Chemoselective Covalent Coupling of Oligonucleotide Probes to Self-Assembled Monolayers

Neal K. Devaraj, Gregory P. Miller, Wataru Ebina, Boyko Kakaradov, James P. Collman, Eric T. Kool, Christopher E. D. Chidsey

Department of Chemistry, Stanford University

Stanford, California, 94305-5080

Supporting Information

Experimental

Reagents:

Dry toluene was distilled over sodium benzophenone ketyl. Glacial acetic acid was dried over molecular sieves. 1-Azidoundecan-11-thiol was synthesized as described previously.[1] Azidotrimethoxyundecylsilane synthesis was adopted from a previously published procedure.[2] All other reagents were purchased commercially and used as received.

Synthesis of Acetylene-Bearing Oligodeoxyribonucleotides

Oligonucleotides were prepared using standard phosphoramidite chemistry on an ABI 394 oligonucleotide synthesizer.

5'-Iodo-modified oligonucleotides were prepared as previously described using the "manual" procedure. A small portion of the unmodified oligonucleotide and the iodinated product were retained for analysis by reverse-phase HPLC.

The 5'-pentyne thioether-modified oligonucleotides were prepared using a previously described procedure with minor modifications. A 1.0mL syringe filled with dry DMF was attached to one end of a DNA synthesis column containing 5'-iodo-oligonucleotide and an empty 1.0mL syringe was attached to the opposite end. The DMF was pushed through the column into the other syringe to remove any water adsorbed to the resin. To prepare the thiolate solution, 100μL of a 0.5M solution of trimethyl phosphine in THF was added to 500 μL of a 1.0M solution of 4-pentyne 1-thiol. After 15 minutes, the solution was added to a suspension of 5.5 mg (50 μmol) sodium pentoxide in 500μL DMF and was agitated for a further 30 seconds. The syringe containing the DMF was then removed from the synthesis column and a syringe containing the thiolate solution was attached. The solution was then passed back and forth between the two syringes for five minutes, followed by a 10mL rinse with DMF and a 5mL CH_2Cl_2 rinse. Cleavage and deprotection

of the alkyne-modified oligonucleotide was effected by incubation with concentrated NH_4OH at 60°C overnight.

Products of the 5'-modification reactions were analyzed by reverse-phase HPLC. Oligonucleotides were eluted using a 0-36% gradient of CH_3CN in 50mM triethylammonium acetate buffer (TEAA) over 20 minutes. Comparison of the chromatograms of the unmodified and iodinated oligonucleotides with that of the reaction product indicate that nearly all of the full-length DNA was converted to a single product. Mass spectral data revealed the mass of the product corresponds to that of the expected 5'-alkyne modified oligonucleotide.

Substrate Preparation:

The gold substrates were prepared by electron-beam evaporation of a titanium adhesion layer (99.99% purity) followed by gold (99.99% purity) onto 4-inch silicon wafers. Silicon was pre-cleaned for 10 minutes in hot piranha (1:3 H_2O_2/H_2SO_4), and rinsed in deionized water (**Warning**: Piranha solution reacts violently, even explosively, with organic materials. It should not be stored or combined with significant quantities of organic material.)

The gold deposition was carried out in a cryogenically pumped deposition chamber. Titanium thicknesses (monitored with a quartz oscillator) were on the order of 10-20nm and gold thicknesses were on the order of 50-100nm. After deposition, the chamber was backfilled with purified argon.

Gold Seal® glass slides were cleaned in hot piranha (1:3 H_2O_2/H_2SO_4), rinsed with deionized water, and dried with nitrogen.

Formation of Mixed SAMs on Gold

Freshly evaporated gold substrates were immersed in depositions solutions made by dissolving the desired ratio of 1-azidoundecan-11-thiol and diluent thiol (either octanethiol or mercaptohexanol) in ethanol. The total thiol concentration was always 5mM. Cleaned gold substrates were then immersed in the deposition solution for 24-36 hours. After deposition, SAM's were rinsed in ethanol and water in order to remove excess absorbate and dried with N_2 to remove residual solvent.

Formation of Azidoundecyltrimethoxysilane SAMs on Silica

Deposition solutions were prepared by first forming a toluene solution consisting of 85% by volume water-saturated toluene and 15% by volume dry toluene. To this solution azidotrimethoxyundecylsilane (2μM) and dry acetic acid (80μM) were introduced. Clean glass slides were immersed in deposition solutions for >15 hours or until a water sessile contact angle of 78±2^{o} was reached indicating complete formation of an azidotrimethoxyundecylsilane monolayer.

Derivatization of gold and silica substrates with oligonucleotide probes

Conjugation solutions consisted of 1:1 Water/DMSO mixtures containing 53μM oligonucleotide probe (5'-TCGATGGCGTCAA-3'), ~400μM $Cu(I)(BF_4)$ ~400μM *tris*-(benzyltriazolylmethyl)amine (TBTA) and ~400μM sodium ascorbate ($Cu(I)BF_4$ was stored in DMSO and used when needed. We noticed color changes indicative of oxidation over a period of several days. One equivalent of Ascorbate was added in order to ensure the presence of Cu(I) at the start of reaction). Azide SAMs on either gold or silica were covered with the deposition solution for thirty minutes in the absence of light. After thirty minutes, the surfaces were rinsed with copious amounts of water, 5% Tween 20 detergent solution, ethanol, and hexane and then again in the reverse order. The samples were finally dried with nitrogen and immediately used.

Electrochemical measurements:

The electrochemical cell area was defined by pressing down on the sample with a cylindrically bored Teflon^{TM} cone (4mm or 8mm inner diameter) pressed against the sample. The bore was filled with 1M aqueous perchloric acid. A platinum counter electrode and a glass frit-isolated Ag/AgCl/KCl reference electrode were suspended above the cell. The cell potential was controlled and the cell current converted to a potential signal by a conventional potentiostat (BAS CV-50W, Bioanalytical systems).

Chronocoulometric determination of oligonucleotide coverages on gold surfaces

A previously published method was adopted in order to determine the oligonucleotide coverages on gold.[3] Briefly, oligonucleotide derivatized surfaces were immersed in a 10mM solution of Tris buffer (pH 7.4) that had been purged with nitrogen for roughly 5 minutes. A potential step was applied (typically +100mV to -400mV vs. Ag/AgCl/3M KCl) and the charge as a function of time was recorded. The charge per area of electrode was then plotted as a function of the square root of time and the linear region fitted by a least squares linear regression. The y-intercept of the fit was taken as

the double layer charge of the given sample. Next, the derivatized electrode was immersed in a 10mM Tris buffer (pH 7.4) containing 50μM ruthenium hexamine ($Ru(NH_3)_6^{+3}$). A potential step was applied starting at a potential where insignificant electrolysis occurs and ending at a potential where the reduction of $Ru(NH_3)_6^{+3}$ was diffusion limited (typically this was a potential step from +100mV to -400mV vs. Ag/AgCl/3M KCl). Once again the charge per area electrode was plotted as a function of the square root of time and the linear region fitted by a least squares linear regression. The y-intercept of the fit was this time taken as the double layer charge plus the faradaic charge due to the reduction of surface immobilized $Ru(NH_3)_6^{+3}$ that was electrostatically bound to the oligonucleotide phosphate backbone. The previously determined double layer charge was subtracted to obtain the charge due solely to oligonucleotide bound $Ru(NH_3)_6^{+3}$. The number of oligonucleotides was determined from the surface density of bound $Ru(NH_3)_6^{+3}$ by using the following equation:

$$\Gamma_{DNA} = \Gamma_0(z/m)$$

Where Γ_{DNA} is the surface density of oligonucleotide, Γ_0 the surface density of $Ru(NH_3)_6^{+3}$, *z* is the charge of the redox molecule (in this case +3), *m* the number of phophates in the oligonucleotide probe (in this case 12). Hybridization efficiencies were calculated by exposing the oligonucleotide probe surfaces to complementary targets (vide infra) and then repeating the chronocoulometric measurement, this time comparing the increase in $Ru(NH_3)_6^{+3}$ probe coverage to the $Ru(NH_3)_6^{+3}$ probe coverage before hybridization.

Hybridization of oligonucleotide probes to complementary targets

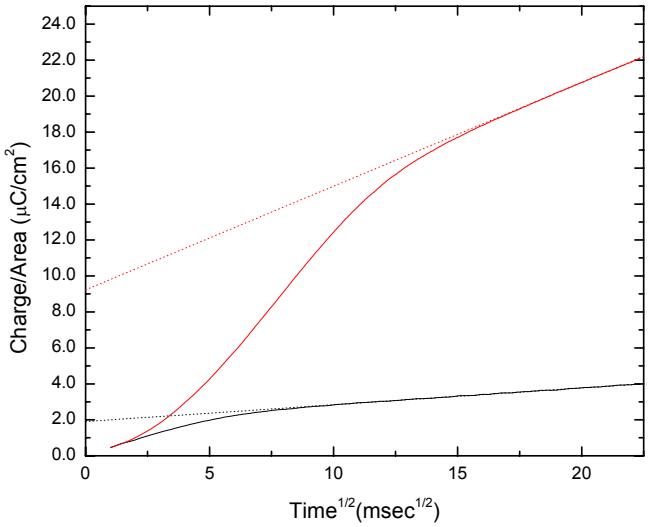
Procedure adapted with modification from previously published work.[4] Briefly, surfaces were first treated with a 10μM solution of a non-complementary oligonucleotide in 1.0M NaCl with 10mM Tris buffer (pH 7.4) and 1mM EDTA for 15-20 minutes. The surface was then rinsed with 10mM NaCl with 5mM Tris (pH 7.4) and water until we were unable to observe non-specific oligonucleotide adsorption via electrochemical assay (for example we noticed that hydrophobic surfaces were more prone to non-specific binding, and thus required more thorough rinses when compared to hydrophilic surfaces). The procedure was then exactly repeated on the same surface but instead using a complementary target oligonucleotide. The amount of hybridized probe was determined electrochemically as described above.

Contact Angle Goniometry

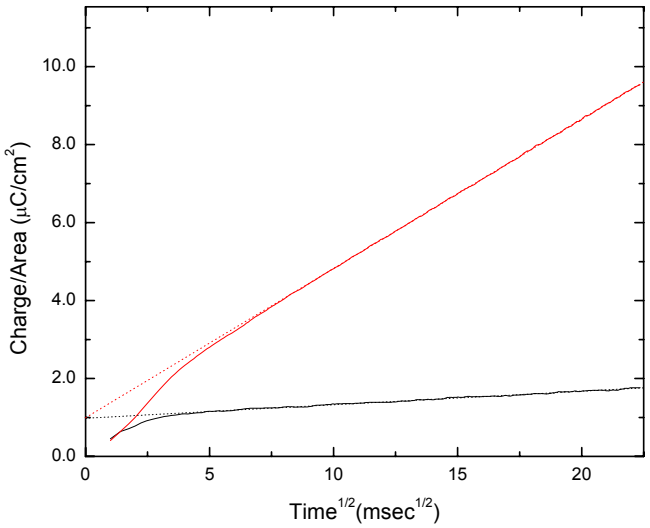
Contact angles were determined on a Rame-Hart model 100 goniometer at room temperature. Contact angles, θ, were measured from sessile drops by lowering a 1μL drop suspended from a fine, blunt syringe needle onto the surface. Both sides of the drop were measured. This was repeated three times and averaged to obtain θ for the surface.

Data/Results

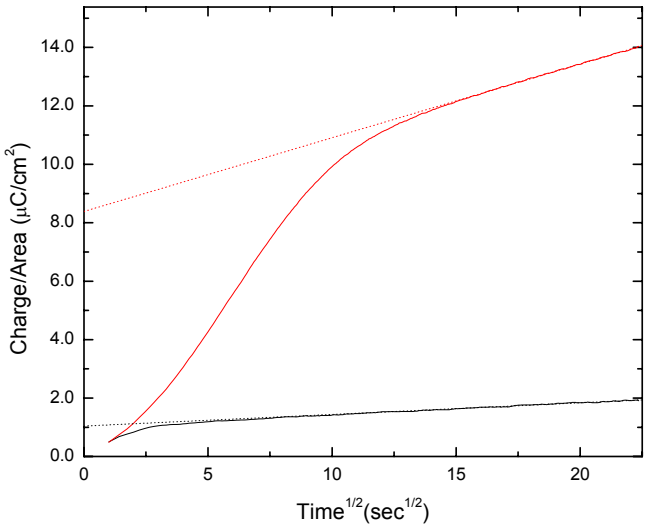
Chronocoulometric Response Curves (solid lines) and fits of the linear region (dotted lines) for SAMs on gold after being exposed to various conjugation solutions. Data was taken in the absence (black) and presence (red) of $Ru(NH_3)_6$ which is known to specifically bind to the phosphate backbone of nucleic acids. The difference in where the black and red linear fits cross the y-axis is directly proportional to the oligonucleotide coverage.



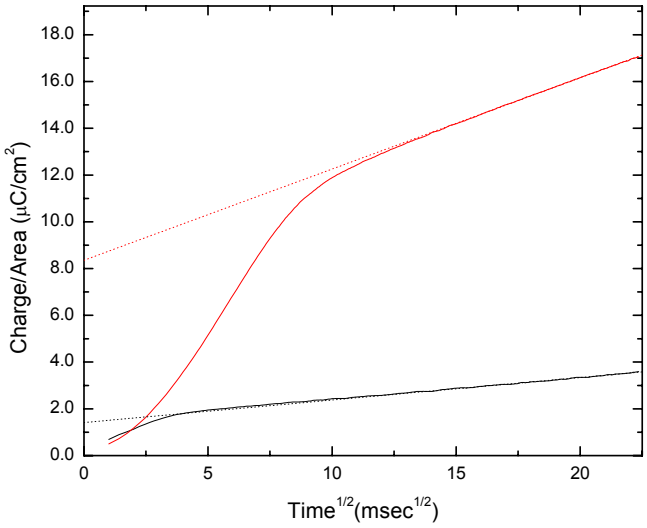
Trial 1: pure azide-derivatized SAM formed from 5mM $N_3(CH_2)_{11}SH$ exposed to standard conjugation solution containing an acetylene functionalized oligonucleotide (13mer). Surface functionalized with $1.2x10^{13}$ oligonucleotides/cm^2.



Trial 2 (control experiment): pure azide-derivatized SAM formed from 5mM $N_3(CH_2)_{11}SH$ exposed to standard conjugation solution containing a control oligonucleotide (13mer) that did not possess an acetylene group. Detected $2.3\pm0.8 \times 10^{10}$ oligonucleotides/cm^2. This is an upper limit to the amount of oligonucleotide nonspecifically bound to the surface. The uncertainty in the measurement is the uncertainty between the extrapolated intercepts in the plot.



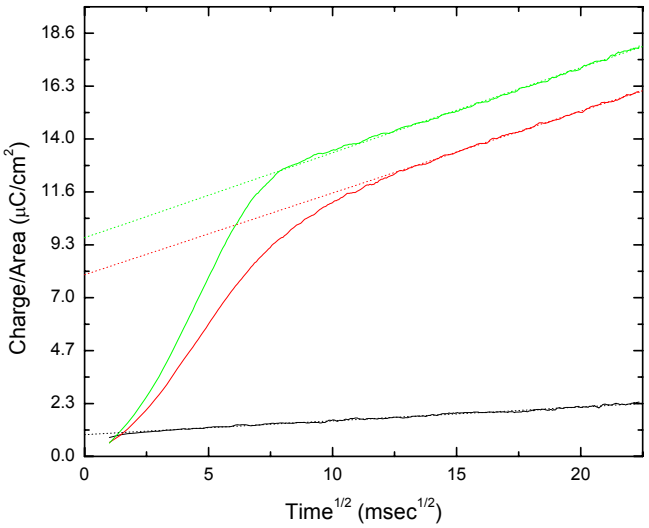
Trial 3 (nucleophilic impurity): pure azide-derivatized SAM formed from 5mM $N_3(CH_2)_{11}SH$ exposed to standard conjugation solution containing an acetylene functionalized oligonucleotide (13mer) and 13x 2-methoxy propyl amine. Surface functionalized with 1.2×10^{13} oligonucleotides/cm^2.



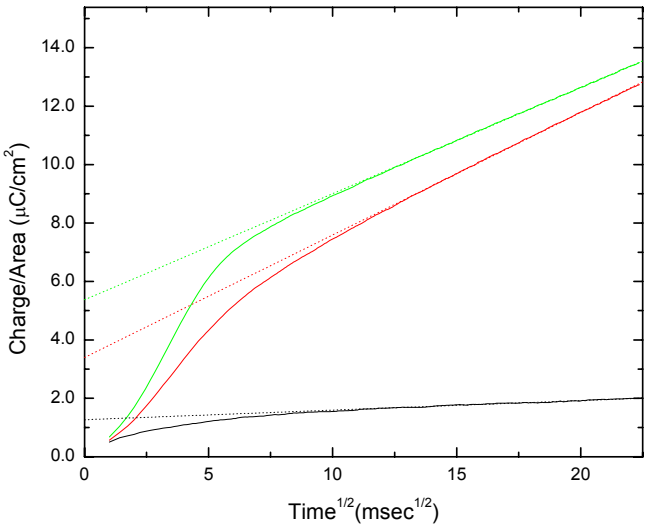
Trial 4 (electrophilic impurity): pure azide-derivatized SAM formed from 5mM $N_3(CH_2)_{11}SH$ exposed to standard conjugation solution containing an acetylene functionalized oligonucleotide (13mer) and 13x acrylonitrile. Surface functionalized with $1.1x10^{13}$ oligonucleotides/cm^2.

Control of Azide density through formation of mixed SAMs and hybridization data

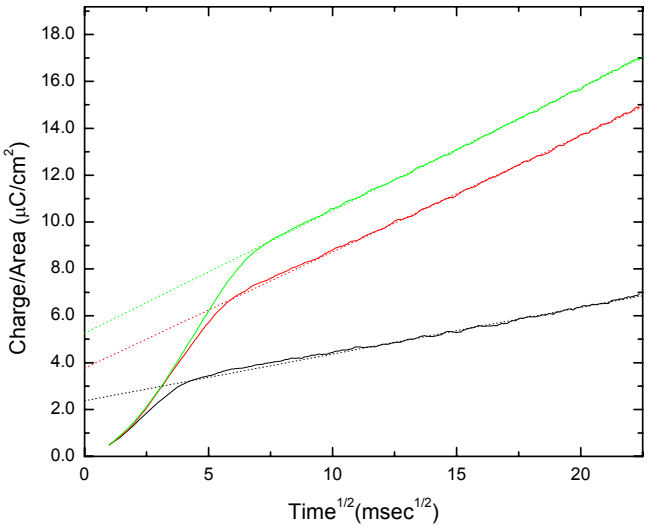
Chronocoulometric response curves (solid lines) and fits of the linear region (dotted lines) for hybridization experiments. Curves were taken in the absence (black) and in the presence of an oligonucleotide specific electroactive marker before (red) and after (green) exposure to a complementary oligonucleotide (13mer). The position at which the linear fits cross the y-axis is indicative of the charge contribution from the double layer and surface bound probe. The difference between the red or green intercept and the black intercept is thus a measure of the amount of surface bound redox active probe.



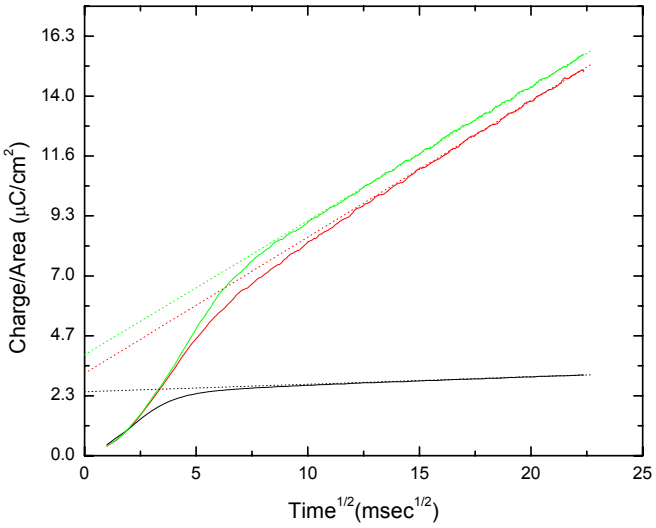
Trial 1: pure azide-derivatized SAM formed from 5mM $N_3(CH_2)_{11}SH$. Surface derivatized with $1.2x10^{13}$ oligonucleotides/cm^2. Hybridization efficiency: 24%.



Trial 2: hydrophobic mixed azide/methyl SAM formed from <0.05mM $N_3(CH_2)_{11}SH$ (sample contained both thiol and the disulfide which is known to adsorb less avidly than the thiol) and 4.95mM $CH_3(CH_2)_7SH$. Surface derivatized with $3x10^{12}$ oligonucleotides/cm^2. Hybridization efficiency: 93%.



Trial 3: hydrophilic mixed azide/alcohol SAM formed from 0.10mM $N_3(CH_2)_{11}SH$ and 4.9mM $HO(CH_2)_6SH$. Surface derivatized with $2x10^{12}$ oligonucleotides/cm^2. Hybridization efficiency: 107%.

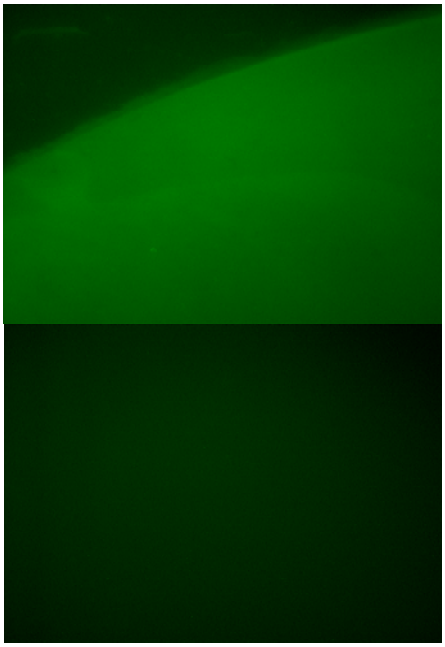


Trial 4: hydrophilic mixed azide/alcohol SAM formed from 0.05mM $N_3(CH_2)_{11}SH$ and 4.95mM $HO(CH_2)_6SH$. Surface derivatized with roughly $1x10^{12}$ oligonucleotides/cm^2. Hybridization efficiency: 103%.

Preliminary Results with Glass Surfaces

Azidotrimethoxyundecylsilane reacts with silicate glass surfaces, forming SAMs with similar properties as those on gold. Glass microscope slides were cleaned with piranha etch (3:1 concentrated sulfuric acid and hydrogen peroxide) and then immersed overnight in a solution made from wet toluene (85% water saturated), 2μM azidotrimethoxyundecylsilane and 80μM acetic acid. The slides were taken out of the deposition chambers and rinsed with copious amounts of organic and aqueous solvents before use. Applying the same reaction conditions as before, we coupled an acetylene-bearing 13mer labeled with fluorescein to pure azidotrimethoxyundecylsilane SAMs on glass slides. After rinsing, we imaged the surface and observe fluorescence due to the fluorescein (see below) and a significant decrease in the contact angle from 78±2^{o} to 24±2^{o}. Deposition solutions containing the fluorescein-labeled oligonucleotide without an appended acetylene did not react as evidenced by the absence of detectable fluorescence and the absence of a detectable change in the contact angle. We are currently exploring the use of mixed azide/methyl-terminated and azide/hydroxyl-terminated silane SAMs to control the density of coupled oligonucleotides.

Fluorescent Images



Fluorescence images of an azidotrimethoxyundecylsilane modified glass surface after exposure to conjugation solutions containing fluorescein labeled oligonucleotide bearing either a reactive acetylene (top) or a 5'-hydroxyl group (bottom). Images taken at the edges of spot where the fluorescein-labeled oligonucleotides were placed. Image parameters were treated identically.

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[1] Collman, J. P.; Devaraj, N. K.; Chidsey, C. E. D. *Langmuir* **2004**, 20, 1051-1053.
[2] Fu, Y.; Yu, S. J. Angew. Chem. Int. Ed. 2001, 40, 437-440.
[3] Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, 70, 4670-4677.