Electronic Structural Dependence of the Photophysical Properties of Fluorescent Heteroditopic Ligands – Implications in Designing Molecular Fluorescent Indicators

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Supporting Information

Experimental Procedures:

(1) CYCLIC VOLTAMMETRIC STUDIES

The cyclic voltammograms (Figures S1-S4) were acquired in CH_3CN (spectroscopic grade) containing Bu_4NPF_6 (0.1 M) as supporting electrolyte using a CHI600C Electrochemical Analyzer. The data were collected at a concentration of ~0.5 mM of a substrate in a single compartment cell with a glassy carbon working electrode, a Pt wire counter electrode, and an Ag/AgCl reference electrode. The cyclic voltammograms at scan rate of 100 mV/s are reported.

(2) COMPUTATIONAL ANALYSIS

The calculations were performed using the Gaussian 03 package of programs at a hybrid density functional theory (DFT) level. The function used to perform the optimizations and computations was the restricted B3LYP/6-31+G (d,p) where the optimized structures were chosen as the stationary points. (Frisch, M. J. et al. Gaussian 03, Revision C.02; Gaussian, Inc. Wallingford CT, 2004).

(3) ABSORPTION AND FLUORESCENCE TITRATION STUDIES

Representative Procedure. The absorption and fluorescence data were collected on a Cary 100 Spectrophotometer and a Cary Eclipse Fluorometer, respectively. An CH_3CN solution of **4** (5.0 μM) and $ZnCl_2$ (64 μM) was titrated into a semi-micro quartz spectrophotometer or fluorometer cuvette (Starna®) containing an CH_3CN solution of **4** (750 μL, 5.0 μM) at rt. In the fluorescence titration experiment, the sample was excited at 336 nm (excitation slit = 1.5 nm; emission slit = 10 nm, PMT = 500 V). $ZnCl_2$ was chosen as the Zn^{2+} source in titration experiments conducted in CH_3CN because of the simple binding stoichiometry of 1:1 between $ZnCl_2$ and the bipy moiety. $Zn(ClO_4)_2$ was chosen under aqueous buffering conditions due to its better solubility.

(4) LIFETIME MEASUREMENTS

The fluorescence lifetimes were measured using the time-correlated single photon counting (TCSPC) technique. **3** and **2a** were excited using a 295 nm LED while **4** and **2b** were excited using 370 nm LED with both LEDs operating at a repetition rate of 1 MHz. The emission decay was observed at emission λ_{max} of respective sample and data were recorded with a band spectral width of 10 nm and 10,000 counts in the peak channel. The timescale of the experiment was 100 ns (115.3 ps/channel). The emission decay data were then analyzed using the DAS6 software. The quality of the exponential fits was judged by both the randomness of the residue distribution, and the χ^2 obtained for different measurements (general acceptable range: $\chi^2 <$ 1.3). Two example decay traces are shown in Figures S14-S15).

(5) FLUORESCENCE QUANTUM YIELD MEASUREMENTS

The absorption spectrum of a substrate was collected first. The excitation wavelength (λ_{ex}) of the substrate was chosen such that (1) the absorbance value at $\lambda_{ex} < 0.1$, and (2) the complete emission spectrum could be collected. Argon gas was bubbled through the sample before the emission spectrum was collected to remove oxygen. The absorbance value at λ_{ex} and integrated emission intensity of the substrate as well as those of a standard sample (quinine bisulfate in 0.05 M sulfuric acid) with known ϕ_f (0.55) were used in Eq. 1 to calculate the ϕ_f of the substrate,

$$\phi_f = [(A_s F_f n^2)/(A_f F_s n_0^2)]\phi_s \qquad \qquad \text{Equation 1}$$

where A_s and A_f are the absorbance of the reference and sample solutions at their respective excitation wavelengths, F_f and F_s are the corresponding integrated fluorescence intensity, and n is the refractive index of the solvent of the sample (n) or of the standard (n_0).

For determining the ϕ_f of the Zn^{2+} complex of a substrate, the concentrations of the substrate and Zn^{2+} in the sample were selected based on the fluorescence titration isotherm so that full saturation of the substrate by $ZnCl_2$, presumably as a 1:2 complex, was guaranteed. The ϕ_f was then determined using the procedure described above.
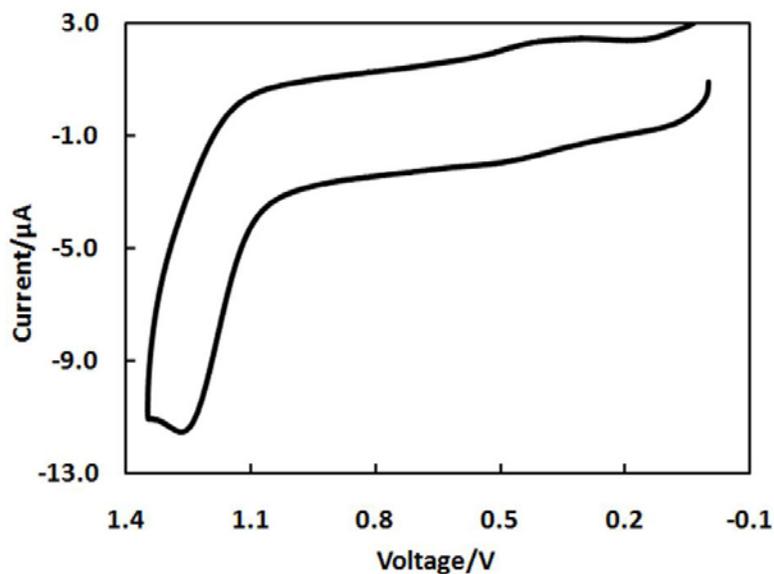
Additional Spectra:

CYCLIC VOLTAMMOGRAMS

Figure S1. Cyclic voltammogram of **2a** (~ 0.5 mM in CH$_3$CN). [Bu$_4$NPF$_6$] = 0.1 M. Reference electrode Ag/AgCl; E$_{1/2}$(Fc/Fc$^+$) = 0.62 V.
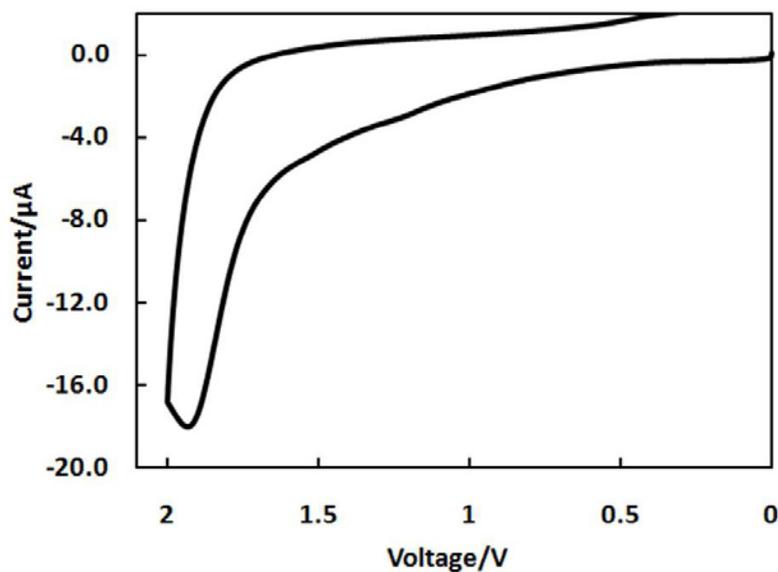

Figure S2. Cyclic voltammogram of **3** (~ 0.5 mM in CH$_3$CN). [Bu$_4$NPF$_6$] = 0.1 M. Reference electrode Ag/AgCl; E$_{1/2}$(Fc/Fc$^+$) = 0.62 V.
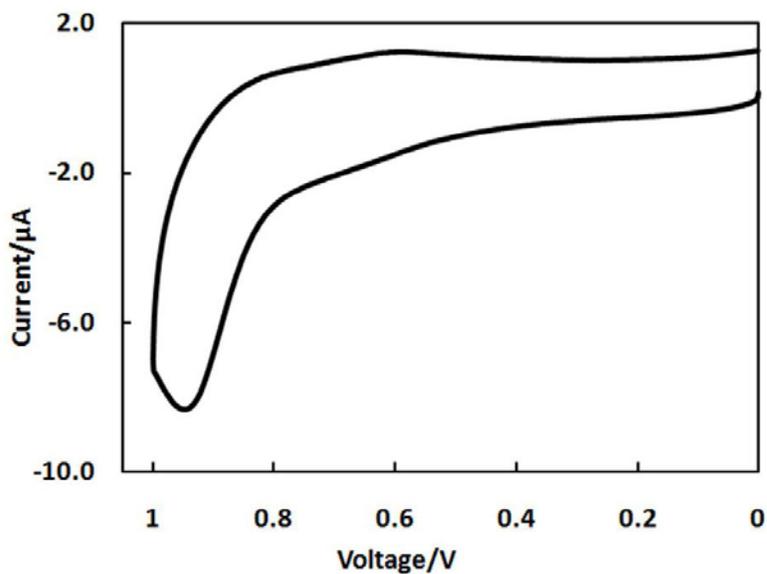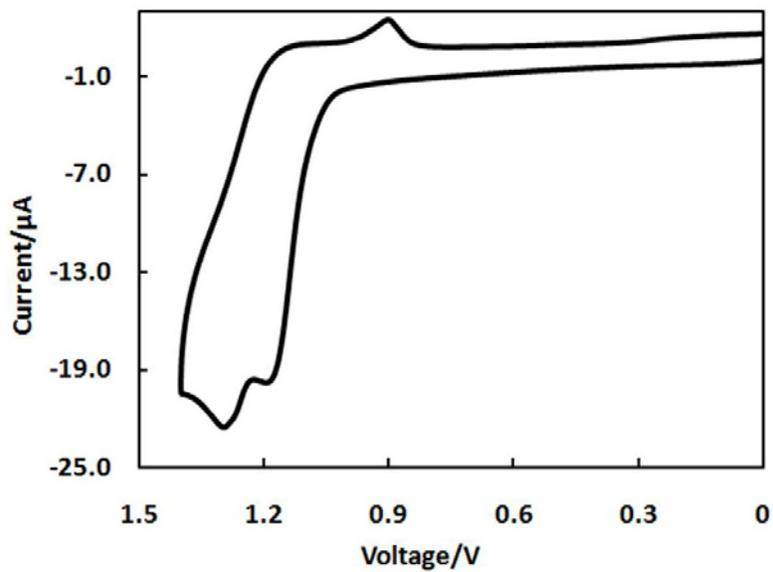
S4

Figure S3. Cyclic voltammogram of **2b** ($\sim$ 0.5 mM in CH_3CN). $[Bu_4NPF_6]$ = 0.1 M. Reference electrode Ag/AgCl; $E_{1/2}(Fc/Fc^+)$ = 0.62 V.

Figure S4. Cyclic voltammogram of **4** ($\sim$ 0.5 mM in CH_3CN). $[Bu_4NPF_6]$ = 0.1 M. Reference electrode Ag/AgCl; $E_{1/2}(Fc/Fc^+)$ = 0.62 V.

ADDITIONAL MOLECULAR ORBITAL DIAGRAMS
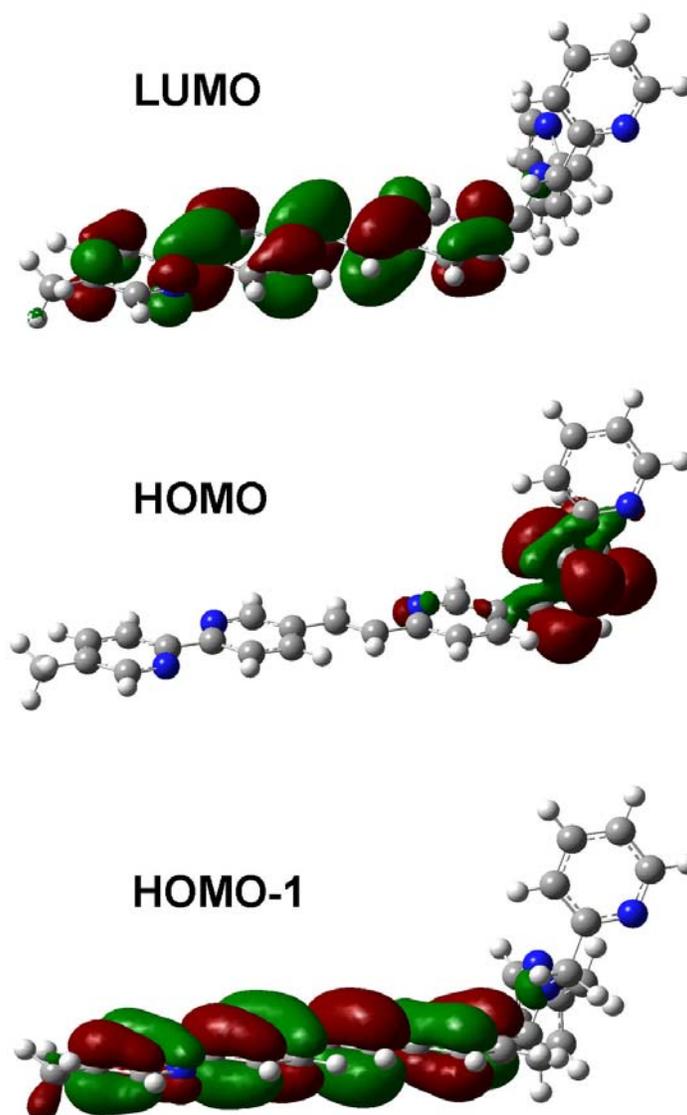
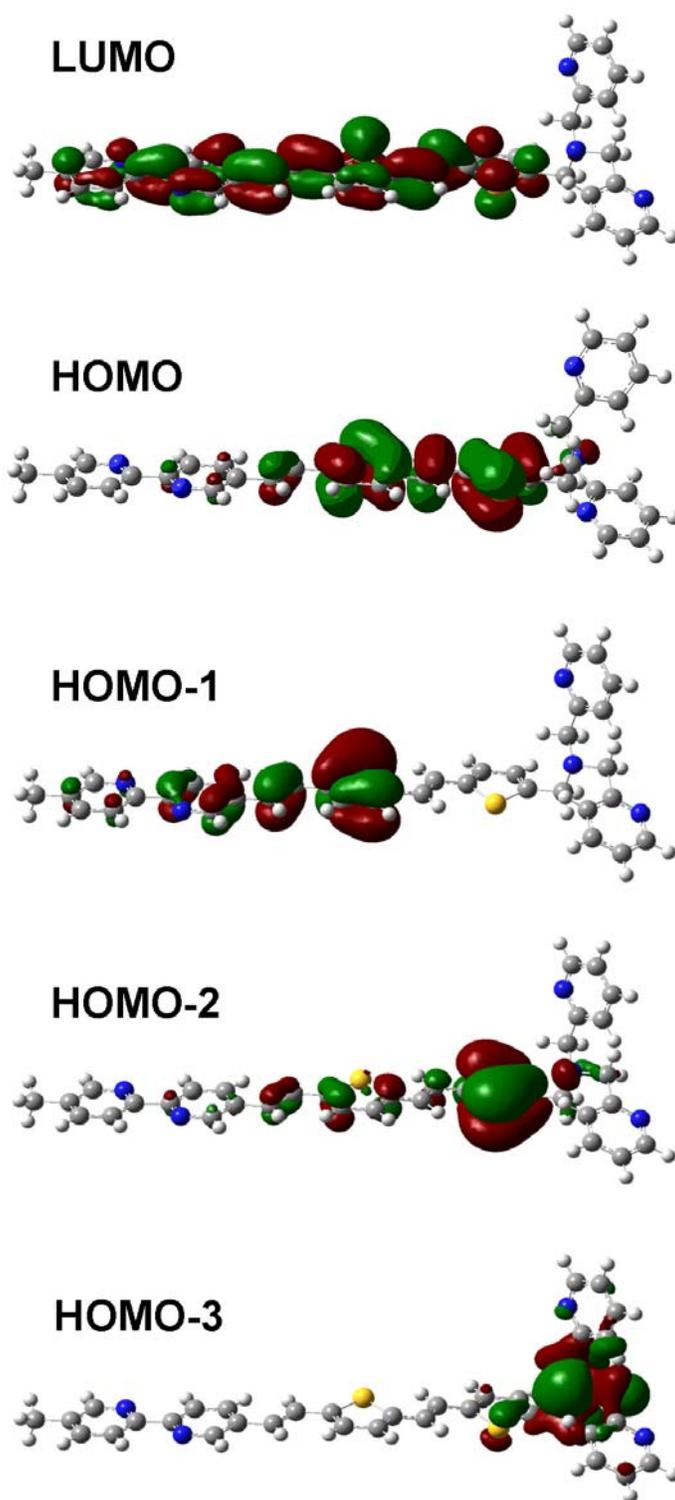**Figure S5**. Frontier molecular orbital diagrams of **2a** (B3LYP/6-31+G (d,p) level).

LUMO

HOMO

HOMO-1

HOMO-2

HOMO-3

Figure S6. Frontier molecular orbital diagrams of **2b** (B3LYP/6-31+G (d,p) level).

ADDITIONAL ABSORPTION AND FLUORESCENCE SPECTRA
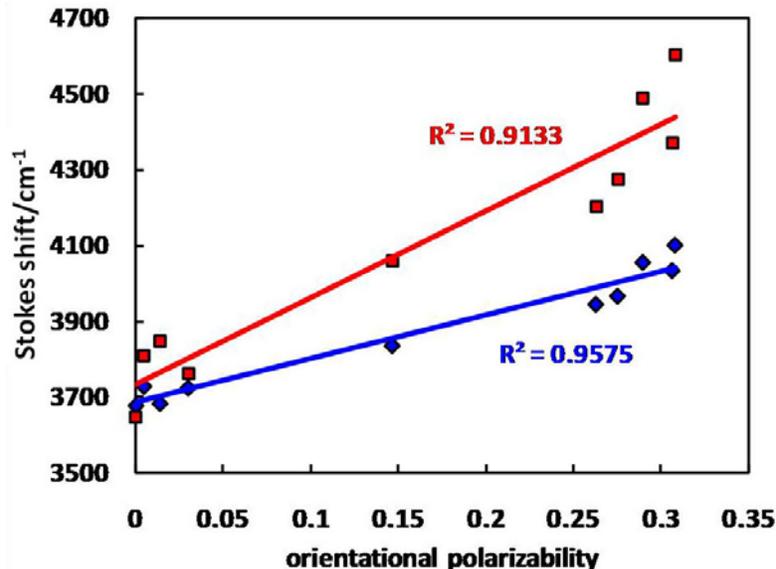
Figure S7. Lippert plots of compounds **3** (blue) and **4** (red). Orientational polarizability $\Delta f = (\varepsilon-1)/(2\varepsilon+1) - (n^2-1)/(2n^2+1)$. ε: relative permittivity; n: refractive index.
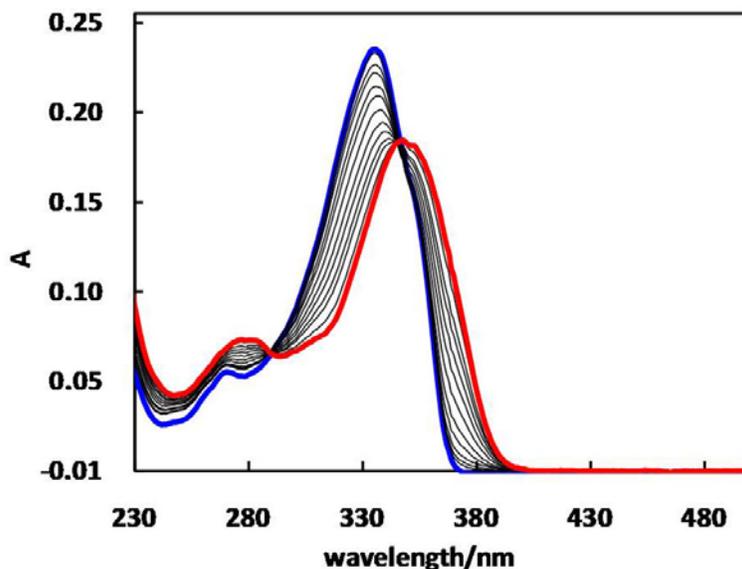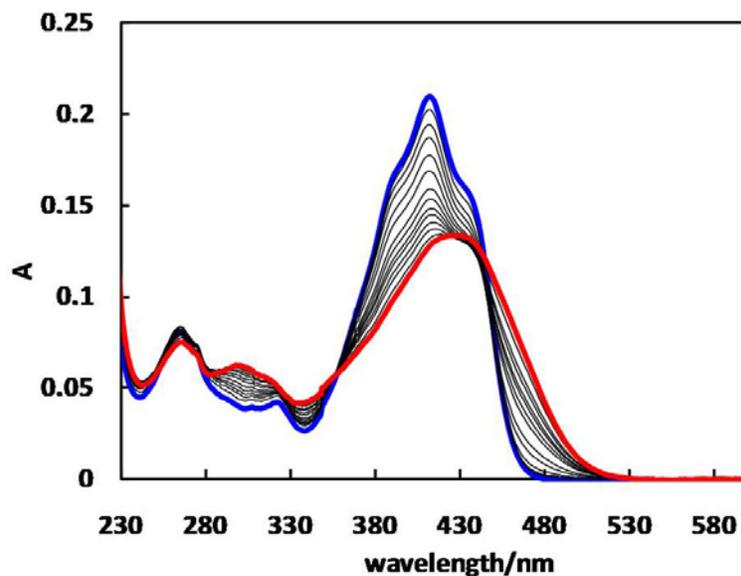

Figure S8. Absorption spectra of **3** (5.0 µM) in CH_3CN upon addition of $ZnCl_2$ (0 – 26.9 µM). The spectra that were collected at the beginning and the end of a titration experiment are coded blue and red, respectively.

Figure S9. Absorption spectra of **4** (3.85 μM) in CH_3CN upon addition of $ZnCl_2$ (0 −25.6 μM). The spectra that were collected at the beginning and the end of a titration experiment are coded blue and red, respectively.
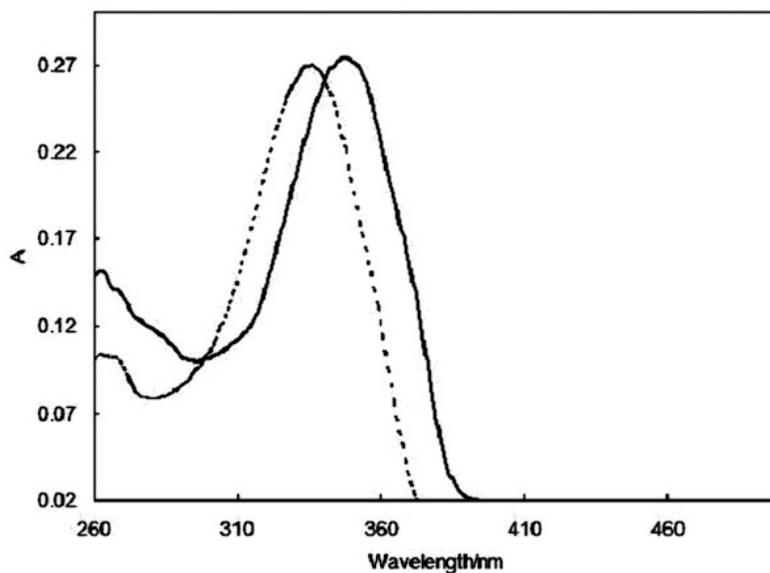

Figure S10. Normalized absorption Spectra of **2a** (dotted) measured in aqueous buffer solution (HEPES: 50 mM, pH =7.4, EGTA: 2.5 mM, HEDTA: 2.5 mM, NTA: 5 mM, KNO_3: 100 mM) with the corresponding zinc coordinated **2a** (dashed).
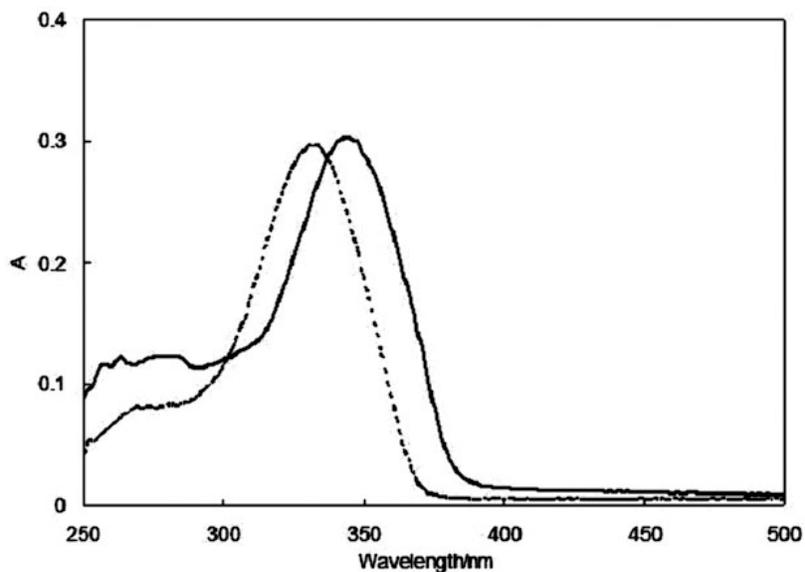
Figure S11. Normalized absorption spectra of **4** (dotted) and its zinc complex (dashed) measured in aqueous buffer solution (HEPES: 50 mM, pH =7.4, EGTA: 2.5 mM, HEDTA: 2.5 mM, NTA: 5 mM, KNO_3: 100 mM).
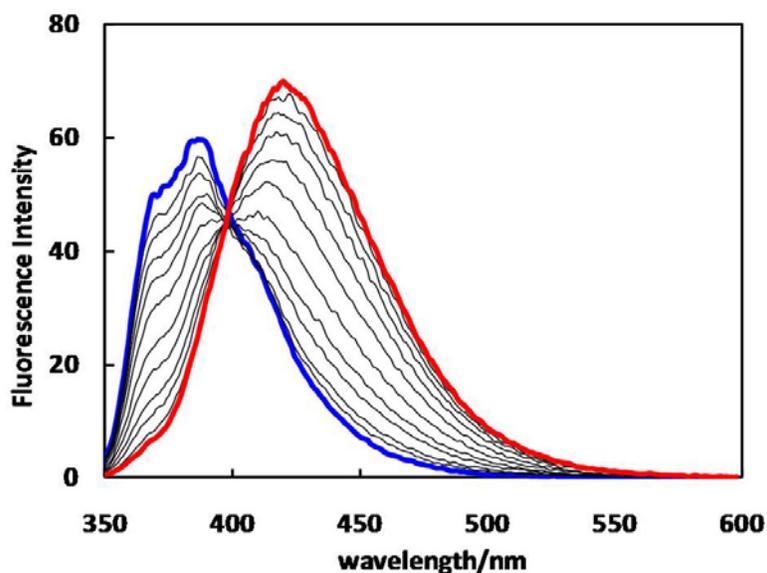

Figure S12. Emission spectra (λ_{ex} = 336 nm) of **3** (5.0 µM) in CH_3CN upon addition of $ZnCl_2$ (0 – 23.4 µM). The spectra that were collected at the beginning and the end of a titration experiment are coded blue and red, respectively.
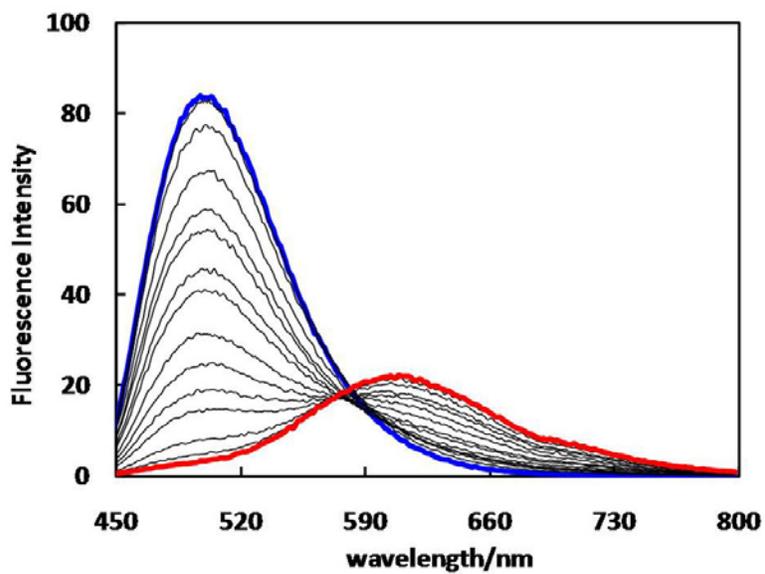
Figure S13. Emission spectra (λ_{ex} = 430 nm) of **4** (3.85 µM) in CH$_3$CN upon addition of ZnCl$_2$ (0 – 40.0 µM). The spectra that were collected at the beginning and the end of a titration experiment are coded blue and red, respectively.

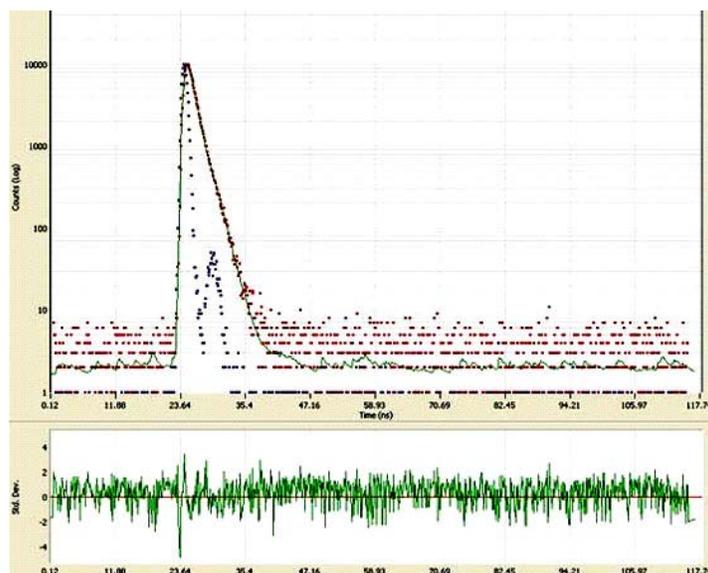### REPRESENTATIVE FLUORESCENCE DECAY TRACES

Figure S14. The fluorescence decay trace of zinc complex of **2a** in CH_3CN. τ_1 = 1.08 ns, τ_2 = 1.98 ns (χ^2 = 1.20).
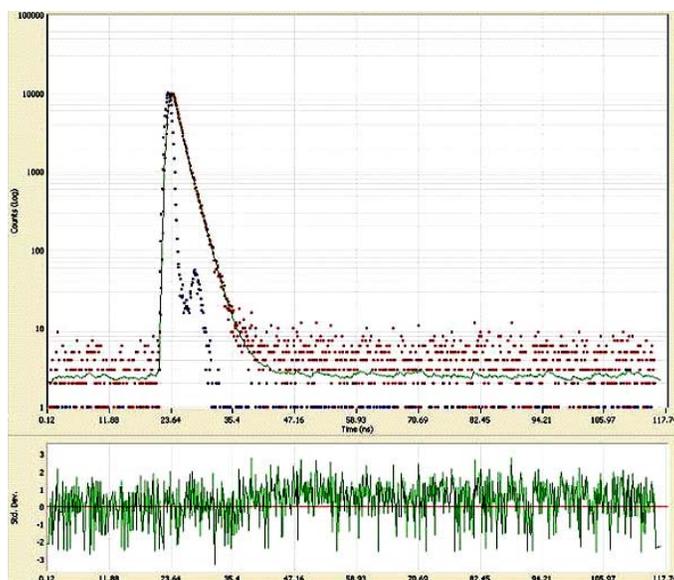

Figure S15. The fluorescence decay trace of zinc complex of **2b** in CH_3CN. τ_1 = 1.39 ns, τ_2 = 2.67 ns (χ^2 = 1.29).

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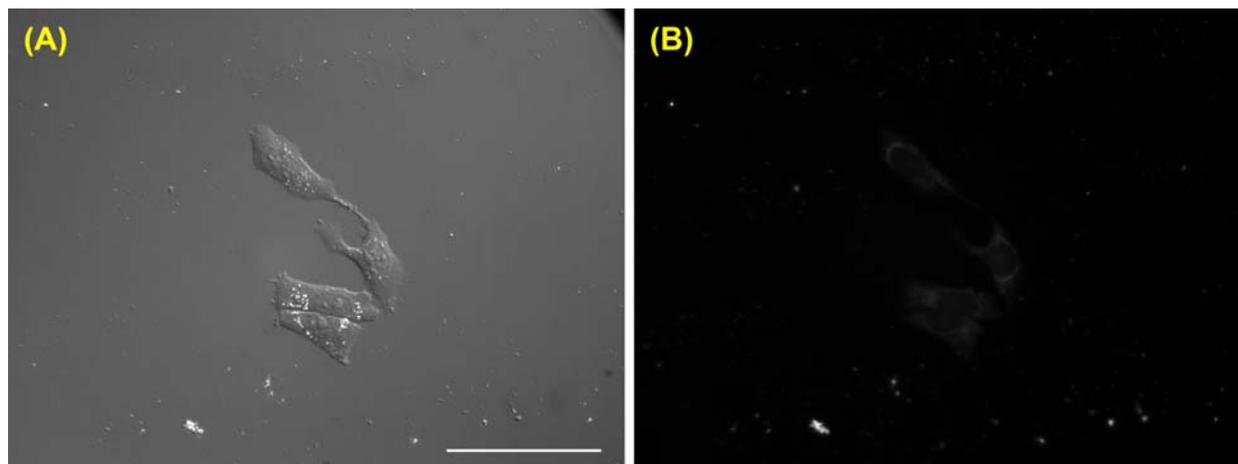**AUTOFLUORESCENCE OF HELA CELLS**

Figure S16. (A) Differential interference contrast (DIC) and (B) fluorescence images (Omega Q-Max Blue filter set; excitation 355 – 405 nm; emission 420 – 480 nm) of live HeLa cells in HBSS buffer at 37 ^{o}C under 5% CO_2. Scale bar: 50 μm.