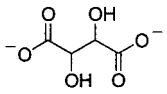
Multicomponent Sensors

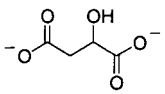
A Multicomponent Sensing Ensemble in Solution: Differentiation between Structurally Similar Analytes**

Sheryl L. Wiskur, Pierre N. Floriano, Eric V. Anslyn, and John T. McDevitt**

One current focus of the field of molecular recognition is to obtain synthetic receptors that rival the affinity and selectivity of natural receptors, such as antibodies or enzymes.[1] While many research groups have had partial success in this endeavor,[2] synthetic receptors are inherently much simpler than their biological counterparts. Therefore, it is quite difficult to achieve a very high degree of selectivity by using synthetic receptors. The difficulties arise both from the challenges of achieving complementary receptors, and from the need to complete time-consuming organic-synthesis routes to make the structurally complicated hosts. As an example, one would expect it to be very difficult to create two different synthetic receptors, each that will bind only one of the two structurally similar guests, such as tartrate and malate.



tartrate



malate

For many applications a single highly selective receptor for each guest is not required.[3] Instead, the goal is to identify the presence of the analytes, with or without quantification of their concentrations. In fact, nature does not use highly selective receptors for each analyte in our senses of taste and smell, and instead uses an array of "differential" receptors.[4] The response from each of these receptors for a particular mixture of stimuli creates a pattern that is stored in the brain. Because the receptors are not highly selective, they are analogous in many ways to synthetic receptors. We have proposed that the combination of differential receptors with pattern recognition may prove to be advantageous in a sensing application where identification and quantification are the main goals.[5] Herein we report that when colorimetric sensors are used, several wavelengths from a solution phase UV/Vis spectrum can serve as the multiple data inputs,

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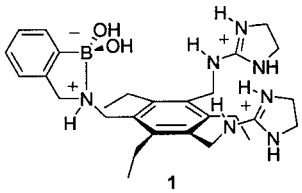
analogous in some respects to array derived information, and the absorbance at each wavelength can be used to create the diagnostic pattern. Unlike conventional array-based systems, which require spatial localization of receptor and/or signaling agents, the present approach uses homogeneous solutions wherein a series of structurally diverse receptor/indicator combinations and their unique spectral features serve to report their local environment changes. In the particular application described herein, we demonstrate that a combination of two receptors, neither of which is very selective, with pattern recognition protocols allows one to achieve a high degree of selectivity in differentiating structurally similar guests. This method obviates the need for complex-specific receptors, making the inherent simplicity of synthetic receptors actually an advantage.

Two previously reported hosts (**1** and **2**), both with affinities for tartrate and malate, were chosen for this study.[3,6] The incorporation of boronic acid binding sites imparts affinity to vicinal diols,[7] while the use of guanidinium groups imparts affinity to carboxylates.[8] Receptor **1** was previously found to have a similar affinity for tartrate and malate,[6] while for receptor **2**, the combination of two boronic acids and one guanidinium was determined to have a greater affinity for tartrate over malate.[9]



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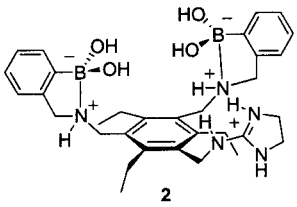
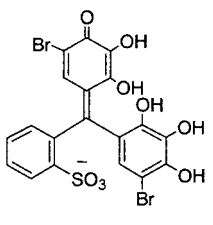


2

We have published numerous examples of the use of indicator-displacement assays for single-analyte sensing.[10] The indicator is first allowed to associate with the host, and upon addition of an analyte the indicator is displaced from the cavity, which results in a colorimetric or fluorimetric change. This same method of signaling is chosen for this multianalyte study. To further exploit the different characteristics of **1** and **2**, two indicators with different affinities for **1** and **2** were chosen. By design, the indicators were chosen so as to exhibit large differences in their wavelength maximums so that their spectral response would be spread over a large wavelength axis. The two indicators chosen were bromopyrogallol red (**3**) ($\lambda_{max} = 567$ nm) and pyrocatechol violet (**4**) ($\lambda_{max} = 445$ nm). Figure 1 shows the change in absorbance of a mixture of **3** and **4** as increasing amounts of **2** are added (75% methanol in water (v/v)). A similar, but reproducibly different response is found upon adding **1**.



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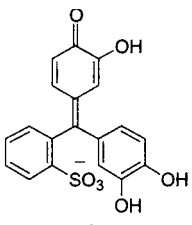

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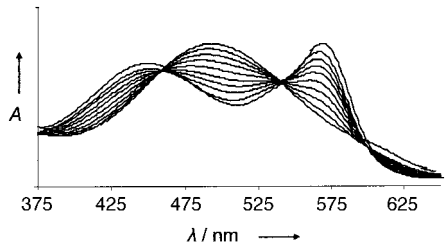

Figure 1. The UV/Vis spectra of **3** (30 μM) and **4** (60 μM) upon adding increasing amounts of **2** (75% methanol in water (v/v), pH 7.4, 10 mM HEPES buffer).

The training set data for the differentiation and quantification of tartrate and malate was created by the analysis of a four component ensemble of hosts and indicators in solution (150 μM of **1**, 150 μM of **2**, 30 μM of **3**, and 60 μM of **4**). Likewise, the UV/Vis spectra were obtained upon addition of various amounts of tartrate and malate, while the concentrations of the hosts and indicators were kept constant. The concentrations of tartrate and malate were altered in 0.2 mM increments ranging between 0 and 1.2 mM, thus resulting in 49 distinct spectra (Figure 2 shows a schematic of the matrix of scans). One representative example of the spectral difference found for the binding of different ratios of tartrate and malate to the two receptors is given in Figure 3. The UV/Vis spectra shown were both taken at a total analyte concentration of 0.8 mM, but for one spectrum the concentration of tartrate was greater than in the other (0.2 mM versus 0.6 mM). These

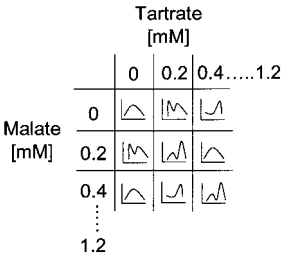

Figure 2. A representation of one experiment performed, where spectra were obtained at various concentrations of tartrate and malate.

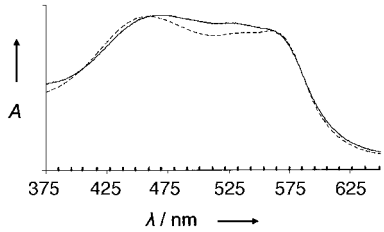

Figure 3. The UV/Vis spectra of the dye-displacement assays formed from the mixture of the indicators **3** (30 μM) and **4** (60 μM) and the receptors **1** (150 μM) and **2** (150 μM) upon the addition of tartrate and malate in various concentrations [(-----) Tartrate (0.6 mM) and malate (0.2 mM)] and [(——) Tartrate (0.2 mM) and malate (0.6 mM)] (75% methanol in water (v/v), pH 7.4, 10 mM HEPES buffer). The inside marks on the *x* axis are representative of the 27 wavelengths chosen for analysis.

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two UV/Vis traces demonstrate that the combination of two differential receptors differentiates mixtures of these two analytes. The reproducible variation in absorbance found for the pure samples and various mixtures of tartrate and malate is the data used as the training set for the supervised learning of the pattern recognition algorithm as described below.

Multilayer perceptron (MLP) artificial neural networks (ANN)[11] were chosen for pattern-recognition analysis. The basic architecture of the MLP that was used here consists of an input, an output, and a hidden layer, which is where the data is processed (or learned) through a back propagation training algorithm. Once the network has been trained, inputs that were not in the original data set are added and the trained algorithm attempts to calculate the output.[12]

Of the 49 spectra obtained, 45 of these were used to train the data set by using 27 wavelengths from each spectrum, while the other four spectral data sets were left out as "unknowns." When the absorbances for the 27 wavelengths were entered for the four unknowns, an absolute error between 1 and 6% was obtained for the output concentrations for both tartrate and malate (Table 1). The good agreement between the internal (i.e. training set) and external data (i.e. unknown set) indicates that solution concentrations of tartrate and malate which normally would be difficult to distinguish with a single receptor and indicator can be identified, and that even the individual concentrations of each analyte can be determined in a single analysis.

Although the maximum error of only 6% would appear to be quite acceptable for the first trial, further attempts to decrease the level of error were completed through the incorporation of more training data. In the second round of data collection, the concentration of malate was held constant (0.2 mM), and the concentration of tartrate was varied between 0 and 1.2 mM moving in increments of only 0.05 mM. In this case, three spectra were withheld from the new training session and these spectra served as the "unknowns." With this training set, the error in the calculated concentration was reduced to consistently less than 2% for all three unknowns (Table 1).

Table 1: The results from the neural network analysis. [mM].

Training NN1	Real [malate]	Pred. [malate]	Real [tartrate]	Pred. [tartrate]
val. case no. 1	0.000	0.0731	1.00	0.971 (3%)
val. case no. 2	0.59	0.568 (3%)	0.80	0.791 (1%)
val. case no. 3	0.99	1.034 (4%)	0.22	0.212 (5%)
val. case no. 4	1.19	1.136 (5%)	1.00	1.013 (1%)
Training NN2	[malate]		Real [tartrate]	Pred. [tartrate]
val. case no. 1	0.2	–	1.00	0.995 (0.6%)
val. case no. 2	0.2	–	0.53	0.527 (0.0%)
val. case no. 3	0.2	–	0.24	0.238 (1.3%)

Pred. = predetermined; val. = validation.

In conclusion, we have shown that the use of differential receptors in concert with pattern recognition can serve as a powerful method for supramolecular chemists to differentiate between very structurally similar guests, thereby avoiding the need for the creation of large numbers of specific receptors. Furthermore, we extended indicator displacement assays to encompass multiple indicators and multiple receptors. This multianalyte-sensing approach allows for the use of a single-solution vessel (i.e. a cuvette) in conjunction with standard spectrophotometry instrumentation. We are currently exploring the limit in the number of analytes that can be confidently detected by using such arrayless solution-phase multicomponent sensing ensembles.

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[1] J. K. M. Sanders, *Pure Appl. Chem.* **2000**, *72*, 2265; A. P. Davis, R. S. Wareham, *Angew. Chem.* **1999**, *111*, 3160; *Angew. Chem. Int. Ed.* **1999**, *38*, 2978; P. D. Beer, P. A. Gale, *Angew. Chem.* **2001**, *113*, 502; *Angew. Chem. Int. Ed.* **2001**, *40*, 486.

[2] T. W. Bell, Z. Hou, *Angew. Chem.* **1997**, *109*, 1601; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1536; C.-T. Chen, W.-P. Huang, *J. Am. Chem. Soc.* **2002**, *124*, 6246; Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813; J. Chin, C. Walsdorff, B. Stranix, J. Oh, H. J. Chung, S.-M. Park, K. Kim, *Angew. Chem.* **1999**, *111*, 2923; *Angew. Chem. Int. Ed.* **1999**, *38*, 2756.

[3] S. L. Wiskur, E. V. Anslyn, *J. Am. Chem. Soc.* **2001**, *123*, 10109.

[4] S. Mierson in *Handbook of Olfaction and Gustation* (Ed.: R. L. Doty), Marcel Dekker, New York, **1995**, p. 597; R. F. Margolskee in *Handbook of Olfaction and Gustation* (Ed.: R. L. Doty), Marcel Dekker, New York, **1995**, p. 575.

[5] J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **2001**, *113*, 3212; *Angew. Chem. Int. Ed.* **2001**, *40*, 3118.

[6] J. J. Lavigne, E. V. Anslyn, *Angew. Chem.* **1999**, *111*, 3903; *Angew. Chem. Int. Ed.* **1999**, *38*, 3666.

[7] C. J. Ward, P. R. Ashton, T. D. James, P. Patel, *Chem. Commun.* **2000**, 229.

[8] B. Linton, A. D. Hamilton, *Tetrahedron* **1999**, *55*, 6027.

[9] S. L. Wiskur, J. J. Lavigne, A. Metzger, S. L. Tobey, V. Lynch, E. V. Anlsyn, unpublished results.

[10] S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne, E. V. Anslyn, *Acc. Chem. Res.* **2001**, *34*, 963.

[11] D. E. Rumelhart, *Parallel Distributed Processing*, MIT Press, Cambridge, **1986**; J. A. Burns, G. M. Whitesides, *Chem. Rev.* **1993**, *93*, 2583; P. A. Jansson, *Anal. Chem.* **1991**, *63*, 357A; P. N. Floriano, A. P. Goodey, A. S. Tsao, J. T. McDevitt, *Anal. Chem.* **2003**, submitted; A. Goodey, J. J. Lavigne, S. M. Savoy, M. Rodriguez, T. Curey, A. Tsao, G. Simmons, J. Wright, S.-J. Yoo, Y. Sohn, E. V. Anlsyn, J. B. Shear, D. P. Neikirk, J. T. McDevitt, *J. Am. Chem. Soc.* **2001**, *123*, 2559.

[12] J. Zupan, J. Gasteiger, *Anal. Chim. Acta* **1991**, *248*, 1; C.-S. Yin, Y. Shen, S.-S. Liu, Q. Yin, W.-M. Guo, Z.-X. Pan, *Comput. Chem.* **2001**, *25*, 239; C.-S. Yin, W.-M. Guo, S.-S. Liu, Y. Shen, Z.-X. Pan, L.-S. Wang, *Chin. J. Chem.* **2001**, *19*, 836; J. Meiler, R. Meusinger, M. Will, *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 1169; Y. Ni, S. Chen, S. Kokot, *Anal. Chim. Acta* **2002**, *463*, 305; F. M. Raymo, S. Giordani, *J. Am. Chem. Soc.* **2002**, *124*, 2004; A. P. de Silva, N. D. McClenaghan, *Chem. Eur. J.* **2002**, *8*, 4935.

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