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Patterning High Surface Area Silica with Lysozyme: Adsorption Kinetics, Fluorescence Quenching, and Protein Readsorption Studies To Evaluate the Templated Surface

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A method was developed for using an inexpensive and widely available protein, hen egg white lysozyme, as a patterning agent for commercial high surface area silicas. The basic patterning methodology involved spontaneous adsorption of the protein from aqueous solution, alkylation of the uncovered surface with an alkylsiloxane, and protein desorption in a slightly alkaline solution of morpholine. Adsorption kinetic studies using Bradford assays assisted in determining protein deposition conditions. These studies were generally consistent with results on more planar silica surfaces and indicated that the protein quickly and strongly adsorbs along its long axis at low surface coverages. A modified fluorescence resonance energy transfer (FRET) technique was developed and employed to evaluate protein spacing. This technique showed that the proteins are well dispersed at low coverages. Readsorption experiments show that the templated regions are robust, retaining the size and shape of the original protein templates.

Introduction

The development of synthetic strategies and analytical tools for controlling structures on the 1–100 nm length scale is fundamental to nanoscience. For planar surfaces, photo- and e-beam lithographic methods are now well established, and a variety of microscopy techniques (e.g., AFM, STM) are readily available to evaluate new structures on the 10 nm length scale. Indeed, the positive feedback loop between the development of lithographic and microscopy techniques has been a major driver for the broader nanoscience field.

In developing their soft lithographic and nanoskiving fabrication methods, Whitesides’ group has highlighted some of the limitations of standard lithographic techniques: they are expensive, cannot be applied to nonplanar surfaces, have narrow specific materials requirements, and provide relatively little control over the chemistry of the patterned surface.5,4 Additionally, it is difficult to build structures in the 1–10 nm size regime, and these techniques cannot be applied to the rough and irregular high surface area materials that are commonly used for applications where a high volumetric density of structures is critical, such as chromatography and catalysis.

One possibility is to combine the intellectual concepts of lithographic techniques with bottom-up surface patterning techniques based on biological nanostructures.5,6 Proteins are potentially excellent masks for high surface area oxides because they can be well characterized, they are reasonably robust, and their adsorption properties may be readily modulated. For example, the surface charge of a protein and/or oxide can be adjusted simply by changing the solution pH. Additionally, because protein–surface interactions play important roles for protein storage, drug delivery devices, contact lenses, tissue culture scaffolds, biosensors, implant materials, and bioreactors, there has been a long-standing general interest in the interactions between solutions containing biological molecules.7–11 This provides a useful literature base with which to choose appropriate proteins and oxides for testing this surface patterning scheme.

The following describes our efforts to apply the intellectual concepts of lithography to a high surface area oxide material. Scheme 1 provides a generic diagram of the surface patterning technique we developed in this study. In the first step, a protein (in this case hen egg white lysozyme) is deposited onto a high surface area oxide. The adsorbed protein serves as a mask while the remaining oxide surface is covered or modified. As a proof of concept, we alkylated the unmasked silica surface; the lysozyme mask served to prevent alkylation in the areas where the protein was strongly adsorbed to the oxide surface. The protein mask can then be removed by a variety of methods, revealing the oxide surface that had been previously masked by the protein.

Ideally, the patterned material will have exposed oxide surfaces that are roughly the size and shape of the mask and spaced throughout the material. Such oxide footprints (derived from the protein mask) can then be used for the controlled preparation of heterogeneous catalysts or as platforms for building larger nanostructures. However, protocols for evaluating the size and spacing of resulting patterns are not well developed. We therefore carefully studied the protein adsorption kinetics, which are well studied on more regular surfaces, as a means of evaluating the

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initial adsorption geometry. We also developed and employed a fluorescence resonance energy transfer (FRET) technique to evaluate the relative spacing of the proteins on the oxide surface. This is the first time that FRET concepts have been applied in such a manner.

**Results and Discussion**

To evaluate the potential for using proteins to template oxide surfaces, we started with a well-characterized industrial silica powder, Davicat SI-1403. This is a relatively high surface area material (BET area = 245 m$^2$/g) that also has a large pore volume (PV = 2 mL/g) and few if any micropores. A large pore volume is important, determine if and how we might be able to control or influence the adsorption process. We therefore set out to study the adsorption capacity and kinetics as a means of studying the adsorption geometry on the high surface area material. By studying the adsorption kinetics, an additional goal is to gain at least a rudimentary understanding of the process and, more importantly, determine if and how we might be able to control or influence the adsorption process. We therefore started with a well-characterized industrial silica. This is the first time that FRET concepts have been applied in such a manner.

Hen egg white lysozyme (Lys) is an excellent protein mask candidate, as it is hard, robust, does not readily denature, and is extremely well characterized. Additional Lys is inexpensive and readily available in kilogram quantities, and its dimensions (30 × 30 × 45 Å) have been known since 1965. The numerous amine residues on the protein surface give Lys a relatively high isoelectric point (pH = 11) and provide a variety of potential adsorption mechanisms on acidic silica (e.g., electrostatic interactions, H-bonding).

**Lysozyme Adsorption.** Lysozyme adsorption onto flat silica surfaces has been examined with a variety of spectroscopic and calorimetric techniques. These studies largely focus on model surfaces such as mica or atomically flat SiO$_2$/Si wafers; relatively few have examined high surface area materials. We therefore undertook a study of the adsorption capacity and adsorption kinetics for Lys on Davicat SI-1403 silica. There are a number of reasons for this study. First, because Lys adsorption onto atomically flat surfaces is well studied, the adsorption kinetics provide an important link between our studies and those under more controlled circumstances. High surface area materials have not been extensively examined, so it is prudent to make sure that protein adsorption behaves similarly to the previous studies, even if they are on less complicated surfaces.

In addition, Lys adsorption is the first step in the patterning methodology, and arguably the most important since it determines the initial size of the mask. Because the adsorption geometry of Lys on planar silica is well characterized, it may be possible to correlate our adsorption kinetics with those on well-defined substrates and, by analogy, discern something regarding the adsorption geometry on the high surface area material. By studying the adsorption kinetics, an additional goal is to gain at least a rudimentary understanding of the process and, more importantly, determine if and how we might be able to control or influence the adsorption process. We therefore set out to study the adsorption capacity and kinetics as a means of studying the first step in the patterning method.

The Davicat silica provides a large surface area to volume ratio (120 m$^2$/mL), so that changes in the solutions protein concentration are large enough to quantify the amount of Lys adsorbed by the silica. This was accomplished by performing Bradford assays, a common method of protein quantification, on small aliquots of the protein solution, as shown in Figure 1. Control experiments in the absence of silica showed no change in Lys concentration over 2 h, so adsorption curves (Figure 1b) were determined directly from the solution concentration data. Moles of adsorbed Lys were then normalized to the total surface area of each sample.

The representative Lys adsorption isotherm (134 μmol of Lys per g of silica) shown in Figure 1 was collected for the purpose of determining the adsorption capacity of this silica. Because the Davicat silica is amorphous and Lys is a relatively large adsorbate, some of the surface may be inaccessible due to protein packing and pore mouth blocking. Hence, it is critical to determine the monolayer adsorption capacity, which represents the surface area of the substrate that is useable for patterning. The data show an initial rapid adsorption event in the first few minutes followed a roughly linear increase in adsorbed protein over time. After 2 h, the silica was thoroughly washed with buffer to remove any weakly adsorbed protein. Bradford assays of the washings yielded 50.0 μmol/g of weakly adsorbed protein. Subtracting this value from the initial conditions left 74.7 μmol lys/g SiO$_2$ as

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strongly bound protein, which corresponds to a surface coverage of 0.305 ± 0.009 μmol/m².

Extrapolating the linear portion of the adsorption isotherm yields a nonzero intercept (0.160 ± 0.009 μmol/m²), suggesting two steps are involved in the adsorption process. The intercept value was highly reproducible (see Supporting Information) and was independent of pH and protein concentration. The intercept (0.160 ± 0.009 μmol/m²) corresponds to an effective Lys footprint (average surface area covered per protein) of 10.5 ± 0.6 nm²/lys. This measured value agrees well with previously values for Lys adsorbed on its long axis (9.7 nm², see Scheme 2).23 The strongly adsorbed Lys corresponds to an effective protein footprint of 5.4 nm², which is similar to previous measurements for Lys adsorbed along its short axis (4.9 nm², see Scheme 2).24 Both of these values measured on the high surface silica are slightly larger than the literature reports, suggesting that a fraction of the surface area is inaccessible to the protein. However, comparison of the values on flat and high surface area silicas indicates that greater than 90% of the total surface area is available for patterning with the protein.

Adsorption kinetics were monitored as a function of Lys concentration (891, 89.1, and 8.91 μM) in solutions buffered to pH 4.0 and 7.2. Representative adsorption isotherms, shown in Figure 2, did not fit to a single first-order exponential, further supporting a two-step adsorption model. The data were well described by two simultaneous Langmuir isotherms (see eq 1), similar to some reports in the literature.25-28 In this adsorption model, \( N_{\text{ads}} \) is the total moles of adsorbed Lys, \( A_1 \) is the intercept value from the linear adsorption regime shown in Figure 1 (0.160 μmol/m²), and \( A_2 \) is the difference between the strongly adsorbed Lys and the intercept value (0.147 μmol/m²). The two area values correspond to two adsorption events with rate constants of \( k_1 \) and \( k_2 \), respectively.

\[
N_{\text{ads}} = A_1(1 - e^{-k_1 t}) + A_2(1 - e^{-k_2 t})
\]  

Values for \( k_1 \) were determined by independently monitoring the adsorption kinetics up to a total surface coverage of 0.16 μmol/m². The determined rate constants are compiled in

**Table 1. Adsorption Kinetics Data**

<table>
<thead>
<tr>
<th>[Lys] (μM)</th>
<th>pH 4.0</th>
<th>pH 7.2</th>
<th>pH 4.0</th>
<th>pH 7.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.91</td>
<td>10</td>
<td>54</td>
<td>115</td>
<td>195</td>
</tr>
<tr>
<td>89.1</td>
<td>35</td>
<td>39</td>
<td>5.9</td>
<td>4.5</td>
</tr>
<tr>
<td>891</td>
<td>25</td>
<td></td>
<td>2.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Typical errors for \( k_1 \) measurements were ±20%. *Typical errors for \( k_2 \) measurements were ±5%.

Table 1. The figure and tabulated data indicate the changes in pH had relatively minor effects on protein adsorption onto our silica. Surprisingly, the data show that lysozyme adsorption rates vary inversely with Lys concentration. This is also readily apparent in the adsorption isotherms, which show that adsorption is effectively complete after 20 min for the lowest protein concentration while higher concentrations required about 2 h to saturate the surface. This is particularly important information for developing a patterning methodology as it defines the time and protein concentrations required in the first patterning step (protein adsorption).

Lysozyme adsorption onto silica has previously been studied by a number of experimental techniques, including polarization interferometry, fluorescence spectroscopy, CD spectroscopy, ellipsometry, reflection fluorescence, optical reflectometry, neutron reflectometry, and atomic force microscopy. These studies indicate that the details of adsorption are relatively sensitive to a number of factors including silica source (particularly particle size), protein concentration, solution pH, and ionic strength. They generally conclude that Lys adsorbs through a multistep process, with the first step being adsorption along the long axis. In the second step, the protein can then undergo a surface reorganization to increase protein—protein interactions (e.g., reorientation along the short axis), thus opening additional adsorption sites and increasing the surface concentration of protein. Alternately, some measurements and systems indicate the second adsorption event is a strongly bound bilayer that does not require reorientation of the protein, while others conclude that additional adsorption steps/geometries are involved.

The adsorption studies on high surface area silica are consistent with both models invoking two adsorption events, and we cannot definitively conclude which is at work under our conditions. Our interest in the current work is in using Lys adsorption for surface patterning, which only deals with protein loadings below the first monolayer. Since our interest is in this submonolayer adsorption regime, we did not further pursue the details of the second adsorption event. Our primary concern was to confirm that the initial adsorption step is well behaved and consistent with the consensus of the literature data. Thus, we conclude that in our patterning scheme with surface coverages substantially below one monolayer, lysozyme adsorbs predominately along its long axis on the high surface area silica.

**Fluorescence Resonance Energy Transfer (FRET) Studies.** The adsorption kinetics experiments provide guidance in choosing deposition conditions and indicate that Lys is adsorbed along its long axis at low surface coverages. If the proteins are to be used for patterning masks, however, it is similarly important to evaluate the protein—protein spacing on the high surface area silica. Ideally, the templates will be well spaced after adsorption, with little or no clustering. Lysozyme is a strongly basic protein which carries a large positive charge at neutral and acidic pH. The strong electrostatic attraction for the negatively charged acidic silica is undoubtedly responsible for the strong and fast adsorption of the protein. The repulsive interactions between strong positive charges on Lys may help to space adsorbed proteins on the surface at low coverage, but this is not guaranteed. Once adsorbed, some of these positive charges will be balanced by the oxide surface, which is likely to reduce the repulsive forces. Since the protein forms a closely packed monolayer on silica, the repulsive forces are likely to be weaker than the energy of adsorption.

If the proteins are to ultimately be used as masks in patterning, it is important to evaluate the surface concentrations at which the proteins begin to approach one another or if they are adsorbed as well-spaced individual proteins or as clusters of proteins. We are unaware of simple methods for evaluating protein spacing on high surface area materials. Therefore, we developed and employed a modified fluorescence resonance energy transfer (FRET) technique to provide some insight into this important factor for surface patterning. In these experiments, two samples of Lys were modified: one with a standard FRET donor (Texas Red; structure in Supporting Information) and the other with a complementary FRET acceptor (CNF; structure in Supporting Information). Each Lys sample was modified with a single FRET donor or acceptor label (Lys-TxRed and Lys-CNf, respectively). Control experiments showed that the modest modifications to the protein surface caused no measurable change in protein adsorption. Additionally, control experiments in solution showed no fluorescence quenching under the conditions used for protein deposition, indicating that there is no protein—protein clustering in solution.

Experiments evaluating potential protein—protein surface interactions involved comparing labeled and unlabeled protein samples at the same surface coverage. In these experiments, 50/50 mixtures of Lys/Lys-TxRed were adsorbed onto the silica and compared to 50/50 mixtures of Lys-TxRed/Lys-CNf. A schematic diagram for these experiments can be found in the Supporting Information. In the control experiment, the unlabeled Lys is included so that the total surface coverage of protein is identical in both experiments. This also controls for any fluorescence quenching due to tryptophan in the protein. Samples were prepared at several surface coverages, ranging from 0.016 to 0.305 μmol/m² of the strongly bound protein monolayer.

Figure 3 shows representative fluorescence data for the 0.016 and 0.306 μmol/m² samples. It is clear from the top spectra that there is essentially no difference between the Lys-TxRed/Lys and the Lys-TxRed/Lys-CNf samples. This conclusively shows that the proteins are not close enough on the surface to allow energy transfer from the fluorescence donor to the acceptor. For the completely covered surface, there is substantial fluorescence quenching, as is expected for proteins packed in a strongly bound monolayer.

The degree of fluorescence quenching can be quantified in terms of the transfer efficiency (% \( E \)):

\[
% E = 1 - \frac{F_{da}}{F_{da}}
\]

the efficiency is also related to both the distance between the donor and acceptor and the strength of the coupling between...
the donor and acceptor absorption. This coupling is often quantified as the Förster radius, \( R_0 \). The Förster radius also has a straightforward physical interpretation: it is the distance required for 50% energy transfer between a donor and acceptor pair (\( R_0 = 5.9 \) nm for the Texas Red–CNF pair). Thus, FRET distances can be evaluated from the transfer efficiency: \[ % E = \frac{R_0^6}{R^6 + R_0^6} \] (3)

Transfer efficiencies for the various samples are shown as a function of surface coverage in Figure 4 and evaluated in terms of the corresponding FRET distances in Table 2. The transfer efficiencies show a steady increase in fluorescence quenching as Lys is packed onto the surface. FRET distances can be calculated from the transfer efficiency; however, the determined values should be evaluated qualitatively rather than interpreted as a direct measure of the interprotein distance. This distance is actually a distribution of distances between fluorescence donors and acceptors and is also a function of the distribution between individual proteins as well as their relative orientations. For the low coverage samples, the transfer efficiencies are so low that the calculated values do not have much direct physical meaning. However, the determined value for the highest coverage samples is 3.2 nm. This value is similar to the dimensions of the protein, so the FRET distance gives reasonable values as the proteins approach one another and the quenching begins to occur.

These experiments provide important information regarding the use of lysozyme as a mask in patterning high surface area silicas.

Figure 3. Fluorescence spectra for FRET labeled Lys with surface coverages of 0.016 \( \mu \)mol/m\(^2\) (A) and 0.305 \( \mu \)mol/m\(^2\) (B). The spectra show no quenching at low protein loading and substantial quenching at high surface coverage.

Most importantly, the points with low surface coverage (< 0.08 \( \mu \)mol/m\(^2\)) show very little quenching, indicating that the proteins are well spaced. Even at a surface coverage of one-fourth of the first monolayer, relatively little quenching is observed.

One possible interpretation is that the proteins are perfectly spaced at low coverages, but at distances far enough beyond the Förster radius to not allow much quenching. Alternately, it is likely that there is some clustering of the protein on certain regions of the high surface area silica and that the quenching results from the close proximity of a few proteins. In the case of the second explanation, the degree of quenching remains small, indicating that clustered proteins do not make up a substantial fraction of the patterned surface. Consequently, it appears that lysozyme proteins are excellent surface patterning masks for high surface area silicas. The adsorption kinetics indicate that the proteins adsorb in a consistent geometry along their long axis and the FRET studies provide substantial evidence that they are largely distributed throughout the material. These are exactly the desired characteristics of a surface patterning agent: deposition is simple and regular, and the masks are well spaced.

**Surface Patterning and Repatterning Experiments.** Beyond providing information regarding orientation of the adsorbed protein, the adsorption kinetics studies also provide important guidance for the protein deposition step in the surface patterning, where protein solution concentration, solution volume, and surface loading can all be varied. On the basis of the adsorption kinetics results, several samples were prepared by adsorbing lysozyme onto 1 g of Davicat silica. The protein solution concentration was held constant (8.9 \( \mu \)M, pH 7.6) to control the adsorption kinetics, and the solution volume was varied to yield samples with surface coverages between 5% and...
After surface alkylation, unpatterned silica and reaction times were optimized to 5 min (see Supporting Information).35 After surface alkylation, unpatterned silica became difficult to suspend in water, indicating that the oxide surface had been rendered largely hydrophobic. As expected, the patterned silicas with low Lys loadings were largely hydrophobic after the alkylation treatment and those with high Lys loadings remained largely hydrophilic.

Removal of the protein masks from the alkylated silica (the last step in Scheme 1) was accomplished with an aqueous solution of morpholine, which is a common reagent in protein purification. Condensation reactions between siloxanes and surface silanol groups are generally straightforward, well understood, and facile at ambient temperatures. For this proof of concept study, we chose to alkylate the protein patterned surface with an ethanol solution of isobutyltrimethoxysiloxane. Lysozyme is insoluble in ethanol, and this medium is unlikely to dramatically affect the solution of isobutyltrimethoxysiloxane. Lysozyme is insoluble in ethanol, and this medium is unlikely to dramatically affect the solution in ethanol. Infrared spectroscopy of the resulting solids (Figure 5) shows that absorbance due to amide bonds increases with surface coverage, and washes of the solids after lysozyme deposition showed no evidence of protein in solution by Bradford assay. This indicates that all of the protein added during the deposition phase of the patterning is strongly bound to the surface.

Numerous methods are available for modifying silica surfaces. Condensation reactions between siloxanes and surface silanol groups are generally straightforward, well understood, and facile at ambient temperatures. For this proof of concept study, we chose to alkylate the protein patterned surface with an ethanol solution of isobutyltrimethoxysiloxane. Lysozyme is insoluble in ethanol, and this medium is unlikely to dramatically affect the strong protein—silica interactions. Nonetheless, this reactive siloxane was used to minimize exposure to the alkylating solution and reaction times were optimized to 5 min (see Supporting Information).35 After surface alkylation, unpatterned silica became difficult to suspend in water, indicating that the oxide surface had been rendered largely hydrophobic. As expected, the patterned silicas with low Lys loadings were largely hydrophobic after the alkylation treatment and those with high Lys loadings remained largely hydrophilic.

Removal of the protein masks from the alkylated silica (the last step in Scheme 1) was accomplished with an aqueous solution of morpholine, which is a common reagent in protein purification. Figure 6 shows infrared spectra of the amide region for the 20% 100% of the strongly bound protein (0.305 μmol/m², includes both strong adsorption events). Infrared spectroscopy of the resulting solids (Figure 5) shows that absorbance due to amide bonds increases with surface coverage, and washes of the solids after lysozyme deposition showed no evidence of protein in solution by Bradford assay. This indicates that all of the protein added during the deposition phase of the patterning is strongly bound to the surface.

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morpholine wash and the same as the amount of protein that was readsorbed from solution. Importantly, the readsorption experiment shows no adsorption beyond what was originally used to template the material, even though an excess of protein was available. This is strong evidence that the patterning method developed here is largely successful, as it indicates that the patterned oxide largely retains the size and shape of the original template yet maintains the chemical properties of the high surface area silica.

Conclusions
A procedure was developed for using hen egg white lysozyme as a surface patterning agent for a commercial high surface area silica. Protein deposition conditions were determined from kinetics studies during the spontaneous adsorption of lysozyme onto the silica. These studies indicated that, at the submonolayer surface coverages that are most important for patterning, lysozyme adsorbs rapidly along its long axis. To evaluate the relative spacing of the adsorbed protein masks, a modified FRET technique was developed and employed. This study showed that the proteins were generally well spaced on the high surface area material, likely because of strong electrostatic repulsions from the positively charged proteins.

The patterning protocol was then developed using a low concentration of lysozyme to disperse protein masks throughout the silica surface. The remaining surface was alkylated with isobutyltrimethoxysiloxane, and the protein masks were removed by treatment in a slightly alkaline morpholine solution. Readsoorption experiments using an excess of protein and the patterned silica showed very similar kinetics. Further, essentially the same quantities of protein adsorbed, removed by morpholine, and readsorbed were measured. We therefore conclude that the patterning was successful and that the patterned oxide retains the size and shape of the original protein template while maintaining the chemical properties of the high surface area silica.

Experimental Section
Materials. Lysozyme from hen egg white (lyophilized, ~95% protein, 42,530 units/mg solid), Coomasie Blue Staining Solution (Sigma Chemical Co.), Na$_3$HPO$_4$·7H$_2$O (Aldrich Chemical Co.), and glacial acetic acid (Fisher Scientific) were purchased and used without further purification. Davicat SI-1403 silica powder (245 m$^2$/g), supplied by Grace-Davison, was pressed, crushed, and sieved to 40/60 and 60/80 mesh particles. The silica was calcined at 500 °C overnight prior to use, and BET surface area measurements were always within 10% of the manufacturer’s value. Acetate buffer (10 mM) was prepared using glacial acetic acid and nanopure water, adjusting the buffer to pH 4.0 using dilute sodium hydroxide solution. Phosphate buffer (0.01 M) was prepared using Na$_2$HPO$_4$·7H$_2$O and nanopure water, adjusting to pH 7.2 using dilute sodium hydroxide. UV–vis absorption data were collected with a Hitachi U-2001 UV–vis spectrophotometer.

Spectroscopy. FTIR spectra were collected on a Thermo Nicolet Nexus 470 FT-IR using a Thermo-Electron ATR accessory. Samples were always dried immediately prior to analysis. Fluorescence spectra were collected using a PTI Model QM-7 fluorescence spectrometer with an excitation wavelength of 280 nm. Solid samples were suspended with methanol (3 mg of silica in 3 mL of methanol) in an ultrasonic bath immediately prior to collecting fluorescence spectra, although the resulting fine slurry showed no signs of settling for several hours.

Bradford Assay. Lys solutions were prepared by adding 128 mg of protein to 10.0, 100, or 1000 mL volumetric flasks, and diluting with nanopure water to give 890, 89, and 8.9 μM solutions, respectively. For solution concentrations less than 100 μM, protein concentration was determined by removing a 1.9 mL aliquot and adding 100 μL of Bradford reagent. After 8 min, the absorbance at 595 nm was determined. For solutions greater than 100 μM, aliquots of 0.4 mL were removed and diluted with 1.3 mL of buffer. Bradford reagent (0.3 mL) was added to each aliquot, and samples were reacted for 8 min before measuring absorbance.

Monolayer Capacity. To determine the monolayer capacity, 890 μM Lys solutions (nominally 0.192 g of Lys in 1.5 L of buffer) were prepared in pH 4.0 and pH 7.2 buffers. Sieved and calcined silica was added and stirred for 2 h, and the solution Lys concentration was periodically measured. The solid was then filtered and washed three times with 5 mL of pure buffer. The washings were combined, diluted to 25.0 mL with buffer, and the Lys concentration was determined. The mass of adsorbed Lys was determined by subtracting the final mass of solution Lys from the initial Lys mass.

Adsorption Kinetics Studies. Lys (128 mg) was added to buffer of the desired pH, giving initial concentrations of 8.9, 89, and 890 μM. Aliquots (1.9 or 0.4 mL) were periodically removed from each sample and Lys concentration determined as described above. To obtain accurate concentration values, a calibration curve was prepared immediately before each kinetics experiment. Adsorption kinetics were studied at 20 °C; the duration of each experiment was 120 min. Rate constants were extracted from the kinetic data using the Solver feature of Microsoft Excel to perform a least-squares analysis. All Kt values were determined using the kinetic data taken immediately after silica was added to the Lys solution.

Preparation of Labeled Proteins. Texas Red was synthesized by preparing a solution of methyl-6-aminohexanoate (0.40 g, 2.20 mmol) and triethylamine (0.60 mL) in chloroform (40 mL). Sulforhodamine 101 acid chloride (1.00 g, 1.60 mmol) was added slowly over 10 min while the reaction mixture was stirred at 0 °C. The reaction mixture was allowed to stir for 15 min at room temperature. This solution was washed three times with 50 mL of water. The organic layer was collected, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Product was purified with silica gel column chromatography using 2.5% methanol in chloroform. The purified product was resuspended in dioxane (15 mL), and 6 M HCl (25 mL) was added dropwise over 5 min. This solution was stirred for 18 h at room temperature and then added to 200 mL of water. The solid product was isolated and purified with silica gel column chromatography using 15% methanol in chloroform.

The purified product was resuspended in DMF (3 mL). O-(N-Succinimidyl)-N,N,N’,N’-tetramethyluronium tetrafluoroborate (150 mg, 0.50 mmol) was added to this solution. Reaction mixture was stirred for 1 h at room temperature and then diluted with chloroform (50 mL). The mixture was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a volume of 10 mL. Ethyl ether (100 mL) was added dropwise to the reaction mixture while stirring vigorously at room temperature. Product was collected by filtration and dried under vacuum.

To couple Texas Red to the protein, Lys (220 mg) was dissolved in pH 8.5 buffer and filtered through a 0.45 μm filter. Texas Red (11 mg) was dissolved in DMF (200 μL) and added dropwise to Lys solution. Reaction mixture was stirred for 2 h at 23 °C. 1.5 M hydroxylamine (2.5 mL) was added to quench the reaction. The solution was dialyzed against 0.25 M NaCl at room temperature overnight. 5-(and 6)-Carboxynaphthofluorescein was anchored to Lys using the methods described above.

FRET Measurements in Solution. A 6.0 mg/mL solution of Texas Red labeled Lys (TXRed-Lys) and a 4.0 mg/mL solution of 5(and 6)-carboxynaphthofluorescein (CNF-Lys) were prepared with pH 7.2 buffer. TXRed-Lys solution (1 mL) was added to unlabeled Lys (32.4 mg) and diluted with pH 7.2 buffer to make a 12.8 mg/mL solution. TXRed-Lys solution (320 μL) was added to a 2 mg/mL unlabeled Lys solution (960 μL) and diluted with pH 7.2 buffer.
7.2 buffer to make a 1.28 mg/mL solution. TxRed-Lys solution (32 μL) was added to a 2 mg/mL unlabeled Lys solution (96 μL) and diluted with pH 7.2 buffer to make a 0.128 mg/mL solution. Fluorescence measurements of the above three solutions were taken; each solution was excited at 563 nm, and the emissions were monitored between 570 and 700 nm.

TxRed-Lys solution (1 mL) was then added to CNF-Lys solution (1.5 mL) and diluted with pH 7.2 buffer to make a 12.8 mg/mL solution. TxRed-Lys solution (320 μL) was added to CNF-Lys solution (480 μL) and diluted with pH 7.2 buffer to make a 1.28 mg/mL solution. TxRed-Lys solution (32 μL) was added to CNF-Lys solution (48 μL) and diluted with pH 7.2 buffer to make a 0.128 mg/mL solution. Fluorescence spectra were collected as described above.

**FRET Measurements on Supported Proteins.** Three solutions of TxRed-Lys and CNF-Lys were added in varying amounts to silica to achieve the surface coverages described in Table 2. TxRed-Lys (50 μL) was added to pH 7.2 buffer (3 mL), and fluorescence was measured. CNF-Lys (215 μL) was added to sample with TxRed-Lys, and fluorescence was measured. 1.00 mg/mL silica slurry suspended in buffer solution (100 μL) was added to sample with TxRed-Lys and CNF-Lys. Sample was sonicated for 20 min, and fluorescence was again measured.

TxRed-Lys (95 μL) was added to pH 7.2 buffer (3 mL), and fluorescence was measured. CNF-Lys (300 μL) was added to sample with TxRed-Lys, and fluorescence was measured. 1.00 mg/mL silica and buffer solution (100 μL) were added to sample with TxRed-Lys and CNF-Lys. Sample was sonicated for 20 min, and fluorescence was again measured.

TxRed-Lys (275 μL) was added to pH 7.2 buffer (2.59 mL), and fluorescence was measured. CNF-Lys (575 μL) was added to sample with TxRed-Lys, and fluorescence was measured. 1.00 mg/mL silica and buffer solution (100 μL) was added to sample with TxRed-Lys and CNF-Lys. Sample was sonicated for 20 min, and fluorescence was again measured.

**Surface Alkylation and Protein Desorption.** Ethanol (47.5 mL) was combined with nanopure water (2.5 mL) and isobutyltrilmethoxysilane (500 μL). Solution was stirred 5 min and brought to pH 5.29 with dilute sodium hydroxide solution. Lys templated silica (200 mg) was added and stirred for 3 min. Solution was decanted off, and silica was washed twice with ethanol. Surface was cured in an oven at 100 °C for 10 min. Lys was desorbed from the surface by stirring with a 0.5 M solution of morpholine in pH 8.2 sodium borate buffer for 2 h.

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**Supporting Information Available:** Table of intercept values, alkylation reaction study, and structures of Texas Red and CNF. This material is available free of charge via the Internet at http://pubs.acs.org.