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A Simple Optoelectronic Tongue Discriminates Amino Acids

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Abstract: A self-assembled nine-element optoelectronic tongue consisting of a positively charged water-soluble poly(*para*-phenyleneethynylene) and three metal ions (Fe²⁺, Co²⁺, and Cu²⁺) at three different pH values (7, 10, and 13) discriminates all of the 20 natural amino acids in water. Unknown identification was not ideal. Addition of a highly positively charged green fluorescent protein in the presence of Fe²⁺, Co²⁺, and Cu²⁺ increased the unknown identification to above 86%. Linear discriminant analysis (LDA) orders the responses according to the amino acid type, that is, hydrophobic, polar, anionic, or cationic.

Sensing of simple and complex analytes by optoelectronic tongues has experienced an upswing due to the work of Anslyn and co-workers,^[1] Suslick and co-workers,^[2] and Severin and co-workers.^[3] They developed powerful concepts for the discrimination of analytes employing sensor fields. The main concepts are dye displacement, a modified lock-and-key approach or arrays of chemically different dyes; all concepts are useful for colorimetric discrimination of a wide variety of analytes, from, for example, red wine to coffee grounds.^[4] Rotello, Bunz and co-workers have employed electrostatic complexes composed of a fluorescent conjugated polymer and positively charged gold nanoparticle to discriminate proteins, bacteria, and cells.^[5]

We have recently started a program, in which we intensively examine chemical/molecular tongues composed of simple fluorescent conjugated polymers alone and with simple adjuvants (other polymers, detergents, peptide etc.) to discriminate and identify analytes, such as carboxylic acids, nonsteroidal *anti*-inflammatories, fruit juices, and white wines.^[6] In all of these cases, a small to medium sized array of fluorophores discriminates the analytes. The fluorescence response (increase of

intensity or quenching) of the single elements is not useful in itself; however, the combined responses, particularly when analyzed by statistical methods, such as linear discriminant analysis (LDA),^[7] allow the identification and discrimination of analytes—be they complex or simple.

An important question is the resolution of these tongues, that is, whether can they discriminate similar analytes. Herein, we tackle that problem and look at amino acids as suitable test beds. We found that poly(*para*-phenyleneethynylene)s (PPEs) or green fluorescent proteins (GFPs) themselves are not enough to discriminate; however, in the presence of different transition-metal ions, discrimination works well.

In a first experiment, we treated **P1** (Scheme 1) with the 20 naturally occurring amino acids (25 mM) at three different pH values (pH 7, 10, and 13); however, we could not find

Scheme 1. Structures of PPEs **P1** and **P2** used for sensing.

strong fluorescence change of amino acids with the PPE, with exception of the aromatic ones and proline, which induce some quenching of the PPEs' fluorescence (Figure 1). In a control experiment, we investigated the interactions of the 20 amino acids with three metal salts (Fe(ClO₄)₂, Cu(ClO₄)₂, Co(ClO₄)₂) in buffer of different pH (7, 10, 13). We found that only cysteine and histidine gave color changes, but none of the other ones (see Figure S1 in the Supporting Information).

Next, we investigated the response of **P1** towards aqueous solutions of metal salts, which are effective fluorescence quenchers,^[8] and we found, despite the fact that the PPE is positively charged, that the metal salts lead to a significant decrease in fluorescence intensity (Figure S2 in the Supporting Information). We assume that either the anions of the metal salts coordinate to the ammonium ions, and/or that the metal ions are coordinated to the branched oligoethylene glycol moieties, attached to the PPE.^[9]

Although neither the PPE nor the metal salts alone are useful for the discrimination of amino acids, the combination of one PPE with three different metal cations at three pH

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Figure 1. Fluorescence response pattern $((I-I_0)/I_0)$ of P1 (1 μM) at pH 7, 10, and 13 (buffered) and GFP-K72 (20 nM) at pH 7 (buffered) after treating with 20 natural amino acids (25 mM; buffer solutions: pH 7 ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$), pH 10 (borax/ NaOH), and pH 13 (KCl/NaOH)). Each value is the average of two independent measurements; each error bar shows the standard deviation (SD) of these measurements.

values generates useful signals (Figure 2). Thus, an optoelectronic tongue was constructed by mixing P1 and three metal

ions (Fe^{2+} , Co^{2+} , and Cu^{2+}) at three different pH values (7, 10, and 13) in water. Figure S5 in the Supporting Information

Figure 2. Fluorescence response pattern $((I-I_0)/I_0)$ of the P1 (1 μM) in the presence of different metal salts (1 mM), treated with solutions of the 20 natural amino acids (25 mM) at pH 7, 10, and 13. Each value is the average of six independent measurements; each error bar shows the standard deviation (SD) of these measurements. Structure of amino acids is shown in the bottom panel. The fluorescence intensities (I_0 or I) were recorded at the peak intensity at 460 nm with an excitation at 410 nm by using a plate reader.

shows a 3D plot of the first three canonical scores obtained by LDA, which converts the training matrix (fluorescence response patterns, nine complexes $\times$ 20 amino acids $\times$ 6 replicates) into canonical scores according to their shortest Mahalanobis distances. Mahalanobis distance, a multidimensional generalization of the idea of measuring how many standard deviations away a point is from the mean of distribution, is widely used in cluster analysis and classification techniques.^[10] The larger the difference between the means of the canonical group is, the better the predictive power of the canonical discriminant function in classifying the observations; all of the amino acids group into different classes. According to the amino acid type, hydrophobic, negatively charged, and aromatic amino acids all group very well. Only His is isolated and does not group with the positively charged amino acids Arg and Lys. A similar scenario results for the polar amino acids. Herein, glutamic acid (Gln) is not grouped with the rest of the polar amino acids Asn, Ser, and Thr. The last three “other” amino acids do not group at all. Starting from this nine-element tongue, we performed a two-stage screening process employing principal component analysis (PCA; see Figure S3 in the Supporting Information) to end up with a four-element tongue identifying all of the 20 amino acids. The four-element tongue identifies 77.5% of all of the amino acids when presented as unknowns (see Table S11 in the Supporting Information and Figure 3 a). To investigate the influence of the cationic moiety, we looked at metal-ion complexes of the Sw-PPE **P2** (Scheme 1) and found that it shows less discriminative power (Figure S7 in the Supporting Information).

Next question to be answered: what is the mechanism of the discrimination? Amino acids form stable metal-ion complexes, and these have been described.^[11] Herein, the quenched metal-ion–PPE complex was decomposed under differential fluorescence turn-on to generate the discriminative signal that led to the identification of the amino acids, by their complexing of the metal ions and releasing the PPE (for schematic illustration, see Figure S11 in the Supporting Information). Because the discrimination of unknowns with the four-element tongue is not satisfactory, we selected a green fluorescent protein (GFP-K72), which is fused to an unfolded positively supercharged polypeptide by genetic engineering,^[12] as a tongue element. Based on our recent experience, the fluorescence of GFP variants was quenched at strongly acidic or basic conditions. Thus, we investigated the GFP in the presence of metal ions (Fe^{2+} , Cu^{2+} , and Co^{2+}) at pH 7 (excitation and emission wavelengths were 480 and 514 nm, respectively); these three-elements tongue also discriminates 20 amino acids (Figure 3 b).

Although the discrimination of each tongue works quite well, the identification of unknowns is improved in the final combined tongue (GFP and **P1**) consisting of seven elements (**P1** four elements, GFP three elements; Figure 4); 69 of 80 unknown samples were correctly identified, representing an accuracy of 86.3% (Table S11 in the Supporting Information). In conclusion, we could identify and discriminate 20 different natural amino acids by using a simple PPE tongue, GFP tongue, or a combined GFP/PPE tongue, composed of a cationic PPE or a

Figure 3. (A) 2D Canonical score plot for the optimized PPE tongue obtained by an array of four elements. (B) 2D canonical score plot for GFP tongue (three elements) obtained by an array of GFP-K72 (20 nM) with Fe^{2+} (pH 7), Cu^{2+} (pH 7), and Co^{2+} (pH 7), 95% confidence ellipses were shown.

Figure 4. 3D canonical score plot for the combined tongue consisted of PPE tongue (four elements) and GFP tongue (three elements). Each point represents the response pattern for a single amino acid in the array.

supercharged polypeptide fused to GFP and several different metal cations at different pH values. The complexes are disrupted by the metal-binding amino acids, and the differential fluorescence turn on is achieved. In future, we will increase both the sensitivity but also the discriminative power of the tongue with respect to the identification and discrimination of amino acids. If performed at high speed and with high precision, it might be an attractive way to investigate and discriminate degradation products of polypeptides, which form a large part of the human proteome. Equally important is the future identification of peptide hormones by simple fluorescence arrays.

Experimental Section

Preparation of polymers **P1** and **P2**^[6a-e,13] has been reported previously. The number-average molecular weights (M_n) of **P1** and **P2** were estimated to be 1.4×10^4 and 1.0×10^4 with polydispersity values ($PDI = M_w/M_n$) of 3.9 and 2.2, respectively. Detailed method for obtaining the fluorescence response pattern, the experimental setup, titration, and linear discriminant analysis is provided in the Supporting Information.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: amino acids • linear discriminant analysis • optoelectronic tongue • poly(*para*-phenyleneethynylene)s • sensors

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