On the Importance of Silane Infusion Order on the Microscopic and Macroscopic Properties of Multifunctional Charge Gradients

Kayesh M. Ashraf,* Kallol Roy,* Daniel A. Higgins,* and Maryanne M. Collinson*

ABSTRACT: Four multicomponent charge gradients containing acidic and basic functionalities were prepared via sol–gel processes and the controlled-rate infusion (CRI) method to more clearly understand how preparation conditions influence macroscopic properties. CRI is used to form gradients by infusing reactive alkoxy silanes into a glass vial housing a vertically oriented modified silicon wafer. The concentration and time of infusion of the silane solutions were kept constant. Only the sequence of infusion of the silane solutions was changed. The first set of samples was prepared by initially infusing a solution containing 3-aminopropyltriethoxysilane (APTES) followed by a mercaptopropyltrimethoxysilane (MPTMS) solution. The individual gradients were formed either in an aligned or opposed fashion with respect to the initial gradient. The second set of samples was prepared by infusing the MPTMS solution first followed by the APTES solution, again in either an aligned or opposed fashion. To create charge gradients (NH$_3^+$, SO$_4^{2−}$), the samples were immersed into H$_2$O$_2$. The extent of modification, the degree of protonation of the amine, and the thicknesses of the individual layers were examined by X-ray photoelectron spectroscopy (XPS) and spectroscopic ellipsometry. The wettability of the individual gradients was assessed via static contact angle measurements. The results demonstrate the importance of infusion order and how it influences the macroscopic and microscopic properties of gradient surfaces including the surface concentration, packing density, degree of protonation, and ultimately wettability. When the gradient materials are prepared via infusion of the APTES sol first, it results in increased deposition of both the amine and thiol groups as evidenced by XPS. Interestingly, the total thickness evaluated from ellipsometry was independent of the infusion order for the aligned gradients, indicative of significant differences in the film density. For the opposed gradients, however, the infusion of APTES first leads to a significantly thicker composite film. Furthermore, it also leads to a more pronounced gradient in the protonation of the amine, which introduces a very different surface wettability. The use of aminosilanes provides a viable approach to create gradient surfaces with different functional group distributions. These studies demonstrate that the controlled placement of functional groups on a surface can provide a new route to prepare gradient materials with improved performance.

INTRODUCTION

A multicomponent gradient surface is one where two or more functional groups are spatially distributed in an increasing or decreasing fashion along the length of a substrate.$^{1−6}$ As described in several reviews, gradient materials can be prepared in different ways that include self-assembly, dip coating, cross diffusion, electrochemical deposition, microfluidic mixing, and immersion.$^{1−6}$ Silane, polymer, and thiol chemistry have all been used to make these materials on a variety of surfaces including siloxane surfaces, electrodes, polymeric scaffolds, hydrogels, and even monoliths.$^{1−6}$ The close proximity of the different functional groups allows for specific interactions to take place between them and/or a reagent in solution. These interactions are critical in many fields of science including separation science,$^{7,8}$ catalysis,$^{9−11}$ and imprinted materials,$^{12}$ and they are thus important to study and understand. By strategically varying the positions of the different functionalities with respect to each other, such as what takes place on a gradient surface, these intermolecular interactions can be studied in a time-efficient fashion.

One approach to prepare multicomponent gradients containing three or more chemical functionalities is via controlled-rate infusion (CRI).$^{13,14}$ In this method, a solution containing a functionalized reactive silane is slowly infused into a vial housing a vertically aligned modified silicon wafer. The bottom of the wafer is exposed to the reactive silane longer than the top, thus yielding a chemical gradient in that particular functional group.$^{13,14}$ A second gradient can be added by repeating the infusion process.$^{15,16}$ An aligned,
multicomponent gradient is produced when the two individual gradients are formed in the same direction, whereas an opposed multicomponent gradient is obtained when they are created in opposing directions.\(^{15,16}\) Cooperativity, both positive and negative, can only take place when the different functional groups are adjacent to each other such as that obtained along the entire length of an aligned gradient. In contrast, in an opposed gradient, the mutual mixing of the individual functionalities only takes place near the middle of the substrate where the two gradients intersect.

In previous work,\(^{16,17}\) we explored cooperativity between acidic and basic functionalities (SiOH, SiO\(^-\), NH\(_2\), NH\(^+\), and SO\(_3\)) on a multicomponent charge gradient prepared using the CRI method. The distribution of charge was determined by zeta potential measurements on the surface and found to be very different for opposed versus aligned multicomponent gradients. As a result, materials with very different surface chemistry were obtained. The gradients in that work were prepared by first depositing an amine gradient followed by a gradient in thiol, which was subsequently oxidized to form a gradient in sulfonic acid moieties.\(^{16}\) It is known that amines having a lone pair of electrons can catalyze hydrolysis and condensation reactions of alkoxysilanes.\(^{18}\) It has also been shown that the presence of surface-adsorbed amine (e.g., ethylenediamine) can help accelerate condensation reactions leading to increased loading onto a surface.\(^{19}\) With this in mind, we hypothesized that by simply changing the order of infusion, we could alter the distribution and placement of functional groups on the gradient surface, thus affecting both thickness, degree of protonation, and surface wettability. The aim of this work is to determine what effect the order of deposition (amine=thiol or thiol=amine) has on the properties of multicomponent charge gradients. Such a comparative study will provide a deeper understanding of how to alter the spatial positioning of multiple functional groups on a gradient surface and optimize gradient performance for their intended applications.

### RESULTS AND DISCUSSION

**Gradient Fabrication.** In this work, four different gradients were prepared from sol–gel-derived organically modified silica sols using the controlled-rate infusion (CRI) method\(^{13,14}\) to evaluate the importance of infusion order on the molecular-scale properties of multicomponent charge gradients. Scheme 1 pictorially demonstrates the process.

The multicomponent gradients were prepared on tetramethoxysilane (TMOS)-derived base layers from individual sols containing either aminosilane or mercaptosilane precursors. Due to the versatility associated with the sol–gel route, materials with very different physical and chemical properties can be made.\(^{1}\) In the present work, the base layer provides a uniform surface for attachment of the reactive silanol groups particularly for substrates that lack such reactive groups;\(^{15}\) it can also be used to provide additional functionality to the surface.\(^{20}\) Aligned gradients are formed when the individual gradients in amine and thiol are in the same direction, and opposed gradients are formed when the individual gradients run in opposite directions. These gradients are converted to charge gradients (NH\(^+\)=SO\(_3\)^-) upon immersion in a 30% H\(_2\)O\(_2\) solution. As previously described,\(^{16,17}\) the oxidation of thiol to SO\(_3\)H subsequently leads to the protonation of adjacent amines (NH\(_2\) → NH\(^+\)). Amine groups can also be protonated by nearby silanol groups.\(^{17}\) Scheme 2 depicts this process.

**Gradient Characterization.** X-ray Photoelectron Spectroscopy (XPS). The presence and extent of modification of the surfaces with amine and thiol were initially evaluated using XPS. Figure 1 shows an overlay of five N1s and S2p XPS spectra for the aligned and opposed (SO\(_3\)+NH\(^+\)) gradients acquired at ∼3 mm intervals from the high amine end (position S) to the low amine end (position 1). The N1s peak in the XPS spectra shown in Figure 1A,C appears as a doublet with binding energies (BE) ∼399.5 and ∼401.8 eV, corresponding to free amine and protonated/H-bonded amines, respectively, in agreement with literature.\(^{13,14,21–23}\) For S2p, the spectrum consists of one peak at a binding energy of 168.5 eV (see Figure 1B,D) in agreement with the presence of the sulfonate group.\(^{24–26}\) For the aligned gradient, both the N1s and S2p signals increase from the low amine end to the high amine end (position 1 → S); while for the opposed gradient, the N1s signal increases while the S2p decreases with position. These trends indicate the presence of multicomponent gradients in the same or opposing directions, respectively. Similar results were observed for the aligned and opposed (NH\(^+\)=SO\(_3\)^-) gradients, Figure S1, with the exception that the peaks are noticeably larger when the amine is deposited first and the relative amounts of the free

---

**Scheme 1.** Fabrication of Aligned (A) and Opposed (O) (NH\(_3\)=SO\(_3\)) (Right Side) and (SO\(_3\)=NH\(^+\)) (Left Side) Charge Gradients by Controlled-Rate Infusion (CRI)\(^{14}\)

**Scheme 2. Simplified Cartoon of the Surface of the Modified Silica Surface Before and After Oxidation by Hydrogen Peroxide. The Weakly Acidic Silanol Groups on the Surface Can Protonate Amine Groups; Likewise, Sulfonic Acid Can Protonate Nearby Amine Groups**
amine to protonated/hydrogen-bonded amines are also very different.

To better evaluate the intensity differences and the gradient profile, the integrated areas under the N1s and S2p peaks for both the NH$_3^{+}$SO$_3^{-}$ and SO$_3^{-}$+NH$_3^{+}$ charge gradients are plotted as a function of distance along the length of the substrate, Figure 2. The area under the N1s peak increases from top to bottom for all the gradients. The area under the S2p peak either decreases or increases with distance depending on whether the individual gradients were made in an aligned or opposed fashion. As can be clearly seen in Figure 2A, the order of deposition is an important variable. When the amine is deposited first (black lines in Figure 2A), a greater amount of nitrogen is found on the surface compared to the situation when the thiol is deposited first (red lines). Not only is there a plethora of free silanol groups on the base-layer-coated substrate but also the amine groups can act as a self-catalyst.

Likewise, it is also noteworthy that the amount of S on each sample is larger when the amine is deposited first (Figure 2B, black vs red). In this case, the amine acts as a surface-bound catalyst to catalyze the condensation of thiol to the surface leading to greater deposition of thiol on an amine-terminated surface relative to the base-layer-coated surface. This result is in agreement with prior work, which showed that one way to double the amount of 3-(aminopropyl) dimethylethoxysilane on glass slides was to first adsorb a layer of ethylenediamine to catalyze the condensation reaction. Immobilization of an amine can also increase the amount of phenylalkoxysilane deposited on thin-layer chromatography plates.

**Extent of Protonation.** To obtain details regarding the interactions between neighboring groups, the deconvolution of the N1s peak into two-component peaks (NH$_3^{+}$ and NH$_2$) was undertaken. The results are shown in Figure 3 for the aligned and opposed gradients. The amine groups will be protonated by the strongly acidic sulfonic acid groups and weakly acidic silanols ($pK_a$ varies from ~4–8) when they are in close proximity to each other (Scheme 2). The amines can also be protonated by surface silanol groups when closely associated with the interface. Thus, the relative placement of the acidic and basic functional groups on the surface will affect the degree of protonation in these materials.

It is evident in Figure 3A that, when the amine is deposited first followed by the thiol for the aligned gradient, protonation of the amine groups increased (64% vs 50% at the high amine end). The greater degree of protonation of the amine is attributed to a closer association with the surface silanol groups (because the amine was deposited first) and the increased deposition of sulfonic acid groups (evident in Figure 2). It can also be noted that the %protonation increases with increasing surface density of the amines, which is attributed to a greater association with silanol groups on the surface and elsewhere.
Other possibilities include a change in pKₐ or the dependence of %ionization with concentration.

For the opposed gradients, the infusion order also matters. At the high amine, low thiol end, the degree of protonation is much larger (66% vs 42%) when the aminosilane is deposited before the thiol silane. Because the surface density of sulfonate groups is fairly low at this end, the greater degree of protonation of the amine indicates a closer association with silanol groups on the surface and neighboring molecules. In contrast, on the opposing end (high thiol-low amine), a greater degree of protonation is noted when the thiol silane is deposited first (66% vs 56%). The presence of a significant number of sulfonic acid groups at the low amine end ensures a high degree of protonation of the amine even though the amine was deposited second and slightly further away from the surface.

Wettability. Water contact angles (WCA) can provide important information about hydrophobicity and hydrophilicity along the length of gradient samples. The surface polarity, and hence the WCA, is determined by the net effects of the various component functional groups present on the surface. The degree to which the water wets the surface will depend on the localized charge, which varies along the length of the substrate for these gradients. In Figure 4, the static deposited first, the contact angle is significantly larger across the entire length of the substrate and a clear gradient in wettability can be noted. When the amine is deposited first (black solid line), however, no gradient in wettability is observed. Water completely wets the surface, and the contact angle is ≈10°. The presence of a significant quantity of charged groups at both ends (%protonation ranges from 55 to 65%) leads to the near constant contact angle with distance. When thiol is deposited first, the high amine end exhibits a lower degree of protonation (42%), thus leading to a larger contact angle at this end relative to the high amine end, which exhibits ~64% protonation. A large gradient in wettability is observed. Both aligned gradients, regardless of the infusion order, exhibit an increasing WCA along the length of the substrate from the highly modified end to the less modified end, which is expected.

Film Thickness. Spectroscopic ellipsometry was used to evaluate the total thickness of the gradient and control samples as well as the thicknesses of the individual amine and thiol layers formed on the base-layer-coated substrate. An X−Y map (0.2 cm × 1.5 cm) consisting of 24 points was collected after each step in the gradient formation process. Representative data of Psi (ψ) and Delta (Δ) as a function of wavelength for the thiol+amine gradients are shown in Figure S2. A representative two-dimensional color map of the film thickness for the amine-thiol gradient substrate is shown in Figure S3. This map was collected in the middle of the sample slide to avoid edge effects. At each position along the length of the substrate, the average thickness and corresponding standard deviation were obtained for the aligned and opposed gradients and uniformly modified substrates. The film thickness of each individual layer was obtained by taking the difference between thicknesses of the amine gradient and base layer and then the amine-thiol gradient from the amine gradient at a given point (within ±0.5 mm) on the substrate.

Figure 5 shows a plot of the total film thickness for the aligned and opposed gradients as a function of distance along the length of the substrate. For the aligned gradient (Figure 5A), it is evident that a thickness gradient is formed irrespective of whether the amine or the thiol is deposited first. Given that a monolayer of the aminopropylsilane is ~7−10 Å, and assuming that a monolayer of mercaptopropylsilane is similar in thickness, the aligned gradient starts out at a ~monolayer thick at the least-modified end and slowly increases to 2−3 monolayers near the highly modified end. The thicker film observed at the highly modified amine end of the gradient results from the polymerization of the aminosilane in the sol during the 30-min infusion time leading to the deposition of a thicker film. Because the condensation of aminooxysilanes is very fast due to the presence of the amine group, cross-linking takes place quickly amongst the different precursors.

It is interesting that the total thickness of the aligned gradient is independent of the infusion order while XPS shows a greater deposition of both N and S when the aminosilane is deposited first. This result suggests that packing density is different for the two films. When the amine is deposited first, a more condensed film is produced due to the catalytic effects of the amine. When thiol is deposited first, the molecular density of the deposited silane molecules is less than when the amine goes first. Under these circumstances, the total thickness of the films would be the same but there would be less overall thiol on the surface as evident in the XPS data, Figure 2. Further...
supporting evidence comes from refractive index measurements. Representative values are shown in Tables S1 and S2. Qualitatively, the refractive index can be related to packing density for nonabsorbing films although some other effects may also come into play. The refractive index of the film when the amine is deposited first is significantly larger (~1.53) as compared to that obtained (~1.42) when the thiol silane is deposited first, consistent with a more dense film in the former.

For the opposed gradient and uniform sample (Figure S4), however, the infusion order is important. Clear differences in “total” film thickness are observed depending on whether amine or thiol silane is deposited first. When the aminosilane is deposited first, the film is 2–3 monolayers thick. When thiol silane is deposited first, the film is initially ~1.5 monolayers thick at the high thiol-low amine end. The film increases to ~2 monolayers at the high amine-low thiol end. The uniformly modified substrate shows similar behavior in that when aminosilane is deposited first, the film is about ~2X as thick as when thiol goes down first. The uniformly modified sample was obtained by soaking the base layer-coated substrate in the respective silane solutions for 30 min each. Thus, they mimic the high amine and high thiol end of the gradients. In this case, it can be seen that the film is thicker when the substrate is modified with the aminosilane first (~1.5 monolayers) compared to when the thiol silane is deposited first (monolayer) consistent with that observed in the opposed gradient. The total film thickness is slightly smaller than that observed on the high-amine end of the gradients because there are fewer available silanol groups on the surface after the thiol silane is deposited for 30 min. Again, a very large difference in refractive index (1.52 (amine first) versus 1.41 (thiol first)) was noted, suggesting a difference in packing density.

To obtain more detailed information, the distance-dependent thickness of “each individual layer” was evaluated by subtracting the different layers from each other. Figure 6 shows the thickness of the “amine layer” alone (after subtraction of the appropriate layers) when the aminosilane was deposited first (black curve with solid squares) and when the thiol silane was deposited first (red curve with solid circles) for the gradient samples. The amine layer thickness for both aligned and opposed gradients also comes out to be about the same, regardless of the infusion order, again suggesting a difference in film density.

It can be noted in Figure 6 that the amine layer exhibits a gradient in thickness from the low amine end (about a monolayer thick) to the high amine end, which is about 2–3 monolayers thick. This gradient in thickness is independent of the infusion order and suggests that the thicker film (~2–3 monolayers) found at the high amine end of the substrate results from the polymerization of the amine in the deposition solvent (water and ethanol).

The thickness of the amine layer on the uniformly modified substrates (Figure S5A), however, depends on the deposition order. Because of the 30-min deposition time, the control sample mimics the high amine-high thiol end of the gradients (e.g., the high modification end of the aligned gradient). In this case, it can be seen that the film is thicker when the substrate is modified with the aminosilane first (~1.5 monolayers) compared to when the thiol silane is deposited first (monolayer). The amine layer is not as thick (2–3 monolayers) as that noted on the gradient samples. The reduction in available silanol groups on the surface once thiol is deposited for 30 min may explain these results.
Figure 7 shows the thickness of the individual thiol layer (after subtraction of the appropriate layers) when the amine was deposited first (black curve with solid squares) and when thiol was deposited first (red curve with solid circles) for the gradient samples. Unlike the aminoalkoxysilanes, the thiol silane does not have the propensity to condense and cross-link rapidly in solution. Not surprisingly, the thiol layer is significantly thinner than the amine layer. For the aligned gradient, the thickness of the thiol layer is ~0.5 monolayer thick, and it is just slightly larger when the aminosilane is deposited first. Similar results are observed for the uniformly modified substrate, Figure S5B.

The importance of infusion order is most apparent for the opposed gradient. As can be seen, the thiol layer exhibits a gradient in thickness when the aminosilane is deposited first. The thiol layer is slightly less than a monolayer at the high amine end and reaches almost two monolayers at the high thiol end. The submonolayer of aminosilane on the surface coupled with readily available silanol groups leads to greater deposition of the thiol silane on the surface.

**Mechanism.** Surface modification via aminosilanes has been well studied as such materials have shown to be good supports for the immobilization of biomolecules, the formation of heterogeneous acid–base catalysts, and carbon dioxide sorption among other applications. These previous investigations help us understand how aminosilanes influence gradient structure and properties, a subject that is significantly less understood. The presence of the amine group ensures high reactivity both with surface-bound silanol groups along with itself via self-condensation reactions leading to cross-linked networks. Siloxane bond formation is possible between two neighboring surface-bound molecules or upon the reaction of an aminosilane in solution to one on the substrate. It is also possible for APTES to form H-bonds among the primary amines leading to a “head-to-head” conformation and thus creating additional points of attachment for the thiol silane by exposing hydrolyzed alkoxide groups.

The condensation of amine to the surface is very fast, and this is evident by a nonzero intercept in the gradient profile. This initial reaction takes place in seconds, and it is followed by slower processes. Over the 30-min deposition period, the aminosilane monomers and oligomers in the silica

---

**Scheme 3. Simple Representation of a Small Section of the Bifunctional Gradient layers on the Base-Layer-Coated Silica Film.**

**A. Amine+Thiol**

**B. Thiol+Amine**
sol continue to react forming more oligomers as condensation is rapid due to the presence of the amine functionality. Likewise, as the exposure time of the silica surface to the aminosilane sol increases, multilayer deposition and/or agglomeration also take place. The net result is an increase in thickness noted on the high amine ends of the gradients. In this work, the aminosilane layer ranges from a monolayer to about 2–3 monolayers, which is consistent with other reports. Because the thiol silane does not act as a self-catalyst, its reactivity toward surface silanols is much slower, and thus in evidence the thiol tends to form a submonolayer catalyst, its reactivity toward surface silanols is much slower, and thus in evidence the thiol tends to form a submonolayer.

### CONCLUSIONS

Functionalized materials where the amount and placement of the individual functional groups can be strategically modified are very important in fields such as catalysis, separations, and chemical sensing. Gradient materials are particularly valuable due to the ability of the aminosilane to act as a self-catalyst and condense in the sol, forming oligomers. The amine groups can thus bond to each other and surface silanol groups and thus influence the structure of the film. Condensation is much slower for the thiol silane, and hydrogen bonding is weaker; thus, its reactivity is much different. Submonolayer formation occurs when the thiol silane is deposited first. More deposition takes place when the amine is present on the surface. The reactivity of the amine strongly influences the film thickness, degree of protonation, and wettability of these gradient materials.

### EXPERIMENTAL SECTION

#### Materials.

The silanes (tetramethoxysilane (TMOS, 99%, Acros Organics) mercaptopropyltrimethoxysilane (MPTMS, 95%, VWR), aminopropyltriethoxysilane (APTES, 99%, Acros Organics)), and trim(hydroxymethyl) aminomethane (Sigma-Aldrich, ACS reagent) were used as received. Absolute ethanol was used in the experiments as was Millipore 18 Megaohm cm water. Hydrogen peroxide was 30% in water from Fisher.

**Sample Preparation.** To provide a uniform silica layer upon which to graft the functionalized silane precursors, a precleaned silicon wafer was first spin coated at 5000 rpm for 30 s with an aged silica sol to form a base layer. The silica sol contains ethanol, TMOS, 0.1 M HCl, and water in a 0.4:0.2:0.06:0.06 volumetric ratio. The base-layer coated silicon wafers were then dried in a desiccator overnight. The aminosilane solution used for infusion was prepared by mixing ethanol, APTES, and water in a 5:0.25:0.06 volumetric ratio. The mercaptopropylsilane solution was prepared by mixing ethanol, MPTMS, and 0.1 M HCl (water) in an 11.8:0.125:0.125 volumetric ratio. The solution was vigorously stirred for 5 min after which 0.125 mL of 0.3 M NH₄OH solution was added to neutralize the acid and ultimately increase the rate of silanol condensation. The pH of the resultant solution was estimated to be ~6. The solution was stirred for an additional 25 min and sat quiescent for 90 min before use in CRI. The gradient surfaces were prepared by slowly infusing (~0.3 mL/min) the functionalized silane solution into a vial containing the vertically aligned base-layer-coated silicon wafer by CRI using a New Era syringe pump. The total infusion time was ~30 min. When forming an aligned gradient, the slide is flipped so that the least modified end is located at the bottom of the glass vial. For the formation of an opposed gradient, the slide is placed so that the end that is most modified is located at the bottom of the glass vial. After CRI, the samples were rinsed with ultrapure water, dried with a stream of N₂, and stored in a desiccator. To oxidize the thiol groups, the materials were exposed to 30% H₂O₂ at 70 °C for 20 min. The duration of the reaction time with H₂O₂ was optimized as described previously. A flow chart outlining the steps associated with gradient formation is depicted in Scheme 1. The charge gradients are referred to as NH₄⁺SO₃⁻ if the amine was deposited first and SO₃⁻NH₄⁺ if the thiol was deposited first. In the figures given throughout this work, “A” stands for aligned and “O” stands for opposed. Uniform samples were made by soaking the base-layer-coated substrates in the amine and thiol sols for 30 min each and oxidized as described.

**Characterization.** Water contact angle (WCA) measurements were made with a Rame-Hart contact angle goniometer by the sessile drop method. The volume of the drop of water was 1 μL. The thickness and refractive index of aligned and opposed gradients on the base-layer-coated silicon wafer prior to oxidation was determined using an M-2000 ellipsometer (J.A. Woollam Co.) at a fixed incident angle 60° from a xenon.
orcid.org/0000-0001-6839-5334; Kallol Roy

Fullerenes were spin coated on the plasma-cleaned silicon substrates (2 M HCl, and 2.5 mL of ethanol. After aging for 12 h, the sol was spin coated on the plasma-cleaned silicon substrates (2 × 1 cm²) at 5000 rpm for 30 s. Dried base-layer-coated substrates were treated with 0.5 M tris (hydroxymethyl) aminomethane in ethanol for 30 min and dried for 2 days. Gradient formation was then performed by CRI as described earlier. We find that a very thin, stable base layer improves the quality of the ellipsometric results.

XPS spectroscopy was performed with a Thermo Fisher ESCALAB250 imaging X-ray photoelectron spectrometer (Al Kα (1486.68 eV), 500 μm spot size, 50 eV pass energy, and 0.1 eV step size). The ESCALAB250 has 45 degree X-ray incident angle, and 90 degree position of entrance for the electron kinetic energy analyzer. Samples were placed on top of conducting tape on a 5 cm × 3 cm sample holder. XPS spectra were acquired at constant intervals (typically every 3 mm) across the wafer and starting ~3 mm from the edge. The spectra were calibrated by taking the C1s peak as 284.6 eV. The deconvolution of the N1s peak areas was done using CASA smart background subtraction.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03068.

N1s and S2p XPS spectra for the aligned and opposed amine+thiol gradients, spectroscopic ellipsometry raw data for thiol+amine gradients and uniform samples, a two-dimensional map of the thickness of the di-thiol layer on the uniformly modified substrate, and tables of refractive index values obtained from ellipsometry (PDF)

AUTHOR INFORMATION

Corresponding Author
Daniel A. Higgins — Department of Chemistry, Kansas State University, Manhattan, Kansas 66506-0401, United States; orcid.org/0000-0002-8011-2648; Email: higgins@kstu.edu

Maryanne M. Collinson — Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284-2006, United States; orcid.org/0000-0001-6839-5334; Email: mmcollinson@vcu.edu

Authors
Kayesh M. Ashraf — Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284-2006, United States
Kallol Roy — Department of Chemistry, Virginia Commonwealth University, Richmond, Virginia 23284-2006, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03068

Author Contributions
K.M.A. and K.R. contributed equally to this work.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We gratefully acknowledge support by the U.S. National Science Foundation (DMR-1404805 and DMR-1404898). The authors acknowledge NSF CHE-0820945 MRI Program for the acquisition of an X-ray photoelectron spectrometer (XPS) for research and education at VCU. We also acknowledge the support of the VCU Nanomaterials Core Characterization (NCC) facility and Dr. Dmitry Pestov for his help with the XPS data acquisition.

REFERENCES

(1) Collinson, M. M.; Higgins, D. A. Organosilane Chemical Gradients: Progress, Properties, and Promise. Langmuir 2017, 33, 13719–13732.
(2) Genzer, J. Surface-Bound Gradients for Studies of Soft Materials Behavior. Annu. Rev. Mater. Res. 2012, 42, 435–468.
(3) Genzer, J.; Bhat, R. R. Surface-bound soft matter gradients. Langmuir 2008, 24, 2294–2317.
(4) Morgenthaler, S.; Zink, C.; Spencer, N. D. Surface-chemical and -morphological gradients. Soft Matter 2008, 4, 419.
(5) Wu, J.; Mao, Z.; Tan, H.; Han, L.; Ren, T.; Gao, C. Gradient biomaterials and their influences on cell migration. Interface Focus 2012, 2, 337–355.
(6) Krabbenborg, S. O.; Huskens, J. Electrochemically Generated Gradients. Angew. Chem., Int. Ed. 2014, 53, 9152–9167.
(7) Yu, X. D.; Lin, L.; Wu, C. Y. Synergistic effect of mixed stationary phase in gas chromatography. Chromatographia 1999, 49, 567–571.
(8) Jeong, L. N.; Rutan, S. C. Simulation of elution profiles in liquid chromatography—III. Stationary phase gradients. J. Chromatogr. A 2018, 1564, 128–136.
(9) Margelefsky, E. L.; Zeidan, R. K.; Davis, M. E. Cooperative catalysis by silica-supported organic functional groups. Chem. Soc. Rev. 2008, 37, 1118–1126.
(10) Notestein, J. M.; Katz, A. Enhancing heterogeneous catalysis through cooperative hybrid organic-inorganic interfaces. Chem. - Eur. J. 2006, 12, 3954–3965.
(11) Brunelli, N. A.; Venkatasubbaiah, K.; Jones, C. W. Cooperative catalysis with acid–base bifunctional mesoporous silica: impact of grafting and co-condensation synthesis methods on material structure and catalytic properties. Chem. Mater. 2012, 24, 2433–2442.
(12) Chen, L.; Wang, X.; Lu, W.; Wu, X.; Li, J. Molecular imprinting: perspectives and applications. Chem. Soc. Rev. 2016, 45, 2137–2211.
(13) Kannan, B.; Dong, D.; Higgins, D. A.; Collinson, M. M. Profile control in surface amine gradients prepared by controlled-rate infusion. Langmuir 2011, 27, 1867–1873.
(14) Kannan, B.; Higgins, D. A.; Collinson, M. M. Aminoalkoxysilane Reactivity in Surface Amine Gradients Prepared by Controlled-Rate Infusion. Langmuir 2012, 28, 16091–16098.
(15) Dewoolkar, V. C.; Kannan, B.; Ashraf, K. M.; Higgins, D. A.; Collinson, M. M. Amine-phenyl multi-component gradient stationary phases. J Chromatogr A 2015, 1410, 190–199.
(16) Ashraf, K. M.; Giri, D.; Wynne, K. J.; Higgins, D. A.; Collinson, M. M. Cooperative Effects in Aligned and Opposed Multicomponent Charge Gradients Containing Strongly Acidic, Weakly Acidic, and Basic Functional Groups. Langmuir 2016, 32, 3836–3847.
(17) Ashraf, K. M.; Khan, M. R. K.; Higgins, D. A.; Collinson, M. M. pH and Surface Charge Switchability on Bipolar Charge Gradients. Langmuir 2018, 34, 663–672.

(18) Brinker, C. J. Hydratosis and condensation of silicates: Effects on structure. J. Non-Cryst. Solids 1988, 100, 31–50.

(19) Kanan, S. M.; Tse, W. T.; Tripp, C. P. Method to double the surface concentration and control the orientation of adsorbed (3-aminopropyl)dimethylethoxysilane on silica powder and glass slides. Langmuir 2002, 18, 6623–6627.

(20) Ashraf, K. M.; Wang, C.; Nair, S. S.; Wynne, K. J.; Higgins, D. A.; Collinson, M. M. Base Layer Influence on Protonated Aminosilane Gradient Wettability. Langmuir 2012, 28, 26621–26627.

(21) Metwalli, E.; Haines, D.; Becker, O.; Conzone, S.; Pantano, C. G. Surface characterization of mono- and tri-aminosilane treated glass substrates. J. Colloid Interface Sci. 2006, 298, 825–831.

(22) Zhang, F.; Srinivasan, M. P. Self-Assembled Molecular Films of Aminosilanes and Their Immobilization Capacities. Langmuir 2004, 20, 2309–2314.

(23) Vandenbark, E. T.; Bertilsson, L.; Liedberg, B.; Udval, K.; Erlandsson, R.; Elwing, H.; Lundstrom, L. Structure of 3-aminopropyl triethoxy silane on silicon oxide. J. Colloid Interface Sci. 1991, 147, 103–118.

(24) Corum, L. E.; Hlady, V. Screening platelet-surface interactions using negative surface charge gradients. Biomaterials 2010, 31, 3148–3155.

(25) Ding, Y. X.; Streitmatter, S.; Wright, B. E.; Hlady, V. Spatial variation of the charge and sulfur oxidation state in a surface gradient affects protein adsorption. Langmuir 2010, 26, 12140–12146.

(26) Chen, C. H.; Lin, J. C. Surface characterization and platelet compatibility evaluation of binary mixed self-assembled monolayers containing novel sulfonic acid terminated alkanethiol. Surf. Interface Anal. 2010, 42, 156–163.

(27) Ong, S.; Zhao, X.; Eisenthal, K. B. Polarization of water molecules at a charged interface: second harmonic studies of the silica/water interface. Chem. Phys. Lett. 1992, 191, 327–335.

(28) Saini, G.; Trenchevska, O.; Howell, L. J.; Boyd, J. G.; Smith, D. P.; Jain, V.; Linford, M. R. Performance Comparison of Three Chemical Vapor Deposited Aminosilanes in Peptide Synthesis: Effects of Silane on Peptide Stability and Purity. Langmuir 2012, 28, 12405–12409.

(29) Zhang, F.; Sautter, K.; Larsen, A. M.; Findley, D. A.; Davis, R. C.; Samha, H.; Linford, M. R. Chemical vapor deposition of three aminosilanes on silicon dioxide: surface characterization, stability, effects of silane concentration, and cyanine dye adsorption. Langmuir 2010, 26, 14648–14654.

(30) Zhou, M.; Lerum, M. Z.; Chen, W. How to prepare reproducible, homogeneous, and hydrolytically stable aminosilane-derived layers on silica. Langmuir 2012, 28, 416–423.

(31) Salin, M.-C. B.; Bardet, M.; Belgacem, M. N. Solvolysis—hydrolysis of N-bearing alkoxysilanes: Reactions studied with 29 Si NMR. Silicon Chem. 2008, 3, 335–350.

(32) Picc, C.; Argento, C.; Drazan, G.; Frechette, J. Micropatterned charge heterogeneities via vapor deposition of aminosilanes. Langmuir 2015, 31, 10725–10733.

(33) Asenath Smith, E.; Chen, W. How to prevent the loss of surface functionality derived from aminosilanes. Langmuir 2008, 24, 12405–12409.

(34) Sastry, M. A note on the use of ellipsometry for studying the kinetics of formation of self-assembled monolayers. Bull. Mater. Sci. 2000, 23, 159–163.

(35) Wasserman, S. R.; Whitesides, G. M.; Tisdwell, I. M.; Ocko, B. M.; Pershan, P. S.; Axe, J. D. The structure of self-assembled monolayers of alkylsiloxanes on silicon: a comparison of results from ellipsometry and low-angle x-ray reflectivity. J. Am. Chem. Soc. 1989, 111, 5852–5861.

(36) McCrackin, F. L.; Passaglia, E.; Stromberg, R. R.; Steinberg, H. L. Measurement of the thickness and refractive index of very thin films and the optical properties of surfaces by ellipsometry. J. Res. Natl. Bur. Stand., Sect. A 1963, 67A, 363.