Pattern-Based Sensing with Metal−Dye Complexes: Sensor Arrays versus Dynamic Combinatorial Libraries

Sébastien Rochat and Kay Severin*

Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

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The dyes Methyl Calcein Blue, Arsenazo I, and Xylenol Orange, and the metal salts $CuCl_2$ and $NiCl_2$ were used to generate colorimetric sensors for peptides. Two different approaches were followed: (1) Sensors based on dynamic combinatorial libraries of metal−dye complexes were created by mixing dyes with metal salts in one pot. The optical response of these libraries was analyzed by measuring the spectral changes of the mixtures upon addition of the peptide analytes at six selected wavelengths. (2) Sensor arrays were created from six different metal−dye combinations. The six individual sensors were analyzed at one wavelength, and the resulting data was used as the input for a multivariate analysis. Both types of sensors were evaluated for their ability to differentiate 13 different di- and tripeptides. The sensors based on dynamic combinatorial libraries gave in most cases better results than the sensor array. Furthermore it was found that libraries of intermediate complexity perform best as sensors.

Introduction

A complex between a synthetic receptor and a dye can be employed as a chemosensing ensemble, given that the displacement of the dye by an analyte leads to a change in color or fluorescence. Sensors of this type are commonly referred to as indicator displacement assays (IDAs).[1] Transition metal complexes have been used very successfully as receptor units in IDAs.[2] In the presence of a dye and an analyte, a dynamic mixture of metal−dye and metal−analyte complexes is established (Figure 1a). Fluorescence or UV/vis spectroscopy can then be used to obtain information about the equilibrium, and thus about the identity and/or quantity of the analyte.

The analytical power of IDAs can be increased if they are performed in an array format.[3] In such an array, several IDAs are performed in parallel to each other, and the recognition of the analyte is then achieved by a pattern-based analysis of the response of the entire array. Sensor arrays of metal-based IDAs have been created by mixing different dyes with different metal complexes (Figure 1b),[4] or by utilization of one metal−dye combination at different pH values.[5,6]

Another way to increase the analytical power of displacement assays is to perform several IDAs simultaneously in one pot (Figure 1c).[7] This approach results in the formation of a dynamic combinatorial library (DCL)[8] of metal−dye complexes. The optical response of a DCL is likewise analyzed with pattern-recognition protocols.

The sensors described above have fundamental differences. In a sensor array, each metal−dye combination is analyzed separately by UV/vis or fluorescence spectroscopy. This measurement is typically done at the wavelength, where the largest changes are observed. For a DCL sensor, on the other hand, the information about the analyte is distributed over the entire spectrum, and the absorption (or emission) at multiple wavelengths is used as the data input for a pattern-based analysis. Consequently, it is sufficient to record a single UV/vis or fluorescence spectrum for a DCL sensor (once the sensor is calibrated). Sensor arrays and DCL sensors based on metal−dye complexes may also differ on the molecular level. Each sensing unit of an array will contain homoleptic complexes of type $(M)_n(Dye)_m$. A DCL sensor, on the other hand, may also contain heteroleptic complexes such as (M1)(Dye)(M2) or (M)(Dye1)(Dye2). These heteroleptic complexes can participate in displacement reactions with analytes and can help to differentiate them. DCL sensors therefore have two distinct advantages: only one optical measurement is needed and heteroleptic complexes can contribute to the analysis. However, there is also one drawback: since multiple IDAs are performed simultaneously

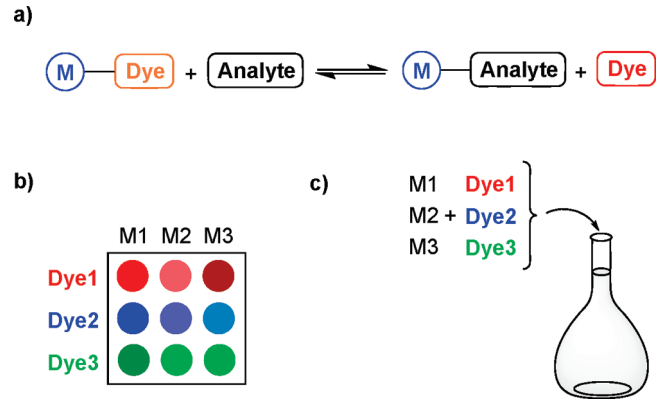

Figure 1. (a) Basic principle of a metal-based indicator displacement assay. (b) A sensor array based on the combination of different metal complexes with different dyes. (c) A sensor based on a dynamic combinatorial library of metal−dye complexes.

* To whom correspondence should be addressed. Fax: +41(0)21 6939305. Phone: +41(0)21 6939302. E-mail: kay.severin@epfl.ch.

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in one pot, it is likely that the spectra of the different dyes and of the metal−dye complexes show significant overlap. This spectral overlap is expected to result in some loss of resolution.

From the arguments outlined above it is clear that both type of metal−dye sensors, arrays and DCLs, have advantages and disadvantages. It thus appears interesting to perform a direct comparison of the two approaches for a particular sensing problem. The results of such an investigation are reported below. As a case study, we chose to compare the discriminating abilities of a sensor array and DCL sensors toward a series of di- and tripeptides composed of only four different amino acids. Interestingly, DCL sensors were found to perform significantly better than sensor arrays for most cases studied.

Experimental Section

Chemicals and Procedures. All the chemicals were commercially available, and used as received: Xylenol Orange sodium salt, NaOH (Acros), Methylcalcein Blue, Met-Gly, Gly-Leu, Gly-Met, Leu-Gly, Leu-Met, Met-Leu (Sigma-Aldrich), $CuCl_2(H_2O)_2$, Gly-Gly (Fluka), Arsenazo I (Alfa Aesar), CHES (AppliChem), $NiCl_2(H_2O)_6$ (Strem Chemicals), Gly-Gly-Met, Gly-Met-Gly, Met-Leu-Gly, Gly-Phe, Met-Phe, Met-Phe-Gly (Bachem). CHES buffer (286 mM, pH 8.4) was prepared with bidistilled H_2O and used for all experiments (the pH was adjusted by addition of NaOH). Stock solutions of the dyes (MCB: 0.60 mM; AI: 0.30 mM; XO: 0.15 mM), the metal salts ($NiCl_2$: 1.0 mM; $CuCl_2$: 1.0 mM), and the peptides (5.0 mM) were prepared in bidistilled water and stored at 4 °C. UV/vis measurements were performed at room temperature with a Perkin-Elmer Lambda 40 spectrometer.

Sensing of Peptides with DCL Sensors. Aliquots of stock solutions of Methylcalcein Blue (83.3 μL), Arsenazo I (83.3 μL), Xylenol Orange (83.3 μL), $CuCl_2$ (100 μL), $NiCl_2$ (100 μL), CHES buffer (350 μL), and the respective peptide (200 μL) were mixed in a UV/vis cuvette. The final concentrations were [MCB] = 50 μM, [AI] = 25 μM, [XO] = 12.5 μM, [$NiCl_2$] = [$CuCl_2$] = 100 μM, [peptide] = 1.0 mM, and [CHES] = 100 mM, and the final volume was 1.0 mL. The solution was equilibrated for 30 min at 60° C, cooled to room temperature, and its UV/vis spectrum was recorded between 332 and 700 nm. The experiment was repeated 10 times for each peptide. An analogous procedure was employed for DCL sensors of reduced complexity (smaller number of dyes and/or metal complexes). In these cases, the library components to be omitted were replaced by bidistilled water.

Data Analysis. The UV/vis data obtained for each DCL sensor were analyzed as follows: For each measurement, 93 data points (absorbance values in the region λ = 332−700 nm, with intervals of 4.0 nm) were used as input. To determine the wavelengths, which contribute most to the differentiation of the peptides, a variable selection algorithm was applied. Six wavelengths were selected for each data set. The selected variables were then utilized to calculate the LDA classification functions and to generate the score plots. The commercial software package Systat[9] (version 11) was employed for the variable selections, the linear discriminant analyses, and the calculation of the *F*-values.

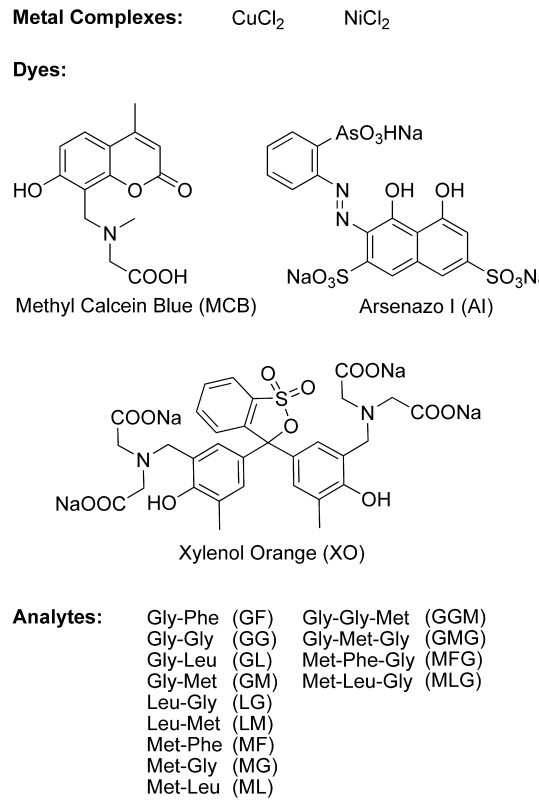

Figure 2. Metal complexes, the dyes, and the peptide analytes used in this study.

Sensing of Peptides with the Sensor Array. First, we have determined the wavelengths, at which the largest spectral changes occurred for each metal−dye combination. For that purpose, the UV/vis spectra of buffered aqueous solutions containing the free dye were compared with the spectra of solutions containing a mixture of MCl_2 (100 μM) and the dye (50 μM for MCB, 25 μM for AI, or 12.5 μM for XO). The largest differences were observed at the following wavelengths: MCB/Cu: 372 nm, AI/Cu: 492 nm, XO/Cu: 584 nm, MCB/Ni: 376 nm, AI/Ni: 532 nm, and XO/Ni: 592 nm. The assays were carried out in a similar manner as the measurements with the DCL sensors: mixtures of the respective dye (50 μM for MCB, 25 μM for AI, or 12.5 μM for XO), metal chloride (100 μM), and peptide (1.0 mM) in water (pH 8.4, CHES buffer) were heated at 60 °C for 30 min. After cooling to room temperature, UV/vis measurements were performed (10 independent measurements for each peptide).

Data Analysis. The six individual sensors were analyzed at the wavelengths given above. The absorbances for the 2 × 3 × 13 metal−dye−peptide combinations (10 replicates per combination) were used as input to calculate the LDA classification functions with Systat.

Results and Discussion

For our study we decided to use a colorimetric DCL sensor, which was recently developed by our group.[7a] It is composed of the dyes Methyl Calcein Blue (MCB), Arsenazo I (AI), and Xylenol Orange (XO), and the metal salts $CuCl_2$

and $NiCl_2$ (Figure 2). When the five components are mixed in buffered aqueous solution, a dynamic mixture of homo- and heteroleptic metal−dye complexes is formed. The sensor can be used for the colorimetric detection of peptides as evidenced by an analysis of the peptide hormones angiotensin I and angiotensin II.[7a]

For the creation of a sensor array, we used the same five components to make a total of six sensors composed of all possible metal−dye combinations. The readout of the six sensors was performed at the wavelength, where the largest spectral change was observed upon complexation of the dye to the respective metal (spectrophotometric titrations of the dyes and the metal salts are described in ref 7a). The following values were used: MCB/Cu, 372 nm; AI/Cu, 492 nm; XO/Cu, 584 nm; MCB/Ni, 376 nm; AI/Ni, 532 nm; and XO/Ni, 592 nm.

The DCL sensor and the sensor arrays were then employed for the sensing of di- and tripeptides. The analytical task was the differentiation of the 13 peptides listed in Figure 2 at a concentration of 1.0 mM.

For the analysis with the DCL sensor, the UV/vis spectrum of the library was recorded for each of the 13 analytes after equilibration (10 repetitions each). The initial data contained 93 values for each measurement (absorbance values in the region $\lambda = 332-700$ nm, with intervals of 4 nm). To determine the wavelengths which contribute most to the differentiation of the peptides, an internal variable selection algorithm of the software package Systat[9] (version 11) was applied. This allowed reducing the data set to 6 values for each measurement. The reduced data was then used as the input for a linear discriminant analysis (LDA).[10] LDA was chosen as a method because it allows to obtain quantitative information about the quality of the classification. This information is very useful for comparing different sensors. One should note, however, that LDA tends to give "over-optimistic" results when compared to unsupervised methods such as principal component analysis (PCA). For the analysis of the sensor array, the optical response of the six different sensors was recorded for each of the 13 peptides. As in the case of the sensor array, 10 independent measurements were performed for each peptide. The resulting data was also classified by an LDA.

A graphic representation of the two analyses in form of two-dimensional score plots is shown in Figure 3. It is evident that the resolution of the DCL sensor is superior to that of the sensor array. The qualitative assessment is confirmed by a cross-validation analysis, in which 33% of the cases are removed from the data set and then reclassified using the remaining data as a training set. For the sensor array, the cross-validation procedure results in a correct classification in 96% of the cases, whereas 98% is obtained for the DCL sensor (averaged results of 10 independent executions of the cross-validation routine).

One can note that the first two scores of the sensor array contain 89% of the total variance. In the case of the DCL sensor, 97% of the total variance is found for the first two scores. The higher dispersion of the sensor array data is not unexpected, since the displacement assays are performed independently from each other and not in one pot. Still, the

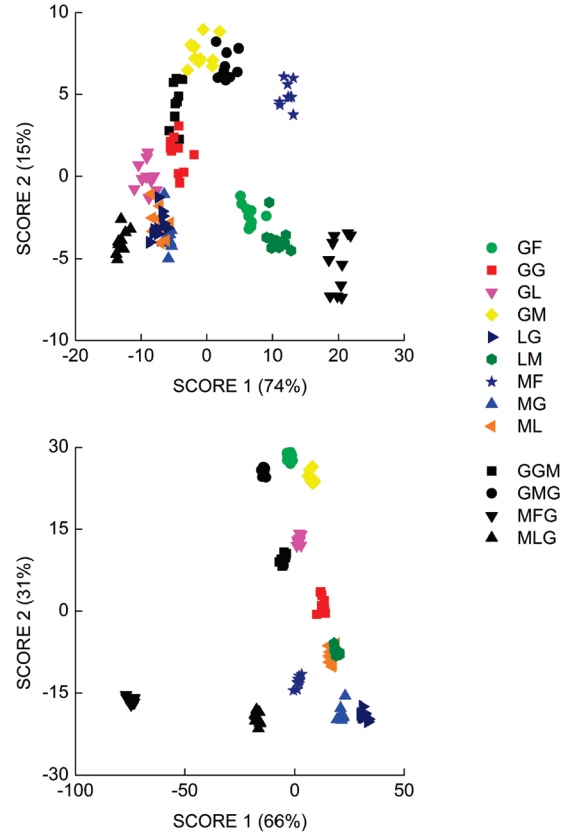

Figure 3. LDA score plots generated from the data of the sensor array composed of six individual metal−dye sensors (top), or the data of the DCL sensor made by mixing the dyes MCB, AI, and XO with the metal complexes $CuCl_2$ and $NiCl_2$ (bottom). Both sensors were used for the differentiation of 13 di- and tripeptides in buffered aqueous solution at a concentration of 1.0 mM.

dimensionality of the sensor array data is low compared to what has been observed for some other systems.[6b,e,h,i,l]

The complexity of a DCL sensor, that is, the number of its constituent components, is expected to influence the analytical power of the system. Reducing the complexity substantially should lead to a loss in resolution. However, it is not clear whether DCL sensors of high complexity (more dyes and metals) are necessarily better. To address this point, we have examined the performance of DCL sensors containing fewer components than our original 3-dye-2-metal sensor. Systematically, we have omitted one, two, or three components, and the resulting DCLs were then evaluated for their ability to differentiate the 13 peptides used before. To characterize the quality of the sensor, we have performed cross-validation procedures in all cases. As a second criterion for comparison, we have examined the Wilks' lambda values for the different analyses. Wilks' lambda represents the ratio between residual variance (left unexplained by the model) over the total variance. A small ratio (i.e., close to zero) is an indication of a strong model. A so-called *F*-value (or *F*-ratio) can be calculated from Wilks' lambda, taking into account the number of samples, classes and replicates. *F*-values can be used to determine whether a statistical model is significant, and larger *F*-values indicate a better model and analysis.[11] A summary of the results is given in Table 1.

Interestingly, the quality of the analysis was found to increase in most cases when one component was removed

Table 1. Analysis of 13 Di- and Tripeptides with DCL Sensors of Different Composition and with the Sensor Array Described in the Main Text

entry	dye(s)	metal salt(s)	% cross-validation	*F*-value
1	AI + XO	$CuCl_2$ + $NiCl_2$	100	510
2	MCB + AI + XO	$NiCl_2$	100	423
3	MCB + XO	$CuCl_2$ + $NiCl_2$	100	325
4	XO	$NiCl_2$	100	238
5	MCB + XO	$CuCl_2$	100	220
6	AI	$CuCl_2$ + $NiCl_2$	100	209
7	MCB + AI	$NiCl_2$	100	154
8	AI + XO	$NiCl_2$	100	154
9	AI	$NiCl_2$	100	141
10	MCB + XO	$NiCl_2$	100	133
11	MCB + AI	$CuCl_2$ + $NiCl_2$	99	661
12	MCB + AI + XO	$CuCl_2$	99	230
13	MCB + AI	$CuCl_2$	99	190
14	MCB	$CuCl_2$	99	121
15	MCB + AI + XO	$CuCl_2$ + $NiCl_2$	98	254
16	AI	$CuCl_2$	98	209
17	XO	$CuCl_2$ + $NiCl_2$	98	194
18	AI + XO	$CuCl_2$	98	105
19	MCB	$CuCl_2$ + $NiCl_2$	96	161
20	sensor array		96	92
21	XO	$CuCl_2$	87	63
22	MCB	$NiCl_2$	81	60

from the original 5-component DCL sensor. All sensors made from two dyes and two metal salts, for example, gave a better discrimination than the full library (Table 1, entries 1, 3, and 11 vs entry 15). Further reduction in complexity to 3 or 2 components typically resulted in a loss of quality as shown by a comparison of the *F*-values (Figure 4).

Quite surprising was the performance of some of the sensors composed of just one metal and one dye. The combination of $NiCl_2$ with XO, for example, resulted in a sensor which gave 100% correct classification in the cross-validation procedure and an *F*-value of 238 (Table 1, entry 4 and Figure 5). Consequently, it performs significantly better than the sensor array with only 96% correct classification and an *F*-value of 92 (Table 1, entry 20).[12]

At first hand, it may appear paradoxical that a mini-DCL sensor composed of just $NiCl_2$ and XO is superior to an array of six individual sensors, one of which is based on a mixture of the very same components: $NiCl_2$ and XO. However, only one wavelength is taken into account for each of the six sensors of the array (592 nm for XO/Ni), whereas six

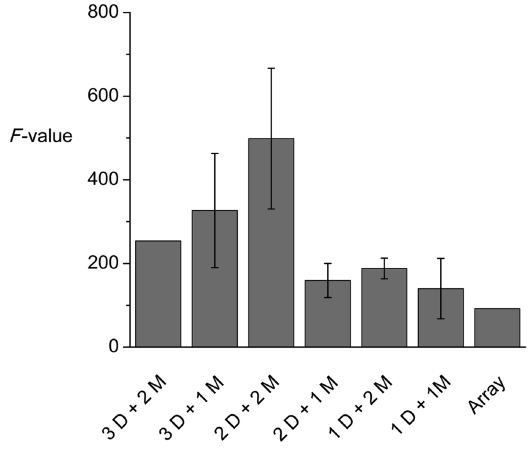

Figure 4. Comparison of the *F*-values for multivariate analyses of DCL sensors composed of a different number of dyes (D) and metal salts (M). The *F*-value of the sensor array is given for comparison.

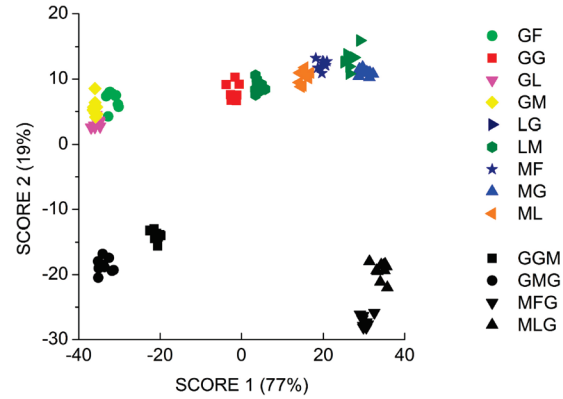

Figure 5. LDA score plot generated from the data of a sensor made from XO and $NiCl_2$. The sensor was used for the differentiation of 13 di- and tripeptides in buffered aqueous solution at a concentration of 1.0 mM.

wavelengths are used for the analysis of the mini-DCL sensor. A buffered aqueous solution of $NiCl_2$, XO, and the peptide analyte is expected to contain metal−dye complexes of the stoichiometry [Ni(XO)] and [Ni_2(XO)],[13] metal-peptide complexes, and possibly heteroleptic metal−dye−peptide complexes. The system is thus more complex than the idealized displacement assay shown in Figure 1a, which assumes that the dye and a 1:1 metal−dye complex are the only colored species. An analysis of the XO/Ni sensor at only one wavelength neglects the information that is provided by the inherent complexity of the system (multiple colored species, the concentration of which depends on the nature of the analyte).[14] In this regard it is easy to understand that a pattern-based analysis of the XO/Ni sensor is better than a single-wavelength analysis. It is surprising, however, that the information provided by the five other sensors of the array (MCB/Cu, AI/Cu, XO/Cu, MCB/Ni, and AI/Ni) is not sufficient to outperform the simple DCL sensor composed of $NiCl_2$ and XO.

Conclusion

DCL sensors containing one, two, or three dyes (XO, AI, MCB) as well as one or two metal salts ($CuCl_2$, $NiCl_2$) were used to differentiate short peptides. The analytical power of the DCL sensors was compared with that of a sensor array made from six separate metal−dye mixtures (MCB/Cu, AI/Cu, XO/Cu, MCB/Ni, AI/Ni, XO/Ni). DCL sensors of intermediate complexity (e.g., 4-component sensors) were found to give better results than the full library made from all five components. Interestingly, most DCL sensors including very simple 2-component systems performed better than the sensor array. We are aware of the fact that these results cannot be easily generalized. For example, we have used chemically rather homogeneous analytes, and different results may be obtained with a more diverse set of analytes. However, the results clearly show that the one-pot-one-spectrum approach of DCL sensors represents a potentially very attractive alternative to the more common sensor array approach.

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(12) A comparison of the *F*-factors is legitimate because the format of the data input is the same for the DCL sensors and for the sensor array (13 analytes, 10 repetitions, 6 wavelengths).

(13) Spectrophotometric titrations had shown that the dominant species in mixtures of XO and $NiCl_2$ are complexes with the stoichiometry [Ni(XO)] and [Ni_2(XO)] (see ref 7a).

(14) For a discussion about the advantages of higher order complexes in IDAs, see ref 4d.

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