Supporting Information For

A Bimodal Multianalyte Simple Molecule Chemosensor for Mg\(^{2+}\), Zn\(^{2+}\), and Co\(^{2+}\)

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Contents:

Determination of binding constants for Mg\(^{2+}\) and Zn\(^{2+}\)

Determination of binding constants for Co\(^{2+}\)

Scheme S1 Synthetic route to HL.

Fig. S1 The linear dynamic response of HL for Mg\(^{2+}\) and the determination of the detection limit (LOD) for Mg\(^{2+}\) in CH\(_3\)CN.

Fig. S2 Fluorescence Job’s plot for HL with Mg\(^{2+}\) in CH\(_3\)CN.

Fig. S3 ESI-MS spectrum of MgL\(_2\) complex.

Fig. S4 Competition experiments. The black bars represent the addition of an excess of metal ions
to an acetonitrile solution of HL (50 µM) in the presence of Mg^{2+} (25 µM). \( \lambda_{em} = 546 \) nm, [Metal] = 100 µM, [Ca^{2+}] = 500 µM, [K^+] = 500 µM, [Na^+] = 500 µM, [Al^{3+}] = 500 µM.

**Fig. S5** Competition experiments. The black bars (A) represent the addition of an excess of metal ions to an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution of HL(50 µM), and the red bars (B) represent the subsequent addition of 50 µM Zn^{2+} ions to the foregoing solution. \( \lambda_{em} = 558 \) nm.

**Fig. S6** The linear dynamic response of HL for Zn^{2+} and the determination of the detection limit (LOD) for Zn^{2+} in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution.

**Fig. S7** Absorbance Job’s plot for HL with Co^{2+} in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution.

**Fig. S8** ESI-MS spectrum of CoL\textsubscript{2} complex.

**Fig. S9** The linear fitting (absorbance at 476 nm) of CoL\textsubscript{2}.

**Fig. S10** Competition experiments (absorbance at 476 nm). The black bars (A) represent the addition of an excess of metal ions to an acetonitrile/Tris-HCl (50 mM, pH 7.40) (1:1, v/v) solution of HL(50 µM), and the chromatic bars (B) represent the subsequent addition of 25 µM Co^{2+} ions to the foregoing solution.

**Fig. S11** The different metal salts toward HL for sensing Mg^{2+}, Zn^{2+}, and Co^{2+}.

**Fig. S12** \(^1\)H-NMR spectrum of HL (\(d_6\)-DMSO) at room temperature.

**Fig. S13** \(^{13}\)C-NMR spectrum of HL (\(d_6\)-DMSO) at room temperature.

**References**
**Determination of binding constants for Mg\(^{2+}\) and Zn\(^{2+}\).**

The association constant and stoichiometry for the formation of the complex were evaluated using the Benesi-Hildebrand (B-H) plot [Eq. (1)].

\[
\frac{1}{F - F_0} = \frac{1}{K} \frac{1}{F_{\text{max}} - F_0} \frac{1}{[M^{n+}]} + \frac{1}{F_{\text{max}} - F_0}
\]  

(1)

Binding stoichiometry for the complex formation is confirmed from experimental data. In the case of evaluation of the binding constant from the results of fluorescence titration, a modified B-H equation [Eq. (1)] is used, where \(F_0, F_{\text{max}},\) and \(F\) represent the emission intensity of HL, the maximum emission intensity observed in the presence of Mg\(^{2+}\) or Zn\(^{2+}\), and intensity at a certain concentration of the metal ion, respectively. \(K\) is the association constant (M\(^{-2}\)) and was determined from the slope of the linear plot, and \([M^{n+}]\) is the concentration of the Mg\(^{2+}\) or Zn\(^{2+}\) ion added during titration studies.

**Determination of binding constants for Co\(^{2+}\).**

Assuming a 1: \(n\) stoichiometry for interaction between L and Co\(^{2+}\), the equilibrium is given by the following equation:

\[
L + n\text{Co}^{2+} \rightleftharpoons \text{LCo}_n^{2+}
\]  

(2)

The association constant, \(K\), is therefore expressed as:

\[
K = \frac{[\text{LCo}_n^{2+}]}{[L][\text{Co}^{2+}][n]} = \frac{[\text{LCo}_n^{2+}]}{[[L]_0 - [\text{LCo}_n^{2+}]]([\text{Co}^{2+}]_0 - n[\text{LCo}_n^{2+}])}
\]  

(3)

\([\text{LCo}_n^{2+}], [L],\) and \([\text{Co}^{2+}]\) represent the equilibrium concentrations of the complex, free L, and free Co\(^{2+}\), respectively. \([L]_0\) and \([\text{Co}^{2+}]_0\) are the initial concentrations of L and Co\(^{2+}\), respectively.

If \([\text{Co}^{2+}]_0 \gg [\text{LCo}_n^{2+}],\) the equation 3 can be simplified as follows:

\[
K = \frac{[\text{LCo}_n^{2+}]}{[[L]_0 - [\text{LCo}_n^{2+}]]([\text{Co}^{2+}]_0^n)}
\]  

(4)

Then it can be transformed to:

\[
K[\text{Co}^{2+}]_0^n = \frac{[\text{LCo}_n^{2+}]}{[L]_0 - [\text{LCo}_n^{2+}]}\]

(5)

Absorbance intensity is given by following equations:
\[
\frac{A - A_{\text{min}}}{A_{\text{max}} - A} = \frac{[\text{LCo}_n^{2+}]}{[\text{LCo}_n^{2+}]_{\text{max}} + [\text{LCo}_n^{2+}]} = \frac{[\text{LCo}_n^{2+}]}{[\text{L}]_0 - [\text{LCo}_n^{2+}]} \quad (6)
\]

\(A_{\text{min}}\) is the absorbance intensity of \(\text{L}\) without cations, \(A\) is the absorbance intensity of \(\text{L}\) obtained with \(\text{Co}^{2+}\), \(A_{\text{max}}\) is the absorbance intensity of \(\text{L}\) in the presence of excess amount of \(\text{Co}^{2+}\). In the presence of excess amount of \(\text{Co}^{2+}\), \([\text{LCo}_n^{2+}]_{\text{max}}\) is almost equal to \([\text{L}]_0\). Using equations 5 and 6, the following equation is given:

\[
\frac{A - A_{\text{min}}}{A_{\text{max}} - A} = K[\text{Co}^{2+}]_0^n \quad (7)
\]

\[
\lg \frac{A - A_{\text{min}}}{A_{\text{max}} - A} = \lg K + n \lg [\text{Co}^{2+}]_0 \quad (8)
\]

When assuming the 2:1 stoichiometry \((n = 0.5)\), equation 1 is obtained.

\[
\lg \frac{A - A_{\text{min}}}{A_{\text{max}} - A} = \lg K + 0.5 \lg [\text{Co}^{2+}] \quad (1)
\]
Scheme S1 Synthetic route to HL

Fig. S1 The linear dynamic response of HL for Mg$^{2+}$ and the determination of the detection limit (LOD) for Mg$^{2+}$ in CH$_3$CN.

Fig. S2 Fluorescence Job’s plot for HL with Mg$^{2+}$ in CH$_3$CN. The total [HL] + [Mg$^{2+}$] = 100 µM.
ESI-MS exhibited the formation of a complex between two deprotonated HL and an Mg$^{2+}$ \([\text{m/z } 661.3057 (2L+Mg^{2+}); \text{calcd for C}_{40}\text{H}_{40}\text{MgN}_{6}\text{O}_{2} \text{m/z 661.09}].\]

The physiologically important cations, such as K$^+$, Na$^+$, Ca$^{2+}$, and Al$^{3+}$, which exist at high concentrations in living cells, have negligible interference for forming the Mg-complex. In addition, Cd$^{2+}$ and Hg$^{2+}$ quenched the fluorescence intensity due to the heavy metal effect. Even though some biologically relevant metal ions, like Zn$^{2+}$, Cu$^{2+}$ and so on, showed various extend responses in the fluorescence intensity, these cations would have little influence, since they exist at low concentrations compared to Mg$^{2+}$. These results suggested HL could be a fluorescence sensor for Mg$^{2+}$ without interference of other physiologically important cations.

**Fig. S4** Competition experiments. The black bars represent the addition of an excess of metal ions to an acetonitrile solution of HL (50 µM) in the presence of Mg$^{2+}$ (25 µM). $\lambda_{em} = 546$ nm, [Metal] = 100 µM, [Ca$^{2+}$] = 500 µM, [K$^+$] = 500 µM, [Na$^+$] = 500 µM, [Al$^{3+}$] = 500 µM.
Via monitoring the fluorescence intensity at 558 nm, zinc ions could be distinguished from other metal ions, such as: Na⁺, K⁺, Ag⁺, Fe²⁺, Al³⁺, Ca²⁺, Cr³⁺, Fe³⁺, Cd²⁺, Hg²⁺, Mg²⁺, and Mn²⁺, indicating excellent selectivity for Zn²⁺ over these competing cations. But Ni²⁺, Cu²⁺ and Co²⁺ had some extent the influence on the $F_{558\text{nm}}$ value of the zinc complex, which indicated that Ni²⁺, Cu²⁺, and Co²⁺ could form complexes with HL and thus quenched the fluorescence. There were many other Zn²⁺ sensors, which had exhibited similarly depressed responses due to the competition from these ions. However, these free cations would have little influence in vivo because they exist at a very low concentration.⁴

**Fig. S5** Competition experiments. The black bars (A) represent the addition of an excess of metal ions to an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution of HL (50 µM), and the red bars (B) represent the subsequent addition of 50 µM Zn²⁺ ions to the foregoing solution. $\lambda_{\text{em}} = 558$ nm.

**Fig. S6** The linear dynamic response of HL for Zn²⁺ and the determination of the detection limit (LOD) for Zn²⁺ in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution.
**Fig. S7** Absorbance Job’s plot for HL with Co\(^{2+}\) in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution. The total [HL] + [Co\(^{2+}\)] = 100 µM.

**Fig. S8** ESI-MS spectrum of CoL\(_2\) complex.

ESI-MS exhibited the formation of a complex between two deprotonated HL and a Co\(^{2+}\) [m/z 695.2476 (2L+Co\(^{2+}\)); calcd for C\(_{40}\)H\(_{40}\)CoN\(_6\)O\(_2\) m/z 695.25].

**Fig. S9** The linear fitting (absorbance at 476 nm) of complexes. \[ \log\left(\frac{A - A_{\text{min}}}{A_{\text{max}} - A}\right) = \log K + \log[\text{Co}^{2+}] \] \( K \) is the stability constant, \( A_{\text{min}} \) is absorbance of HL without any cations, \( A \) is absorbance of HL obtained with Co\(^{2+}\), \( A_{\text{max}} \) is absorbance of HL in the presence of excess amount of Co\(^{2+}\).
**Fig. S10** Competition experiments (absorbance at 476 nm). The brown bars (A) represent the addition of an excess of metal ions to an acetonitrile/Tris-HCl (50 mM, pH 7.40) (1:1, v/v) solution of HL (50 µM), and the chromatic bars (B) represent the subsequent addition of 25 µM Co^{2+} ions to the foregoing solution.

**Fig. S11** The different metal salts toward HL for sensing Mg^{2+}, Zn^{2+}, and Co^{2+}. (a) Fluorescent ($\lambda_{ex} = 470$ nm) spectral changes of HL (1.0 µM) upon titration with MgCl$_2$, Mg(ClO$_4$)$_2$, and Mg(NO$_3$)$_2$ in CH$_3$CN. (b) Fluorescence ($\lambda_{ex} = 470$ nm) responses of HL (50 µM) in the presence of ZnCl$_2$, Zn(ClO$_4$)$_2$, and Zn(NO$_3$)$_2$ in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (9:1, v/v) solution. (c) UV/Vis absorption spectra of HL (50 µM) in the presence of CoCl$_2$, Co(ClO$_4$)$_2$, and Co(NO$_3$)$_2$ in an acetonitrile/Tris-HCl (50 mM, pH 7.40) (1:1, v/v) solution.
**Fig. S12** $^1$H-NMR spectrum of HL ($d_6$-DMSO) at room temperature.

**Fig. S13** $^{13}$C-NMR spectrum of HL ($d_6$-DMSO) at room temperature.
References


