayer with an electric potential followed by N_3-GRGDS, ascorbate, and copper(II) resulted in RGD immobilization and initiated cell migration from regions of fibronectin onto the remaining regions. Scale bar = 250 μm.

mediates the adhesion of cells.[22,23] As expected, electric activation followed by the click reaction afforded bright fluorescence and cell adhesion only from the activated region (Figure 4). Next, to examine the robustness of the masking group and the multiplexing capability of our approach, we tested the sequential activation and immobilization of a surface with two different molecules. As shown in Figure 5a, we prepared a gold-patterned substrate having six electrodes and presenting acetylene dicobalt hexacarbonyl groups at a density of 50%. Four of the six electrodes (blue squares) were activated by an electric potential, after which the interior gold region was exposed to the click reaction with tetramethylrhodamine (λ_{ex} = 555 nm; λ_{em} = 580 nm). Subsequently, two additional electrodes of the identical substrate (red squares) were activated, and rhodamine 123 (λ_{ex} = 505 nm; λ_{em} = 534 nm) was immobilized. The fluorescence image of the substrate showed two distinctive colors from the electrodes as intended (Figure 5b). This result indicates that our strategy allows selective and noninvasive activation of the click reaction and furthermore provides multiplexing capability. The lift-off method with transparent Scotch tape was used for the fluorescence observation, as described in the literature.[24]

We next demonstrated the biological relevance of this dynamic substrate by applying our strategy as a model system for the study of migration of mammalian cells.[25] The monolayer patterned with stripe features was prepared using a well-known microcontact printing (μCP) method. Briefly, a polydimethylsiloxane stamp containing stripe features was inked with a hexadecanethiol solution. The stamp was applied to the gold-coated slide, after which the hexadecanethiol on the stripe features was transferred to the substrate. The remaining region was then filled with electroactive acetylenic $Co_2(CO)_6$-terminated disulfide and tri(ethylene glycol)-terminated disulfide in a ratio of 5:95. The patterned monolayer was treated with fibronectin (25 μg/mL in PBS), an extracellular matrix protein that induces integrin-mediated cell adhesions, and then incubated with HeLa cells. After a brief washing with sterilized PBS, the monolayer was photographed. An optical micrograph showed selective adherence of cells to the stripe features, indicating selective adsorption of fibronectin only to the stripe regions and the inertness of the remaining regions presenting acetylene dicobalt hexacarbonyl

groups (data not shown). Next, an electric potential was applied to the monolayer, which was subsequently treated with N_3-GRGDS peptide (1 μM in PBS), copper(II) sulfate (0.6 μM), and sodium ascorbate (0.6 μM) at 37 °C for 1 h and then briefly washed. An optical micrograph of this monolayer showed that cells on the fibronectin-coated stripe regions were not affected by the electric potential and the click reagents (Figure 6, 0 h). Following electrochemical activation and the click reaction for immobilization of the RGD peptide, cells migrated progressively from the stripe regions into the previously inert, electrochemically activated regions of the monolayer (Figure 6). As a control experiment, we repeated this experiment using a patterned monolayer that was filled with only tri(ethylene glycol)-terminated disulfide. As expected, cells did not migrate and remained attached to the stripe pattern (Figure S2). An additional control experiment using the patterned monolayer presenting 5% acetylene dicobalt hexacarbonyl groups without the click reaction after an electric potential treatment showed no cell migration (Figure S3). These results clearly demonstrate that cell migration was mediated by RGD ligands that were immobilized through the click reaction and that the electric potential and click reagents did not compromise the inertness of the substrate.

Strategies for protection and deprotection of acetylene groups for the click reaction have been reported.[26,27] However, in those reports, the protecting groups were used because the acetylene group was not compatible with the chemistry employed for introduction of the acetylene groups onto a graphite electrode or a Si(111) surface. Orski et al.[28] recently described a strategy for on-demand activation of click chemistry wherein a cyclooctyne was masked with cyclopropenone, which revealed the cyclooctyne group upon irradiation with UV light. The dynamic property of the SAM-based biointerface enables modulation of surface activities and has many applications in various areas of biology and medicine. For preparation of dynamic substrates, common candidates for induction of surface reactions on demand include light, electric potential, and temperature.[29] Among them, electrochemical induction is advantageous over the other induction strategies because it can provide a simple, inexpensive, easy-to-miniaturize platform with independently addressable electrodes. This work has demonstrated dynamic SAMs that can be electrochemically activated to undergo the click reaction for selective surface modifications. In addition, the electroactive acetylenic $Co_2(CO)_6$ complex used in the current study is biocompatible and stable in contact with water and therefore allows in situ activation under very mild conditions in the presence of biomaterials and cells. In combination with a well-known photolithographic method for fabrication of a microdevice, this strategy can be expanded to multiplexed in situ activation systems. We believe that our method, which utilizes the click reaction by allowing "dynamic" modulation of the surface property at the molecular level, can make an important contribution to many applications.

■ ASSOCIATED CONTENT

Supporting Information. Synthesis, experimental section, and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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