Published in final edited form as:

Langmuir. 2008 September 2; 24(17): 9198–9203. doi:10.1021/la800991t.

Aldehyde-Terminated Amorphous Carbon Substrates for the Fabrication of Biomolecule Arrays

Matthew R. Lockett, Michael R. Shortreed, and Lloyd M. Smith*

Department of Chemistry, University of Wisconsin-Madison, 1101 University Avenue, Madison, Wisconsin 53706

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 chemical and biomolecule array fabrication. Aldehyde-terminated amorphous carbon substrates were fabricated via the attachment of a 2-(10-undecen-1-yl)-1,3-dioxolane molecule. The surfaces were then deprotected in 1.5 M HCl to yield an aldehyde-terminated surface that is readily reactive with amine containing molecules. An array of amine-modified oligonucleotides was prepared on aldehyde-terminated surfaces prepared on both amorphous carbon and on gold self-assembled monolayers, and the fluorescence background, feature signal-to-noise ratio, and hybridization densities were compared. The aldehyde-terminated amorphous carbon substrates offer inherently lower background fluorescence intensity and a greater number of hybridization-accessible sites.

Introduction

Functionalized carbon substrates such as glassy carbon, diamond, and amorphous carbon thin films are attractive materials for the fabrication of chemical and biomolecule arrays. Yang et al. evaluated carbon-based substrates, comparing oligonucleotide arrays fabricated on functionalized ultra-nanocrystalline diamond thin films, glass, and silicon surfaces as well as gold self-assembled monolayers (SAMs).¹ In their study each oligonucleotide array was subjected to 30 serial hybridization and dehybridization cycles and the array stability was evaluated. Only the functionalized diamond substrates remained intact, as evidenced by the number of oligonucleotides accessible to hybridization after the thirtieth hybridization. Phillips et al. have recently shown that functionalized glassy carbon and diamond thin film substrates not only withstand the conditions necessary for *in situ* oligonucleotide synthesis, but also yield oligonucleotide arrays that outperform their commonly used glass analogues when incubated at high temperatures, exposed to basic conditions, or serially hybridized and dehybridized.² Oligonucleotide arrays prepared on functionalized amorphous carbon films have shown equivalent stability to that of their diamond thin film analogues.³

Several means of functionalizing carbon substrates have been developed.^{4–7} However, the direct functionalization of carbon substrates^{3,8,9} with alkene-containing molecules is of particular interest because of its utility for fabricating biomolecule arrays. In this reaction, alkene-containing molecules are covalently attached to a hydrogen-terminated carbon substrate through the UV light mediated formation of a carbon–carbon bond between the substrate and the molecule of interest. First described as a method of modifying hydrogen-terminated silicon substrates,^{10,11} the photochemical attachment of alkene-containing molecules has been

*smith@chem.wisc.edu.

applied to hydrogen-terminated nanocrystalline diamond films,^{1,9,12} glassy carbon,^{1,2} carbon nanofibers,¹³ and most recently amorphous carbon thin films.^{3,14}

Amorphous carbon thin films are particularly attractive substrates, as they can be reproducibly fabricated at room temperature,^{3,15,16} offering the ability to integrate the chemical stability of carbon with substrates that are not amenable to surface modification and/or array fabrication. Two such examples include the application of amorphous carbon thin films to quartz crystal microbalances for the fabrication of a biocompatible electromechanical device³ and to silicon nitride electrodes to fabricate microelectrode arrays.^{16,17}

A variety of alkene-containing molecules have been employed in carbon substrate functionalization. Colavita et al. functionalized amorphous carbon substrates with 1-dodecene, methyl-10-undecenoate, 10-*N*-Boc-amido-dec-1-ene, and trifluoroacetic acid protected 10-aminodec-1-ene (TFAAD).¹⁸ Alcohol-terminated carbon substrates (9-decene-1-ol) have been utilized in the *in situ* synthesis of oligonucleotide arrays.² To date, the TFAAD molecule has been the most utilized alkene-containing molecule for the functionalization and fabrication of biomolecule arrays on carbon surfaces.^{1,3,5,9,19–21} The deprotection of the TFAAD molecule yields an amine-terminated surface, which has been used to attach biomolecules. For example, oligonucleotides containing a 5'- or 3'-terminal thiol have been attached to these surfaces via a heterobifunctional linker capable of reacting with both the surface amine and oligonucleotide thiol.²² For efficient coupling to the surface, the biomolecules of interest must contain a reduced thiol group. Thus, each thiol-modified oligonucleotide must be chemically reduced and then purified with reverse-phase chromatography. Due to reformation of the disulfide bonds over time, this process must be performed just prior to the surface-coupling reaction, making array fabrication arduous.

The development of new surface chemistries that react with other functional groups commonly occurring on biomolecules would alleviate the preparation needed with the TFAAD modified surface. Amines commonly occur in proteins and are also easily attached to the terminal ends of oligonucleotides. An amine is a desirable functional group, as it does not readily undergo oxidation, eliminating the need for the immediate preparation and purification that is required for thiols.

Here, we report an aldehyde-terminated amorphous carbon substrate formed from the photochemical functionalization and deprotection of the 2-(10-undecen-1-yl)-1,3-dioxolane molecule. Aldehyde modified glass^{23,24} and gold²⁵ surfaces have also shown utility for attaching biomolecules. Amine-modified oligonucleotides were attached to this surface as well as to aldehyde-terminated gold self-assembled monolayers^{26,27} and the two were compared with respect to their background fluorescence intensity, the intensity of their oligonucleotide features upon hybridization with fluorescently labeled complement, signal-to-noise ratio, and spot radius. Both surfaces yielded similar spot radii, indicating the aldehyde functional group and not the substrate material dictates this parameter. Oligonucleotide arrays prepared on amorphous carbon substrates yielded a signal-to-noise ratio 40% higher than those prepared on gold SAMs. This is due to both lower background fluorescence intensity and brighter oligonucleotide features on the amorphous carbon substrate.

Experimental Section

Materials and Reagents

All chemical reagents were purchased from Sigma-Aldrich, 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 a 3'-amine (3AmM) 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.²⁸ Complementary oligonucleotides to Probes 1 and 2 were synthesized with a 3' 6-carboxyfluorescein moiety (6-FAM). The probe and complementary oligonucleotides were purified, prior to use, by reverse-phase, binary gradient elution HPLC (SCL-10ADVP Shimadzu; Columbia, MD). Purified oligonucleotides were stored dry, under nitrogen at -20 °C, until needed. All oligonucleotide concentrations were determined by absorption measurements at 260 nm (HP8453 UVVIS; Santa Clara, CA).

Preparation of 2-(10-Undecen-1-yl)-1,3-dioxolane

The 2-(10-undecen-1-yl)-1,3-dioxolane molecule was prepared by dissolving 1.0 g of 10-undecenal (5.9 mmol, 95% purity) in 10.0 mL of anhydrous toluene. A catalytic amount of *p*-toluenesulfonic acid (95 mg, 0.5 mmol, 98.5% purity) was dissolved in 1.0 mL of anhydrous toluene and added to the undecenal. To this mixture, 1.2 mol equiv of anhydrous ethylene glycol (0.4 mL, 99.8% purity) was added and the reaction refluxed for 1 h. The product was purified via vacuum distillation, yielding 0.82 g of the protected aldehyde (3.9 mmol, 82% yield). ¹H NMR (CDCl₃) δ: 5.83 (m, 1H), 5.03 (s, 1H), 4.98 (s, 1H), 4.86 (t, 1H), 4.02 (m, 2H), 3.85 (m, 2H), 2.05 (d, 2H), 1.62 (m, 2H), 1.28 (m, 12H). HRMS (ESI) for C₁₃H₂₄O₂ (M + H⁺) *m/z* 213.3364; found 213.3362.

Preparation of Aldehyde-Terminated, Amorphous Carbon Substrates

The preparation of aldehyde-terminated substrates from 2-(10-undecen-1-yl)-1,3-dioxolane is outlined in Scheme 1. Amorphous carbon thin films (15 nm) were deposited onto commercially prepared gold-coated glass slides by DC magnetron sputtering a graphite source at a base pressure of 2×10^{-6} Torr and an Ar 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 100 nm thick gold film with a 5 nm chromium under layer to provide better adhesion to the glass substrate (Evaporated Metal Films Co, Ithaca, NY). Prior to use, the gold-coated slides were rinsed with deionized (DI) water and dried under a stream of nitrogen gas.

The amorphous carbon substrates were photochemically functionalized using a modified procedure for the functionalization of diamond thin film substrates, developed in our laboratory.⁹ First, each amorphous carbon thin film was hydrogen-terminated in a 13.56 MHz inductively coupled hydrogen plasma for 12 min (30 Torr H₂, room temperature). Next, 30 μL of the 2-(10-undecen-1-yl)-1,3-dioxolane molecule (neat) was placed directly onto the newly hydrogen-terminated surface and covered with a quartz coverslip. The substrates were then irradiated with a low-pressure mercury lamp ($\lambda_{\text{max}} = 254$ nm, 0.35 mW/cm²), under nitrogen purge. After the photoreaction the substrates were rinsed with ethanol and DI water, dried under a stream of nitrogen gas, and stored in a desiccator until needed.

The functionalized substrates were deprotected in a 60 °C, 1.5 M HCl solution to yield the aldehyde-terminated, amorphous carbon surface.

Surface Characterization

Fourier transform infrared reflection-absorption spectra (FTIRRAS) of the substrates were collected on a Bruker Vertex 70 FTIR spectrometer equipped with a VeeMaxII variable angle specular reflectance accessory (Pike Technologies, Madison, WI) and a wire grid polarizer. The presence of the metal underlayer (100 nm gold) enhanced the reflectance of the sample and defined the surface selection rules, which are analogous to those for metal substrates.²⁹ Spectra were collected using p-polarized light at a 55° angle of incidence from the surface

normal; 500 scans at 4 cm^{-1} resolution were collected for both the background and the sample. The background spectra were collected on hydrogen-terminated amorphous carbon substrates that were not functionalized.

Functionalization and deprotection measurements were made by polarization modulation FTIR reflection absorption spectroscopy (PM-FTIRRAS)³⁰ on a Bruker PMA50 spectrometer with real-time interferogram sampling electronics (GWC Technologies Inc.; Madison, WI). Spectra were collected at 85° from surface normal. PM-FTIRRAS integration values reported are the average of measurements on 3 separate amorphous carbon substrates.

FTIR spectra of the neat molecules were collected in transmission mode at a 1 cm^{-1} resolution using a PIKE MIRacle micro ATR accessory with a germanium crystal on the Bruker Vertex 70 instrument.

Contact angles of the amorphous carbon substrates were measured with a Ramé-Hart goniometer (model 200-F1) equipped with *DroplImage Standard* software. The advancing angles were measured with DI water as the probe liquid, and are reported as the average from 3 different substrates with 3 measurements taken at different positions on each substrate.

Preparation of Aldehyde-Terminated SAMs on Gold

Gold substrates were modified with aldehyde terminated self-assembled monolayers (SAMs). The synthesis of di(undecanal) disulfide and its use in the fabrication of gold-thiol SAMs for the fabrication of biomolecule arrays has been previously reported.^{23,24} Commercially prepared gold substrates were rinsed with DI water and ethanol and then dried under a stream of nitrogen. The substrates were then soaked in a 10 mM ethanolic solution of di(11-undecanal) disulfide for 12 h. The aldehyde-terminated surfaces were washed with copious amounts of ethanol and DI water, and then dried under a stream of nitrogen gas.

Coupling of Amine-Terminated Oligonucleotides to the Aldehyde-Terminated Substrates

Amine-terminated probe oligonucleotides (Probes 1 and 2, Table 1) were diluted to 1 mM in 100 mM sodium carbonate (pH = 10.0) buffer. Before spotting the oligonucleotides (0.3 μL) onto the aldehyde-terminated surfaces, 50 mM NaBH_3CN was added to the buffered oligonucleotide solutions. This reducing agent is used to convert the newly formed Schiff base to a more stable, secondary amine. Each surface was incubated at room temperature for 12 h. Each surface was then washed with DI water and incubated in $1 \times \text{SSPE}$ (10 mM NaH_2PO_4 , 150 mM NaCl , 1 mM EDTA, pH 7.4) for 30 min at 37°C , removing any nonspecifically bound oligonucleotides.

Oligonucleotide Hybridization Density

The procedure for determining the hybridization density of oligonucleotides attached to a surface has been described previously.²³ The current work uses a modified procedure. Aldehyde-terminated surfaces modified with single-stranded oligonucleotides were incubated with a 2 μM solution of fluorescently labeled Complement 1 or 2 (Table 1) in $1 \times \text{SSPE}$ buffer for 30 min (40 μL total volume). The surfaces were then rinsed with 10 mL of $1 \times \text{SSPE}$ buffer and incubated in $1 \times \text{SSPE}$ at 37°C for 5 min to remove nonspecifically bound complements. Fluorescence intensities of the hybridized oligonucleotide features were measured with a GeneTAC UC4 $\times 4$ scanner (Genomic Solutions; Ann Arbor, MI). Each substrate was then placed in 2 mL of an 8 M 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} to 10^{-8} M) of the fluorescently labeled complements in 8 M urea. The hybridization densities were calculated from these values.

Discussion

Characterization of Aldehyde-Modified Amorphous Carbon Substrates

Amorphous carbon substrates were photochemically functionalized with a protected aldehyde (2-(10-undecen-1-yl)-1,3-dioxolane) molecule, which was synthesized and purified prior to use. Protection of the aldehyde group was necessary, as directly functionalizing the substrate with 10-undecenal resulted in an unwanted and unknown side reaction. During UV photofunctionalization the neat alkene is placed on the substrate and then covered with a quartz coverslip. UV irradiation of the 10-undecenal resulted in the quartz coverslip sticking to the amorphous carbon substrate. The force required to remove the quartz coverslip resulted in the removal of the amorphous carbon, rendering the substrate unusable. A series of FTIRRAS measurements were taken to characterize the amorphous carbon substrate after (1) it was functionalized with the 2-(10-undecen-1-yl)-1,3-dioxolane molecule; (2) it was deprotected to yield terminal aldehyde groups; and (3) a primary amine molecule (methylamine) was coupled to the substrate. The reaction of a primary amine with an aldehyde is known to proceed via formation of a Schiff base, which is water-labile. The presence of a reducing agent (NaBH_3CN) results in formation of a secondary amine (Scheme 1).

The functionalization of the amorphous carbon substrate with the 2-(10-undecen-1-yl)-1,3-dioxolane molecule was monitored by the appearance of asymmetric (2931 cm^{-1}) and symmetric (2856 cm^{-1}) methylene stretches. A weaker set of peaks corresponding to the dioxolane stretches (1128 and 1018 cm^{-1}) was also observed, indicating the molecule remained intact during the reaction. The disappearance of the alkene stretching modes (3078 , 1640 cm^{-1}), present in the neat molecule, also suggested covalent attachment to the substrate (Figure 1a).

Deprotection of the surface with 1.5 M HCl led to replacement of the 1,3-dioxolane stretches with the aldehyde carbonyl (1719 cm^{-1} , Figure 1b). The aldehyde-terminated surface was then placed in a $100\text{ }\mu\text{M}$ solution of methylamine in 100 mM sodium carbonate ($\text{pH} = 10.0$) buffer containing $50\text{ mM NaBH}_3\text{CN}$. This resulted in disappearance of the carbonyl stretch and appearance of a new peak corresponding to the C–N stretch of a secondary amine (1146 cm^{-1}) (Figure 1c) stretch. The position and intensity of the methylene stretches remain constant after deprotection and the methylamine coupling reaction, indicating that the functionalized substrate remains intact under these reaction conditions. Table 2 lists the frequencies of the IR modes observed for the neat and surface-attached molecules.

The functionalization of the 2-(10-undecen-1-yl)-1,3-dioxolane molecule to a hydrogen-terminated amorphous carbon substrate was monitored with PM-FTIRRAS measurements of the methylene stretch ($\sim 2840\text{--}2980\text{ cm}^{-1}$) intensities as a function of illumination time. Here, a series of amorphous carbon substrates were reacted with the neat molecule for varying amounts of illumination ($0\text{--}24\text{ h}$) and the integrated intensities of the methylene stretches were measured (Figure 2a). A plateau in the methylene stretch intensities is obtained after 16 h of illumination. Contact angle measurements were used as a complementary technique to characterize surface functionalization as a function of illumination time (Figure 2b). These measurements supported the PM-FTIRRAS data, yielding similar results and a plateau in contact angle after 12 h of reaction time. Figure 2 is the average of four substrates followed over a 24-h functionalization period.

Next, the optimal deprotection time for the protected aldehyde surface was investigated with PM-FTIRRAS by monitoring the appearance of the C=O stretch (1719 cm^{-1}). Prior to deprotection, infrared spectra of the functionalized amorphous carbon substrates were obtained to serve as a baseline reference. The data presented in Figure 3 are the average of four substrates followed over a 24 h deprotection period. Each substrate was incubated in a 1.5 M HCl solution

at 60 °C, removed from the solution, thoroughly washed, dried under a stream of nitrogen gas, and an infrared spectrum taken. This process was repeated for a 24 h period. The integrated methylene stretches ($\sim 2840\text{--}2980\text{ cm}^{-1}$) were measured at each time point and compared to the baseline spectra to monitor the fidelity of the substrate. For each time point the integrated intensity of the C=O (1719 cm^{-1}) stretch was measured. The results indicate that a minimum of 14 h is required to fully deprotect the aldehyde surface under the conditions employed. The intensity and position of the methylene stretches remained consistent throughout the deprotection process, indicating the surface withstood the deprotection conditions.

Attachment of Amine-Modified Oligonucleotides

Oligonucleotides modified with a 3'-primary amino group were attached to the aldehyde substrate by hand-spotting 0.3 μL of oligonucleotide solution as described in the Experimental Section. This means of attachment has been previously described in detail for aldehyde terminated, gold SAMs.²³ Small oligonucleotide arrays consisting of two probe oligonucleotides (5 features of Probes 1 and 2 on each array; Table 1) were prepared in triplicate on the following: unmodified, 2-(10-undecen-1-yl)-1,3-dioxolane-modified, and aldehyde-terminated amorphous carbon substrates. Oligonucleotide arrays were also prepared in triplicate on aldehyde-terminated gold SAMs. Each array was exposed to fluorescently labeled Complements 1 and 2 (separately) and hybridization determined with fluorescent images taken with a GENE TAC4 $\times$ 4 fluorescent scanner. Figure 4 shows a representative fluorescence image obtained from one section of an array prepared on the aldehyde-terminated amorphous carbon substrate.

The fluorescence images of the oligonucleotide arrays prepared on the aldehyde-terminated amorphous carbon and gold substrates were used to determine the average fluorescence background intensity, the average oligonucleotide fluorescence intensity, and the average feature size. This information is summarized in Table 3.

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. Aldehyde-terminated amorphous carbon substrates had an overall background fluorescence that was half the intensity of the gold SAM analogue. The unmodified amorphous carbon displayed the lowest fluorescence background of 415 RFU (Table 3).

The average fluorescence intensity and spot radius of the oligonucleotide features on the aldehyde-terminated amorphous carbon and gold arrays were measured after hybridization with the fluorescently labeled complements. To serve as a control for the specificity of the coupling chemistry, oligonucleotides were attached to bare and 2-(10-undecen-1-yl)-1,3-dioxolane modified amorphous carbon substrates. The feature fluorescence intensity for the bare amorphous carbon substrate was equal to that of the background fluorescence intensity, indicating that no oligonucleotides were coupled to the surface. The 2-(10-undecen-1-yl)-1,3-dioxolane modified surface contained oligonucleotide features with an average fluorescence intensity of 1460 RFU, yielding a signal/noise ratio of 12 (see below). This fluorescence could be attributed to the nonspecific adsorption of the probe oligonucleotides to the surface or from a small number of the aldehyde molecules becoming deprotected during the functionalizing and/or washing steps of surface preparation. The average feature fluorescence intensity for the amorphous carbon aldehyde substrate (14710 RFU) yields a signal/noise ratio of 233, which is 1.4-fold greater than that of the ratio found for the gold SAM analogue (10733 RFU, signal/noise ratio of 171). Signal to noise ratios were calculated using the following equation:

$$\frac{S}{N} = \frac{(\text{average feature signal, RFU}) - (\text{average background signal, RFU})}{(\text{standard deviation of background signal, RFU})} \quad (1)$$

The average feature size for the aldehyde-terminated amorphous carbon and gold SAM substrates were similar in size (875 and 850 μm , respectively), indicating that these two aldehyde-modified substrates are similar in their surface properties.

Hybridization Density

The number of accessible oligonucleotide probes coupled to the aldehyde-terminated substrates was measured and the hybridization densities for both the amorphous carbon and gold SAMs were obtained using previously described methods.²³ Each surface was hybridized with fluorescently labeled complement and then dehybridized in 8 M urea, the wash-off collected, the fluorescence intensities for each surface measured and compared to a calibration constructed from known concentrations of the fluorescently labeled complementary oligonucleotides in 8 M urea solutions. Once the number of oligonucleotides was obtained, the hybridization density was determined by dividing the number of oligonucleotides by the total area of the oligonucleotide features (molecules/ cm^2). The hybridization density measurements for the two substrates are in agreement with the feature fluorescence intensities, with densities of 4.4×10^{12} and 3.5×10^{12} molecules/ cm^2 for the amorphous carbon and gold SAM substrates, respectively, which is commensurate with previously published results.^{2,23}

Conclusion

Amorphous carbon substrates are readily functionalized with alkene-containing molecules through a UV photochemical reaction, providing a robust surface capable of supporting biomolecule array fabrication. A 2-(10-undecen-1-yl)-1,3-dioxolane molecule can be attached to amorphous carbon surfaces, yielding an aldehyde-terminated surface that is readily reactive with amine containing molecules. Here, we determine the photofunctionalization conditions necessary for maximal surface coverage using both PM-FTIRAS and contact angles for surface characterization. The presence of the aldehyde functional group on the surface and its ability to react with amine-containing molecules is shown. Also, amine-modified oligonucleotides were attached to the amorphous carbon surfaces and compared with analogous surfaces prepared on gold SAMs. The aldehyde-terminated amorphous carbon substrates offer inherently lower background fluorescence intensity and a greater number of hybridization-accessible sites.

Acknowledgment

The authors would like to thank Prof. Robert Hamers for the use of his PM-FTIRAS instrument and Prof. David Beebe for the use of his goniometer. This work was funded by NIH grant R01HG002298, the University of Wisconsin Technology Transfer Grant Program, and the University of Wisconsin Industrial and Economic Development Research program.

References

1. Yang WS, Auciello O, Butler JE, Cai W, Carlisle JA, Gerbi J, Gruen DM, Knickerbocker T, Lasseter TL, Russell JN, Smith LM, Hamers RJ. *Nat. Mater* 2002;1(4):253–257. [PubMed: 12618788]
2. Phillips MF, Lockett MR, Rodesch MJ, Shortreed MR, Cerrina F, Smith LM. *Nucleic Acids Res* 2008;36(1):e7. [PubMed: 18084027]
3. Sun B, Colavita PE, Kim H, Lockett M, Marcus MS, Smith LM, Hamers RJ. *Langmuir* 2006;22(23):9598–9605. [PubMed: 17073485]
4. Kuo T, McCreery R, Swain G. *Electrochem. Solid State Lett* 1999;2(6):288–290.

5. Baker SE, Tse K-Y, Hindin E, Nichols BM, Lasseter TL, Hamers RJ. *Chem. Mater* 2005;17(20):4971–4978.
6. Miller JB. *Surf. Sci* 1999;439(1–3):21–33.
7. Liu Y, Gu ZN, Margrave JL, Khabashesku VN. *Chem. Mater* 2004;16(20):3924–3930.
8. Ohta R, Saito N, Inoue Y, Sugimura H, Takai O. *J. Vac. Sci. Technol. A* 2004;22(5):2005–2009.
9. Strother T, Knickerbocker T, Russell JN, Butler JE, Smith LM, Hamers RJ. *Langmuir* 2002;18(4):968–971.
10. Strother T, Hamers RJ, Smith LM. *Nucleic Acids Res* 2000;28(18):3535–3541. [PubMed: 10982873]
11. Strother T, Cai W, Zhao XS, Hamers RJ, Smith LM. *J. Am. Chem. Soc* 2000;122(6):1205–1209.
12. Nebel CE, Uetsuka H, Rezek B, Shin D, Tokuda N, Nakamura T. *Diamond Relat. Mater* 2007;16(8):1648–1651.
13. Baker SE, Colavita P, Metz K, Nichols BM, Sun B, Uhlrich J, Wang X, Kuech TF, Hamers RJ. *Chem. Mater* 2006;18:4415–4422.
14. Colavita PE, Sun B, Tse K-Y, Hamers RJ. *J. Am. Chem. Soc* 2008;129(44):13554–13565. [PubMed: 17927179]
15. Robertson J. *Adv. Phys* 1986;35(4):317–374.
16. Sreenivas G, Ang SS, Fritsch I, Brown WD, Gerhardt GA, Woodward DJ. *Anal. Chem* 1996;68(11):1858–1864.
17. Fiaccabrino GC, Tang XM, Skinner N, deRoij NF, KoudelkaHep M. *Sens. Actuators, B* 1996;35(1–3):247–254.
18. Colavita PE, Sun B, Tse K-Y, Hamers RJ. *J. Am. Chem. Soc* 2007;129(44):13554–13565. [PubMed: 17927179]
19. Lu MC, Knickerbocker T, Cai W, Yang WS, Hamers RJ, Smith LM. *Biopolymers* 2004;73(5):606–613. [PubMed: 15048784]
20. Knickerbocker T, Strother T, Schwartz MP, Russell JN, Butler J, Smith LM, Hamers RJ. *Langmuir* 2003;19(6):1938–1942.
21. Nichols BM, Metz KM, Tse K-Y, Butler JE, Russell JN, Hamers RJ. *J. Phys. Chem. B* 2006;110:16535–16543. [PubMed: 16913787]
22. Sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SSMCC) is typically used.
23. MacBeath G, Schreiber S. *Science* 2000;289:1760. [PubMed: 10976071]
24. Feng J, Gao CY, Wang B, Shen JC. *Colloids Surf., B* 2004;36:177.
25. Yeo WS, Mrksich M. *Adv. Mater* 2004;16:1352.
26. Peelen D, Smith LM. *Langmuir* 2005;21(1):266–271. [PubMed: 15620313]
27. Lee J, Didier DN, Lockett MR, Scalf M, Greene AS, Olivier M, Smith LM. *Anal. Biochem* 2007;369(2):241–247. [PubMed: 17624292]
28. Guo Z, Guilfoyle RA, Thiel AJ, Wang RF, Smith LM. *Nucleic Acids Res* 1994;22(24):5456–5465. [PubMed: 7816638]
29. Tolstoy, VP. *Handbook of Infrared Spectroscopy of Ultrathin Films*. Hoboken, NJ: Wiley-VCH; 2003. p. 710
30. Barner BJ, Green MJ, Saez EI, Corn RM. *Anal. Chem* 1991;63:55–60.

Figure 1.

FT-IRRAS spectra of photofunctionalized, hydrogen-terminated amorphous carbon surfaces. Dashed lines correspond to the peak frequencies found in Table 2. (a) 2-(10-undecen-1-yl)-1,3-dioxolane modified surface after 12 h of photoattachment. (b) The same surface then subjected to 15 h of deprotection in 1.5 M HCl, yielding the aldehyde functionality. (c) Methylamine attached to the surface through formation of a secondary amine.

Figure 2. Functionalization of 2-(10-undecen-1-yl)-1,3-dioxolane molecule as a function of time on hydrogen-terminated amorphous carbon surfaces. The reaction progress was monitored by (a) integrating the methylene stretching peaks in PM-FTIRRAS spectra taken at various time points between 0 and 24 h. The functionalization reaction plateaus after 16 h of illumination time. (b) Contact angle measurements were used as a complementary method for monitoring the functionalization reaction. Contact angle measurements show a plateau occurring after 12 h of functionalization.

Figure 3.

Amorphous carbon substrates functionalized with 1,3-dioxolane protected undecanal (15 h) were deprotected in 1.5 M HCl. Deprotection was monitored by integrating the C=O (◆) and C—H (■) stretches of PM-FTIRRAS data. The appearance of the C=O stretches corresponding to the aldehyde carbonyl was used to determine the amount of deprotection. Integration of the C—H stretches was monitored throughout the course of the deprotection as a measure of stability. The data show that the surface was fully deprotected after 14 h.

Figure 4.

Fluorescence intensity images of oligonucleotides coupled to an aldehyde-terminated amorphous carbon substrate, hybridized with their fluorescein-labeled complements: (a) Probe 1 hybridized with complement 1, (b) Probe 2 hybridized with complement 2. Parts (a) and (b) are the same array; the white circles in each represent the position of probe oligonucleotides that were not hybridized. (c) Fluorescence intensity profile of hybridized Probe 2.

Scheme 1.
Array Preparation

Table 1
Oligonucleotides

Probe 1:CCACTGTTGCAAAGTTATT-(T)₁₅-NH₂**Probe 2:**CGCTTCTGTATCTATATTCATCA-(T)₁₅-NH₂**Complement 1:**AATAACTTTGCAACAGTGG- F**Complement 2:**TGATGAATATAGATACAGAAGCG- F

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

Table 2

assignments ^a	peak frequencies (cm ⁻¹)			
	protected aldehyde		aldehyde	
	neat	surface	neat	surface
ν (H ₂ C=)s	3078	-	3078	-
ν (CH ₂)a	2924	2931	2925	2931
ν (CH ₂)s	2854	2856	2854	2856
ν (CHO)s	-	-	2715	-
ν (C=O)	-	-	1725	1719
ν (C=C)	1640	-	1640	-
β (CH ₂)	1464	1462	1463	1460
ν (1,3-dioxolane ring)	1125	1128	-	-
	1035	1018	-	-
	942	-	-	-

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

Table 3

	average background (RFU)	average signal (RFU)	average spot size (μm)	average hybridization density (10^{12} molecules/ cm^2)
Aldehyde aC	713 ± 60	14710 ± 840	850 ± 75	4.4 ± 0.12
Aldehyde Au SAM	1340 ± 55	10733 ± 920	875 ± 70	3.5 ± 0.23
Protected Aldehyde aC	730 ± 60	1460 ± 80	-	-
Bare aC	415 ± 30	432 ± 50	-	-