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Electrochemical Release of Fluorescently Labeled Thiols from Patterned Gold Surfaces

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Abstract

Reductive desorption of alkanethiols is a tool for spatially and temporally controlled release of small molecules or particles from individually addressable gold electrodes. Here we report on the dynamics of release using fluorophore-terminated C6 or C11 thiols. We show that the release kinetics for C6 thiols are determined solely by diffusive transport, whereas for C11 thiols the release kinetics are attenuated by the low solubility that limits the rate at which the desorbed thiols can diffuse away from the surface. The release of multiple different molecules from the same electrode is demonstrated using red- and green-emitting fluorophores. The fraction of the monolayer released is dependent on the electrode potential.

The reductive desorption of thiols can be employed for spatially and temporally controlled release of molecules or particles tethered to individually addressable electrodes. Although reductive desorption is often viewed as a limitation to the use of thiol monolayers,^{1–3} this process has been exploited for cell spreading,^{4–6} study of cell detachment at the subcellular level,⁷ and spatially and temporally controlled release of proteins and nanoparticles.⁸

Here we use fluorescence microscopy to visualize the release process and to study the dynamics of release of fluorophore-terminated thiols. We show that the release kinetics of a fluorophore-terminated C6 thiol are controlled by diffusive transport from the electrode surface. For a longer chain, fluorophore-terminated C11 thiol, the release kinetics are complicated by the fact that transport into the bulk solution is limited by the bulk solubility. We demonstrate spatial and temporal control of release of different molecules with red- and green-emitting fluorophore-terminated thiols. Finally, we show that the visible fluorescence on release is dependent on the release potential. These results provide new insight into the electrochemically programmed release of thiol modified surfaces.

Figure 1 shows a schematic illustration of the release process (see the Supporting Information for details). First, an amine-terminated thiol, either C6 or C11, is conjugated to an NHS-terminated fluorophore. The fluorophore-terminated thiol is then incubated on the surface of a lithographically patterned electrode array for at least 2 h followed by extensive rinsing. The electrode array consists of individually addressable 10 μm wide gold lines with 10 μm spacing. Release of the fluorophore-terminated thiol is achieved by applying a

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Supporting Information Available: Detailed description of the materials and methods, and videos of programmed release. This material is available free of charge via the Internet at <http://pubs.acs.org>.

potential pulse of -1.3V (with respect to a Ag/AgCl wire) to an individual line in the electrode array. The release process is observed using fluorescence microscopy.^{9,10}

Cyclic voltammetry can be used to characterize the presence and desorption of thiol on the surface. Figure 1g shows a cyclic voltammogram for a gold line functionalized with a fluorophore-terminated C11 thiol in phosphate-buffered saline (PBS) at pH 7.4. Initially, in the forward scan, the current is very low as the SAM acts as a molecular resist to block current flow. At about -0.6V , the current begins to increase due to the onset of reductive desorption. In neutral solution, the desorption process can be written as $\text{R-S-Au} + \text{H}^+ + \text{e}^- \rightarrow \text{R-S-H} + \text{Au}$.^{1,11} As the thiols begin to desorb, the resistive nature of the monolayer begins to break down, causing an increase in current. The small peak at about -0.9V corresponds to the point where approximately half of the monolayer has desorbed. At about -1.1V , the entire monolayer has desorbed and the current increases due to hydrogen evolution. The formation of hydrogen bubbles is not observed at potentials down to -1.3V . The corresponding cyclic voltammogram for a bare gold line (Figure 1h) is the same as that for the reverse scan on the electrode with the fluorophore-terminated thiol, confirming that the thiol is completely removed from the surface.

Figure 2a–f shows a sequence of fluorescence images from a typical release experiment. In this experiment, a red fluorophore (Alexa Fluor 568, em. 610 nm) attached to a C6 thiol is tethered to the surface. Prior to release, there is very little visible emission from the fluorophore due to fluorescence quenching by the gold electrodes (Figure 2a). The fluorophore is about 1.5 nm from the gold surface, sufficiently close for efficient quenching.^{12–15} The background signal on the electrodes is primarily due to reflection of the excitation source (ex. 568 nm) that is not filtered out due to its proximity to the emission wavelength. This is confirmed in control experiments on bare gold lines (see Supporting Information Figure 3). On applying a voltage pulse of -1.3V , the fluorophore-terminated thiol is desorbed from the surface. Shortly after release, a bright flash is seen on the release electrode (Figure 2b) as the thiols move away from the surface and the fluorescence is no longer quenched by the gold electrode. The remaining four panels show the decrease in intensity as the fluorophore-terminated thiol diffuses radially away from the release electrode (Figure 2c–f). After about 100 ms, the adjacent electrodes appear bright due to fluorophore that has diffused laterally from the release electrode. Note that the fluorescence intensity appears brighter over the electrodes due to reflection from the gold. After 1.84 s, the fluorophore-terminated thiol has diffused across several of the adjacent electrodes (see Supporting Information Movie 1). Supporting Information Figure 2 shows the average fluorescence intensity for the images shown in Figure 2a–f as well as the standard deviation of the emission intensity over the electrode area.

Figure 2g shows a plot of the normalized intensity over the release electrode plotted versus time for the C6 fluorophore-terminated thiol shown in Figure 2a–f. The raw data for this release are given in Supporting Information Figure 1. The sampling rate of the camera was insufficient to capture the initial rise in intensity; however, after the first frame, the intensity over the release electrode decreases with time. The inset of Figure 2g shows normalized intensity versus time curves for 11 different electrodes, illustrating the high reproducibility of these release experiments. Hypothesizing that the dynamics of release are controlled by diffusive transport of the fluorophore, we fit the normalized intensity profile to the solution to Fick's second law for a plane source with finite thickness¹⁶ with the boundary conditions imposed by the electrode:

$$\frac{C}{C_0} = \frac{1}{2} \left[\text{erf} \left(\frac{h-x}{2\sqrt{Dt}} \right) + \text{erf} \left(\frac{h+x}{2\sqrt{Dt}} \right) \right] \quad (1)$$

where C is the concentration of the fluorophore, C_0 is the initial concentration at $t=0$, h is half the line width ($5\ \mu\text{m}$), and D is the diffusion coefficient. Taking the normalized fluorescence intensity as C/C_0 , the experimental data were fit with the diffusion coefficient D as the only adjustable parameter. The solid line in Figure 2g shows excellent agreement to the experimental data, with $D=1.25 \times 10^{-6}\ \text{cm}^2\ \text{s}^{-1}$. This value is at the low end of the range of 1×10^{-6} – $1 \times 10^{-5}\ \text{cm}^2\ \text{s}^{-1}$ reported in the literature for alkanethiols,^{17–20} presumably due to the presence of the fluorophore. From the diffusion coefficient, the time required for the fluorophores to diffuse beyond the quenching distance (about 10 nm) is estimated ($t \approx x^2/D$) to be a few microseconds. This is much faster than our sampling interval, and hence, we can ignore effects due to quenching in our analysis.

The image sequence in Figure 2a–f shows release of a C6 thiol; however, similar behavior is observed with a C11 thiol. Figure 2h shows the normalized intensity versus time for both C6 and C11 fluorophore-terminated thiols. In contrast to the C6 thiol, the C11 thiol does not follow the diffusion model and interestingly shows a faster decay. This effect is due to the significantly lower solubility of the C11 thiol and the greater hydrophobic interactions as compared to the C6 thiol. In previous work, we showed that reductive desorption can be explained by a model that takes into account diffusion of the thiolate into the bulk solution at a rate that is determined by the bulk solubility.²¹ Our results here suggest that, at pH 7.4, the C6 fluorophore-terminated thiol is sufficiently soluble that the release kinetics are determined solely by diffusive transport away from the electrode surface, but that the release kinetics of C11 fluorophore-terminated thiol are attenuated by the low solubility that limits the rate at which the desorbed thiols can diffuse away from the surface.

To demonstrate spatial and temporal control of programmed release of different molecules, we performed an experiment with red and green fluorophore-terminated thiols. A schematic illustration of the regeneration process necessary to achieve conjugation with different molecules is shown in Figure 1. After release of a red-emitting fluorophore (Figure 1c), the device is cleaned by quickly rinsing three times with PBS. Cyclic voltammetry is then performed to verify that the gold surface is bare (Figure 1d). Finally, the device is incubated in solution containing a green-emitting fluorophore-terminated thiol (Figure 1e). At this point, the device is loaded with both red- and green-emitting fluorophores and either one can be selected for release.

Figure 3 shows sequential release of red- and green-emitting fluorophores from the same electrode. Figure 3a shows a bright field microscope image of an electrode array with the release stripe indicated by the arrow. Initially, the device is incubated with a red fluorophore, and as it is released, a bright red flash is seen (Figure 3b). The electrode is then cleaned and incubated with a green fluorophore, as described above. This same stripe is then released, and the bright green flash is seen (Figure 3c). This process can be repeated several times, as illustrated in Figure 3d, which shows the peak emission intensity for seven release experiments from the same electrode. Supporting Information Movie 2 provides a movie of sequential release of red-emitting and green-emitting fluorophore-terminated C6 thiol.

The release experiments described above were performed at $-1.3\ \text{V}$ where the complete monolayer is desorbed. To determine the influence of potential on fluorophore release, we performed a series of experiments with a red-emitting fluorophore-terminated C6 thiol. Figure 4b shows representative fluorescence intensity versus time plots from independent experiments at different potentials. As the release potential becomes more negative, the peak intensity increases and the peak becomes sharper. Figure 4c shows the peak intensity for release at potentials from -0.4 to $-1.3\ \text{V}$. The onset in observable fluorescence intensity occurs at about $-0.6\ \text{V}$ and increases sigmoidally up to a maximum at about $-1.2\ \text{V}$, close to the potential corresponding to the completion of reductive desorption in the voltammogram

(Figure 4a). Since the fluorescence represents the amount of fluorophore-terminated thiol released from the electrode, the normalized intensity represents the complement of the fractional coverage. The coverage θ is related to the desorption current by $\theta = q/(q_{\text{total}}F)$, where q is the charge density in the desorption peak, q_{total} is the total charge in the peak, and F is Faraday's constant. For a symmetrical peak, after background subtraction, $\theta = 0.5$ at the potential of the current peak. As expected, the coverage is close to one at potentials positive to -0.6 V. As the potential becomes more negative, the thiol coverage progressively decreases, reaching zero coverage (complete removal) at about -1.1 V.

In summary, we have used fluorescence microscopy to study programmed release of fluorophore-terminated thiols from individually addressable gold electrodes. For C6 thiol, the release kinetics are determined solely by diffusive transport, whereas for C11 thiol the release kinetics are attenuated by the low solubility that limits the rate at which the desorbed thiols can diffuse away from the surface. The release of multiple different molecules from the same electrode was demonstrated using red- and green-emitting fluorophores. The fraction of the monolayer released is dependent on the electrode potential. This work demonstrates the spatial and temporal control of small molecules or particles. Microfabrication techniques are used to spatially locate molecules or particles via a thiol linkage. By triggering the release from individually addressable electrodes, temporal control is achieved. The regeneration technique allows different electrodes to be loaded with different molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic illustration of spatially and temporally programmed release of a fluorophore-terminated thiol from individually addressable gold electrodes. (a) Electrode array consisting of individually addressable gold lines. (b) The electrode array is incubated in a solution of red-emitting fluorophore-terminated thiol. No fluorescence is seen due to quenching from the gold electrodes. (c) Applying a sufficiently negative potential results in release of the tethered thiol from the electrode. (d) Regeneration of the release electrode. (e) Incubation in a solution of green-emitting fluorophore-terminated thiol. This regeneration and reloading process allows release of different molecules. (f) Release of the green-emitting fluorophore-terminated thiol. Cyclic voltammograms for a 13 mm long gold line functionalized with (g) fluorophore-terminated C11 thiol and (h) bare gold, in PBS at pH 7.4 at a scan rate of 100 mV s^{-1} .

Figure 2.

(a–f) Fluorescence images for release of a red-emitting fluorophore-terminated C6 thiol in PBS. (g) Normalized fluorescence intensity after background subtraction versus time. Solid line shows a fit to the diffusion equation for a plane source with finite thickness. Inset shows the normalized intensity versus time for release experiments from 11 different electrodes. (h) Normalized intensity versus time for C6 thiol (blue) and C11 thiol (orange).

Figure 3.

Bright field microscope image of an electrode array. All electrodes are conjugated with a red-emitting fluorophore-terminated C6 thiol. (b) Fluorescence image immediately after release of the red-emitting fluorophore from the indicated electrode. (c) Fluorescence image immediately after release of a green-emitting fluorophore from the same electrode. The device was cleaned by rinsing in PBS and performing a cyclic voltammogram to verify that the surface was bare. The device was then incubated with the thiol for 2 h prior to triggering release. (d) Plot of maximum fluorescence intensity for sequential release of red- and green-emitting fluorophore-terminated C6 thiols from the same electrode. The error bars represent the standard deviation of the intensity for three independent experiments.

Figure 4.

(a) Cyclic voltammogram (negative scan only) for fluorophore-terminated C6 thiol in PBS at a scan rate of 100 mV s^{-1} . (b) Fluorescence intensity versus time for release of a fluorophore-terminated C6 thiol at different potentials. (c) Peak fluorescence intensity versus release potential.