Electroactive Self-Assembled Monolayers that Permit Orthogonal Control over the Adhesion of Cells to Patterned Substrates†

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This article describes an electroactive substrate that displays two independent dynamic functions for controlling the adhesion of cells. The approach is based on self-assembled monolayers on gold that are patterned into regions presenting the Arg-Gly-Asp peptide cell adhesion ligand. The patterned regions differ in the electrochemical properties of the linkers that tether the peptides to the monolayer. In this work, three distinct chemistries are employed that provide for release of the ligand on application of a negative potential, release of the ligand on application of a positive potential, and no change in response to a potential. Cells were allowed to attach to a monolayer patterned into circular regions comprising the three chemistries. Treatment with electric potentials of 650 or −650 mV resulted in the selective release of adherent cells only from regions that display the relevant electroactive groups. This example establishes the preparation of dynamic substrates with multiple functions and will be important to preparing model cultures derived from multiple cell types, with control over the temporal interactions of each cell population.

Introduction

The development of strategies for controlling the interface between materials and adherent cells remains an important challenge in materials science.[1,2] Surfaces that are tailored to influence the behaviors of cells are important in cell-based sensors, drug screening, and fundamental studies of cell migration.[3−5] We have introduced an approach for creating dynamic substrates, based on self-assembled monolayers that present electroactive groups, wherein the activities of immobilized ligands can be switched on and off in response to applied potentials.[5−12] These methods give real-time control over the molecular interactions that mediate the adhesion of cells and, together with related strategies using polymeric substrates, give unprecedented control over assembling and manipulating the positions of one or more cell types on a common substrate.[13−15] This article extends previous work by demonstrating a monolayer that is patterned into distinct regions that present cell-adhesive ligands that are tethered to the monolayer by way of different redox-active tethers. By using tethers that respond to positive or negative applied potentials, the adhesion of different populations of cells can be manipulated independently.[9] Furthermore, we report the design and synthesis of alkanethiol reagents that incorporate these electroactive moieties and that are substituted with a maleimide group. These reagents permit a broad class of biologically active ligands to be immobilized onto a monolayer and thereby increase the scope of applications that can be addressed with dynamic substrates. The work that follows in this article concerns the application of the patterned monolayers to control the adhesion of cells.

Our approach utilizes self-assembled monolayers (SAMs) on gold that incorporate alkanethiolates terminated in electroactive moieties that respond to electrical potentials by releasing attached ligands (Figure 1). We prepare monolayers from alkanethiolates that incorporate the electroactive groups and maleimide groups, which can be used to immobilize ligands. In this way, we introduce the ligands after the monolayer has been assembled.[16] The tri(ethylene glycol) groups of the monolayer serve to prevent the nonspecific attachment of cells to the monolayer.[17] The demonstration in this article is based on two electroactive groups that release the tethered ligands in response to either reductive or oxidative potentials (Chart 1). In the first case, an electroactive quinone ester (**QE**) undergoes reduction to give the corresponding hydroquinone, which then undergoes a cyclization reaction to give a lactone with release of the ligand (Figure 1A). In the second case, an O-silyl hydroquinone (**SHQ**) undergoes electrochemical oxidation to give a benzoquinone, with hydrolysis of the silyl ether and selective release of the ligand (Figure 1B). In all cases, we used as the ligand the peptide Cys-Gly-Arg-Gly-Asp-Ser (CGRGDS). This peptide contains the RGD sequence that serves as a ligand for the receptor-mediated adhesion of cells.[18] We have shown that monolayers that present this ligand support the adhesion and spreading of mammalian cells.[19,20]

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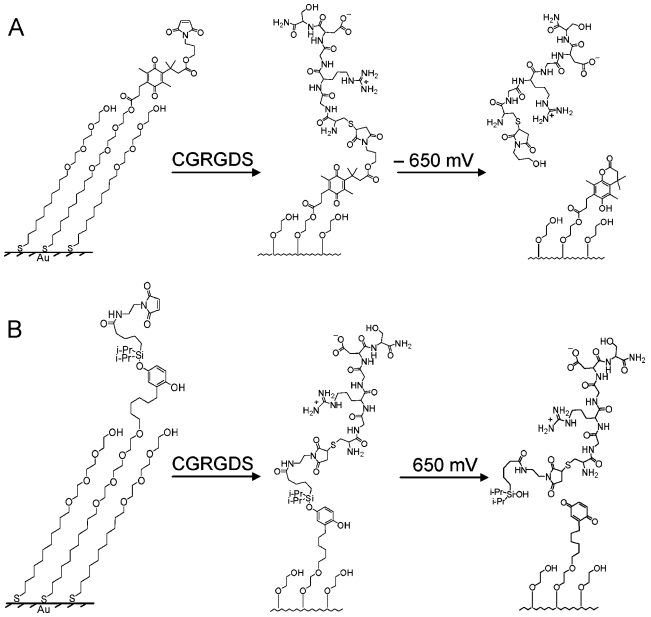
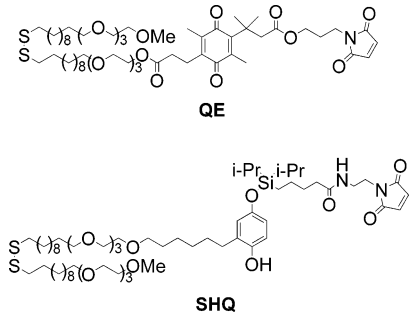
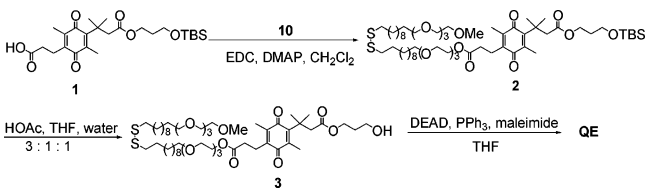


Figure 1. Molecular strategies used to prepare dynamic substrates that can release tethered ligands in response to applied potentials. (A) A monolayer presenting a maleimide tethered to an electroactive quinone ester reacts with a cysteine-terminated RGD peptide to immobilize the ligand. Upon electrochemical reduction of the quinone to the corresponding hydroquinone, a cyclization reaction ensues to give a lactone with the release of the RGD ligand. (B) A monolayer presenting a maleimide group tethered to an electroactive *O*-silyl hydroquinone is used to immobilize a cysteine-terminated RGD peptide and undergoes electrochemical oxidation to give a benzoquinone, with the hydrolysis of the silyl ether and the selective release of RGD ligands.

Chart 1



Scheme 1. Synthesis of Electroactive Quinone Ester QE



Results and Discussion

Synthesis. The synthesis of electroactive quinone ester **QE** started with intermediate **1**, which was converted to ester **2** by coupling with hydroxyl-terminated disulfide **10**.[21] The *tert*-butyldimethylsilyl (TBS) protecting group was removed under acidic conditions to reveal the alcohol, which was then coupled to the maleimide with a Mitsunobu reaction to give **QE** (Scheme 1). Separately, **4** was treated with trifluoroacetic acid (TFA) and a triethylsilane scavenger to deprotect the thiol. The resulting thiol was coupled with activated disulfide **9** to give disulfide **5**. The hydroquinone was treated with a chlorosilane reagent to provide **6,** which was then reacted with the aminoethylmaleimide to provide **SHQ** (Scheme 2). To prepare intermediate **9**, the terminal alcohol of **7** was methylated by treatment with sodium hydride followed by iodomethane. The trityl group was removed to provide thiol **8**, which was then activated with aldrithiol affording **9**. Intermediate **10** was accessed by treatment of **9** with a tri(ethylene glycol)-terminated alkanethiol (Scheme 3).

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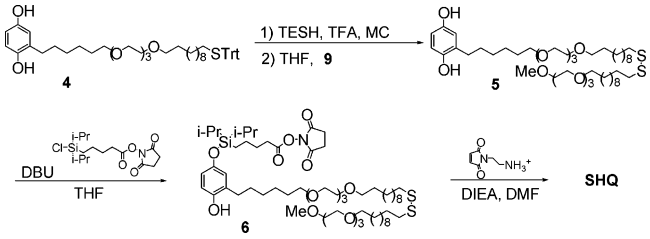
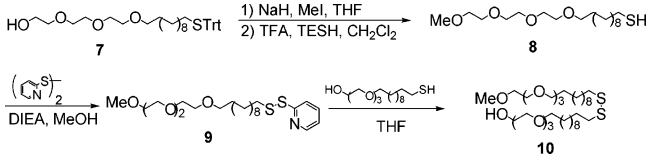
Scheme 2. Synthesis of Electroactive *O*-Silyl Hydroquinone SHQ



Scheme 3. Synthesis of Intermediates 9 and 10



Characterization of Electrochemical Reactions. We prepared a self-assembled monolayer from a mixture of a QE-terminated and a tri(ethylene glycol)-terminated alkanethiol in a ratio of 1:3. The mass spectrum of this monolayer showed two major peaks at *m*/*z* 1134.5 and 1548.3 (Figure 2A, top).[22] These peaks correspond to the mixed disulfide derived from a tri(ethylene glycol)-terminated and a QE-terminated alkanethiolate and a symmetric disulfide derived from the QE-terminated alkanethiolate, respectively. After treatment with the CGRGDS peptide (1 mg/mL in phosphate-buffered saline (PBS) at pH 7.4) for 30 min, the original peaks were absent and gave rise to new peaks corresponding to the peptide-conjugated products at *m*/*z* 1357.1 and 1706.4 (Figure 2A, middle). An identical monolayer was subjected to an electrical potential of −650 mV (vs a Ag/AgCl reference) for 4 min and analyzed by mass spectrometry, which gave two peaks corresponding to electrochemical reaction products at *m*/*z* 982.0 and 1242.0 (Figure 2A, bottom).

In a separate experiment, we prepared a monolayer presenting SHQ groups and repeated the same line of experiments. The mass spectrum of the initial monolayer displayed peaks at *m*/*z* 1236.2 and 1752.6 corresponding to the SHQ-containing disulfides (Figure 2A, top). Treatment of the monolayer with the CGRGDS peptide gave new peaks at *m*/*z* 1457.9 and 1806.9 that corresponded to the peptide-conjugated products (Figure 2B, middle). Upon application of electrical potentials of 650 mV for 4 min and −550 mV for 30 s, the original peaks were absent and gave rise to a new peak that represents the hydroquinone-terminated disulfide at *m*/*z* 899.9 (Figure 2B, bottom).

Preparation of Electroactive Substrates. To demonstrate that this chemistry can be used to prepare a dynamic substrate that releases ligands and therefore adherent cells at two different potentials, we patterned a monolayer into several circular regions with the two electroactive alkanethiolates and a nonelectroactive

(22) Sodium adducts of disulfides are the major species that are observed in MALDI spectra of SAMs of alkanethiolates. See Su, J.; Mrksich, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4715 and Trevor, J. L.; Lykke, K. R.; Pellin, M. J.; Hanley, L. *Langmuir* **1998**, *14*, 1664.

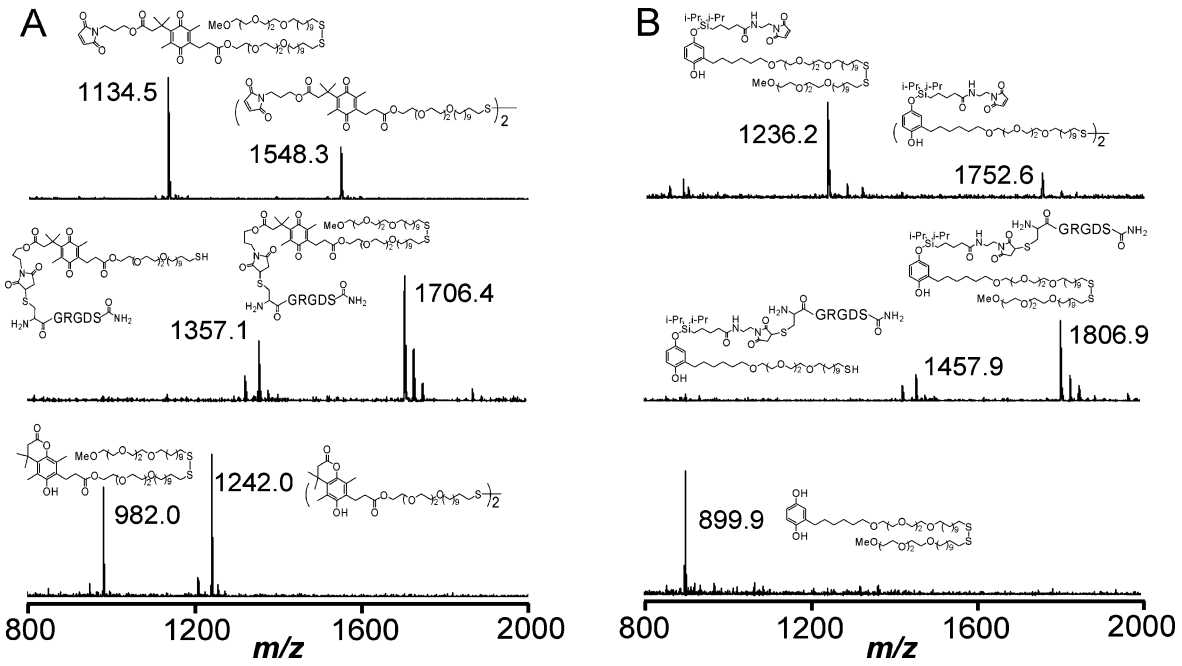


Figure 2. Characterization of the immobilization and electrochemical release of RGD ligands using MALDI-TOF MS. (A) A monolayer presenting QE groups gave two molecular ion peaks corresponding to disulfides as shown above the peaks (top). After treatment with a CGRGDS solution, the original peaks were absent and gave rise to new peaks corresponding to the peptide-conjugated products (middle). The identical monolayer was treated with an electrical potential of −650 mV for 4 min and analyzed by mass, which gave two peaks corresponding to electrochemical reaction products. (B) The mass spectrum of the monolayer presenting SHQ groups displayed peaks at *m*/*z* 1236.2 and 1752.6 corresponding to the SHQ-containing disulfides (top). Treatment of the monolayer with the CGRGDS peptide gave new peaks at *m*/*z* 1457.9 and 1806.9 corresponding to the peptide-conjugated products (middle). After the application of electrical potentials of 650 mV for 4 min and −550 mV for 30 s, the original peaks disappeared and gave rise to a new peak corresponding to the hydroquinone-terminated disulfide at *m*/*z* 899.9 (bottom).

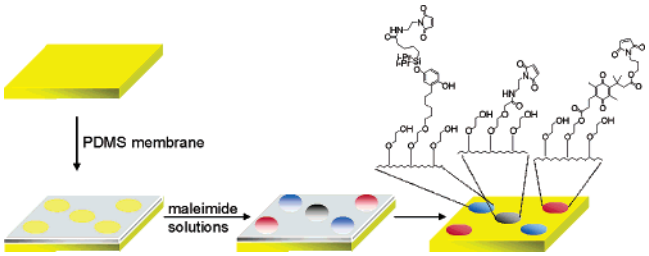


Figure 3. Preparation of an electroactive substrate that combines two dynamic functions. A monolayer patterned into several regions was prepared as described in the text.

alkanethiolate, all of which present the RGD ligand for cell adhesion. We prepared a thin PDMS membrane with five holes (500 μm in diameter) and applied this structure to a gold-coated glass slide. Three different solutions of maleimide-containing disulfides (0.5 μL, mixed with tri(ethylene glycol)-terminated disulfide in a ratio of 1:200, total concentration of 0.2 mM in EtOH) were applied to the holes according to the scheme in Figure 3. After 5 min, the membrane was removed, and the monolayer was immersed in an ethanolic solution of tri(ethylene glycol)-terminated disulfide (1 mM) for 12 h. The RGD peptide was then immobilized on this monolayer by treatment with a solution of CGRGDS for 30 min. With this procedure, the initial assembly of the monolayers in the wells was not complete, but the final incubation in a solution containing the tri(ethylene glycol)-terminated disulfide resulted in complete monolayers. Furthermore, we used disulfide reagents rather than the more common alkanethiols because the latter are not compatible with the maleimide groups of the reagents.

Selective Release of Adherent Cells. Swiss 3T3 fibroblast cells were harvested and allowed to attach to the patterned monolayer. An optical micrograph 2 h after incubation showed

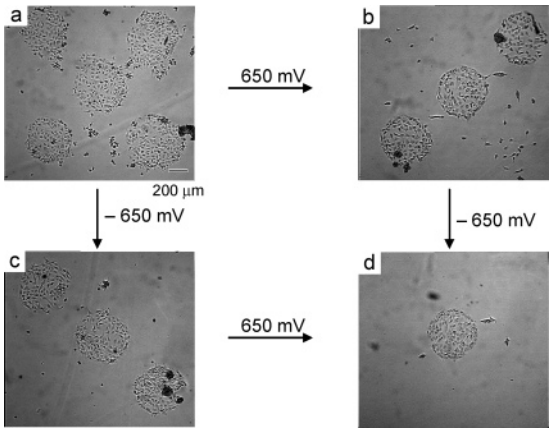


Figure 4. Demonstration of the selective release of adherent cells under electrochemical control. An optical micrograph showed that Swiss 3T3 fibroblast cells adhered to the circular regions that presented RGD ligands (a). After treatment with an electrical potential of 650 mV, cells were efficiently released only from regions presenting the electroactive *O*-silyl hydroquinone (b), whereas an electrical potential of −650 mV resulted in the release of cells only from regions presenting the electroactive quinone ester (c). The subsequent application of electrical potentials of −650 mV to the monolayer in panel b and 650 mV to the monolayer in panel c caused an additional release of cells from regions that present the remaining electroactive groups (d). Note that the image shown in panel c was taken from a separate experiment.

that cells adhered only to the circular regions that present RGD ligands (Figure 4a). An electrical potential of 650 mV was applied to the monolayer, and cells were efficiently released only from regions that present peptides tethered by way of the electroactive O-silyl hydroquinone groups (Figure 4b). In this experiment, the substrate was incubated for 1 h after the electrochemical treatment,

and then the medium was exchanged prior to acquiring the image. A separate culture prepared in the same manner was subjected to an electrical potential of −650 mV and resulted in the release of cells only from regions that present the electroactive quinone ester groups (Figure 4c). For this experiment, the cells were incubated for 5 min after application of the potential, after which the medium was exchanged and the image was acquired. In both cases, the monolayers were not rinsed or otherwise treated to assist the detachment of the cells. Subsequent application of an electrical potential of −650 mV to the monolayer in panel b or 650 mV to the monolayer in panel c caused an additional release of cells from regions that present remaining electroactive groups (Figure 4d). In both cases, cells remained adherent to the monolayer only in the regions presenting the nonelectroactive RGD ligands. This example illustrates that electrochemical strategies can be used to release cells selectively and noninvasively.

This article emphasizes the development and combination of surface chemistry that provides for orthogonal control of the presentation of two ligands on a monolayer. By utilizing electrochemically active tethers that respond to either positive or negative potentials, it is possible to trigger the release of either ligand without manipulating the second ligand (even in cases where the second ligand is structurally identical to the first). We used relatively simple PDMS templates to pattern the assembly of monolayers in regions that are 500 μm in size. The PDMS templates can be made to have smaller regions, but at sizes below 200 μm it is difficult to apply solutions of alkanethiols to the wells manually. For the preparation of surfaces that are patterned with different monolayers on smaller scales, the use of dip-pen nanolithography[23] or microfluidic flow cells[24] represents effective approaches. Finally, the electroactive alkanethiol reagents described in this article are substituted with maleimide groups and therefore provide a general route toward the immobilization of a broad range of ligands, provided that the ligands can be prepared with a primary thiol functional group.

Conclusions

The development of dynamic substrates that can modulate the ligand−receptor interactions with adherent cells has brought about new opportunities for fundamental studies of cell behavior and for integrating cells with microfabricated devices. The protein matrix to which cells adhere not only provides a structural scaffold for organizing cells into tissue but also provides cells with several molecular signals that are required to direct or maintain regulatory functions. The development of dynamic substrates has now made it possible to mimic the signal modulation from the protein matrix and therefore to study these complex problems.[25,26] In applications, it is clear that materials that can modulate their biological properties will be important in the development of scaffolds for tissue engineering and perhaps for the surfaces of medical devices.[27,28] Indeed, with the acceleration of research in stem cell biology, these active surfaces may prove instrumental in directing cell maturation.[30] Other applications include the development of assays that require control over cell migration and the patterning of multiple cell types to build functional models of multicellular organs.

Experimental Section

Preparation of SAMs. Titanium (5 nm) and then gold (15 nm) were evaporated onto glass coverslips (25 mm × 25 mm) using an electron beam evaporator (Thermionics VE-100) at a rate of 0.2−0.4 nm/s and at a pressure of 9×10^{-7} Torr. A thin PDMS membrane with five holes (500 μm in diameter) was placed on the gold-coated coverslip. Solutions of maleimide-terminated disulfides (0.5 μL, mixed with tri(ethylene glycol)-terminated disulfide in a ratio of 1:200, total concentration of 0.2 mM in EtOH) were applied to the monolayer. After 5 min, the membrane was removed, and the monolayer was immersed in an ethanolic solution of tri(ethylene glycol)-terminated disulfide (1 mM) for 12 h. The monolayer was rinsed with ethanol, dried under a stream of nitrogen, and treated with a solution of the CGRGDS peptide(1 mg/mL, in PBS, at pH 7.4) for 30 min. The monolayer was rinsed with deionized water and ethanol and dried under a stream of nitrogen.

MALDI-TOF Analysis. Mass analysis was performed using a Voyager DE-PRO Biospectrometry mass spectrometer (Applied Biosystems, Framingham, MA). A 337 nm nitrogen laser was used as a desorption−ionization source, and all of the spectra were acquired with a 20 kV accelerating voltage using the reflector mode in positive ions with 2,4,6-trihydroxyacetophenone (1 μL, 5 mg/mL in acetonitrile) as a matrix.

Cell Culture. A PDMS cylinder (inner diameter of 130 mm, outer diameter of 220 mm, and height of 150 mm) was placed on the monolayer. Swiss Albino 3T3 cells (25 000−50 000) were seeded in the PDMS cylinder, and all cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Cells were grown in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin.

Electrochemistry. Electrochemistry was performed with a Bioanalytical Systems CV-50W potentiostat using PBS (10 mM phosphate and 150 mM NaCl, pH 7.4) or cell culture media as the electrolyte. A custom-designed electrochemical cell was used for measurement with the monolayer as the working electrode, a Pt wire as the counter electrode, and a Ag/AgCl reference electrode.

3-{2,5-Dimethyl-3,6-dioxo-4-[tri(ethylene glycol)undecane disulfide]-cyclohexa-1,4-dienyl}-3-methyl-butyric Acid 3-(*tert*-Butyl-dimethyl-silanyloxy)-propyl Ester (2) To a solution of 3-[4-(2-carboxy-ethyl)-2,5-dimethyl-3,6-dioxo-cyclohexa-1,4-dienyl]-3-methyl-butyric acid 3-(*tert*-butyl-dimethyl-silanyloxy)-propyl ester (**1**) (62 mg, 0.13 mmol) dissolved in CH_2Cl_2 were added disulfide **10** (84 mg, 0.13 mmol), EDC (27 mg, 0.13 mmol), and DMAP (2 mg). The reaction mixture was stirred for 2 h, washed with 10 mL of water and 10 mL of brine, and then dried over $MgSO_4$. After concentration, the residue was purified by column chromatography (1:1 hex/EtOAc) to give 46 mg (31%) of the adduct as a pale-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.03 (s, 6H), 0.87 (s, 9H), 1.0−1.3 (m, 28H), 1.41 (s, 6H), 1.57 (quint, J = 7.2 Hz, 2H), 1.66 (quint, J = 7.4 Hz, 2H), 1.75 (quint, J = 6.25 Hz, 2H), 1.99 (s, 3H), 2.12 (s, 3H), 2.48 (t, J = 8.0 Hz, 2H), 2.67 (t, J = 7.3 Hz, 4H), 2.73 (t, J = 7.6 Hz, 2H), 2.95 (s, 2H), 3.38 (s, 3H), 3.40−3.65 (m, 32H), 4.05 (t, J = 6.4 Hz, 2H), 4.23 (t, J = 4.9 Hz, 2H).

3-{2,5-Dimethyl-3,6-dioxo-4-[tri(ethylene glycol)undecane disulfide]-cyclohexa-1,4-dienyl}-3-methyl-butyric Acid 3-Propyl Ester (3). Conjugate **2** (46 mg, 0.04 mmol) was dissolved in 10 mL of 3:1:1 AcOH/THF/H_2O. The reaction mixture was stirred overnight. Evaporation of the solvent gave 40 mg (97%) of product **3** as a pale-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.0−1.3 (m, 28H), 1.41 (s, 6H), 1.57 (quint, J = 7.2 Hz, 2H), 1.66 (quint, J = 7.4 Hz, 2H), 1.78 (quint, J = 6.25 Hz, 2H), 1.99 (s, 3H), 2.12 (s, 3H), 2.48 (t, J = 8.0 Hz, 2H), 2.67 (t, J = 7.3 Hz, 4H), 2.72 (t, J = 7.6 Hz, 2H), 2.97 (s, 2H), 3.37 (s, 3H), 3.40−3.65 (m, 32H), 4.12 (t, J = 6.4 Hz, 2H), 4.22 (t, J = 4.9 Hz, 2H).

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QE. To a solution of alcohol **3** in THF (5 mL) were added DEAD (2 μL), triphenylphosphine (3.5 mg), and maleimide (2 mg). The reaction mixture was stirred for 1 h and reduced to give an oil. Silica gel chromatography (1:1 hex/EtOAc) gave 4 mg (34%) of the adduct as a pale-yellow oil.^{1}H NMR (500 MHz, $CDCl_3$): δ 1.0−1.3 (m, 28H), 1.41 (s, 6H), 1.57 (quint, J = 7.2 Hz, 2H), 1.66 (quint, J = 7.4 Hz, 2H), 1.87 (quint, J = 6.25 Hz, 2H), 1.99 (s, 3H), 2.12 (s, 3H), 2.48 (t, J = 8.0 Hz, 2H), 2.67 (t, J = 7.3 Hz, 4H), 2.72 (t, J = 7.6 Hz, 2H), 2.97 (s, 2H), 3.37 (s, 3H), 3.40−3.65 (m, 32H), 3.96 (t, J = 6.4 Hz, 2H), 4.22 (t, J = 4.9 Hz, 2H), 6.70 (s, 2H).

2-(6-(2-(2-(2-(11-(2-(11-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)undecyl)disulfanyl)undecyloxy)ethoxy)ethoxy)ethoxy)hexyl)-benzene-1,4-diol (5). To a solution of 2-(6-(2-(2-(2-(11-(tritylthio)-undecyloxy)ethoxy)ethoxy)ethoxy)hexyl)benzene-1,4-diol (**4**) (167 mg, 0.22 mmol) in 10% TFA/methylene chloride (v/v, 5 mL) was added triethylsilane (50 μL) as a carbocation scavenger. The reaction mixture was stirred for 2 h and then concentrated to an oil. The residue was purified by silica gel chromatography (1:1 hex/EtOAc) to give 105 mg (92%) of 2-(6-(2-(2-(2-(11-mercaptoundecyloxy)-ethoxy)ethoxy)ethoxy)hexyl)benzene-1,4-diol as a clear oil.^{1}H NMR (500 MHz, $CDCl_3$): δ 1−1.6 (br, 26H), 2.52 (t, J = 7.3 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 3.40−3.65 (m, 16H), 6.54 (m, 1H), 6.63 (m, 2H).

The resulting product (105 mg, 0.20 mmol) was dissolved in THF (5 mL) and 92 mg (0.20 mmol) of 2-(2-(11-(2-(2-(2-methoxyethoxy)-ethoxy)ethoxy)undecyl)disulfanyl)pyridine (**9**) was added. The reaction mixture was stirred for 2 days and purified with silica gel chromatography (1:1 hex/EtOAc then EtOAc only) to give 156 mg (90%) of the adduct as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1−1.6 (br, 44H), 2.52 (t, J = 7.3 Hz, 2H), 2.67 (t, J = 7.5 Hz, 4H), 3.37 (s, 3H), 3.40−3.65 (m, 30H), 6.54 (m, 1H), 6.63 (m, 2H).

1-Hydroxy-4-diisopropylsiloxy Pentanoic NHS Ester-2-(6-(2-(2-(2-(11-(2-(11-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)undecyl)-disulfanyl)undecyloxy)ethoxy)ethoxy)ethoxy)hexyl)benzene (6). To a solution of **5** (68 mg, 0.078 mmol) in THF (10 mL) was added DBU (23 μL, 0.155 mmol), followed by 5-(chlorodiisopropylsilyl) pentanoic NHS ester (60 mg, 0.11 mmol). The reaction mixture was stirred for 4 h and then diluted with EtOAc, washed with saturated NH_4Cl and then brine, and dried over $MgSO_4$. The organic layer was concentrated and purified by column chromatography with 2:1 ethyl acetate/hexane to give 55 mg (52%) of **6** as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (m, 2H), 0.88 (m, 2H), 1.04 (m, 12H), 1.1−1.6 (br, 48H), 2.54 (m, 4H), 2.66 (t, J = 7.5 Hz, 4H), 2.83 (brm, 4H), 3.37 (s, 3H), 3.40−3.65 (m, 30H), 6.53 (m, 1H), 6.60 (m, 2H).

SHQ. To a solution of NHS ester **6** (4 mg, 0.003 mmol) in THF (2 mL) was added DIEA (2.5 μL), followed by 1-(2-aminoethyl)-1H-pyrrole-2,5-dione (1.5 mg). The reaction mixture was stirred overnight at 50 °C and then diluted with EtOAc, washed with saturated NH_4Cl and then brine, and dried over $MgSO_4$. The organic layer was concentrated and purified by column chromatography with 2:1 ethyl acetate/hexane to give 2 mg (55%) of the adduct as a pale-yellow oil.^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (m, 2H), 0.87 (m, 2H), 1.04 (m, 12H), 1.1−1.6 (br, 48H), 2.07 (t, J = 7.5 Hz, 2H), 2.54 (t, J = 7.5 Hz, 2H), 2.67 (t, J = 7.5 Hz, 4H), 3.38 (s, 3H), 3.40−3.65 (m, 34H), 6.53 (m, 1H), 6.60 (m, 2H), 6.71 (s, 2H).

11-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)undecane-1-thiol (8). To a solution of a *S*-trityl tri(ethylene glycol)-terminated alkanethiol **7** (939 mg, 1.62 mmol) in THF (10 mL) was added NaH (162 mg) portionwise at 0 °C. The reaction mixture was stirred for 2 h at room temperature, followed by the addition of iodomethane (300 μL), and stirred for 30 min at room temperature. The reaction mixture was quenched with water, diluted with 30 mL of EtOAc, washed with saturated NH_4Cl and then brine, and dried over $MgSO_4$. The organic layer was concentrated and purified by column chromatography with 2:1 hexane/ethyl acetate to give 938 mg (98%) of (11-(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)undecyl)(trityl)sulfane as a color-less oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1−1.6 (br, 18H), 2.10 (t, J = 7.3 Hz, 2H), 3.37 (s, 3H), 3.40−3.65 (m, 14H), 7.1−7.4 (m, 15H).

To a solution of the resulting product above (936 mg, 1.58 mmol) in 10% TFA/methylene chloride (v/v, 10 mL) was added triethylsilane (500 μL) as a carbocation scavenger. The reaction mixture was stirred for 2 h. The residue was purified by silica gel chromatography (2:1 hex/EtOAc) to give 530 mg (96%) of the thiol as a clear oil.^{1}H NMR (400 MHz, $CDCl_3$): δ 1−1.6 (br, 18H), 2.48 (q, J = 7.5 Hz, 2H), 3.37 (s, 3H), 3.40−3.65 (m, 14H).

2-(2-(11-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)undecyl)-disulfanyl) Pyridine (9). To a solution of thiol **8** (258 mg, 0.736 mmol) in MeOH was added aldrithiol (178 mg, 0.808 mmol) followed by DIEA (650 μL). The reaction mixture was stirred overnight and purified by column chromatography with 2:1 hexane/ethyl acetate to give 253 mg (78%) of the adduct as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1−1.6 (br, 18H), 2.73 (t, J = 7.5 Hz, 2H), 3.37 (s, 3H), 3.40−3.65 (m, 14H), 7.02 (m, 1H), 7.60 (m, 1H), 7.68 (m, 1H), 8.40 (m, 1H).

2-(2-(2-(11-(2-(11-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)un-decyl)disulfanyl)undecyloxy) ethoxy)ethoxy)ethanol (10). To a solution of 2-(2-(2-(11-mercaptoundecyloxy)ethoxy)ethoxy)ethanol (270 mg, 0.80 mmol) in THF (10 mL) was added disulfide **9** (368 mg, 0.8 mmol). The reaction mixture was stirred for 2 days, reduced by evaporation of solvent, and purified by column chromatography with 1:1 hexane/ethyl acetate to give 422 mg (80%) of the adduct as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1−1.6 (br, 36H), 2.68 (t, J = 7.4 Hz, 4H), 3.37 (s, 3H), 3.40−3.65 (m, 28H).

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