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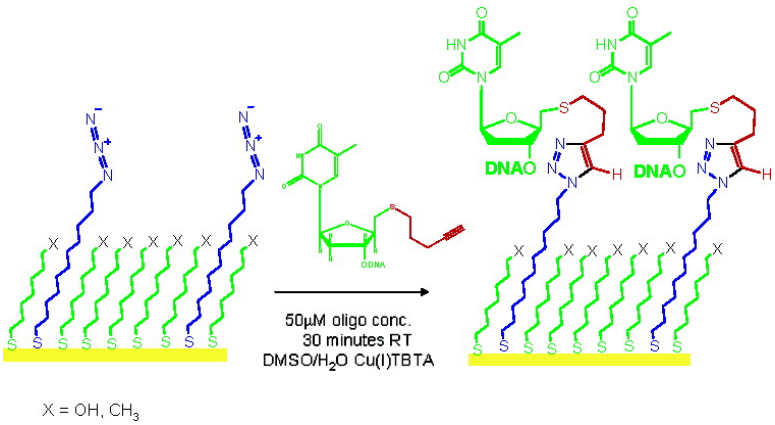

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Chemoselective Covalent Coupling of Oligonucleotide Probes to Self-Assembled Monolayers

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Surface arrays of single-stranded DNA are at the center of some of the most active areas in biological research. These include conventional applications in genome sequencing and disease diagnostics, as well as more novel emerging examples, such as combinatorial drug and reaction discovery.[1] Most methods of oligonucleotide immobilization rely on traditional nucleophilic−electrophilic reactions to achieve coupling of the oligonucleotide to the surface.[2] Unfortunately, this strategy is susceptible to side reactions, for instance, with amino groups on the nucleotides or the small molecule contaminants inherent to oligonucleotide synthesis.[3] Additionally, popular reactive electrophiles, such as *N*-hydroxysuccinimide esters, are prone to hydrolysis before and during the coupling reaction, which both reduce coupling yields and can make the yields irreproducible.[4] Accurate and reproducible detection of target oligonucleotides depends on the accuracy and reproducibility with which the surface can be functionalized. In this paper, we report a chemoselective approach to the formation of oligonucleotide probe surfaces using copper(I) tris(benzyltriazolylmethyl)amine (TBTA)-catalyzed triazole formation between a controlled density of azide groups on densely packed self-assembled monolayers (SAMs) and acetylene groups on the oligonucleotide probe to be immobilized. We have found this strategy to be highly predictable, very fast, and resistant to side reactions, unaffected even by the presence of excess nucleophilic or electrophilic impurities.

Recently, we have demonstrated that Sharpless "click" chemistry can be used to covalently attach acetylene-bearing molecules to azide-terminated SAMs.[5,6] The surface reaction is quantitative and regioselective, exclusively yielding a single product at a single orientation. The chemistry is orthogonal to most typical organic transformations and thus is chemoselective.[7] Recent studies have demonstrated that the chemistry is well suited for the coupling of biomolecules to surfaces.[8] Although application of this chemistry to the attachment of oligonucleotides may appear straightforward, the majority of past work used free Cu(I), typically generated and maintained in aqueous solution by an excess of reducing agent. Unfortunately, in the presence of dioxygen, Cu(I) rapidly damages DNA via the generation of reactive oxygen species.[9] In order for a surface array of oligonucleotides to be useful as a sensor, the structure of the oligonucleotides must be preserved. Recently, the Sharpless group has introduced a triazolylamine copper ligand, tris-(benzyltriazolylmethyl)amine, that can accelerate the cycloaddition reaction.[10] At the same time, this ligand was found to significantly deter the redox chemistry of Cu(I) with oxygen, which is essential for preventing damage to the oligonucleotides. Encouraged by the highly desirable features of the Cu(I)TBTA-catalyzed azide−alkyne cycloaddition, we studied its applicability to the attachment of oligonucleotide probes onto well-defined SAMs.

Oligodeoxyribonucleotides (13mers) bearing reactive acetylene linkers on the 5′-end were synthesized by adapting a previously reported procedure.[11] Briefly, 5′-iodo oligonucleotides were generated on the DNA synthesis column by application of $(PhO)_3PCH_3I$ to the 5′-deprotected oligomers. Reaction of the resulting support-bound iodo oligonucleotide with sodium 4-pentyne 1-thiolate yielded the desired product in 5 min. The modified oligonucleotides were cleaved and deprotected using standard ammoniacal conditions and were used without purification.

Table 1. Oligonucleotide Coverages for Various Thiol SAM Deposition and Oligonucleotide Coupling Conditions

thiol solution	coupling solution	coverage (oligos/cm^2)	hybridization efficiency
5 mM $N_3(CH_2)_{11}SH$	standard	$1.1 \pm 0.1 \times 10^{13}$	$24 \pm 11\%$
5 mM $N_3(CH_2)_{11}SH$	no acetylene on oligo	$2.3 \pm 0.8 \times 10^{10}$	N/A
5 mM $N_3(CH_2)_{11}SH$	standard + 13 × 2-methoxy propylamine	$1.1 \pm 0.2 \times 10^{13}$	N/A
5 mM $N_3(CH_2)_{11}SH$	standard + 13 × acrylonitrile	$1.1 \pm 0.1 \times 10^{13}$	N/A
<0.05 mM $N_3(CH_2)_{11}SH$[a] 4.95 mM $CH_3(CH_2)_7SH$	standard	$3.6 \pm 0.2 \times 10^{12}$	$97 \pm 7\%$
0.05 mM $N_3(CH_2)_{11}SH$ 4.95 mM $HO(CH_2)_6SH$	standard	$1.1 \pm 0.1 \times 10^{12}$	$96 \pm 13\%$

[a] Azide thiol had partially oxidized to the azide disulfide, which is known to adsorb less avidly than the azide thiol.

Azide-terminated SAMs on gold were formed as previously described by soaking silicon wafers, freshly coated with evaporated gold, in SAM deposition solutions of 1-azidoundecane-11-thiol in ethanol either without any other "diluent" thiol to form pure azide-terminated thiol SAMs or with octane thiol or 6-mercaptohexanol to form mixed azide/methyl-terminated or azide/hydroxyl-terminated thiol SAMs (Table 1).[6] The azide-terminated thiol SAMs were exposed to a coupling solution of the 13mer (50 μM) and Cu(I) (400 μM) complexed by TBTA in aqueous dimethyl sulfoxide for 30 min at room temperature, after which the surfaces were rinsed thoroughly with both aqueous and organic solvents. This procedure is both simple and fast in comparison to many previously reported modifications that covalently attach oligonucleotides to surfaces. This method requires a single covalent coupling step (does not require a bifunctional linker), does not make use of buffers or humidification chambers, and occurs in just minutes as opposed to hours.[12]

The oligonucleotide coverage was measured with an assay developed by Tarlov and coworkers.[13] Oligonucleotide surfaces are exposed to dilute solutions of a redox probe ($Ru(NH_3)_6^{+3}$), which electrostatically binds to the phosphate backbone and is electrochemically quantified by a chronocoulometric method that distinguishes bound from soluble $Ru(NH_3)_6^{+3}$. On the basis of this assay, we found that pure azide-terminated thiol SAMs were routinely functionalized with 1.1×10^{13} oligonucleotides/cm^2. To rule out nonspecific adsorption, control reactions were run using a 13mer of the same sequence without the appended acetylene. After

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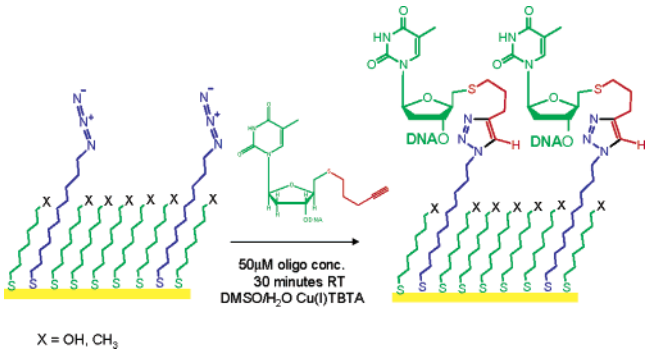

Figure 1. Surface modification of a mixed SAM on gold by chemoselective coupling of acetylene-bearing oligonucleotides.

identical rinsing, roughly 500 times less oligonucleotide could be detected electrochemically. To demonstrate the tolerance of our procedure to impurities, reactant solutions were prepared containing a 13-fold molar excess of either the nucleophile 2-methoxy propylamine or the electrophile acrylonitrile. Neither impurity affected the amount of oligonucleotide attached to the surface during the coupling reaction.

Attempts to hybridize the 13mers on the pure azide-terminated thiol SAMs to the complementary 13mer target resulted in incomplete and variable levels of hybridization. This is not surprising given the high surface density of the 13mers on the pure azide-terminated thiol SAMs. Prior studies have demonstrated that hybridization efficiencies drop as the surface density of oligonucleotides increases due to increasing steric crowding and electrostatic repulsion.[13,14] Fortunately, oligonucleotide probe density can be easily controlled by use of mixed azide/methyl-terminated or azide/hydroxyl-terminated SAMs. Oligonucleotide probe density was reduced by coupling to SAMs formed by immersing gold substrates in deposition solutions containing 1 mol % azidoundecanethiol and 99 mol % of either octanethiol or mercaptohexanol. These surfaces were able to hybridize greater than 90% of all probes to the complementary targets (Table 1).

In summary, we have developed a rapid and straightforward procedure for creating structurally well-defined surface arrays of oligonucleotides. Acetylene-bearing oligonucleotides were reacted with azide-functionalized self-assembled monolayers on gold, and the chemistry was shown to be extremely selective and is, thus, immune to typical yield-reducing side reactions, such as hydrolysis or deactivation by electrophiles or nucleophiles. Specific oligonucleotide coverages can be reproducibly achieved simply by controlling the amount of reactive azide in mixed monolayers. In principle, this technique can be extended to other surfaces on which self-assembled monolayers readily form. For instance, in preliminary work, we have used azidotrimethoxyundecylsilane monolayers on silicate glass surfaces to immobilize the oligonucleotides used here (see Supporting Information).[15] We are working to develop mixed monolayers in this system, so that the density of oligos can be controlled in the same way as that we have shown above for gold substrates. We believe this strategy will be useful in the formation of diagnostic microarrays, where the predictable, reproducible, and fast formation of oligonucleotide probe surfaces is highly desirable.

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Supporting Information Available: Detailed experimental procedures, chronocoulometric response curves, and fluorescent images of oligonucleotide arrays on silica substrate. This material is available free of charge via the Internet at http://pubs.acs.org.

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