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Orthogonally Reactive SAMs as a General Platform for Bifunctional Silica Surfaces

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We report the synthesis and self-assembly of azide and amine trimethoxysilanes that result in mixed monolayers on silica. The amine and azide functional groups can be independently reacted with acid chlorides and terminal alkynes, respectively. Consequently, these orthogonally reactive monolayers represent a general starting point for making bifunctional surfaces. Using X-ray photoelectron spectroscopy, we determined the azide/amine surface ratio as well as the reactivity of the azide and amine functional groups in the mixed self-assembled monolayer (SAM). Significantly, the surface azide/ amine ratio was much lower than the azide/amine ratio in the self-assembly mixture. After determining the self-assembly mixture composition that would afford 1:1 azide-amine mixed monolayers, we demonstrated their subsequent functionalization. The resulting bifunctional surface has a similar functional group ratio to the azide/amine precursor SAM demonstrating the generality of this approach.

1. Introduction

With advances in materials and polymer chemistry, multifunctional materials are becoming increasingly accessible.¹ Such materials that combine targeting, imaging, and therapeutic functions onto one scaffold have the potential to revolutionize the treatment of disease.² In addition to materials,³ controlling the combination of specific functional groups in self-assembled monolayers (SAMs) is desirable for research requiring well-defined surfaces⁴ such as biodiagnostics,^{5–7} cell biology,^{8,9} and mechanistic studies of immobilized catalysts.^{10,11} With multifunctional monolayers, synergistic and cooperative effects can be explored between neighboring immobilized functional groups.¹²⁻¹⁴ For example, mixed adlayers containing two types of catalysts have been synthesized on silica where each immobilized catalyst activates a different reactant in the catalytic cycle.¹⁰ Such cooperative catalysts have garnered much attention because they can increase the scope and efficiency of catalytic transformations.¹⁵ In this and other cooperative interfacial systems, the distance between the two

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immobilized groups must be on molecular length scales.^{10,13} Unlike nanometer-scale surface patterns that can be fabricated from lithographic techniques, to immobilize pairs of functional groups within subnanometer distances, the groups must be mixed within the monolayer.11,16,17

Such mixed monolayers are typically synthesized by introducing the surface to a mixture of two surface-reactive molecules.¹⁶ In many examples, one molecule with a functional group of interest is diluted in the mixed monolayer by another molecule that does not have any specific function.^{4,11,18} For example, receptor molecules in a monolayer are often spaced out from one another with an excess of ethyleneglycol-terminated surface reactive groups.^{16,18} This "dilution" molecule can minimize nonspecific interactions with the monolayer. Alkyl terminated surface reactive groups are also often used to vary the surface density in mixed monolayers.¹⁶ To quantify the surface density in such mixed monolayers, Collman, Chidsey and coworkers used cyclic voltammetry to measure the absolute surface coverage of an electrochemically active group diluted in an alkyl monolayer.¹⁹ The ratio of the electrochemically active molecule and the dilution molecule was inferred from IR measurements but could not be directly measured because the dilution molecule lacked any electrochemical or IR signature. Indeed, it is often difficult to find unique spectroscopic or electrochemical signatures for each component in a mixed monolayer. As a result, the solution ratio of the two components is often used as a guide for the surface ratio.⁹

Other groups have characterized the composition of bifunctional monolayers where the two head groups have distinguishable signatures in IR absorbance or X-ray photoelectron spectroscopy (XPS). These experiments explored the influence of the headgroup, the alkyl chain length, and the solvent polarity on the ratio of the two components in the resulting monolayer.^{20,21}

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Scheme 1. Generating Bifunctional Surfaces from Orthogonally Reactive SAMs



Significantly, it was found that the surface ratio could vary drastically from the solution ratio depending on the experimental conditions. Consequently, this early work in the field of selfassembly led by the Whitesides group established that surface ratio determination in mixed monolayers requires quantitative surface characterization methods.²⁰

Combining multiple functions into a monolayer will be critical for understanding cooperative effects in biological and materials systems.¹² The difficulty in characterizing and controlling the distribution and ratio of various functional groups, however, has slowed the development of well-defined and structurally complex bifunctional surfaces. Therefore, the development of general methods for making controlled multifunctional monolayers is needed to accelerate progress in this area.¹⁷ In the following, we outline a general strategy for accessing different combinations of functions on silica based on a mixed SAM containing two orthogonally reactive groups. By characterizing and controlling their surface ratio, the ratio of virtually any mixture of surface-bound molecules can be controlled (Scheme 1). Additionally, by combining self-assembly with orthogonal reactivity, bifunctional mixed monolayers become available that are not easily accessed through direct self-assembly methods. This concept was very recently employed by Hudalla and Murphy in a trifunctional mixed monolayer on gold consisting of a small percentage of azides and carboxylicacid-terminated thiols mixed within an ethylene glycol-terminated monolayer. The azide and an activated form of the acid could then be reacted with two peptides containing alkynyl and nucleophilic groups, respectively. Although the amount of azide and acid was varied, the absolute ratio of these two orthogonal groups was not determined.¹⁴ Moreover, the acid and azide represented only a small fraction of the monolayer, which is not ideal for applications requiring a high density of surface reactive sites like cooperative catalysis. Therefore, to develop densely functionalized orthogonally reactive surfaces of controlled ratios, a new system must be developed. Herein, we demonstrate a quantitative approach to control the ratio of functional groups in monolayers post-SAM formation, starting from a well-defined azide-amine mixed monolayer.

2. Experimental Section

Materials. 3-{2-[2-(2-Methoxy–ethoxy)-ethoxy]-ethoxy}propyne²² was synthesized following a literature procedure, as was 4-(trifluoromethyl)phenyl acetylene, which was synthesized from the commercially available trimethylsilyl-protected analog.²³ All other reagents or solvents were purchased from Aldrich, VWR, Fisher, or Gelest and used without further purification. Silica gel (70–230 mesh, 100 Å) was purchased from Sigma-Aldrich, and silicon (100) wafers (prime grade, 0.5 mm thickness) were purchased from Fluoroware.

General Procedure for Preparing Azide-Substituted Trimethoxysilanes. The following procedure was modified from a procedure noted in a patent application for synthesizing propyl azido trimethoxysilane.²⁴ NaN₃ (1.34 g, 20.7 mmol) was weighed in a 50 mL Schlenk flask containing a magnetic stirbar. The flask was placed under nitrogen, followed by the addition of dimethylformamide (DMF) (6 mL). After stirring for 15 min, chloridesubstituted benzyl or propyl trimethoxysilane (10.2 mmol) was added to the suspension via syringe. The reaction flask was then equipped with a water condenser and allowed to stir for 48 h at 70 °C under N₂. After the reaction was cooled to room temperature, dry hexane (10 mL) was cannula-transferred to the reaction mixture. This biphasic mixture was stirred for 2 h, then allowed to settle for 1 h. At this point, the upper hexane layer was decanted via cannula to another 25 mL Schlenk flask under N₂, and the hexane was removed under reduced pressure on a Schlenk line to yield the desired product as a colorless liquid. Because of the possible explosive nature of the hydrolyzed silyl azides, dry toluene (3-5 mL) was immediately transferred to the product, which was thereafter kept as a solution under nitrogen. The concentration and yield were calculated from the ¹H NMR data for a sample made of 50 μ L of the reaction mixture using 10 μ L of ethyl acetate in acetone- d_6 as an internal standard. No chloride starting material was visible in the ¹H NMR.

4-(Trimethoxysilyl)benzyl Azide. Final concentration in toluene: 0.54-0.96 M. Yield: 48.2%. ¹H NMR (acetone-*d*₆): δ 3.60 (s, 9H, CH₃-OSi), 4.48 (s, 2H, CH₂-N₃), 7.56 (dd, 4H, Ar-*H*). IR cm⁻¹: 2944.4, 2842.1, 2099.7, 1464.6, 1414.2, 1345.0, 1277.9, 1242.9, 1193.1, 1089.0.

3-(Trimethoxysilyl)propyl Azide. Final concentration in toluene: 0.96-1.83 M. Yield 73.1%. ¹H NMR (acetone- d_6): δ 0.69 (t, 2H, $-CH_2-CH_2-CH_2-N_3$), 1.68 (m, 2H, $CH_2-CH_2-N_3$), 3.28 (t, 2H, $-CH_2-N_3$), 3.55 (s, 9H, CH_3 -OSi). IR cm⁻¹: 2944.4, 2842.5, 2100.9, 1605.5, 1455.3, 1399.7, 1342.2, 1277.4, 1248.6, 1193.0, 1124.4, 1085.9.

Synthesis of 4-Bromobenzoyl Chloride. 4-Bromobenzoic acid (2.02 g, 10.1 mmol) and thionyl chloride (5.0 mL, 69 mmol) were combined in a 25 mL round-bottomed flask equipped with a stirbar and a water condenser, at which point the mixture was refluxed for 5 h. After cooling to room temperature, the thionyl chloride was removed under reduced pressure, and the crude product was purified by vacuum distillation (50–52 °C, 100 mTorr) to yield a white solid (1.66 g, 7.06 mmol, 70%). mp 38.8–40.3 °C (matched with manufacturer (TCI America) data). ¹H NMR (CDCl₃): δ 7.67 (d, 2H, Ar-*H*), 7.98 (d, 2H, Ar-*H*). ¹³C NMR (CDCl₃): δ 131.31 (s, ArC-COCl), 132.27 (s, ArC-Br), 132.54 (s, ArC), 132.76 (s, ArC), 167.79 (s, -COCl). High-resolution EI-MS (M⁺⁺): 219.91139.

Synthesis of 4,4,4-Trifluorobutanoyl Chloride. 4,4,4-Trifluorobutanoic acid (6.88 g, 4.85 mmol) was taken in a 25 mL round-bottomed flask and dissolved in the minimum volume of dichloromethane (DCM) (\sim 3.0 mL). A solution of oxalyl chloride in DCM (2.0 M, 3.0 mL, 6.0 mmol) was added slowly via syringe; then, the reaction mixture was placed in an ice bath. A catalytic amount of DMF (1 drop) was added, and the reaction was stirred for 15 min. It was then allowed to come to room temperature and stirred for an additional 6 h. DCM and excess oxalyl chloride were removed slowly under moderately reduced pressure to avoid evaporation of the product. We then purified the

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crude product by collecting the acid chloride (colorless liquid) in a liquid-nitrogen-cooled trap under reduced pressure (0.459 g, 2.86 mmol, 59%). ¹H NMR (CDCl₃): δ 2.54 (m, 2H, $-CH_2$ – CF₃). 3.19 (t, 2H, $-CH_2$ –COCl), ¹³C NMR (CDCL₃): δ 29.37 (q, $-CH_2$ –CF₃), 39.59 (q, $-CH_2$ –COCl), 125.78 (q, $-CF_3$), 171.75 (s, -COCl). IR cm⁻¹: 2917.4, 2849.1, 1829.7, 1446.3, 1424.3, 127.9, 1232.3, 1143.5, 1114.5, 1063.5, 1008.1, 985.1.

Synthesis of 2,2,2-Trifluoroethyl Hept-6-ynoate. Hept-6ynoic acid (0.996 g, 7.89 mmol) was weighed in a 25 mL roundbottomed flask and dissolved in the minimum volume of DCM (4.0 mL). An oxalyl chloride solution in DCM (2.0 M, 5.0 mL, 10.0 mmol) was added slowly via syringe; then, the reaction mixture was placed in an ice bath. After the reaction mixture was cooled to 0 °C, DMF (1 drop) was added, and immediate effervescence of gas was observed. After the reaction mixture was stirred in an ice bath for 15 min, it was allowed to come to room temperature and stirred for 2 h. DCM and excess oxalyl chloride were then rotovaped off, and the reddish brown crude product was used in the next step. ¹H NMR (CDCl₃): δ 1.55–1.63 (m, 2H, CH=C-CH₂-CH₂-), 1.81–1.88 (m, 2H, CH=C-CH₂-CH₂-CH₂-), 1.97 (t, 1H, HC=CH₂C-), 2.21–2.25 (d of t, 2H, HC=C-CH₂-), 2.93 (t, 2H, CH₂-CO).

Hept-6-ynoyl chloride was taken in a 25 mL flask and dissolved in DCM (4 mL). 2,2,2-Trifluoroethanol (0.7 mL, 9.1 mmol) and triethylamine (TEA, 0.9 mL, 9.0 mmol) were dissolved in DCM (3 mL) and added to the flask containing the acid chloride. The resulting mixture was stirred for 4 h at room temperature. The reaction mixture was then diluted with DCM (5 mL), washed with water (5 \times 3 mL), dried over Na₂SO₄, and filtered in a 25 mL round-bottomed flask. The solvent and any remaining trifluoroethanol starting material were removed from the filtrate by rotary evaporation. The crude product was purified by vacuum distillation (65-70 °C, 48 mTorr) to yield a clear oil (1.17 g, 5.55 mmol; overall yield in two steps 70.3%). ¹H NMR (CDCl₃): δ 1.58 (m, 2H, CH=C-CH₂-CH₂-), 1.79 (m, 2H, CH=C- $CH_2-CH_2-CH_2-$), 1.95 (t, 1H, $CH=CCH_2-$), 2.23 (d of t, 2H, CH=C-C H_2 -), 2.44 (t, 2H, C H_2 -CO), 4.47 (q, 2H, O-C H_2 CF₃). ¹³C NMR (CDCl₃): δ 18.06 (s, CH=C-C H_2 -), 23.69 (s, $CH \equiv C - CH_2 - CH_2 -)$, 27.62 (s, $CH \equiv C - CH_2 - CH_2 -)$, 27.62 (s, $CH \equiv C - CH_2 -)$, 60.21 (q, $CF_3 - CH_2 -)$, 68.78 (s, $CH \equiv C -)$, 83.65 (s, $CH \equiv C -)$, 123.01 (q, CF₃-CH₂-), 171.72 (s, C=O). IR cm⁻¹: 3311.1, 2928.6, 2869.9, 2119.3, 1760.9, 1414.6, 1283.1, 1171.3, 1141.5, 1083.3, 978.4.

Substrate Cleaning Procedure. Silicon wafers containing a native oxide layer were used as the substrates for the XPS and AFM measurements. Glass slides (Fisher, Microscope Slides) were used for the contact angle measurements. Prior to surface functionalization, both types of samples were cleaned with methanol (~15 mL), followed by drying in an oven for 10 min at 90 \pm 5 °C. The cleaned wafers were reacted with "piranha solution" (3:1 mixture of sulfuric acid and 30% hydrogen peroxide) in a glass beaker to make the surface rich with hydroxyl groups.²⁵ (Caution: Piranha solution reacts explosively with organic materials, so it should not be combined with any organic compounds or solvents. Use caution.) The beaker containing the samples was rinsed copiously with deionized water ($5 \times 100 \text{ mL}$); the individual samples were then each rinsed with a stream of Millipore water (100 mL), sonicated in Millipore water (100 mL) for 10 min, and blown dry with a stream of nitrogen gas. The substrates thus obtained were placed in an oven for 30 min $(90 \pm 5 \text{ °C})$. In some cases, the silicon wafers were next cleaned using a reactive ion etcher (μ Etch RIE, Plasma Lab) with oxygen as reactive gas in a radio frequency (RF)-induced plasma to remove residual fluorine from the packing material. Process pressure was 500 mTorr, and process time was 1 min for plasma cleaning.

Self-Assembled Monolayer Formation on Silica Surfaces. Cleaned samples were rinsed with a stream of methanol (15 mL), a mixture of methanol and toluene (1:1 v/v, 15 mL), and finally a stream of toluene (15 mL) and then immersed in 3 mM solution of the trimethoxysilane in dry toluene with 0.1% water (v/v) for 4 h. The same protocol was used for SAM formation in hexane, with hexane substituted for toluene in the above steps. To make the orthogonally reactive SAMs, we combined different proportions of amino and azido trimethoxysilanes to reach a total concentration of 3 mM trimethoxysilane. After the 4 h of reaction time, the samples were rinsed thoroughly with toluene (15 mL), and methanol (15 mL) and then blown dry with a stream of nitrogen gas. The samples were either used immediately in a subsequent reaction or stored under vacuum.

Modification of Powdered Silica for the IR Absorbance Experiments. SiO₂ powder (0.5 g, 70–230 mesh, 100 Å) was added to a 25 mL flask containing a solution of the respective trimethoxysilane in toluene (5 mL, 50 mM). This slurry was stirred for 4 h at room temperature. The higher concentration of trimethoxysilane was used because of the high surface area of the silica gel (surface area = $300 \text{ m}^2/\text{g}$). After reaction, the functionalized silica powder was filtered in a Buchner funnel to remove the filtrate. The powder was then washed with toluene (4 × 15 mL) and methanol (4 × 15 mL). Finally, the powder was annealed for 30 min in an oven at 90 ± 5 °C. The subsequent acylation or [3 + 2] cycloaddition reaction was performed on the functionalized powder using the same procedure as the silica surfaces with 0.1 g powdered silica samples.

General Method for Acylation of Amino SAMs. The acid chloride (0.5 mmol) was dissolved in a 20 mL vial in acetonitrile (ACN) (5 mL), followed by the addition of triethylamine (0.05 mL, 0.36 mmol). Freshly prepared amino SAMs were immersed in the vial containing the reaction mixture, which was then capped and allowed to react overnight. The acylated amino SAMs were then taken out of the vial and rinsed with ACN (10 mL) and toluene (10 mL), followed by 1 min sonication in toluene (10 mL) and methanol (10 mL) each and then blown dry with a stream of nitrogen gas.

General Method for [3 + 2] Cycloaddition Reaction of Alkynes with Azido SAMs. The following procedure was used for all of the alkynes <u>except</u> for the reaction of benzyl azido SAM with propargyl-substituted triethyleneglycol monomethyl ether (TEG-alkyne). Copper sulfate (6.2 mg, 0.025 mmol) and ascorbic acid (8.8 mg, 0.050 mmol) were transferred to a 20 mL vial and dissolved in DMF (5 mL) by sonication. The alkyne (0.25 mmol) was dissolved in that solution at which point freshly prepared azido SAMs were immediately immersed in the vial, which was then capped, wrapped in foil, and allowed to react overnight. The reacted azido SAMs were then taken out of the vial and rinsed with DMF (10 mL) and toluene (10 mL), followed by sonication for 1 min each in toluene (10 mL) and methanol (10 mL). Finally, the samples were blown dry with a stream of nitrogen and stored under vacuum.

[3 + 2] Cycloaddition of the Benzyl Azido SAM with TEG-Alkyne. AFM analysis suggested that different cycloaddition conditions were required for the benzyl azido SAM with TEG-alkyne. Therefore, copper(I) iodide (9.5 mg, 0.050 mmol) and triethylamine (0.1 mL, 0.7 mmol) were transferred to a 20 mL vial containing water (2.5 mL) and methanol (2.5 mL). TEG-alkyne (0.05 g, 0.25 mmol) was dissolved in that solution, and freshly prepared benzyl azido SAMs were immersed in the vial, which was then capped. The SAM sample was taken out of the vial after overnight reaction and rinsed with water (10 mL), methanol (10 mL), toluene (10 mL), and methanol (10 mL), followed by sonication in methanol (10 mL) for 1 min. The samples were blown dry with nitrogen and stored under vacuum.

Bifunctional Modification of Orthogonally Reactive SAM. The orthogonally reactive SAMs were reacted via acylation and

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cycloaddition, sequentially, following the same modification procedures detailed above.

XPS Analysis. XPS measurements were performed on samples that had been prepared within 4 days using the AXIS ULTRA spectrometer (Kratos Analytical). The base pressure in the analytical chamber was $< 3 \times 10^{-8}$ Pa. The monochromatic Al K α source (h ν = 1486.6 eV) was used at a power of 210 W. The photoelectron exit angle was 90°, and the incident angle was 35.3° from the plane of the surface. The analysis spot was $400 \times 700 \,\mu\text{m}$. The resolution of the instrument was 0.55 eV for Ag 3d and 0.70 eV for Au 4f peaks. Survey scans were collected for binding energies from 1100 to 0 eV with an analyzer pass energy of 160 eV and a step of 0.35 eV. The high-resolution spectra were run with a pass-energy of 20 eV and a step of 0.1 eV. Relative sensitivity factors (RSFs) for different elements were as follows: 1 for F (1s), 0.477 for N (1s), 0.955 for Br (3d). Only one set of XPS scans was performed on a given sample; therefore, XPS analysis before and after surface reactions was performed on different samples.

AFM Measurements. AFM experiments were performed on samples no more than 2 days old using a MFP-3D (Asylum) operated in tapping mode with commercially available Si cantilevers (Olympus, 300 kHz frequency). The force constant of the cantilevers was 42 N/m. The oscillation frequency used for tapping mode was 310 ± 5 kHz.

Contact Angle. Contact angles of water were measured on samples that had been prepared within 2 days using a FTA200 (First Ten Angstroms) system for contact angle and drop shape analysis fitted with manufacturer software. Contact angles were determined from sessile water drops of 8.0 μ L volume. The average of at least six contact angle measurements was determined from, at minimum, two separately prepared slide samples.

Calculation of Azide/Amine Ratio from N-Peak Fitting. The high-resolution N (1s) scan for the monofunctional azide and azide-amine mixed monolayers was analyzed using the multipeak fitting program in Igor Pro 6.05. Two Gaussian peaks, one centered at 401 eV and another at 405 eV, resulted after fitting the N (1s) region of the high-resolution XPS spectrum. The peak areas were determined from the following equation

peak area = $\sqrt{\pi}$ (amplitude × width)

The calculated ratio of the 401/405 eV peak areas for the benzyl azido SAM was 3.07(7), whereas the corresponding peak ratio for the propyl azido SAM was found to be 4.1(1). Substituting these values and the peak areas calculated for the mixed monolayers, the azide/amine ratio was determined from the corresponding equation

$$\frac{\text{benzyl azide}}{\text{amine}} = \frac{(1+3.07)\text{peak area}_{405\text{eV}}}{3}$$

$$\times \frac{1}{\text{peak area}_{401\text{eV}} - (\text{peak area}_{405\text{eV}} \times 3.07)}$$

$$\frac{\text{propyl azide}}{1} = \frac{(1+4.1)\text{peak area}_{405\text{eV}}}{2}$$

amine
$$3 \times \frac{1}{\text{peak area}_{401\text{eV}} - (\text{peak area}_{405\text{eV}} \times 4.1)}$$

For peak fitting, the peak center, width, and amplitude values were allowed to vary with the exception of the 1:1 propyl azide amine sample. For this spectrum, the small peak at 405 eV was difficult to fit without holding any parameters. Therefore, a peak width of 0.74 eV was held for the smaller peak at 405 eV, because this value was generated for the peak at 405 eV from fitting both the monofunctional propyl azido SAM and the 5:1 propyl azide amine SAM spectra.

Calculation of the Azide/Amine and Triazole/Amide Ratios. Atomic compositions of the surfaces were calculated from Scheme 2. Synthesis of Azido Trimethoxysilanes



the elemental peak areas in the XPS spectra of the amine-azide mixed monolayers adjusted with their corresponding RSFs. Specifically, the measured Br/N and F/Br ratios were substituted into the following equations to determine the azide/amine and triazole/amide surface ratios.

From Br/N ratio (assuming 87% conversion) :
$$\frac{\text{azide}}{\text{amine}} = \frac{1 - (\text{Br/N})/0.87}{3 \times (\text{Br/N})/0.87}$$

From F/Br ratio : $\frac{\text{triazole}}{\text{amide}} = \frac{\text{F/Br}}{3}$

3. Results and Discussion

Our strategy requires the selection of two reactive groups that exhibit orthogonal reactivity; in other words, they should not react with one another or the other's reactive partner (Scheme 1). One obvious choice of reaction is the Cu-mediated cycloaddition of surface-bound azides with terminal alkynes owing to the functional group tolerance, specificity, and high reactivity associated with this click reaction.^{1,19,26-29} As a complementary reaction, amine-terminated monolayers have been used to introduce substituted electrophiles onto surfaces and other materials.⁴ Commercially available *p*-aminophenyl trimethoxysilane was selected because it is known to make uniform reactive SAMs.²⁵ For the azide monolayer, most azido SAMs on silica are synthesized postself-assembly by reaction of an alkylhalide SAM with sodium azide.^{26,30} Because alkyl halides and amines react with one another, we had to develop a method for the direct assembly of azido SAMs to generate mixed monolayers with amino silanes. Therefore, we synthesized two azido trimethoxysilanes from the reaction of sodium azide with p-(trimethoxysilyl)benzylchloride and *n*-(trimethoxysilyl)propylchloride (Scheme 2). With the corresponding benzyl and propyl azido silanes in hand, we were able to explore the influence of the azide structure on monolayer selfassembly. Additionally, by directly incorporating the azide into the monolayer, we prevented the incorporation of unreacted alkyl halides, which is a potential problem in post-self-assembly modification strategies.³⁰

The synthesis and reactivity of the monofunctional SAMs were first explored by exposing silica surfaces to 3.0 mM solutions of the corresponding trimethoxysilane in toluene (0.1% water v/v) for 4 h. The presence of the amine and azide functional groups was confirmed by AFM and water contact angle measurements (vide infra). To quantify the extent of reaction between the amino or azido SAM with their solution-phase reactive partners, we elected to use XPS and CF₃-labeled acid chlorides and alkynes, respectively. From the XPS spectra, the percent completion can easily be determined, where 100% surface conversion of the amines leads to a F/N ratio of three, and conversion of all azides leads to a F/N ratio of one. Quantification of the XPS F1s and N1s signal

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intensities indicated that exposing the aminophenyl SAM overnight to CF₃-substituted benzoyl chloride led to quantitative formation of the amide (Table 1, entry 1; Figure 1A). To determine the amino SAM reactivity with aliphatic acid chlorides, we performed the same experiment with CF₃-butanoyl chloride (Table 1, entry 3). High yields were also observed for this reactant, which supports previous reports indicating that different substituted acid chlorides can be attached to these aminophenyl monolayers.³¹ Next, we examined the reactivity of the propyl azido SAM and benzyl azido SAM in the overnight reaction between

 Table 1. XPS Data and Percent Conversions for the Amine and

 Azide Monofunctional SAMs before and after Reaction with

 Various Reactants^a

	Surface	Reactant	F:N	%			
	Sunace		(Br:N)	Conversion			
1	-IINH2		3.0 ± 0.3	100 ± 10			
2	-I-NH2		(0.87 ± 0.02)	87 ± 2			
3	-I-NH2		2.68 ± 0.05	89 ± 2			
4	-H		1.06 ± 0.04	106 ± 4			
5	- -{\		0.9 ± 0.1	90 ± 10			
6	1~~_ _{N3}		1.2 ± 0.2	120 ± 20			
7	1~~_ _{N3}		0.9 ± 0.2	90 ± 20			

^{*a*} Data represent the average of at least two samples made from separate batches. The error indicates the range of measured values.

the azido monolayers and *p*-(trifluoromethyl)phenyl acetylene. We were pleased to note that quantitative conversion to the triazole occurred with azido SAMs directly assembled on silica (Table 1, entries 4 and 6; Figure 1B,C). High conversions were also observed with an aliphatic alkyne, indicating that our new azido SAMs have broad reactivity (Table 1, entries 5 and 7). The ability of all of our SAMs to react with aromatic and aliphatic reactants is important because it indicates that a wide variety of substituted alkynes and acid chlorides can be attached using these monolayers, which supports the generality of our strategy. Finally, control experiments were performed that indicated that neither the azido SAMs nor the amino SAM exhibited cross reactivity with acid chlorides and alkynes, respectively, under appropriate reaction conditions. (See the Supporting Information.)

To confirm the structural changes of the azides and amines after undergoing their respective reactions, infrared absorbance experiments were performed on powdered silica using the reactants shown in Table 1 in a similar manner as the silica-coated wafers used in the XPS experiments. In all IR absorbance spectra, a broad absorption was observed from 2800 to 3700 cm⁻¹ attributed to water and H-bonded silanol.^{32,33} For the benzyl azide- and propyl azide-modified powdered silica, the intense absorption peak at 2107 cm⁻¹, characteristic of the azide stretch, confirmed the attachment of the azides (Figure 2A, blue traces). In general, this peak disappeared after reaction with alkynes, indicating quantitative conversion to the triazole product. The one exception was the reaction of the aliphatic alkyne, 2,2,2-trifluoroethyl heptynoate with propyl azide-modified silica (Figure 2A (ii), black trace), where the azide peak was diminished but still observable. However, for both azide samples, a new peak appeared at 1740 cm⁻¹ after reaction with this ester substituted alkyne, confirming the presence of the carbonyl (C=O stretch, Figure 2A, black traces). Similarly new



Figure 1. N1s and F1s XPS data for the monofunctional (A) amino, (B) propyl azido, and (C) benzyl azido SAMs before and after reaction with their corresponding CF_3 -labeled reactive partner. The intensity of the N1s XPS signal has been multiplied by 3.5 to allow comparison with the stronger fluorine signal.



Figure 2. IR absorption spectra of powdered silica modified with: (A) (i) Propyl azido silane and (ii) benzyl azido silane, before (blue) and after reaction with an aromatic alkyne (red) or an aliphatic alkyne (black); (B) Phenylamino silane before (blue) and after reaction with an aromatic acid chloride (red) and an aliphatic acid chloride (black).



Figure 3. Tapping-mode atomic force microscopy images of SAMs before and after reaction with alkyne-substituted triethyleneglycol (TEG). (A) Benzyl azido SAM. (B) Amino SAM. (C) Benzyl azido SAM reacted with TEG. (D) Mixed monolayer synthesized from a 5:1 molar mixture of benzyl azido and amino trimethoxysilanes after reaction with TEG. (E) Mixed monolayer synthesized from a 1:1 molar mixture of benzyl azido and amino trimethoxysilanes after reaction with TEG. Table: Contact angles of water droplets on SAMs. The contact angle decreases with increasing hydrophilicity of the monolayer.

peaks were visible in the IR after reaction with the aromatic alkyne, *p*-trifluoromethylphenyl acetylene. Specifically, a new peak appeared at 1328 cm⁻¹ attributed to the CF₃ antisymmetric stretching vibration mode of the aromatic 1,4-isomer (Figure 2A, red traces).³⁴

The reactivity of amine-modified silica with acid chlorides was similarly explored using changes in the IR absorbance spectra. After reaction of the amino silica and either the aliphatic or aromatic acid chloride, a new band at 1653 cm⁻¹ appeared (corresponding to the C=O), confirming the formation of amide groups (Figure 2B, red trace). This band overlapped with the band at ~1630 cm⁻¹ present for both the amine- and amide-modified silica that is attributed to the vibration of the adsorbed water on silica.³⁵ The peak at 1329 cm⁻¹ corresponding to the aromatic CF₃ on the substituted benzoyl chloride was also visible after amide formation.³⁴ In addition to confirming the structural changes associated with forming and reacting these SAMS, the high reactivity observed for the modified silica powder suggested that our strategy is amenable to functionalizing porous materials, with potential uses in multicomponent catalyst¹⁰ or therapeutic² applications requiring high surface area materials.

We next synthesized mixed monolayers from mixtures of azido and amino trimethoxysilanes with solution mole ratios of 1:1 and 5:1, respectively. For the benzyl azide system, the resulting mixed monolayers exhibited contact angles with water of $55 \pm 1^{\circ}$ for the 1:1 mixture and $58 \pm 1^{\circ}$ for the 5:1 mixture, in between the values for the corresponding monofunctional SAMs (Figure 3, table; see Figure S-1 of the Supporting Information for characterization of propyl azide—amine SAMs). To verify that the surface azide/ amine ratio increased with increasing amounts of azide trimethoxysilane in solution, we reacted the azide in these mixed monolayers with alkyne-substituted triethyleneglycol (TEG). The resulting AFM images are shown in Figure 3. The surface roughness exhibited by the root-mean-square (rms) height values for the

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mixed monolayers after reaction with TEG increased from 1.07 to 1.53 nm with increasing amounts of azide in solution (Figure 3A–D). This increase in roughness can be explained by the reaction of the long-chain alkyne with the surface-bound azides. Additionally, the correlation between surface roughness and the solution fraction of azido trimethoxysilane is consistent with an increase in the amount of azide in the monolayer. Differences in hydrophilicity of the SAMs were also used to characterize the mixed monolayers. The water contact angle of the benzyl azide SAM decreased markedly from 60.7 ± 0.9 to $36 \pm 2^{\circ}$ upon incorporating the hydrophilic TEG chain. For the mixed monolayer, the trend in contact angle also confirmed that increasing the azide content led to greater incorporation of the hydrophilic TEG group (Figure 3, table).

After establishing that we had successfully synthesized mixed monolayers with varying azide ratios, one question that remained was how closely the azide/amine surface ratio matched the ratio of azide and amine trimethoxysilanes in solution. To correlate the solution and surface composition, we took advantage of the signature double peak in the N1s region of the XPS spectrum that corresponds to the azide (Figure 1B,C).³⁶ The distinctive two peaks and the unique position of the smaller peak at 405 eV provide a handle for characterizing the amount of azide in our mixed monolayers. Fitting the high-resolution N1s scan to two Gaussian peaks resulted in a large peak centered at 401 eV and a smaller peak centered at 405 eV for both azido SAMs. Interestingly, the peak area ratios differed for the two azido SAMs; the benzyl azido SAM exhibited a peak ratio of 3.07 ± 0.07 , and the propyl azido SAM exhibited a ratio of 4.1 \pm 1. The different ratios for the two substituted azides suggest that the oxidation state of the azide nitrogen atoms depends on azide substitution, which is consistent with different resonant contributions for the propyl and benzyl azides.

Next, we measured the ratio of the peak areas from the highresolution XPS data of monolayers synthesized from 1:1 molar mixtures of the azido and amino trimethoxysilanes. As expected, the 401 to 405 eV peak ratios were much greater than that of the monofunctional azido SAMs, owing to overlap of the amine and azide signals at 401 eV. Specifically, peak area ratios of 13 ± 2 and 12 ± 1 were observed for the benzyl azide—amine and the propyl azide—amine SAMs, respectively. Using these values, the area of the peak at 405 eV, and the peak ratio for the corresponding monofunctional azido SAM, the relative amount of azide was determined for each mixed monolayer. To illustrate, the following equation was used to determine the azide/amine ratio for the benzyl azide—amine mixed monolayers

$$\frac{\text{benzyl azide}}{\text{amine}} = \frac{(1+3.07)\text{peak area}_{405\text{eV}}}{3} \times \frac{1}{\frac{1}{\text{peak area}_{401\text{eV}} - (\text{peak area}_{405\text{eV}} \times 3.07)}}$$

We also attempted a three-peak fit of the XPS data to resolve the amine and azide contributions at 401 eV, but the similarities in the peak positions (400.8, 400.9, and 401.2 eV for the amine, propyl azide, and benzyl azide, respectively) led to azide/amine values that were inconsistent with the peak area at 405 eV.

The peak ratios reveal that only $12 \pm 3 \mod \%$ azide has been incorporated into the monolayer for a 1:1 molar mixture of benzyl azido and amino trimethoxysilane (50 mol % benzyl azide in solution) (Figure 4A, Table 2). Similarly, the propyl azide—amine mixed monolayer exhibited a much smaller amount of azide on the



Figure 4. N1s high-resolution scans of (A) benzyl azide—amine and (B) propyl azide—amine mixed monolayers synthesized from solutions with varying mol % azide (total [trimethoxysilane] = 3.0 mM). By fitting each spectrum to two Gaussian peaks (red dashed lines) and comparing the peak ratios of the mixed and monofunctional azido SAMs, the mole percent of azide in each mixed monolayer was determined.

surface compared with the solution composition (18 \pm 2 vs 50 mol %, respectively, Figure 4B). From these results, we conclude that the solution ratio differs substantially from the surface ratio. To achieve 52 mol % of azide in the benzvl azide-amine mixed monolayer, benzyl azido trimethoxysilane must be five times more concentrated than aminophenyl trimethoxysilane in solution (i.e., 83 mol % azide). For the propyl azide-amine mixed SAM, a solution azide/amine ratio of 8 was needed to achieve 53 mol % of the propyl azide in the mixed monolayer. (See the Supporting Information.) These results reveal that the composition of azide-amine mixed monolayers varies significantly from the ratio of silanes in solution. Although it has long been recognized that surface reactivity and solubility³ can influence surface ratios in mixed monolayer systems²⁰ because of the difficulty in quantifying surface ratios, the solution ratio is often used to interpret the structure and behavior of the mixed monolayer.^{7,14} In our mixed monolayer system, such an assumption would introduce significant error to the interpretation because of the disproportionate amount of amine groups versus azides.

One explanation for the larger amount of aminophenyl groups in our mixed monolayers is the ability of aminophenyl silanes to interact with the silica surface through hydrogen bonding and self-associate via pi stacking.²⁵ The former should enhance their presence at the interface and has been observed in flexible aminosilanes as the first step prior to surface reaction.³⁸ The latter has been inferred from the well-ordered monolayers that result from phenylamino silanes.²⁵ Additionally, the ability of amines to act as base catalysts capable of cross-linking trimethoxysilanes or condensing them with surface silanol groups could also increase the incorporation of aminosilanes into the monolayer, particularly if they are self-associating.^{38,39} In our mixed monolayers, the azide/amine surface ratio was similar regardless of the azide structure, which is consistent with the proposed surface concentration enhancement of the amines due to hydrogen bonding. Moreover, the similarity suggested that neither azide interacted strongly with the phenylamine, which was surprising for the benzylazido silane.

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Table 2. Azide/Amine Surface R	atio for Mixed M	onolayers Assembled i	in Different Solvents
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	t	oluene	hexane	
solution azide mol %	N1s XPS ratio ^a	surface azide mol %	N1s XPS ratio ^a	surface azide mol %
83% BnAz	4.3 ± 0.2	52 ± 6	4.5 ± 0.3	48 ± 4
50% BnAz	13 ± 2	12 ± 3	23 ± 11	9 ± 5
83% PrAz	6.3 ± 0.2	44 ± 2	5.1 ± 0.1	63 ± 2
50% PrAz	12 ± 1	18 ± 2	10.0 ± 0.9	23 ± 3

^a Ratio represents the XPS peak areas for signals centered at 401 and 405 eV, respectively.



Figure 5. (A) Functionalization of orthogonally reactive SAMs with (1) Br-labeled acid chloride, followed by (2) CF₃-labeled alkyne. (B) Surface ratios determined from the XPS data of mixed monolayers prepared from 1:1 or 5:1 molar mixtures of azido and amino trimethoxysilane (1 Az:Am and 5 Az:Am, respectively). The azide/amine ratio was determined from the Br/N ratio (Br/N ratio) and the N peak ratio prior to functionalization (N Peak Fit). The triazole/amide product ratios were determined from the F/Br ratio (F/Br ratio). *Denotes reversal of reaction sequence, that is, the mixed monolayer was reacted first with the alkyne and then the acid chloride.

We reasoned that toluene might minimize any pi-stacking interactions between the benzyl azides and phenylamines, thereby impacting their surface ratio. Therefore, we repeated the mixed monolayer experiments in hexane. Interestingly, changing the solvent from toluene to hexane had no effect on the benzylazide/ amine surface ratio, suggesting that pi stacking of the solvent did not play a substantial role. To see whether the same held true for the propylazide-amine mixed monolayer, we also measured the role of solvent on its azide/amine surface ratio. For this system, a greater amount of the propyl azide was observed for monolayers prepared in hexane rather than toluene $(63 \pm 2 \text{ vs } 44 \pm 2 \text{ mol } \%)$ azide, respectively). We expected hydrogen bonding between the aminosilane and the silica to be enhanced by the hexane; instead the decrease in amine content indicated either enhanced reactivity of propylazide in hexane or a change in monolayer stability. Kang and colleagues observed that the more polar of two surfacereactive species tended to make up a larger component in the mixed monolayer, despite the solution ratio. Yet the *difference* between the surface and solution ratios systematically decreased with decreasing solvent polarity.²¹ The resulting decrease in polar content of the propyl azide-amine monolayer was thus attributed to a change in monolayer stability with solvent polarity. For our benzyl azide-amine mixed monolayer, the polarity of the benzyl azides should be closer to that of the phenylamines, minimizing the effect of solvent polarity on surface ratio. Future work will address how the solvent influences the spatial distribution of the azides and amines,⁴ which might provide more clues as to the origin of monolayer stability for these mixed SAMs.

A key aspect of the orthogonally reactive-SAM strategy is that the ratio of azides to amines will determine the ratio of functional groups that are subsequently attached. To characterize the ratio of the triazole and amide groups that result from reacting the azides and amines, respectively, we once again turned to XPS. Figure 5A illustrates the functionalization of benzyl azide-amine SAMs with *p*-bromobenzoyl chloride, followed by the fluorine-labeled alkyne used in previous experiments. As a reference, the Br/N ratio for the monofunctional amino SAM after reaction with *p*-bromobenzoyl chloride yielded a ratio of 0.87 ± 0.02 , indicating that the maximum conversion was only $87 \pm 2\%$ with this reactant (Table 1, entry 2). Taking this slightly lower yield into consideration, the calculated azide/amine surface ratios from XPS analysis of the Br/N ratio after functionalization are very similar to those calculated from the peak-fitting of the highresolution N1s scan before functionalization (Figure 5B). More importantly, the ratio of the triazole and amide products determined from the F/Br ratio was very consistent with the azide/ amine surface ratios, indicating that the ratio of orthogonally reactive groups dictated the amount of surface products. In contrast, efforts to synthesize 1:1 CF₃ and Br mixed monolayers from monofunctional SAMs led to CF₃/Br surface ratios much greater than one. Specifically, when a 1:1 mixture of CF₃- and Br-labeled acid chlorides was reacted with an amino SAM, the resulting CF₃/Br surface ratio was 3.2 (Figure 6A). Similarly, a 1:1 mixture of CF₃- and Br-labeled alkynes reacted with a benzyl azido SAM led to CF₃/Br ratios of 2.3 (Figure 6B). The substantial difference between the observed ratios and the desired ratio of one emphasizes the difficulty in controlling surface ratios with post-SAM modification strategies using monofunctional SAMs. Once again, these experiments highlight the utility of orthogonally reactive SAMs in controlling surface ratios.

We did, however, observe some sensitivity in our orthogonally reactive-SAM strategy to the sequence of functionalization steps. When we performed the cycloaddition reaction first and the acylation second, the amount of surface bromine, quantifying the efficiency of amide formation, decreased (Br/N of 0.17 ± 0.01 vs 0.23 ± 0.02 for samples acylated second and first, respectively). The smaller Br/N ratio led to a larger calculated azide/amine ratio (Figure 5B, Br/N ratio: 5 Az:Am*). Instead, the lack of bromine is most likely due to lower conversion of the amines to the bromine-labeled amides. This lower conversion suggests that amide formation



Figure 6. (A) Monofunctional amino SAM reacted with a 1:1 molar mixture of 4-trifluoromethyl- and 4-bromo-benzoyl chloride. The resulting CF_3/Br surface ratio was more than three times higher than the solution ratio. (B) Monofunctional benzyl azido SAM reacted with a 1:1 molar mixture of 4-trifluoromethyl- and 4-bromo-phenyl acetylene. The resulting CF_3/Br surface ratio was more than twice the solution ratio. These experiments indicate that generating mixed monolayers post SAM formation does not improve the correlation between the solution and surface ratio.

is sensitive to the local environment around the amine. The formation of the triazoles might sterically block the amine groups, preventing them from reacting with the *p*-bromobenzoyl chloride. In contrast, the amount of fluorine-labeled alkyne was similar whether the click reaction happened first or second (F/N of $0.79 \pm$ $0.04 \text{ vs } 0.78 \pm 0.05$, respectively). These results suggest that the alkyne-azide click reaction is not affected by changes in the local environment around the azide. This similarity in conversion indicates that the high reactivity of the azido monolayer with this alkyne can compensate for difficulty in accessing the surface sites. Future work will explore other reactions orthogonal to the click reaction that are more tolerant of reaction sequence than acylation by acid chlorides. For example, the reaction of thiols with maleimides or other olefinic electrophiles should be orthogonally reactive to the cycloaddition reaction and perhaps less sensitive to the order of reaction.¹

4. Conclusions

Mixed monolayers represent an important avenue for combining functions in materials and in model systems.^{4,16} In the field of self-assembly, many early reports on SAMs demonstrated that solvent and interactions among assembling molecules could influence the resulting mixed monolayer composition.^{20,37} As a result, the surface ratios do not often reflect the composition of the assembly solution. Despite this long-held wisdom in SAM formation, the difference in surface versus solution composition is often overlooked in bifunctional monolayer systems because of the difficulty in characterizing the functional group ratio. The challenge in identifying surface ratios underscores the need for general strategies, like orthogonally reactive SAMs, that provide a well-defined starting point for making complex mixed monolayers of controlled composition.

that can be directly assembled with aminophenyl trimethoxysilane to yield azide-amine mixed monolayers. Using XPS, we have demonstrated how to quantify the amount of orthogonally reactive azides and amines in our monolayers and tune their surface ratio. Through subsequent cycloaddition and acylation reactions, this azide-amine mixed monolayer can be used to anchor combinations of functional groups to silica that are not necessarily amenable to silane chemistry. Moreover, by controlling the amine/azide ratio in the SAM, the ratio of subsequent groups reacted with the azides and amines can be controlled independently of their surface reactivities. The many examples of surface functionalization reactions based on click chemistry or amine modification indicate that there are many combinations of molecules available for forming mixed monolayers with this strategy.¹⁶ The high reactivity observed for the azide and amine groups with different substituted reactive partners suggests that this platform should be general for attaching a wide variety of molecules. Furthermore, this strategy of making mixed monolayers on silica containing orthogonally reactive groups should work with other reactive pairs besides azides and amines; for example, azides or alkynes should be orthogonally reactive with other nucleophiles besides amines like thiols or other electrophilic groups besides acid chlorides like maleimides, epoxides and N-hydroxysuccinimidyl esters.¹ Finally, our strategy for characterizing orthogonally reactive mixed monolayers containing azides should be accessible to other substrates besides silica as well as other reactive group pairings besides azides and amines.¹⁴ Current work is aimed at determining the surface distribution of the two reactive groups in the orthogonally reactive SAM.

We have synthesized two azide-substituted trimethoxysilanes

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Supporting Information Available: Additional IR absorbance, XPS, and AFM data. This material is available free of charge via the Internet at http://pubs.acs.org.