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A molecular self-assembled colourimetric chemosensor array for simultaneous detection of metal ions in water†

Yui Sasaki,[ab] Tsukuru Minamiki,[a] Shizuo Tokito[b] and Tsuyoshi Minami *[a]

We propose a novel strategy for a high-throughput sensing of metal ions using a molecular self-assembled colourimetric chemosensor array. The proposed colourimetric assay has been achieved by only using the combination of commercially available materials. Importantly, the easy-to-prepare assay can be utilised to quantitatively detect metal ions under competitive conditions.

The popularity of chemosensor arrays is intensively increased because of their capability to recognise various analytes with high classification accuracy.[1] More recently, chemosensor array systems have achieved not only simultaneous qualitative analysis but also the quantitative one based on pattern recognition algorithms.[2] Hence, it could be said that the array-based sensory system opens up an avenue for practical applications in supramolecular analytical chemistry. However, we need great effort to synthesise a number of chemosensors for an array. Too complicated and multi-step syntheses of chemosensors may prevent generalisation and mass production in industry.

To avoid the above-mentioned problem, we have focused our attention to artificial molecular self-assembled systems. Such systems have been widely employed in the fields of organic electronics,[3] bioimaging,[4] drug delivery[5] and catalysis,[6] because we do not need to spare time for troublesome organic synthesis and are able to obtain smart systems. Among them, phenylboronic acid (PBA) derivatives and their boronate esterification are of remarkable interest. PBA derivatives not only serve as Lewis acid metal centres, but also form cyclic boronate esters with *cis*-diol moieties of biomolecules in aqueous media.[7] Thus, many groups have attempted to use PBA and its derivatives for various supramolecular architectures. Especially, in the field of analytical chemistry, it believes that PBA-based chemosensors are some of the most promising candidates for the establishment of practical supramolecular sensory systems in aqueous media. For example, Anslyn *et al.* reported PBA-based chemosensors using enantioselective indicator displacement assays for α-hydroxycarboxylates and diols.[8] Kubo *et al.* used alizarin–PBA interactions in a strategy for self-assembled fluorescent chemosensors of metal ions such as Cu^{2+}.[9] Inspired by these pioneering works, Anzenbacher and Minami, who is one of the authors of this communication, reported self-assembled chemosensor array systems based on PBA for anions such as ATP.[10] Although such intensive efforts have made significant progress in chemosensor arrays, multi-analyte detection in water using only simply synthesised materials is still in its early stage. Toward that end, we decided to fabricate a chemosensor array system by only using a combination of generally commercially available compounds. As target analytes, we selected metal ions because the development of simple, inexpensive, and high-throughput analytical methods for the detection of metal ions is highly important from the viewpoint of environmental monitoring.[11] To achieve simultaneous detection of metal ions in water, we employed commercially available catechol dyes (alizarin red S: ARS, bromopyrogallol red: BPR, pyrogallol red: PR) and 3-nitrophenylboronic acid (3-NPBA), which acts as a colour manipulator accompanied by boronate esterification (Fig. 1). These materials are simply mixed in water, which offers the construction of colourimetric chemosensors for metal ions. In other words, the boronate esters can be cleaved in the presence of metal ions, resulting in colour change. A chemosensor array fabricated using such self-assembled boronate esters allowed us to detect metal ions qualitatively and quantitatively.

Initially, the self-assembled complexation of catechol dyes and 3-NPBA was investigated in a 50 mM HEPES buffer solution containing 10 mM sodium chloride at pH 7.4 at r.t. 3-NPBA, which has an electron-withdrawing group (= nitro group) for increasing Lewis-acidity of boronic acid, was utilised as a colour manipulator for dyes. Fig. 2 shows the changes in the UV-vis absorption when 3-NPBA was stepwise added into the aqueous solution of ARS, BPR, or PR. The addition of 3-NPBA caused

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[a] *Institute of Industrial Science, the University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan. E-mail: tminami@iis.u-tokyo.ac.jp; Fax: +81-3-5452-6365; Tel: +81-3-5452-6364*

[b] *Research Center for Organic Electronics, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan*

† Electronic supplementary information (ESI) available: General information of experiments, UV-vis titrations, FAB-MS analysis, linear discriminant analysis (LDA) and details of regression analysis. See DOI: 10.1039/c7cc03218h

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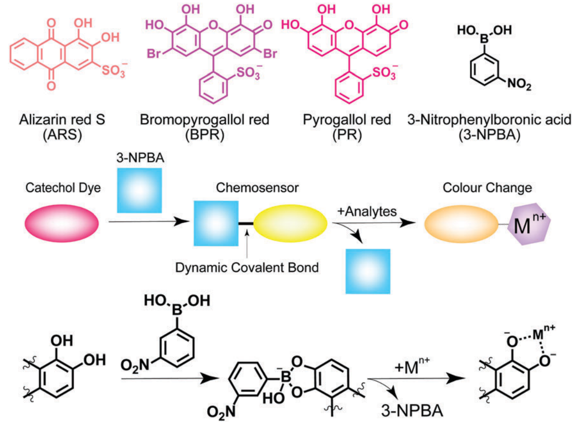

Fig. 1 Top: Structures of ARS, BPR, PR and 3-NPBA. Bottom: Illustrated concept and scheme of the self-assembled chemosensor utilising the building blocks (a catechol dye and 3-NPBA) for the easy-to-prepare colourimetric assay.

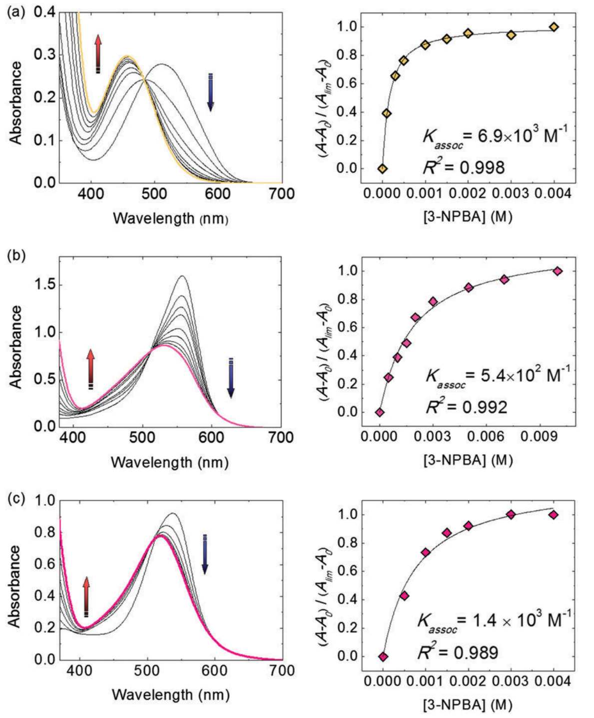

Fig. 2 UV-vis spectra of the catechol dye (40 μM) upon the addition of 3-NPBA in a HEPES buffer solution (50 mM) at pH 7.4 at r.t. (a) ARS, (b) BPR and (c) PR.

significant blue shifts in the absorption spectra with isosbestic points. The shifts of the absorption band in each dye were 55 nm (ARS), 27 nm (BPR), 19 nm (PR), respectively. Such shifts are attributed to boronate esterification between dyes and 3-NPBA. The association constants (K_{assoc}) of 3-NPBA and each dye which are estimated from the UV-vis titration are as follows: ARS: $(6.9 \pm 0.4) \times 10^3$ M^{-1}, BPR: $(5.4 \pm 0.6) \times 10^2$ M^{-1}, and PR: $(1.4 \pm 0.2) \times 10^3$ M^{-1}. Similar K_{assoc} values for dyes and phenylboronic acids were reported in earlier works.[9,12] The formation of the proposed dye–3-NPBA complex was also evaluated by fast-atom-bombardment (FAB) mass spectrometry (see the ESI†). Judging from these results, the binding model of the complexation between dyes and 3-NPBA was estimated to be a 1 : 1 complex. According to the titration isotherms, we estimated the complexation ratios of boronate esters at 100 eq. of 3-NPBA. These ratios were estimated to be ARS: ∼100%, BPR: ∼80%, and PR: ∼100%, respectively.

Next, the UV-vis titration experiment for the detection of metal ions using such complexes was carried out. By considering the impact of environmental and health-related issues, we did the titrations for the following 11 analytes: Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Hg^{2+}, Al^{3+}, Pb^{2+}, Ga^{3+} and Ca^{2+}. The addition of metal ions into the solutions containing the dye–3-NPBA complexes induced red-shifts of the absorption maxima in the UV-vis spectra (Fig. 3 and see the ESI† for other titration results). For example, the maximum absorbance wavelength of the ARS–3-NPBA complex in a HEPES buffer solution was shifted from 457 nm to 540 nm upon the addition of Ni^{2+} (Fig. 3(a)). On the other hand, there was no change in the UV-vis spectra of 3-NPBA upon the addition of metal ions (see the ESI†), suggesting that the observed shift arose from the dynamical displacement of 3-NPBA in the complexes and the generation of metal-coordinated dyes. This displacement mechanism has contributed to provide the distinct red-shifts of the UV-vis spectra as well as various response patterns of the isotherms. In other words, 3-NPBA plays a key role in abundant colour changes although the stand-alone dyes respond to

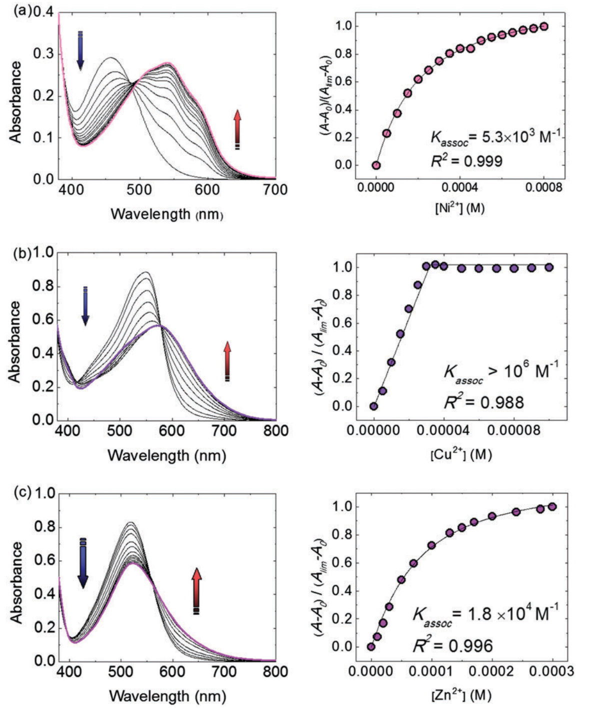

Fig. 3 UV-vis spectra of the catechol dye–3-NPBA complex upon the addition of metal ions in HEPES buffer (50 mM) at pH 7.4 at r.t. [Dye] = 40 μM. [3-NPBA] = 4 mM. The indicated titration results were obtained from various self-assembled chemosensor systems. (a) ARS–3-NPBA and Ni^{2+}, (b) BPR–3-NPBA and Cu^{2+} and (c) PR–3-NPBA and Zn^{2+}.

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Table 1 The apparent association constants (K_{assoc}, M^{-1})[a] obtained from UV-vis titration

	Analyte										
Dye	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}	Co^{2+}	Fe^{2+}	Hg^{2+}	Al^{3+}	Pb^{2+}	Ga^{3+}	Ca^{2+}
ARS	5300	ND[b]	8300	$< 10^2$	1200	$< 10^2$	ND[b]	6500	ND[b]	1100	ND[c]
BPR	40 000	$> 10^6$	ND[b]	9900	24 000	3100	ND[c]	ND[b]	$> 10^6$	ND[b]	8700
PR	22 000	$> 10^6$	18 000	5400	66 000	ND[b]	28 000	ND[b]	310 000	3400	4300

[a] The apparent K_{assoc} values were calculated using the change in UV-vis absorption upon the addition of each analyte in a HEPES buffer (50 mM) solution at pH 7.4 at r.t. The errors of the curve fitting were $< 20\%$. [b] ND means not determined due to the biphasic nature of the isotherm. [c] The apparent K_{assoc} values could not be calculated owing to the insufficient change in the UV-vis response.

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metal ions. Table 1 summarises the apparent K_{assoc} values for metal ions. The apparent K_{assoc} values of the 1 : 1 binding system can be estimated from the sufficient change of the maximum wavelengths in the UV-vis spectra. The binding ratios were supported by FAB mass spectrometry (see the ESI†), Job's plots and earlier works.[13] Meanwhile, we were not able to determine apparent K_{assoc} in some cases due to its biphasic behaviour (probably due to more complicated binding modes) or insufficient change in the UV-vis response. The binding affinities that correspond to analyte-induced colour changes indicate the cross-reactivity of the present assay for metal ions. Additionally, the assay showed almost no responses to Na^+, K^+, and Mg^{2+}, which represent families of metal ions generally present in environmental water (see the ESI†). Thus, these information-rich responses are expected to contribute to the discrimination of metal ions in complex mixtures quantitatively (*vide infra*).

The multi-responsiveness in the self-assembled sensor systems encouraged us to fabricate a sensor array for metal ions. We employed conventional 384 well microplates and a robotic dispenser to demonstrate high-throughput analysis. Each microwell contained a solution of 3-NPBA (4 mM) and the diol-dye (40 μM), and then the target analytes were dispensed to the solutions (see the ESI† for a detailed description). By using a microplate reader, the absorption spectra were recorded from 400 nm to 700 nm. Such recorded changes in absorption spectra were analysed using pattern recognition models.

To achieve the qualitative assay, we employed linear discriminant analysis (LDA). LDA is frequently used for a supervised pattern recognition tool to reduce dimensionality and classification of the multivariate data.[14] In this assay, the leave-one-out cross-validation protocol (= the Jackknife method) was performed to determine the level of correct classification of the observations within the clusters (see the ESI†). As a result, LDA showed that the array based on the molecular self-assembly can clearly discriminate 11 analytes and control (Fig. 4). Fig. 4 presents the response space specified by the first three canonical factors (F1–F3, see also the ESI†), showing that 95.2% of variance is adequate to discriminate the analytes. The Jackknife cross-validation outputs 100% correct classification of all 240 data-sets (corresponding to 11 analytes and control). Notably, the obtained results reveal that the assay can discriminate several metal ions (= 10 μM) in the presence of the competing interferent (= NaCl, 10 mM). Conversely, a dye-array (ARS, BPR and PR) under the same conditions in the absence of 3-NPBA could not discriminate 11 analytes and control correctly, indicating that 3-NPBA significantly increased the discriminatory power in the sensing system. The achievement of the qualitative analysis for the identification of 11 analytes validated that the self-assembled chemosensor array has high potential to be a powerful tool for the simultaneous detection of metal ions in water.

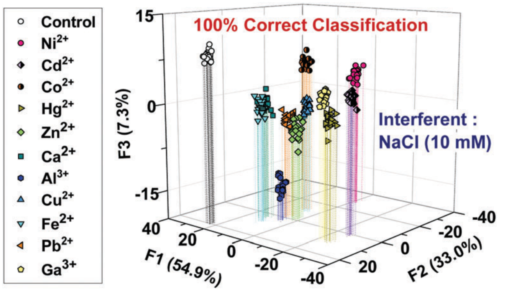

Fig. 4 LDA plots for the response of the sensor array to 11 analytes (and control) in a HEPES buffer (50 mM) solution with sodium chloride (10 mM). [Metal ion] = 10 μM. 20 repetitions were measured for each analyte. The cross-validation routine shows 100% correct classification.

Because of the gravity of the effect of mixture toxicity[15] of metal ions on ecological systems, we decided to attempt a quantitative analysis using the proposed array system. According to risk assessments for plating wastewater, the inflow of multiple metal ions (*e.g.* the combination of Cu^{2+}, Zn^{2+} and Ni^{2+}) into environmental water systems may affect the growth of microorganisms such as algae.[16] In that regard, a semi-quantitative analysis was performed to evaluate the detectability of the present assay for Cu^{2+}, Zn^{2+} and Ni^{2+}. Furthermore, a regression analysis for the quantitative estimation of concentrations of each analyte in the mixture system was achieved utilising the support vector machine (SVM) algorithm.[17]

To evaluate the potential of the proposed assay toward a monitoring tool for the contaminant level in water, the semi-quantitative analysis for each analyte was carried out (Fig. 5). The clusters clearly depended on each metal ion level (0–3.7 ppm) even in the presence of a large excess of NaCl (10 mM). This strongly encouraged us to attempt regression analysis of metal ions in mixtures.

Fig. 6 shows the results of the quantitative estimation of multiple concentrations of each metal ion in mixture systems. For the analysis, we dissevered the acquired data set into two parts; the first component for calibration and model construction and the second one for utilisation as unidentified samples for cross-validation. As a result, we can see accurate quantitative

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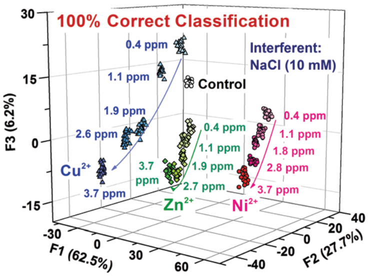

Fig. 5 LDA results of the semi-quantitative assay toward three different metal ions (Cu^{2+}, Zn^{2+} and Ni^{2+}) at a concentration range of 0–3.7 ppm. 20 repetitions were measured for each concentration.

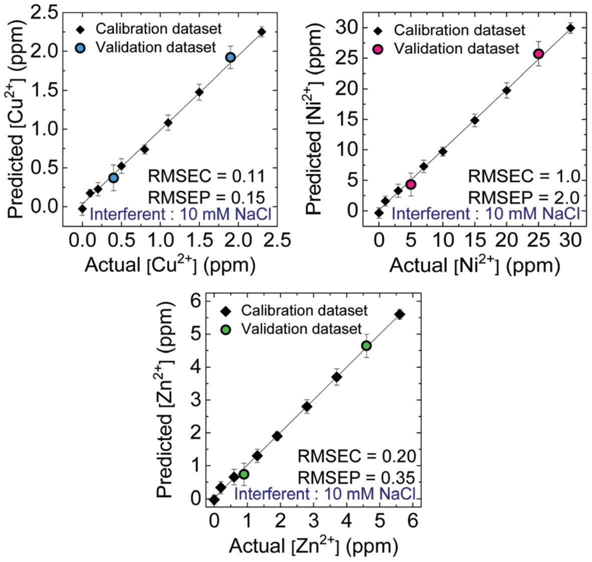

Fig. 6 The results of the regression analysis for quantitative estimation of metal ion concentrations of Cu^{2+}, Ni^{2+} and Zn^{2+} in mixtures. All solutions contain HEPES (50 mM) and NaCl (10 mM). The detailed concentration conditions are summarised in the ESI.† Plots of actual *versus* predicted concentrations show high accuracy of prediction for multiple concentrations of each analyte. The values of root-mean-square error (RMSE) of calibration (C) and prediction (P) attest to the high quality of the model and prediction.

regression analyses for metal ions, meaning that the developed assay can analyse each concentration of Ni^{2+}, Zn^{2+} and Cu^{2+} even in mixture systems. Notably, the obtained results in the concentration range (Ni^{2+}: <30 ppm, Zn^{2+}: <6 ppm, and Cu^{2+}: <2.5 ppm) suggested that the present assay has a potential for an environmental risk assessment of plating wastewater.[18]

In summary, we have developed a colourimetric sensor array with molecular self-assembly systems using only the combination of commercially available materials. The mixtures of catechol dyes and 3-NPBA offered the construction of colourimetric chemosensors for metal ions. Thus, we fabricated the sensor array and detected 11 different species of metal ions simultaneously. As a result, we succeeded in discriminating metal ions with 100% correct classification and quantified a couple of metal ions at sub-ppm levels. Because the proposed array is fast, simple and easy-to-handle, we believe that self-assembled chemosensor arrays would be a new platform for the detection of metal ions in aqueous solutions.

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