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$_3$CN (spectroscopic grade) containing Bu$_4$NPF$_6$ (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$_3$CN 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$_3$CN 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$_3$CN 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 $\lambda_{\text{max}}$ 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 $\chi^2$ 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 ($\lambda_{\text{ex}}$) of the substrate was chosen such that (1) the absorbance value at $\lambda_{\text{ex}} < 0.1$, 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 $\lambda_{\text{ex}}$ 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 $\phi_f$ (0.55) were used in Eq. 1 to calculate the $\phi_f$ of the substrate,

$$\phi_f = \frac{(A_sF_fn^2)/(A_tF_sn_0^2))\phi_s}{\text{Equation 1}}$$

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 $\phi_f$ 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 $\phi_f$ was then determined using the procedure described above.
**Additional Spectra:**

**Cyclic Voltammograms**

![Cyclic voltammogram of 2a](image1)

**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.

![Cyclic voltammogram of 3](image2)

**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.
**Figure S3.** Cyclic voltammogram of 2b (≈ 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 S4.** Cyclic voltammogram of 4 (≈ 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.
**ADDITIONAL MOLECULAR ORBITAL DIAGRAMS**

**Figure S5.** Frontier molecular orbital diagrams of 2a (B3LYP/6-31+G (d,p) level).
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 $\Delta f = \frac{(\varepsilon - 1)}{(2\varepsilon + 1)} - \frac{(n^2 - 1)}{(2n^2 + 1)}$. $\varepsilon$: relative permittivity; $n$: refractive index.

**Figure S8.** Absorption spectra of 3 (5.0 μM) in CH$_3$CN 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 CH₃CN upon addition of ZnCl₂ (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, KNO₃: 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, KNO₃: 100 mM).

**Figure S12.** Emission spectra ($\lambda_{ex} = 336$ nm) of 3 (5.0 µM) in CH₃CN upon addition of ZnCl₂ (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 ($\lambda_{ex} = 430$ nm) of 4 (3.85 $\mu$M) in CH$_3$CN upon addition of ZnCl$_2$ (0 – 40.0 $\mu$M). 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 CH₃CN. \( \tau_1 = 1.08 \) ns, \( \tau_2 = 1.98 \) ns (\( \chi^2 = 1.20 \)).

**Figure S15.** The fluorescence decay trace of zinc complex of 2b in CH₃CN. \( \tau_1 = 1.39 \) ns, \( \tau_2 = 2.67 \) ns (\( \chi^2 = 1.29 \)).
**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 °C under 5% CO₂. Scale bar: 50 μm.