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## Mixed Azide-Terminated Monolayers: A Platform for Modifying Electrode Surfaces

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### Abstract

We have prepared and characterized mixed self-assembled-monolayers (SAM) on gold electrodes from azido alkane thiols and various  $\omega$ -functionalized alkane thiols. In the presence of copper(I) catalysts these azide-modified surfaces are shown to react rapidly and quantitatively with terminal acetylenes forming 1,2,3-triazoles, via “click” chemistry. The initial azide substituents can be identified and monitored using both grazing-angle infrared (IR) and X-ray photoelectron spectroscopies. Acetylenes possessing redox-active ferrocene substituents react with the azide-terminated mixed SAMs and electrochemical measurements of the ferrocene-modified SAM electrodes have been used to quantify the redox centers attached to these platforms. Time-resolved electrochemical measurements have enabled us to follow the formation of these ferrocene centers and thus to measure the rate of the surface “click” reaction. Under optimal conditions this well-behaved second-order reaction takes place with a rate constant of  $1 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ . Typical reaction times of several minutes were realized using micromolar concentrations of acetylene. These techniques have been used to construct well-characterized, covalently-modified monolayers that can be employed as functional electrode surfaces.

### Keywords

Self-Assembled Monolayers; Azide; Click Chemistry; Kinetics; Surface Reaction

### Introduction

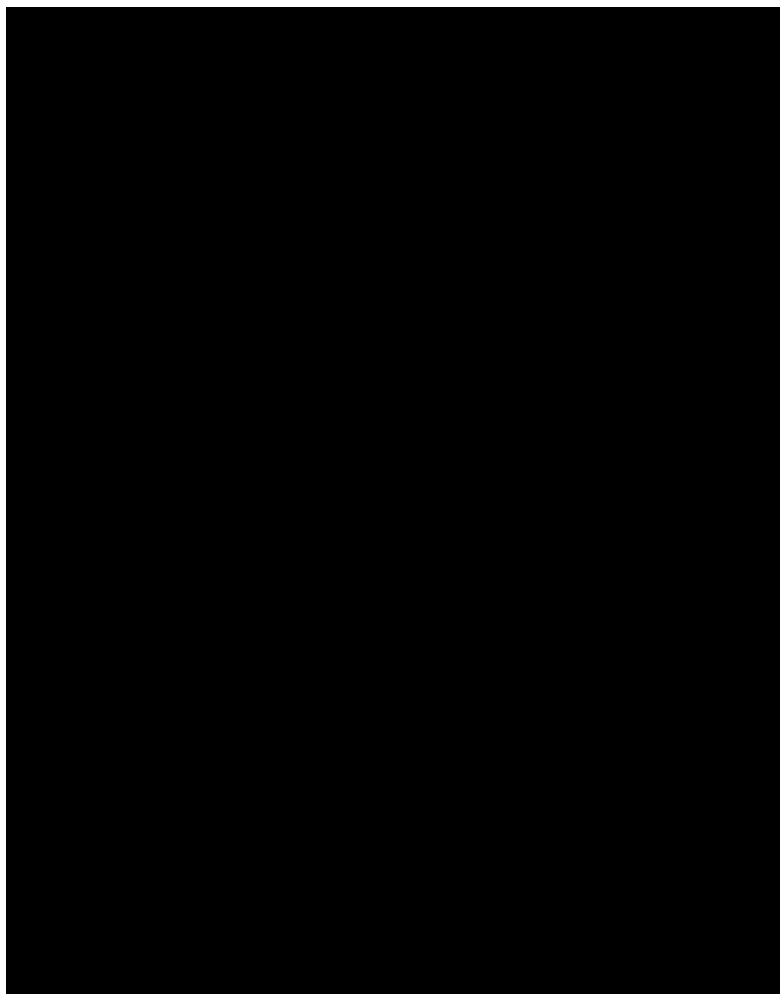
Self-assembled monolayers (SAMs) offer a chemically well-defined interface between surfaces and species of interest such as molecular catalysts or biomolecules. Although monolayers form on a variety of different surfaces, such as silica, silicon, alumina and silver, monolayers on gold are perhaps the most common due to their ease of formation and the ability to use the gold substrate as an electrode<sup>1</sup>. SAMs on gold are routinely functionalized with a variety of redox-molecules, oligonucleotides, and nanomaterials. Typically one synthesizes the species of interest with a pendant thiol group and then forms a mixed SAM on a gold surface from the thiolated species and a diluent thiol. Although this approach is straightforward, it is not ideal if one wishes to study a variety of different species and linkers. Not only must additional time be invested in the synthesis of each thiolated species and in the characterization of its surface coverage, but there is also no guarantee that a particular species will form a well ordered SAM simply because it is tethered to a thiol.<sup>2</sup>

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A more versatile and convenient approach is to first assemble a SAM containing terminal reactive groups that can serve as a well-defined platform onto which one can subsequently couple a wide range of species to the surface. Unfortunately, most coupling methods are hampered by lack of specificity, difficulty in introducing the reactive functional groups or low yields.<sup>3-5</sup> Ideally, one desires an extremely high-yield coupling reaction that uses chemistry and functional groups that are easy to introduce, highly specific, and compatible with a wide range of species and solvents. Recently we reported the use of Sharpless “click” chemistry, specifically the Cu(I)-catalyzed azide-alkyne cycloaddition, as a method for preparing such monolayers.<sup>6, 7</sup> Mixed monolayers of azidoundecanethiol and decanethiol were first formed and then coupled with two different ferrocene acetylenes. The coupling reaction gives a high yield and proceeds under very mild conditions. Both the azide and the acetylene can be conveniently incorporated into a wide variety of species and neither reactant reacts with itself, the solvent, or the catalyst.<sup>8</sup> Coupling only occurs between the two reactants. SAMs are known to be more stable in polar aqueous solvent systems, and thus it is fortunate that the chemistry works best in aqueous and mixed aqueous-organic solvents. By monitoring the intense infrared stretching mode of organic azides, we have been able to demonstrate that the surface conversion is quantitative. Since our initial report, other groups have demonstrated that “click” methodology is applicable to azide-terminated SAMs on silica, as well as acetylene terminated SAMs on gold.<sup>9, 10</sup> We and others have used this technique to modify SAMs with complex functional molecules such as single-stranded DNA, porphyrin redox catalysts, and receptors for gold nanoparticles<sup>11, 12</sup>. Although our interest is in SAMs, recent reports have revealed numerous other materials applications of “click” chemistry.<sup>13-19</sup>

The purpose of the present work is to further demonstrate the utility of these azide-containing monolayers as platforms for surface immobilization. There are several requisites for an ideal immobilization strategy. In order to control the coverage of species coupled to the surface, the reactive group should be easily assayed. It should also be simple to modify the surface rapidly with a complementary reactive group. Surface chemistry is hampered to a greater degree than solution chemistry by incomplete reactions and side reactions because unreacted and byproduct materials cannot be separated from the surface. Moreover, the type and sensitivity of surface-characterization methods are severely limited whereas a wide range of methods are available to characterize reactions in solution. Therefore, a useful surface immobilization strategy should be quantitative, fast, resistant to side reactions, and easily monitored by routine surface characterization techniques. Azide-terminated surfaces subsequently modified by copper-catalyzed azide-alkyne cycloaddition completely satisfy these requirements.

In the current work, the surface coverage of reactive azides has been quantified electrochemically by using a simple redox-active probe. These surface coverages correlate with easily measured physical properties such as contact angles. Furthermore, the azide group can be characterized by using two common surface analysis techniques, grazing-angle infrared (IR) spectroscopy and X-ray photoelectron spectroscopy (XPS). We have used these techniques to measure reaction yields and to look for side reactions. Azide coverages were monitored during functionalization, and the rate of reaction on this surface was studied. By combining results from the electrochemical and spectroscopic techniques we demonstrate that azide-containing mixed monolayers are a nearly ideal reactive surface onto which acetylene bearing molecules can be “clicked.”



## Experimental Details

### Reagents

Methylene chloride, ethanol, octanethiol **3**, decanethiol **4**, dodecanethiol **5**, hexadecanethiol **6**, mercaptoundecanoic acid **7**, mercaptoundecanol **8**, and Tetrakis(acetonitrile)copper(I) Tetrafluoroborate were purchased from commercial sources and used as received. *Tris*-(benzyltriazolylmethyl)amine (TBTA) was received as a gift from the Sharpless group.

The synthesis of 1-azido-11-undecanethiol **1**, 1-ferrocenyl-2-propyn-1-one **10**, and ethynylferrocene **11** have been described elsewhere.<sup>6, 20</sup> 1-azido-11-undecanethiol **2** was synthesized in an identical manner to 1-azido-11-undecanethiol with the exception that 1-bromohexadecanol was used as the starting material and during the last step (thioacetate deprotection) ethanol was substituted for methanol to maintain solubility. In a typical preparation of bis(11-azidoundecyl) disulfide **9**, 100 mg of 1-azido-11-undecanethiol and 1 equivalent of MnO<sub>2</sub> was added to 10 mL of methylene chloride. The mixture was stirred in air overnight and then filtered through a Celite pad. Excess solvent was removed by rotary evaporation. Typical yields were 80% <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): 3.25 (t, 4H); 2.68 (t, 4H); 1.57 (m, 8H); 1.22-1.4 (m, 28H).

Copper(I) *tris*-(benzyltriazolylmethyl)amine tetrafluoroboride (Cu(TBTA)BF<sub>4</sub>) was formed by adding equimolar amounts of tetrakis(acetonitrile)copper(I) tetrafluoroborate (stored in an inert atmosphere box) and *tris*-(benzyltriazolylmethyl)amine to DMSO. The resulting solution was stored outside the inert atmosphere box where it slowly oxidizes with time. For this reason, we added a mild reducing agent to the coupling solution to ensure that the catalyst was in the active Cu(I) oxidation state.

### Substrates

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 vol 30% by mass aqueous H<sub>2</sub>O<sub>2</sub>; 3 vol H<sub>2</sub>SO<sub>4</sub>), 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 deposition was carried out in a cryogenically pumped deposition chamber. Titanium thicknesses (monitored with a quartz oscillator) were on the order of 15-20nm and gold thicknesses were on the order of 150-200nm. After deposition, the chamber was backfilled with argon.

### Formation of Mixed SAMs

Deposition solutions were made by dissolving the desired ratio of decanethiol and 1-azidoundecan-11-thiol in ethanol. The total thiol concentration was kept between 0.1-1mM. Freshly evaporated gold substrates were immersed in the deposition solution for 24-36 hours. After deposition, SAMs were rinsed with copious amounts of ethanol, methylene chloride, and water to remove excess adsorbate and then dried with N<sub>2</sub> to remove residual solvent. Mixed SAMs were formed from azidoundecanethiol N<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>SH **1** and various diluent thiols; octanethiol CH<sub>3</sub>(CH<sub>2</sub>)<sub>7</sub>SH **3**, decanethiol CH<sub>3</sub>(CH<sub>2</sub>)<sub>9</sub>SH **4**, hexadecanethiol CH<sub>3</sub>(CH<sub>2</sub>)<sub>15</sub>SH **6**, mercaptoundecanoic acid HO<sub>2</sub>C(CH<sub>2</sub>)<sub>10</sub>SH **7**, and mercaptoundecanol HO(CH<sub>2</sub>)<sub>11</sub>SH **8**. In order to compare thiols with disulfides, mixed monolayers were also prepared from bis(11-azidoundecyl) disulfide (N<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>S)<sub>2</sub> **7**. For kinetic measurements, mixed SAMs were formed from azidohexadecanethiol N<sub>3</sub>(CH<sub>2</sub>)<sub>16</sub>SH **2** and either dodecanethiol **5** or hexadecanethiol **6** as diluents. Additionally, SAMs used for kinetic measurements were exchanged overnight in 100mM concentration diluent solutions in order to ensure that azide groups located at defect sites were eliminated.

### Formation of Ferrocene-Terminated Monolayers:

In a typical preparation, mixed monolayers of N<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>SH and CH<sub>3</sub>(CH<sub>2</sub>)<sub>9</sub>SH were completely submerged in a 1mM solution of ferrocene propynone **10** in a solvent comprised of 2 vol. water : 1 vol. ethanol). To catalyze the “click” reaction two procedures were routinely used. In the first procedure 1 mol% CuSO<sub>4</sub>·5H<sub>2</sub>O was added followed by 15 mol% sodium ascorbate to generate a catalytically active Cu<sup>I</sup> species. In the second procedure (which was used for all the kinetic trials) deposition solutions consisted of 3:1 DMSO/water containing 400μM copper(I) *tris*-(benzyltriazolylmethyl)amine tetrafluoroboride (Cu(TBTA)BF<sub>4</sub>), 8mM hydroquinone (to ensure that the catalyst was in the active Cu(I) oxidation state), and 1-20μM ethynylferrocene **11**. In order to prevent the known photo-oxidation of the thiol monolayers during reaction, the reaction container was covered in foil and stored inside a drawer in the absence of light but still in the presence of air for the duration of the reaction (for the first procedure this was roughly 4-6 hour and for the second procedure 5-20 minutes)<sup>21</sup>. After reaction, monolayers were rinsed with copious amounts of ethanol, water, methylene chloride, and then again with water to ensure that any adventitiously absorbed ferrocenes were washed off.

## Contact Angle Goniometry

Contact angles were determined on a Rame-Hart model 100 goniometer at room temperature. Contact angles,  $\theta$  were measured from sessile drops by lowering a 1  $\mu$ L 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  $\theta$  for the surface.

## Infrared External Reflection Spectroscopy:

Spectra were obtained using a Mattson RS 10000 FT-IR spectrometer with external optics enclosed in a plexiglass box and bathed by dried, CO<sub>2</sub>-depleted air. A narrow-band MCT detector cooled with liquid nitrogen was used to detect the light. The light was incident on the sample at 86° from the normal. Only p-polarized light was collected (light polarized in the plane of incidence). 64 scans were recorded at 8 cm<sup>-1</sup> resolution. The moving mirror speed was 3.1 cm/sec (HeNe laser modulation frequency = 50 kHz) in the forward and reverse directions. The SAM spectra were ratioed against a background spectrum of freshly evaporated gold.

## Electrochemical Measurements:

The electrochemical cell area was defined by a cylindrically bored Teflon™ cone (4 mm inner diameter) pressed against the sample. The bore was filled with 1 M aqueous perchloric acid. A platinum counter electrode and a glass frit-isolated Ag(s)/AgCl(s)/3 M NaCl(aq) aqueous 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 (PAR 273). The potential program signal to the potentiostat was generated by a Wavetek 395 function generator, and the current response signal from the potentiostat was recorded using a Tektronix TDS 520 digital oscilloscope. All measurements were performed at room temperature without exclusion of air.

## X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were obtained on a Surface Science model 150 XPS spectrometer equipped with an Al K $\alpha$  X-ray source, quartz monochromator, concentric hemispherical analyzer operating in constant analyzer mode, and a multichannel detector. The pressure in the analytical chamber during analysis was approximately 2  $\times$  10<sup>-8</sup> Torr. A takeoff angle of 5° or 35° from the surface was employed. Spectra were obtained at 0.1 eV resolution and areas under the unsmoothed peaks were measured (after a Shirley background subtraction) and corrected for the number of scans.

## Results and Discussion

### Electrochemical Determination of Azide Surface Concentration

A common problem in characterizing mixed monolayers is determining the surface coverages of various components. Although the mole fraction of each absorbent is known in the deposition solution ( $\chi_{\text{soln}}$ ), these are typically not the surface mole fractions of the absorbents after monolayer formation ( $\chi_{\text{surf}}$ ). Differing solubilities of the adsorbants in the deposition solvent is a common reason for this disparity.<sup>22</sup>

In this work, we sought to quantify the mole fraction of azide-terminated adsorbates on the surface as a function of the mole fraction in the ethanolic solutions for different diluent thiols. In a previous report we used infrared spectral analysis to show that the reaction between a ferrocene acetylene and the organic azide group of mixed azidoundecanethiol-decanethiol SAMs proceeds quantitatively<sup>6</sup>. Taking advantage of this property, we determined the surface coverages of azides for a number of different monolayers by modifying the monolayer with a redox probe **10** whose coverage can be electrochemically determined after the reaction.

Assuming that a monolayer of well packed alkyl thiols is entirely composed of the  $\sqrt{3} \times \sqrt{3} R30^\circ$  structure on Au(111) and so consists of  $4.67 \times 10^{14}$  molecules/cm<sup>2</sup>,<sup>23</sup> one can find the fractional coverage of azide by ratioing the electrochemically determined ferrocene coverage to the assumed total coverage of alkyl thiol chains.

Figure 1 depicts the electrochemically determined ferrocene coverage as a function of  $\chi_{\text{soln}}$ . When forming mixed monolayers from ethanolic deposition solutions consisting of two alkyl thiols, the adsorption of the longer alkyl thiol is known to be preferred<sup>22</sup>. This was confirmed by the surface coverage of ferrocene versus  $\chi_{\text{soln}}$  when octanethiol **3** and decanethiol **4** were used as diluents. In the extreme case that monolayers were formed from solutions containing hexadecanethiol **6**, a diluent thiol with an alkyl chain much longer than that of the azide, no ferrocene could be detected electrochemically (Figure 1A). Hydrophilic monolayers formed with either mercaptoundecanoic acid **7** or mercaptoundecanol **8** as diluents both showed amounts of azide incorporation similar to that seen with using decanethiol as diluent (Figure 1B).

Although the plots in Figure 1 are well behaved at low  $\chi_{\text{soln}}$  values, at high  $\chi_{\text{soln}}$  values a limit to the amount of ferrocene that can be immobilized is reached. When reacting the acetylene modified redox probe on pure azide surfaces with no diluting adsorbate ( $\chi_{\text{soln}}=1.0$ ) roughly 55% of a monolayer can be functionalized with ferrocene. One interpretation of this limit is that the steric bulk of the ferrocenes prevent further conversion of azides to triazoles. Previous studies have estimated the maximum ferrocene coverage to be about 58% of a monolayer which is in excellent agreement with our experimentally determined limit.<sup>2</sup> To test this hypothesis, we sought to compare the remaining azide after reaction for both a pure azidoundecanethiol monolayer and a mixed monolayer of azidoundecanethiol and octanethiol  $\chi_{\text{soln}}=0.5$  for which  $\chi_{\text{surf}}=0.45$  is obtained by the electrochemical measurement of the ferrocene coverage. After reacting both monolayers with an acetylene-modified ferrocene, we searched for the presence or absence of the strong azide asymmetric stretch by grazing-angle reflection-absorption spectroscopy (see section on grazing-angle IR spectroscopy). The azide signal disappeared completely from the mixed monolayer indicating complete conversion whereas the azide signal persisted in the 100% azidoundecanethiol monolayer after reaction indicating that unreacted azide groups were present on the surface (Figure 2). This result is consistent with our hypothesis and supports our claim that azide disappearance is due to triazole formation and that the reaction is quantitative for coverages lower than the steric limit.

### Coadsorption of Thiol vs. Disulfide

It is well established that both alkyl thiols and disulfides will adsorb onto gold surfaces to form SAMs. Under prolonged exposure to air, azidoundecanethiol slowly oxidizes to the disulfide. We were therefore interested in the rate of adsorption of the disulfide relative to the thiol as the former can be expected to be a common contaminant in adsorption solutions having varying concentrations. Using XPS, Whitesides showed that the relative rates of adsorption of thiols and disulfides differ significantly, with the thiol adsorbing roughly 75 times faster than the corresponding disulfide.<sup>24</sup> However, using electrochemical methods, He *et al.* observed that ferrocene alkyl thiols and related disulfides adsorb at the same rate.<sup>25</sup> Since thiol surface exchange with thiols in solution has been shown to be extremely slow, the surface composition of mixed monolayers can be used to draw conclusions about the relative rates of adsorption.<sup>2</sup>

Using the click reaction to modify the azide containing SAMs with ferrocenes after adsorption, we were able to measure electrochemically the relative amounts of adsorption without interference from the redox-active ferrocene during the adsorption event.

Figure 3 shows azide coverages for mixed monolayers formed from solutions containing either azidoundecanethiol **1** or bis(11-azidoundecyl) **9** disulfide. In both cases, decanethiol **4** is the diluent. The results indicate that the azidoundecanethiol adsorbs significantly faster compared to bis(11-azidoundecyl) disulfide when in competition with decanethiol. Disulfides adsorb to a significant surface mole fraction only at high solution mole fractions. When the disulfide was the only adsorbent present in the deposition solution, the resulting monolayers yielded nearly identical ferrocene coverages as monolayers formed from azidoundecanethiol. Contact angles and infrared spectra are consistent with these results.

### Contact Angles

Contact angle goniometry is a convenient and straightforward method to measure the wetting properties of monolayers.<sup>26</sup> By measuring the sessile contact angles of water on mixed monolayers, we were able to reproducibly determine the behavior of water droplets as a function of the composition of  $\chi_{\text{soln}}$  ( $\chi_{\text{soln}}$  equals the molar ratio of azide to diluent in solution). Figure 1 depicts the sessile contact angle of water versus the composition of the deposition solution from which the monolayer was made. All monolayers consisting of entirely methyl-terminated alkyl thiols exhibited contact angles around 110°. On the other hand, sessile drops on monolayers terminated entirely by hydrophilic acid or alcohol groups were irregular and had contact angles of about 15°. Finally, an entirely azide-covered surface, whether made from azidoundecanethiol or the corresponding disulfide gave reproducible contact angles of 77°. By comparing this information with the surface-coverage information obtained from using redox probes, we were able to relate the surface concentration of reactive azidoundecanethiol **1** to the contact angle of water. For example, a mixed monolayer of azidoundecanethiol and octanethiol **3** with a contact angle of 83° would be predicted to have a surface composition of roughly 32% azide. Because contact angles are easy to obtain, this serves as a useful calibration to check the fidelity of monolayers made from diverse deposition solutions. As explained in a previous section, this would only be applicable to coverages below 50% due to the steric limit of ferrocene coverage on the surface.

As expected, mixed monolayers exhibited contact angles in between those observed for pure monolayers of either component. For a given mole fraction of azidoundecanethiol in the adsorption solution, mixed monolayers consisting of azidoundecanethiol and methyl-terminated alkyl thiols were more hydrophobic (larger contact angle) the longer the alkyl chain of the diluent. As previously mentioned, this is likely due to preferential absorption of longer-chain alkyl thiols from ethanolic solutions onto the gold surfaces.<sup>22</sup> Hydrophilic monolayers composed of either mercaptoundecanoic acid **7** or mercaptoundecanol **8** show similar increases in contact angle as a function of the mole fraction of azidoundecanethiol.

### Grazing Angle Infrared (IR) Spectroscopy of Azide-Containing Surfaces

Grazing angle infrared (IR) spectroscopy is a convenient technique for determining the chemical functionality on gold surfaces. The grazing-angle IR spectra for a monolayer consisting entirely of azidoundecanethiol is shown in Figure 5. Carbon-hydrogen stretches at 2921cm<sup>-1</sup> and 2850cm<sup>-1</sup> are observed and correspond to the methylene asymmetric and symmetric stretches respectively.<sup>26</sup> The asymmetric methylene stretch location indicates that the azidoundecanethiol (**1**) is densely packed and well-ordered.<sup>27</sup> The prominent asymmetric stretch of the organic azide group occurs at 2105cm<sup>-1</sup>.<sup>28</sup> This azide stretch is easily detectable for all mixed azidoundecanethiol monolayers, and therefore serves as a convenient measure of the presence or absence of the reactive functional group.

The integrated intensity of the azide stretch versus the mole fraction of ferrocene-derivitized azidoundecanethiol for hydrophobic diluents is shown in Figure 6a. Figure 6b displays similar data using hydrophilic diluents. In this case, the integrated intensity is roughly similar for both

diluents with respect to solution concentration. Most of the data follow similar trends with respect to the contact angle and electrochemical coverage data. A notable exception is the IR intensity of the azidoundecanethiol thiol + hexadecanethiol **8** mixed monolayers. Azidoundecanethiol has definitely been incorporated into the SAM as evidenced by the integrated IR intensity. However, as mentioned in the previous section, we were unable to detect the azide since it failed to react with the acetylene modified redox-active probe. The use of hexadecanethiol as a diluent was motivated by the question of whether a longer diluent would inhibit the reaction between the azides and ferrocene acetylenes. Because the azide is clearly present, we conclude that steric crowding of the longer hexadecanethiol molecules acts as a kinetic barrier to the cycloaddition and shuts down the reaction.

The IR spectra of a  $\chi_{\text{soln}}=0.5$  azidoundecanethiol and octanethiol (**3**) monolayer before and after functionalization with propynone ferrocene are displayed in Figure 7. The azide asymmetric stretch around  $2100\text{cm}^{-1}$  disappears to the noise level, and, in its place, new signals emerge in the region between  $1700$  and  $1900\text{cm}^{-1}$  which are associated with the carbonyl CO and aromatic CC stretches.

### X-ray photoelectron spectroscopy (XPS) Analysis

X-ray photoelectron spectroscopy (XPS) offers another spectroscopic method useful for surface analysis. Whereas IR gives information about the types of chemical functionality present on a surface, XPS allows analysis of the elemental composition of surfaces. In this case, we used XPS to observe changes due to reaction of surface azides with acetylenes. High resolution spectra of the nitrogen 1s binding energies of azidoundecanethiol **1** monolayers revealed two distinct peaks, one centered at 400 eV and another centered at 405 eV, with a 2:1 ratio of the peak areas.<sup>4</sup> The less intense, higher energy, 405 eV peak is unusual for an organic nitrogen and we assign this peak to the central, electron-deficient nitrogen in the azide group.

Monitoring the 405 eV peak offers an alternative method to grazing-angle IR to gauge the reaction of azides. As an example, we looked at a mixed monolayer made from azidoundecanethiol and octanethiol **3** with  $\chi_{\text{soln}}=0.5$  (Figure 9A). Using previously determined escape depths for high energy electrons in a SAM, we estimated the azide coverage in the monolayer and found it to agree with the electrochemical data.<sup>29, 30</sup> Reaction of this monolayer with the ferrocene acetylene linked via the click reaction reduced the 405 eV peak to the level of noise (Figure 9B). The nitrogen 1s signal at 400 eV broadens indicating the presence of chemically distinct nitrogen atoms. Assuming that the azide disappearance is correlated only to product formation, this provides additional evidence that the reaction is nearly quantitative.

### Quantification of Azide Functionality via Grazing-angle Infrared (IR) Reflectance Spectroscopy

It would be useful if the integrated azide IR intensity tracked linearly with the quantity of azide thiol adsorbed from solution. If this were true, then IR spectroscopy would offer a fast and non-invasive method to quantify the number of azides on a surface. We were unsure whether or not the adjacent azides would interact with each other and affect the infrared signal, resulting in a nonlinear relationship. To explore this, we plotted the amount of ferrocene on the surface determined electrochemically against the percentage of azide on the surface determined by IR intensity (fractional coverages determined by taking the integrated intensity for the sample with  $\chi_{\text{soln}}=1.0$  as 100%). As mentioned earlier, we expected a steric limit of about 58% to the amount of ferrocene that one could possibly immobilize on a surface. Even if the IR intensity was linear with the azide coverage at low coverages, it should become non-linear at coverages close to the steric limit. In light of this we excluded any data indicating coverages above 50% and then attempted a linear fit with no intercept to the rest of the data (Figure 8). The resulting



line showed a high correlation coefficient, 0.98, strongly suggesting that the relationship is linear, and that the amount of azide on the surface can be tracked by the IR stretch within reasonable experimental error. Furthermore, the slope of the line,  $0.99 \pm 0.04$  indicates that the integrated IR intensity for a given mixed monolayer can be simply divided by the integrated intensity of a homogenous azidoundecanethiol monolayer in order to approximate the ratio of azide on the surface

### Kinetic Measurements

The rate of copper(I) catalyzed 1,2,3-triazole formation on surfaces is both of fundamental and practical importance. The applicability of this chemistry to the functionalization of surfaces has been demonstrated by several groups<sup>6, 9-11, 31-35</sup>. A reaction that is nearly quantitative and occurs under mild conditions is particularly useful in surface chemistry since side products or unreactive groups cannot be separated easily and the surface or group to be immobilized might not be stable under harsh conditions. The issue of quantitatively how fast the reaction occurs on a surface has to our knowledge not been previously addressed. One of the challenges in studying such a question involves the method of monitoring the surface reaction. Fortunately, our reaction can be easily monitored electrochemically by using ferrocene redox probes. In order to probe how fast azide-terminated surfaces can be functionalized, we first formed mixed monolayers of azidohexadecanethiol **2** with dodecanethiol **5** as diluent. To ensure that the reactive azides were surrounded by diluent and not located at defect sites, these monolayers were exchanged in 100mM diluent solution overnight. Coverages were kept to below 10% of a monolayer in order to prevent the product of one reaction interfering with a successive reaction. As described above, ferrocene acetylenes are excellent redox probes and we chose to use the redox signal from reacted ethynylferrocene **11** to monitor the reaction. We also wanted to ensure that we were only measuring a catalyzed reaction. Ferrocene propynone is able to slowly react with surface azides without a copper catalyst, therefore making it an unsuitable redox probe for this study<sup>6</sup>. For this reason, we used ethynylferrocene which does not react spontaneously with surface azides ensuring that any signal due to reaction could be attributed directly to the catalytic reaction.

Specific reaction conditions are given in the supporting information. The Sharpless group has recently introduced a copper complex of *tris*-(benzyltriazolylmethyl)amine (TBTA) that is an extremely active catalyst for 1,2,3-triazole formation and inhibits the oxygen reduction by copper(I) which in certain cases can produce reactive oxygen species which could potentially damage the functionality one wishes to attach to a surface. This is particularly the case if one wishes to attach oligonucleotides to the surface<sup>36, 37</sup>. We used this catalyst in excess of ethynylferrocene in order to ensure that all acetylenes were activated by the catalyst. Freshly prepared monolayers were attached to the electrochemical cell and a cyclic voltammogram was taken. The monolayer was rinsed and then dried under nitrogen and the deposition solutions were placed in contact with monolayer surface. After a given time had elapsed, the deposition solution was rinsed off, the surface cleaned with ethanol, methylene chloride, ethanol and water and a cyclic voltammogram was taken. This process was repeated till the cyclic voltammogram changed insignificantly from the previous run indicating completion of the reaction.

A typical set of cyclic voltammograms is depicted in Figure 10. The concentration of ethynylferrocene used was 10 $\mu$ M. The area under the oxidation or reduction peaks can be used to calculate the number of ferrocenes immobilized onto the surface. In the previous section we showed that the ferrocene coverage is a good probe of the number of reactive azides on the surface for coverages lower than roughly 55%. Taking the final value of ferrocene coverage as the total amount of surface azide, one can calculate the decay of the azide species with time from the voltammetric data. This is shown in Figure 11 (circles). The data fit reasonably well to a first-order exponential (solid line), indicating pseudo first order kinetics. This is not

surprising since the ethynylferrocene is present in great excess with respect to the azide which makes up only a small fraction of a monolayer. The acetylene concentration is not expected to change during reaction and can be treated as a constant. From the exponential fit, a pseudo first order rate constant ( $k_{\text{obs}}$ ) can be derived. For the run depicted in Figure 11,  $k_{\text{obs}}$  was determined to be  $0.010 \text{ sec}^{-1}$ .

This procedure was repeated for other concentrations of ethynylferrocene ranging from  $1\text{-}20\mu\text{M}$  and in each case the azide decay could be fit by an exponential and a pseudo first order rate constant was extracted. By plotting  $k_{\text{obs}}$  versus ferrocene concentration and fitting to a straight line (Figure 12), one can estimate a second order rate constant of  $k=1\times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ . This is a very fast surface reaction, the second-order rate constant being several orders of magnitude higher than most reported values for covalent modification of a surface<sup>3, 38, 39</sup>. The fastest reported second-order rate constant we could find was the surface reaction of a pentamethyl cyclopentadiene and an immobilized benzoquinone that was recently reported by Gawalt and Mrksich to have a second order rate constant of  $k=220 \text{ M}^{-1}\text{sec}^{-1}$ . That reaction is strongly influenced by substituents on the diene. In contrast, a simple cyclopentadiene reacts with a second-order rate constant that is over two orders of magnitude lower<sup>40</sup>. The high speed of copper(I) catalyzed azide-alkyne cycloaddition is particularly valuable if one wishes to immobilize species that are limited due to difficulties in preparation and thus low concentrations must be used. Practically speaking, we found that under optimum conditions, a  $20\mu\text{M}$  solution of acetylene could fully functionalize a submonolayer azide-covered surface in 3 minutes while a  $1\mu\text{M}$  solution of acetylene could fully functionalize a submonolayer of azide in less than 100 minutes. The speed of this reaction is not without precedent. Zhou et. al. have shown that in solution, under conditions of one reagent in large excess to the other and copper in a ligand environment similar to that used here, copper-catalyzed azide-alkyne cycloaddition can be driven to completion on the timescale of minutes<sup>41</sup>.

It should be noted that there are likely to be a number of factors that affect the kinetics of this reaction. As we earlier showed, the reaction is prone to steric effects. Preliminary results with longer diluent molecules indicate that the second-order rate constant diminishes as the diluent length increases. Furthermore, in an earlier section we showed that if the azide-terminated thiol is shorter than the diluent thiol the reaction can be inhibited. A previous study in solution has shown that the choice of catalyst and even the concentration of copper ligand can affect the kinetics of triazole formation<sup>42</sup>. Finally, the steric and electronic properties of the acetylene to be immobilized should have a dramatic effect on the reaction rate. For instance, it is well known that acetylenes with electron-withdrawing groups adjacent to the acetylene react rapidly with organic azides, so much so that a catalyst is not required<sup>7</sup>. Nonetheless, this present work demonstrates that an acetylene bearing a redox molecule can rapidly functionalize a SAM containing a dilute amount of azide.

## Conclusion

Mixed azide-terminated monolayers have been characterized as platforms for the modification of surfaces through “click” chemistry. The reactive azide surface coverage can be determined electrochemically by reaction with an acetylene redox-active probe. The azides themselves can be directly monitored, either by using grazing-angle IR spectroscopy, or with XPS. Both techniques are commonly used to analyze surfaces. Using these methods we have observed that the azides are free of side reactions and can react quantitatively and rapidly with acetylene-bearing species. The major limitation on these reactions is steric. We were able to make the azide sterically inaccessible for reaction by forming mixed monolayers having longer chain diluents. Similarly, when we attempted to couple species that are unable to pack as dense as alkyl chains, the reaction was limited to the maximum possible surface coverage. However, this is a fundamental limit that would exist for any surface reaction. By combining results from

the electrochemistry and grazing-angle IR studies, we have shown that the integrated IR stretch is directly proportional to the amount of azide on the surface for coverages below 50%. Electrochemical probes were used to show that the kinetics of the reaction are well behaved and very fast, reaching completion on the time scale of minutes.

Surfaces containing organic azides are excellent platforms for further modification. One can control and measure the amount of a surface-bound azide that, once incorporated, reacts quantitatively with an acetylene. In addition the presence of the azide can be monitored to ensure completeness of the reaction. Although we have used azido-terminated mixed thiol SAMs on gold due to the utility, ease of preparation, and large experience base of thiol SAMs on gold, in principle other surfaces bearing azide containing groups should exhibit similar properties. For these reasons we conclude that azide monolayers are nearly ideal interfaces for the immobilization of functionality onto surfaces.

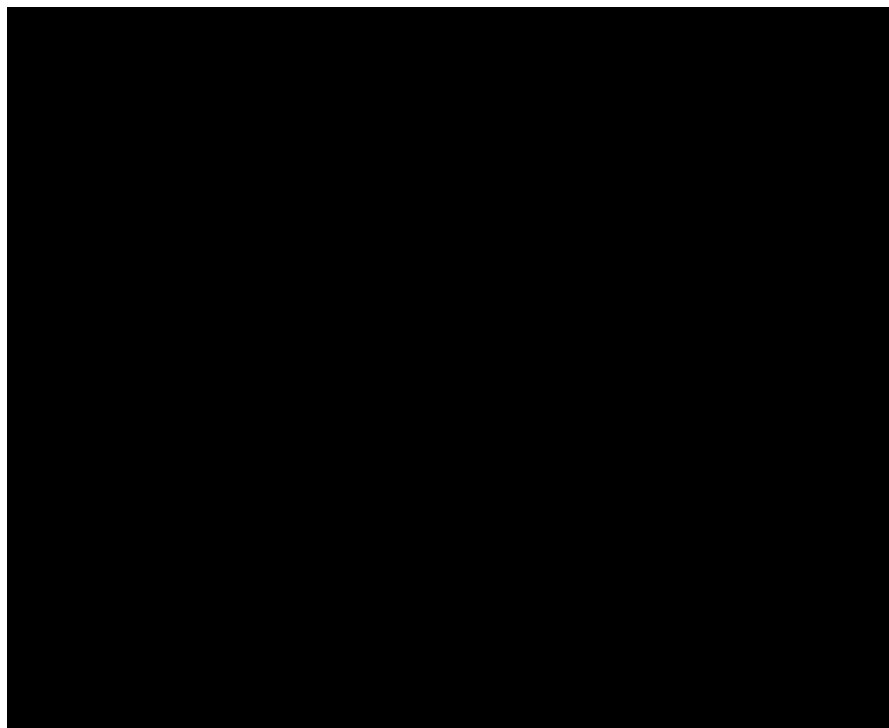
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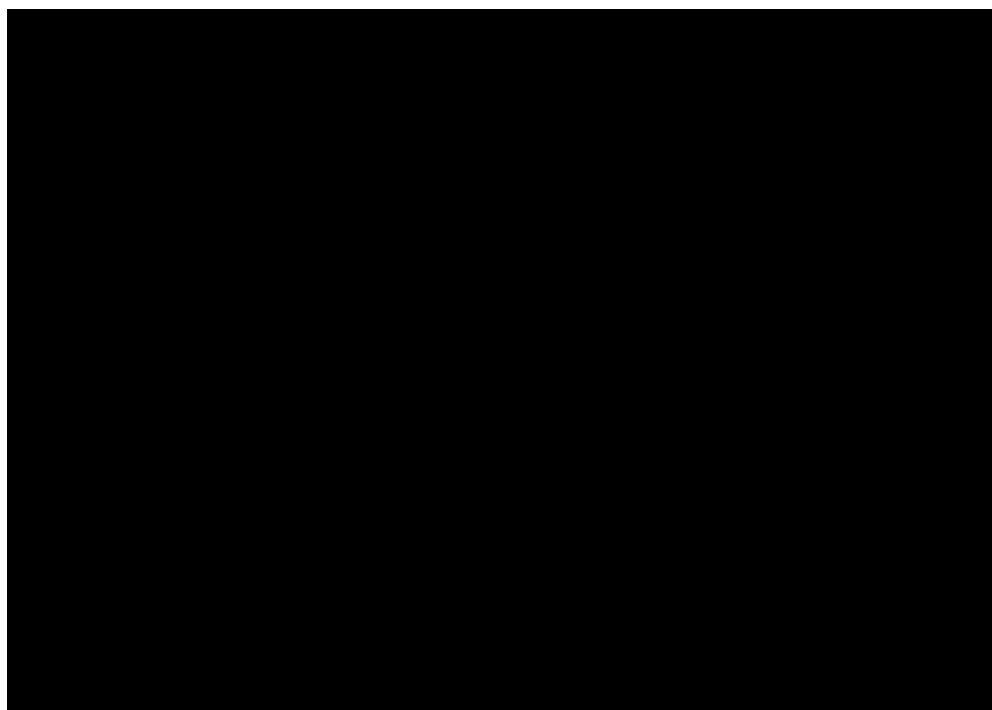
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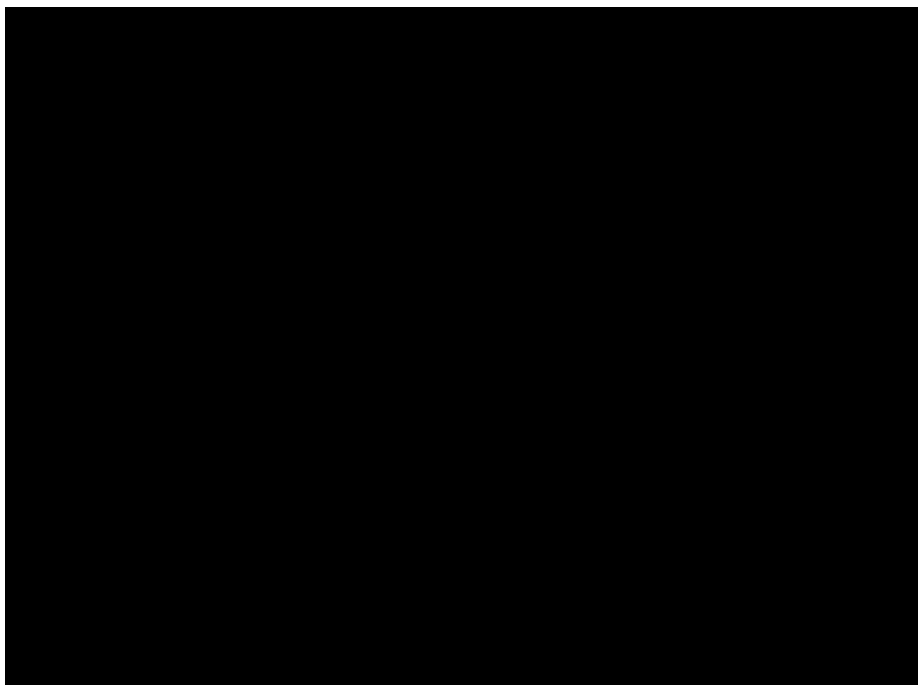
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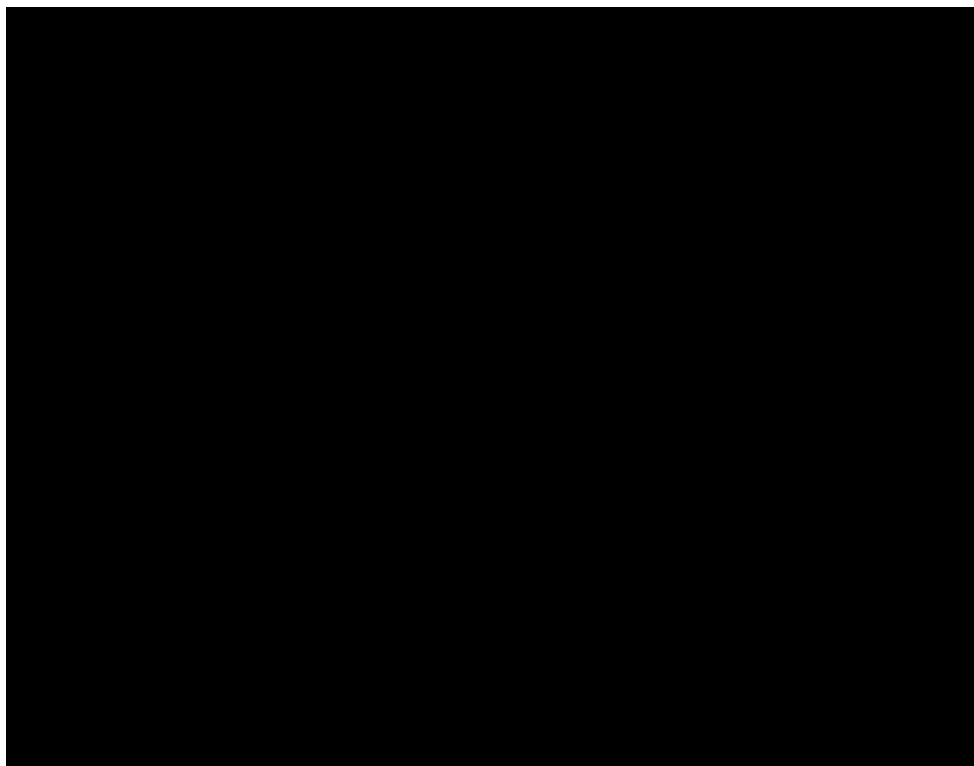
**Figure 1:**  
 Electrochemically determined coverage of ferrocene after reaction of mixed monolayers with ferrocene propynone (10). (A) Hydrophobic diluents  $[\text{unk}] \text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_7\text{SH}$ ,  $[\text{unk}] \text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_9\text{SH}$ ,  $[\text{unk}] \text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_{15}\text{SH}$ . (B) Hydrophilic diluents  $[\text{unk}] \text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{HCO}_2(\text{CH}_2)_{10}\text{SH}$ ,  $[\text{unk}] \text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{HO}(\text{CH}_2)_{11}\text{SH}$ .



**Figure 2:**  
Grazing angle IR spectra of  $\chi_{\text{soln}}=1.0$  Azidoundecanethiol (solid line) and  $\chi_{\text{soln}}=0.5$  azidoundecanethiol/octanethiol (dashed line) after reaction with ferrocene propynone (10).

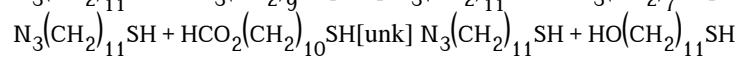
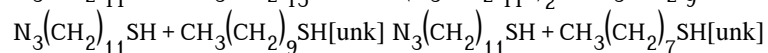
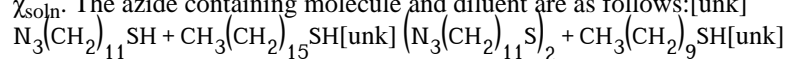


**Figure 3:**  
Ferrocene coverage vs.  $\chi_{\text{soln}}$  for: [unk]  $(\text{N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_9\text{SH})$  and [unk]  
 $((\text{N}_3(\text{CH}_2)_{11}\text{S})_2 + \text{CH}_3(\text{CH}_2)_9\text{SH})$

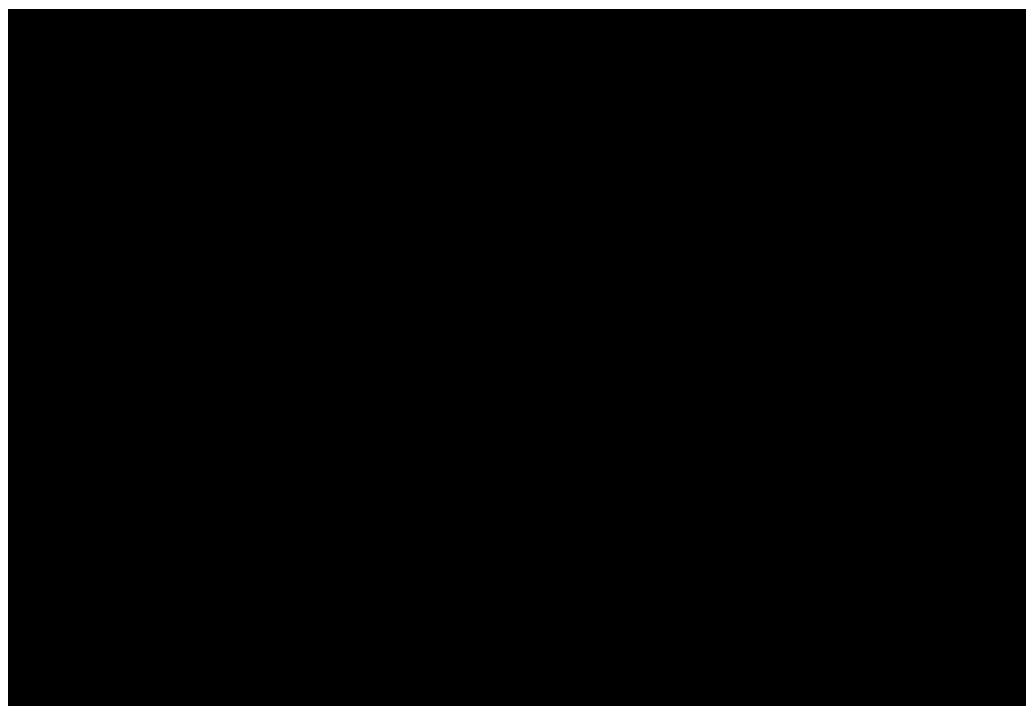
**Figure 4:**

Plot of the sessile drop contact angles for water on mixed azidoundecanethiol monolayers vs.

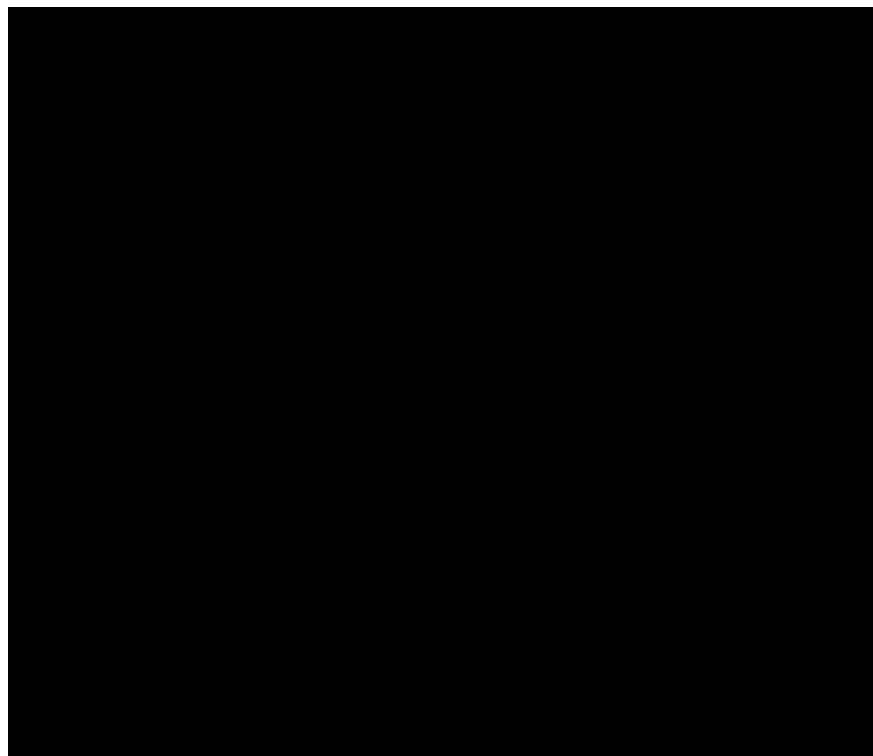
$\chi_{\text{soln}}$ . The azide containing molecule and diluent are as follows:[unk]



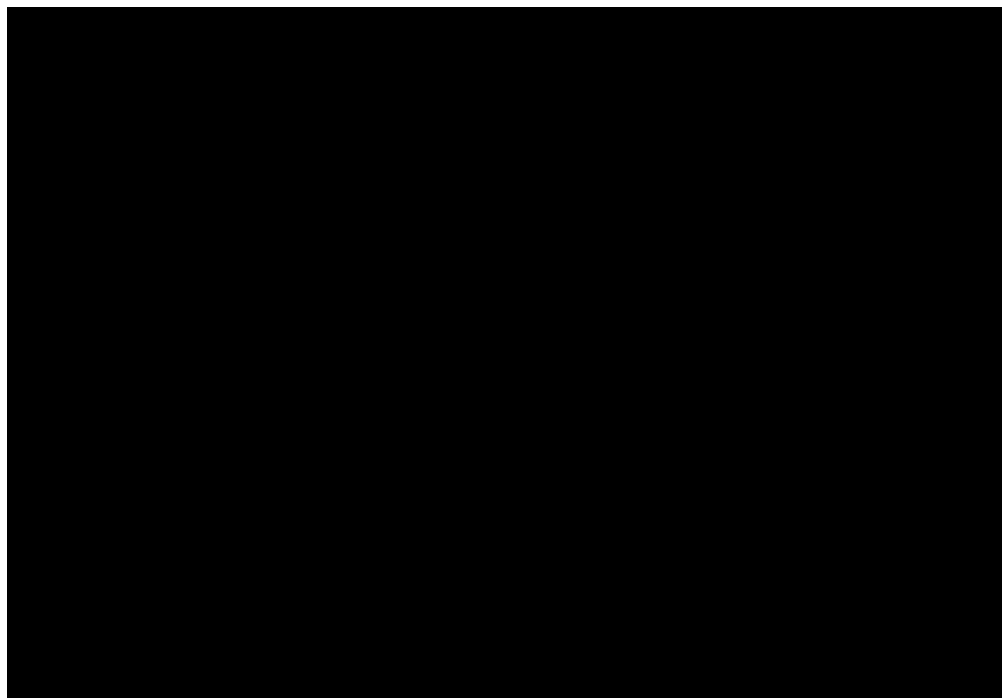




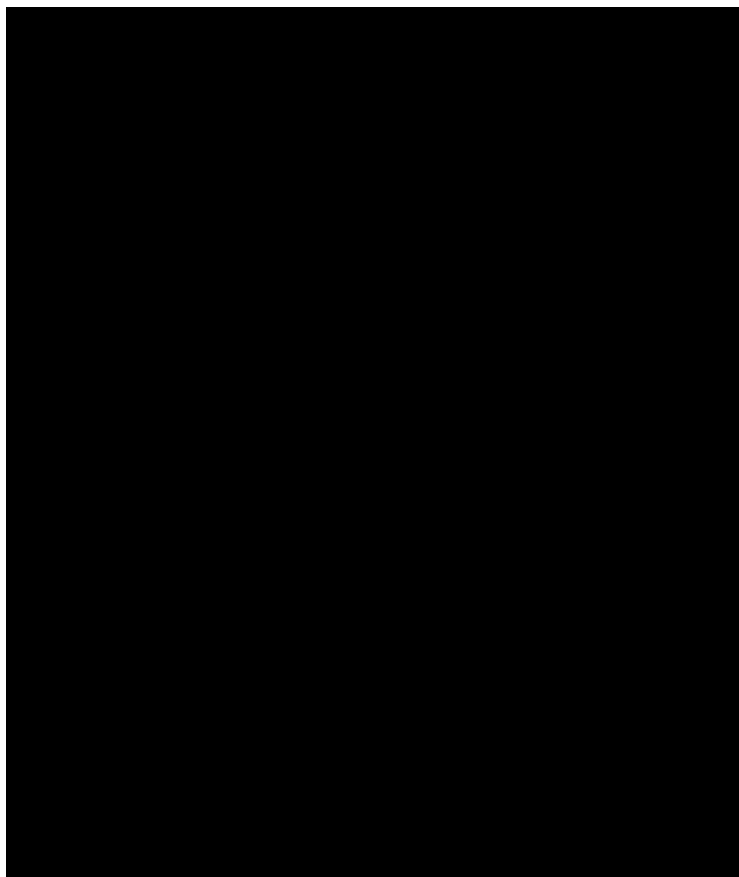
**Figure 5:**  
Grazing angle IR spectra of a  $\chi_{\text{soln}}=1.0$  Azidoundecanethiol monolayer.



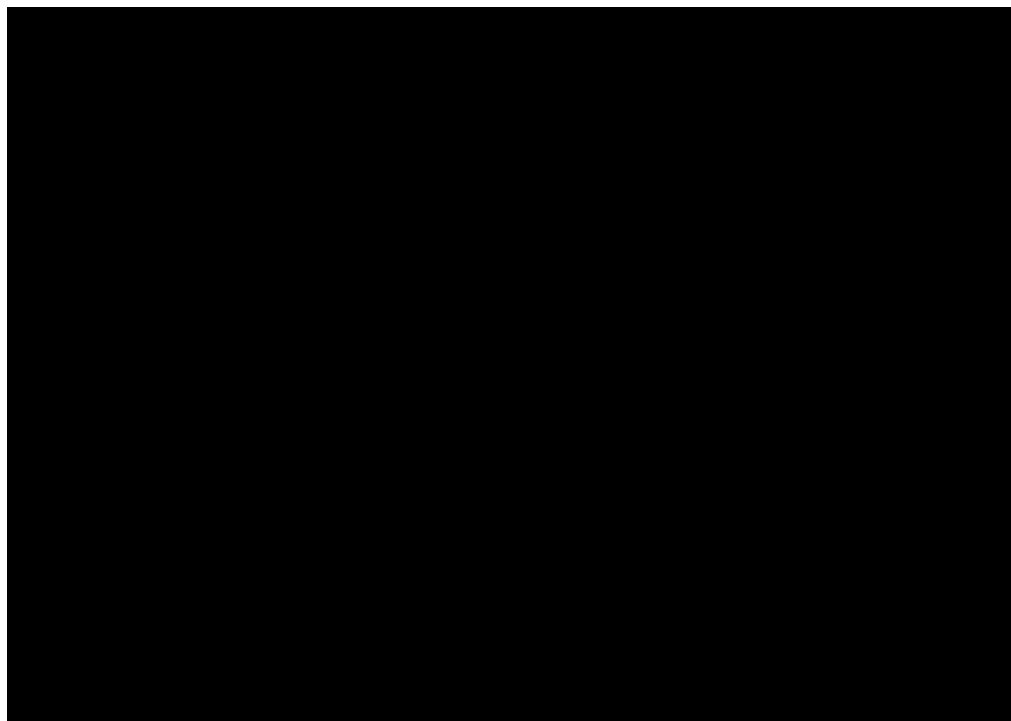
**Figure 6:**  
 Integrated IR intensity vs  $\chi$  for A) Mixed monolayers with Hydrophobic diluents [unk]  
 $N_3(CH_2)_{11}SH + CH_3(CH_2)_7SH$ , [unk]  $N_3(CH_2)_{11}SH + CH_3(CH_2)_9SH$ , [unk]  
 $N_3(CH_2)_{11}SH + CH_3(CH_2)_{15}SH$ . (B) Mixed monolayers with hydrophilic diluents [unk]  
 $N_3(CH_2)_{11}SH + HO_2C(CH_2)_{10}SH$  [unk]  $N_3(CH_2)_{11}SH + HO(CH_2)_{11}SH$



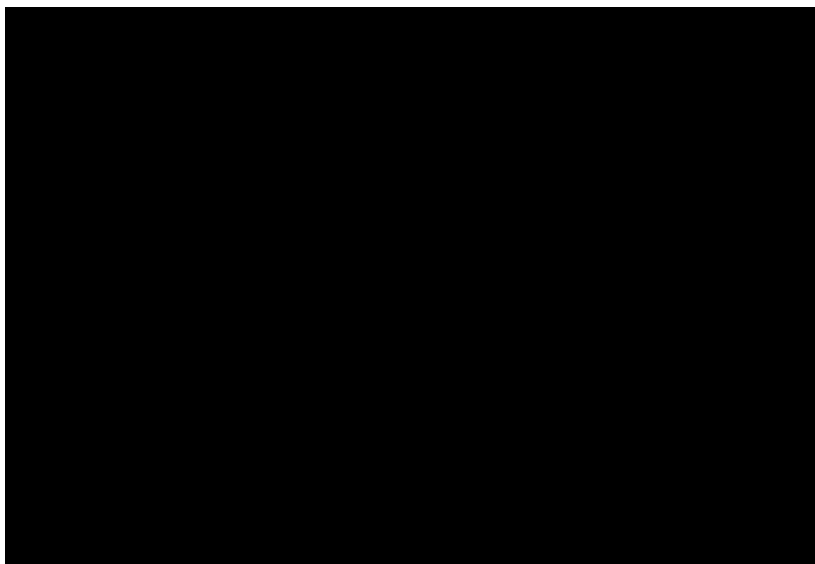
**Figure 7:**  
Grazing angle IR spectra of  $\chi_{\text{soln}} = 0.5 \text{ N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_7\text{SH}$  mixed monolayer before (dashed line) and after (solid line) reaction with **8**.



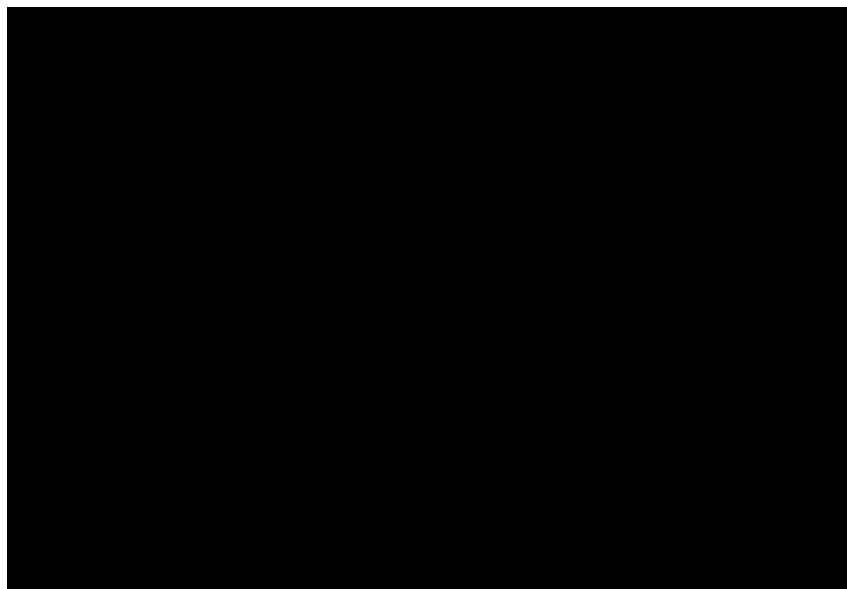
**Figure 8:**  
XPS data for a  $\chi_{\text{soln}} = 0.5 \text{ N}_3(\text{CH}_2)_{11}\text{SH} + \text{CH}_3(\text{CH}_2)_7\text{SH}$  monolayer before (A) and after (B) reaction with ferrocene propynone **10**.



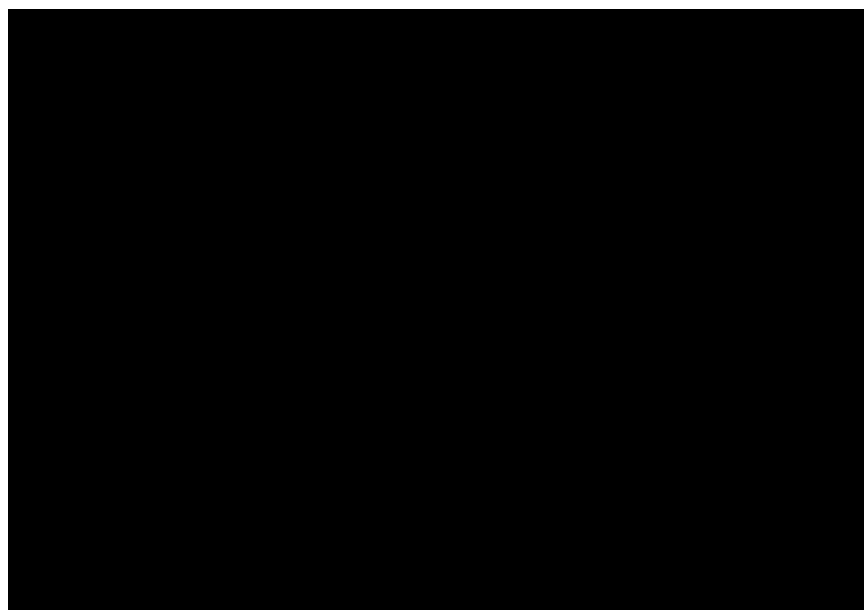
**Figure 9:** Relationship between the ferrocene coverage and the integrated IR intensity as a percentage of the neat azidoundecanethiol monolayer. The solid line is a least-squares fit of all points with ferrocene coverage values of less than 50%. The resulting equation is  $y=0.99\pm 0.04 x$ . The dashed line represents the experimentally observed steric limit to ferrocene coverage of 55%.



**Figure 10:** Electrochemical measurements indicating ferrocene coverage at various timepoints during surface derivitization. Measurements made after [unk] 0, [unk] 60, [unk] 120, [unk] 180, [unk] 240, [unk] 300, and [unk] 360 seconds. Mixed monolayer was formed from  $\text{N}_3(\text{CH}_2)_{16}\text{SH}$  and dodecanethiol as diluent. Electrolyte was 1 M  $\text{HClO}_4$  and scan rate was 0.3V/sec. Ethynylferrocene concentration in the derivitization solution was 10  $\mu\text{M}$ .



**Figure 11:** Plot of the surface density of azides versus time during a reaction between a mixed monolayer of  $\text{N}_3(\text{CH}_2)_{16}\text{SH}$  and dodecanethiol with a derivitization solution containing  $10 \mu\text{M}$  ferrocene acetylene. Azide quantity is back calculated from the electrochemically determined ferrocene coverage (see Figure 10). The solid line is a first order exponential fit of the data from which one can derive a pseudo first-order rate constant of  $k_{\text{obs}} = 0.010 \text{ sec}^{-1}$ .



**Figure 12:**  
Plot of  $k_{\text{obs}}$  versus the concentration of ferrocene acetylene for mixed monolayers of  $\text{N}_3(\text{CH}_2)_{16}\text{SH}$  and dodecanethiol. The solid line is a linear fit of the data. The slope of the linear fit allows estimation of a second-order rate constant for the reaction of  $k=1 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$ .