Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 7 | Number 22 | 21 November 2009 | Pages 4549–4800

ISSN 1477-0520

FULL PAPER

Kay Severin *et al.*

Sensing of peptide hormones with dynamic combinatorial libraries of metal–dye complexes: the advantage of time-resolved measurements

Highlights in Chemical Science

In this issue...

1477-0520(2009)7:22;1-C

RSC Publishing

Sensing of peptide hormones with dynamic combinatorial libraries of metal–dye complexes: the advantage of time-resolved measurements†

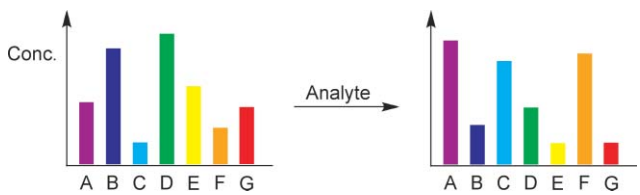
Friederike Zaubitzer, Thomas Riis-Johannessen and Kay Severin*

Received 23rd June 2009, Accepted 24th July 2009
First published as an Advance Article on the web 3rd September 2009
DOI: 10.1039/b912400d

A dynamic combinatorial library of metal–dye complexes was obtained by reacting aqueous solutions of the dyes Methyl Calcein Blue, Arsenazo I, and Xylenol Orange with $CuCl_2$ and $NiCl_2$. The mixture gave a characteristic UV-Vis response upon addition of the peptide hormones angiotensin I and angiotensin II. This allowed distinguishing pure samples of peptide hormones from mixtures. The discriminatory power of the sensor was enhanced when the several UV/Vis measurements were performed during the equilibration process of the library.

Introduction

Adaptive chemical networks are formed by mixing molecular building blocks, which are able to bind to each other *via* reversible interactions. If there is cross-reactivity between the various building blocks, the assemblies are formed in a combinatorial fashion and the resulting network is referred to as a dynamic combinatorial library (DCL).[1] The concentrations of the different members of a DCL depend on the environment of the respective system (pH, solvent, temperature, *etc.*). It is possible to alter the library composition by adding molecules that interact with some members of the library. If the library composition can be transduced into a signal output, the DCL can be used as a sensor (Scheme 1).



Scheme 1 Basic principle of a DCL sensor: a dynamic mixture of colored (or fluorescent) compounds A–G undergoes an analyte-induced re-equilibration. The change in color (or fluorescence) can be used to obtain information about the identity, the quantity, or the purity of the analyte.

For a sensor of this kind, the information about the analyte is distributed over the spectrum. The spectrum therefore represents a 'fingerprint' of the analyte. To correlate the changes at different wavelengths with the analyte properties of interest (identity, quantity, purity), it is advantageous to use multivariate analyses. In this regard, a DCL sensor is related to sensor arrays.[2] However, sensor arrays are analyzed by measuring the response of the independent sensor units, whereas a single UV/Vis or fluorescence measurement is sufficient for a DCL sensor.

The utilization of DCLs for sensing purposes is a relatively new concept and so far there are only few studies in this direction. Guiseppone and Lehn have investigated constitutionally dynamic polymers ("dynamers"),[3] which were obtained by polycondensation of fluorescent and non-fluorescent diamine monomers with 2,7-fluorenebiscarbaldehyde in the presence of variable amounts of Zn^{2+} ions.[4] The polymer composition—and consequently the fluorescence—was found to depend on the concentration of Zn^{2+}. The dynamic polymer can therefore be regarded as a system which is able to sense Zn^{2+} by an analyte-induced constitutional rearrangement.

The group of Anslyn has investigated DCL sensors based on two synthetic receptors for oxoanions and two dyes.[5] Both dyes can bind to both receptors and four different receptor–dye complexes were formed. This system was used to simultaneously determine the concentration of malate and tartrate in aqueous solutions. A related approach was employed to determine the concentration of Ca^{2+} and citrate in flavored vodkas.[6]

The Lavigne group has studied the analyte induced aggregation of carboxylic acid functionalized poly(thiophene)s. The system was used to identify low millimolar concentrations of α,ω-diamines.[7] In a subsequent study, they have shown that a sensor of this kind can be used to quantify histamine in a fish sample.[8]

The most recent investigation about sensing with adaptive chemical systems was published by Margulies and Hamilton.[9] As sensors, they used dynamic libraries of fluorescent G-quadruplexes. The interaction with proteins resulted in characteristic fluorescence emission patterns, which allowed identification of the respective protein.

Our group has shown that combinatorial mixtures of transition-metal complexes and commercially available dyes can be used as powerful sensors for small peptides[10] and nucleotides.[11] In extension of this work, we have reported recently a sensor that is able to determine in retrospect the history of analyte variations.[12] The study demonstrated that the adaptation process of a DCL contains useful information. This inspired us to explore whether time-resolved measurements with DCL sensors are beneficial for the resolution. In the following we will demonstrate that this is indeed the case. A DCL sensor, which is analyzed in a

Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), 1015, Lausanne, Switzerland. E-mail: kay.severin@epfl.ch; Fax: +41(0)21 6939305

† Electronic supplementary information (ESI) available: Mass spectrometry and UV/Vis data; details about the multivariate analyses. See DOI: 10.1039/b912400d

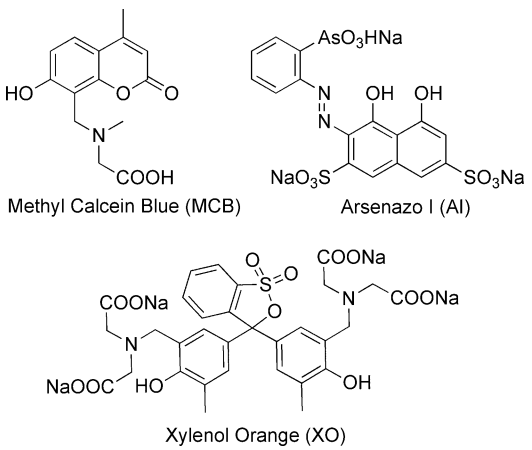
Table 1 Fitted cumulative formation constants ($\log \beta_{m,l}$) for the complexes $[M_m(dye)_l]$ ($M = Cu^{2+}$, Ni^{2+}) observed during UV/Vis titrations in buffered aqueous solution (CHES buffer, 0.1 M, pH 8.4, 298 K). The numbers in parentheses give the calculated error of the fitting procedure

	Xylenol Orange		Arsenazo I			Methyl Calcein Blue	
M^{2+}	$\log \beta_{1,1}$	$\log \beta_{2,1}$	$\log \beta_{1,2}$	$\log \beta_{1,1}$	$\log \beta_{2,1}$	$\log \beta_{1,2}$	$\log \beta_{1,1}$
Cu^{2+}	8.75(3)	15.98(6)	—	8.69(4)	15.53(7)	—	7.34(3)
Ni^{2+}	7.75(5)	13.86(5)	12.80(8)	7.53(4)	—	12.52(3)	7.19(2)

time-resolved fashion, is able to distinguish mixtures of the hormones angiotensin I and angiotensin II with a significantly improved resolution compared to a sensor that is analyzed under pseudo-equilibrium conditions.

Results and discussion

First, a small screening was performed in buffered aqueous solution (2-(*N*-cyclohexylamino)ethanesulfonic acid (CHES) buffer, 0.1 M, pH 8.4), in which the stability and solubility of fourteen commercially available dyes (50 μM) and their binding towards Ni^{2+} and Cu^{2+} (0.1 mM each) were investigated by UV/Vis spectroscopy (for details see experimental section). In order to obtain optimal signal output from a dynamic combinatorial library consisting of a mixture of dyes and metals, complexation induced absorbance changes must be in different regions of the UV/Vis spectrum. Methyl Calcein Blue (MCB, $A_{max} = 360$ nm), Arsenazo I (AI, $A_{max} = 500$ nm) and Xylenol Orange (XO, $A_{max} = 580$ nm) best fulfilled these requirements under the chosen experimental conditions (Scheme 2).



Scheme 2 Molecular structures of the three dyes used to construct the sensor.

Binding of the three selected dyes to Cu^{2+} and Ni^{2+} cations was then further investigated by performing a series of UV/Vis spectrophotometric titrations. In all cases, adding sequential aliquots of aqueous metal solutions to buffered aqueous solutions of the dyes caused marked absorbance changes in distinct regions of the UV/Vis spectrum (overall range 300–700 nm). Plots of absorbance *vs.* metal/dye ratio for selected wavelengths were examined to identify possible stoichiometries for the participating species. In the case of XO, clear inflection points at metal/dye ratios of 1.0 and 2.0 were observed for both Cu^{2+} and Ni^{2+}, indicating the successive fixation of two ions to the two pendant bis(*N*-carboxylate)amine binding sites on the dye. The spectral variations ceased at metal/dye ratios of ≥ 2.0 and, accordingly, the absorbance changes for integer wavelengths in the range 400–650 nm could be satisfyingly modeled by the two equilibria leading to the formation of [M(XO)] and $[M_2(XO)]$ ($M = Cu^{2+}$, Ni^{2+}) complexes (Table 1 and Fig. S1 and S2, ESI†). Electrospray ionization mass spectra (ESI-MS) of solutions containing $[XO]_{tot} = 0.1$ mM (pH 8.4) and metal/dye ratios in the range 0.5–2.0 reflect these observations. With low relative concentration of metal, the spectra are dominated by peaks corresponding to complexes of the type [M(XO)]. Increasing the metal concentration to $[M]_{tot} = 0.2$ mM then results in the appearance of peaks for dinuclear species with $[M_2(XO)]$ formulations ($M = Cu^{2+}$, Ni^{2+}; Table S1, ESI†).

Titrations with AI suggest markedly different speciation behavior for the two metals. For Cu^{2+}, clear inflections occur at metal/dye ratios of 1.0 and 2.0 (Fig. 1), and the data can be excellently modeled by considering similar [Cu(AI)]- and $[Cu_2(AI)]$-type complexes to those observed for XO (Table 1). For Ni^{2+}, however, although perhaps less apparent to the naked eye, inflection points are found at metal/dye ratios of 0.5 and 1.0. Formation of the implied $[Ni(AI)_2]$ species is also supported by evolving factor analysis,[13,14] which strongly invokes the presence of *three* absorbing species to account for the spectral variations at metal/dye ratios of ≤ 1.0. After a ratio of ~1.0 is attained, however, no further changes occur and the data can thus be modeled by sequential formation of the binary complex $[Ni(AI)_2]$ and the 1 : 1 complex [Ni(AI)] (Table 1 and Fig. 1). Parallel ESI-MS titrations showed intense peaks for the free dye and 1 : 1 complexes of AI with Cu^{2+} and Ni^{2+}, but we were unable to observe either the higher nuclearity $[Cu_2(AI)]$ complex or the binary complex

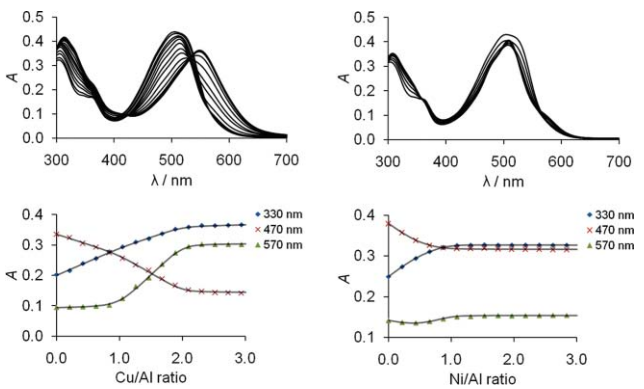


Fig. 1 Changes in absorbance observed during titrations of Cu^{2+} (left) and Ni^{2+} (right) into solutions of AI ($[dye]_{tot} = 15$ μM, CHES buffer, 0.1 M, pH 8.4, 298 K). Below the spectral overlays are corresponding plots of absorbance *vs.* metal/dye ratio for three selected wavelengths (the lines represent best fits to the models discussed in the text).

$[Ni(AI)_2]$ suggested by the UV/Vis data (Table S2, ESI†). We note, however, the persistence of intense peaks corresponding to the free dye, even in the presence of excess metal. It is thus plausible that observation of the latter complexes is impeded by gas-phase fragmentation processes operating in the spectrometer.

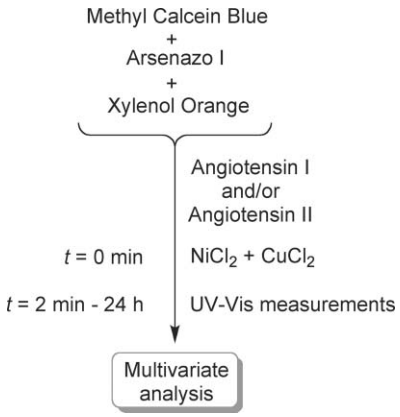
Analogous titrations with MCB and Cu^{2+} showed the exclusive formation of a mononuclear [Cu(MCB)] complex with one absorbance inflection occurring at a metal/dye ratio of 1.0 (Fig. S3, ESI†). Once again, however, the same titration with Ni^{2+} showed biphasic behavior and the data could only be modeled satisfactorily by considering the formation of a binary complex $[Ni(MCB)_2]$, in addition to the 1 : 1 complex [Ni(MCB)] (Table 1 and Fig. S4, ESI†). The mass spectra show complexes of the latter formulation in abundance for both metals (Table S3, ESI†), but as for the AI/Ni^{2+} system, no binary complexes were detected in the gas phase for solutions containing *e.g.* a 1 : 2 ratio of Ni^{2+} : MCB ($[MCB]_{tot} = 0.1$ mM, pH 8.4).

We stress that these complex formulations are limited to the basic stoichiometries of the metal/dye complexes: no concrete conclusions regarding chemical structure, charge and/or protonation state of the various species can be drawn from the current observations. The fitted values listed in Table 1 may thus be considered effective stability constants, whose significance hold strictly within the conditions chosen for the current investigation. They are also subject to error arising from *e.g.* salt impurities in the dye precursors and (in some cases) high correlation between the calculated spectra of the participating species.

These considerations aside, combining all three dyes with both Cu^{2+} and Ni^{2+} will clearly yield a dynamic combinatorial library of complexes, metal ions and free dyes, with the metal–dye complexes being not only homometallic and homoleptic, but ideally comprising mixed species. To provide evidence of the variety of the species in this DCL, we studied an aqueous mixture (pH 8.4) of the three dyes ([MCB] = 50 μM, [AI] = 25 μM and [XO] = 12.5 μM) and Ni^{2+} and Cu^{2+} (0.1 mM each) by ESI mass spectrometry. Homo- and heterometallic, as well as homo- and heteroleptic compounds (Table S4, ESI†) comprising all dyes and metal ions could indeed be identified. These results give an insight into the complexity of such a system. In fact, it is very likely that in reality this DCL contains even more species, as due to instability in the gas-phase some metal complexes might not be detectable by mass spectrometry. It should be noted that the formation of heterometallic and heteroleptic complexes is a unique feature of the DCL sensor approach. In a more classical sensor array with individual metal–dye combinations as sensor units, such species are not formed and can thus not contribute to the resolution of the sensor.[15]

The DCL was subsequently studied for sensing of the peptide hormones angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) and angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). Human angiotensin I and angiotensin II are ten- and eight-amino acid peptides respectively, with angiotensin I being the physiologically inactive precursor of angiotensin II. Angiotensin II is involved in several physiological and pathophysiological effects in the human body, such as vasoconstriction, which leads to the increase of blood pressure. Permanent hypertension can be the cause for chronic renal failure and cardiac failure, amongst others.[16] Angiotensin I is transformed to angiotensin II through removal of the terminal histidine and leucine residues by the exopeptidase Angiotensin-converting enzyme (ACE). Since the side chain of histidine is a good ligand for transition metals, it was expected that the two peptides bind differently to Cu^{2+} and Ni^{2+}.

The basic concept of the DCL sensor is illustrated in Scheme 3. MCB (50 μM), AI (25 μM) and XO (12.5 μM)[17] were mixed with either one or both peptides ($[peptide]_{tot} = 20$ μM), followed by the addition of a 1 : 1 mixture of Ni^{2+} and Cu^{2+} ($[M^{2+}] = 0.2$ mM) at $t = 0$ min (CHES, 0.1 M, pH 8.4). The solutions were analyzed by UV/Vis spectroscopy at different times and the data were evaluated by multivariate analyses.



Scheme 3 Experimental procedure for the sensing of the peptide hormones angiotensin I and angiotensin II. At $t = 0$ min the metal salts were added to a solution containing the three dyes and either one or a mixture of both peptides. The data from UV/Vis measurements at different times were evaluated by multivariate analysis.

Time-resolved UV/Vis studies of the sensing ensembles showed that the major absorbance changes occurred in the first 20 min following addition of the metal ions (Fig. 2). After this initial rapid phase, the system took a further 4 days to fully equilibrate. The slow equilibration kinetics of the DCLs contrast with those characterizing the bimolecular reactions of any two of

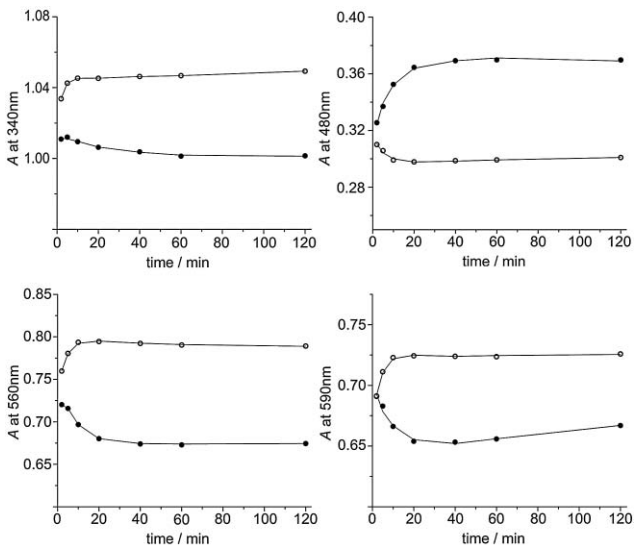


Fig. 2 UV/Vis absorbance data at different wavelengths showing the equilibration over time of DCLs containing Ni^{2+}, Cu^{2+}, Methyl Calcein Blue, Arsenazo I, Xylenol Orange and angiotensin I (filled symbols) or angiotensin II (empty symbols).

the (metal/dye) components, which are typically complete within minutes. Such effects are, however, to be expected since the true energy minimum of the complex network of reactions operating in the DCL is undoubtedly reached *via* numerous close-lying minima, each with a corresponding kinetic barrier to be overcome. In this respect, equilibration of the DCL is therefore slow enough to be followed by simple UV/Vis measurements. Furthermore, the kinetic profile of the equilibrating system was found to vary according to the analyte. This phenomenon is especially appealing for systems where different analytes give rise to the same signal when the sensor is read out at thermodynamic equilibrium.

Our DCL sensor was first implemented to differentiate between angiotensin I, angiotensin II and a 1 : 1 mixture of the two peptides. The absorbance changes (300–800 nm) were recorded at different times ($t = 2, 5, 10, 20, 40, 60$ min) until the system reached a pseudo-equilibrium defined as $t = 24$ h. For each peptide, the experiment was repeated five times. The data were then evaluated by linear discriminant analysis (LDA).[18] The results of the LDA suggested that the data from four wavelengths and at $t \leq 10$ min was enough to tell apart the three sample types.

The DCL sensor was then used to distinguish between 11 mixtures (six repetitions each) of angiotensin I and II ([angiotensin I] = $(20 - x)$ μM; [angiotensin II] = x μM; with $x = 0, 2, 4, 6, \ldots, 20$). The sensing experiments were performed under the conditions described above and the samples were analyzed by UV/Vis spectroscopy (330–650 nm) at $t = 2$, 5, 10 min and 24 h. The absorbance data at $\lambda = 590$, 560, 480 and 340 nm was then evaluated by a LDA. In order to determine to what extent the kinetic profiles contributed to the discriminative power of the DCL, the data was analyzed twice. First, only the data obtained from the DCL at pseudo equilibrium ($t = 24$ h) was included in the LDA (Fig. 3, top). In a succeeding analysis, all the data was used for evaluation (Fig. 3, bottom). The corresponding results are displayed as score plots.

For both analyses, most of the variance of the data was contained in score 1 (94.8 and 93.5%, respectively). Important differences were found for the resolution. Analysis (a) including only the data obtained at pseudo-equilibrium yields a score plot with considerable overlap of most of the scores of the mixtures. With a correctness of classification of 83%,[19] the data is not sufficient to tell apart all sample types. It should be noted, however, that the scores for the pure samples (angiotensin I: dark blue crosses, angiotensin II: maroon diamonds) are well clustered and separated. Therefore, this method is still good enough to discriminate the pure samples. In analysis (b) where the entire data set, *i.e.* also the data from the equilibration kinetics of the DCL, is considered, the scores of all sample types are properly classified and well separated. In spite of some score clusters being in close proximity, the correctness of classification is 100%.[20] The supplementary data used in analysis (b) led to an improvement of the classification of the scores and consequently an increase in the discriminative power of the DCL sensor.

Conclusions

A DCL of metal–dye complexes was prepared by mixing the dyes Methyl Calcein Blue, Arsenazo I and Xylenol Orange with $CuCl_2$ and $NiCl_2$. Detailed UV/Vis titration experiments and ESI mass spectrometry studies of the library and its components provided insight into the complexity of the resulting chemical network. The DCL was used as a sensor for the peptide hormones angiotensin I and angiotensin II. Despite its simplicity, the DCL sensor was found to display a remarkable discriminative power: mixtures of the two peptides were distinguished from pure samples at low micromolar concentrations (20 μM). The resolution of the sensor was significantly enhanced when the measurements were performed in a time-resolved fashion. It should be noted that so far, work with DCLs has primarily focused on systems under thermodynamic control.[1] The findings described above are evidence that the kinetics of a DCL adaptation process may contain useful information, in particular if dynamic networks are used for challenging sensing problems.

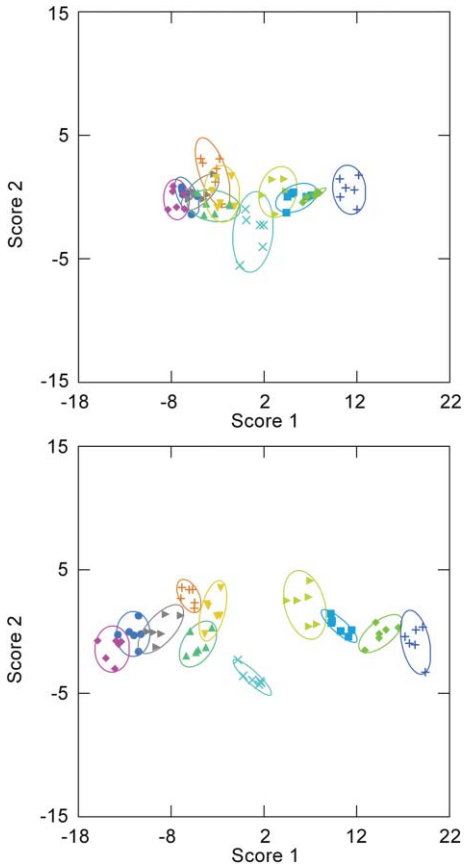


Fig. 3 Score plots of LDA treating only the data after 24 h at pseudo-equilibrium (top) and including the data after 2, 5, 10 min and 24 h (bottom): $[\text{angiotensin I}]_{tot} = (20 - x)$ μM, $[\text{angiotensin II}]_{tot} = x$ μM; $x = 20$ (maroon, ◆), 18 (blue, ●), 16 (grey, ▶), 14 (orange, +), 12 (green, ▲), 10 (yellow, ▼), 8 (light blue, ×), 6 (light green, ▶), 4 (light blue, ■), 2 (green, ◆), 0 (dark blue, +).

Experimental

General

Alizarin Red S (Fluka), Arsenazo I (Lancaster), Azophloxine (Fluka), Gallocyanine (Acros Organics), Methyl Calcein Blue (Sigma), Methylene Blue (Sigma), Methyl Orange (Ciba), Methyl Red (Acros Organics), Mordant Blue 9 (Acros Organics), Mordant Blue 13 (Acros Organics), Naphthol Blue Black (Fluka), Orange G (Acros Organics), Pyrocatechol Violet (Acros Organic), Xylenol

Orange (Fluka), $NiCl_2 \cdot 6H_2O$ (Strem Chemicals), $CuCl_2 \cdot 2H_2O$ (Fluka), CHES buffer (Fluka), and angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) and angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) (both Bachem) were used as received. Stock solutions of all dyes (1 mM, except Xylenol Orange: 0.5 mM), the metal salts (10 mM), the peptides (0.1 mM) and CHES buffer (pH 8.4, 0.2 M) were prepared in bidistilled water. All UV/Vis spectra were recorded on a Lambda 35 spectrometer (Perkin Elmer). Electrospray-ionisation MS data were acquired on a Q-Tof Ultima mass spectrometer (Waters) fitted with a standard Z-spray ion source and operated in the negative ionization mode. Experimental parameters were set as follows: capillary voltage: 3 kV, sample cone: 50 V, source temperature: 80 °C, desolvation temperature: 200 °C, acquisition window: m/z 50-1000 in 1 s. 5 μL of the sample was introduced into the mass spectrometer by infusion at a flow rate of 20 μL min^{-1} with a solution of ACN–H_2O 50 : 50 (v/v). External calibration was carried out with a solution of phosphoric acid at 0.01%. Data were processed using the MassLynx 4.1 software.

Dye screening

For each dye, UV/Vis spectra of the free dye and the metal–dye combinations (final conc.: $[dye]_{tot} = 50$ μM; $[M^{2+}]_{tot} = 0.1$ mM) in buffered aqueous solution (CHES, 0.1 M, pH 8.4) were recorded after standing for 1 h at room temperature. The following dyes were studied and not considered for further investigation due to precipitation with either one of the metal ions: Azophloxine, Methylene Blue, Naphthol Blue Black. Orange G and Pyrocatechol Violet were not suitable, as they decompose in the presence of one of the metal ions. Methyl Orange and Methyl Red do not display sufficient binding affinity towards the metal ions and Alizarin Red S, Mordant Blue 9 and Mordant Blue 13 gave weak signals, when tested in a dynamic combinatorial library.

Spectrophotometric titrations

For titrations of Xylenol Orange with $CuCl_2 \cdot 2H_2O$ and $NiCl_2 \cdot 6H_2O$, two series of measurements were performed: one in which solutions of $[M^{2+}]_{tot} = 5$ mM were added to solutions of $[XO]_{tot} = 7.5$ μM (3 mL, + CHES, 0.1 M, pH 8.4, 298 K) in 1 μL increments, and one in which solutions of $[M^{2+}]_{tot} = 1$ mM were added to solutions of $[XO]_{tot} = 15$ μM (1.5 mL, + CHES, 0.1 M, pH 8.4, 298 K) in 5 μL increments. For titrations of Arsenazo I with $CuCl_2 \cdot 2H_2O$ and $NiCl_2 \cdot 6H_2O$, solutions of $[M^{2+}]_{tot} = 10$ mM were added to solutions of $[AI]_{tot} = 15$ μM (3 mL, + CHES, 0.1 M, pH 8.4, 298 K) in 1 μL increments, whereas for Methyl Calcein Blue, solutions of $[M^{2+}]_{tot} = 10$ mM were added to solutions of $[MCB]_{tot} = 25$ μM (3 mL, + CHES, 0.1 M, pH 8.4, 298 K) in 1 μL increments. After each addition, solutions were equilibrated for 10 min at 298 K before measuring the absorption spectra in the range 300–700 nm (XO and AI) and 300–500 nm (MCB). The speciation models described in the main text were fitted to the spectral data using an in-house built routine in MATLAB® which implements evolving factor analysis and a Newton–Gauss multi-non-linear least squares fitting algorithm.[13,14] For XO, the speciation models were fitted to the data from both series of measurements (for each metal) in a global multi non-linear least-squares fit. In all cases the fits converged with sum-of-squared residuals of < 0.005, indicating that the data was excellently modeled. Complete spectral overlays, calculated spectra and speciation diagrams for all titrations are shown in the ESI.† The standard deviations for the fitted parameters (listed in parentheses in Table 1) were estimated using the sum-of-squared residuals and inverted curvature matrices as obtained from the iterative fitting processes (see ref. 13 for details).

Mass spectrometric titrations

Samples were prepared by mixing appropriate amounts of stock solutions of the dye ($[dye]_{tot} = 0.1$ mM) and the metal salt ($0.05 \leq [M^{2+}]_{tot} \leq 0.2$ mM) in water. The pH was then adjusted to 8.4 (±0.2) using $NaOH_{(aq)}$ (0.01 M).

Mass spectrometry of the DCL

For sample preparation appropriate amounts of stock solutions of the dyes and the metal salts were mixed to give final concentrations of: $[MCB]_{tot} = 50$ μM, $[AI]_{tot} = 25$ μM and $[XO]_{tot} = 12.5$ μM; $[Ni^{2+}]_{tot} = [Cu^{2+}]_{tot} = 0.1$ mM. $NaOH_{(aq)}$ (0.01 M) was then added to adjust the pH to 8.4 (±0.2).

Sensing experiments

The DCL sensors were prepared by mixing appropriate amounts of stock solutions containing the dyes, the metal salts and the buffer. After addition of either one peptide or a mixture of both peptides, UV/Vis spectra were recorded at $t = 2$, 5, 10, 20, 40, 60 min and 24 h or $t = 2$, 5, 10 min and 24 h. Each series of measurements was repeated five or six times. The final concentrations were: $[MCB]_{tot} = 50$ μM, $[AI]_{tot} = 25$ μM and $[XO]_{tot} = 12.5$ μM, $[Ni^{2+}]_{tot} = [Cu^{2+}]_{tot} = 0.1$ mM, and $[CHES]_{tot} = 0.1$ M. The peptide concentration was varied in 0.1 eq. increments with $[angiotensin\ I]_{tot} = (20 - x)$ μM and $[angiotensin\ II]_{tot} = x$ μM. The data was analyzed with the commercially available statistics program SYSTAT (version 11.0) by a linear discriminant analysis algorithm.

Acknowledgements

We would like to thank Dr Laure Menin for performing mass spectrometry and Dr Geoffrey Wood for help with data treatment. The work was supported by the Swiss National Science foundation, by the COST action CM0703 on *Systems Chemistry*, and by the EPFL.

References

1 (*a*) S. Ladame, *Org. Biomol. Chem.*, 2008, **6**, 219–226; (*b*) R. F. Ludlow and S. Otto, *Chem. Soc. Rev.*, 2008, **37**, 101–108; (*c*) J.-M. Lehn, *Chem. Soc. Rev.*, 2007, **36**, 151–160; (*d*) P. T. Corbett, J. Leclaire, L. Vial, K. R. West, J.-L. Wietor, J. K. M. Sanders and S. Otto, *Chem. Rev.*, 2006, **106**, 3652–3711.

2 (*a*) A. T. Wright and E. V. Anslyn, *Chem. Soc. Rev.*, 2006, **35**, 14–28; (*b*) K. L. Albert, N. S. Lewis, C. L. Schauer, G. A. Sotzing, S. E. Stizel, T. P. Vaid and D. R. Walt, *Chem. Rev.*, 2000, **100**, 2595–2626.

3 J.-M. Lehn, *Prog. Polym. Sci.*, 2005, **30**, 814–831.

4 (*a*) N. Guiseppone, G. Fuks and J.-M. Lehn, *Chem.–Eur. J.*, 2006, **12**, 1723–1735; (*b*) N. Guiseppone and J.-M. Lehn, *J. Am. Chem. Soc.*, 2004, **126**, 11448–11449.

5 S. L. Wiskur, P. N. Floriano, E. V. Anslyn and J. T. McDevitt, *Angew. Chem., Int. Ed.*, 2003, **42**, 2070–2972.

6 S. C. McCleskey, P. N. Floriano, S. L. Wiskur, E. V. Anslyn and J. T. McDevitt, *Tetrahedron*, 2003, **59**, 10089–10092.
7 T. L. Nelson, C. O'Sullivan, N. T. Greene, M. S. Maynor and J. J. Lavigne, *J. Am. Chem. Soc.*, 2006, **128**, 5640–5641.
8 M. S. Maynor, T. L. Nelson, C. O'Sullivan and J. J. Lavigne, *Org. Lett.*, 2007, **9**, 3217–3220.
9 D. Margulies and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2009, **48**, 1771–1774.
10 (*a*) A. Buryak and K. Severin, *J. Comb. Chem.*, 2006, **8**, 540–543; (*b*) A. Buryak and K. Severin, *Angew. Chem., Int. Ed.*, 2005, **44**, 7935–7938.
11 A. Buryak, A. Pozdnoukhov and K. Severin, *Chem. Commun.*, 2007, 2366–2368.
12 A. Buryak, F. Zaubitzer, A. Pozdnoukhov and K. Severin, *J. Am. Chem. Soc.*, 2008, **130**, 11260–11261.
13 M. Maeder and Y.-M. Neuhold, in *Practical Data Analysis in Chemistry*, ed. S. Rutan and B. Walczak, Elsevier Publishing Co, Amsterdam, 2007.
14 (*a*) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **23**, 1133–1139; (*b*) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1986, **33**, 943–951.
15 For a discussion about the advantage of complex stoichoimetries for sensors based on metal dye complexes, see: M. Kitamura, S. H. Shabbir and E. V. Anslyn, *J. Org. Chem.*, 2009, **74**, 4479–4489.
16 M. G. Nicholls and J. I. S. Robertson, *J. Hum. Hypertens.*, 2000, **14**, 649–666.
17 Different dye concentrations were chosen to account for differences in the absorption coefficient.
18 P. C. Jurs, G. A. Bakken and H. E. McClelland, *Chem. Rev.*, 2000, **100**, 2649–2678.
19 A standard jackknifed validation routine, in which one measurement at a time was randomly omitted was performed: 70% of the measurements were classified correctly.
20 82% of the measurements were classified correctly in a jackknifed validation routine.