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Acyl chloride-modified amorphous carbon substrates for the attachment of alcohol-, thiol-, and amine-containing molecules

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Abstract

Amorphous carbon thin films are easily deposited at room temperature, readily functionalized with alkene-containing molecules through a UV photochemical reaction, and provide a robust surface capable of supporting array fabrication. Relatively few attachment chemistries for the fabrication of small organic and/or biomolecule arrays on carbon substrates have been described to date. Here, acyl chloride-terminated amorphous carbon substrates were fabricated, characterized, and used to attach alcohol-, thiol-, and amine-containing small molecules. Oligonucleotide arrays of thiol- and amine-modified oligonucleotides were also prepared on these substrates. The hybridization density, average fluorescence signal of hybridized features, and average background fluorescence of oligonucleotide arrays prepared on acyl chloride-modified substrates were compared to the same parameters for oligonucleotide arrays prepared on maleimide- and aldehyde-modified amorphous carbon substrates.

Introduction

Small molecule and/or biomolecule arrays allow multiple chemical reactions to be monitored in a parallel and multiplexed manner. Oligonucleotide arrays ranging from as few as 4 features to as many as several hundred thousand features have been utilized to determine the sequence-specific binding patterns of transcription factors,^{1–3} detect the presence of single nucleotide polymorphisms,^{4, 5} and quantify gene expression and/or copy number.⁶ Combinatorial synthesis efforts have proven useful in preparing libraries of structurally complex molecules from relatively simple starting materials.^{7, 8} High-throughput, array screening of these libraries is advantageous when identifying potential small molecule substrates for individual targets that are not well-characterized.^{8, 9} For example, the compound uretupamine, which modulates the glucose-sensitive genes responsible for regulating the Ure2p protein, was discovered from a microarray containing 3,780 different 1,3-dioxolane molecules.¹⁰

The success of any array-based experiment begins with choosing a suitable substrate and attachment chemistry. An ideal substrate is chemically robust, easily functionalized, and compatible with a wide variety of analytical modalities (fluorescence, surface plasmon resonance, microscopy, electrochemistry, etc). The attachment chemistry must provide a stable interface between the substrate and molecules of interest. Glass and gold substrates are commonly used due to their well-defined functionalization methods: the silanization of glass and the formation of self-assembled monolayers (SAMs) on gold. Carbon-based materials (glassy carbon, nanocrystalline diamond thin films, amorphous carbon thin films, etc.) are

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Supporting Information: Detailed description of the small molecule synthesis and characterization as well as the oligonucleotide array fabrication (PDF) is available free of charge via the Internet at <http://pubs.acs.org>.

attractive alternative substrates, as they offer superior stability over their glass, gold, and silicon analogs when exposed to prolonged incubations in aqueous solutions at elevated temperatures and/or serial hybridizations.^{11–14}

Recently, amorphous carbon substrates functionalized with terminal maleimide, hydroxyl, and aldehyde groups have been employed in the fabrication of oligonucleotide arrays.^{11, 14, 15} Maleimide- and aldehyde-modified substrates limit the types of molecules that can be attached to the surface requiring a thiol or amine group, respectively. A surface chemistry capable of reacting with a variety of functional groups would permit many different molecule types to be arrayed on a single substrate. Acyl chlorides are an attractive option as they readily react with alcohols, thiols, and amines to form ester, thioester, and amide linkages. Previously, silicon surfaces were modified with acyl chloride groups, each method requiring special reaction conditions and/or equipment. Hussani *et al.* reported a photochemical means of forming acyl chloride terminated silicon surfaces by adding gaseous oxalyl chloride to an evacuated quartz cell containing an alkyl-modified Si(111) surface and irradiating with 367nm light.¹⁶ Lua *et al.* modified silicon surfaces with suberoyl chloride under dry conditions, in which one of the acyl chlorides reacted with the silicon surface while the second acyl chloride was reacted with amine-containing molecules.¹⁷ Duevel, *et al* have also fabricated acyl chloride-modified gold SAMs.¹⁸

Here a method of preparing amorphous carbon substrates terminated with acyl chloride moieties is reported. First, hydrogen-terminated amorphous carbon substrates were functionalized with undecylenic acid, yielding terminal carboxylic acid groups. The substrates were placed in a sealed glass vial, heated, thionyl chloride introduced, and the acyl chloride groups formed via interaction of the gaseous thionyl chloride with the carboxylic acid-modified surface. This method of forming acyl chloride-modified substrates does not require the use of specialized glassware, ultraviolet irradiation, and/or dry box conditions making it accessible to many laboratories. Scheme 1 summarizes the process.

Three different 4-(trifluoromethyl)benzyl molecules (Scheme 2) as well as thiol- and amineo-modified oligonucleotides were attached to the acyl chloride-modified substrates. The small molecules served as model compounds to confirm the formation of ester, thioester, and amide linkages. Simple oligonucleotide arrays fabricated on these substrates were compared to oligonucleotide arrays prepared on maleimide- and aldehyde-modified amorphous carbon substrates using previously published attachment schemes.^{11, 19}

Materials and methods

Materials and Reagents

All chemical reagents were purchased from Sigma-Aldrich and used without further purification unless otherwise noted.

Oligonucleotides used in this work (Table 1) were synthesized by Integrated DNA Technologies (Coralville, IA) using standard phosphoramidite chemistries. Surface-bound probe oligonucleotides were modified with either a 3'-amine (3AmM) or 3'-thiol (C3 S-S) separated from the oligonucleotide sequence of interest with 15 thymidine (dT) residues. The 15 dT spacer increases the hybridization efficiency by providing sufficient distance between the surface and the oligonucleotide region of interest.²⁰ Complementary oligonucleotides were synthesized with a 3' 6-carboxyfluorescein moiety (6-FAM). The amine-modified probe and complementary oligonucleotides were purified, prior to use, by reverse-phase, binary gradient elution HPLC (SCL-10ADVP Shimadzu; Columbia, MD). Thiol-modified probe oligonucleotides were reduced with dithiothreitol (DTT) prior to HPLC purification. Purified oligonucleotides were stored dry, under nitrogen at -20 °C, until needed. All oligonucleotide

concentrations were determined by absorption measurements at 260nm (HP8453 UVVIS; Santa Clara, CA).

Substrate preparation

Amorphous carbon thin films (15nm) were deposited on commercially prepared gold-coated glass slides by DC magnetron sputtering a graphite source at a base pressure of at least 2×10^{-6} Torr and an argon pressure of 3 mTorr (Denton Vacuum, Moorestown, NJ). The amorphous carbon film thicknesses were measured with a calibrated quartz crystal microbalance, located in the sputtering instrument. The gold-coated slides contained a 100nm thick gold film with a 5nm chromium underlayer to provide better adhesion to the glass substrate (Evaporated Metal Films Co, Ithaca, NY). Prior to use, the gold-coated slides were thoroughly rinsed with deionized (DI) water and dried under a stream of nitrogen gas.

Substrate functionalization

Prior to photochemical functionalization, each amorphous carbon thin film was hydrogen-terminated in a 13.56MHz inductively coupled hydrogen plasma for 12 minutes (10 Torr H₂, room temperature). Next, 30 μ L of neat alkene liquid was placed directly onto the newly hydrogen-terminated surface and covered with a quartz coverslip. The substrates were irradiated with a low-pressure mercury lamp ($\lambda_{\text{max}} = 254\text{nm}$, 0.35mW/cm^2) under nitrogen purge, rinsed with ethanol and DI water, dried under a stream of nitrogen gas, and stored in a desiccator until needed. Undecylenic acid, trifluoroacetic acid protected 10-aminodec-1-ene (TFAAD), and 2-(10-undecen-1-yl)-1,3-dioxolane molecules were used to prepare carboxylic acid-, amine-, and aldehyde-modified substrates, respectively. The TFAAD and 2-(10-undecen-1-yl)-1,3-dioxolane molecules were synthesized and purified according to previously reported methods.^{19, 21}

The acyl chloride-modified substrates were prepared by reacting the carboxylic acid groups with gaseous thionyl chloride (Scheme 1). Carboxylic acid-modified substrates were placed in a 5-milliliter glass vial, sealed with a rubber stopper and purged with nitrogen prior to the addition of thionyl chloride (30 μ L). The vial was heated to 40°C, causing the thionyl chloride to enter the vapor phase and react with the substrate (15 minute reaction time). The vial was purged with nitrogen prior to removing the stopper.

Surface characterization

Polarization modulation Fourier transform infrared (PM-FTIRRAS)²² spectra were obtained on a Bruker PMA50 spectrometer equipped with real-time interferogram sampling electronics (GWC Technologies, Madison WI). Spectra were collected at 85° from surface normal. All PM-FTIRRAS integration values reported are the average of measurements made on 3 separate amorphous carbon substrates.

X-ray Photoelectron (XP) spectra were collected on a Perkin Elmer PHI5400 ESCA spectrometer with a magnesium K α (1253. eV) source 45° from the surface normal at a 10^{-9} Torr base pressure. Atomic area ratios were determined with CASA XPS software by fitting the raw data to Voigt functions after Shirley baseline correction²³ and applying predetermined atomic sensitivity factors.²⁴ Reported atomic composition values are the average of measurements made on 3 separate amorphous carbon substrates.

Small molecule coupling

The alcohol, thiol, and amine analogs of the 4-(trifluoromethyl)benzyl molecule (Scheme 2) were attached to the acyl chloride-modified surfaces. A 1mM dichloromethane solution (100 μ L) of each molecule was spotted onto separate substrates and allowed to react for 1 hour

at room temperature. Prior to PM-FTIRAS and XPS analysis, each substrate was thoroughly rinsed with dichloromethane followed by ethanol and dried under a stream of nitrogen. To validate the infrared assignments, solution analogs of the 4-(trifluoromethyl)benzyl ester, thioester, and amide molecule were synthesized, purified, and analyzed with ^1H NMR, IR, and ESI MS. A detailed description of the synthesis conditions as well as the NMR and ESI MS data can be found in the Supporting Information section.

Oligonucleotide coupling

Scheme 3 outlines the general methods for attaching modified oligonucleotides to the acyl chloride- (3a), aldehyde- (3b), and maleimide-modified surfaces (3c). A detailed description of the reaction conditions used for each substrate is provided in the Supporting Information section. The probe oligonucleotides were hand-spotted (1mM, 0.3 μL) onto the substrates and incubated for 12 hours.

Thiol-modified probe oligonucleotides (Table 1) were prepared as described above and attached to the acyl chloride- and maleimide-modified surfaces. The amine-modified probe oligonucleotides were prepared as described above and attached to the acyl chloride- and aldehyde-modified substrates. Prior to spotting the aldehyde substrates, 10mM NaBH_3CN was added to reduce the Schiff base formed between the surface and the oligonucleotide.

Oligonucleotide hybridization and hybridization density

The procedure for determining the hybridization density of oligonucleotides attached to a surface has been described previously.²⁵ Substrates modified with single-stranded oligonucleotides were incubated with a 2 μM solution of fluorescently labeled Complement 1 or 2 (Table 1) in 1xSSPE buffer for 30 minutes (40 μL total volume). The surfaces were then rinsed with 10mL of 1xSSPE buffer and incubated in 1xSSPE at 37°C for 5 minutes to remove nonspecifically bound complements. Fluorescence intensities of the hybridized oligonucleotide features were measured with a GeneTAC UC4X4 scanner (Genomic Solutions; Ann Arbor, MI). Each substrate was then placed in 2mL of an 8M urea solution to elute the complementary oligonucleotides. The urea solutions were collected and placed in a 96-well plate, and their fluorescence intensities were determined and compared to calibration solutions (10^{-11} – 10^{-8} M) of the fluorescently labeled complements in 8M urea. The hybridization densities were calculated from these values.

Results and Discussion

Surface characterization

The substrates used throughout this work were prepared by depositing a thin layer of amorphous carbon onto a gold-coated slide. Amorphous carbon thin films provide a chemically robust interface and are deposited at room temperature, allowing them to be utilized with a wide variety of materials.^{11, 14, 26} In this work, the gold underlayer enhanced the overall reflectance of the substrate and defined the surface selection rules for PM-FTIRAS measurements.²⁷ It also provided a conductive surface, alleviating surface charging during XPS measurements. In previous work, the gold underlayer provided a means for monitoring reactions at the amorphous carbon surface with surface plasmon resonance imaging techniques.¹⁴

The acyl chloride-modified amorphous carbon substrates were prepared by: hydrogen terminating the amorphous carbon substrates, covalently modifying the substrate via a light-mediated reaction with undecylenic acid, and synthesizing the acyl chloride groups via a gas phase reaction with thionyl chloride (Scheme 1). The amorphous carbon substrates were monitored throughout this process with both PM-FTIRAS and XPS methods.

Freshly prepared amorphous carbon substrates contain a substantial amount of carbon, nitrogen and oxygen ($C1s : N1s : O1s = 1.00 \pm 0.054 : 0.019 \pm 0.005 : 0.394 \pm 0.019$). The nitrogen and oxygen content decreased after plasma treatment, leaving only C1s and O1s signals. The decrease in the oxygen-to-carbon ratio (0.435 ± 0.012 before and 0.072 ± 0.009 after plasma treatment) and the elimination of the nitrogen from the surface indicates that the thin film is predominantly carbon with little-to-no adsorbed/interstitial oxygen or nitrogen. The PM-FTIRRAS spectra remained unchanged during this process, showing no detectable infrared-active groups.

Next, the surface was functionalized with undecylenic acid yielding a terminal carboxylic acid. The photofunctionalization of the substrate was monitored by the appearance of the asymmetric (2927 cm^{-1}) and the symmetric (2855 cm^{-1}) methylene stretches as well as the carbonyl (1712 cm^{-1}) stretch. The disappearance of the alkene stretching modes (3091 and 1641 cm^{-1}) present in the neat molecule suggest covalent attachment. A series of amorphous carbon substrates were functionalized with neat undecylenic acid for varying amounts of time (0–24 hours) and the integrated intensities of the methylene and carbonyl stretches measured (Figure 1). A plateau in both stretches is obtained after 16 hours of illumination. Contact angle measurements were used as a complementary technique to characterize surface functionalization as a function of illumination time. These measurements supported the PM-FTIRRAS data, yielding similar results and a plateau in contact angle after 16 hours.²⁸ Figure 1 is the average of three substrates followed over the entire 24-hour functionalization period.

To form the acyl chloride groups, carboxylic acid-modified amorphous carbon substrates were reacted with $30\mu\text{L}$ of gaseous thionyl chloride in a sealed glass vial and the reaction progress was monitored with PM-FTIRRAS measurements. To determine the minimum reaction time, a series of such substrates were reacted with the thionyl chloride for varying amounts of time (0–30 minutes) and the integrated intensities of both the carboxylic acid (1712 cm^{-1}) and acyl chloride (1801 cm^{-1}) carbonyl peaks measured. The carboxylic acid carbonyl peak is completely replaced by the acyl chloride carbonyl peak after 15 minutes, indicating that the reaction is carried to completion. The methylene stretching intensities were compared before and after the thionyl chloride reaction to ensure the substrate remained intact. The above measurements were made in triplicate. Figure 2 shows the PM-FTIRRAS spectra for the carboxylic acid-modified surface before exposure to thionyl chloride and the acyl chloride-modified surface after a 15-minute reaction. Table 2 contains the peak assignments for Figure 2. The carboxylic acid- and acyl chloride-modified substrates were also analyzed by XPS. Figure 3a contains the survey XP spectra for the carboxylic acid-modified substrate and Figure 3b contains similar information for the acyl chloride-modified surface.

Small molecule attachment

Acyl chlorides are reactive with a number of nucleophilic functional groups, reacting with molecules containing alcohol, thiol, and/or amine groups. Three different 4-(trifluoromethyl) benzyl R molecules (where R = alcohol, thiol, or amine) were attached to the acyl chloride substrates and then analyzed with PM-FTIRRAS. In each reaction $100\mu\text{L}$ of a 1mM solution of the trifluoromethyl benzyl molecule in dichloromethane was spotted onto a surface and allowed to react for 1 hour. Each surface was thoroughly rinsed prior to analysis. PM-FTIRRAS spectra revealed that the acyl chloride carbonyl stretch was replaced with the corresponding ester, thioester, or amide (1742 cm^{-1} , 1695 cm^{-1} , 1637 cm^{-1} respectively) carbonyl stretch. The spectra of the substrates after attachment of the different 4-(trifluoromethyl)benzyl molecules are shown in Figure 4. The Supporting Information section contains infrared peak assignments for the ester, thioester, and amide molecules prepared on the surface as well as analogs prepared in solution. The absence of a carbonyl peak corresponding to the acyl chloride and/or the carboxylic acid suggests the reaction was complete.

Oligonucleotide array fabrication

Arrays of thiol- and amine-modified oligonucleotides were prepared on acyl chloride-modified amorphous carbon substrates and compared to oligonucleotide arrays prepared with previously published attachment methods: Sun *et al.* attached thiol-modified oligonucleotides to an amine-modified amorphous carbon substrate that was first reacted with the heterobifunctional linker sulfosuccinimidyl-4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (SSMCC);¹¹ Lockett *et al.* attached amine-modified oligonucleotides to an aldehyde-modified amorphous carbon substrate.¹⁹

Prior to attaching the thiol-modified probe oligonucleotides, the maleimide and acyl chloride substrates were prepared as described above. The oligonucleotides were reduced and purified with reverse phase HPLC, spotted onto the substrates (0.3 μ L of 1 mM oligonucleotide in 10 mM TEA buffer, pH=7.0), and incubated for 12 hours. Prior to attaching the amine-modified probe oligonucleotides, the aldehyde- and acyl chloride-modified amorphous carbon substrates were prepared as described above. The amine-modified probe oligonucleotides were hand-spotted (0.3 μ L, 1 mM oligonucleotides in 10 mM NaHCO₃ buffer, pH=10) onto each of the substrates and incubated for 12 hours; 50 mM NaBH₃CN was added to the oligonucleotide solutions prior to spotting the aldehyde surface. The reducing agent ensures that the oligonucleotides are covalently attached to the substrate via secondary amine formation. A 12-hour incubation period was chosen to match the previously published protocols for attaching oligonucleotides to the maleimide- and aldehyde-modified substrates, although as little as 30 minutes has been shown to be sufficient to obtain full surface coverage on the acyl chloride-modified substrates.²⁹

Once fabricated, each array was hybridized with fluorescently labeled Complement 1 (Table 1) and the array was imaged with a GENE TAC4x4 fluorescent scanner. The arrays were then incubated in 8M urea for 30 minutes to remove all of the complement. Each array was then exposed to Complement 2 and fluorescence images were again obtained. Figure 5 shows a representative fluorescence image obtained from one section of an acyl chloride substrate containing amine-modified probe 1 (5a) and thiol-modified probe 2 (5b) oligonucleotides. In both images, the features are uniform in nature. Oligonucleotide arrays (aldehyde substrate with amine-modified probe 1 and 2, acyl chloride substrate with amine-modified probe 1 and 2, acyl chloride substrate with thiol-modified probe 1 and 2, and maleimide substrate with thiol-modified probe 1 and 2) were prepared, in triplicate and used to determine the average fluorescence background intensity, the average oligonucleotide fluorescence intensity, the calculated signal/noise ratio, and the hybridization density. This information is summarized in Table 3. The signal/noise ratio was determined using:

$$S/N = \frac{(\text{average feature fluorescence} - \text{average background fluorescence})}{\text{standard deviation background fluorescence}}.$$

The average fluorescence background intensity was determined by averaging the fluorescence intensities of areas on the array not containing oligonucleotide features. High fluorescence background intensities are often caused by nonspecific interaction between the surface and the fluorescently labeled molecule of interest. The ability to prevent such unwanted interactions can lead to an overall higher sensitivity. The overall fluorescence background intensity for each amorphous carbon substrate was similar in value, ranging from 420 – 590 relative fluorescence units (RFU) for the acyl chloride and maleimide substrates and ~1030 RFU for the aldehyde substrate.

The average fluorescence intensity for oligonucleotide features prepared from amine-modified oligonucleotides was 12230 ± 120 and 13200 ± 620 for the acyl chloride and aldehyde substrates, respectively. The arrays prepared from thiol-modified oligonucleotides yielded average feature fluorescence intensities of 10240 ± 190 and 11090 ± 150 for the acyl chloride and maleimide substrates, respectively. The fluorescence intensities correspond to the hybridization density values obtained for each substrate (Table 3). The hybridization density of a given oligonucleotide feature is the number of oligonucleotides accessible to hybridization per unit area. The hybridization densities obtained for arrays fabricated with amine-modified oligonucleotides are similar ($2.03 \pm 0.20 \times 10^{12}$ molecules/cm² for the acyl chloride substrate and $2.24 \pm 0.15 \times 10^{12}$ molecules/cm² for the aldehyde substrate). The acyl chloride substrates showed lower hybridization densities than the maleimide substrate for the attachment of thiol-modified oligonucleotides ($1.11 \pm 0.52 \times 10^{12}$ molecules/cm² for the acyl chloride substrate and $2.26 \pm 0.48 \times 10^{12}$ molecules/cm² for the aldehyde substrate). This decrease in the number of oligonucleotides attached is attributed to side reactions of the acyl chloride with water.

Conclusion

Amorphous carbon substrates are readily functionalized with alkene-containing molecules through a UV photochemical reaction, providing a robust surface capable of supporting array fabrication. A substrate capable of reacting with multiple molecules provides the flexibility needed to attach a variety of different molecules to a single surface. Here, we fabricated an acyl chloride-modified amorphous carbon substrate and attached model small organic molecules containing alcohol, thiol, or amine groups. Arrays of thiol- or amine-modified oligonucleotides were also prepared on the acyl chloride substrates. The preparation and reaction of the acyl chloride substrates was monitored using PM-FTIRRAS, XPS, and fluorescence measurements.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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28. Data not Shown.
29. Data not shown

Figure 1.

The photofunctionalization reaction of neat undecylenic acid with hydrogen terminated amorphous carbon surfaces as a function of time. The reaction progress was monitored by integrating the carbonyl stretch peak (■) as well as the methylene stretching peaks (◆) of PM-FTIRRAS spectra taken at various time points between 0 – 24 hours. The functionalization reaction plateaus after 16 hours.

Figure 2. PM-FTIR spectra of (a) carboxylic acid-modified amorphous carbon substrate and (b) acyl chloride-modified amorphous carbon substrate after a 15-minute reaction with 30 μL (0.4 mmol) of gaseous thionyl chloride.

Figure 3. XPS survey spectra of a (a) carboxylic acid-modified amorphous carbon substrate and an (b) acyl chloride-modified amorphous carbon substrate.

Figure 4. PM-FTIRRAS spectra of an acyl chloride-modified substrate after reaction with 4-(trifluoromethyl)benzyl R molecule where (a) R = alcohol and the corresponding ester spectra, (b) R = thiol and the corresponding thioester spectra, (c) R = amine and the corresponding amide spectra. Infrared assignments for each substrate can be found in the Supporting Information section.

Figure 5.

Fluorescence intensity images of thiol- and amine-modified oligonucleotides coupled to the same acyl chloride-modified amorphous carbon substrate, hybridized with their fluorescein-labeled complements: (a) amine-modified oligonucleotide Probe 1 hybridized with its complement, (b) thiol-modified oligonucleotide Probe 2 hybridized with its complement. The white circles in each represent the position of the probe oligonucleotide that was not hybridized. The line drawn across each hybridized feature corresponds to the intensity profiles.

Scheme 1.
Formation of acyl chloride-modified surface

Scheme 2.
4-(trifluoromethyl)benzyl molecules

Scheme 3.
DNA-Surface coupling reactions

Table 1

Oligonucleotides (5' → 3') used in this work were synthesized using standard phosphoramidite chemistry. Probes 1 and 2 were synthesized with either a 3'-primary amine or a 3'-thiol group (where X = NH₂ or SH) separated from the oligonucleotide sequence of interest by 15 thymidine (dT) residues. Complementary oligonucleotides were modified with a 3'-fluorescein moiety.

Probe 1:CCACTGTTGCAAAGTTATT-(T)₁₅-X**Probe 2:**CGCTTCTGTATCTATATTCATCA-(T)₁₅-X**Complement 1:**

AATAACTTTGCAACAGTGG-F

Complement 2:TGATGAATATAGATACAGAAGCG-F

Table 2
PM-FTIRRAS data

Assignments ^a	Peak Positions (cm ⁻¹)			
	Undecylenic Acid		Undecenoyl Chloride	
	<i>neat</i>	<i>surface</i>	<i>neat</i>	<i>surface</i>
V(H ₂ C=) s	3091	---	3091	---
V(CH ₂) a	2928	2927	2928	2927
V(CH ₂) s	2856	2855	2856	2854
V(C=O)	1709	1712	1801	1801
V(C=C)	1641	---	1641	---
B(CH ₂)	1465	1464	1465	1464
	1405	1413	1405	1413

^a ν =stretching, β =bending
a =asymmetric, s =symmetric

Table 3
Oligonucleotide Array Comparisons

Comparison of the average fluorescence background signal, average oligonucleotide feature fluorescence signal, average signal-to-noise ratio, and average hybridization density for oligonucleotide arrays prepared on acyl chloride-, aldehyde-, and maleimide-terminated amorphous carbon substrates. Amine-modified oligonucleotides were coupled to the acyl chloride- and aldehyde-terminated substrates. Thiol-modified oligonucleotides were coupled to the acyl chloride- and maleimide-terminated substrates. Each substrate was prepared in triplicate as discussed in the Materials and Methods section of the manuscript. The average background fluorescence signal was determined after the arrays were hybridized with fluorescently labeled complements and is the average signal obtained from areas not containing oligonucleotide features. The average fluorescence signal is the average of oligonucleotide features after hybridized with their fluorescently labeled complement. The hybridization densities were obtained by collecting the hybridized complements and comparing their fluorescence intensities to a calibration curve.

<i>Substrate</i>	Amine-Modified Oligonucleotides		Thiol-Modified Oligonucleotides	
	Acyl Chloride	Aldehyde	Acyl Chloride	Maleimide
Average Fluorescence Background Intensity (RFU)	520 ± 30	1030 ± 50	590 ± 20	420 ± 30
Average Fluorescence Signal (RFU)	12230 ± 120	13200 ± 620	10240 ± 190	11090 ± 150
S/N ratio	390 ± 23	243 ± 16	482 ± 19	356 ± 26
Average Hybridization Density (10 ¹² molecules/cm ²)	2.03 ± 0.20	2.24 ± 0.15	1.11 ± 0.52	2.26 ± 0.48