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An Optimized Sensor Array Identifies All Natural Amino Acids

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Supporting Information

ABSTRACT: Wet-chemical discrimination of amino acids is still a challenge due to their structural similarity. Here, an optimized self-assembled eight-member sensor array is reported. The optimized sensor array stems from the combination of elements of different tongues, containing poly(para-phenyleneethynylene)s (PPE) and a supercharged green fluorescent protein (GFP) variant. The responsivity of the sensor dyes (PPEs and GFP) is enhanced in elements that contain adjuvants, such as metal salts but also cucurbit[7]uril (CB[7]) and acridine orange; a suitable and robust eight element array discriminates all of the 20 natural amino acids in water at 25 mM concentration with 100% accuracy. The results group well to the amino acid type, i.e., hydrophobic, polar, and aromatic ones.

KEYWORDS: sensor array, amino acid, poly(para-phenyleneethynylene), cucurbiturils, acridine orange, green fluorescent protein

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RESULTS AND DISCUSSION

Figure 1 shows the structures of poly(p-phenyleneethynylene)s P1–P7, the macrocyclic host cucurbiturils (CB[n], n = 7 or 8), the dye acridine orange (AO), and the GFP variant GFP-K72 with a high positive net charge induced by recombinant fusion of an unfolded, supercharged polypeptide chain.19,27 Figure 2 displays the four starting arrays. Array 1 consists of a positively

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charged GFP-K72 in the presence of different metal cations at pH 7. Array 2 employs the positively charged P1 also in the presence of the metal cations, while arrays 3 and 4 are supramolecular arrays in which cucurbiturils[8] and PPEs in the presence of acridine orange and cucurbiturils[7] form complex fluorescence-responsive arrays.19 We note that the fluorescence of GFP-K72 or P1 was quenched by metal ions.

In arrays 3 and 4, cucurbit[n]urils (n = 7 or 8) are used for detection and recognition of amino acids as these interact with the CB host cavity.28–30 Array 3 and its function have been discussed.27 Array 3 using the larger CB[8] (vide infra) is not efficient for the discrimination of amino acids, and it will not be discussed in detail. In addition to our published array 2, arrays 1 and 4 impart additional selectivity to the array. Attempts to discriminate amino acids just with the cationic GFP were not very successful, but analogously to our published array 1, addition of metal salts unlocked the sensitivity of the GFP toward amino acid analytes. Here we also assume that the GFP forms a nonfluorescent complex with the metal salt, which is reversed by the addition of the analytes. While the used GFP is overall positively charged at pH 7, there will be still a significant number of negatively charged residues that coordinate to the metal salts.

The most remarkable array is the ternary one, composed of acridine orange (AO), P1, and CB[7]. Control experiments show that CB[7] enhances FRET between the AO and P1. Interestingly, both species bind to the cavity of CB[7], as shown by NMR titration experiments (Figures S4 and S5); in the case of AO, CB[7] forms a 1:1 complex with the dye (Figure S6). AO exhibits an emission peak at 530 nm; upon addition of CB[7], a blue shift to 510 nm occurs (Figures 3 and S6).31,32 Sensor elements were constructed through in situ assembly of the PPEs P1−P7, CB[7], and AO at a molar ratio of 1:2:1 (based on a per

Table 1. Results of Unknown Detection Using a LDA Algorithm

<table>
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<tr>
<th>Amino Acids</th>
<th>Number of Samples</th>
<th>Correctly Identified</th>
<th>Accuracy (%)</th>
<th>Correctly Identified</th>
<th>Accuracy (%)</th>
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<td>90</td>
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</tbody>
</table>

Figure 1. Chemical structures of poly(p-phenyleneethynylene)s P1−P7, cucurbiturils CB[7] and CB[8], tricyclic dye acridine orange AO, and green fluorescent fusion protein GFP-K72.

Figure 2. Fabrication of a multiple sensor array for the discrimination of amino acids.
repeat unit of the PPEs). Amino acids form hydrogen bonds with CB[7] and dyes due to the amine and carboxylate groups. Tryptophan (Trp) or histidine (His) displace AO or P1 from the cavity of CB[7], shutting down FRET and the emission of AO at 510 nm (Figure 3c and d) decreases. Figure 4 shows the proposed schematic illustration of PPE-CB[7]-AO tongue working with amino acids.

Figure 3. Fluorescence emission spectra of (a) P1, AO, the mixture of AO and CB[7] (1:1), P1 and AO (1:1), and P1, CB[7], and AO (1:2:1); (b) P1-CB[7] in the presence of different concentrations of AO (from 0 to 20 μM), [P1] = 2 μM, [CB]7 = 4 μM; changes in fluorescence intensity of P1-CB[7]-AO (2.0 μM/4.0 μM/2.0 μM) upon gradual addition of (c) Trp and (d) His (from 0 to 30 mM). All spectra were performed in water upon excitation at 410 nm.

As a control, we first treated P1−P2 and P5−P7 (Figure 1) with the 20 naturally occurring amino acids (25 mM). The results (Figure S7) indicate that the simple PPE tongue alone is useful for the discrimination of Tyr and Trp but does not discriminate the other amino acids with polar and hydrophobic residues. However, the PPE-CB[7]-AO assembly induces better sensitivity for these analytes (Figure S8). According to the two-dimensional linear discriminant analysis (2D-LDA; Figure 5d, Tables S3 and S4), the PPE-CB[7]-AO tongue discriminates all 20 amino acids. A more simple tongue omitting CB[7] (Figure S9) shows less discriminatory power.

In the next experiment, we tested all four tongues against the 20 amino acids. Figure 5 shows the 2D-LDA plots for the first two factors obtained by the individual sensor arrays 1−4, i.e. the GFP-metal salt tongue, the PPE-metal salt tongue, the PPE-CB[8] tongue and the PPE-CB[7]-AO tongue. The discrimination is fairly poor in the PPE-CB[8] sensor array, while the other arrays work quite well.

Figure S10 shows a two-dimensional canonical score plot obtained by 28 sensor elements; all of the 20 amino acids are reliably discerned. We then performed a screening process,
employing principal component analysis (Figure 6): This loading plot finds the elements most useful for discrimination and allows to remove weakly performing ones. Excellent discrimination results with a pruned eight-element tongue (all the elements that are marked with red in Figure 6) that identifies all 20 amino acids after LDA (for more details, see the Supporting Information, Figures S2, S3 and Tables S2, S5). None of the high performing elements came from the PPE-CB[8] tongue; control experiments show that the addition of AO does not improve signal of these array-elements (Figure S11).

The fluorescence modulation data of the pruned final tongue were recorded. LDA was performed and converted the training matrix (8 factors $\times$ 20 amino acids $\times$ 6 replicates) into canonical scores. The canonical scores are clustered into 20 different groups. The jackknifed classification matrix with cross-validation reveals a 100% accuracy (Figure S14 and Tables S6, S7). According to the amino acid residue, hydrophobic, polar and aromatic amino acids all grouped very well (Figure 7a). By zooming into a specific part, the discrimination of hydrophobic and polar amino acids becomes quite clear (Figures 7b and 7c). The testing was performed at 25 mM concentration of the amino acid. To see if we could lower the concentration we also investigated 10 mM solutions. We still discriminate the amino acids, but amino acids with hydrophobic and polar residues do not group well; especially Gln, Ser, and Thr are quite close to the hydrophobic amino acids (Figure 8 and Table S8). Solutions of 5 mM were also investigated; however, the discrimination is not satisfactory (Figure S12).

To validate the efficiency of the optimized sensing system, we performed tests with 80 randomly chosen amino acids. The new cases are classified into groups, generated through the training matrix, based on their shortest Mahalanobis distance to the respective group.34,35 We have used our old six-element metal salts based sensor array as comparison and 8 of 80 unknown samples of amino acids were misclassified, representing an accuracy of 90% (Table 1 and Table S9). In stark contrast, the identification of unknowns is improved to 100% when employing the final 8-element tongue (Table 1 and Table S10).

**CONCLUSIONS**

In conclusion, we have dramatically improved our PPE-based amino acid array by addition of further sensor elements. We have...
Figure 6. Loading plot of the principal component analysis plot by the four arrays, identifying the contribution of each element to an axis. The selected eight elements are labeled in red.

Figure 7. (a) Two-dimensional canonical score plot for the first two factors obtained by eight optimized sensor elements treated with 20 amino acids ($c = 25$ mM) with 95% confidence ellipses. Each point represents the response pattern for a single amino acid to the optimized array. Amino acids with hydrophobic, polar, and aromatic residues are given in blue, green, and pink, respectively. (b,c) Detailed view of the amino acids with polar and hydrophobic residues, respectively.
investigated four different arrays, plucked the best elements from three of the arrays, and created a new, much more powerful array, containing six elements of our old array and two additional elements gleaned from other tongues. We are currently aiming to lower the concentration of detectable amino acids and will investigate microfluidic-type approaches to identify and discriminate amino acids with our hypothesis free arrays. Overall, this is an encouraging development, which shows that simple tongues and sensor arrays discriminate tightly related analytes.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.8b00371.

General information, synthetic details and analytical data for P1−P7, detailed method for obtaining the fluorescence response pattern, experimental setup, additional screening, and linear discriminant analysis data (PDF)

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Author Contributions
The paper was written through contributions of all authors. All authors have given approval to the final version of the paper.

Notes
The authors declare no competing financial interest.

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REFERENCES

Figure 8. (a) Two-dimensional canonical score plot for the first two factors obtained by eight optimized sensor elements treated with 20 amino acids (c = 10 mM) with 95% confidence ellipses. Each point represents the response pattern for a single amino acid to the optimized array. Amino acids with hydrophobic, polar, and aromatic residues are given in blue, green, and pink, respectively. (b,c) Detailed view of the amino acids with polar and hydrophobic residues, respectively.
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