An Interfacial Oxime Reaction To Immobilize Ligands and Cells in Patterns and Gradients to Photoactive Surfaces

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We report a molecularly controlled interfacial chemoselective methodology to immobilize ligands and cells in patterns and gradients to self-assembled monolayers on gold. This strategy is based on reacting soluble ketone or aldehyde tethered ligands to surface-bound oxyamine alkanethiols to generate a covalent oxime linkage to the surface. We characterize the kinetic behavior of the reaction on the surface with ferrocenecarboxaldehyde (FcCHO) as a model ligand. The precise extent of immobilization and therefore surface density of FcCHO on the SAM is monitored and determined by cyclic voltammetry, which shows a pseudo-first-order rate constant of 0.13 min^{-1}. In order to generate complex surface patterns and gradients of ligands on the surface, we photoprotected the oxyamine group with nitroveratryloxycarbonyl (NVOC). We show that ultraviolet light irradiation through a patterned microfiche film reveals the oxyamine group and we characterize the rate of deprotection by immobilization of ketone containing redox active groups. Finally, we extend this strategy to show biospecific cell attachment of fibroblast cells by immobilizing ketone-GRGDS peptides in patterns. The interfacial oxime reaction is chemoselective and stable at physiological conditions (pH 7.0, 37 °C) and may potentially be used to install ligands on the surface in the presence of attached cells to modulate the cell microenvironment to generate dynamic surfaces for monitoring changes in cell behavior in real time.

Introduction

In nature, cells exist in a highly evolving complex environment where numerous spatial input cues, such as gradients or patterns of ligands, are dynamically presented, which can then directly modulate a range of cellular behavior.[1,2] In order to generate new in vitro model systems to study complex cell behavior, there has been tremendous interest in interfacing material science with cell biology. For fundamental cell biological investigations, self-assembled monolayers (SAMs) on gold have been the model system of choice for a variety of cell adhesion,[3] migration,[4] coculture,[5] dynamics of intracellular protein activation,[6] subcellular nanoarchitecture formation,[7] cell polarization,[8] and cell patterning studies.[9] There are several advantages of using SAMs on gold as a platform for preparing model systems to study cell phenomena over other materials: (1) SAMs are synthetically flexible (routine organic synthesis to generate almost any alkanethiol tethered molecule); (2) many surface spectroscopies to characterize interfacial reactions or associations are available (for example, surface plasmon resonance spectroscopy, atomic force microscopy, scanning tunneling microscopy); (3) monolayers are electroactive (can modulate and/or characterize surface

properties by electrochemistry); (4) for biointerfacial studies, SAMs can become inert to nonspecific protein adsorption (incorporation of ethylene glycol-terminated alkanethiols renders the surface inert); and (5) the method is compatible with cell culture conditions (pH 7, 37 °C) and live-cell high resolution fluorescence microscopy.[6] Taken together, these features allow for the generation of well-defined surfaces for biospecific interactions between ligands presented on the SAM and cell surface receptors.

To generate model surfaces to investigate complex biospecific cell phenomena, a synthetically simple method for immobilizing ligands in spatially controlled patterns and gradients would lead to the design of more sophisticated model surfaces for interrogating cell behavior. Although several methodologies for biomolecule conjugation to SAMs on gold have been developed, including acid–base chemistry,[10–12] Staudinger ligation,[13–15] Click chemistry,[16,17] Diels–Alder reaction,[18] Michael addition,[19] disulfide–thiol exchange reaction,[20] as well as other methods,[21]

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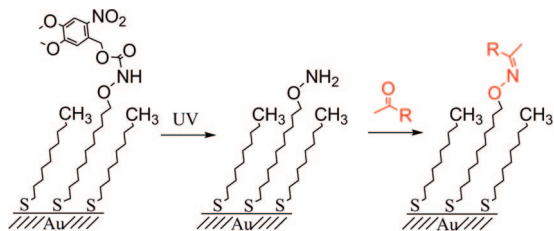
Figure 1. Scheme describing a chemoselective interfacial oxime reaction to immobilize ligands to a photoactive surface. Illumination with ultraviolet light (365 nm) onto a NVOC-oxyamine-terminated SAM yields an oxyamine group that subsequently reacts with a ketone-functionalized ligand forming a stable interfacial oxime linkage.
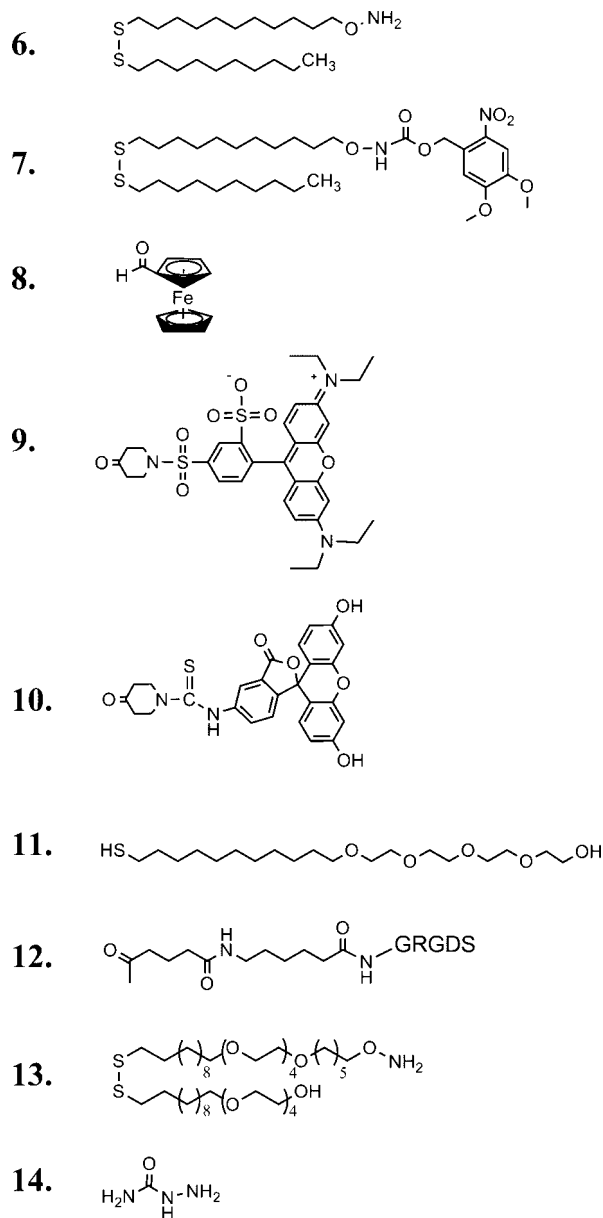
only a few are ideal as an immobilization method to generate patterns, multiple ligand patterns, and gradients on SAMs for cell biological applications. To access biospecific dynamic cell behavior, the model surface must be able to change its properties by either releasing ligands or immobilizing ligands rapidly and in the presence of attached cells.

Herein, we report an interfacial oxime reaction as a conjugation strategy to immobilize ligands and cells in patterns and gradients to a surface. We characterize the reaction by electrochemistry and demonstrate the utility of this method by patterning multiple ligands and show it is amenable to generating surfaces for biospecific studies of cell adhesion and migration. We show that the reaction between surface-bound oxyamine groups and soluble carbonyl tethered groups is kinetically well behaved, fast, and generated in high yield under physiological conditions.[22] Also, the reaction is chemoselective in the presence of cell lysates,[23] and the resulting oxime is chemically stable for a range of pH's and temperatures on a gold surface. From a synthetic perspective, the ketone moiety can also be easily incorporated into peptide ligands using solid-phase peptide synthesis.[24] We further demonstrate that photochemical lithography can be applied to produce patterns and gradients at the microscale on the surface. Nitroveratryloxycarbonyl (NVOC)-oxyamine surfaces are deprotected by UV illumination through a photolithographic mask to reveal oxyamine functional groups in spatially controlled patterns. Ketone-GRGDS peptide is chemoselectively immobilized and shown to support biospecific ligand mediated cell adhesion.

Results and Discussion

The general scheme to photochemically unveil a reactive oxyamine surface in patterns and gradients and subsequent ketone tethered ligand immobilization is shown in Figure 1. A Photoprotected oxyamine alkanethiol (**7**) was synthesized and assembled on a gold surface (Scheme 1). The photochemical protecting group NVOC effectively hides the oxyamine group from reacting with carbonyl-functionalized ligands. Upon UV illumination through a microfiche mask, the NVOC group is effectively removed to reveal the oxyamine group, which can subsequently chemoselectively react with carbonyl-functionalized ligands to generate an immobilized oxime linkage to the surface.

We first showed an oxyamine alkanethiol surface can react with carbonyl-bearing ligands (Figure 2A) rapidly and in high yield. To characterize the real-time kinetics of the interfacial reaction, we immobilized a redox active ferrocenecarboxaldehyde [FcCHO (**8**)] to a surface containing a 1:1 mixture of oxyamine alkanethiol (**6**) and decanethiol. The extent and kinetics of the

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reaction were characterized by cyclic voltammetry. The surface initially has no redox peaks, but after the addition of FcCHO (150 mM, in ethanol), a peak at 348 mV steadily increased with reaction time, indicating that ferrocene is immobilized to the surface. Analysis of the rate of increase in peak currents of the ferrocene monolayer followed pseudo-first-order kinetics, because the FcCHO was present in large excess relative to the immobilized oxyamine. The data was fit to an exponential decay (eq 1) to give a pseudo-first-order rate constant (k') of 0.13 min^{-1}, where I_t is the peak current at time t and I_o is the initial peak current.

$$I_t = I_o(1 - \exp^{-k't}) \qquad (1)$$

This result shows that the oxyamine monolayer reacts chemoselectively with carbonyl containing ligands to give the corresponding oxime on the surface. To show the reaction went to completion, we compared the theoretical and calculated amount of ferrocene immobilized to a surface containing 50% oxyamine (a surface presenting molecule **6** should have 1:1 ratio of oxyamine to decanethiol, since it is a disulfide). After 60 min of reaction time, the redox peak for the ferrocene does not increase, indicating complete conversion of the surface from the oxyamine to the oxime conjugate. To determine the amount of ferrocene–oxime

Scheme 1. Synthesis of NVOC-Protected Oxyamine-Terminated Alkanedisulfide (7)
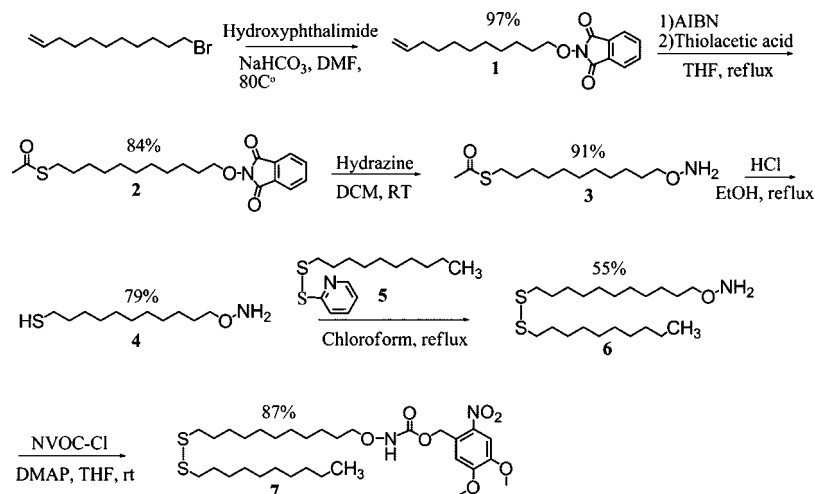

conjugate on the surface, we used the equation $Q = nFA\Gamma$, where Q represents the charge and was calculated to be 6.38 μC, n represents the number of moles of electrons and is 1 for the redox interconversion of ferrocene and ferrocenium, A is the area of working electrode and was determined to be 1.3 cm^2, and F is the Faraday constant and is 96485 C/mol. The surface coverage (Γ) was determined to be 4.7×10^{13} molecules/cm^2 and is experimentally close to the theoretical coverage of a 50% surface of 5.0×10^{13} molecules/cm^2. This result shows that the interfacial reaction is fast, kinetically well-behaved, and can potentially tailor surfaces with a variety of carbonyl containing ligands. As controls, the redox peaks characterizing ferrocene are not observed when the substrate is first treated with a 10% aqueous acetone solution for 3 h (acetone will react with oxyamines to generate an oxime, effectively eliminating the oxyamine group from the surface) before the addition of the FcCHO solution.

To generate molecularly controlled complex patterns and gradients on the surface, we synthesized a photoprotected oxyamine alkanethiol (**7**). By illuminating the surface with ultraviolet (UV) light of 365 nm through a patterned microfiche,

we demonstrate spatial unveiling of the oxyamine group and subsequent immobilization of oxime ligands. We chose NVOC as the photoprotecting group because of the high efficiency of deprotection and UV compatibility with SAM surfaces. However, upon UV illumination the photobyproduct of the NVOC-oxyamine bears an aldehyde functionality that immediately reacts with the oxyamine surface to essentialy quench its subsequent reactivity. In order to eliminate the competing aldehyde photobyproduct, the photodeprotection was performed in a saturated solution of semicarbazide (**14**) (in H$_2$O and N$_2$ purged for 30 min before usage), which acts as an efficient carbonyl scavenger. Under these conditions, UV irradiation of a SAM composed of molecule **7** revealed the oxyamine group for subsequent reactivity. The substrate was then immersed in FcCHO solution to show ligand immobilization. For complete photodeprotection, the surface was illuminated with UV light for 60 min and revealed approximately 95% of the oxyamine functional groups relative to a control substrate made of oxyamine disulfide (**6**) (Figure 2B). These results show the oxyamine can be revealed and immobilized with ligands with no deterioration in the surface or the oxyamine molecule. No immobilization was detected by cyclic
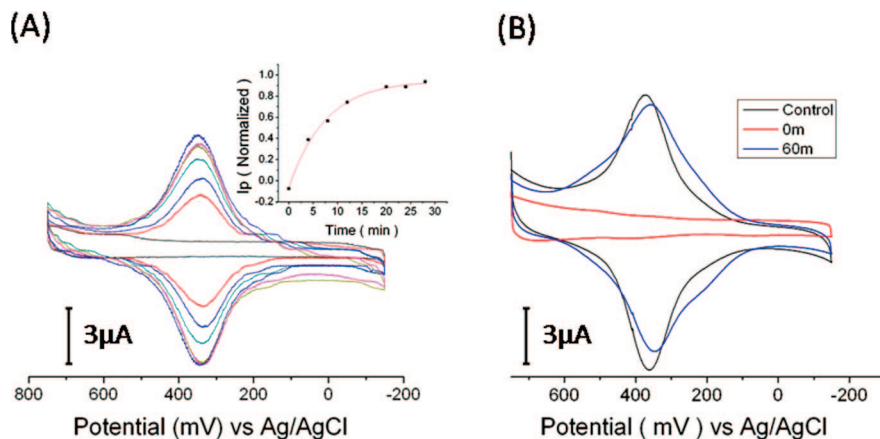
Figure 2. Cyclic voltammetry characterization of the kinetics of the interfacial oxime reaction and the photodeprotection of NVOC-oxyamine SAMs. All cyclic voltammetry was performed in 1 M HClO$_4$ solution against Ag/AgCl reference electrode with a scan rate of 100 mV s^{-1}. (A) A SAM of molecule **6** was immersed in a FcCHO (**8**) solution (150 mM) to determine the rate of interfacial oxime reaction. (Inset) The plot of time versus normalized peak current (Ip) at 348 mV was fit to an exponential decay and gave a pseudo-first order rate constant of 0.13 min^{-1}. (B) A SAM of NVOC-oxamine (**7**) was immersed into a FcCHO solution (150 mM) after UV deprotection (365 nm) for 0 and 60 min. After 60 min of illumination the FcCHO immobilized to the surface shows a cyclic voltammogram similar to a control substrate composed of molecule **6** showing no desorption or damage to the surface due to UV exposure. The photodeprotection was performed in a saturated semicarbazide solution to quench the aldehyde functional group of the photobyproduct.
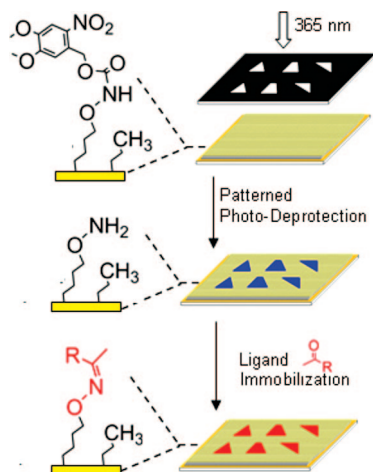
Figure 3. Scheme describing the spatial unveiling of oxyamine groups for ligand immobilization. A mixed monolayer of NVOC-protected oxyamine-terminated SAMs is illuminated through a patterned microfiche mask to reveal the oxyamine group for subsequent chemoselective immobilization of ketone-tethered ligands.

votammetry when the substrate was treated with a 10% aqueous acetone solution for 1 h before introducing the FcCHO solution.

To demonstrate that the methodology can immobilize ligands in a spatially controlled manner, we illuminated the photoprotected oxyamine surface with UV light through a patterned microfiche mask and added ketone-functionalized fluorescent dyes. Figure 3 shows the general scheme of photopatterning and ligand immobilization. To introduce the ketone group to the fluorescent dyes (fluorescein isothiocyanate and sulforhodamine B acid chloride), we modified the structures by reacting with 1,4-dioxa-8-azaspiro[4.5]decane, followed by treatment with 15% HCl solution (Scheme 2). To generate complex surface patterns and multiple ligand immobilizations, we illuminated the surface through a microfiche patterned mask consecutively to pattern two different fluorescent dyes. The ketone-functionalized dyes **9** and **10** are immobilized after each round of photodeprotection through a microfiche mask of pentagon and circle patterns, respectively. The fluorescence image shows that the dyes were immobilized onto only selected regions conforming to the photolithographic patterns (Figure 4) after two separate rounds of photodeprotection and immobilization.

To create molecularly defined gradients on a surface that can immobilize a variety of ligands, we extended the photodeprotection and capture strategy. Figure 5A shows the fidelity of the microfiche gradient mask and the corresponding immobilization of fluorescent dye **9**. Analysis of the fluorescent intensity profile along the axis of the dumbbell shape gradient surface shows that the relative fluorescence intensity closely corresponds with the gradient slope of the microfiche mask and confirms that the ligand is immobilized in a gradient on the surface.

To demonstrate that this strategy is capable of immobilizing biologically relevant molecules and is compatible with cell culture conditions, we selectively immobilized the GRGDS peptide as a cell-binding ligand to an otherwise inert surface. The GRGDS peptide motif is found in the extracellular matrix protein fibronectin and is known to bind to cell surface integrin receptors to promote cell attachment, migration, and proliferation.[25] A ketone functional group was introduced to the GRGDS peptide as a 4-acetylbutylic acid without any protecting groups during routine solid-phase peptide synthesis (**12**).[24] To show a biospecific

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interaction between the cells and the surface, SAMs consisting of a 2:98 ratio of molecules **13** and **11** were generated and exposed to the ketone-peptide (**12**) for immobilization (10 mM, in PBS, 2 h) (Scheme 3). The major component of the surface consists of the ethylene glycol group due to its ability to resist nonspecific protein adsorption and cell attachment, a critical requirement for biospecific studies of mechanistic cell adhesion and migration. Fibroblasts were then added, and after adhering to the surface, they migrated and proliferated to eventually become a lawn of cells that are contact inhibited. To show the interaction was due only to the peptide, addition of soluble GRGD peptide (without the ketone group, 10 μM, 1 h) caused the cells to detach from the surface, indicating that the cells were adhered only via a ligand−receptor interaction. Furthermore, surfaces that were not exposed to the peptide, and therefore only present the oxyamine group and ethylene glycol groups, showed no cell attachment.

To attach cells in patterns biospecifically, we prepared a mixed SAM of molecule **7** and tetra(ethylene glycol)-terminated alkanethiol (**11**) 2:98 and immobilized ketone-GRGDS (**12**) (10 mM in PBS, 2 h). Ultraviolet light illumination through a patterned microfiche mask followed by peptide immobilization and cell seeding shows cells patterned on the surface (Figure 6). To demonstrate that the cells were attached biospecifically via their integrin receptors to RGD-presenting surfaces, we showed that the addition of soluble RGD peptides (10 μM, 1 h) detached the cells from the surface. Furthermore, when a scrambled GRD-ketone peptide was immobilized, no cells attached to the surface.

Conclusion

We have developed an interfacial oxime reaction that chemoselectively immobilizes ligands to photoactive surfaces in patterns and gradients. We showed the sequential immobilization of two fluorescent dyes in patterns and also the immobilization of ligands in gradients. The interfacial oxime reaction is fast, kinetically well-behaved, high-yielding, and can be characterized by electrochemistry. Furthermore, we extend the strategy to immobilize peptide ligands (ketone-GRGDS) for biospecific cell attachment in patterns. The strategy presented here can be used to immobilize a variety of ligands containing the ketone group and may be used for spatial and temporal control of ligands to generate dynamic surfaces where the cell microenvironment is modulated in real time to monitor and study changes in cell behavior. This methodology is compatible with attached cell culture and provides molecular level control of patterned ligands and gradients on surfaces. This strategy can be extended to generate high-throughput arrays, and in combination with high-resolution live-cell microscopy, it can be used to investigate a variety of cell adhesion, cell signaling, and cell migration phenomena.

Experimental Section

All amino acids were purchased from Anaspec, Inc. (La Jolla, CA). Rink amide MBHA resin was purchased from NovaBiochem. All reagents and solvents were purchased from Aldrich and used as received. THF was distilled from sodium benzophenone under nitrogen before use. All reagents used in cell culture were obtained from Gibco BRL.

Synthesis. *2-(Undec-10-enyloxy)isoindoline-1,3-dione (**1**).* A solution of *N*-hydroxyphthalimide (9.9 g, 60.7 mmol) and sodium bicarbonate (4.95 g, 59 mmol) in DMF (100 mL) was heated to 80 °C. After the mixture turned dark brown, bromoundecene (5.26 mL, 23 mmol) was added and the reaction mixture stirred for 10 h. The reaction mixture was extracted with water and ethyl acetate. The organic phase was concentrated and purified by column chromatography with 1:3 ethyl acetate/hexane to afford 7.1 g (97%) of

Scheme 2. Synthesis of Ketone-Functionalized Fluorescent Dyes (9, 10)
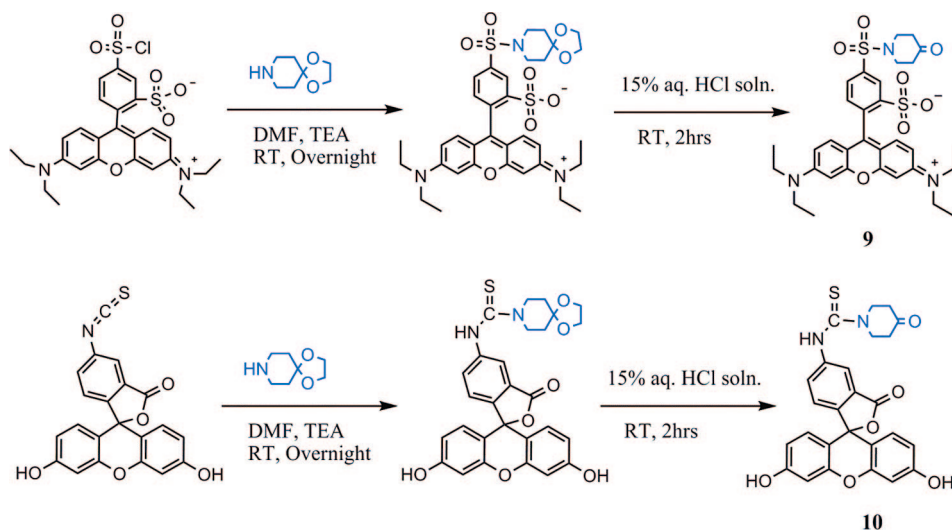
product as a colorless oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2H), 7.72 (m, 2H), 5.80 (m, 1H), 4.95 (m, 2 H), 4.17 (t, 2 H, J = 6.8), 2.03 (m, 2H), 1.77 (m, 2 H), 1.32 (m, 2 H), 1.19−1.27 (br s., 10 H).

S-11-(1,3-Dioxoisoindolin-2-yloxy)undecyl Ethanethioate (2). A solution of **1** (2.0 g, 6.4 mmol) and a catalytic amount of azobis(isobutylnitrile) in dry THF was heated under reflux. Upon observing N$_2$ bubbles, thioacetic acid (0.91 mL, 12.7 mmol) was added to the reaction mixture and the mixture was kept stirring under reflux for 6 h. The reaction mixture was concentrated and separated by column chromatography with 1:3 ethyl acetate/hexane to afford 2.1 g (84%) of product as a white solid: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2 H), 7.72 (m, 2 H), 4.17 (t, 2 H, J = 6.8), 2.84 (t, 2 H, J = 6.8), 2.30 (s, 3H), 1.54 (br s, 4 H), 1.34 (m, 2 H) 1.28 (br s, 12 H).

S-11-(Aminooxy)undecyl Ethanethioate (3). To a solution of **2** (2.8g, 7.1mmol) in DCM (30 mL) was added hydrazine (23 mL of 1.0 M solution in THF, 23 mmol) and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtered and the filtrate was concentrated and then separated by column chromatography with 1:3 ethyl acetate/hexane to afford 1.7 g (91%) of product as colorless oil: ^{1}H NMR (CDCl$_3$) δ 3.65 (t, 2 H, J = 6.8), 2.84 (t, 2H, J = 6.8), 2.30 (s, 3 H), 1.54 (br s, 4 H), 1.28 (br s, 14 H).

11-(Aminooxy)undecane-1-thiol (4). To a solution of **3** (1.5 g, 5.7 mmol) in EtOH (22 mL) was added 12 N HCl (36 mmol, 3 mL) and the mixture was heated under reflux for 3 h. The reaction mixture was concentrated and dissolved in ethyl acetate. The organic phase was washed with a saturated aqueous solution of NaHCO$_3$, water, brine, and sodium sulfate. The organic phase was concentrated to afford 1.0 g (79%) of a yellowish oil (4.5 mmol): ^{1}H NMR (CDCl$_3$) δ 3.65 (t, 2 H, J = 6.8), 2.51 (q, 2 H, J = 6.8), 1.54 (m, 4 H), 1.28 (br s, 14H).

2-(Decyldisulfanyl)pyridine (5). A reaction mixture of decanethiol (1.0 g, 5.7 mmol), dipyridyl disulfide (1.9 g, 8.6 mmol), and dimethylaminopyridine (1.1 g, 8.7 mmol) in methylene chloride (30 mL) was stirred at room temperature for 8 h. The reaction mixture was concentrated and separated by column chromatography with 1:2 ethyl acetate/hexane to afford 1.5 g (92%) of product as a colorless oil (5.3 mmol): ^{1}H NMR (CDCl$_3$) δ 8.44 (d, 1 H, J = 3.8 Hz), 7.72 (d, 1 H, J = 3.8 Hz) 7.61 (t, 1 H, J = 2 Hz), 2.78 (t, 2 H, J = 7.4 Hz), 1.64 (m, 2 H), 1.34 (m, 2 H), 1.22 (m, 12 H), 0.85 (t, 3 H, J = 6.8 Hz).

O-(11-(Decyldisulfanyl)undecyl)hydroxylamine (6). A reaction mixture of **4** (465 mg, 2.12 mmol) and **5** (723 mg, 2.55 mmol) in 15 mL of chloroform was heated under reflux overnight. The reaction mixture was concentrated and separated by column chromatography with 1:3 ethyl acetate/hexane to afford 450 mg (55%) of white solid: ^{1}H NMR (CDCl$_3$) δ 3.65 (t, 2H, J = 6.8 Hz), 2.65 (t, 4 H, J = 7.2 Hz) 1.64 (m, 4 H), 1.52 (m, 6 H), 1.28 (br s, 24 H), 0.86 (t, 3 H, J = 6.6 Hz).

4,5-Dimethoxy-2-nitrobenzyl 11-(Decyldisulfanyl)undecyloxycarbamate (7). A reaction mixture of **7** (150 mg, 0.38 mmol), dimethylaminopyridine (61 mg, 0.5mmol), and 4,5-dimethoxy-2-nitrobenzyl chloroformate (140 mg, 0.50 mmol) in 10 mL of dry THF was stirred at room temperature for 12 h. The reaction mixture was then concentrated and separated by column chromatography with 1:2 ethyl acetate/hexane to afford 120 mg (87%) of product as white solid: ^{1}H NMR (CDCl$_3$) δ 7.70 (s, 1 H), 7.39 (s, 1 H), 7.01 (s, 1 H), 5.57 (s, 2 H), 3.96 (s, 3 H), 3.94 (s, 3 H) 3.88 (t, 2 H, J = 6.8 Hz), 2.65 (t, 4 H, J = 7.2 Hz), 1.64 (m, 6 H), 1.24 (28 H), 0.86 (t, 3 H, J = 6.6 Hz); ESI mass in CH$_2$Cl$_2$ calcd 630.3, found 630.3.

Synthesis of Ketone-Terminated Fluorescent Dyes (9, 10). To a mixture of rhodamine sulfonyl chloride and triethylamine (1:3 mol
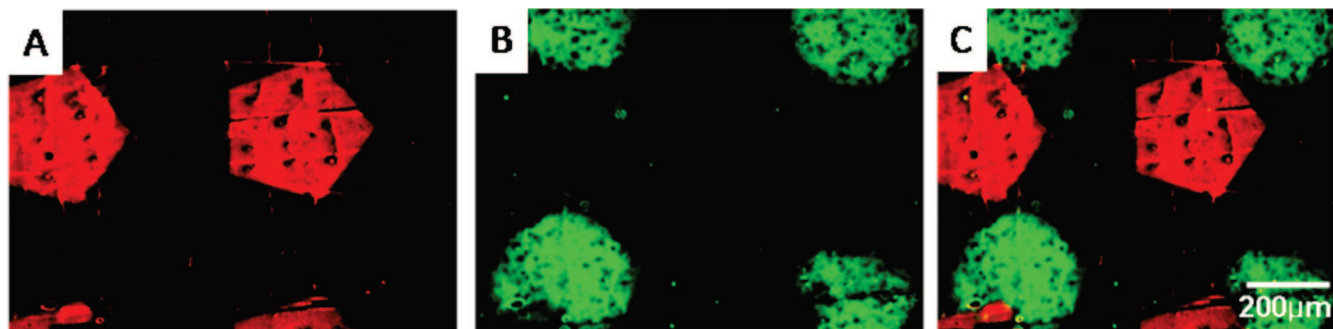

Figure 4. Fluorescent micrographs demonstrating multiple ligand immobilization in patterns to a surface. Two consecutive rounds of photodeprotection with different photolithographic patterned masks followed by the immobilization of ketone-tethered rhodamine [red (**9**)] (A) and fluorescein [green (**10**)] (B) fluorescent dyes, respectively. (C) A combined image of A and B.
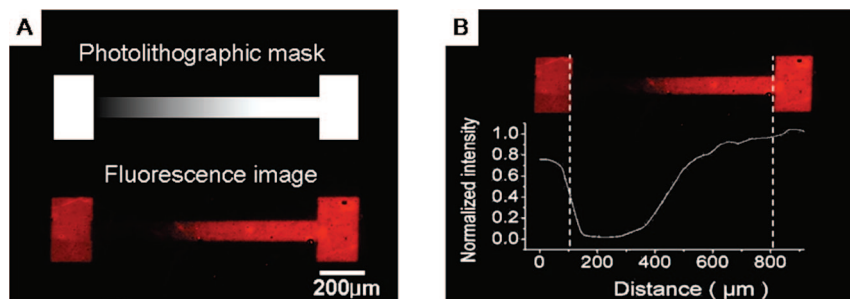
Figure 5. Fluorescent micrographs and line profiles of molecularly defined gradients on the SAM surface. (A) Microfiche film with patterned dumb-bell gradient (above) and the corresponding fluorescent image of the photodeprotected and oxime-immobilized ketone-rhodamine (**8**) dye. (B) Intensity profile along the axis of the patterned surface showing a gradient fluorescent pattern.

Scheme 3. Synthesis of an Ethylene Glycol-Incorporated Oxyamine-Terminated Alkanedisulfide (13).
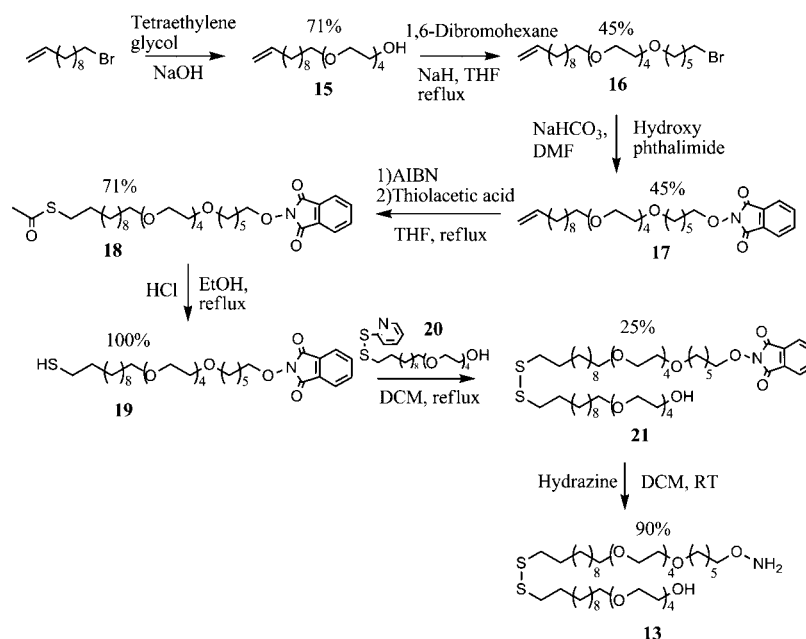

equiv) in DMF (just enough to dissolve the two molecules), 1,4-dioxa-8-azaspiro[4.5]decane (1.2 mol equiv) was added and stirred at room temperature for 4 h. The product is then precipitated by ethyl ether. The dark purple precipitate is treated with HCl solution (15%) and stirred for 3 h at room temperature. Triethylamine was added until the solution reached pH 7. The reaction mixture was then concentrated to afford a dark purple powder (**9**): ESI mass in H_2O calcd 639.2, found 639.1. The same procedure was used to synthesize a fluorescein-ketone dye molecule (**10**): ESI mass in H_2O calcd 488.1, found 488.2.

Molecule **11** was prepared as previously described.[26]

Solid-Phase Peptide Synthesis of GRGDS-ketone (12). Ketone-functionalized GRGDS peptide was synthesized using a peptide synthesizer (CS Bio) at 0.1 mmol scale. 4-Acetylbutylic acid was used without protection of the ketone group. The peptide was obtained from the resin after treating with 10 mL of TFA containing 5% water and 5% methylene chloride for 2 h under stirring and filtration. The filtrate was mixed with ethyl ether (40 mL) and the mixture was centrifuged at 3000 rpm for 15 min to obtain a white precipitate (2×). The precipitate was lyophilized overnight to obtain a white solid. ESI mass H_2O calcd 714.4, found 714.3.

1-Bromo-7,10,13,16,19-pentaoxatriacont-29-ene (16). A solution of **15** (2.4 g, 6.9 mmol) in 40 mL of dry THF was stirred in a ice bath for 30 min. NaH (1.34 g, 56 mmol) was then added to the solution and stirred at room temperature for 30 min. After further
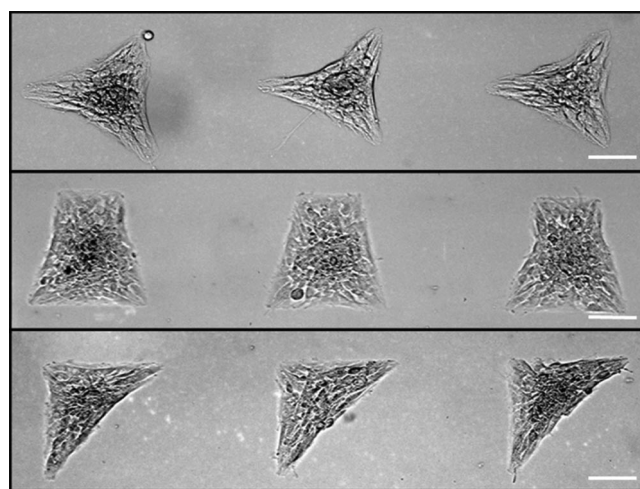
Figure 6. A phase-contrast image of patterned attached Swiss 3T3 Albino fibroblast cells on a SAM photodeprotected and immobilized with ketone-GRGDS peptide. The patterns are triangle, trapezoid, and right triangle from the top row. The scale bar indicates 100 μm. The micrograph was taken 3 days after cell seeding.

stirring in room temperature for 1 h, 1,6-dibromohexane (5.6 g, 23 mmol) in 5 mL of dry THF was added. The reaction mixture was stirred at room temperature overnight and the reaction mixture was concentrated in vacuo and separated by column chromatography

(26) (a) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714–10721. (b) Pale-Grosdemange, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 12–20.

with EA to afford 1.6 g (45%) of product that was a transparent oil: ^{1}H NMR (CDCl$_3$) δ 5.75 (m, 1 H), 4.95 (m, 2 H), 3.5−3.7 (m, 18 H), 3.32−3.45 (m, 4 H), 2.0 (m, 2 H), δ 1.80−1.85 (m, 2 H), δ 1.5−1.59 (m, 4 H), δ 1.2−1.45 (br. s., 16 H).

2-(7,10,13,16,19-Pentaoxatriacont-29-enyloxy)isoindoline-1,3-dione (17). A solution of *N*-hydroxyphthalimide (1.08 g, 9.4 mmol) and sodium bicarbonate (789 mg, 9.5 mmol) in DMF (70 mL) was heated to 80 °C. After the mixture turned dark brown, **16** (1.6 g, 3.1 mmol) was added and the reaction mixture stirred for 10 h. The reaction mixture was extracted with water and ethyl acetate. The organic phase was concentrated and purified by column chromatography with 1:1:1 ethyl acetate/ethyl ether/ hexane to afford 1.3 g (45%) of product as a colorless oil: ^{1}H NMR (CDCl$_3$) δ (CDCl$_3$) 7.82 (m, 2 H), 7.72 (m, 2 H), 5.75 (m, 1 H), 4.95 (m, 2 H), 4.18 (t, 2 H, *J* = 6.8 Hz), 3.5−3.7 (m, 16 H), 3.32−3.45 (m, 4 H), 2.0 (m, 2 H), 1.80−1.85 (m, 2 H), 1.5−1.59 (m, 4 H), 1.2−1.45 (br, 16 H).

S-11-(1,3-Dioxoisoindolin-2-yloxy)undecyl Ethanethioate (18). A solution of **17** (1.3 g, 2.2 mmol) and a catalytic amount of azobis(isobutylnitrile) in dry THF was heated to reflux. After observing N$_2$ bubbles, thioacetic acid (0.33 mL, 4.4 mmol) was added to the reaction mixture and the mixture was stirred under reflux for 6 h. The reaction mixture was concentrated and separated by column chromatography with 1:0.7:0.3 ethyl acetate/hexane/ ethyl ether to afford 400 mg (71%) of product as a pale yellow oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2 H), 7.72 (m, 2 H), 4.37 (t, 2 H, *J* = 6.8 Hz), 3.84 (t, 2 H, *J* = 2 Hz), 3.63 (t, 2 H, *J* = 2 Hz), 3.50−3.62 (m, 14 H), 3.41 (t, 2 H, *J* = 2 Hz), 2.83 (t, 2 H, *J* = 6 Hz), 2.30 (s, 3 H), 1.80−1.85 (m, 2 H), 1.5−1.59 (br, 6 H), 1.2−1.45 (br, 18 H).

2-(29-Mercapto-7,10,13,16,19-pentaoxanonacosyloxy)isoindoline-1,3-dione (19). To a solution of **18** (400 mg, 0.61 mmol) in EtOH (10 mL) was added 12 N HCl (12 mmol, 1 mL) and the mixture was heated under reflux for 3 h. The reaction mixture was concentrated and dissolved in ethyl acetate. The organic phase was washed with a saturated aqueous solution of NaHCO$_3$, water, brine, and sodium sulfate. The organic phase was concentrated to afford 370 mg of product (~100%) as a transparent oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2 H), 7.72 (m, 2 H), δ 4.17 (t, 2 H, *J* = 6.8 Hz), 3.67 (t, 2 H, *J* = 2 Hz), 3.50−3.62 (m, 16 H), 3.41 (t, 2 H, *J* = 2 Hz), 2.5 (q, 2 H, *J* = 6 Hz), 1.80−1.85 (m, 2 H), 1.5−1.59 (br, 6 H), 1.2−1.45 (br, 18 H).

2-(60-Hydroxy-7,10,13,16,19,42,45,48,51,54-decaoxa-30,31-dithi-ahexacontyloxy)isoindoline-1,3-dione (21). To a solution of **19** (370 mg, 0.60 mmol) in methylene chloride (20 mL) was added **20** (280 mg, 0.50 mmol). The reaction was stirred under reflux for 3 h. The reaction mixture was concentrated and separated by column chromatography with 1:1 ethyl acetate/ethyl ether (5% MeOH) to afford 134 mg of product (25%) as a transparent oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2 H), 7.72 (m, 2 H), 4.17 (t, 2 H, *J* = 6.8 Hz), 3.67 (t, 2 H, *J* = 2 Hz), 3.50−3.65 (m, 32 H), 3.41 (t, 4 H, *J* = 2 Hz), 2.65 (t, 4 H, *J* = 6 Hz), 1.80−1.85 (m, 2 H), 1.5−1.59 (m, 10 H), 1.2−1.45 (br s., 32 H).

60-(Aminooxy)-7,10,13,16,19,42,45,48,51,54-decaoxa-30,31-dithi-ahexacontan-1-ol (13). To a solution of **21** (134 mg, 0.12 mmol) in DCM (10 mL) was added hydrazine (0.4 mL of a 1.0 M solution in THF, 0.4 mmol) and the mixture stirred at room temperature for 3 h. The reaction mixture was filtered and the filtrate was concentrated followed by washing with a saturated NH$_4$Cl aqueous solution. The reaction mixture was concentrated to afford 102 mg (90%) of product as a colorless oil: ^{1}H NMR (CDCl$_3$) 3.67 (t, 2 H, *J* = 2 Hz), 3.50−3.65 (m, 34 H), 3.41 (t, 4 H, *J* = 2 Hz), 2.65 (t, 4 H, *J* = 6 Hz), 1.80−1.85 (m, 2 H), 1.5−1.59 (m, 10 H), 1.2−1.45 (br s., 32 H); ESI mass in CH$_2$Cl$_2$ calcd 873.6, found 873.6.

Other Methods. *Preparation of Monolayers.* The glass substrate (75 mm × 25 mm) from Fisher was immersed into a piranha solution (1:1 volume ratio of H$_2$SO$_4$ and 31.6% hydrogen peroxide) for 4 h and cleaned thoroughly with deinoized water and absolute ethanol. Electron-beam deposition of Titanium (10 nm for electrochemical measurements and 3 nm for cell adhesion studies) and gold (100 nm for electrochemical measurements and 12 nm for cell patterning studies) on the glass substrate was performed consecutively. The prepared gold substrate was cut into 1.25 × 1.5 cm^2 and washed with absolute ethanol. All substrates were treated with a solution of the corresponding alkanethiols in absolute ethanol (1 mM) for 12 h and then rinsed with ethanol.

Electrochemical Measurements. A Bioanalytical Systems CV-100W potentiostat was used for all electrochemical measurements. HClO$_4$ solution (1 M) was used as an electrolyte solution. A platinum wire was used as the counter electrode and Ag/AgCl was used as a reference electrode. All cyclic voltammograms were scanned at 100 mV/s. The substrates of SAM molecule **6** were immersed in the FcCHO solution (150 mM) and then transferred to 1 M HClO$_4$ solution in order to measure every 4 min. A SAM of **7** was immersed in a FcCHO solution (150 mM) after UV deprotection for 0 and 60 min.

Photodeprotection of NVOC-oxyamine (7) Monolayers. The photodeprotection was performed in a saturated semicarbazide solution to quench the aldehyde containing photobyproduct. The saturated solution was purged by N$_2$ for 30 min before reaction.

Cell Culture. Swiss Albino 3T3 cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (Gibco) supplemented with 10% calf bovine serum and penicillin/streptomycin. The cells on the flask bottom were detached by treating with a solution of 0.05% trypsin/0.53 mM EDTA for 2 min in the incubator (37 °C in a humidified 5% CO$_2$ atmosphere). Then serum-free media was added and cells were precipitated by centrifugation at 1000 rpm for 10 min. The precipitate was resuspended in serum-free culture medium (~50 000 cells/mL) and added onto the substrates for 2 h. The substrate was then transferred to serum-containing media.