Transformation of a Modest Luminescent Chemosensor into a Light-Up Probe: The Overload Displacement Approach

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As a proof of principle for the overload displacement strategy a luminescent ruthenium(II) azacrown ether phenanthroline with restrained sensoric properties has been converted into a light-up chemosensor for lead(II) ions by overloading the system with copper(II) ions.

Highly selective cation or anion chemosensors are indispensable to various areas of application, such as screening toxic residues in the environment, analysing biological and clinical samples, and controlling waste management.[1] Often sophisticated analytical techniques, such as atomic absorption or emission spectroscopy, are used to detect the presence of heavy metal ions,[2] but the alternative use of luminescent chemosensors offers many advantages in terms of sensitivity, selectivity, and low cost.[3] Hence, substantial efforts have been made in the last two decades to improve sensitivity, selectivity, and dynamic working range[4] of luminescence probes for metal ions.[1,5,6] However, there still remains a significant need to design new chemosensors for trace amounts of metal ions. Herein, we would like to illustrate how the performance of a known luminescence probe operating along the reporter–receptor design (Chart 1a) can be vastly improved at no extra "synthetic cost" by the overload displacement strategy thus leading to a light-up probe.[7]

New concepts for chemosensor development, such as the lab-on-a-molecule[8] and the indicator displacement strategy,[4c,9] have been pressing the field ahead. The latter protocol, persuasively used for the detection of anions,[10] relies on the displacement of an indicator, "dark" in the bound state but well visible in its free state, from the recognition site of a receptor due to the competitive binding of the analyte (Chart 1b). An apparent disadvantage of this protocol is that the observed emission of the released indicator is not directly documenting the actual state of the receptor. While in most cases it is a reasonable guess that the indicator has been displaced by the putative analyte, the receptor site could have equally been destroyed.

In contrast to the classical design of luminescent chemosensors, sensoric approaches addressing biological analytes have made ample use of competitive binding assays,[11] quite often measuring the displacement of a competitor with a known affinity from the binding site or the degree of inhibition.[12] Herein, we describe a variant of a displacement assay, the overload

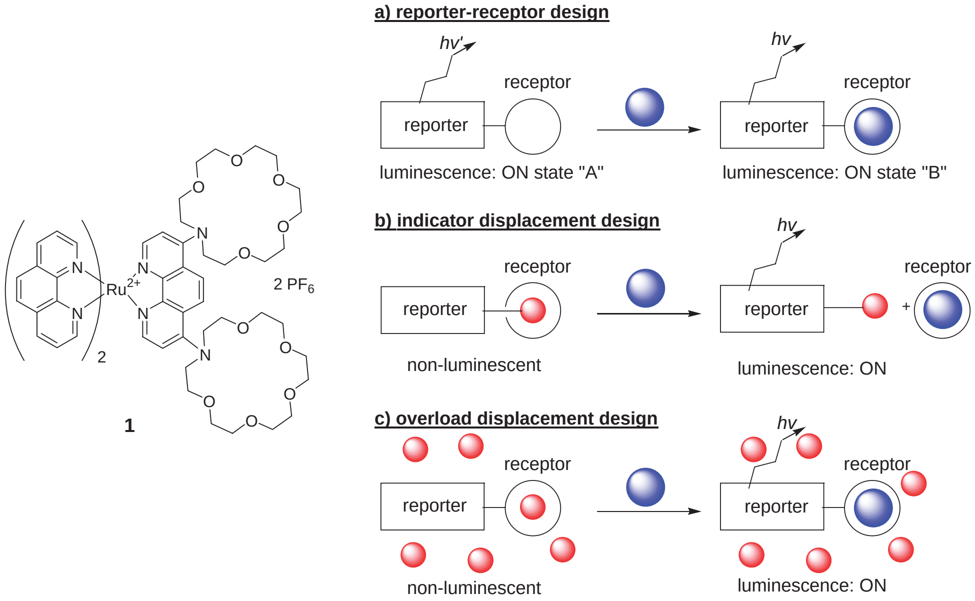

Chart 1. Chemical structure of compound **1** and various designs of luminescent sensors with analyte (blue) and competitor (red).

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displacement strategy, which is based on perturbing the equilibrium between the analyte and the receptor through the addition of a competitively binding guest (competitor) in large excess. Unlike many displacement approaches we do not follow the amount of released competitor or the degree of inhibition, but analyse directly the receptor as it is either bound to the competitor or the analyte. Our approach to a light-up probe requires a system, luminescent in its free state, that upon preloading with a dynamically and weakly binding guest (=competitor) has a strongly quenched emission (Chart 1c) and after displacement of the competitor by the analyte has a restored strong luminescence. Importantly, unlike the indicator displacement strategy and many other displacement designs, this protocol provides constant information about the situation in the receptor site of our reporter system.

The preparation and full characterisation of ruthenium(II) azacrown ether phenanthroline **1** (Chart 1) has been described recently.[8b,13] The detailed study of its emission and absorption in presence of a range of metal ions, e.g. Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Hg^{2+}, and Pb^{2+} (20 equiv each) as perchlorate salts in acetonitrile[8b] revealed that only Pb^{2+} and Cu^{2+} ions induced pronounced changes in the emission of **1**. Presence of Pb^{2+} (20 equiv) entailed a moderate 70% enhancement in emission intensity along with a blue shift of $\lambda_{\max}$(em) from 672 to 624 nm, while Cu^{2+} ion addition quenched the luminescence efficiently. Based on emission and UV–vis titration experiments of **1** against Pb^{2+} and Cu^{2+}, association constants were evaluated and assigned to specific binding models by using SPECFIT.[14] According to emission titration data the analysis revealed a two-step binding sequence for Pb^{2+} corresponding to **1**–(Pb^{2+}) and **1**–$(Pb^{2+})_2$ with $\log K_1{}^{Pb^{II}} = 6.4 \pm 0.2$ and $\log \beta_2{}^{Pb^{II}} = 11.3 \pm 0.3$, respectively. The association constant $\log K_1{}^{Pb^{II}}$ fully agrees with a value obtained with another luminescent probe containing the same azacrown ether.[15] The association constants for the binding of Cu^{2+} to **1** were evaluated using UV–vis titration rather than emission data since the quenching of the luminescence is not entirely due to Cu^{2+} binding but also due to intermolecular quenching.[16] The binding model only revealed a one-step association sequence with $\log \beta_2{}^{Cu^{II}} = 10.7 \pm 0.2$, which corresponds to the formation of **1**–$(Cu^{2+})_2$. It is important to stress that experiments and simulations, both in the emission and absorption mode (vide infra), probing the competitive binding of Pb^{2+} and Cu^{2+} for the receptor sites in **1** indicated that $\Delta \log \beta_2 = \log \beta_2{}^{Pb^{II}} - \log \beta_2{}^{Cu^{II}}$ is not 0.6 but around 2.[17] Such distinctly different association constants suggested the possibility to replace the bound Cu^{2+} ions in **1** with Pb^{2+} thus boosting its emission in line with the overload displacement strategy.

In line with the above rationale, addition of Pb^{2+} ions into **1**–$Cu^{2+}{}_{excess}$, i.e. the overloaded **1** (10 µM of **1** with 20 equiv of Cu^{2+}), led to a strong emission enhancement (≈4000%) along with a slight $\lambda_{\max}$(em) blue shift (Figure 1). This has to be compared to the modest 70% enhancement of the luminescence of "unloaded" **1** in the presence of Pb^{2+} ions under otherwise identical conditions. The emission response of **1**–$Cu^{2+}{}_{excess}$ was equally tested in the presence of a large range of other metal ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Hg^{2+}). As is evident from the emission curves in Figure 1, the **1**–$Cu^{2+}{}_{excess}$ exhibited a very high selectivity for Pb^{2+} ions over all other metal ions.

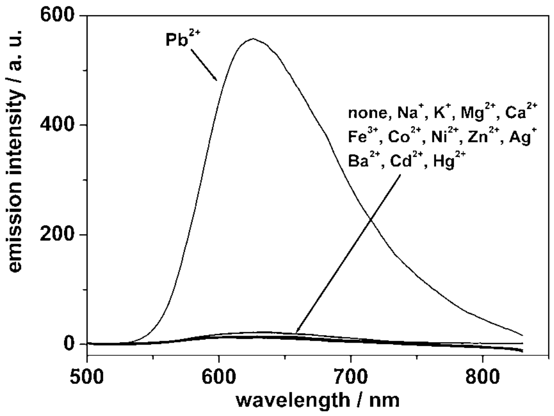

Figure 1. Emission of **1**–$Cu^{2+}{}_{excess}$ (10 µM of **1**, 20 equiv of Cu^{2+}) upon addition of metal ions in MeCN (Pb^{2+} and Ba^{2+}: 20 equiv each, all other metal ions: 100 equiv). The excitation wavelength was 429 nm.

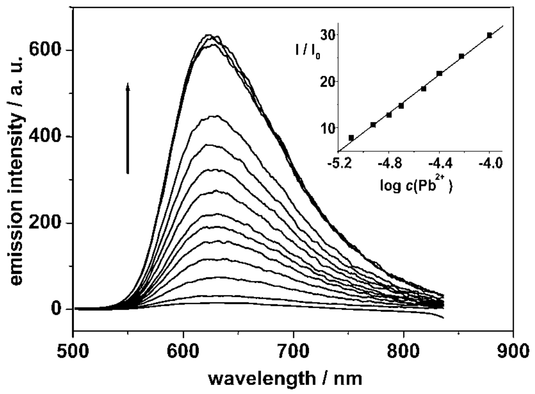

Figure 2. Emission of the **1**–$Cu^{2+}{}_{excess}$ ensemble (10 µM of **1**, 20 equiv of Cu^{2+}) upon addition of 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 6.0, 10, 20, 40, and 100 equiv of Pb^{2+}. Inset: Relationship between emission intensity of **1**–$Cu^{2+}{}_{excess}$ and $\log c(Pb^{2+})$ with I and I_0 referring to the emission intensity of **1**–$Cu^{2+}{}_{excess}$ with and without added Pb^{2+} ions, respectively. The wavelength of excitation was 429 nm.

The utility of the overloaded system **1**–$Cu^{2+}{}_{excess}$ for the quantitative detection of Pb^{2+} was further interrogated through titration experiments (Figure 2). When a Pb^{2+} solution was titrated into a solution of **1**–$Cu^{2+}{}_{excess}$ (10 µM of **1** and 20 equiv of Cu^{2+}), the emission intensity was enhanced rapidly with increasing additions of Pb^{2+}, levelling off at around 20 equiv (Figure 2). A linear correlation between emission intensity of the **1**–$Cu^{2+}{}_{excess}$ probe and $\log c(Pb^{2+})$ was established for an intermediate concentration range (Figure 2, inset). The increase of emission intensity of the **1**–$Cu^{2+}{}_{excess}$ chemosensor upon addition of Pb^{2+} seems to arise from the formation of the **1**–$(Pb^{2+})_2$ complex, as advocated by the identical emission wavelength of the independently generated complex,[8b] indicating that the Pb^{2+} ions replaced the Cu^{2+} ions in the azacrown ether receptor sites of **1**.

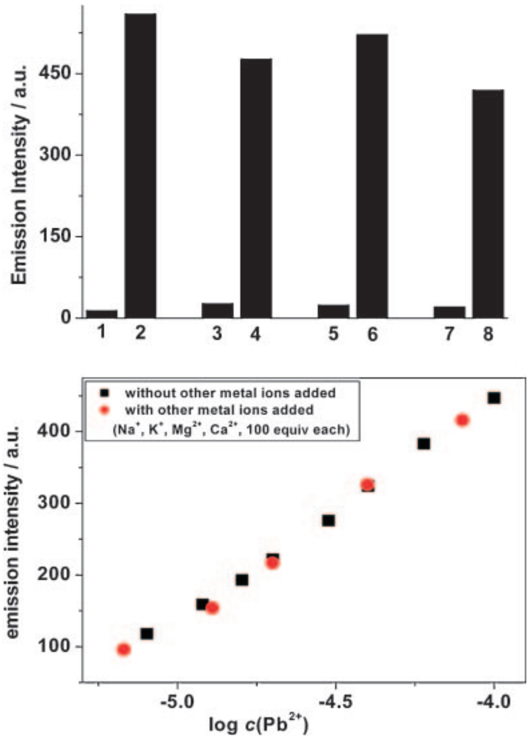

Figure 3. (Top) Luminescence of the **1**–$Cu^{2+}{}_{excess}$ ensemble in presence of interfering metal ions. (1) **1**–$Cu^{2+}{}_{excess}$ (20 equiv), (2) "(1)" + Pb^{2+} (20 equiv); (3) **1**–$Cu^{2+}{}_{excess}$ (20 equiv) + (Na^+, K^+, Mg^{2+}, Ca^{2+}, 100 equiv each), (4) "(3)" + Pb^{2+} (20 equiv); (5) **1**–$Cu^{2+}{}_{excess}$ (20 equiv) + (Co^{2+}, Ni^{2+}, Ag^+, Fe^{3+}, 100 equiv each), (6) "(5)" + Pb^{2+} (20 equiv); (7) **1**–$Cu^{2+}{}_{excess}$ (20 equiv) + (Zn^{2+}, Cd^{2+}, Hg^{2+}, 100 equiv each), (8) "(7)" + Pb^{2+} (20 equiv); (bottom) Relationship between emission intensity of **1**–$Cu^{2+}{}_{excess}$ and $\log c(Pb^{2+})$ in the absence and in the presence of Na^+, K^+, Mg^{2+}, and Ca^{2+} (100 equiv each).

The above emission results revealed that **1**–$Cu^{2+}{}_{excess}$ behaves as a highly selective ensemble chemosensor for Pb^{2+}, constituting a convincing example of metal ion detection using the overload displacement strategy. However, a true chemosensoric system has to equally work quantitatively in the presence of competing metal ions. As displayed in Figure 3, the **1**–$Cu^{2+}{}_{excess}$ ensemble chemosensor exhibited a linear relationship of the emission and $\log c(Pb^{2+})$ even in the presence of 100 equiv of other metal ions. Rewardingly, identical linear relationships of the emission intensity of **1**–$Cu^{2+}{}_{excess}$ and $\log c(Pb^{2+})$ were observed, both in the absence and presence of 100 equiv of Na^+, K^+, Mg^{2+}, and Ca^{2+} (Figure 3, bottom). Similarly, the Pb^{2+} concentration could also be determined in the presence of 100 equiv of Co^{2+}, Ni^{2+}, Ag^+, and Fe^{3+} and in that of Zn^{2+}, Cd^{2+}, and Hg^{2+} (see Supporting Information, Figures S1–S6). With reference to **1**,[8b] the results described herein therefore demonstrate **1**–$Cu^{2+}{}_{excess}$ to be a far superior luminescent chemosensor for Pb^{2+} ions.

The Pb^{2+} concentration may even be determined in samples containing large amounts of Cu^{2+}. In such a scenario, measurements need to be run with **1**–$Cu^{2+}{}_{excess}$ ensembles (10 µM of **1**) at various increasing overloads of Cu^{2+} (see Supporting Information, Figure S7). Once the overload exceeds significantly the amount of Cu^{2+} in the sample, the correct lead(II) ion concentration will be furnished (seen as a steady value independent of the overload).

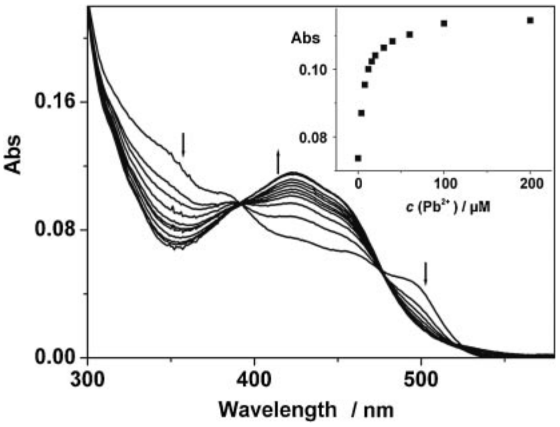

Figure 4. UV–vis titration of the **1**–Cu^{2+} ensemble sensor (10 µM of **1**, 20 equiv of Cu^{2+}) with 0, 0.4, 0.8, 1.2, 1.6, 2.0, 3.0, 4.0, 6.0, 10, and 20 equiv of Pb^{2+} added. Inset: The relationship between absorbance at 429 nm of **1**–$Cu^{2+}{}_{excess}$ and $c(Pb^{2+})$.

To further understand the metal ion exchange process, UV–vis titrations of **1**–$Cu^{2+}{}_{excess}$ against Pb^{2+} were performed. An increase in the MLCT absorbance of **1** upon addition of Pb^{2+} was observed (Figure 4) with the final absorption bands being identical to those obtained after a titration of free **1** (i.e. no Cu^{2+} present) with Pb^{2+}. Fitting[18] of the experimental data (see Figure 4, inset) to the model of the overload displacement strategy requiring rapid equilibration between

$$\begin{aligned} &\mathbf{1}\text{–}(Cu^{2+})_2 + Pb^{2+} \rightleftharpoons \mathbf{1}\text{–}(Cu^{2+})(Pb^{2+}) \\ &\qquad\qquad\qquad\qquad \text{in presence of excess of } Cu^{2+} \\ &\mathbf{1}\text{–}(Cu^{2+})(Pb^{2+}) + Pb^{2+} \rightleftharpoons \mathbf{1}\text{–}(Pb^{2+})_2 \\ &\qquad\qquad\qquad\qquad \text{in presence of excess of } Cu^{2+} \end{aligned} \tag{1}$$

was accomplished with $\log K_1{}^{Pb^{II}} = 6.4$, $\log \beta_2{}^{Pb^{II}} = 11.3$, $\log \beta_2{}^{Pb^{II}Cu^{II}} = 10.0$, and $\log \beta_2{}^{Cu^{II}} = 8.9$ (see Supporting Information, Figure S9). Notably, the same data set also allowed fitting of the experimental fluorescence titration data (see Supporting Information, Figure S9) providing strong support for the suggested model.

It is rewarding to see that the overload displacement strategy can be used as a quantitative analytical protocol despite the complexity of the system. The main reason for the increased performance is straightforward: starting out from **1**–$Cu^{2+}{}_{excess}$ assures low emission intensity at the onset of the analysis, whereas starting out from the bright **1** only results in a marginal increase of the emission as **1**–$(Pb^{2+})_2$ is forming. As soon as Pb^{2+} ions are added, Cu^{2+} is displaced from **1**–$Cu^{2+}{}_{excess}$ to furnish the complexes **1**–$(Pb^{2+})(Cu^{2+})$ and **1**–$(Pb^{2+})_2$ as well as further free copper(II) ions. The latter, however, are not innocent and quench to some extent the emission from the luminescent complex **1**–$(Pb^{2+})_2$ due to unspecific quenching.[16] In order to prevent copper(II) ions, which are released from their binding sites upon lead addition, to add substantially to the unspecific quenching of free copper(II) ions, it is recommended to use a large excess of Cu^{2+}, i.e. 20 equiv.

In conclusion, we have demonstrated the utility of the overload displacement strategy, a variant of competitive binding assays, to improve a known chemosensor's properties. As a proof of concept, the ruthenium(II) azacrown ether phenanthroline **1** with limited fluorosensor properties has been converted into a light-up chemosensor for lead(II) ions by overloading the system with copper(II) ions. In order to apply this strategy, the fluorosensor has to be preloaded with a weaker binding guest inducing a strong quenching of the emission. Upon addition of the analyte the preloaded guest is replaced by the analyte and fluorescence is restored.

Experimental

Preparation. The preparation and full characterisation of bis(1,10-phenanthroline)[4,7-bis(1,4,7,10,13-pentaoxa-16-azacyclooctadec-16-yl)-1,10-phenanthroline]ruthenium(2+) hexafluorophosphate (**1**) has been described recently.[13]

Luminescence and UV–Vis Titrations. UV–vis spectra were measured on a Varian Cary 100 Bio UV–Visible Spectrophotometer and emission spectra on a Varian Cary Eclipse Fluorescence Spectrophotometer with excitation and emission slit widths at 5 nm. All emission spectra were corrected. Stock solutions of **1** (1 mM) and the metal ions (1–100 mM, all as perchlorate salts) in dry MeCN were used to set up the required solutions. 3 mL of the **1**–Cu^{2+} ensemble in MeCN, i.e. complex **1** (10 µM) and Cu^{2+} (200 µM; 20 equiv), were placed in a quartz cuvette (optical length: 1 cm) thermostated at 298 K. In the titration a stock solution of Pb^{2+} in MeCN was added with an Eppendorf pipette in amounts of 2–20 µL, insuring that the total addition was less than 1% of the original volume (3 mL). Spectra were recorded immediately after addition and proper mixing. If not mentioned otherwise, equivalents of metal ions are always denoted according to the complex.

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Supporting Information

Experimental, luminescence titration of **1**–Cu^{2+} complex with Pb^{2+} in presence of interfering metal ions and titration of **1** with Cu^{2+}. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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