Multi-readout logic gate for selective metal ions detection in parts per billion levels

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Abstract: Optical sensors utilizing visual responses for metal ions identification require reliable molecular systems able to selectively operate in multicomponent solutions. Herein we report water soluble terpyridyl based ligand that demonstrates effective quantification of ppb to ppm levels of Fe2+, Fe3+, Zn2+, and Ru3+. While Fe2+ and Ru3+ ions bind to the ligand to form monometallic complex, 2:1 ligand to metal binding stoichiometry was found for Zn2+ and Fe3+ complexes. Corresponding metal binding events are directly translated into distinct colorimetric and spectroscopic logic outputs. Applying molecular logic (Boolean logic operations) to describe these binding events, selective discrimination between the ions was demonstrated.

Introduction

Transition metals play a vital role in biology and chemistry. Among the various transition metals, iron is the most abundant essential trace element in the human body, which plays crucial roles in physiological processes such as oxygen transport, electron transfer, and enzymatic catalysis.[1] Iron deficiency may lead to anemia, organ dysfunction, and tumorigenesis. Paradoxically, too much iron is equally hazardous to health due to its ability to generate free radicals that increase risk of cancer[2] and Alzheimer Disease.[3] Zinc is the second most abundant transition metal in human body after iron.[4] Unlike iron, which is required for certain specific functions, zinc is required for general metabolism.[5] However, increased dietary zinc exhibits significant memory deficits and may cause Alzheimer’s disease,[6] a failure of zinc homeostasis is linked to development of prostate cancer,[7] and finally, accumulation of zinc to toxic levels leads to cell death.[8] Extensive use of ruthenium catalysts in industrial processes, generation of e-wastes where Ru is employed as a hardener in Pt/Ru alloys, and use of ruthenium in medicine[9] result in raising of ruthenium content in the environment.[10] Recent success in development of water splitting process catalyzed by ruthenium complexes may potentially become an additional supply of ruthenium to the environment, since metal leaching is a typical issue for many catalytic processes.[11] Even though ruthenium is not an essential trace element in the human body, it may have significant impact on human health; in particular, ruthenium complexes were shown to form cross-links with nucleic acids to halt DNA replication.[12] Although significant progress has been made in monitoring of iron(II),[13] iron(III),[14] zinc(II),[15] and ruthenium(III)[16] as single-analyte ions, as well as in detection of iron(II)-iron(III) pair,[17] recent research efforts have been focused on the development of assays that allow simultaneous multi-analyte detection in ppb to ppm level,[17-19] which is especially important in environmental testing, food chemistry and molecular biology.[19] Since the seminal work by de Silva who invented the first two-input molecular logic gate,[20] many types of logic gates responsive to two physical and/or chemical signals have been successfully demonstrated.[21] Implementation of multianalyte assays in molecular-scale devices resulting in sensor arrays in solution remains challenging.[22] Here we report on development of a water-soluble ligand able to simultaneously detect several metal ions.

Results and Discussion

The ligand 2,2’:6’,2”-terpyridine (terpy) is known to form complexes with variety of metals.[23] However, low solubility of terpy in water constrains the use of this ligand for direct metal detection in aqueous media. To avoid this problem, we decorated terpy by polar diethylphosphonate group resulting in terpyPO(Et)2, ligand 1 (Scheme 1) as previously described.[24] Being more polar than terpy, 1 is soluble, and can form soluble metal complexes in alcohol-water mixtures. For example solubility of 1 in deionized (DI) water: ethanol mixture (80:20 v/v) was found to be 28 mM. Yet, solubility of 1 in 100% DI water (0.1 mM saturated) remains quite low. Further hydrolysis of 1 in acidic conditions results in terpyPO(H)2, ligand 2, which is readily soluble in DI water at concentrations up to 66 mM.

![Scheme 1](image)

Scheme 1. Ligands terpyPO(Et)2, (1), terpyPO(H)2, (2) and their Fe2+ complexes, [Fe(terpy-PO(Et))2]3+ (3) and [Fe(terpy-PO(H))2]3+ (4).

To probe metal sensing ability in water, UV-vis absorbance and fluorescence emission spectra of 2 (4.7 ppm, 0.015 mM) with various metal cations (15 ppm) were measured. Presence of

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[c] Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejic.201600606. It can be obtained from The Cambridge Crystallographic Data Centre.
Fe²⁺, Fe³⁺, Ru³⁺, and Zn²⁺ resulted in significant changes of either the absorption or emission spectrum (Figs. 1, S2), but no distinct changes were observed for Al³⁺, Ba²⁺, Ca²⁺, Cs⁺, Co²⁺, Cr³⁺, Cu²⁺, Ir⁴⁺, K⁺, Li⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, Pd²⁺, and Sn²⁺. When Fe was mixed with Fe²⁺ ion in water, colourless solutions immediately turned pink (Fig S1) due to the formation of complex 3. A characteristic metal to ligand charge transfer band is observed at 560 nm (ε₅₆₀=1.197·10⁴ M⁻¹ cm⁻¹). Presence of Fe²⁺ and Ru³⁺ ions in relatively high concentrations (15 ppm) in limiting (4.7 ppm, 0.015 mM) water solutions of 2 can be tracked by the appearance of characteristic bands at λₐbs = 560 nm and λₐbs = 400 nm, respectively. Significant increase in intensity of the absorption λₐbs = 290 nm is due to either Ru³⁺ or Fe²⁺. Comparing the change in intensities of λₐbs = 290 nm and λₐbs = 400 nm, Ru³⁺ or Fe³⁺ ions can be discriminated. Furthermore, mixtures of 2 with Zn²⁺ show strong fluorescence emission at λ = 360 nm and λ = 700 nm upon excitation at 326 nm. Thus, by combination of the UV-vis and fluorescence data, it is possible to detect and differentiate between all four metal ions (Fig. 1). To get inside the formation of metal complexes of these four target metal cations with 2 in aqueous media, UV-vis and fluorescence emission spectra of DI water solutions containing excess of 2 and limiting amounts of metal salts were recorded.

Using the UV-vis absorbance response at λₐbs = 560 nm, we could detect as low as 50 ppb of Fe²⁺ in aqueous solutions in presence of 15 ppm of 2. Increasing Fe²⁺ concentration up to 1000 ppb results in a linear increase of the response (Fig 2A). Importantly, detection of Fe²⁺ ions was found to be independent on the nature of anion (Cl⁻ vs ClO₄⁻). Identical linear correlations were found for detection of FeCs (black line, R²=0.997) and Fe(CIO₄)₂ (red line, R²=0.997). Fig 2A. Similar experiments performed with 1 in DI water : ethanol (20%-80% v/v) (Figure 2B) allow to detect as low as of 50 ppb of Fe²⁺ with the linear increase of the response up to 1000 ppb of Fe²⁺. The similarity of these results suggests that PO₃Et and PO₃H₂ groups in 1 and 2 do not participate in coordination with Fe²⁺.

**Figure 1.** Representative UV-vis absorbance changes upon addition of metal salts (15 ppm) to 2 (4.7 ppm) in DI water: A) at 290 nm; B) at 400 nm; C) at 560 nm. D) Representative fluorescence emission changes upon addition of metal salts (15 ppm) to 2 (4.7 ppm) in DI water at 360 nm.

**Figure 2.** UV-vis absorbance of A) mixed aqueous solution containing 2 (15 ppm, 0.048 mM), and B) water : ethanol solution containing 1 (15 ppm, 0.041 mM), (right) with different concentrations of Fe²⁺.

Linear increase of absorbance intensities of aqueous solutions of 2 in presence of Fe²⁺ or Ru³⁺ ions up to 1:1 ligand to metal stoichiometric ratio, suggests the formation of a monometallic complex in both cases (Figs. S10, S11). On the contrary, increase of Fe²⁺ concentration in an aqueous solution of 2 results in relatively narrow linear area of the absorbance followed by deviation from linearity and saturation of the signal. This suggests the formation of complexes where more than one ligand (L) per Fe²⁺ (M) ion is involved. Indeed, HRMS analysis of Fe²⁺ to 2 adduct (complex 3) gives molar mass that corresponds to L:M structure (see SI). Moreover, slow evaporation of isopropanol-water mixture containing excess of Fe²⁺ and 1 as a limiting reagent, results in X-Ray quality L:M crystals of complex 4 (Fig. 3A). The complex displays a slightly distorted octahedral geometry around Fe (II) center formed by coordination of two molecules of ligand 1 via terpyridine moieties situated in almost orthogonal fashion.

**Figure 3.** A) Crystallographically determined structure of [Fe(terpy-PO₃Et)₂](ClO₄)₂ (4). Thermal ellipsoids drawn at the 30% confidence level. Hydrogen atoms, counter anions (ClO₄⁻) and solvent molecules (H₂O) are omitted for clarity. B) Fluorescence emission at 360 nm of a mixed aqueous solution containing compound 2 (1 ppm, 3.19·10⁻³ M) with different concentrations of Zn²⁺.

Presence of Zn²⁺ ions in aqueous solutions of 2 can be detected by the appearance of two fluorescence emission bands (λᵢ = 360 nm and λᵢ = 700 nm). Band at 360 nm has quantum
yield (QY) of 0.75, which is much higher than QY of a common fluorescence standard tryptophan (0.14), and thus can be used to quantify ultra-small concentrations of Zn$^{2+}$ (as low as 0.25 ppb of Zn$^{2+}$ can be detected by 1 ppm solution of 2, Fig. 3B). On the contrary, band at 700 nm is lower in intensity and can be used to detect and quantify significantly higher concentrations of Zn$^{2+}$ (Figs. S14). To establish stoichiometry of the coordination of 2 with all four metal ions, continuous variation analysis procedure (Jobs plot) was performed with the corresponding absorbance and fluorescence intensity data (Fig. 4). Examination of the Jobs plot suggests 2:1 ligand (L) to metal (M) binding stoichiometry for Zn$^{2+}$ and Fe$^{3+}$ complexes that is consistent with literature reports for structurally related complexes.\(^ {25} \) Performing titration of 2 by Zn$^{2+}$ as well as by Fe$^{3+}$ in phosphate buffer (pH 7.0) results in similar (within the measurement error) results. Fe$^{3+}$ and Ru$^{3+}$ form 1:1 L:M complex.

However, the intensity of both fluorescence emission maxima at 360 nm and 700 nm of water solutions of Zn$^{2+}$ (1 ppm) and 2 (15 ppm, 0.048 mM) decrease significantly (yet does not quench completely) in presence of 1 ppm of Fe$^{3+}$ (Fig. 5). Moreover, presence of Fe$^{3+}$, Fe$^{2+}$, Ru$^{3+}$, and Zn$^{2+}$ ions (1 ppm each) in water solutions containing an excess of ligand (15 ppm), results in a complex optical and fluorescence response that can be analyzed utilizing molecular logic gates concept.

To discover ability of 2 for simultaneous detection of few metal cations, absorbance and fluorescence of 2 (excess, 15 ppm) in presence of target cation and competing metal ion or mixtures of few metal ions (1 ppm each) were studied. When Fe$^{3+}$ is present in the solution, intensity of the characteristic absorbance band at 560 nm is almost not affected by the presence of Zn$^{2+}$, Ru$^{3+}$, and Fe$^{3+}$ in different combinations (Fig 5). To probe if our system is applicable to real objects, samples of apple juice (Rougemont) and apple cider (Wellesley) were analyzed. The amount of Fe$^{3+}$ determined by $\lambda_{abs}$ = 560 nm using standard addition method in juice (0.0210 mM) and cider (0.0321 mM) is consistent with the results of total iron determined by atomic absorption spectroscopy (0.0242 mM and 0.0344 mM, respectively). The same (within experimental error) results were obtained when the samples were treated with hydroxylamine that is known to reduce Fe$^{3+}$ to Fe$^{2+}$ thus suggesting that most of the iron in both solutions consists in Fe$^{2+}$ form and confirming the statement.

In the following set of experiments, four chemical inputs Fe$^{3+}$, Ru$^{3+}$, Fe$^{2+}$, and Zn$^{2+}$ were used to address the optical outputs of the Fe$^{3+}$ (15 ppm) in water, 0.048 mM to various metal ions. Black bars represent the absorption of 2 in the presence of selected metal ion(s) (1 ppm each) without Fe$^{3+}$ (A) and without Zn$^{2+}$ (B) after 5 min, respectively. Blue and green bars represent the absorption of 2 at 560 nm in presence of Fe$^{3+}$ and a competitive ion or a mixture of competitive metal ions (1 ppm each) after 5 min and 2 h, respectively. Red bars represent the absorption of 2 at 700 nm (excitation on 360 nm) in presence of Zn$^{2+}$ (1 ppm) and a competitive ion or a mixture of competitive metal ions (1 ppm each) after 5 min.

Figure 4. Jobs plot for 2 and A) Zn$^{2+}$ (in phosphate buffer, pH 7.0), B) Fe$^{3+}$ (in phosphate buffer, pH 7.0), C) Ru$^{3+}$, and D) Fe$^{2+}$, suggesting the formation of a 2:1 (L:M) complex in cases of Zn$^{2+}$ and Fe$^{3+}$; and 1:1 (L:M) complex in cases of Ru$^{3+}$ and Fe$^{2+}$.

To establish stoichiometry of the coordination of 2 with all four metal ions, continuous variation analysis procedure (Jobs plot) was performed with the corresponding absorbance and fluorescence intensity data (Fig. 4). Examination of the Jobs plot suggests 2:1 ligand (L) to metal (M) binding stoichiometry for Zn$^{2+}$ and Fe$^{3+}$ complexes that is consistent with literature reports for structurally related complexes.\(^ {25} \) Performing titration of 2 by Zn$^{2+}$ as well as by Fe$^{3+}$ in phosphate buffer (pH 7.0) results in similar (within the measurement error) results. Fe$^{3+}$ and Ru$^{3+}$ form 1:1 L:M complex.

Figure 5. UV-vis absorbance intensity at 560 nm (A) and fluorescence emission at 700 nm (B) responses of 2 (15 ppm in water, 0.048 mM) to various metal ions. Black bars represent the absorption of 2 in the presence of selected metal ion(s) (1 ppm each) without Fe$^{3+}$ (A) and without Zn$^{2+}$ (B) after 5 min, respectively. Blue and green bars represent the absorption of 2 at 560 nm in presence of Fe$^{3+}$ and a competitive ion or a mixture of competitive metal ions (1 ppm each) after 5 min and 2 h, respectively. Red bars represent the absorption of 2 at 700 nm (excitation on 360 nm) in presence of Zn$^{2+}$ (1 ppm) and a competitive ion or a mixture of competitive metal ions (1 ppm each) after 5 min.

Figure 6. Logic circuit for the 4-input and 4-output logic gate system. Each output is separated for clarity. Since output $\lambda_{abs}$ = 560 nm depends only on presence of Fe$^{3+}$, the logic circuit is not shown.
The response at 400 nm (output B) is positive (here Δ400 ≥ 0.012 a.u.) in presence of Ru3+ (trial 3). Presence of Zn2+ together with Ru3+ results in the signal disappearance, i.e. output B = 0 (trial 10). Thus using Zn2+ ions as an input, NOT gate was implemented followed by AND gate joining Zn2+ and Ru3+ inputs and resulting in an INHIBIT logic gate. On the other hand, mixture of Ru3+ with either Fe2+ or Fe3+ (trials 6 and 9, respectively) as well as mixture of Ru3+ with both Fe2+ AND Fe3+ (trial 12), and even Ru3+ with Fe2+, Fe3+, and Zn2+ (trial 16) result in a positive (output B = 1) response. Presence of Zn2+ together with either Fe2+ (trial 8) or Fe3+ (trial 11) result in insignificant optical response (output B = 0, Δ400 < 0.012 a.u.). Output at 290 nm is positive (Output C = 1, Δ290 ≥ 0.17 au) when Fe3+ and/or Ru3+ is/are present in the system (trials 3, 4, and 9). Presence of either Fe2+ or Zn2+ or both Fe2+ AND Zn2+ inhibits output C. Fluorescence emission output at 360 nm is positive (Output D = 1, if Δ360 ≥ 2000 au) in presence of Zn2+ (trial 5). However, presence of Fe2+ AND at least one of the other ions (Fe3+ or Ru3+) INHIBITs fluorescence.

Conclusions

In summary, we have developed a promising sensing platform for rapid and sensitive detection of Fe2+, Fe3+, Ru3+, and Zn2+ in aqueous solutions, which is potentially applicable for analysis of environmental, food, biological and biomedical systems. Unique responses from these four metal cations could be detected by the combination of UV-vis and fluorescence spectrometry. To get fundamental understanding of this sensing system, metal-ligand stoichiometries were studied. Corresponding metal binding events are directly translated into distinct colorimetric and spectroscopic logic outputs. By utilizing all acquired knowledge we were able to apply molecular logic (Boolean logic operations) to describe the interaction between the ligand (as a logic gate) and four target cations (four logical inputs). We believe that the combination of these logic gates with other novel technologies, such as ‘lab-on-a-chip’ and even ‘lab-on-a-molecule’, could bring about a broad range of applications in analytical and material sciences.

Acknowledgements

Research supported by the University of Ontario Institute of Technology start-up grant and NSERC Discovery grant.

Keywords: Metal-ligand recognition • sensors • molecular logic • X-ray • terpy


Entry for the Table of Contents (Please choose one layout)

Layout 1:

Sensing platform for rapid and sensitive (parts per billion) detection of Fe$^{2+}$, Fe$^{3+}$, Ru$^{3+}$, and Zn$^{2+}$ in aqueous solutions was proposed.

Boolean logic operations were applied to describe the interaction between the ligand (as a logic gate) and four target cations (four logical inputs).

Crystallographically determined structure of [Fe(terpy-PO$_3$Et$_2$)$_2$](ClO$_4$)$_2$ complex was presented.

Simultaneous detection

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Multi-readout logic gate for selective metal ions detection in parts per billion levels

*one or two words that highlight the emphasis of the paper or the field of the study