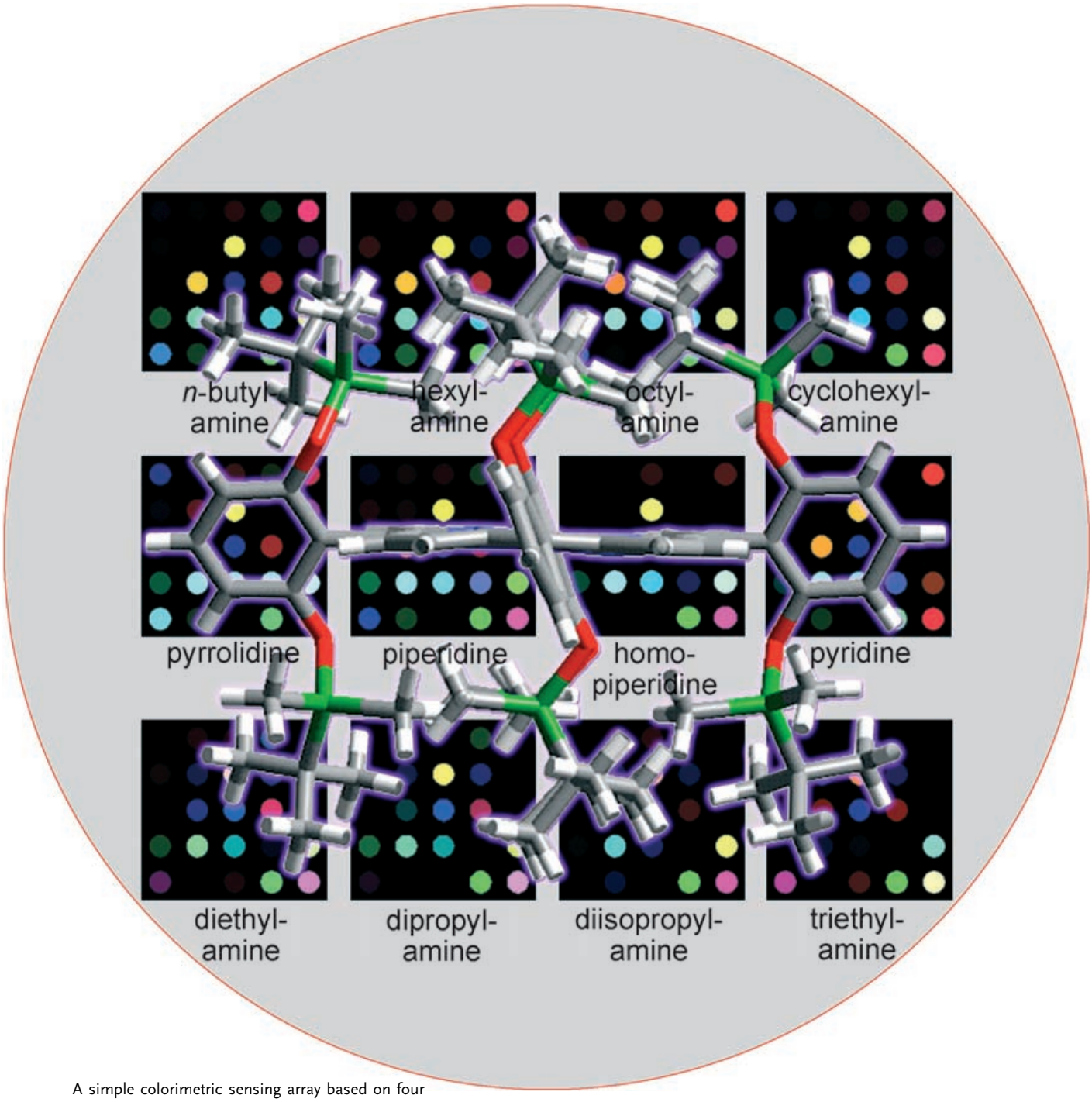
Communications



A simple colorimetric sensing array based on four different types of molecular recognition allows the discrimination of functionalized organic vapors. Sensitivities well below 1 ppmv have been observed for amines and the technique even allows the discrimination of isomers. More about this approach can be found in the Communication by K. S. Suslick and co-workers on the following pages.

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Sensors

Molecular Recognition and Discrimination of Amines with a Colorimetric Array**

*Neal A. Rakow, Avijit Sen, Michael C. Janzen, Jennifer B. Ponder, and Kenneth S. Suslick**

Electronic nose technology for the generalized detection of volatile organic compounds (VOCs) has generally employed arrays of sensors based either on adsorption into a set of polymers or on oxidations at a set of heated metal oxides.[1,2] While such systems generally allow for distinction between analytes of different chemical functionality, the discrimination of compounds within a given chemical class remains a challenging goal. We have previously reported the colorimetric array detection of a wide range of odorants using a family of metalloporphyrins immobilized on reverse-phase silica[3] and on hydrophobic membranes.[4] Here we report the use of an expanded colorimetric array detector[4] that is capable of the highly sensitive and highly selective discrimination of amines.

Volatile amines are common by-products of rapidly growing cells. As a consequence, the human nose is well-adapted for the detection of amines, with sensitivities approximately 10^4 higher for these compounds than for most alkanes, alcohols, or ketones.[5] For example, cadaverine and putrescene are bacterial metabolic products relevant to food freshness,[6] isobutylamine is diagnostic of bacterial vaginosis,[7] and aniline and *o*-toluidine are biomarkers in lung cancer.[8] While sensitive detection of individual amine compounds has been previously reported,[9] the selective discrimination within a larger family of amines is not easily achieved. Previous approaches to selectively discriminate between amines include sensors based on molecular imprinting,[10,11] functionalized mesoporous silica,[12] and chromogenic reagents.[13] Amines span a wide range of molecular shapes, sizes, and electronic properties; molecular recognition of these compounds poses a unique scientific challenge with important technological consequences.

Molecular recognition relies on the ability to differentiate on the basis of the chemical properties of analytes. Four families of chemically responsive dyes were incorporated into a colorimetric sensor array (Figure 1 and see the Supporting Information) to probe a wide region of "chemical properties space": 1) a series of various metalated tetraphenylporphyrins was used to differentiate analytes on the basis of Lewis acid/base interactions (namely, metal-selective coordination); 2) bis-pocketed Zn porphyrins[14] were used to differentiate on the basis of the size and shape of the analyte; 3) pH acid/base indicators (for example, methyl red and Nile red) were used to differentiate on the basis of Brønsted basicity; and finally 4) highly solvatochromic dyes (for example, Reichardt's ET_{30} betaine dye) were used to indicate the polarity of the analyte. Both the dyes and substrate were chosen to be hydrophobic; as a consequence, changes in humidity over a wide range (10 to >95% relative humidity) do not affect either the initial color of the dyes or the response of the array to analyte vapors.

Responses to a set of very closely related analytes were recorded to provide a stringent test of the molecular recognition by this sensor array: specifically, 12 amines comprising linear, branched, and cyclic structures of similar molecular weight were chosen to provide subtle distinctions in electronic structure and molecular shape (Figure 2). As seen, each amine gives a unique color-difference map. The closest analyte pairs were, not surprisingly, *n*-butylamine and *n*-hexylamine. Even here, however, the array responses are distinct even by eye, and statistical comparisons shows that the variance (that is, the sum of the squares of the differences between the changes in red, green, and blue (RGB) values) among multiple runs of hexylamine is 4.5-fold less than the variance between *n*-hexylamine and *n*-butylamine.

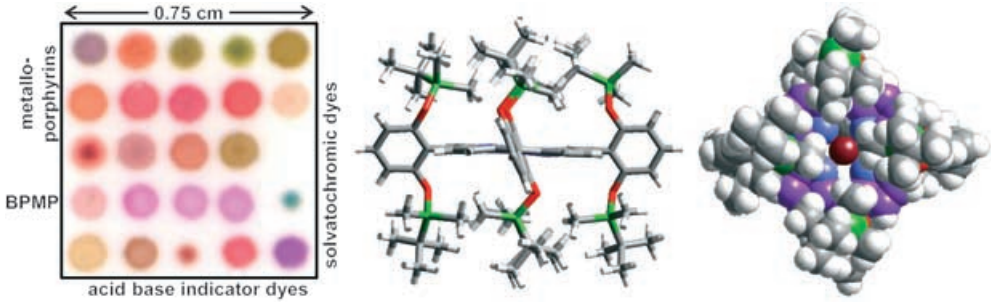

Figure 1. Colorimetric sensor array and the structure of the bis-pocket Zn porphyrin[9a] 5,10,15,20-tetrakis(2′,6′-bis(*tert*-butyldimethylsilyloxyl)phenyl)porphyrinatozinc(II), shown in framework side view (center) and space-filled top view (right). BPMP = bis-pocket metalloporphyrins.

[*] Dr. N. A. Rakow, Dr. A. Sen, Dr. M. C. Janzen, J. B. Ponder, Prof. K. S. Suslick
School of Chemical Sciences
University of Illinois at Urbana-Champaign
601 South Goodwin Avenue, Urbana, Illinois 61801 (USA)
Fax: (+1) 217-333-2685
E-mail: ksuslick@uiuc.edu

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

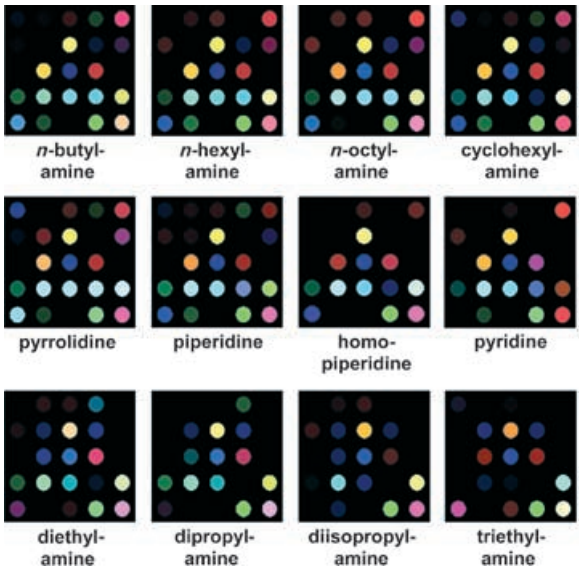

Figure 2. Color-difference maps for a family of 12 amines. Maps are generated from the absolute values of the differences of the red, green, and blue values of each dye spot before and after equilibration with the analytes; saturated analyte vapor at 20 °C. The images shown are in eight bit color expanded from five bit.

The images are digitally obtained and thus detailed statistical analysis of the difference maps is straightforward (all digital data and statistical analyses are provided as Supporting Information): each analyte is represented as a 72-dimensional vector (namely, the red, green, and blue difference values of 24 dyes). Principal component analysis (PCA) provides a quantitative evaluation of the analytical dispersion (that is, selectivity) of a technique based on its number of independent dimensions of discrimination.[11] Other techniques for electronic nose technology generally have very few independent dimensions (typically only two or three for 99% of the total discrimination, with a single dimension highly predominant (>90%)), and consequently only have very limited ability to discriminate between similar analytes.[2] In contrast, because the colorimetric dye array probes a wide range of intermolecular interactions, we observe a very high level of dispersion with the colorimetric dye array. When PCA is applied even to this family of closely related analytes, there are six dimensions necessary for 90% discrimination, eight for 95%, and ten for 99% (Figure 3).

A hierarchical cluster analysis[15] (HCA) was performed and a response dendrogram generated to examine the multivariate distances between the analyte responses in this 72-dimensional RGB color space (Figure 4). Remarkably, the dendrogram is consistent with the qualitative structural and electronic properties of the amines: the branched amines produce one cluster, linear amines another, the cyclic structures are tightly associated, and the lone aromatic amine (pyridine) separate from any of the other clusters. In contrast to prior electronic nose technologies, the colorimetric array is able to easily resolve very similar compounds and even structural isomers. For example, the C_6 amines (*n*-hexylamine, homopiperidine, dipropylamine, diisopropylamine, and triethylamine) have clearly distinct color patterns.

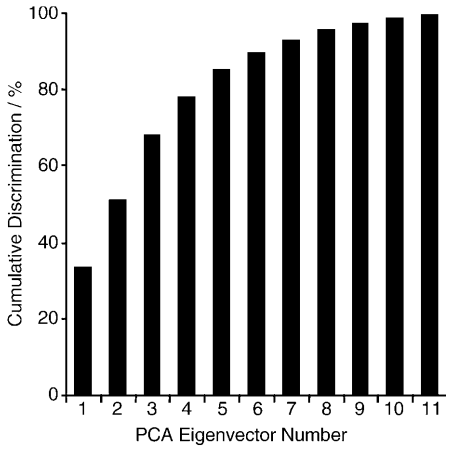

Figure 3. Principal component analysis from 12 very closely related amines shows that the 24-dye sensor array has a very high level of dispersion.

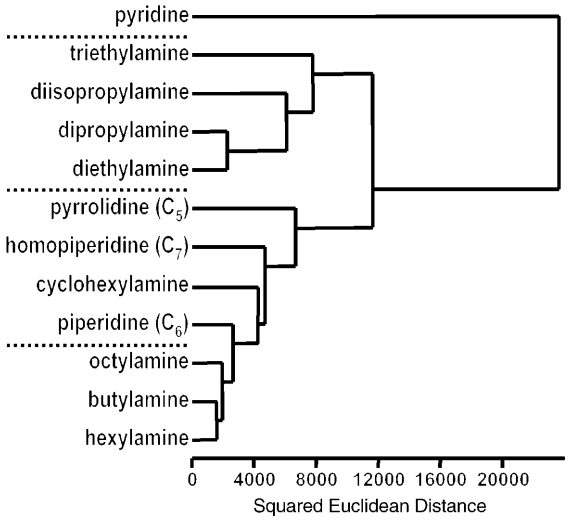

Figure 4. Dendrogram representation of the hierarchical cluster analysis of amines at their saturation vapor pressure from HCA (minimum variance).

The origin of this high level of discrimination lies in the broad range of chemical properties space being probed by the colorimetric array.

In addition to excellent analyte discrimination, high sensitivity to odorants is essential for practical applications. these amine analytes can be readily distinguished at concentrations well below 1 ppmv by using the array. Color-difference maps for a series of amines as a function of concentration are shown in Figure 5, where vivid and easily distinguishable responses are even seen at 600 ppbv. It is important to recognize that the same analyte at different concentrations gives different color change patterns: some dyes change color at low concentrations and then saturate, others do not begin to change until high concentrations. Quantitative analysis is therefore most easily accomplished by quantitative comparison to a library of analyte patterns at various concentrations (see the Supporting Information). Given the magnitude of the color changes observed at

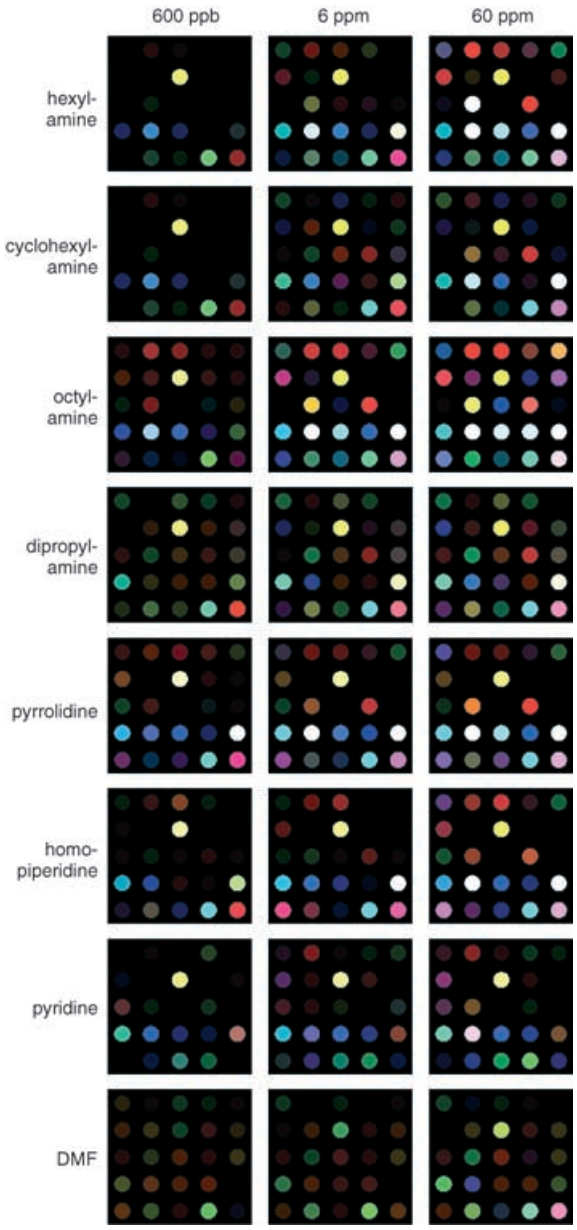

Figure 5. Color difference maps for a family of amines at 600 ppbv, 6 ppmv, and 60 ppmv at 20 °C. The images shown are in eight-bit color expanded from four bit.

600 ppbv, it is clear that detection at even lower concentrations is possible. Indeed, the limits of detection (3 × signal/noise) for several amines are well under 100 ppbv. Even GC-MS techniques typically achieve only 100 to 1000 ppbv sensitivities for such compounds in the absence of preconcentration. In many cases, these colorimetric arrays are in fact more sensitive than the human nose.[4,5] The origin of this high sensitivity to amines is based on strong and relatively specific interactions (for example, metal ligation): this is in marked contrast to previous electronic nose technologies that rely on weak, nonspecific interactions (for example, physical adsorption onto metal oxides or into polymers). Interestingly, it has been argued recently that the active site of many mammalian olfactory receptors are metalloproteins.[16]

A second 36-dye sensor array was designed without any bis-pocketed metalloporphyrins to test the role of bis-pocketed Zn porphyrins in selective differentiation between closely related amines. The new 36-dye sensor array contained various nonpocketed metalated tetraphenylporphyrins, pH acid/base indicators, and solvatochromic dyes. HCA of the response of 29 amines reveals a clustering of the aromatic and aliphatic amines into distinct branches. Class differentiation between primary, secondary, tertiary, and cyclic amines is reduced (see the Supporting Information), although there remains no possibility of confusion among individual amines. PCA shows our 36-dye array has an extraordinarily high level of dispersion: 11, 14, and 21 dimensions are required to define 90, 95, and 99% of the total variance, respectively.

In summary, we have demonstrated the facile discrimination of a range of functionalized organic vapors by using a simple colorimetric sensing array. Sensitivities well below 1 ppmv have been observed for amines, and in other work, thiols, phosphines, and carboxylic acids. Even distinction between isomeric amines is readily feasible by incorporating dyes that allow for distinction based on metal coordination, molecular size/shape, analyte acidity/basicity, and polarity.

Experimental Section

Methods: A 24-dye colorimetric array[3] composed of metalloporphyrins, free-base porphyrins, pH indicators, and solvatochromic dyes were spotted onto reverse-phase silica gel (Whatman KC2, 200 μm thickness, 350 $m^2 g^{-1}$ surface area) using 0.1 μL microcapillary tubes and employed for the analyses described herein (Figure 1 and see the Supporting Information). The 36-dye arrays used for data given in the Supporting Information were spotted on hydrophobic membranes and are commercially available from ChemSensing, Inc. (www.chemsensing.com, Array no. 031). Images were obtained at 1200 dots per inch in RGB color mode using an ordinary flatbed scanner (Epson Perfection 1200S). Color-difference maps were obtained from the scanned RGB images by digitally subtracting the image before exposure to analytes from the image after exposure, using a 314-pixel average from the center of each dye spot (thus avoiding subtraction artifacts at the periphery of the spots). Statistical analysis was performed with MVSP 3.1 (Kovach Co.). Gas streams containing the vapor of interest at known vapor pressure[17] were generated by flowing nitrogen through the neat liquid analyte in a thermostated, glass-fritted bubbler. To vary analyte concentrations, serial dilution in nitrogen using MKS digital mass-flow controllers was utilized. In all cases, full equilibration of the array by the analyte vapor was confirmed by monitoring images versus time. All liquid analytes were obtained from Aldrich or Fisher Scientific and used as received.

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