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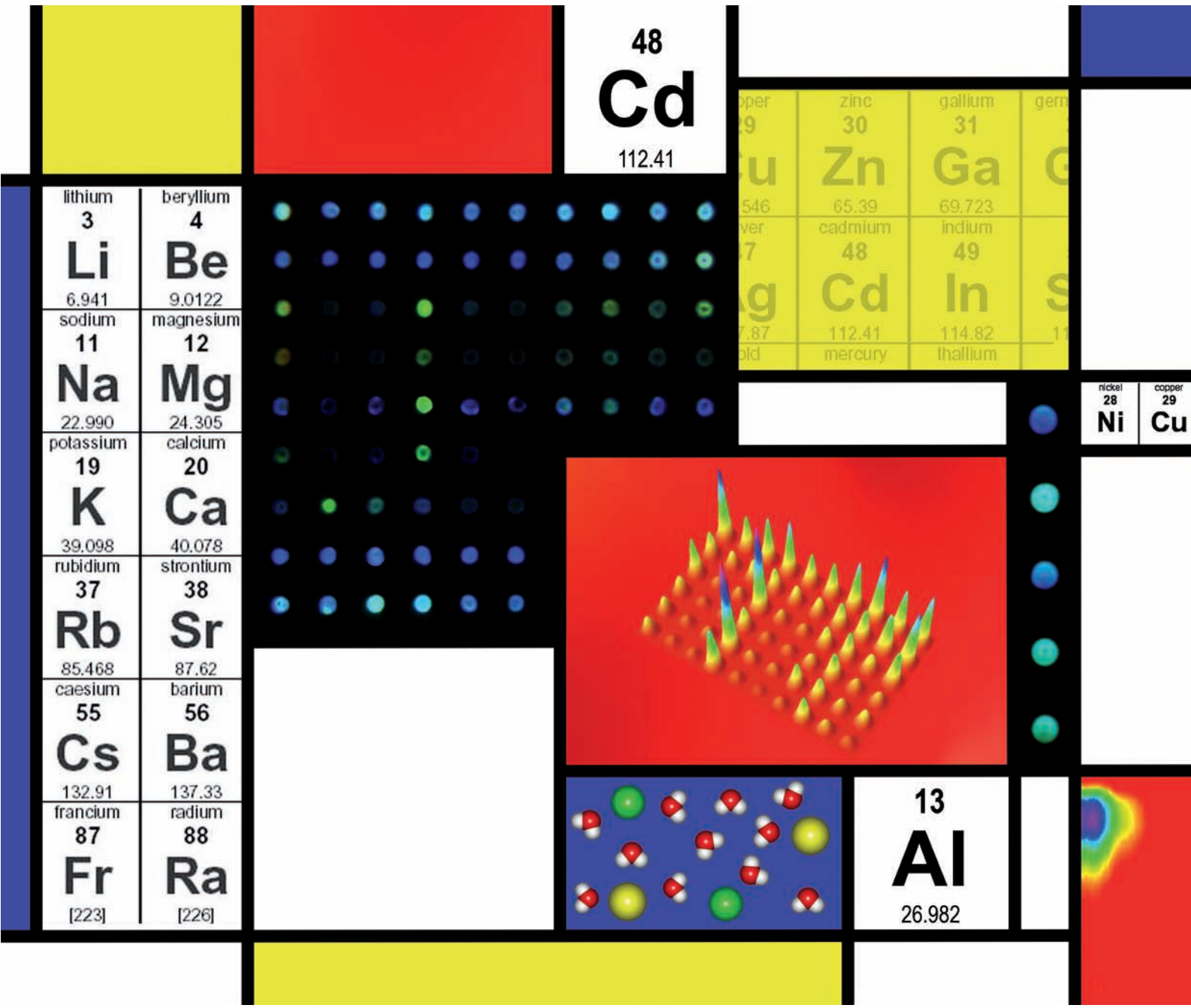

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Hydroxyquinolines with extended fluorophores: arrays for turn-on and ratiometric sensing of cations†

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8-Hydroxyquinoline-based ligands with extended conjugated fluorophores were designed to provide turn-on and ratiometric signal output optimized for use in fluorescence-based sensor arrays, where the changes in blue and green channels of the RGB signal are used to distinguish between cationic analytes.

Growing environmental concerns, as well as scientific enquiries on the participation of metal ions in biological processes, mandate the development of materials and methods for the detection and sensing of heavy- and transition-metal cations in aqueous environments. Sensors based on metal-induced changes in fluorescence appear to be particularly attractive as they offer the potential for high sensitivity at a low analyte concentration, coupled with the obvious ease of use.[1]

Numerous fluorescent sensors for cations are known from the literature.[2] These include various chelators and even off-the-shelf materials.[2,3] Among them, the sensors with *turn-on*[2] or ratiometric[4] fluorescence signalling are particularly valuable. Here, derivatives of 8-hydroxyquinoline (oxine or 8-HQ), such as 8-hydroxyquinoline-5-sulfonic acid (8-HQS), are among the most important chelators and sensors.[5] This is why we have used this chelator to demonstrate the advantage of our sensing approach. In the past, we have shown how the performance of fluorescent[6] and colorimetric[7] anion sensors may be improved *via* a careful design, as well as multi-channel analysis of the photonic response,[8] as opposed to tuning the analyte–ligand association.

With the arrival of array-based sensors,[9] the focus has shifted to signalling in a cross-reactive fashion. Cross-reactive arrays utilize small analyte-triggered perturbations arising from a large number of low-specificity sensor elements that show a wide range of responses, resulting in the formation of a pattern specific to a given analyte.[10] In luminescence array sensors, the light emitted by the sensors can be deconvoluted into its respective colors (*e.g.* red–green–blue, RGB), and the intensity of each color compared to the analyte-specific patterns stored in the device's memory.[9]

Here, we show how the performance of a chemosensor in general can be easily improved and, more importantly, adapted for use in cross-reactive arrays by modification of the signalling chromophore to induce a ratiometric response by varying the emission intensity in blue and green channels. As a receptor, we decided to use 8-HQ, as 8-HQ and its derivatives form luminescent chelates with a number of metal ions, including Cd^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+}, Ga^{3+}, In^{3+}, Sn^{4+}, Ti^{4+}, *etc.*[11] Perhaps the most interesting feature of 8-HQ is its lack of fluorescence in aqueous or organic solutions, and luminescence arising from metal chelation (*off–on* signalling). The non-fluorescent character of 8-HQ is due to the excited state proton transfer/intramolecular charge transfer and non-radiative relaxation.[12] Formation of a chelate prevents this non-radiative relaxation, and as a result, many metal chelates of 8-HQ exhibit blue–green luminescence. The respective color depends on the 8-HQ substituents[13] and the electropositivity of the metal.[14] 8-HQ has great potential in the design of cross-reactive arrays as it shows a *turn-on* signal and is highly cross-reactive, *i.e.* binds a number of metals while emitting light of slightly different luminescence quantum yields and wavelengths.

For applications in sensor arrays,[10b] it is desirable that sensors have a strong luminescence, a wide dynamic range of response and a strong signal output in at least two of the RGB channels utilized as a signal output. Because metalloquinolinolates are only moderately emitting ($\Phi < 0.15$),[15] it is desirable to attach a conjugated chromophore to boost their luminescence output. We present here 8-HQ-based sensors **S2–S6** (Fig. 1), which comprise of the ligand and blue-emitting residues, *i.e.* 2-pyrenyl or fluorene bridges. The varying conjugated fluorophores will ensure variable proportions of blue to green emissions of the quinolinolate complex. The reason why we decided to utilize ditopic ligands was to obtain a wider dynamic range of response during the sensing.[16]

Sensors **S2–S6** were synthesized by the Suzuki–Miyaura cross-coupling[17] reaction described previously.[18] **S2–S6** show varying

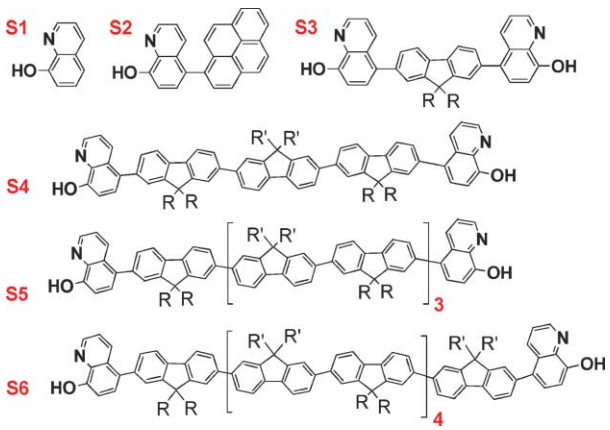

Fig. 1 Structures of sensors **S1** (8-hydroxyquinoline, 8-HQ) and **S2–S5** (8-HQ-based). R = *rac*-2-ethylhexyl, R′ = *n*-hexyl.

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fluorescence in solution ($\Phi_{S2} \sim 0.19$, $\Phi_{S3} \sim 0.01$, $\Phi_{S4} \sim 0.03$, $\Phi_{S5} \sim 0.29$ and $\Phi_{S6} \sim 0.77$) as the intramolecular quenching also deactivates the excited state in the fluorene bridges by fast energy migration in **S4**, **S5** and **S6**. The degree of fluorescence quenching is reduced for the longer oligofluorenes. Based on the degree of intramolecular quenching, one can roughly define two cases (Fig. 2, A and B), depending on the length of the conjugated chromophore. While the smaller fluorophores **S2** and **S3** show simpler turn-on behavior, the longer fluorene fragments are partially uncoupled from the quenching 8-HQ moiety and provide blue fluorescence even in the *off-state* (A). The green channel is then turned on upon cation complexation. Finally, septifluorene in **S5** and novifluorene in **S6** show strong fluorescence in both blue and green channels (B), while the green–blue balance depends on the cation. This scheme allows for varying the output in both the green and blue channels, which may then be utilized in array evaluation.

Prior to evaluation in arrays, we performed solution studies to ascertain the binding behavior of **S2–S6** in solution. The solution fluorescence of **S2–S6** was investigated in THF, as this solvent dissolves both the ligands and the resulting complexes. The experiments revealed a moderate–strong increase in fluorescence intensity, particularly for Ca^{2+}, Cd^{2+}, Mg^{2+}, Al^{3+} and also, to a lesser extent, Zn^{2+}. In the case of Cu^{2+}, Ni^{2+} and Co^{2+}, we observed attenuation of the fluorescence. Fig. 3 shows the excitation–emission map for **S4**, with a green highlight on the excitation axis to show the broadband excitation, where the UV scanner excites both the sensor and the array. Here, we also show two titration experiments of **S4** with Al^{3+} to show how the fluorescence output changes, depending on the excitation wavelength. Most importantly, the excitation–emission maps (Fig. 4) generated for the respective sensor–metal complexes show that the ratio of blue and green emission is likely to change, which can then be used for the ratiometric output.

A comparison of luminescence signatures in the excitation range of the scanners (300–410 nm) shows that each of the sensors **S1–S6**

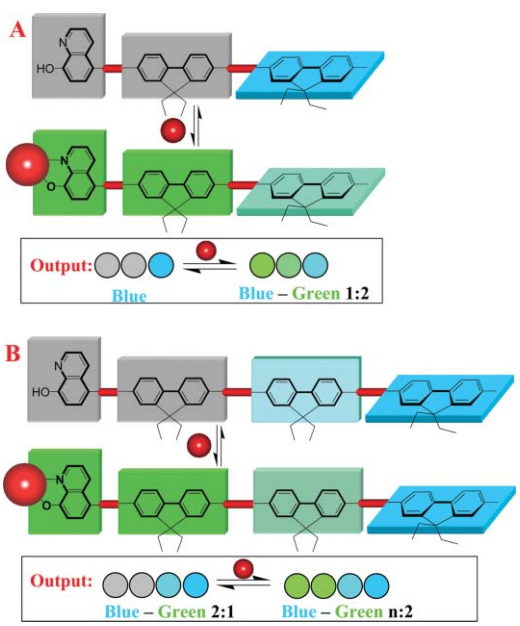

Fig. 2 Fluorescence output modulation in sensors **S2–S6** is achieved by using various types of extended conjugated chromophore.

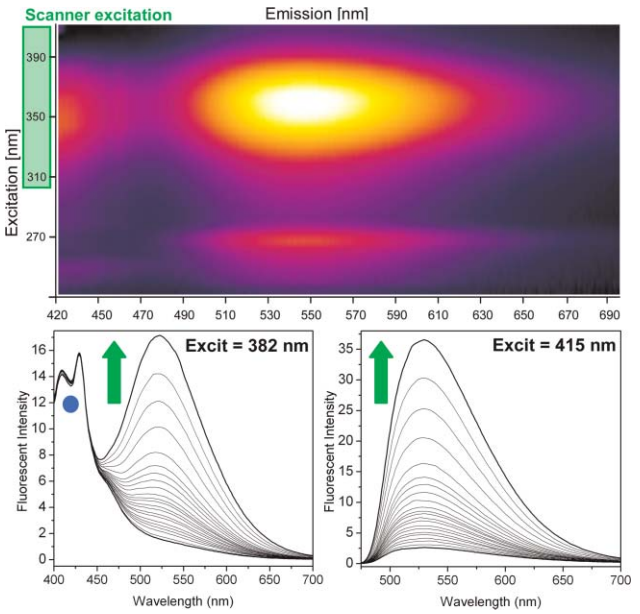

Fig. 3 Top: Fluorescence excitation-emission map generated for the **S4**–Al^{3+} complex. Bottom left: Titration of **S4** with $AlCl_3$ shows a blue emission and growing green band. Bottom right: Titration of **S4** with $AlCl_3$ shows only green emission.

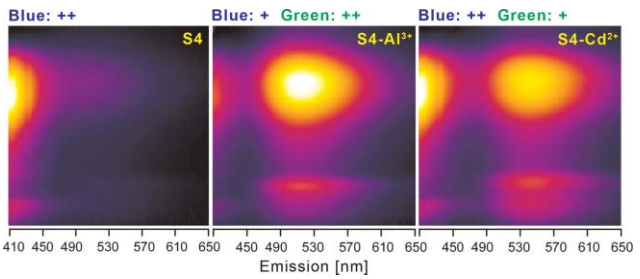

Fig. 4 Excitation–emission maps showing the relative intensity in blue and green emission to be used in the reading of the array. Left: Fluorescence of **S4**. Center: **S4**–Al^{3+} complex. Right: **S4**–Cd^{2+} complex.

will react differently to various cations (see ESI†). For example, in most sensors, Zn^{2+} and Cd^{2+} ions induced a varying degree of red shift in emission compared to Al^{3+} ions, while Ca^{2+} induced a blue shift. This is illustrated in Fig. 4, which shows emission from **S4** and its response to Al^{3+} and Cd^{2+} ions.

In the solid state array, the sensors are immobilized in a polyurethane carrier matrix to achieve uniform quality of films, regardless of the film-forming properties of the individual sensors. Presumably, immobilizing **S2–S6** in the polymer matrix precludes formation of coordination polymers due to high sensor–polymer dilution (0.07% **S2–S6** in polyurethane, w/w). This simplifies the binding processes as each quinolinolate moiety forms a 1 : 1 complex with any cation. Therefore, each of the ditopic sensors **S3–S6** can form only 1 : 1 or 1 : 2 (cation) complexes.

The arrays utilizing **S1–S6** dispersed in polyurethane and solution-cast into micro-well plates (1000 μm wide, 250 μm deep; sample volume = 400 nL) are shown in Fig. 5. The role of the hydrophilic polyurethane is to act as a mechanical support, and to draw the liquid analyte into the sensor material and ensure the formation of homogeneous sensor films in all wells of the assay,

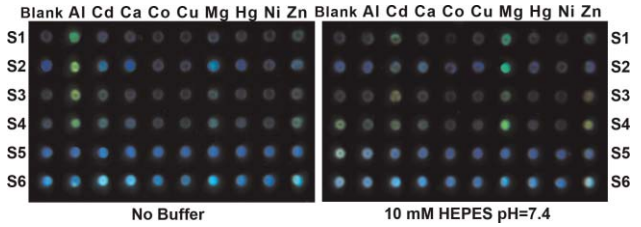

Fig. 5 Fluorescence output from two arrays using sensors **S1–S6** in the presence of chloride salts of various metal ions. Left: Metal ions (1 mM, 400 nL) were administered in de-ionized water. Right: Metal ions were administered in HEPES buffer (pH = 7.4).

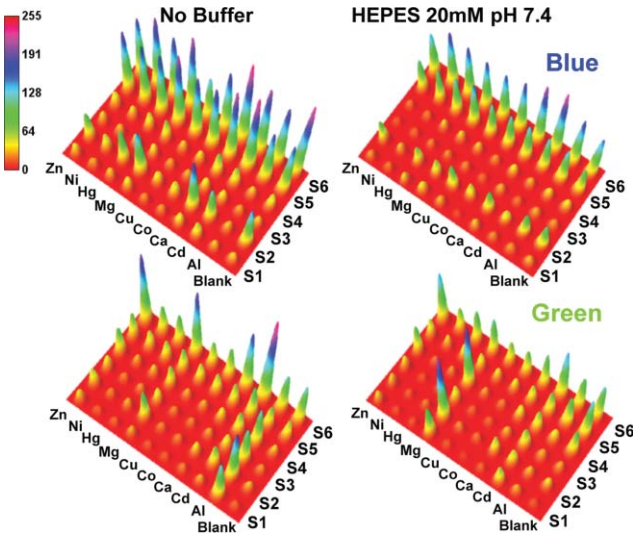

Fig. 6 Deconvolution of the array images of sensors **S1–S6** allows integration of the pixel intensities into the green and blue channels.

regardless of the relative hydrophilicity/lipophilicity of the individual materials. Most importantly, the polyurethane allows us to circumvent the incompatibility in solubility of the sensors and cations, which can be administered in water or buffer without causing aggregation or precipitation of the sensors.

The response of the array changes depends on the pH of the analyte. This feature can be further used to increase the data density used for discrimination between the analytes (Fig. 5).[19]

While the majority of cations tested provide a response discernible by the naked eye at 50–5000 μM concentration (Fig. 5), the scanned images, deconvoluted into their respective RGB channels, allow the construction of response patterns. Fig. 6 shows a quantitative representation of the changes of the grey pixel value (8-bit/channel) in their respective RGB channels.

The channel deconvolution of the array images confirms spectroscopic observations suggesting that sensors **S1–S6** will show varying emission intensities in the blue and green channels, thus generating response patterns useful for metal ion analysis. The combination of the turn-on response in the green channel with the analyte-specific intensity changes in the blue–green channels allows for ratiometric sensing in solid state arrays.

In summary, sensors **S2–S6**, utilizing extended conjugated chromophores, were designed to display luminescence turn-on, as well as ratiometric, responses. Luminescence measurements performed in solution confirmed the ability to differentiate between metal ions such as Ca^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+} and Al^{3+}, and show a strong response to Ca^{2+}, Cd^{2+}, Mg^{2+} and Al^{3+}. Conversely, cations such Zn^{2+} and Hg^{2+} showed only a weak response, while Co^{2+}, Cu^{2+} and Ni^{2+} showed emission quenching. Both luminescence turn-on and signal attenuation can be used to identify the respective cations on a qualitative level in aqueous solutions using a **S2–S6** micro-well array. Partial selectivity with variable intensity responses and changes in emission wavelengths make sensors **S2–S6** excellent candidates for use in cross-reactive luminescence-based arrays. Finally, preliminary experiments suggest that quantitative determination of metal ions is possible at a concentration range between 10 μm and 10 mM.‡

Notes and references

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