

## Covalently Modified Silicon and Diamond Surfaces: Resistance to Nonspecific Protein Adsorption and Optimization for Biosensing

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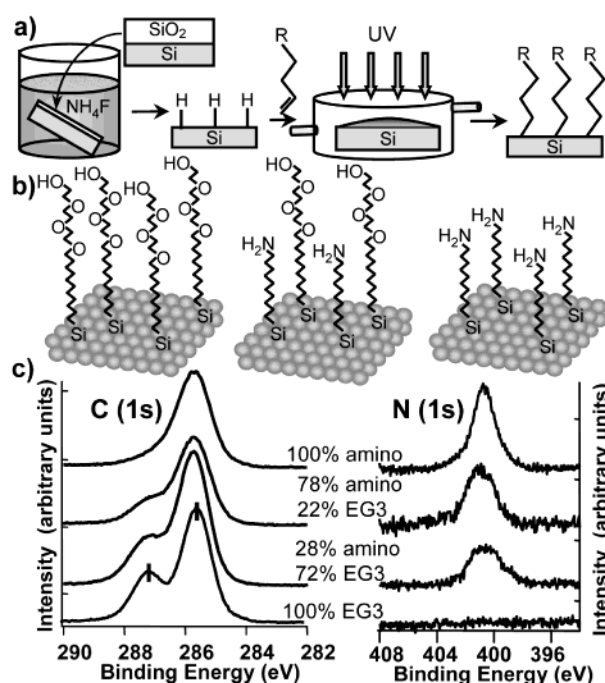
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Oligoethylene glycol monolayers on gold<sup>1,2</sup> and SiO<sub>2</sub> surfaces<sup>3,4</sup> have been used to resist the nonspecific adsorption of proteins and cells. This ability to resist biofouling<sup>5</sup> is important for the design of biocompatible coatings (e.g., diamond and diamond-like carbon) for implants and for biosensors capable of detecting analytes in complex protein mixtures.<sup>6</sup> Covalently modified surfaces of silicon and of diamond thin films are now emerging as useful materials for the direct electrical detection of biomolecules.<sup>7–9</sup> We report here the direct covalent functionalization of silicon and of diamond with short ethylene glycol (EG) oligomers via photochemical reaction of the hydrogen-terminated surfaces with terminal vinyl groups of the oligomers. Our results show that the functionalized surfaces effectively resist the nonspecific adsorption of proteins. We also demonstrate the preparation of mixed monolayers on silicon and diamond and apply these surfaces to optimize the ratio of specific to nonspecific binding in a model protein sensing assay.

Recent studies have reported that monolayers on gold and SiO<sub>2</sub> can be unstable when used over the span of many days,<sup>5,9</sup> while monolayers on silicon<sup>8,10</sup> and carbon-based materials<sup>9</sup> show promise for longer-term stability. We explored covalent modification of Si(111) surfaces through Si–C bond formation<sup>11</sup> because vinyl groups will photochemically react directly with the surface,<sup>10,12,13</sup> producing covalently linked monolayers that can serve as stable anchor points for tethering biological molecules to the surface. Diamond surfaces can be modified similarly, producing DNA layers exhibiting higher stability than those on gold, silicon, and SiO<sub>2</sub>.<sup>9</sup> However, methods for reducing nonspecific binding on silicon and diamond surfaces have remained relatively unexplored.<sup>11</sup>

We prepared mixed monolayers presenting both amine and triethylene glycol (EG3) functionalities. The incorporation of amines into the monolayer allowed for subsequent chemical modification of these interfaces. Mixed monolayers were formed by applying solutions of various mole percentages of triethylene glycol undec-1-ene (EG3-ene)<sup>1</sup> and *t*-Boc 10-aminodec-1-ene (Boc-N-ene) onto hydrogen-terminated silicon (111) surfaces<sup>8</sup> or TFA-protected 10-aminodec-1-ene (TFA-N-ene) onto hydrogen-terminated polycrystalline, p-type diamond thin films.<sup>9</sup> Deposition of the liquids onto the surface followed by UV illumination at 254 nm for 3 h (silicon) or 12 h (diamond) links the molecules to the surface via the vinyl group.<sup>8,9</sup> Single-crystal and polycrystalline diamond samples show nearly identical reactivity, indicating that defects and grain boundaries do not control the reaction of the polycrystalline films. Finally, the amino group is generated by the deprotection of the Boc or TFA group under acidic conditions. To facilitate comparison of our results on silicon and diamond with previous studies, we also formed mixed monolayers of amino-terminated and EG3-terminated alkanethiols on gold.<sup>14,15</sup> Clean Au surfaces were immersed in 2 mM mixed solutions of 11-amino-



**Figure 1.** (a) Covalent modification of silicon. (b) The resulting monolayers of 100% EG3, 50% amino–50% EG3, and 100% amino. (c) XPS spectra of the carbon (1s) and nitrogen (1s) regions of mixed amino and EG3 monolayers on silicon.

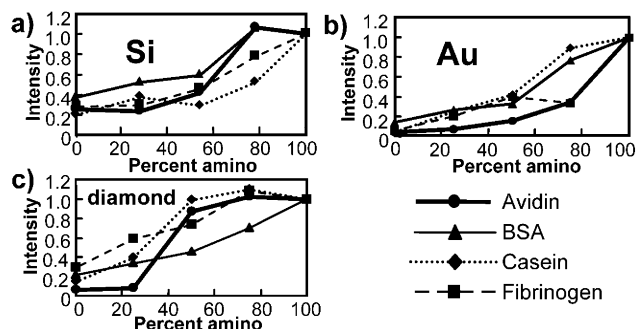
**Table 1.** Composition of the Mixed Monolayers on Silicon, Based upon the Areas of the N(1s) and 287.2 eV C(1s) XPS Peaks

mol % amino in liquid	mol % amino by XPS
78	56
54	42
28	31

undecanethiol (Dojindo) and triethylene glycol undecanethiol (Prochimia) for at least 12 h.

Monolayers of short EG oligomers on silicon have not been reported previously. Therefore, we characterized the monolayers using X-ray photoelectron spectroscopy (XPS), shown in Figure 1c. The areas of the N(1s) peak and the high binding energy C(1s) peak at 287.2 eV<sup>1</sup> (Figure 1c) were used to calculate the percentages of Boc-N-ene and EG3-ene in the mixed monolayers on silicon. Competitive binding experiments showed that, although the OH group and the vinyl group of the EG3-ene both can react with silicon, the vinyl group reacts approximately 3 times faster, so that ~75% of EG3-ene molecules are bonded via the vinyl group, and 25% via the terminal O atom. At high amino concentrations the surface and solution compositions differ slightly (Table 1). This difference likely arises from steric effects associated with the bulky *t*-Boc protecting group on the amine.

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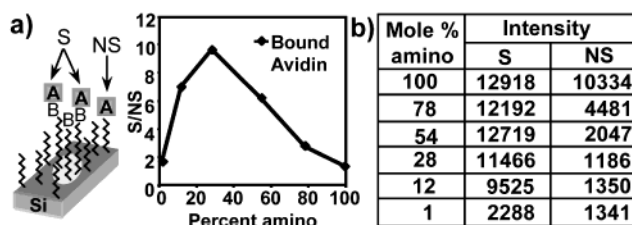


**Figure 2.** Use of mixed monolayers to minimize the nonspecific adsorption of four fluorescently labeled proteins on silicon (a), gold (b), and diamond (c) surfaces.

Fluorescence imaging was used to study the binding of fluorescently tagged avidin, bovine serum albumin (BSA), casein, and fibrinogen to these surfaces. High protein concentrations (0.2 mg/mL in 0.1 M  $\text{NaHCO}_3$ , pH 8.3), long binding times (1 h), and short rinsing times ( $1 \times 15$  min  $2 \times \text{SSPE}$  buffer (Promega) + 1% Triton-X 100) were chosen to challenge the resistance to nonspecific binding. Fluorescence intensities were measured at 512 nm for fluorescein-labeled avidin, BSA, and casein, and at 550 nm for AlexaFluor546-conjugated fibrinogen using a Genomic Solutions UC4  $\times$  4 fluorescence scanner. No significant lateral variations in intensity were detectable, indicating that adsorption occurred uniformly on optical length scales. We note that the fluorescence intensities cannot be used to directly compare the absolute amount of nonspecific binding on the different substrates because of differing amounts of fluorescence quenching. The fluorescence intensities were normalized to those of the 100% amino-terminated monolayers.

The plots in Figure 2 show that the EG3 oligomers on silicon (Figure 2a), gold (Figure 2b), and diamond (Figure 2c) efficiently reduce nonspecific adsorption of all of the proteins studied. The nonspecific adsorption can be reduced by at least 60% on silicon, by 70% on diamond, and by 90% on gold surfaces. Since there are a number of mechanisms by which ethylene glycol oligomers can reduce nonspecific adsorption, understanding the molecular origin of these differences will require additional investigation.<sup>16</sup>

We exploited the properties of these new interfaces in the optimization of a standard protein assay. Utilizing the reactivity of the deprotected amino groups in mixed monolayers, we incorporated biotin into the interface using the amine-reactive biotin linker, sulfosuccinimidyl-6'-(biotinamido)-6-hexamido hexanoate (Pierce Endogen).<sup>7</sup> Avidin was allowed to bind to the entire surface for 10 min at 4 °C, the surface was briefly rinsed and then soaked for 15 min in  $2 \times \text{SSPE}$  buffer + 1% Triton-X 100. Because EG3 reduces the amount of nonspecific binding to the surface, the ratio of specifically bound avidin (the avidin that is retained on the biotinylated spot, S) to nonspecifically bound avidin (the avidin that is retained on the rest of the monolayer, NS), S/NS, can be improved by using mixed monolayers containing EG3 as shown in Figure 3a. The improvement in S/NS by forming mixed monolayers containing EG3 is a factor of 8, which was obtained using approximately 30% Boc-N-ene and 70% EG3-ene. The intensity data in Figure 3b show that this composition optimizes the ability to detect specifically bound avidin while simultaneously reducing the nonspecific adsorption.



**Figure 3.** Specific to nonspecifically (S/NS) bound avidin (A) ratio on mixed monolayers of amino and EG3-terminated alkane, which was reacted in one spot with an NHS-biotin linker (B), on silicon. The intensity data for the plot in 3a is shown in Figure 3b.

Our results show that mixed monolayers containing EG3 functionality on silicon and diamond largely resist the nonspecific adsorption of proteins. The highest S/NS was achieved using a mixed monolayer that allowed for specific binding while reducing nonspecific binding.<sup>17</sup> While previous work has shown that EG oligomers can reduce nonspecific binding on gold,<sup>14</sup> in many applications covalently functionalized materials such as silicon or diamond are advantageous because of their stability under a wide range of chemical and electrochemical conditions<sup>9</sup> and because semiconductor provide a pathway for direct electrical sensing via field-effect devices.<sup>8</sup> The work presented here establishes a method for minimizing nonspecific binding that can significantly enhance the ability to integrate biological molecules, especially proteins, with microelectronic materials.

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