J|A|C|S ARTICLES

Published on Web 11/02/2010

Building Addressable Libraries: Site-Selective Use of Pd(0) Catalysts on Microelectrode Arrays

Libo Hu,[†] Melissae Stuart,[†] Jun Tian,[†] Karl Maurer,[‡,§] and Kevin D. Moeller*[,†]

Department of Chemistry, Washington University, St. Louis, Missouri 63130, United States, and CombiMatrix Corporation, 6500 Harbor Heights Parkway, Suite 301, Mukilteo, Washington 98275, United States

Received August 18, 2010; E-mail: moeller@wustl.edu

Abstract: Site-selective Pd(0)-catalyzed reactions have been developed to functionalize a microelectrode array. Heck, Suzuki, and allylation reactions have all been accomplished. The reactions are compatible with both 1K and 12K arrays and work best when a nonsugar porous reaction layer is used. Suzuki reactions are faster than the Heck reactions and thus require more careful control of the reactions in order to maintain confinement. The allylation reaction requires a different confining agent than the Heck and Suzuki reactions but can be accomplished nicely with quinone as an oxidant for Pd(0).

Introduction

Microelectrode arrays hold great promise as analytical platforms for detecting ligand−receptor interactions in "real time".[2−5] Capitalizing on this promise requires tools for placing or constructing molecules proximal to the microelectrodes in the arrays. Electrochemical impedance experiments can then be used to monitor the molecules (Figure 1).[5]

The impedance experiments work by cycling a redox couple between oxidation at the array and reduction at a remote electrode. The current for this process is measured at each microelectrode in the array. When a receptor binds a molecule on the array, a drop-off in this current is recorded at the associated microelectrode. For example, when a receptor that recognizes M_1 binds to M_1 (Figure 1), the current at the corresponding microelectrode drops relative to the current at the neighboring microelectrode. For this to work, the molecules being probed must be selectively located only next to the microelectrode being used to monitor them. If any M_1 is located next to the microelectrode used to monitor M_2, then differentiating the binding of M_1 and M_2 to the receptor becomes impossible.

Hence, to use the arrays as analytical tools we need to develop "site-selective" reactions that allow us to first functionalize and then conduct syntheses next to any single microelectrode or pattern of microelectrodes in an array. These reactions must be carefully confined to the region of the array immediately surrounding a selected electrode without any migration of reagents to the neighboring electrodes, even when the array has a density of 12 544 microelectrodes/cm^2. Given these constraints, traditional synthetic protocols become impossible. One cannot simply buy a reagent and then add it to the surface of an array next to only one microelectrode. Instead, strategies must be developed for making reagents on the arrays proximal to selected microelectrodes and then confining those reagents to those, and only those, locations. To do this, one needs to take advantage of the microelectrodes themselves for initiating the synthetic reactions.

With this in mind, we have begun moving traditional synthetic methods to the microelectrode array platform by taking advantage of a competitive reaction strategy.[6−10] To this end, the microelectrodes on the array are used to generate a reactive chemical reagent or catalyst. At the same time, a substance (typically referred to as a confining agent) is added to the solution above the array in order to destroy whatever reagent or catalyst is being generated. By balancing the rate at which the reagent or catalyst is generated relative to the rate at which it is consumed in solution, the distance the reagent or catalyst can migrate away from the electrode where it is generated can be controlled. Different molecules are then placed at different

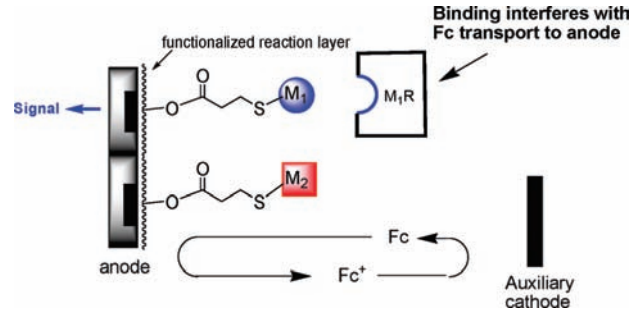


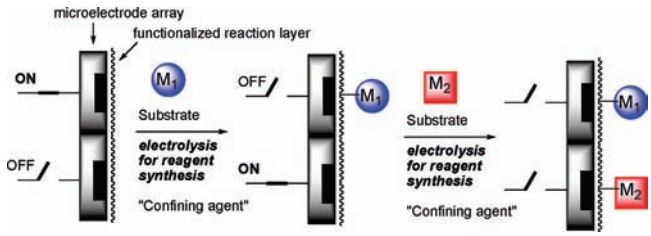
Figure 1. Plan for signaling on a microelectrode array.

[†] Washington University.
[‡] CombiMatrix Corporation.
[§] Current address: CustomArray Inc., 6500 Harbour Heights Parkway, Suite 202, Mukilteo, WA 98290.

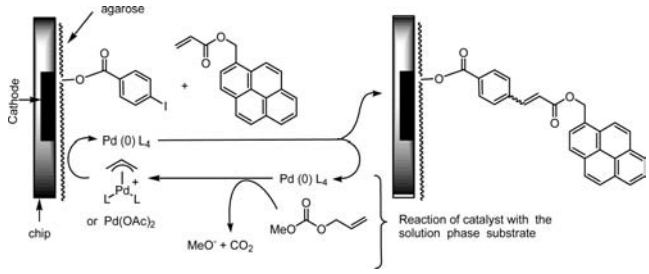
(1) Note deleted in proof.
(2) For a description of the chips used here, see: Dill, K.; Montgomery, D. D.; Wang, W.; Tsai, J. C. *Anal. Chim. Acta* **2001**, *444*, 69. 1K chips: electrode diameter = 92 μm; distance between the Pt electrodes (rectangular cells) = 245.3 and 337.3 μm. 12K slide: diameter = 44 μm; distance between the Pt electrodes (square cells) = 33 μm.
(3) Microelectrode arrays can be purchased from CustomArray, Inc.
(4) For alternative approaches, see: (a) Sullivan, M. G.; Utomo, H.; Fagan, P. J.; Ward, M. D. *Anal. Chem.* **1999**, *71*, 4369. (b) Zhang, S.; Zhao, H.; John, R. *Anal. Chim. Acta* **2000**, *421*, 175. (c) Hintsche, R.; Albers, J.; Bernt, H.; Eder, A. *Electroanalysis* **2000**, *12*, 660.
(5) For real time signaling on an array, see: (a) Tesfu, E.; Roth, K.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2006**, *8*, 709. (b) Stuart, M.; Maurer, K.; Moeller, K. D. *Bioconjugate Chem.* **2008**, *19*, 1514.

10.1021/ja107490t

Scheme 1



Scheme 2



locations on the array by utilizing a new set of microelectrodes for generating the desired chemical reagent (Scheme 1). This is best understood with an example.

Due to the tremendous synthetic versatility of Pd(0) catalysts, we have been working to develop them as tools for synthesis on the arrays.[11] Particularly attractive is the potential that Heck and Suzuki-type reactions hold as strategies for coupling new molecules to the surface of an array. For example, consider the chemistry highlighted in Scheme 2.[11a] In this experiment, the surface of the array was coated with agarose. The agarose is used as a porous reaction layer to attach molecules to the surface of the array. The layer needs to be porous so that reagents can reach the electrodes below. 4-Iodobenzoic acid was then placed on the agarose next to every microelectrode in the array using a base-catalyzed esterification reaction.[7,9−11] $Pd(OAc)_2$ and allyl methyl carbonate were placed in the solution above the array (a DMF/MeCN/water mixture containing triphenylphosphine as a ligand for the Pd and tetra-*n*-butylammonium bromide as an electrolyte) along with a pyrene-labeled acrylated derivative for the Heck reaction. Selected microelectrodes in the array were then used as cathodes to reduce $Pd(OAc)_2$ and form a Pd(0) catalyst for effecting the Heck reaction. The allyl methyl carbonate "confining agent" was placed in solution to scavenge the Pd(0) catalyst being generated by reacting with it to form a π-allyl−Pd(II) species. In so doing, the allyl methyl carbonate prevented the Pd(0) catalyst from migrating to microelectrodes on the array that were not used to form the catalyst. The success

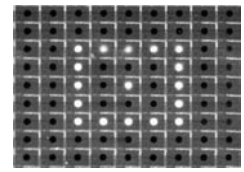


Figure 2. A "confined" Heck reaction on a 1K array.

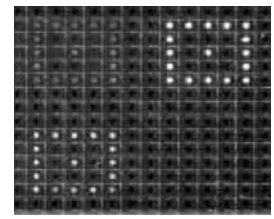


Figure 3. Fluorescence image of Heck reaction: −2.4 V, time on 0.5 s, time off 0.1 s, allyl methyl carbonate confined. Lower right: methyl acrylate substrate for 6 min, a blank with no fluorescence for comparison. Upper right: 1-pyrenemethyl acrylate, reaction running for 3 min. Lower left: 1-pyrenemethyl acrylate, reaction running for 6 min. Upper left: 1-pyrenemethyl acrylate, reaction running for 12 min.

of this strategy can be seen in Figure 2. The figure shows a 1K array (1024 microelectrodes/cm^2) with a dot in a box pattern of microelectrodes used as cathodes (−2.4 V relative to a Pt counter electrode for 300 cycles of 0.5 s on and 0.1 s off) to accomplish the reaction illustrated in Scheme 1. Following this reaction, a different pattern could be placed on the same array by simply repeating the reaction while using a new set of electrodes for the reduction of Pd(II).

Interestingly, the Heck reactions worked beautifully with either the aryl iodide or the acrylate derivative on the surface of the array. The "inverse" Heck reaction (acrylate on the surface) worked in spite of the arylpalladium intermediate for the reaction being generated in solution where it would be free to migrate.[12] Apparently, the Heck reaction on the surface is fast enough to prevent the migration.

Although the reactions worked well and confinement was easy to obtain, there was an underlying problem with reactions requiring longer reaction times. As the reaction time was increased, the intensity of fluorescence from the selected microelectrodes decreased (Figure 3). In this image, an array is shown with four experiments having been run on its surface. The first is shown in the lower right portion of the array. It utilized methyl acrylate instead of the pyrene-derived substrate for the Heck reaction and served as a control showing no fluorescence. The second experiment is shown in the upper right. This experiment was identical to the one illustrated in Figure 2. The reduction was run for 300 cycles. In the third experiment, shown in the lower left, the reduction was run for 600 cycles. In the fourth, shown in the upper left, the reduction was run for 1200 cycles. Clearly, the intensity of the fluorescence decreased with increasing reaction time.

At the time, we wondered if the methoxide generated from reaction of the confining agent with the Pd(0) catalyst was cleaving either the ester linkage between the molecule on the surface of the array and the agarose polymer or the acrylate ester.

These initial findings left us with three questions: Were the conditions developed for initiating Pd(0)-catalyzed reactions general? Did all Pd(0)-catalyzed reactions have the problem

(6) For Pd(II) reactions, see: (a) Tesfu, E.; Roth, K.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2006**, *8*, 709. (b) Tesfu, E.; Maurer, K.; Ragsdale, S. R.; Moeller, K. D. *J. Am. Chem. Soc.* **2004**, *126*, 6212. (c) Tesfu, E.; Maurer, K.; McShae, A.; Moeller, K. D. *J. Am. Chem. Soc.* **2006**, *128*, 70.

(7) For examples of the site-selective generation of base, see ref 4b and the work of Maurer et al. [Maurer, K.; McShea, A.; Strathmann, M.; Dill, K. *J. Comb. Chem.* **2005**, *7*, 637].

(8) For the site-selective generation of acid, see: Kesselring, D.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2008**, *10*, 2501.

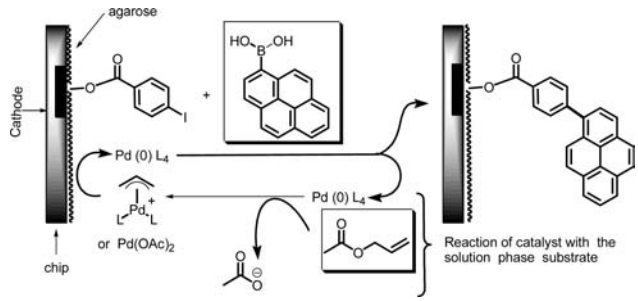
(9) For the use of CAN in a site-selective fashion, see: Kesselring, D.; Maurer, K.; Moeller, K. D. *J. Am. Chem. Soc.* **2008**, *130*, 11290.

(10) For the site-selective use of Sc(III), see: Bi, B.; Maurer, K.; Moeller, K. D. *Angew. Chem., Int. Ed.* **2009**, *48*, 5872.

(11) For preliminary accounts of this work, see: (a) Tian, J.; Maurer, K.; Tesfu, E.; Moeller, K. D. *J. Am. Chem. Soc.* **2005**, *127*, 1392. (b) Hu, L.; Maurer, K.; Moeller, K. D. *Org. Lett.* **2009**, *11*, 1273.

(12) Tang, F.; Chen, C.; Moeller, K. D. *Synthesis* **2007**, 3411.

Scheme 3



associated with longer reaction times? How could the decrease of material on the surface of the array with greater reaction time be stopped?

A Test for Generality: The Suzuki Reaction

The Suzuki reaction offers a potentially powerful strategy for placing molecules onto arrays. Hence, it was selected as a test for examining the generality of site-selective Pd(0) catalyst formation (Scheme 3).[11b] To this end, 4-iodobenzoic acid was again placed proximal to every microelectrode in an array. Two changes to the previously studied Heck reaction were made. First, the acrylate substrate for the Heck reaction was replaced with a pyrenylboronic acid nucleophile. Second, in an attempt to avoid any complications with the generation of methoxide during the reaction, the allyl methyl carbonate was replaced with allyl acetate as the confining agent. Allyl acetate reacts with Pd(0) to generate the π-allylpalladium(II) species and acetate. The result would be a significantly less basic solution than when the carbonate is used. The electrochemical part of the reaction was kept identical to the earlier Heck reaction with the selected electrodes (a checkerboard pattern) held at -2.4 V vs the remote Pt electrode for 0.5 s followed by 0.1 s off. This was continued for 300 cycles. The image generated is shown in Figure 4a. The checkerboard pattern can be clearly seen, but the confinement of the reaction was not perfect. Weaker fluorescent spots can be observed by the microelectrodes not utilized for the reaction. This loss of confinement is consistent with the Suzuki reaction being significantly faster than the Heck reaction. To address this issue, either the rate of Pd(0) catalyst generation at the electrodes needs to be decreased or the rate of catalyst destruction in solution needs to be increased. In this case, the former approach was chosen. The potential at the selected microelectrodes was reduced to -1.7 V, thereby reducing the current flow and the rate at which Pd(0) was generated. This change led to complete confinement of the reaction to the selected microelectrodes (Figure 4b).

The Suzuki reaction could also be confined nicely with air as the solution-phase oxidant. However, since the oxidation of Pd(0) with air is slower than the reaction between Pd(0) and

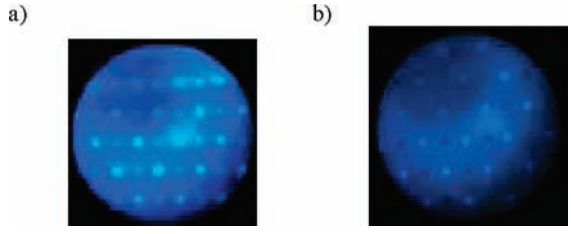


Figure 4. Fluorescence image of a site-selective Suzuki reaction: (a) checkerboard pattern run at -2.4 V vs a remote Pt electrode and (b) checkerboard pattern run at -1.7 V.

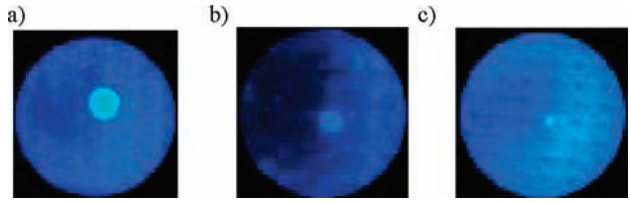


Figure 5. Fluorescence image of air-confined Suzuki reaction run at (a) -2.4 V, (b) -1.7 V, and (c) -1.4 V relative to a remote Pt electrode.

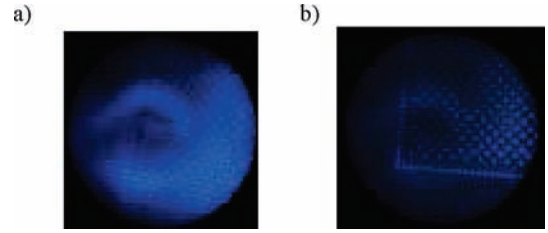


Figure 6. Fluorescence image of the site-selective Suzuki reaction on a 12K chip: (a) checkerboard pattern run with 1K conditions and (b) checkerboard pattern run with double the amount of confining reagent.

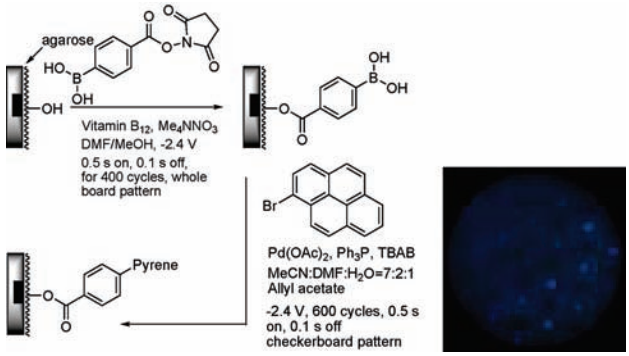
allyl acetate, the rate at which Pd(0) was generated had to be reduced even further. In the experiment illustrated in Figure 5, the Suzuki reaction was run at a single microelectrode in an array. Air was bubbled through the reaction mixture prior to the electrolysis. As can be seen in Figure 5a, when the reaction was run at -2.4 V relative to the remote Pt electrode, confinement was completely lost. As the current was reduced and the rate of Pd(0) generation decreased, confinement was regained. When the voltage at the selected microelectrode was set at -1.4 V, the reaction was nicely confined to the single electrode being used.

Confinement of the Suzuki reaction could also be gained by increasing the concentration of the confining agent. A nice example of this approach is illustrated in Figure 6. In this experiment, a 12K array (12 544 microelectrodes/cm^2) was used. Initially, the experiment was run in a fashion identical to that used successfully on the 1K array with allyl acetate as the confining agent (Figure 6a). In other words, the reaction was run at a voltage of -1.7 V vs the remote Pt electrode. The pattern selected for the electrolysis was a checkerboard inside of a box. Although the pattern can be seen on the right-hand side of the image, the reaction was not confined to the selected electrodes. To bring the reaction back into confinement (Figure 6b), the amount of allyl acetate was doubled from a concentration of 0.54 M for the experiment illustrated in Figure 6a to 1.08 M for the experiment illustrated in Figure 6b.

Both of the previous examples illustrate the nature of the competition that leads to site selectivity on the arrays. Every site-selective reaction on a microelectrode array involves this balancing of the rate at which a reagent or catalyst is generated at the electrodes with the rate at which it is destroyed in the solution above the array.

An inverse-Suzuki reaction having the nucleophile on the surface of the array and the aryl bromide in solution could also be confined to selected microelectrodes in an array (Scheme 4). To this end, a phenylboronic acid was placed next to each microelectrode in a 1K array. This was accomplished by using a base-catalyzed esterification reaction as illustrated.[5b,9−11] Once the boronic acid was in place, the array was treated with a solution containing bromopyrene and $Pd(OAc)_2$. Allyl acetate was used as the confining agent. A checkerboard pattern of

Scheme 4



microelectrodes was then selected as cathodes for reducing the Pd(II) species and generating the catalyst. The reaction was confined to the selected electrodes, even when the microelectrodes were held at −2.4 V relative to the remote Pt electrode. In this case, the reaction on the surface of the array was fast enough, relative to migration of the pyrenyl Pd(II) species away from the selected electrode, to allow confinement even with the more rapid generation of Pd(0).

With the Suzuki reaction in place, we utilized it to probe the generality of observation made with the Heck reaction concerning the relationship between spot intensity and reaction time. Would extended reaction times also lead to a decrease in the intensity of fluorescence resulting from the Suzuki reaction? To address this question, the reaction outlined in Scheme 2 was repeated at three different microelectrodes on a 1K array, varying the reaction time at each of the sites (Figure 7). Following the reactions, the amount of fluorescence relative to background was measured for each site.

The setup for the reactions was identical. The array was coated with agarose, 4-iodobenzoic acid was placed by each of the microelectrodes on the array, a voltage of −1.7 V vs the remote Pt electrode was applied to each of the selected electrodes for 0.5 s followed by 0.1 s with the electrode turned off, and allyl acetate was used as the confining agent. The reactions at the three different microelectrodes were run for 200 (2 min), 400 (4 min), and 600 (6 min) cycles, respectively. After 600 cycles, the reaction began to lose confinement, a very curious observation that initially defied explanation. From the experiment, it was clear that the Suzuki reactions were very fast and approach saturation of the surface after only 6 min. During the time of the experiment before loss of confinement, there did not appear to be a loss in fluorescence at the reaction sites. But how did the reaction lose confinement? With a large excess of confining agent being used, the rate of Pd(0) generation at the electrode relative to the rate of Pd(0) destruction by the confining agent in solution should not vary significantly as the reaction progressed.

Spot	Relative intensity*
200	100 ± 8
400	187 ± 11
600	204 ± 8

Figure 7. Fluorescence image of Suzuki reaction: −1.7 V, time on 0.5 s, time off 0.1 s for 200, 400, 600 cycles, allyl acetate confined. Lower left: reaction running for 2 min. Lower right: reaction running for 4 min. Upper middle: reaction running for 6 min. *Relative intensities are normalized to a spot obtained with 200 cycles.

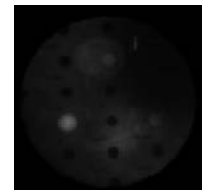
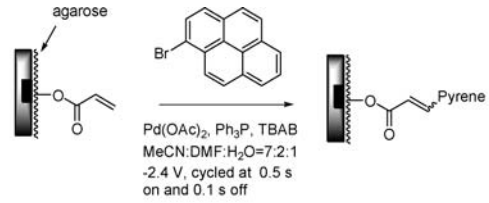
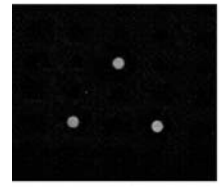


Figure 8. Fluorescence image of Heck reaction run at −2.4 V for 0.5 s followed by 0.1 s with the electrode off. The reaction was run for 300, 600, and 900 cycles with allyl acetate as the confining agent. Lower left: reaction time = 3 min. Lower right: reaction time = 6 min. Upper middle: reaction time = 9 min.





Spot	Relative intensity with green filter	Relative intensity with blue filter
300	100 ± 9	100 ± 2
600	210 ± 15	116 ± 2
900	197 ± 10	95 ± 2

Figure 9. Fluorescence image of the "inverse-Heck" reaction: −2.4 V; time on 0.5 s; time off 0.1 s; 300, 600, 900 cycles; allyl acetate confined. Lower left: reaction running for 3 min. Lower right: reaction running for 6 min. Upper middle: reaction running for 9 min.

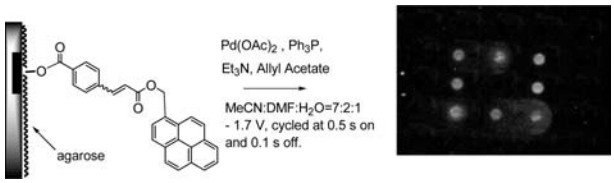
Revisiting the Heck Reaction

The result highlighted in Figure 7 led to questions about how the change from allyl methyl carbonate to allyl acetate as the confining agent influenced the reaction. Was this change the reason that the Suzuki reaction did not appear to lose fluorescence with time? To test this idea, the Heck reaction was repeated using allyl acetate as the confining agent. Everything else was kept the same as the reaction outlined in Figure 1 (electrode voltage of −2.4 V relative to a remote Pt electrode, etc.). As in the Suzuki time trial, three microelectrodes in a 1K array were selected for use (Figure 8). The three reactions were run for 300, 600, and 900 cycles. As in the earlier Heck reaction, the most intense spot was obtained for the reaction run for 300 cycles (lower left). As the reaction ran longer, the fluorescent spot indicating product grew less intense. Clearly, the change in confining agent did not alter the reaction. The methoxide generated when allyl methyl carbonate was used was not the reason for the decrease in product intensity with time.

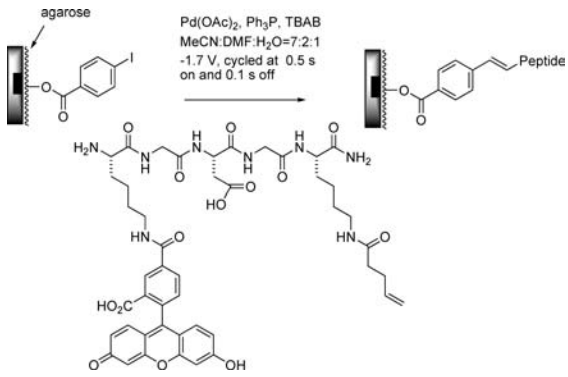
An inverse-Heck reaction appeared to show similar behavior (Figure 9), although in this case the decrease in intensity was small enough to preclude a definitive conclusion. The reaction was slower, leading to an increase in intensity from 3 to 6 min of reaction time. This increase dropped off at the 9-min mark (900 cycles), but again the effect was small. The reaction could not be continued past 900 cycles because of decomposition of the agarose polymer coating the surface of the array.

Interestingly, when the product was independently synthesized, placed on the array, and then exposed to the reaction

Scheme 5



Scheme 6



conditions, the image shown in Scheme 5 was obtained. The product was placed in a box pattern on the array. After the Heck reaction conditions were applied to the array, the box pattern was still evident, but the fluorescence had begun migrating away from the microelectrodes. Like the Suzuki reaction, confinement was being lost. Since the only fluorophore in the reaction was the product placed by the microelectrodes, the loss of confinement in this experiment provided evidence that the product from the reaction was being cleaved from the surface of the array and then migrating to other locations.

Peptides, Agarose, and the Role of Pd(II)

A much clearer picture of what was happening with the Heck reaction came to light when the reaction was utilized for placing a peptide substrate onto the array (Scheme 6). As in the earlier experiments, the microelectrode array was coated with an agarose polymer and then 4-iodobenzoic acid placed by each microelectrode in the central region of a 12K array using a base-catalyzed esterification reaction.[5b,9−11]

The Heck reaction was then conducted using the conditions described above with the only change being the olefin substrate used for the transformation. In this case, an unactivated olefin was used for the Heck reaction to avoid polymerization of the peptide triggered by the N-terminal amine. Although Heck reactions are slower with unactivated olefins, 4-pentenoic acid derivatives are known to undergo the reaction.[13]

Surprisingly, the reaction could not be confined at all (Figure 10). The product wound up being placed by every microelectrode in the array where the iodobenzoic acid had been placed. The result surprised us since we know that Pd(0) is confined under these conditions (see Figure 2 above).

Attempts to place the peptide on an array using an inverse-Heck reaction met with the same loss of confinement (Figure 11).

In this experiment, acrylate was placed on a 12K array in two patterns, one a checkerboard within a box and one a series of lines in a box. The peptide was functionalized with an aryl

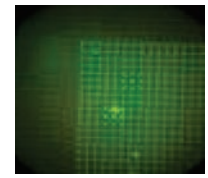


Figure 10. Heck reaction using a peptide substrate.

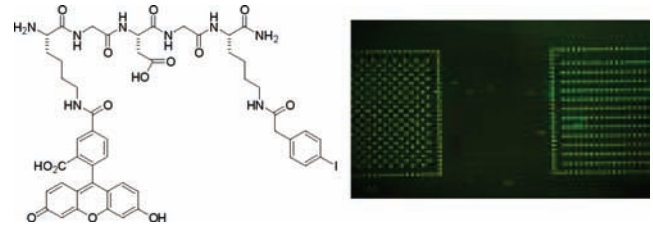


Figure 11. Inverse-Heck reaction using a peptide substrate.

iodide, as shown in the figure. The inverse-Heck reaction was then performed using only the microelectrodes in the lines within a box pattern. The image shows that the peptide was not only placed by the microelectrodes used for Pd(0) generation but also by each of the microelectrodes in the unused checkerboard within a box pattern. There was no evidence of confinement, even though once again we know Pd(0) is confined under these conditions (Scheme 4).

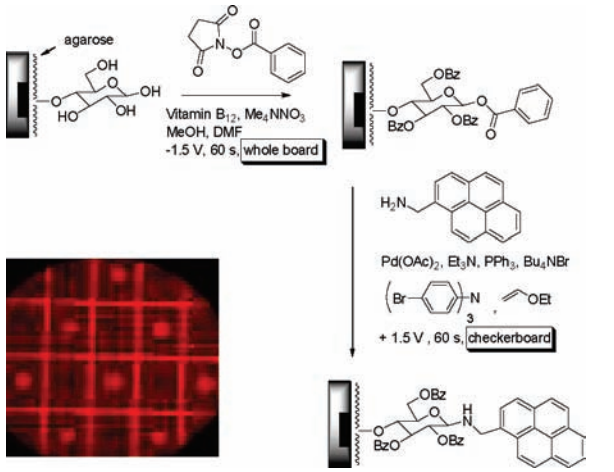
Clearly, a side reaction was placing the peptide on the array. For the inverse-Heck reaction it was easy to suggest a Michael-type reaction between the amine nucleophile at the N-terminus of the peptide and the acrylate on the surface of the array. However, no such possibility exists for the Heck reaction illustrated in Scheme 6. Suggestions that the reaction was catalyzing an addition of the amine nucleophile to the aryl iodide were ruled out with solution-phase control reactions showing that this reaction did not occur.

An alternative explanation was that placing the initial reaction substrates on the agarose surface using an ester linkage generated leaving groups on the anomeric carbon of the sugar. Pd(II) could then serve as a Lewis acid to generate oxonium ions on the surface of the array, leading to addition of the N-terminus of the peptide to the sugar polymer coating the array. Such a reaction would only occur at sites having been functionalized with the initial substrate, giving rise to the patterns seen in Figure 11.

To test this idea, a control experiment was performed by taking advantage of the chemistry developed for conducting site-selective Pd(II) reactions on the arrays.[6] The experiment started by taking an agarose-coated array and functionalizing the sugars by each of the microelectrodes with a benzoyl group (Scheme 7). The functionalized array was then treated with a solution of $Pd(OAc)_2$, ethyl vinyl ether, a triarylamine, triphenylphospine, triethylamine, and tetra-*n*-butylammonium bromide in a DMF, acetonitrile, water mixture. The ethyl vinyl ether was used as a confining agent to rapidly reduce any Pd(II) in solution by means of a Wacker oxidation. The triethylamine was present to scavenge the protons generated during this oxidation. Previous site-selective Wacker oxidations have shown this method to be extremely effective for confining Pd(II) on an array to only regions surrounding microelectrodes used as anodes.[6b] Pyrenemethylamine was then added to the solution above the array and selected microelectrodes (a checkerboard pattern) used to oxidize Pd(0) and generate Pd(II). As can be seen in the image shown, the amine nucleophile was added to the functionalized

(13) Lambert, J. D.; Rice, J. E.; Hong, J.; Hou, Z.; Yang, C. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 873.

Scheme 7



agarose surface by each of the microelectrodes selected for Pd(II) generation. Clearly, Pd(II) catalyzes the addition of amine nucleophiles to the functionalized agarose, an observation that explains the lack of confinement shown in Figures 10 and 11. In these previous "Pd(0) experiments", the whole array was covered with a Pd(II) species that was then reduced at selected electrodes. Hence, a Pd(II)-catalyzed reaction would occur everywhere on the array.

Although it is tempting to suggest that a Pd(II)-catalyzed addition can be used to add peptides to an array using a lysine side chain, the addition reaction proved to be reversible. When an array covered with agarose was functionalized with the benzoyl groups in two regions and then the pyrenylmethylamine placed on one of the patterns using the site-selective generation of Pd(II), a fluorescence image of the array showed fluorescence only by the pattern of microelectrodes selected for the Pd(II) reaction (the benzoyl group on the anomeric carbon is essential for oxonium ion formation and nucleophilic addition to the surface). However, when the array was re-exposed to the reaction conditions minus the pyrenylmethylamine and the second pattern used to generate Pd(II), the image of the array showed fluorescence at the second pattern. With no fluorescent amine nucleophile in solution, the fluorescence observed at the second pattern must have originated from the first pattern. This led to a conclusion that the attachment was not stable enough for use in generating isolated patterns of molecules on the arrays.

In the end, we concluded that both the loss of confinement during some Pd(0)-catalyzed reactions on the arrays and the decreasing amount of product by the selected microelectrodes in others were the result of the surface not being stable to the Pd(II) solutions used.

Use of a New, Non-Sugar-Based Surface

Further support for this conclusion was obtained by running the reactions on an alternative, more stable surface. Recently, we reported the use of a diblock copolymer as a porous reaction layer for coating microelectrode arrays.[14] The polymer consists of a methacrylate block functionalized with a cinnamoyl group and a *p*-bromo-substituted polystyrene block (Figure 12). The methacrylate block is used for coating the array. The cinnamoyl groups are then photo-cross-linked in order to add stability to the surface. The bromo-substituted polystyrene block is used

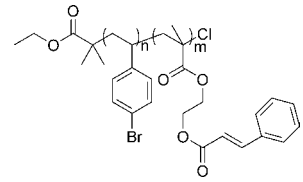
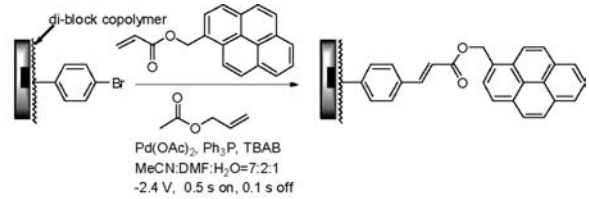
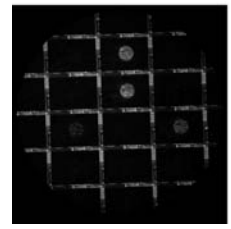


Figure 12. Diblock copolymer for coating the microelectrode arrays.





Spot	Relative intensity*
300	100 ± 5
600	118 ± 3
900	235 ± 10
1800	296 ± 9

Figure 13. Fluorescence image of Heck reactions run on poly-4-bromostyrene-*block*-cinnamoyloxyethyl methacrylate. The reactions were run by cycling selected electrodes on at −2.4 V for 0.5 s and then off for 0.1 s. Lower left: reaction run time = 3 min (300 cycles). Lower right: reaction run time = 6 min (600 cycles). Upper middle: reaction run time = 9 min (900 cycles). Middle: reaction run time = 18 min (1800 cycles).

to provide attachment points for fixing molecules to the surface of the arrays. Using this polymer, substrates are attached to the surface in a manner that cannot be readily cleaved. Hence, if the issues with the Heck reaction are due to cleavage of the product from the surface of the array, then they should not be a problem when the diblock copolymer is employed as the porous reaction layer.

This proved to be the case (Figure 13). When the Heck reaction was repeated, varying the number of cycles used for the electrolysis, the intensity of product fluorescence by the selected electrodes continued to increase with increasing reaction time. There was no decrease in intensity, even after an 18-min reaction. Previous reactions could not be conducted for this length of time because of agarose decomposition (delamination from the surface). A nearly identical result was obtained when the same experiment was repeated using the Suzuki reaction.

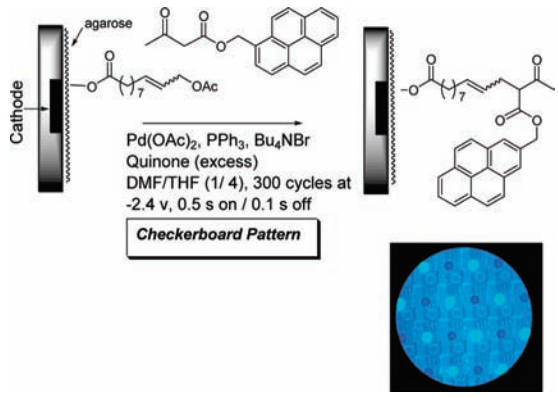
Allylations and a Final Point about Confining Agents

Of course, Heck and Suzuki reactions are not the only synthetically valuable transformations that are triggered with the use of Pd(0) catalysts. Another powerful synthetic tool is the Pd-catalyzed allylation reaction. A site-selective version of this reaction (Scheme 8) presented a unique challenge for the microelectrode array chemistry.[15] Although air can be and often was used as the solution phase confining agent to keep Pd(0) from migrating to unwanted sites on an array, reactions run on the 12K arrays often benefited from the use of allyl acetate as the confining agent. When a reaction lost confinement, doubling the amount of confining agent used (as in Figure 6) was not a problem. However, for a surface allylation reaction,

(14) Hu, L.; Bartels, J. L.; Bartels, J. W.; Maurer, K.; Moeller, K. D. *J. Am. Chem. Soc.* **2009**, *131*, 16638.

(15) For a preliminary report of this chemistry, see: Tian, J.; Maurer, K.; Moeller, K. D. *Tetrahedron Lett.* **2008**, *49*, 5664.

Scheme 8



the use of allyl acetate as the confining agent is not possible. By design, the reaction generates a π-allylpalladium(II) species on the surface of the array, which then undergoes attack from a nucleophile in solution to regenerate the Pd(0) catalyst. The use of allyl acetate as a solution-phase confining agent would generate a related π-allylpalladium(II) species in solution. This Pd(II) intermediate would also react with the nucleophile in solution to remake the Pd(0) catalyst. The net result is that the Pd(0) catalyst would not be consumed in solution and confinement of the electrode reaction would not be possible.

To solve this problem, a new confining agent was needed. To this end, quinone turned out to be an excellent choice. As illustrated in Scheme 8, when a Pd(0)-catalyzed allylation was conducted on a microelectrode array using a surface-bound allyl acetate, a solution-phase acetoacetate nucleophile, a checkerboard pattern of electrodes as cathodes, and quinone as an oxidant to scavange Pd(0), the reaction was perfectly confined to the electrodes used for the reduction of Pd(II).

Although quinone performed beautifully in this reaction, it would be a terrible choice as an oxidant for confining a Heck reaction. Quinone is itself a Heck reaction substrate. This leads to an important point. The key to developing reagents and catalysts for effecting a variety of site-selective reactions on a microelectrode array is always the proper choice of the confining agent. The electrochemistry part of the experiment does not vary from one type of transformation to the next. One may need to adjust the rate at which a reagent or catalyst is generated at the electrode surface. However, once a strategy is in place for electrochemically generating a reagent or catalyst, it can be employed for each new reaction developed. What changes is how new substrates interact with the confining agent. For any given reaction, the confining agent must rapidly and irreversibly scavenge the reagent or catalyst generated at the electrode while remaining inert to the substrate on the surface of the array. Pd(0) chemistry gives us examples of both problems. When allyl acetate is used as a confining agent for an array-based allylation reaction, the acetoacetate in solution reverses the scavenging reaction, regenerates Pd(0) in solution, and destroys confinement on the array. When quinone is to confine a Heck reaction, the quinone competes with the solution-phase olefin substrate being added to the surface of the array.

Conclusions

Three different Pd(0)-catalyzed reactions have been conducted site-selectively on microelectrode arrays: Heck, Suzuki, and allylation reactions. For the Heck and Suzuki reactions, it was found that the Suzuki reaction is faster and requires either lower currents to reduce the rate of Pd(0) generation or greater amounts of a solution-phase oxidant to maintain confinement of the reaction. Although both reactions proceeded well at short reaction times, in the initial studies both had problems when the reactions were run for longer periods. In the case of the Heck reaction, the product was cleaved from the surface of the array with longer reaction time. For the Suzuki reaction, confinement on the array was lost with time. The use of a peptide substrate containing an N-terminal amine shed light on the chemistry involved with these changes. When an agarose-coated array was functionalized with substrates using an ester linkage, Pd(II) catalyzed the formation of oxonium ions on the surface of the array. This allowed for addition of the amine nucleophile to the agarose on the array, a reaction that could be accomplished site-selectively by controlling the synthesis of Pd(II). With this knowledge, a non-sugar-based porous reaction layer was used to coat and functionalize the array. Using this more stable surface, both the Heck and Suzuki reactions showed normal behavior with longer reaction times, leading to greater amounts of product on the array with no loss of confinement. Finally, development of the site-selective allylation reaction required identification of a new confining agent. For this purpose, quinone proved to be very effective.

It is clear that Pd(0) catalysts are effective tools for functionalizing microelectrode arrays in a site-selective fashion. Since generation of the Pd(0) catalyst by a microelectrode is independent of the substrates used in the subsequent reaction, the chemistry developed should work for any Pd(0)-catalyzed transformation. The only change needed from one reaction to the next is the identification of a confining agent that rapidly converts Pd(0) to Pd(II) in an irreversible fashion and does not interfere with the desired surface reaction by providing a competitive solution-phase substrate. The use of Pd(0) on the microelectrode arrays is quickly becoming one of the main synthetic tools available for developing addressable molecular libraries.

Acknowledgment. We thank the National Science Foundation (CHE-0809142) for their generous support of our work. We also gratefully acknowledge the Washington University High Resolution NMR facility, partially supported by NIH grants RR02004, RR05018, and RR07155, and the Washington University Mass Spectrometry Resource Center, partially supported by NIHRR00954, for their assistance.

Supporting Information Available: Full experimental and characterization data are provide for all substrates and products. Copies of proton and carbon NMR are included. This material is available free of charge via the Internet at http://pubs.acs.org.

JA107490T