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A Facile Approach to Cross-Reactively Colorimetric Sensor Arrays: An Application in the Recognition of 20 Natural Amino Acids

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Abstract

A very facile approach for the design and fabrication of a colorimetric sensor array through taking only a single indicator-receptor couple with various ratios and concentrations is described for the first time. As a proof-of-concept application, discrimination and identification of the 20 natural amino acids are successfully accomplished. Classification analyses demonstrate that the as-fabricated colorimetric sensor array has a high dimensionality and, consequently, has a capability of recognition among the 20 natural amino acids. Moreover, qualitative and semi-quantitative detection of the amino acids are able to be realized through combining classification analyses, recognition patterns and corresponding fitting curves. The developed strategy in this current study represents probably a "maximally" simplified approach for design and fabrication of colorimetric sensor arrays, and thus allowing for taking their full advantages in sensing application field.

Introduction

The superior properties of cross-reactively colorimetric sensor arrays, i.e. capable of rapid, efficient and simultaneous detection of multiple chemical structure and/or property similar analytes, have made them to be very attractive in the past two decades.[1-5] These systems of which based on cross-responsive sensor elements aim to mimic the mammalian olfactory or gustatory system by producing composite responses unique to each analyte.[1,2] This kind of molecular recognition does not have to possess specific or selective binding to a target analyte, but instead occurs through a pattern of response from an array of indicators.[1,3] The design and fabrication of a colorimetric sensor array, however, has to usually select a diverse range of chemically responsive indicators based on detection targets, and this is frequently a laborious and tedious process. Therefore, it is essential to develop a facile approach for design and fabrication of colorimetric sensor arrays in order to take full of their advantages in sensing applications.

Indicator displacement assays (IDAs) with simple metal ions or complexes as receptors are typically not very selective, but they are well suited for building colorimetric sensor arrays.[4-5] The arrays that previously reported based on IDA were generally designed and fabricated via a number of combinations of selected receptors and indicators to achieve sufficient cross-reactivities for discrimination of multiple chemical structures and/or properties analogous analytes.[4] Recently, we noticed that the sensitivity and selectivity of an IDA can be modulated by simply altering ratios and concentrations of a receptor-indicator couple.[5] These findings can be understood that each combination of a receptor/indicator ratio at a specific concentration may be regarded as a cross-reactive indicator. This consideration inspired us to explore the possibility, for the first time, to fabricate a colorimetric sensor array through only simply varying concentrations and ratios of a single indicator-receptor couple. If this assumption works, the design and fabrication of a colorimetric sensor array would be "maximally" simplified. Therefore,

a proof-of-concept of the above proposed strategy was carried out by investigating the recognition and discrimination capability for the 20 natural amino acids as an example.

As key constituents of proteins, amino acids play important roles in many physiological processes,[6] and their abnormal levels are usually implicated in a variety of diseases.[7] The affinity of metal ions to amino acids has long been recognized,[8] and we found that the couple of pyrocatechol violet (PV) and Cu^{2+} can response to all the 20 natural amino acids in pH 7.2 buffer. Thus, the PV-Cu^{2+} couple is an ideal candidate to explore the "maximally" simplified design strategy to fabricate a colorimetric sensor array. Herein, four ratios (total twelve combinations) of PV and Cu^{2+} at different concentrations were taken to fabricate a 1×12 array, and its capabilities for recognition of the 20 natural amino acids were fully investigated. Experimental results exhibited that the such-fabricated array could excellently discriminate and recognize the 20 natural amino acids in aqueous solution at neutral pH at their micromolar concentrations. More importantly, these findings evidenced an applicability of the proposed design concept to fabricate a colorimetric sensor array, i.e. simply varying concentrations and ratios of a single indicator-receptor couple.

Experimental

Reagents and materials: Pyrocatechol violet, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) and 20 natural amino acids were obtained from Aladdin. Cu^{2+} salt (as perchlorate) was purchased from J&K Scientific. All reagents were of analytical reagent grade and were used as received without any further purification unless otherwise specified. Deionized water was used throughout the whole experiments.

The stock solutions of pyrocatechol violet and Cu^{2+} were 1.0 mM. The stock solutions of 20 natural amino acids were as follows: 0.5 mM cysteine (Cys), 0.5 mM histidine (His), 0.5 mM

aspartic acid (Asp), 0.5 mM asparagine (Asn), 0.5 mM glutamic acid (Glu), 3.0 mM glycine (Gly), 3.0 mM alanine (Ala), 3.0 mM valine (Val), 3.0 mM leucine (Leu), 3.0 mM isoleucine (Ile), 3.0 mM phenylalanine (Phe), 3.0 mM proline (Pro), 3.0 mM tryptophane (Trp), 3.0 mM serine (Ser), 3.0 mM methionine (Met), 3.0 mM glutamine (Gln), 3.0 mM threonine (Thr), 3.0 mM lysine (Lys), 3.0 mM arginine (Arg) and 200 μM tyrosine (Tyr). A 50 mM stock solution of HEPES at pH 7.2 was prepared by dissolving 1.1916 g HEPES in 80 mL deionized water, and then adjusting the pH to 7.2 by adding an appropriate amount of 1.0 M NaOH; the solution was then diluted to 100 mL with deionized water and mixed thoroughly. The working solutions of all these reagents were diluted from the stock solutions with deionized water

Instrumentation: For all sensing experiments, imaging of the arrays was performed with a flatbed scanner (Epson Perfection V300). The pH measurements were performed using a PHS-3C pH meter. 96-well plates (Corning 3632) were obtained from Genetimes Technology.

Experimental procedures: Control solutions containing various concentrations of pyrocatechol violet and Cu^{2+} in 10 mM HEPES at pH 7.2 were prepared. Then, 300 μL of each control solution was added to a 96-well plate and the "before" images were acquired with an Epson Perfection V300 flatbed scanner. Working solutions composed of corresponding concentrations of pyrocatechol violet and Cu^{2+}, 10 mM HEPES at pH 7.2 and diverse concentrations of amino acids were prepared. Similarly, 300 μL of each working solution was added to a 96-well plate and the "after" images were then obtained.

All the analyses of amino acids samples were conducted in quadruplicate experiments. For each experiment, a color change profile was obtained by subtracting the "before" image from the "after" image using Photoshop. Difference maps were acquired by taking the difference of the red, green and blue (RGB) values from the centre of every indicator spot from the "before" and

"after" images. Subtraction of the two images yielded a difference vector of 3N dimensions, where N is total number of spots (for our 1 × 12 array, this difference vector is 36-dimensional). The color change profiles were then compiled into a database library of 36-dimensional vectors (twelve RGB values) and represented a unique fingerprint for each amino acid. Hierarchical cluster analysis and principal component analysis were performed on the database library using the minimum variance for classification.

Results and discussion

Design concept and fabrication of colorimetric sensor arrays. Previous reports have shown that the sensitivity and selectivity of an IDA can be modulated by simply altering ratios and concentrations of a receptor-indicator couple.[5] Consequently, we decide to examine the possibility, for the first time, to fabricate a colorimetric sensor array through only simply varying concentrations and ratios of a single indicator-receptor couple. Such a design and fabrication would "maximally" simplify the process of development of a colorimetric sensor array for specific sensing purpose.

To demonstrate an applicability of the above proposed design concept, pyrocatechol violet (PV) and Cu^{2+} couple was employed, of which had been found to response to all the 20 natural amino acids in neutral aqueous solution (pH 7.2 buffer). Herein, a 1×12 array was fabricated simply by mixing PV and Cu^{2+} buffer solution at different concentrations and ratios and loaded into a commercial available 96-well plate (see Figure 1 for details). To further simply the sensing process, an ordinary flatbed scanner was applied to obtain the array's images and a detailed description for data taking and analysis refer to the Experimental section.[9]

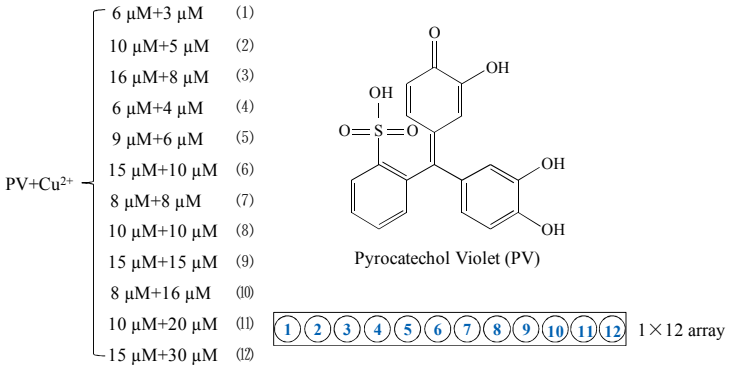

Figure 1. The chemical structure of pyrocatechol violet (PV) and the selected ratios and concentrations of PV and Cu^{2+} for fabricating the 1×12 array.

Array's responses to the 20 natural amino acids at 100 μM. Extensive test of the array's responses to the 20 natural amino acids (their chemical structures and names abbreviations are illustrated in the Electronic Supplementary Information (ESI) Figure S1) at 100 μM was firstly carried out. As demonstrated by the difference maps of the 20 natural amino acids that shown in ESI Figure S2, this colorimetric sensor array is sufficiently successful in identifying all the 20 natural amino acids at 100 μM. Even by naked eye (without statistical analysis), the array response to each amino acid with a unique pattern. For quantitative comparison of the color changes of the array, however, we define a 36-dimensional vector (i.e. twelve changes in RGB values of the 1×12 array) for each experiment. The high dispersion of the colorimetric sensor array data requires a classification algorithm that uses the full dimensionality of the data. Herein, hierarchical cluster analysis (HCA),[10] which is a model-free method based on the grouping of the analyte vectors according to their spatial distances in their full vector space, is employed. Based on the clustering of the array response data in the 36 dimensional ΔRGB color space, dendrograms formed by HCA are depicted in ESI Figure S3. Remarkably, in quadruplicate experiments, 20 natural amino acids at 100 μM and a control are accurately identified against one

another with no error or misclassifications out of 84 cases. Moreover, principal component analysis (PCA)[11] was also performed to provide further evidence for the array's identification capability. As can be seen in Figure 2, a two-dimensional plot is obtained with excellently separated the 20 natural amino acids, i.e. every amino acid locates in a particular area.

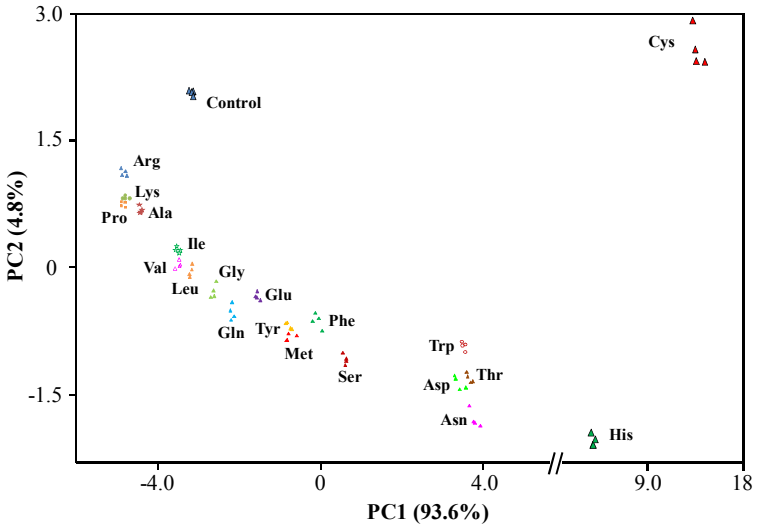

Figure 2. Two-dimensional principal component analysis plot of the array for the 20 natural amino acids at 100 μM and a control. All of the experiments were performed in quadruplicate in aqueous solution at neutral pH (10 mM HEPES buffer at pH 7.2).

Array's responses to the 20 natural amino acids at 50 μM. To further evaluate the capability of the array in the recognition of the 20 natural amino acids, their lower concentrations (i.e. 50 μM) were as well as investigated. ESI Figure S4 shows the colorimetric response difference maps of the array to the 20 natural amino acids, and these difference maps again provide unique patterns that can effectively identify all the amino acids at this concentration. The HCA and PCA (see ESI Figures S5 and S6) results are similar with that of at 100 μM, i.e. all amino acids and a control are accurately identified against one another with no error or misclassifications out of 84 cases (HCA), and locate clearly in particular areas (PCA).

Array's discrimination capability to the 20 natural amino acids. The response of a colorimetric sensor array depends primarily on equilibrium interactions between the indicators and the analytes. Consequently, different concentrations of the same analyte present different array responses. By combining the data sets at 50 and 100 μM, we can distinctly differentiate the array responses to the same analytes at different concentrations. The HCA for the full set of database at 50 and 100 μM (ESI Table S1) indicates that the array can accurately identify all the amino acids at 50 and 100 μM against one another except Cys, which is believed due to reaching the equilibrium interaction between Cys and the array at 50 μM (Figure 3). All the above described results of statistical analysis (HCA and PCA) demonstrate that the as-fabricated colorimetric sensor array can be effectively applied for identification and discrimination of the 20 natural amino acids at 50 and 100 μM.

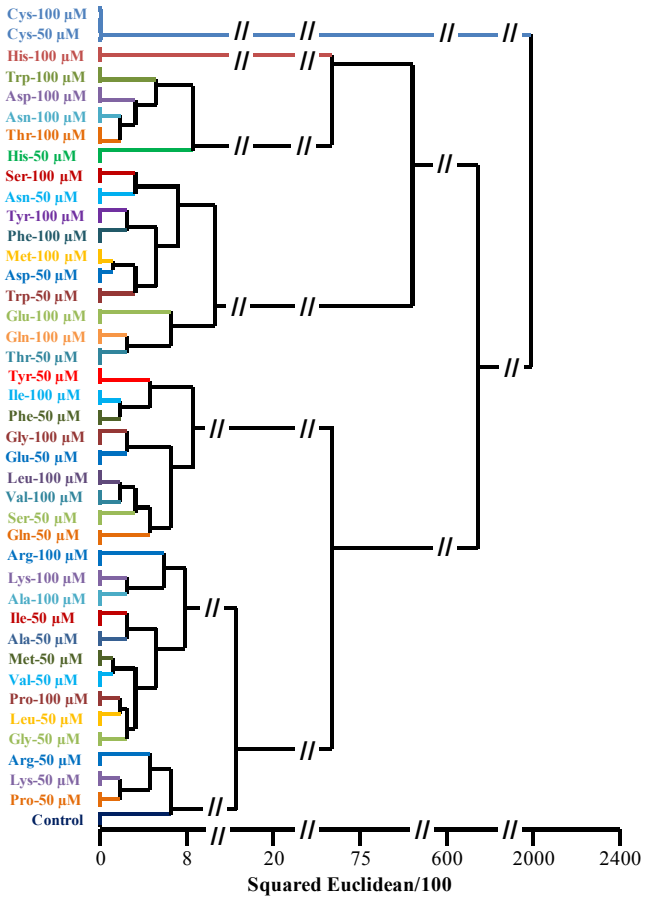

Figure 3. Hierarchical cluster analysis for 20 natural amino acids at 50, 100 μM and a control. All of the experiments were performed in quadruplicate in aqueous solution at neutral pH (10 mM HEPES buffer at pH 7.2).

Array's sensing properties to a single natural amino acid. In colorimetric sensor array assay, each concentration of an analyte generally has a separately unique pattern. Color change profiles for 20 amino acids, His and Cys are taken as examples, as a function of concentrations are further observed. As a result, His and Cys at all the investigated concentrations exhibit different recognition patterns except Cys at 50-100 μM (*vide supra*, ESI Fig. S7). HCA shows that His at 5, 10, 20, 30, 50, 80, 100 μM and a control are accurately identified against one another with no error or misclassifications out of 32 cases (Figure 3). Moreover, PCA reveals that all the investigated concentrations of His and a control locate well in particular areas in the plot (ESI Figure S8). We next examined the relationship between the color changes of the array (illustrated by the total Euclidean distances (ED), i.e. square root of the sums of the squares of the ΔRGB values) as a function of His concentrations. As shown in Fig. 4, the ED increases gradually with the concentrations of His increasing, and a nice sigmoidal fitting curve can be obtained.

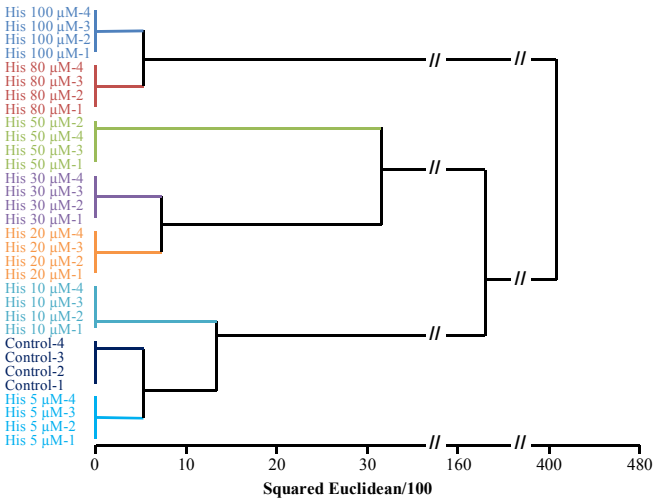

Figure 4. Hierarchical cluster analysis for His at different concentrations and a control. All of the experiments were performed in quadruplicate in aqueous solution at neutral pH (10 mM HEPES buffer at pH 7.2).

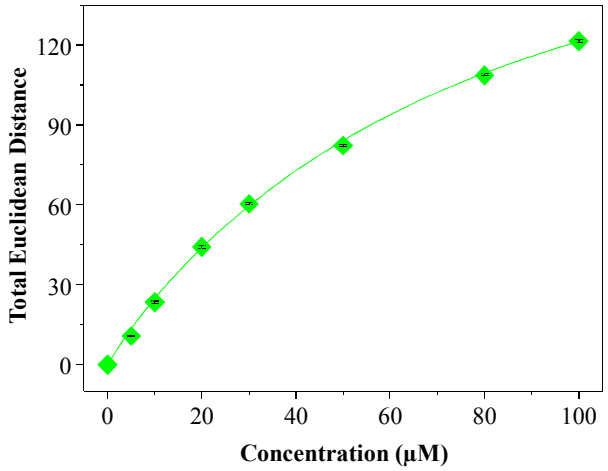

Figure 5. The total Euclidean distances of the array plotted versus different concentrations of His. All of the experiments were performed in quadruplicate in aqueous solution at neutral pH (10 mM HEPES buffer at pH 7.2); the error bars shown are the standard deviation of quadruplicate experiments.

The responses of the array for different concentrations of Cys are similar with that of for His (but only at a smaller concentrations range of 0-50 μM, and higher concentrations of 50-100 μM are highly alike due to reaching the array's equilibrium interaction), i.e. HCA shows 5, 10, 20, 30 and 50 μM of Cys and a control are accurately identified against one another with no error or misclassifications out of 24 cases (ESI Figure S9); PCA displays 5, 10, 20, 30, 50 μM of Cys and a control locate well in particular areas in the plot (ESI Figure S10); and the color changes of the array (total ED) increase gradually with Cys concentrations increasing from 0 to 50 μM, and a

nice sigmoidal fitting curve is as well as obtained (SI Figure S11). These results indicate that the as-fabricated colorimetric sensor array may be employed for recognition of the 20 amino acids in a broad range of concentrations, and even allowing for a semi-quantitative analysis (based on the corresponding fitting curve between the color changes of the array (total ED) and the concentrations of an amino acid).

Conclusions

In conclusion, we have successfully developed a very facile approach for the design and fabrication of a colorimetric sensor array through taking only a single indicator-receptor couple (CV-Cu^{2+} herein) with various ratios and concentrations. As a proof-of-concept application of this design strategy, discrimination and identification of the 20 natural amino acids in aqueous solution at neutral pH are successful. Classification analyses (HCA and PCA) demonstrate that the as-fabricated colorimetric sensor array has a high dimensionality and, consequently, has a capability for recognizing among the 20 natural amino acids. Moreover, qualitative and semi-quantitative detection of the amino acids, e.g. His and Cys, are able to be realized through combining HCA/PCA, recognition patterns and corresponding fitting curves. The developed strategy in this current study represents probably a “maximally” simplified approach for design and fabrication of colorimetric sensor arrays, and thus allowing for taking their full advantages in sensing community. Further applications of this strategy such as for peptides and proteins detection are undergoing in our laboratory.

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Notes and references

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†Electronic Supplementary Information (ESI) available: Chemical structures of the 20 natural amino acids; Colorimetric difference maps; HCA; PCA; database for 20 natural amino acids at different concentrations and a control, Figure S1 to S11 and Table S1. See DOI: 10.1039/c000000x/

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