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Pattern-Based Discrimination of Enantiomeric and Structurally Similar Amino Acids: An Optical Mimic of the Mammalian Taste Response

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The senses of taste and smell serve to detect myriad discreet and highly diverse chemical structures. Across the animal kingdom, the challenges inherent to chemical sensing are addressed by distinctly different mechanisms than those used to perceive continuously variable stimuli, such as electromagnetic wavelength or acoustic frequency. Organisms ranging from *Drosophila* to humans have evolved chemosensory strategies in which arrayed receptor proteins recognize analytes to provide differential interaction patterns that are interpreted as characteristic odors and flavors.[1] The implementation of this general principle in the design of synthetic sensors has recently been the focus of considerable effort. Good progress has been reported using both optical[2] and electrochemical[3] sensor arrays to generate diagnostic response patterns for chemoselective analysis. On the other hand, very few studies[3e] have applied this approach to chiral differentiation, which is a key feature of gustation and olfaction.[4] Herein, we report an enantioselective differential array comprised of optical sensors. The system employs a series of indicator displacement assays (IDAs) to discern hydrophobic α-amino acids with high enantio- and chemoselectivity, in a manner that parallels the response of the mammalian gustatory system.

Our laboratories and others have developed multicomponent optical sensors that function on the basis of indicator displacement signaling. This approach relies upon competition between an optical indicator and the analyte of interest for the binding site of a molecular receptor.[5] Primarily, IDAs have been used to create sensors specific for a particular substrate. In the context of differential array sensing, the modular nature of the approach may be exploited for the purposes of sensor diversification. The use of various combinations of indicators and receptors provides access to many distinct sensing ensembles that are easily organized into an array format using multi-well plate spectroscopy.

We have recently expanded the scope of IDAs by incorporating single chiral receptors to allow for the quantification of enantiomeric excess.[6] It was thus envisioned that a series of enantioselective IDAs could be arrayed to achieve multi-analyte differential analyses with discrimination on the basis of molecular structure *and* absolute configuration. In light of current interest in the gustatory response to α-amino acids,[2c,d,4a−c] and the use of their metal affinity in previous IDAs,[2d,5a−c,6c] we decided to target amino acids through a series of IDAs based on dynamic metal coordination. Such systems can be realized in aqueous solvents and generally exhibit good air stability and kinetic lability.

Preliminary screening experiments identified the Cu(II) complexes of bidentate N-donor ligands **1**−**3** and the catechol and salicylate-derived chromophores pyrocatechol violet (**PCV**), chromoxane cyanin R (**CCR**), and chrome azurole S (**CAS**) as promising receptors and indicators, respectively (Figure 1A). The selected indicators undergo large red shifts in their absorbance spectra upon metal coordination, thus providing a highly sensitive colorimetric output.

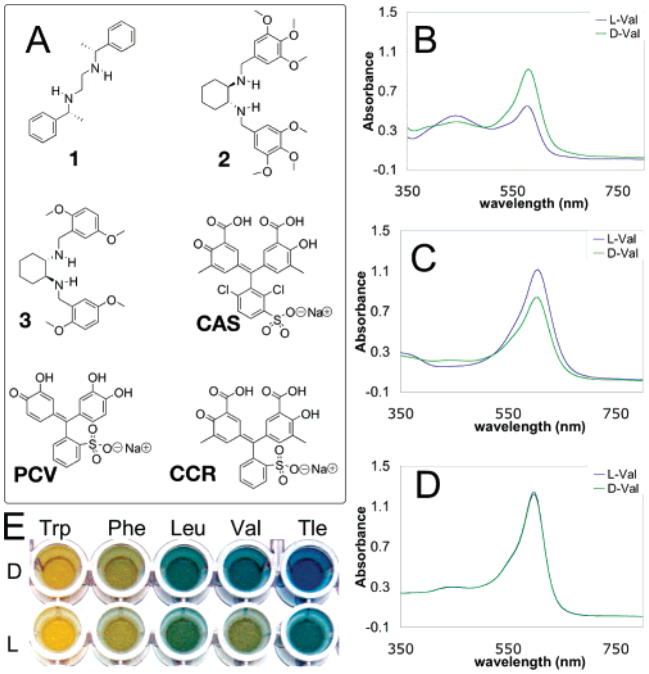

Figure 1. (A) Ligands and indicators used to construct sensor array. Absorbance spectra for (B) **1** [35 mM], $Cu(OTf)_2$ [157 μM], **CCR** [75 μM], and Val [200 μM]; (C) **3** [1.2 mM], $Cu(OTf)_2$ [393 μM], **CAS** [36 μM], and Val [2.5 mM]; (D) *N*,*N*′-tetramethylethylenediamine [4.5 mM], $Cu(OTf)_2$ [200 μM], **CAS** [55 μM], and Val [200 μM]. (E) Colorimetric output for **1** [35 mM], $Cu(OTf)_2$ [235 μM], **CAS** [35 μM], and amino acid [200 μM]. All studies carried out in 1:1 MeOH:H_2O, 50 mM HEPES buffer, pH = 7.8.

Taking different combinations of the ligands and indicators with $Cu(OTf)_2$ (OTf = trifluoromethanesulfonate) and varying the concentrations of the species, we created a library of IDAs. Both enantiomers of the naturally occurring amino acids Leu, Val, Trp, and Phe, as well as the unnatural amino acid *tert*-leucine (Tle), were examined, giving a total of 10 analytes. For each analyte, absorbance spectra were recorded under a set of 21 different conditions (see Supporting Information). To ensure reproducibility, each experiment was performed in triplicate. Absorbance spectra produced by D- and L-Val in two different IDAs are shown in Figure 1B and C. The output of a control IDA using an achiral diamine ligand is shown in Figure 1D. The sense of enantioselectivity exhibited in each IDA was found to be a general property of the chiral receptor, with complexes **1**−Cu(II) and **2**−Cu(II) preferring L configurations and **3**−Cu(II) favoring D. The colorimetric responses of an IDA involving **CAS** and **1**−Cu(II) to each analyte are shown in Figure 1E. Because free **CAS** is yellow in color and Cu(II)-bound **CAS** is blue, the ratio of bound to free indicator, which depends on the stability of the receptor-amino acid complex, can be assessed by visual inspection. All of the IDAs

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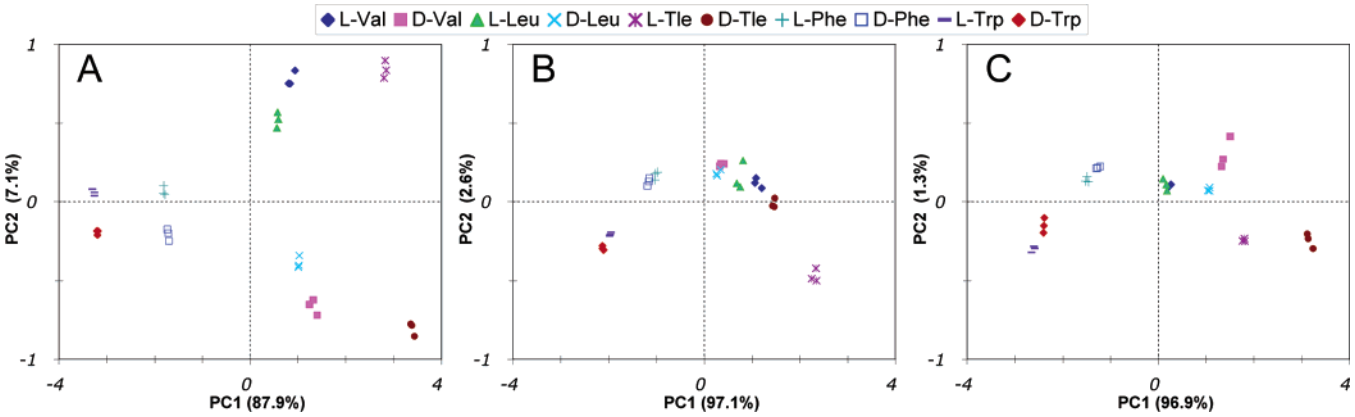

Figure 2. Two-dimensional PCA plots for D and L amino acids prepared (A) from data for all 21 enantioselective indicator displacement assays (IDAs), (B) from data for 8 IDAs selective for D amino acids, and (C) from data for 13 IDAs selective for L configuration.

conformed to the relative chemoselective ordering for complex stability of Trp > Phe > Leu ~ Val > Tle, which can be seen in Figure 1E.

To identify diagnostic patterns present in the array, principle component analysis[3d] (PCA) was performed using the entire dataset to give the two-dimensional plot shown in Figure 2A. The tight clustering of repetitious data relative to the separation of each amino acid demonstrates good spatial resolution. The aliphatic amino acids (Leu, Val, Tle), which vary only by side chain methylene groups, are clearly separated. Importantly, within each enantiomeric subset, all of the analytes are chemoselectively ordered along PC1, with scores inversely related to the sequence of affinities for receptor complexes. The chiral information is predominantly expressed along PC2, with negative scores for D amino acids and positive scores for L amino acids.

The near orthogonality of enantio- and chemoselective variance exhibited in Figure 2A arises from the use of sensors with opposite enantiomeric preferences. The chemoselective response for every sensor is qualitatively constant, but the enantioselective response varies depending on the chiral receptor used, and so PCA interprets each as a distinct component of variance. This rationalization is confirmed by splitting the dataset into two groups so that each group contains only data from sensors of a single enantiomeric preference. PCA of either group fails to separate chemo- and enantioselective information along two PC axes (Figure 2B and C). Instead, only one statistically relevant PC can be identified in each case, with analytes falling along this dominant PC1 axis in the established chemoselective order and with enantioselective sequence determined by receptor preference (i.e., preferred enantiomer assigned lower PC1 score). Thus, in the absence of oppositely biased enantiosensors, chemo and enantiomeric characteristics are combined in the output, and their distinction is lost.

The ability of the sensor array to orient enantiomeric variance along PC2 (Figure 2A) draws analogy to the human taste response, which for the natural amino acids studied, classifies tastants of D configuration (negative PC2 score, Figure 2A) as sweet and L configuration (positive PC2 score) as bitter.[4b,c] This response capability arises from the use of oppositely biased chiral receptors in the same array. It has been suggested that this same strategy is operational in human chemosensory systems,[4d] and this notion has recently gained support by the discovery that mammalian amino acid taste receptor proteins do in fact respond with opposite senses of enantioselectivity.[4a] More broadly, the present results show that by integrating oppositely biased enantioselective sensors into a differential array, variance describing chirality may be effectively isolated from chemoselectivity, and structurally similar and enantiomeric substrates may be simultaneously distinguished.

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Supporting Information Available: Experimental and data processing procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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