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Chemoselectivity and the Chan–Lam Coupling Reaction: Adding Amino Acids to Polymer-Coated Microelectrode Arrays

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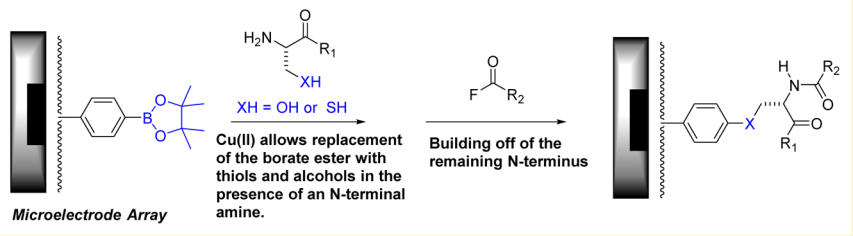
Supporting Information



ABSTRACT: The placement of a peptide onto a microelectrode array is frequently complicated by the presence of multiple nucleophiles in the peptide. In the work reported here, we have found that the Chan–Lam coupling reactions used to site-selectively place thiol, alcohol, and amine nucleophiles onto diblock-copolymer-coated surfaces are chemoselective for the placement of thiol and alcohol nucleophiles on the arrays. This means that cysteine- and serine-containing peptides can be placed onto an array without any need to protect the N terminus of the peptide. Furthermore, it was found that placement of thiol groups onto an array with the Chan–Lam reaction using optimized reaction times leads to complete coverage of the electrodes. The extent of this coverage can be controlled by varying the reaction time in a manner that allows for the construction of arrays with a gradient of peptide concentrations.

INTRODUCTION

Microelectrode arrays are intriguing devices for monitoring binding events between small molecules and biological targets.[1−14] They work by placing or building the molecules to be monitored proximal to individually addressable electrodes in the arrays and then using those electrodes to detect binding events with the biological target. Central to these efforts is the organic chemistry used to manipulate the surface of the array so that the molecules are located only by the electrodes selected. It is our ability to control the surface of the array that ultimately determines the nature of the biological studies that can be conducted on the arrays and the quality of the data that are obtained from those studies. For example, consider the chemistry shown in Scheme 1.[15]

In this reaction, a cysteine-terminated RGD-peptide was placed by blocks of 12 electrodes each in a microelectrode array having over 12000 electrodes/cm^2. This was accomplished by using the electrodes in the array to generate a Cu(I) catalyst that then mediated a coupling reaction between the thiol in the peptide and an aryl bromide on the surface of the array.[16] The Cu(I) catalyst generated at the electrode was then destroyed in the solution above the array by oxygen, which oxidized the Cu(I) species back into an inactive Cu(II) precursor. The location of the reaction at the selected electrodes and only the selected electrodes was controlled by adjusting the rate of Cu(I) generation at the electrodes relative to the rate of reoxidation in solution. Once the peptide was located by the electrodes selected for the study, the electrodes were then used to detect interactions between the peptide and its integrin target.[15,16] The chemistry nicely demonstrated both the synthetic and analytical capabilities of the microelectrode arrays used.[17,18] However, the signaling studies were "curious" in that the RGD-integrin binding event on the array occurred with picomolar concentrations of the integrin receptor even though the RGD-peptide is known to bind integrin with nanomolar affinity. Others have seen similar amplification of signals in electrochemical experiments when a protein target undergoes an exchange between multiple ligands bound to the surface of the electrode.[19] The amplification occurs because a dissociated protein migrating between ligands on the surface of the electrode is still close to the surface of the electrode. Hence, it still influences the conductivity of the surface. If this is the case for the RGD-peptide/integrin interaction, then the array can be calibrated and the signal moved to a value consistent with the literature value; the concentration of peptide on the surface would need to be reduced. Such an adjustment is necessary if one wants to use the arrays to not only detect a signal but also to measure relative binding constants. Differences between the signals cannot be monitored well if all of the signals are amplified to a picomolar level.

In principle, this issue can be addressed by varying the relative concentration of the ligand on the surface of the electrodes and then choosing a surface concentration that affords a binding signal with an appropriate constant (appropriate being defined as one that matches the solution

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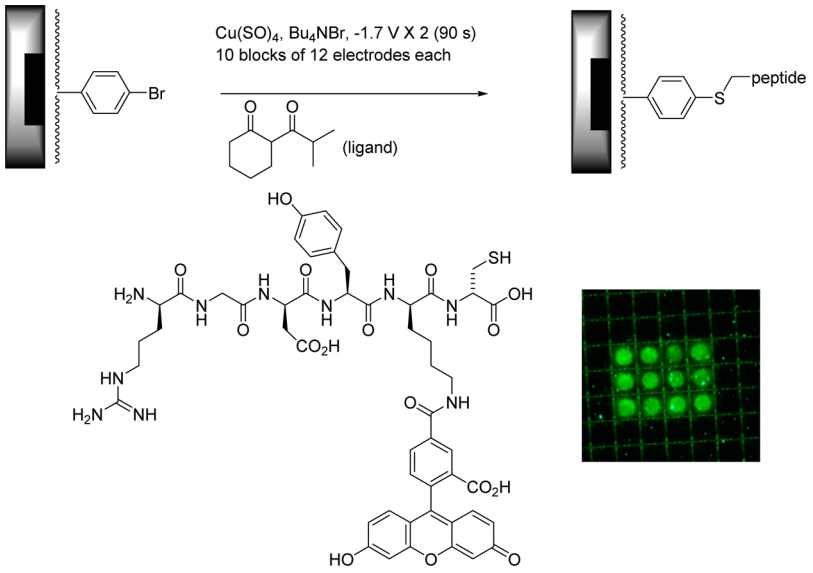
Scheme 1. Placement of an RGD-Peptide onto a Microelectrode Array



binding constant). However, to conduct an experiment requires further development of the synthetic methodology available on the surface of the array. We need to be able to use the chemistry to not only confine the reaction to selected electrodes on the array but also vary the relative amounts of material that are placed on the surface of those electrodes. In addition, we need to know how much of the material placed on a surface is active. This second point requires that we understand the chemoselectivity of the synthetic reaction that takes place on the surface of the array. For the chemistry illustrated in Scheme 1, it was possible that the placement of the peptide onto the array did not proceed with the thiol as planned but rather involved the unprotected N-terminus. Such a binding event would still lead to fluorescence, indicating the presence of the molecule, but would not place the molecule on the array in a manner that would allow it to still bind the integrin receptor. The binding event recorded suggests that at least some of the material was added to the surface through the thiol linker, but how much? Is there a preference for the addition of a thiol nucleophile to the surface relative to an amine or vice versa? As always, the key to developing an analytical experiment on the array is to properly control the organic chemistry of the surface.

While it is tempting to suggest placing the peptide onto the array using a site-selective [3 + 2]-cycloaddition-based "click reaction",[15,20] such reactions require prior functionalization of both the surface of the array and the peptide and are not compatible with the use of the more versatile tunable borate ester surfaces on the arrays.[21] The borate ester based surface undergoes reactions with nucleophiles in the presence of Cu(II),[22] and the use of a Cu(I)-catalyzed click reaction on the array requires treatment of the array with Cu(II) and then generation of the Cu(I) catalyst at the desired electrodes. Hence, for a click-reaction on the borate ester surface, background reactions dominate over the desired transformation. With this in mind, we hoped to develop a more general approach on the arrays that would allow for the tunable borate ester surface.

We report here that the Cu(II)-catalyzed addition of a thiol to a borate ester based surface on a microelectrode array occurs at a much more rapid rate than the corresponding reaction with an amine nucleophile. Similar reactions with alcohol nucleophiles occur at a rate roughly equal to that of the thiol. Hence, cysteine- and serine-containing peptides can be chemoselectively placed by selected electrodes in a microelectrode array without any need to protect the N terminus of the peptide or any need to further modify either the peptide or the surface. In addition, the extent of electrode coverage during the reactions can be controlled by the time allotted for the reaction, an observation that allows for variations in the surface coverage of the electrodes.

RESULTS AND DISCUSSION

Because of the overall utility of tunable borate ester surfaces on the array (made from the diblock copolymer shown in Figure 1),[21] they were employed for all efforts to explore reaction

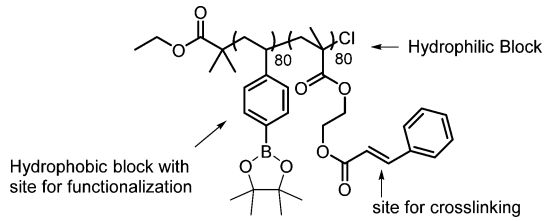


Figure 1. Diblock copolymer used to coat the arrays.

selectivity on the arrays. Nucleophiles were added to the borate ester polymer with the use of a Cu(II) Chan–Lam procedure.[22,23] The procedure is illustrated for a thiol nucleophile in Scheme 2. In the reaction, the array was treated with a catalytic amount of copper acetate and an excess of the thiol. This led to the formation of Cu(I) and a small amount of the dithiane. Selected electrodes in the array were then used as anodes in order to regenerate the Cu(II) catalyst and trigger the Chan–Lam coupling reaction at those specific sites. Any Cu(II) that migrates away from the electrodes selected for its generation was consumed by the excess thiol in solution. The level of confinement for the reaction was monitored with the use of a fluorescent label and can be seen in the image provided. As can be seen, an S pattern of electrodes was used for the Chan–Lam coupling. The reaction was a bit of a

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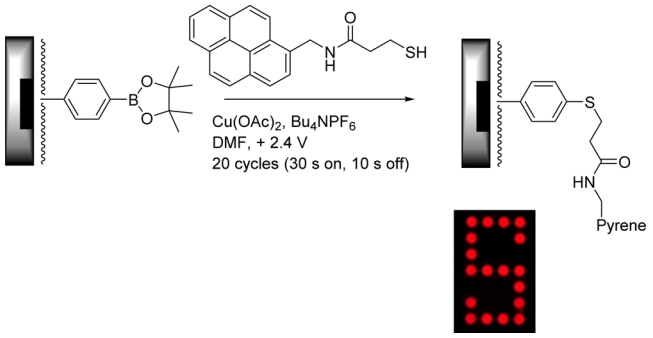
Scheme 2. Chan−Lam Coupling of a Thiol to a Borate Ester Coated Microelectrode Array



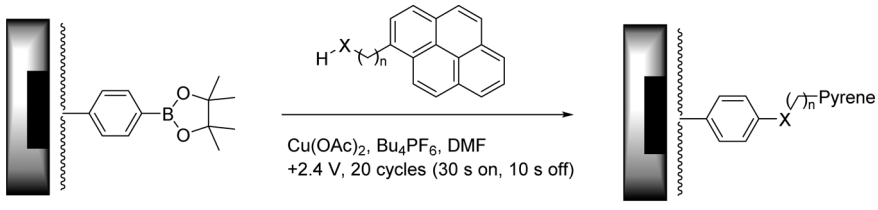
surprise in that it worked so well with the protected borate ester. We had anticipated a need to site-selectively deprotect the surface by the electrodes to be used to form the arylboronic acid species normally used as a substrate for the Chan−Lam coupling. The reaction with the protected surface was welcome because it avoided this step and simplified the overall procedure.

Similar reactions were conducted with commercially available amine and alcohol nucleophiles on the arrays (Scheme 3). The reactions were run in a fashion identical with the reaction shown in Scheme 2 except for the change in nucleophile. The reaction with the alcohol again led to selective placement of the molecule by the electrodes used for the reaction (image b). The reaction with the amine did not (image a). With the amine, very little reaction of any kind was observed. This result was not surprising. Chan−Lam coupling reactions with primary amine nucleophiles are more challenging than their thiol and alcohol counterparts[23] and often benefit from the use of stoichiometric copper or special ligands.[24,25] Their relatively slower reaction with the still protected borate ester surface is consistent with those observations. In addition, the amine reactions are more sensitive to acid. While the array reactions are undivided cells and base is generated at the cathode, it is possible that the region of the reaction next to the anode is acidic enough to inhibit the reaction with the amine.

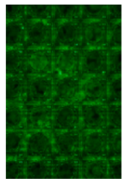
While these initial experiments might not have provided a completely fair comparison of the nucleophiles because of the varying chain lengths between the heteroatom and the pyrene group and the presence of the amide in the thiol substrate, they did provide the motivation for directly testing the chemoselectivity of amino acid placement reactions on the arrays. The experiments suggested that cysteine- and serine-based peptides could be placed onto a borate ester functionalized array without any need to protect the N terminus of the amino acid. This suggestion was tested with the use of cysteine and serine esters and amides as model substrates that contained either a thiol or alcohol nucleophile along with the unprotected N terminal amine. Initially, a fluorescently labeled cysteine was placed by a "C pattern" of electrodes in an array in order to establish the compatibility of the Chan−Lam procedure with the amino acid (Scheme 4). The coupling reaction worked exceptionally well, giving rise to an intense fluorescent pattern by the electrodes used for the generation of Cu(II). No fluorescence was observed anywhere on the array not used for the reaction, indicating that there was no random absorption of the cysteine onto the diblock-copolymer-coated surface.

Having established the utility of the Chan−Lam coupling reaction for placing the amino acid onto the array, attention was turned toward how the cysteine was placed on the array. To this end, the nature of the nucleophile (amine or thiol) participating in the addition reaction was probed with the use of two control experiments (Scheme 5). In the first, cysteine methyl ester was placed by an S pattern of electrodes in the microelectrode array. The array was then treated with a pyrene-labeled thiol in the presence of excess $Cu(OAc)_2$ by submerging the entire array in the reaction solution. In this way, any thiol on the surface of the array would be converted to a dithiane. In this event, no fluorescence of any kind was found on the electrodes used for the placement of the cysteine on the array. The remainder of the array did show some fluorescence due to a background Chan−Lam coupling reaction between the thiol and the borate ester surface in the presence of Cu(II). The fact that no fluorescence could be observed by the electrodes used for placement of the cysteine on the array indicated that there was neither any thiol nor any remaining borate ester present on the surface of the electrodes. The cysteine placement reaction had occurred in high yield, and it proceeded through the thiol nucleophile. The effectiveness of the strategy used for labeling free thiols on the surface of the array was tested by first placing 1,4-dithiobutane on the array in an S pattern and then treating the array with the fluorescent thiol and copper acetate. Formation of the dithiane at the electrodes used for the initial Chan−Lam coupling was clearly evident.

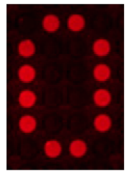
Scheme 3. Amine and Alcohol Nucleophiles and the Chan−Lam Coupling Reaction



a) n = 1, X = NH



b) n = 4, X = O



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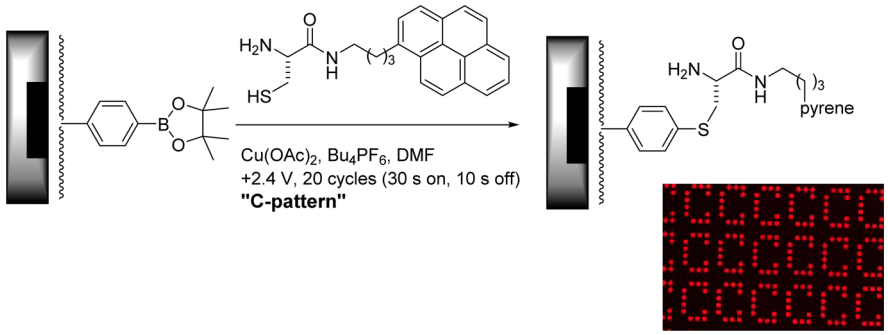
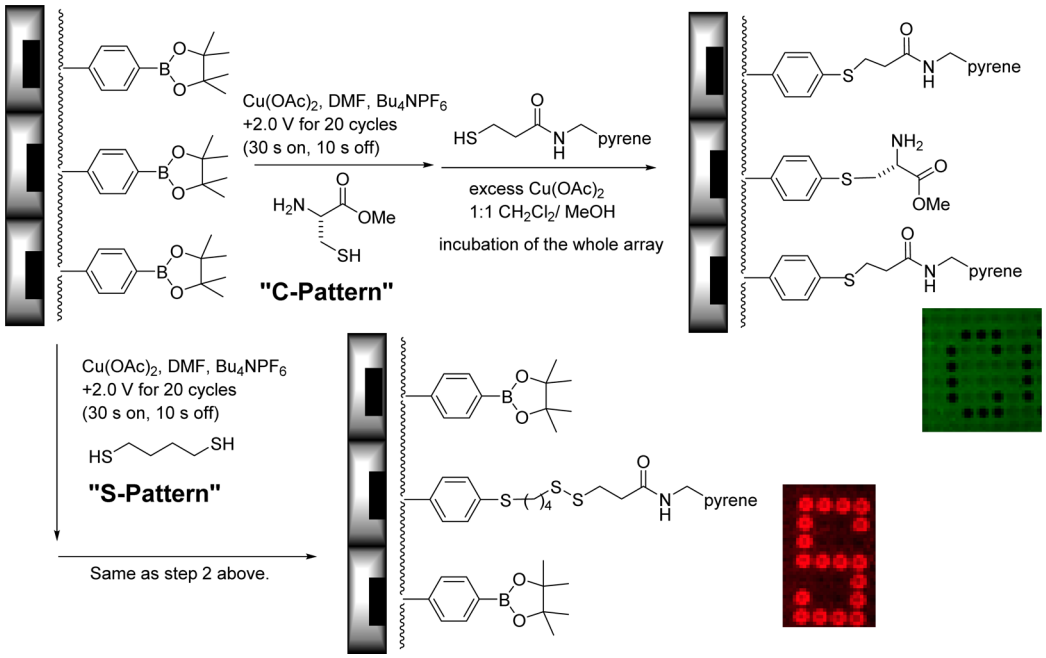
Scheme 4. Placement of Cysteine on an Array

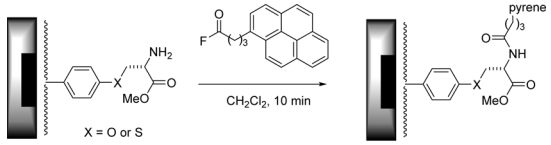


Scheme 5. Evidence for Placement of the Thiol on the Array in the Presence of an Amine



Of course, the discussion of the experiment illustrated in Scheme 5 assumes that the Chan–Lam coupling reaction placed the cysteine onto the array just as it did for the reaction illustrated in Scheme 4. Evidence supporting this assumption and illustrating that the amine was definitely on the surface was obtained by utilizing the amine in a coupling reaction (Scheme 6a). To this end, a cysteine-functionalized array (N pattern) was submerged in a solution containing a fluorescently labeled acid fluoride. The reaction led to the formation of an amide and the associated fluorescence at the electrodes used for the cysteine placement reaction. In addition, the reaction provided its own "control study". The entire surface of the array was treated with the acid fluoride, but the coupling reaction occurred only at electrodes that had been used for the Chan–Lam placement of the cysteine on the array. Hence, the fluorescence at those electrodes was not due to any background reaction between the acid fluoride and the polymer. It was a direct result of the placement reaction and the presence of the cysteine N-terminal amine.

Scheme 6. Availability of the Amines on the Surface of the Array



a) For cysteine (X = S) b) For serine (X = O)

In the related reaction with serine methyl ester, the amino acid placement onto the array (N pattern) proceeded through the alcohol nucleophile (Scheme 6b). We have shown that alcohols on the surface of the electrodes in an array can be imaged by oxidizing them to carbonyls with the use of TEMPO and then forming an imine with a fluorescently labeled amine.[26] A similar experiment with the serine methyl ester functionalized array showed no fluorescence by any of the electrodes in the array. However, treatment of the array with the fluorescently labeled acid fluoride again led to fluorescence by the electrodes used, showing the availability of a free amine on the surface.

When all three nucleophiles were available for the Chan–Lam coupling reactions, a mixture of products was obtained leading to the presence of thiol, amine, and alcohol groups all

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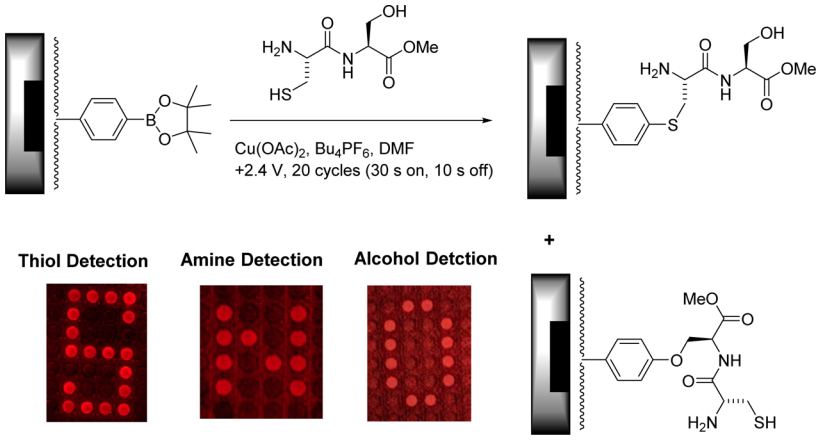
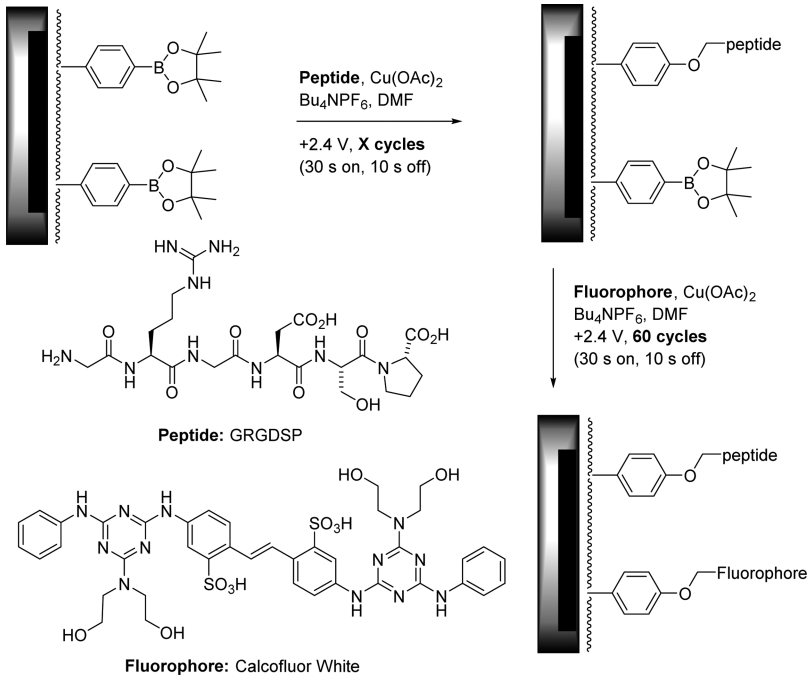
Scheme 7. Placement of a Cysteine−Serine Dimer onto a Microelectrode Array



Scheme 8. Controlling Surface Concentration



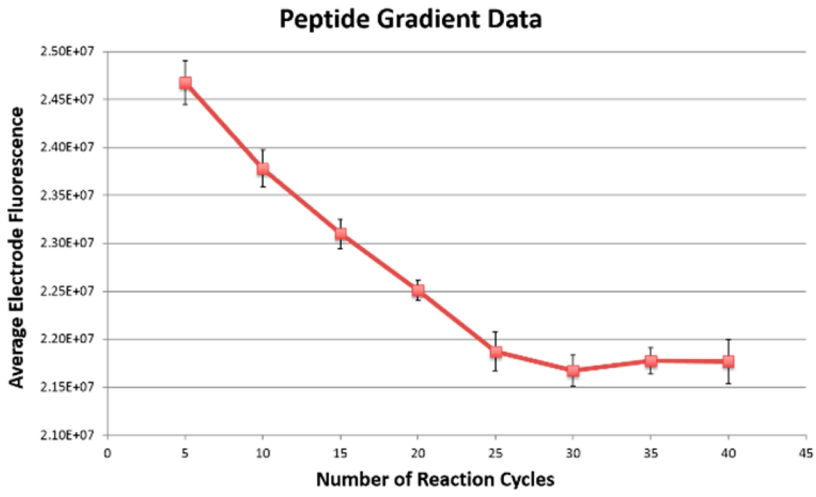


Figure 2. Peptide placement on the arrays.

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being observed on the surface of the electrode used for the coupling reaction (Scheme 7). The methods employed for detecting the presence of the groups on the surface were the same as those described above. Note that, in this case, the method for detecting the alcohol[26] worked just fine for the serine OH. The result of the experiment was consistent with the thiol and alcohol placement reactions being competitive with each other. The placement of either on the surface led to the presence of an amine. When the thiol served as the nucleophile for the reaction, a free alcohol was present on the array, while the addition of the alcohol to the surface left a free thiol on the surface of the electrode. It is clear that, in order to place a peptide on a microelectrode array with a thiol nucleophile, one will first need to protect any free alcohol groups.

Another important question about the Chan−Lam placement reactions described above is whether they can be used to control the quantity of material placed by the electrodes in an array. The ability to do so would allow for the synthesis of arrays with a concentration gradient of molecules by the electrodes. To answer this question, a peptide containing a serine group was placed onto an array along with the fluorescent dye calcofluor white (Scheme 8). An alcohol nucleophile was selected for the peptide placement reaction because the fluorescent dye contained an alcohol nucleophile. The Chan−Lam coupling with the peptide was conducted for varying time periods by controlling the number of electrolysis cycles used for the reaction. Each cycle set the electrode on for a period of 30 s and then off again for a period of 10 s. The electrodes were cycled in this manner in order to slow the generation of Cu(II) so that confinement of the reaction could be optimized. After the peptide was placed on the array, the fluorescent dye was added to the same electrodes using a reaction that was allowed to run for 60 cycles. The amount of peptide placed on the surface was then determined by measuring the amount of fluorescence on the electrode following the second reaction.[27]

As can be seen in Figure 2, the amount of fluorescent dye placed on the electrode in the second step decreased in a linear fashion as the length of time for the initial peptide placement reaction increased up to the point of 25 cycles for the first reaction. Each experiment was conducted at three sites on the array, and the error bars reflect the spread in the data at those three sites. After 25 cycles, the amount of peptide placed on the array leveled off. At that point the initial peptide placement reaction had proceeded to completion.

The experiment made two things clear. First, it is possible to control the relative amount of a peptide placed on the array in a predictable fashion by controlling the reaction time. Second, the narrow error bars in the plot show that the relative amounts of peptide placed at the different sites selected for each reaction were roughly equal. This second point is particularly important because it sets the stage for a second, potentially more useful method for generating arrays with concentration gradients. That method would utilize the same time for every coupling reaction but vary the amount of a desired molecule for study relative to an inert molecule in the coupling reaction. Such an approach can only work if the total amount of material placed by each electrode selected for the reaction is the same. The data in Figure 1 indicate that this is the case. Both the total amount of peptide and the rate at which that peptide is placed on the array remain constant across the array.

CONCLUSION

We have found that the Chan−Lam coupling reaction among thiol, alcohol, and amine nucleophiles with a borate ester coated array favors placement of the thiol and alcohol nucleophiles on the surface over the amine. Hence, peptides containing cysteine and serine amino acids can be placed on an array without any need to protect the N terminus of the molecule. However, the thiol and alcohol nucleophiles function in the Chan−Lam reaction in roughly equal capacity. Selective placement of one group over the other requires protection of the group not wanted for the coupling reaction. Finally, we have shown that the relative amount of a peptide placed on an array can be controlled by the reaction time used for the Chan−Lam coupling reaction. This paves the way for studying the effects of ligand surface coverage on subsequent electrochemical signaling studies.

EXPERIMENTAL SECTION

General Information. All glassware was flame-dried prior to use, and all reactions were conducted under an argon atmosphere unless otherwise noted. Tetrahydrofuran was distilled from sodium benzophenone ketyl, and dichloromethane was distilled from calcium hydride. All other reagents and solvents were used as received from commercial sources unless otherwise noted. Chemical shifts are reported downfield from TMS. NMR yields were obtained using coumarin as an internal standard. High-resolution mass spectra (HRMS) were obtained using electrospray ionization (ESI) with Q-TOF detection. Infrared spectra were obtained using an FT-IR spectrophotometer.

Fluorescence microscopy was carried out with a Nikon Eclipse E200 microscope connected to a Boyce Scientific M-100 burner and a Nikon D5000 camera. Optical filters used were as follows: CFW-BP01-Clinical-000 (Semrock) filter cube excitation 380−395 nm, emission 420−470 nm, ET-GFP (FITC/Cy2) (Chroma) filter cube excitation 450−490 nm, emission 500−550 nm, and TxRed-A-Basic-000 (Semrock) filter cube excitation 540−580 nm, emission 590−670 nm.

The measurement and analysis of the fluorescence intensities were performed using the bioimaging software Icy, version 1.6.1.1 (http://icy.bioimageanalysis.org). Six equiradial circular segments encompassing one functionalized electrode each were selected, and the average intensity value was computed. A baseline fluorescence was determined by using six equally sized segments that each contained an unfunctionalized electrode.

Sample Procedure for Spin-Coating Arrays with the Block Copolymer. The microelectrode arrays were coated with a Model WS-400B-6NPP/LITE spin-coater. The chip was inserted into a socket in the spinner and adjusted to be horizontal, and then three drops of 0.03 g/mL PCEMA-*b*-pBSt solution (4/1.5 DMF/THF) were added onto the chip in order to cover the entire electrode area. The chip was then spun at 1000 rpm for 40 s. The coating was allowed to dry for 15 min and subjected to irradiation using a 100 W Hg lamp for 20 min before use.

General Procedure for Array-Based Chan−Lam Coupling Reactions. The peptide GRGDSP (10.0 mg) and 50.0 mg of tetrabutylammonium hexafluorophosphate were dissolved in 1.0 mL of DMF. To this solution was added 50.0 μL of a 25 mM copper(II) acetate in water solution. The contents were mixed, and then 120 μL of this solution was added to the array. The array was placed into an ElectraSense reader, and all 12544 electrodes were selected and used as anodes. A potential of +2.4 V relative to the auxiliary electrode was used to pulse the selected electrodes for 20 cycles of 30 s on and 10 s off. After completion of the reaction, the array was washed extensively with 95% ethanol.

General Procedure for Conducting Reactions on the Entire Surface of the Array. Reactions on the entire surface of the array were conducted by placing the array on the bottom of a Petri dish with the electrodes facing upward. A 10 mL portion of the appropriate

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reaction solution (described below) was added to the Petri dish in order to cover the entire surface of the array. Once the reaction was complete, the array was washed with methanol, dried, and analyzed with the use of fluorescence microscopy.

Thiol Detection Solution. The reaction solution for detecting thiols on the surface of an array was made by dissolving 50 mg of 3-mercapto-*N*-(pyren-1-ylmethyl)propanamide[15] and 100 mg of $Cu(OAc)_2$ in a mixture of 5 mL of dichloromethane and 5 mL of methanol.

Amine Detection Solution. The reaction solution for detecting amines on the surface of an array was made by dissolving 50 mg of 1-pyrenebutyryl fluoride in 10 mL of dichloromethane.

Alcohol Detection. The method for detecting alcohols on the surface of an array has been reported previously.[26]

Synthesis of Cysteine-NH-butylpyrene. *Boc-cysteine(Trt)-NH-butylpyrene.* In a flame-dried 25 mL round-bottom flask, 1.07 g (2.38 mmol) of Boc-Cysteine(Trt)-OH, 488 mg of *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), 330 mg of ethyl cyano(hydroxyimino)acetate (Oxyma), and 590 mg (2.17 mmol) of pyrenebutylamine (Aldrich) were dissolved in 12 mL of anhydrous DMF. *N*-Methylmorpholine (0.85 mL) was added to the solution, and the reaction mixture was stirred overnight. The reaction solution was quenched with brine and extracted with ethyl acetate. Organic layers were dried, concentrated in vacuo, and purified via column chromatography using 1/1 ethyl acetate/hexanes. The coupling product was obtained in a 64% yield over two steps (998 mg, MW = 718.96 g/mol). ^{1}H NMR (CD_2Cl_2/300 MHz): δ 8.23 (d, J = 9.3 Hz, 1H), 8.16 (d, J = 7.3 Hz, 2H), 8.04 (m, 5H), 7.82 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 7.2 Hz, 6H), 7.27−7.16 (m, 11H), 6.00−5.91 (m, 1H), 4.77−4.69 (m, 1H), 3.85−3.77 (m, 1H), 3.33 (t, J = 7.7 Hz, 2H), 3.26 (m, 2H), 2.77−2.67 (dd, J = 12.6, 7.0 Hz, 1H), 2.49 (dd, J = 12.7, 4.8 Hz, 1H), 1.90−1.80 (m, 2H), 1.63 (dt, J = 15.1, 7.0 Hz, 2H), 1.35 (s, 9H) ppm. ^{13}C NMR (CD_2Cl_2/75 MHz): δ 170.5, 155.5, 144.6, 136.4, 131.5, 130.9, 129.8, 129.7, 128.6, 128.1, 127.6, 127.33, 127.28, 126.9, 126.7, 125.9, 125.10, 125.07, 124.93, 124.88, 124.8, 123.4, 80.1, 67.2, 39.4, 34.2, 33.0, 29.5, 28.9, 28.4 ppm. IR (KBr): 3561, 3428, 3047, 2930, 2253, 2123, 1716, 1680, 1665, 1555, 1502, 1365, 1247, 1166 cm^{-1}. HRMS (ESI/TOF-Q): m/z [M + Na]$^+$ calcd for $C_{47}H_{46}N_2O_3SNa$ 741.3129, found 741.3121.

Cysteine-NH-butylpyrene. In a flame-dried 25 mL round-bottom flask, 970 mg (1.35 mmol) of Boc-Cys(Trt)-NH-butylpyrene was dissolved in 5 mL of dichloromethane. Triethylsilane (1.0 mL) and 0.25 mL of trifluoroacetic acid were then placed in the flask, and the reaction mixture was stirred at room temperature until the starting material was consumed, as evidenced by TLC. The resulting solution was quenched with saturated sodium bicarbonate solution and the crude reaction mixture extracted with dichloromethane. The organic layers were dried and concentrated in vacuo, and the product was purified via column chromatography. The column was initially eluted with 1/1 ethyl acetate/hexanes to remove the faster-moving impurities before switching to 1/1 CH_2Cl_2/MeOH to acquire the desired product in a 90% yield (457 mg; MW = 376.52 g/mol). ^{1}H NMR (CD_2Cl_2/300 MHz): δ 8.23−7.91 (m, 8H), 7.81 (m, 1H), 7.51−7.44 (m, 1H), 3.62−3.55 (m, 1H), 3.36−3.20 (m, 5H), 3.17 (d, J = 4.2 Hz, 1H), 2.69 (dt, J = 13.9, 7.1 Hz, 1H), 1.83 (dt, J = 14.6, 7.4 Hz, 4H), 1.62 ppm (m, 2H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 173.1, 136.4, 131.3, 130.8, 129.7, 128.5, 127.4, 127.2, 126.6, 125.8, 124.98, 124.92, 124.84, 124.75, 124.68, 123.3, 53.8, 43.8, 39.10, 39.06, 33.0, 29.4, 29.0 ppm. IR (KBr): 3284, 3044, 2935, 2863, 2359, 1918, 1651, 1531, 1437, 1181, 1120, 845 cm^{-1}. HRMS (ESI/TOF-Q): m/z [M + Na]$^+$ calcd for $C_{23}H_{24}N_2OSNa$ 399.1509, found 399.1507.

Synthesis of 4-Pyrenebutyryl Fluoride. In a flame-dried 25 mL round-bottom flask, 288 mg (1 mmol) of 1-pyrenebutyric acid was dissolved in 6.8 mL of anhydrous dichloromethane followed by the addition of 0.1 mL of pyridine. Separately, 393 mg (1.2 mmol) of fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate (TFFH) was dissolved in 4.5 mL of DCM. The TFFH solution was then added dropwise to the butyric acid solution, and the mixture was stirred for 3 h. Upon completion, the reaction was quenched with 10 mL of ice water and 10 mL of DCM. The organic layer was removed and washed with ice water (2 × 10 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude acid fluoride was used without further purification. The ^{1}H NMR of the crude mixture is provided in the Supporting Information.

Synthesis of the Cysteine−Serine Methyl Ester Dipeptide. *Boc-Cys(Trt)-Ser(tBu)-OMe.* In a flame-dried 25 mL round-bottom flask, 927 mg (2.0 mmol) of Boc-Cys(Trt)-OH, 420 mg of *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC), and 284 mg of ethyl cyano(hydroxyimino)acetate (Oxyma) were dissolved in 6 mL of anhydrous DMF. To this mixture was added 0.75 mL of *N*-methylmorpholine followed by the addition of a 2 mL DMF solution containing 423 mg (2 mmol) of H-Ser(tBu)-OMe (HCl). The resulting solution was stirred overnight. Upon completion of the reaction, the DMF was removed in vacuo, and the crude mixture was purified by column chromatography through silica gel using 3/1 hexanes/ethyl acetate as the eluent. The desired coupling product was obtained in a 74% yield (918 mg; MW = 620.8 g/mol). ^{1}H NMR (300 MHz; $CDCl_3$): δ 7.42 (d, J = 5.2 Hz, 6H), 7.31−7.19 (m, 9H), 6.71 (d, J = 8.2 Hz, 1H), 4.81−4.77 (m, 1H), 4.61 (dq, J = 7.9, 3.9 Hz, 1H), 3.93−3.89 (m, 1H), 3.77 (dt, J = 9.0, 2.4 Hz, 1H), 3.69 (s, 3H), 3.50 (ddd, J = 9.3, 6.8, 2.9 Hz, 1H), 2.76−2.70 (m, 1H), 2.56 (dt, J = 12.3, 6.0 Hz, 1H), 1.42 (s, 9H), 1.09 ppm (s, 9H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 170.37, 170.23, 144.4, 129.6, 128.0, 126.8, 73.3, 67.1, 61.8, 53.4, 53.0, 52.2, 34.0, 28.3, 27.27, 27.22 ppm. IR (KBr): 3500, 3415, 3056, 2976, 1747, 1669, 1508, 1489, 1363, 1245, 1167, 1099, 1020 cm^{-1}. HRMS (ESI/TOF-Q): m/z [M + Na]$^+$ calcd for $C_{24}H_{44}N_2O_6SNa$ 643.2820, found 643.2800.

Cys-Ser-OMe. In a flame-dried 25 mL round-bottom flask, 745 mg (1.20 mmol) of Boc-Cys(Trt)-Ser(tBu)-OMe was dissolved in 5 mL of dichloromethane. A 1.0 mL portion of triethylsilane and 0.50 mL of trifluoroacetic acid were placed in the reaction flask. The deprotection was monitored via TLC. Upon completion of the reaction, the solution was quenched with saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layers were dried and concentrated in vacuo. The crude mixture was purified via column chromatography using 3/1 CH_2Cl_2/acetone to acquire the desired product in a 90% yield (240 mg; MW = 222.26 g/mol). The spectral data were consistent with previously published data.[28]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02656.

NMR data for all new compounds as evidence for compound purity (PDF)

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Notes

The authors declare no competing financial interest.

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