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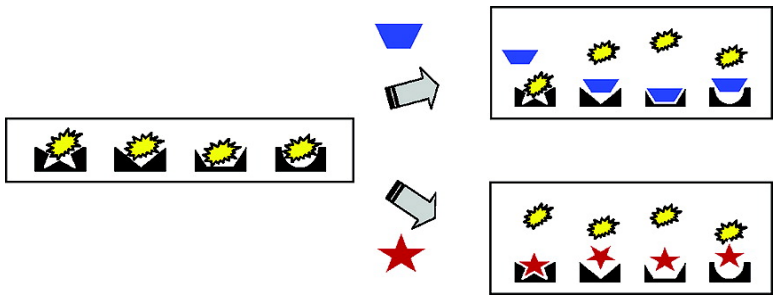

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# Colorimetric Molecularly Imprinted Polymer Sensor Array using Dye Displacement

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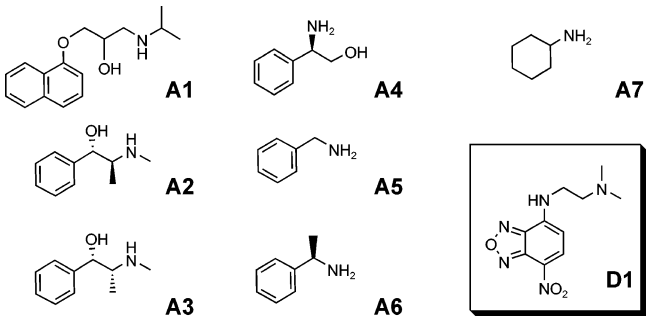
***Abstract:*** A colorimetric sensor array composed of seven molecularly imprinted polymers was shown to accurately identify seven different aromatic amines. The response patterns were systematically classified using linear discriminant analysis with 94% classification accuracy. Analyses of the response patterns of the analytes to the imprinted polymer array suggest that the different selectivity patterns, although subtle, appear to arise from the imprinting process. The molecular imprinting process enabled the rapid preparation of the polymers in the array from ethylene glycol dimethacrylate and methacrylic acid (80:20) in the presence of six different template molecules plus a blank nonimprinted polymer. The response of the imprinted polymer array was coupled to a colorimetric response, using a dye displacement strategy. A benzofurazan dye was selected and shown to give an accurate measure of the binding properties of the imprinted polymer array to all seven analytes. The colorimetric response also enabled the inclusion of analytes that are not spectroscopically active and were not among the original analytes that were used as template molecules. This broadens the potential utility of the imprinted polymer sensor array strategy to a wider range of analytes and applications.

## Introduction

Sensor arrays have been demonstrated to be highly effective formats for sensors that possess high levels of discrimination and accuracy.[1−4] Natural examples include the nose and tongue that are able to differentiate and identify an almost unlimited number of fragrances and flavors, using a finite number of different sensing elements.[5,6] The sensor array format is equally appealing to synthetic chemists as the approach can accommodate sensors that have poor individual selectivity and broad cross-reactivity.[7,8] The individual sensing elements only need to possess sufficient differential selectivity to yield a unique response pattern for each analyte. Herein, we report on the rational and rapid development of a colorimetric molecularly imprinted polymer (MIP)-based sensor array capable of differentiating seven structurally similar amines (**A1**−**A7**) with 94% accuracy. These amine analytes include diastereomers (**A2** and **A3**), pharmaceuticals (**A1**, **A2**, and **A3**), and analytes that differ only by a single methyl group (**A5** and **A6**). When a dye-displacement approach was utilized,[9] the colorimetric signal was readily incorporated into the sensor design. The incorporation of a colorimetric signal greatly enhanced the utility of the MIP sensor array approach without sacrificing the accuracy or the efficiency with which the MIP-based sensor array could be prepared. Finally, the selectivity of the array was shown to arise from the molecular imprinting process as examination of the response patterns of the array for each analyte showed a disproportionally high response to its corresponding imprinted polymer. Sensor arrays have been shown to be effective in a wide range of formats[10,11] and applications.[2,12] A major limitation of the sensor array format is the requirement of a large

***Chart 1*** [a]




[a] Analytes **A1**−**A7** are (±)-propranolol, (+)-pseudoephedrine, (2)-ephedrine, (*R*)-(2)-2-phenylglycinol, benzylamine, α-methylbenzylamine, and cyclohexylamine, respectively. Dye **D1** is *N*,*N*-dimethyl-*N*′-(7-nitro-2,1,3-benzoxadiazol-4-yl)-1,2-ethanediamine.

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number of unique recognition elements. One solution is to use molecularly imprinted polymers (MIPs) as the recognition elements.[13,14] The molecular imprinting process rapidly and rationally generated polymers with the desired differential selectivity. This was accomplished simply by making a series of imprinted polymers using different analyte molecules as template molecules. An array of MIPs generated against structurally similar arylamines (**A1**−**A7**) was shown to yield unique binding patterns with which the amines could be individually differentiated and classified using linear discriminant analysis (LDA).

One difficulty in using MIPs as sensors is that MIPs contain no inherent signaling elements, and thus, our initial studies measured the response from the MIP array using the UV absorbance of the unbound analyte.[13] In this format, the analysis is more similar to a binding assay rather than a sensor and is limited to analytes that were UV-active. To address this deficiency, a common colorimetric response was coupled to the binding events of the analytes to the individual imprinted polymers. This was accomplished using a dye-displacement strategy with the benzofurazan-based amine dye **D1**.[9] The displacement of dye **D1** was demonstrated to be an effective reporter for the binding of analytes to the MIP array. Even though the dye-displacement strategy is an indirect measure of binding, it still gave levels of accuracy in classifying the respective analytes similar to those of direct measurements using UV-active analytes. In addition, the colorimetric dye-displacement strategy enabled the classification of analytes beyond those that were originally used to generate the array. This opens up the possibility of retraining an MIP sensor array to accommodate new analytes.

Sensor arrays can be grouped into two general categories, that is, arrays containing sensor elements with general or specific recognition properties. In the first class, the recognition elements are commonly polymers, polymer blends, copolymers, or mixtures of polymers and small molecule dopants.[15−17] This has the advantage that they are often commercially available or easily prepared. The disadvantage is that there is little control over whether the recognition surfaces will have the necessary differential selectivity to yield unique response patterns for each analyte. The second category of sensor arrays is based on individual receptors that have been specifically tailored with selectivity. This strategy has the advantage that the individual receptors possess the desired differential selectivity.[9] However, significant synthetic effort is necessary to develop a single molecular receptor for a specific analyte, and this effort is multiplied severalfold to generate all of the elements necessary for an array.

Synthesis of the recognition elements in a sensor array by molecular imprinting combines advantages of the two general categories of sensor arrays. The imprinting process enables the rapid preparation of polymers that, at the same time, have been specifically tailored with specificity for the desired set of analytes. The imprinting process involves the formation of a highly cross-linked polymer matrix around a template molecule.[18−21] Removal of the template then leaves behind a cavity with complementary size and shape. The cavity is also lined with complementary functionality, which is provided by a functional monomer. In the more common noncovalent imprinting approach, the cross-linker, functional monomer, and template are all mixed together and polymerized in a single step. The functional monomers are believed to surround the template molecule and are "frozen" into complementary conformations and orientations upon polymerization.

The synthetic ease of the imprinting process, however, is offset by the relatively poor overall affinity and selectivity.[8,22] This most likely arises from binding site heterogeneity in which only a small fraction of the binding sites have high levels of affinity and selectivity for the template molecule.[23] Sensors based on MIPs, in general, have poor selectivity and differentiation, especially in comparison to enzyme and antibody-based sensors. Thus, the sensor array-based approach would appear to be particularly well-suited toward MIPs as the sensor array approach does not require recognition elements with high levels of selectivity. The polymers need only display differential binding that yields unique patterns for each analyte.

## Results and Discussion

To demonstrate the utility of MIP-based sensor array, seven polymers (**P0**−**P6**) were prepared using an ethylene glycol dimethacrylate (EGDMA)/methacrylic acid (MAA) matrix. This copolymer matrix has previously been demonstrated to be effective in imprinting a wide range of analytes, in particular, pharmacologically active arylamines.[24−26] Polymers (**P1**−**P6**) were specifically tailored with selectivity by using analytes **A1**−**A6**, respectively, as templates in their synthesis. In addition, a nonimprinted polymer (**P0**) was synthesized in the absence of any template molecule. The polymers were all synthesized under identical conditions by UV irradiation using a 1:4 mole ratio of MAA/EDMA in toluene with 2 mol % AIBN as the radical initiator.

Analytes propranolol (**A1**), (+)-pseudoephedrine (**A2**), and (2)-ephedrine (**A3**) were chosen for their previously demonstrated ability to be imprinted. Three additional arylamines (**A4**−**A6**) were chosen for they are structurally similar to amines **A1**−**A3**. While MIPs have been generated that can differentiate two or more of these analytes, none have been reported that can differentiate as many as seven structurally similar analytes. An additional analyte **A7**, cyclohexylamine, which was not one of the original template molecules, was added to the analyte pool. **A7** was added to the analyte pool to demonstrate that the colorimetric MIP sensor assay could identify analytes lacking a UV chromophore. The inclusion of **A7** also tested the ability

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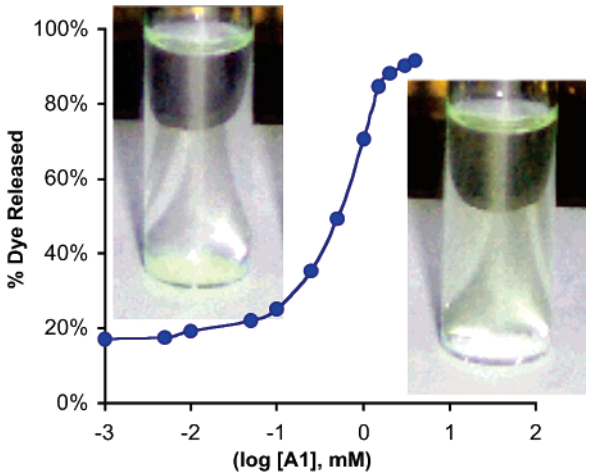


***Figure 1.*** Displacement curve for dye **D1** (10 $\mu$M) and polymer **P1** (25 mg) in acetonitrile (2.5 mL) by addition of analyte **A1** and equilibration for 2 h. The percent of dye released was measured by the absorbance of the solution at 460 nm. The inset pictures show polymer (at the bottom of the vials) equilibrated with dye low (left) and high (right) concentrations of analyte **A1**.

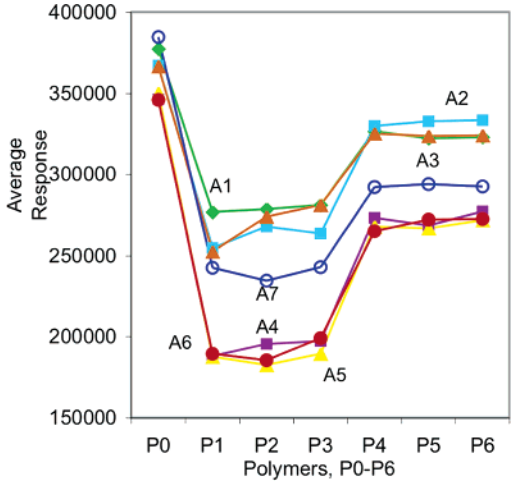


***Figure 2.*** Response patterns of the colorimetric MIP sensor array (**P0**−**P6**) against each of the seven analytes (**A1**−**A7**). The individual binding experiments were carried out by equilibration of 10 $\mu$M dye **D1**, 25 mg of polymer **P1**, and 1.0 mM analyte in 2.5 mL of acetonitrile (2.5 mL) for 2 h. The response was measured as the absorbance at 460 nm remaining in the solution as measured by HPLC. Each point is an average of five different measurements.

of the MIP sensor array to differentiate nontemplate molecules. The potential of an MIP sensor array to identify and differentiate analytes beyond those used in the synthesis of the MIP sensor array greatly expands the pool of potential analytes and the utility of the MIP sensor array strategy.

The key to effectively implementing the colorimetric dye-displacement strategy was the selection of a dye that would act as an accurate reporter for the binding of all the different analytes to the polymers. In this respect, the low selectivity and high cross-reactivity of MIPs was an advantage that enabled a single dye to act as a reporter for the binding of all the analytes to the seven different polymers. Benzofurazan **D1** was selected as the dye molecule because of its similarity to the analyte molecules.[27] Unlike most visible dyes, **D1** is small and is of similar size to those of the analytes **A1**−**A7**. In addition, **D1** contains functionality similar to that of the analytes, including a small aromatic surface and an amino side chain that can hydrogen bond or form electrostatic interactions with the carboxylic acids in the imprinted polymers. These attributes were expected to facilitate the binding of the dye **D1** to the binding sites generated by templates **A1**−**A6**. The dye also had a strong yellow color ($\epsilon_{460} = 2.0 \times 10^4$ $M^{-1}$ $cm^{-1}$) with an absorption maximum at 460 nm. This is in the visible portion of the spectrum where the analytes do not absorb, and thus, the dye was able to act as a reporter for the binding of the analytes without spectroscopic interference from the analytes.

First, the binding characteristics of the dye **D1** were assessed. The polymers all showed strong affinity for the dye **D1** in acetonitrile (Figure 1). For example, 25 mg of polymer **P1** bound more than 80% of the dye from a 10 $\mu$M acetonitrile solution (2.5 mL). This was visually evident as the normally white polymer at the bottom of the vials was bright yellow and the solution was almost colorless. The sequential addition of analyte **A1**, propranolol, to the bound dye gave a broad but clean displacement curve (Figure 1), which was also visually evident as the polymer returned to its white color and the solution became yellow. Similar displacement curves were observed for the other analytes and polymers.

The sigmoidal displacement curves showed the greatest response at an analyte concentration of 1.0 mM, which corresponds to the steepest point of the displacement curve. Therefore, the individual analytes were tested against the array at 1.0 mM. This concentration was lower than that of our previous studies (3 mM) that directly measured the binding of the analyte concentration using UV−vis spectroscopy.[13] Performing the analysis at a lower concentration was expected to have the added benefit of being more selective, as MIPs have been shown to show higher selectivity at lower concentrations.[22]

First, a library of the response patterns for each analyte to the MIP array was generated. This training matrix was generated by individually testing analytes **A1**−**A7** five times against the seven polymer array (**P0**−**P6**). The response of the array was tested by measuring the binding of 1 mM analyte mixed with 0.01 mM dye and 25 mg of each polymer in 2.5 mL of MeCN. The relative binding was measured by UV−vis analysis of the supernatant at 460 nm before and after equilibration of the samples with the polymer, using an HPLC fitted with an autosampler. The dye-displacement strategy yielded a common colorimetric response for all analytes regardless of their extinction coefficients. Even cyclohexylamine (**A6**) that lacked a chromophore gave a measurable and unique response pattern.

The average response patterns for each analyte **A1**−**A7** against the colorimetric MIP sensor array (**P0**−**P6**) are shown in Figure 2. Each point corresponds to the averaged response of five separate measurements for that respective analyte and polymer pair. Overall, the patterns had the same general shape as the response patterns were dominated by the intrinsic affinity of each polymer for the dye molecule. For example, the nonimprinted polymer (**P0**) had the lowest affinity for the dye and, therefore, the highest response. Polymer **P1** had the highest affinity for the dye molecule and the lowest response. Unlike the example shown in Scheme 1, the differences in the response patterns of the colorimetric MIP array were masked by the

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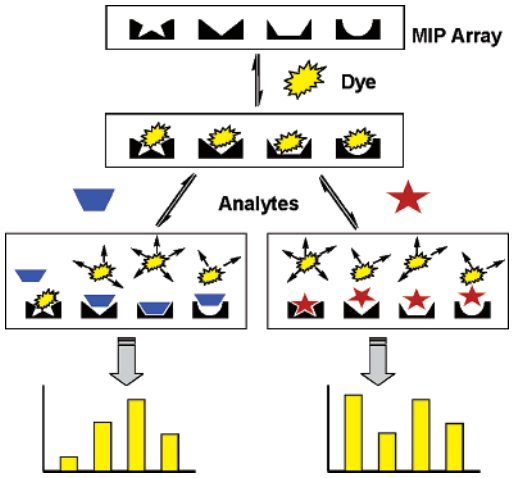
***Scheme 1.*** A Representative Scheme of an MIP Sensor Array that Uses a Dye-Displacement Strategy to Give an Easily Visualized and Unique Colorimetric Response Pattern for Each Analyte




different affinities of the polymers for the dye, and therefore, distinct or unique patterns were not immediately apparent.

Linear discriminant analysis (LDA) was utilized to systematically identify differences in the patterns of the respective analytes. LDA analysis was applied to the raw unprocessed data (Figure 3). LDA reduced the complexity and size of the training

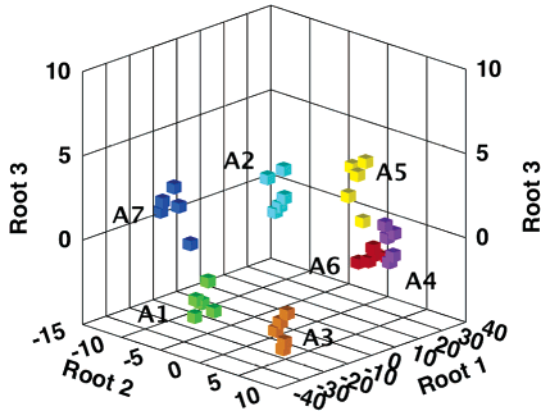



***Figure 3.*** Differences in the response patterns for the seven analytes to the MIP array by LDA.

(7 polymers × 7 analytes × 5 replicates) and transformed them into roots that are linear combinations of the response patterns (6 roots × 7 analytes × 5 replicates). The first three roots contained the majority of the variation (99.7%) in the training matrix with individual values of 93.3, 5.0, and 0.9% of the variation. Thus, the data in the training matrix could be visually represented as a three-dimensional plot of the LDA roots 1, 2, and 3 (Figure 3). In this plot, each point represents the response pattern for a single analyte to the colorimetric MIP sensor array. Patterns that are similar should be close together, and patterns that are dissimilar should be well separated.

The LDA plot suggested that there were distinct repeatable differences in the response patterns for the seven amine analytes against the colorimetric MIP sensor array. The responses for each analyte were clustered into tight distinct groupings, demonstrating the reproducibility of the response for each analyte. The LDA analysis was even able to differentiate the structurally similar analytes within these groups. Diastereomers, pseudoephedrine (**A2**) and ephedrine (**A3**), were well differentiated. Benzylamine (**A5**) and α-methyl benzylamine (**A6**), which differ only by a methyl group, were also well separated. The closest groups are benzylamine (**A6**) and (*R*)-(2)-2-phenylglycinol (**A4**). Qualitatively, the response patterns, as measured by the dye-displacement strategy, had a lower degree of differentiation in comparison to that of previously reported direct measurements of the binding to the MIP array. The dye-displacement strategy required a three-dimensional analysis to spatially differentiate the response patterns for the various amine analytes. By comparison, the direct measurements needed only a two-dimensional multivariate analysis. The lower sensitivity of the dye-displacement strategy is perhaps not surprising as it is an indirect measure of the binding. However, it is evident that the dye-displacement strategy was able to measure distinct and unique patterns for each analyte.

The LDA plot of the first three roots yielded only a qualitative assessment of the ability of the MIP array to classify the seven analytes. To quantitatively test the ability of the MIP array to accurately classify the seven analytes, LDA using all six roots was applied to classify the respective analytes. The accuracy of this classification method was monitored using a jackknife analysis to measure the generalization error.[28] The error analysis was conducted using Systat because this feature was not available in Statistica. The jackknife classification matrix is an iterative method in which one sample pattern at a time is omitted from the LDA and treated as an unknown. The unknown pattern is then classified based on the LDA function generated from the remaining sample patterns. This "leave one out" classification is then repeated for each measured pattern. The jackknife classification matrix correctly classified 33 out of 35 measurements for a 94% accuracy. Even cyclohexylamine (**A8**) was correctly classified, even though it was not one of the original template molecules (**A1**−**A7**). This suggests that once an array has been prepared against one set of analytes, it maybe utilized to classify additional analytes. This greatly extends the range of analytes that can be tested using the MIP array strategy. We are currently examining the generality of this observation.

The 94% classification accuracy for the dye-displacement format was the same as the analysis of the MIP array by directly monitoring the analyte.[13] This suggests that even though the dye-displacement format is an indirect measure of binding, it still gives a fairly accurate measure of the binding efficiency. The two analytes that were misclassified were one replicate of benzylamine (**A5**) that was misclassified as α-methyl benzylamine (**A6**) and one replicate of α-methyl benzylamine (**A6**) that was misclassified as benzylamine (**A5**). The misclassification of these two analytes is consistent with their structural similarity and the proximity of the **A5** and **A6** groups in the three-dimensional LDA plot (Figure 3). Interestingly, the LDA plot of the first three roots suggests that the **A4** and **A6** are closer and, therefore, more similar in pattern than **A5** and **A6**, which were misclassified. Although **A4** and **A6** are closer in the LDA plot, they both have very tightly grouped points and, thus, were still well differentiated. Analyte **A5**, on the other hand, has greater scatter, and thus, there is greater uncertainty when attempting to classify analytes as **A5**.

The question was then posed whether the subtle patterns identified by the multivariate analysis were merely chance

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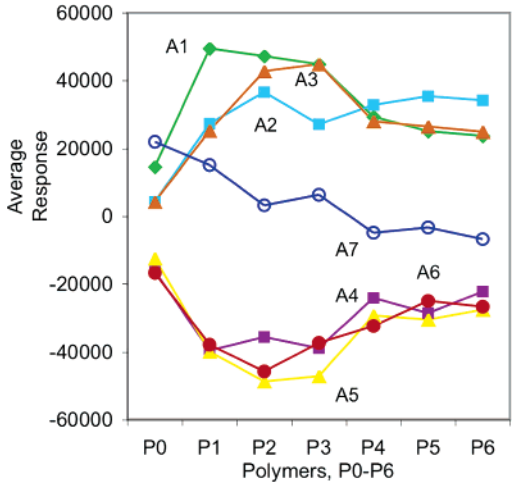


***Figure 4.*** Polymer normalized response to the colorimetric MIP sensor array. Each point represents the average of five individual measurements and was normalized by subtracting from each point the average of all the analytes' responses for that polymer.

differences in the response patterns or whether they arose from the imprinting process. In particular, the concern was that multivariate analyses, such as LDA, are very sensitive to small systematic errors in the concentrations of the stock analyte solutions or in the amounts of polymer, and it could be these systematic differences and not differences in the binding pattern that led to the differentiation of the different analytes by LDA. Therefore, we wanted to see if the differences in the response patterns of the different analytes could be rationally correlated to the structures of the respective analytes. Since each polymer in the array was tailored with selectivity for a specific analyte by the imprinting process, the expectation was that the highest response for an analyte would be observed for the polymer imprinted with that analyte.

However, examination of the raw response patterns for the seven analytes to the MIP array (Figure 2) did not show distinct patterns for each analyte. The patterns all had the same general shape because they were masked by the intrinsic affinity of the respective polymers for the dye molecule. To remove the polymer-based bias and to highlight differences arising from the specific analytes, the raw patterns were normalized with respect to binding affinity for each polymer (Figure 4). This was done by averaging the response for all of the analytes to a polymer and by subtracting this value from each measurement made with that polymer. Examination of these normalized response patterns revealed unique patterns that suggested that the colorimetric MIP sensor array can be used to differentiate the respective analytes. Three clear groupings were evident: the high affinity analytes (**A1**−**A3**), the low affinity analytes (**A4**−**A6**), and the intermediate affinity analyte (**A7**).

Within these three distinct groupings, clear patterns emerge that appear to arise from the imprinting process. In most cases, the highest or close to the highest response for an analyte was to its respective imprinted polymer. For example, in the case of analyte **A1**, the maximum response was with polymer **P1**. Similarly for analytes **A2** and **A3**, the maxima for their response patterns were with polymers **P2** and **P3**, respectively. The observation that the highest responses for analytes **A1**, **A2**, and **A3** were to their corresponding imprinted polymers gave evidence that the distinct patterns identified by the LDA arose from the imprinting process. Furthermore, the results suggest that the MIP array has truly been tailored with selectivity by the imprinting process.

In the cases of the lower affinity analytes **A4**, **A5**, and **A6**, the differences were more subtle. The low affinity analytes all showed the highest response with the imprinted polymer **P0**, and they all had similar levels of response to polymers **P4**−**P6**. However, the low affinity analytes (**A4**−**A6**) all had higher responses to their imprinted polymers (**P4**−**P6**) as a group in comparison to polymers **P1**−**P3** that were imprinted with the high affinity analytes. This was in contrast to the high affinity analytes (**A1**−**A3**), which have higher affinity polymers **P1**−**P3** over polymers **P4**−**P6**. This suggests that the differences in the selectivity of polymers **P4**−**P6** for their respective imprinted analytes were much smaller than those for the polymers **P1**−**P3**. Thus, the patterns for these analytes against the entire MIP array were very similar. This similarity was also apparent in the LDA analysis as these low affinity analytes (**A1**−**A3**) were grouped together in the LDA plot (Figure 3). However, the overall shape of their normalized response patterns were still strongly influenced by the imprinting process as they all had elevated responses for their imprinted polymers as a group.

Finally, the response pattern for the nonimprinted analyte (**A7**) fell between the low and high affinity analytes and had a unique response pattern in comparison to that of the imprinted analytes (**A1**−**A6**). This unique pattern may arise from cyclohexylamine not being one of the template molecules or from cyclohexylamine being an aliphatic and not an aromatic amine like all the other analytes. Regardless of the sources of these differences, the response pattern for the nonimprinted analyte **A7** was well differentiated from the other analytes and, thus, was accurately classified by the LDA. Further examples are necessary to establish whether this is a general phenomenon. However, the ability of nonspecific recognition surfaces to be effectively utilized in sensor arrays suggests that MIP sensor arrays will also be able to classify analytes beyond those that were used as template for their synthesis. This would greatly increase the number of potential analytes that a given MIP array could classify and, therefore, greatly enhance the utility of the approach.

## Conclusion

We have demonstrated the utility of MIP sensor arrays coupled with a dye-displacement strategy to correctly classify seven different amines with high fidelity. The molecularly imprinting strategy enabled the rapid and rational preparation of polymers possessing the differential selectivity necessary in sensor arrays. In addition, a dye-displacement strategy provided an easily measurable colorimetric response. The raw response patterns were dominated by the intrinsic affinity of the polymers for the dye molecules. However, when these polymer-based biases were normalized, distinct patterns emerged that were consistent with those expected from the imprinting process. This suggests that the ability of the colorimetric sensor array to accurately classify the analytes was a product of the imprinting process. The use of MIPs as recognition elements in sensor arrays appears to be a synergistic pairing. The MIPs allow the array to be rapidly and rationally prepared with specificity for the selected analytes. The array format, at the same time, compensates for the low fidelity and high cross-reactivity of

MIPs, allowing them to be used in sensors with high selectivity and accuracy.

## Experimental Section

**Polymer Preparation (P0−P6).** Polymers **P0**−**P6** were synthesized as previously described.[13] Briefly, AIBN (22 g, 0.64 mmol), MAA (1.04 mL, 6.3 mmol), EDMA (9.52 mL, 25.2 mmol), 8 mL of porogen (toluene), and 1.28 mmol of template (**A1**−**A6**) were individually mixed in 7 mL glass vials. The nonimprinted polymer **P0** was prepared using the same mixture but without the addition of the template molecule. The solutions were sonicated under nitrogen and capped. Polymerization was carried out using a Hanovia medium-pressure 450 W mercury arc lamp cooled in a borosilicate immersion well, and the entire apparatus was placed in a UV shielded and refrigerated reaction chamber. The vials were placed 20 cm from the lamp. The initial temperature of the UV reaction chamber was 10 °C, which increased over the course of the polymerization. The final temperature did not increase above 32 °C over the course of the polymerization. The vials are turned 180° after 20 min and once again at 60 and 180 min, then finally removed after 12 h.

After the polymerization was complete, each polymer was ground with a mortar and pestle. Three Soxhlet extractions were preformed on the eight polymers. The first wash used a 28:72 azeotrope of acetic acid/acetonitrile, and the second used a 4:1 ratio of acetonitrile/methanol. Due to residual acetic acid detected within the polymer, it was necessary to repeat the second wash step with the addition of 10 g of $NaHCO_3$ to the receiving flask to adsorb acetic acid. Polymers were dried in vacuo.

**Synthesis of Dye (D1).** A modification of the previously reported synthesis was used to synthesize and purify dye **D1**.[27,29] In a 50 mL round-bottom flask, *N*,*N*-dimethylethyl-1,2-diamine (0.138 mL, 1.26 mmol) and $NaHCO_3$ (103 mg, 1.23 mmol) were dissolved in 5 mL of MeCN. To the stirring mixture was added dropwise a solution of 4-chloro-7-nitrobenz-2-oxa-1,3-diazole (NBD) (250 mg, 1.25 mmol) in 10 mL of MeCN over 1 h. The mixture was heated in an oil bath at 60 °C for 15 min. The reaction mixture was cooled to room temperature, filtered to remove particulates, and concentrated in vacuo. The product was purified via chromatography on silica gel (1:9, $MeOH/CH_2Cl_2$) and concentrated in vacuo to yield a brown solid (200 mg, 64% yield). $^1H$ NMR (300 MHz, $CD_3CN$): $\delta$ 2.1 (s, 6H), 2.5 (t, 2H), 3.4 (t, 2H), (s, 1H), 6.15 (d, 1H), 8.35 (d, 1H).

**Testing the Array.** The analytes were all used in their free base form. Polymers **P0**−**P6** (25 mg) were individually placed into seven separate 6 mL vials. The polymers were equilibrated with 2.5 mL solutions of MeCN solutions containing a single analyte (1mM) and 2.5 mL of a dye, **D1** (0.01 mM), totaling 5 mL for 2 h. This process was repeated for analytes **A1**−**A7** to generate five replicates of each. The response of the array was measured by removal of 1 mL aliquots of supernatant, which was injected into a Varian Pro Star 320 HPLC with autosampler in which the column was removed. The dye concentrations in solution were measured by integrating the entire sample peak at 460 nm. The seven analytes (**A1**−**A7**) were tested against the seven polymer array (**P0**−**P6**) five times to generate a $5 \times 7 \times 7$ data matrix. The raw data matrix was processed using a linear discriminant analysis function as implemented in Statistica and Systat.

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