Sensors

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Dynamic Combinatorial Libraries of Dye Complexes as Sensors**

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Mixtures of compounds which are formed by the combinatorial assembly of molecular building blocks under thermodynamic control are generally referred to as "dynamic

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combinatorial libraries" (DCLs).[1,2] DCLs are adaptive chemical networks. The addition of a target molecule that selectively interacts with some members of the library may result in a re-equilibration, and this adaptation can be used to identify library members with a high affinity for the respective target molecule. So far, DCLs have mainly been used to study receptors, catalysts, enzyme inhibitors, and new materials.[1,2] Herein, we describe a different application: the utilization of DCLs as sensors.[3]

The relative concentrations of the members of a DCL depend on the environment (solvent, pH, absence or presence of target molecules etc.). A certain library composition is thus a characteristic feature of the environment. It is possible to use the DCL as a sensor if the DCL composition can be transduced into a specific signal output. Up to now DCLs have mainly been analyzed by HPLC, mass spectrometry, and NMR spectroscopy.[1,2] For sensing purposes, however, a fast and cheap analysis method such as fluorescence or UV/Vis spectroscopy would be advantageous. To use the latter technique, we have constructed a DCL of metal–dye complexes in which the library members have a different color. Any re-equilibration will, therefore, result in a variation in the UV/Vis spectrum of the mixture (Figure 1). We show herein that such a library can be used to identify dipeptides in aqueous solution with high selectivity.

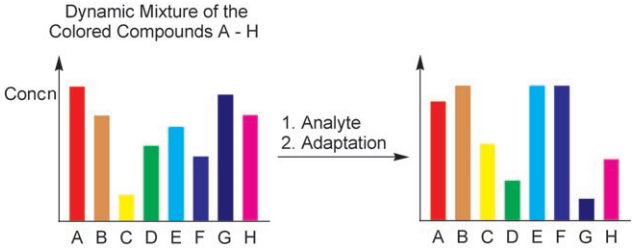
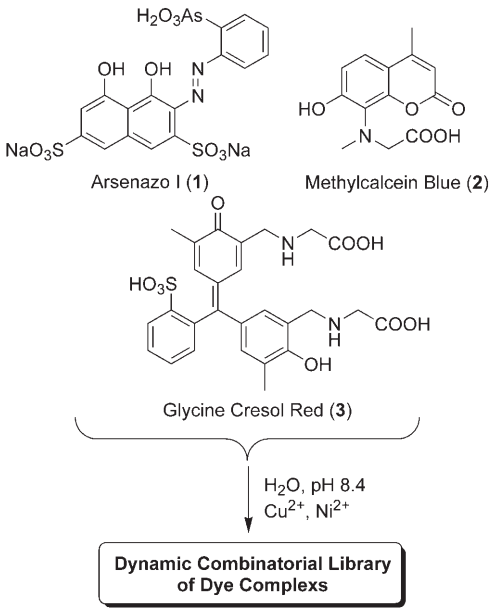


Figure 1. The adaptive behavior of a DCL upon addition of an analyte can be used to identify this analyte by UV/Vis spectroscopy given that the library members possess a characteristic color.

We have used the commercially available dyes arsenazo I (**1**), methylcalcein blue (**2**), and glycine cresol red (**3**) in combination with the metal salts $CuCl_2$ and $NiCl_2$ to generate a DCL in which the library members have a characteristic color (Scheme 1). The dyes form stable complexes with Cu^{2+} and Ni^{2+} ions in buffered aqueous solution (2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) buffer, pH 8.4), as evidenced by UV/Vis titration experiments. This result is in agreement with reports on the high affinity of these dyes for metal ions.[4–6] Job plot analyses[7] revealed that complexes with more than one ligand per metal ion were formed for several combinations in addition to 1:1 complexes (see the Supporting Information).

The individual metal–dye complexes are able to undergo ligand-exchange reactions. This was evidenced by the fact that the UV/Vis spectra of solutions containing a mixture of two dyes and one metal were different from the sum of the spectra of the individual metal–dye mixtures. The same was true for



Scheme 1. Generation of a DCL of metal–dye complexes by mixing arsenazo I, methylcalcein blue, and glycine cresol red with $CuCl_2$ and $NiCl_2$ in buffered aqueous solution.

the experiments with two metal ions and one dye molecule (see the Supporting Information).

This data confirmed that solutions of the dyes **1**–**3** and the two metal salts contain a complex mixture of metal–dye complexes and that these complexes are in a dynamic equilibrium with small amounts of free metal ions and dye molecules. Any disturbance of this equilibrium by addition of an analyte was expected to result in a characteristic change of color.

We decided to use dipeptides as analytes to demonstrate this proposal.[8] Dipeptides are known to form stable complexes with Cu^{2+} and Ni^{2+} ions[9] and may therefore displace some of the dyes from the metal ions.[10] This process should lead to an increase in the free dye concentration as well as to a re-equilibration of the remaining metal–dye complexes. In a first experiment we added aqueous solutions of the dipeptides Val-Phe, Gly-Ala, His-Ala, Ala-His, Phe-Pro, and Pro-Gly to a mixture of the three dyes and the two metal salts (final [peptide] = 1.0 mM; [**1**] = [**2**] = [**3**] = 75 μM; [Cu] = [Ni] = 75 μM; 35 mM CHES buffer, pH 8.4). The changes in the UV/Vis spectra upon addition of the respective peptide are shown in Figure 2.

All six dipeptides can be easily distinguished by UV/Vis spectroscopy. An experiment of this kind can therefore be used to identify the dipeptide. It is interesting to note that His-Ala gave rise to a spectrum which was very different from that of the other peptides. This result can be explained by the fact that the side chain of the N-terminal His residue is able to coordinate to the metal ions. The spectrum of Ala-His, on the other hand, was more similar to the spectra found for simple dipeptides such as Val-Phe. This observation suggests that the side chains of a His residue at the C terminus is less important for metal coordination. Another distinctive spectrum was found for Phe-Pro, with rather weak color changes being

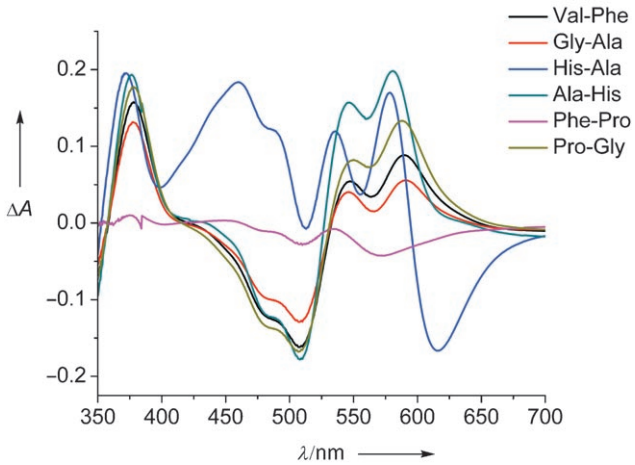


Figure 2. The changes in the UV/Vis spectra upon addition of different dipeptides (1.0 mM) to an aqueous solution containing a DCL sensor composed of the dyes **1**–**3** and the metal salts $CuCl_2$ and $NiCl_2$ ([**1**] = [**2**] = [**3**] = 75 μM, [Cu] = [Ni] = 75 μM, 35 mM CHES buffer, pH 8.4).

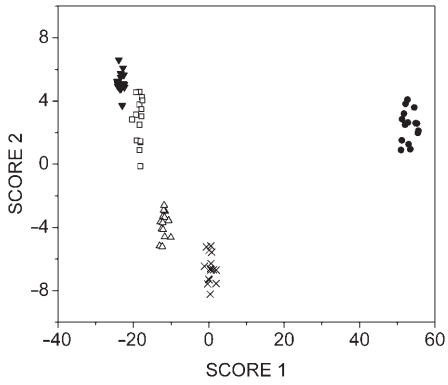


Figure 3. Two-dimensional LDA score plot for the analytes Gly-Ala (●), Val-Phe (□), Ala-Phe (▼), Phe-Ala (△), and D-Phe-Ala (×).

observed. This result highlights the importance of the amide bond for metal coordination.[9]

To test the scope of the DCL sensor we performed a second set of experiments with dipeptides which were structurally more closely related (Gly-Ala, Val-Phe, Ala-Phe, Phe-Ala, and D-Phe-Ala). As expected, the UV/Vis difference spectra were similar to each other, and we therefore used chemometrics[11] to classify the analytes. Fifteen UV/Vis measurements were performed for each dipeptide. To verify that the discrimination between the analytes did not arise from small differences in the concentrations of the peptide stock solutions we varied the peptide concentration for each analyte by ±5%. Thus, five measurements were performed with a peptide concentration of 1.00 mM, five with a concentration of 0.95 mM, and five with a concentration of 1.05 mM (the sensor composition was the same as that described in Figure 2). Data analysis was carried out with the help of the commercial statistics program SYSTAT (version 11.0).[12] A preselection was performed using an automatic variable selection algorithm (see the Supporting Information) to determine which wavelengths in the region between 350 and 700 nm were most relevant for the identification of the peptide. The data from the eight selected wavelengths were then classified by a linear discriminant analysis (LDA).[13] A graphical representation of this analysis in the form of a score plot is shown in Figure 3.

A 100% discrimination was achieved for a "jack-knifed" classification matrix, in which one measurement at a time was treated as an unknown and the rest of the data was used as the training set.[14] This result is quite remarkable, given the fact that none of the dipeptides contain coordinating side chains and that closely related analytes such as the regioisomers Ala-Phe and Phe-Ala as well as the stereoisomers L-Phe-Ala and D-Phe-Ala were used. The discriminative power of the sensor was lower when the complexity of the DCL was reduced by omitting one of the two metal salts: 7 out of the 75 measurements were misclassified for a DCL containing the dyes **1**–**3** and only $CuCl_2$, and 17 misclassifications were obtained for a DCL containing the dyes **1**–**3** and only $NiCl_2$ (see the Supporting Information). This observation shows that a certain library complexity is required to obtain a good differentiation.

The results described above clearly demonstrate the potential of DCLs as sensors. The present library was obtained by mixing commercially available dyes with two transition-metal salts. Despite this simplicity, it was possible to differentiate closely related analytes such as the steroisomers Phe-Ala and D-Phe-Ala. It should be noted that other adaptive systems which show a detectable response upon addition of an analyte can easily be envisioned. DCLs based on compounds with a distinct redox potential or fluorescence, for example, are potentially well suited. The response could then be used to identify a single component or to classify a complex matrix. The modular nature of a DCL makes it easy to optimize the response for a particular sensing problem by variation of the nature, the number, and/or the relative amount of its constituent building blocks. Given these advantages, it seems likely that DCL sensors may find various applications in analytical chemistry.

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[14] A 97% discrimination was achieved in a cross-validation in which 50% of the measurements were taken out randomly and the rest of the data was used as the training set (see the Supporting information).

