Zinc coordination to the bapbpy ligands in homogeneous solutions and at liposomes: zinc detection *via* fluorescence enhancement

Supplementary Information

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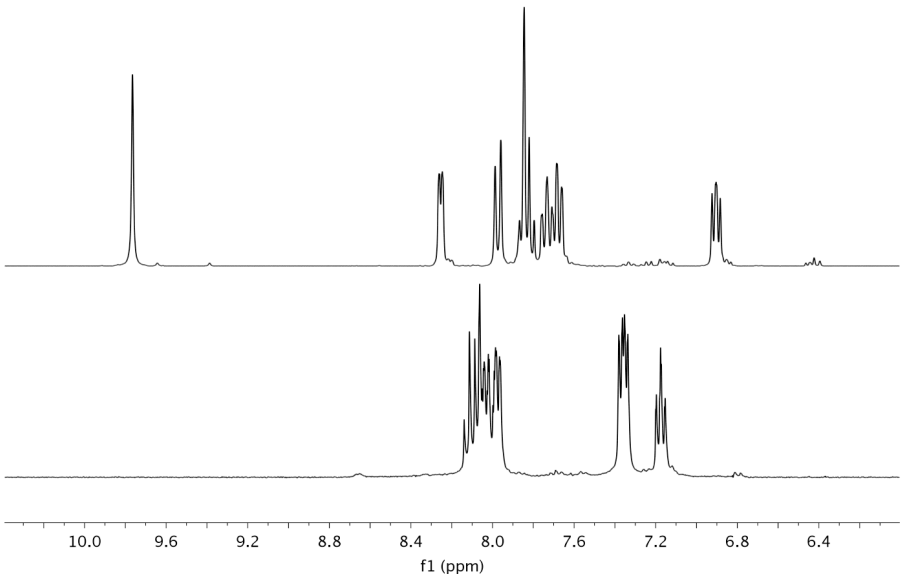


Figure S0. ^{1}H NMR of bapbpy in DMSO-d6 (top) and of compound **2** in CD_3OD (bottom).

Figure S1. LC-MS analysis of (a) the crude mixture and (b) the purified compound **3**.

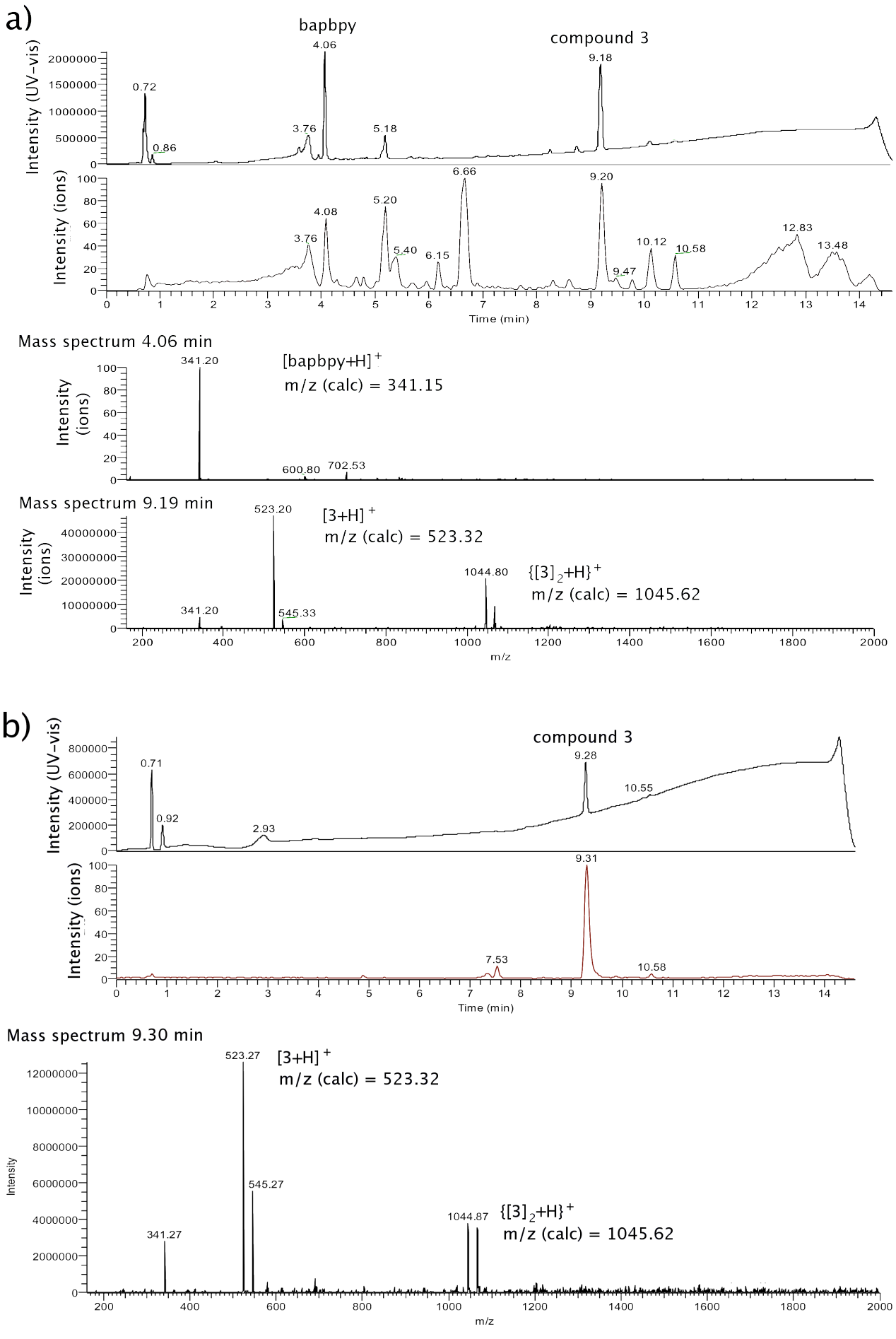
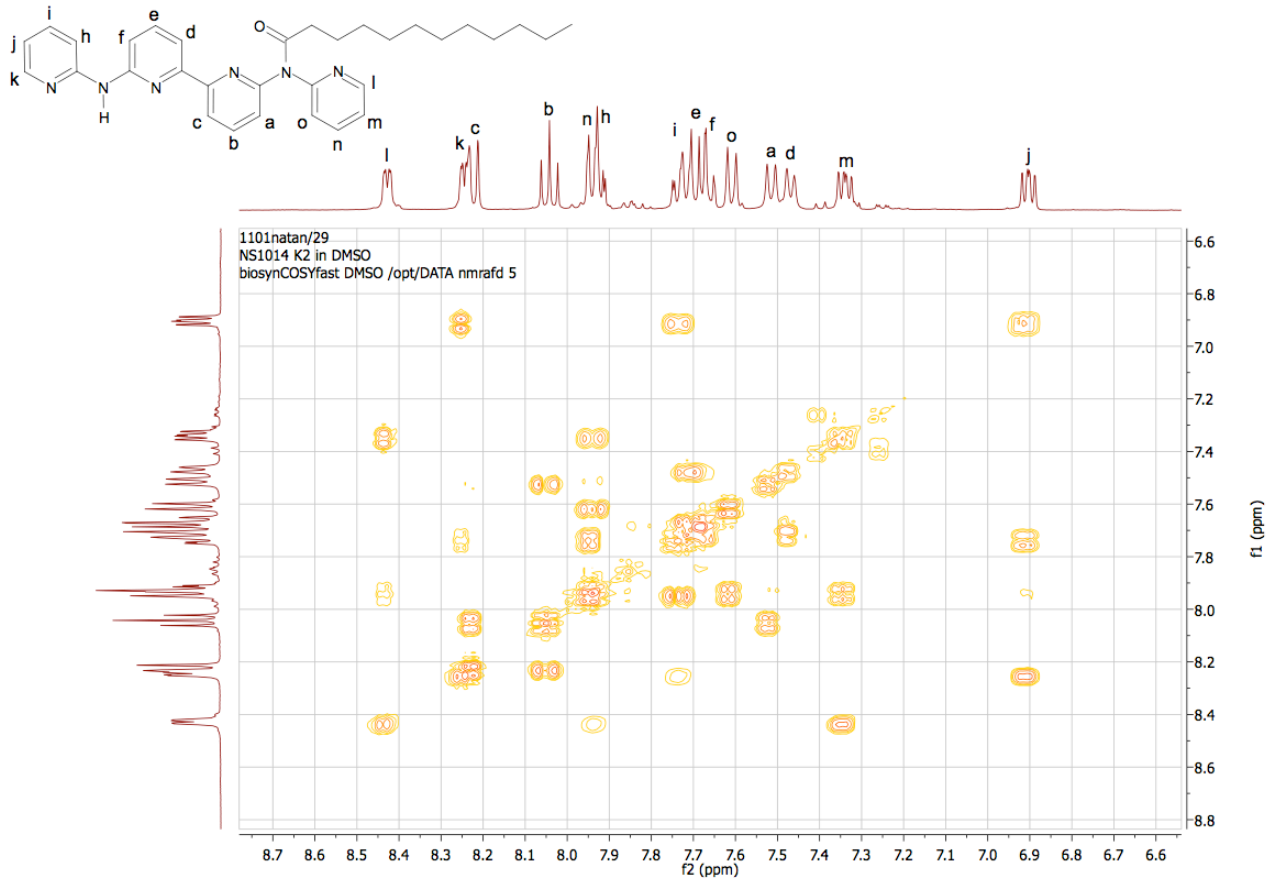


Figure S2. Attribution and COSY NMR spectrum of ligand **3**. Aromatic part (6.5-8.8 ppm in DMSO-d_6).



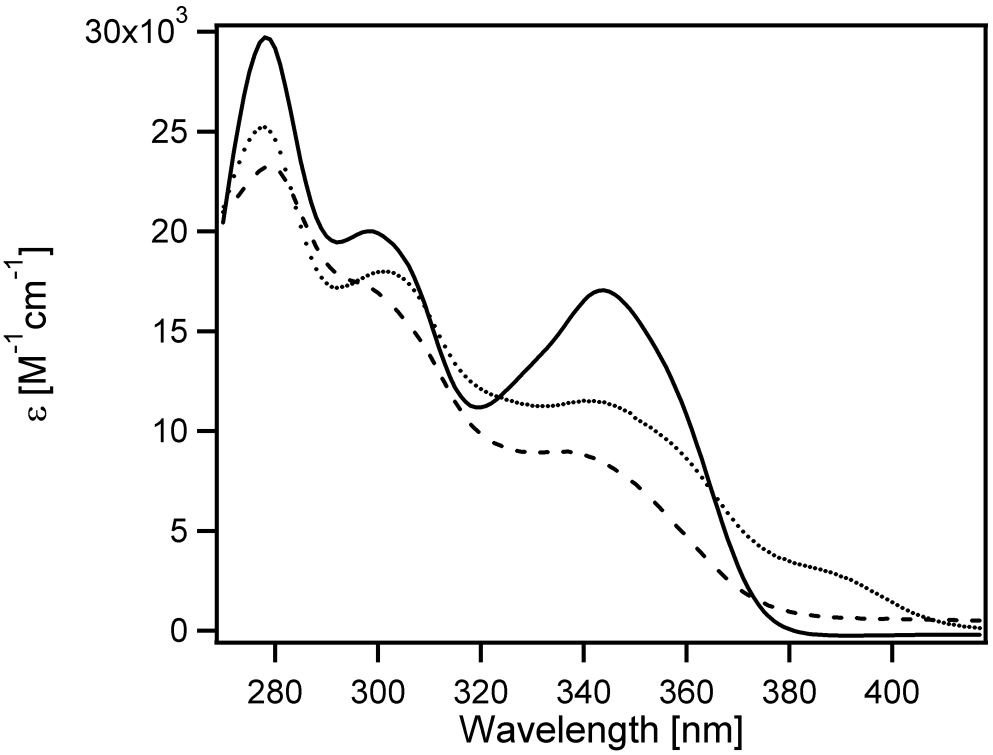


Figure S3. Extinction coefficient for compound **1** (solid), **2** (dotted) and **3** (dashed) in DMF.

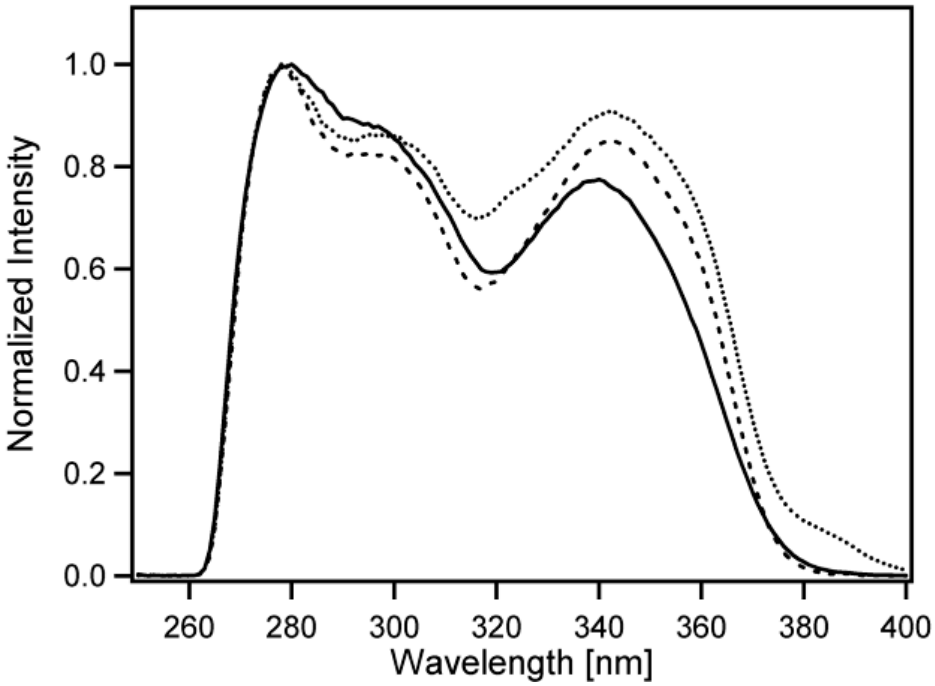


Figure S4. Excitation spectra of compounds **1** (solid), **2** (dotted) and **3** (dashed) in DMF, monitored at 440 nm.

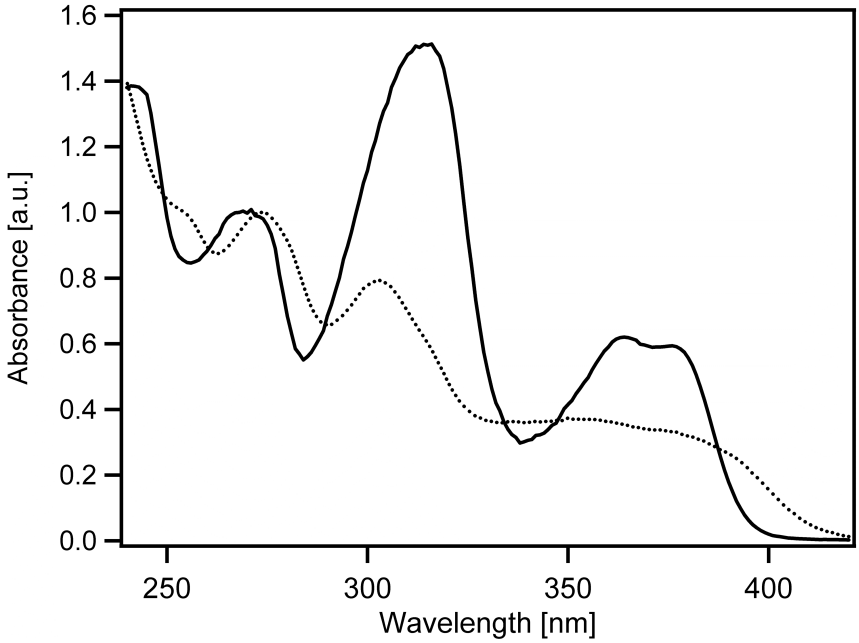


Figure S5. Absorption spectra of **2** in THF (dashed, saturated solution) and water (plain) at room temperature.

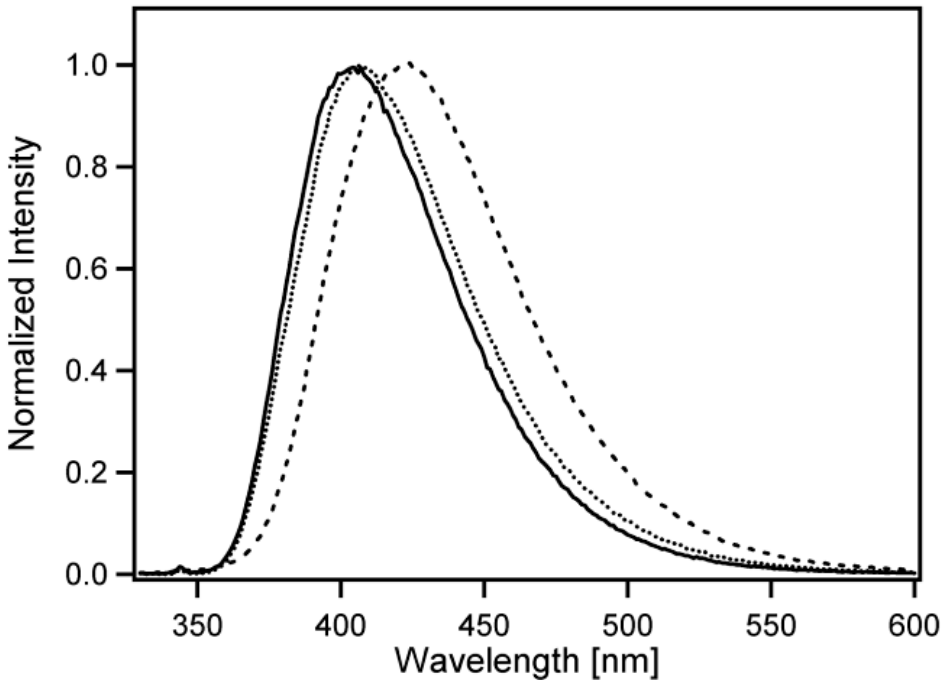


Figure S6. Emission spectra of compound **1** (solid), **2** (dotted) and **3** (dashed) in DMF at room temperature, $\lambda_{exc} = 312$ nm.

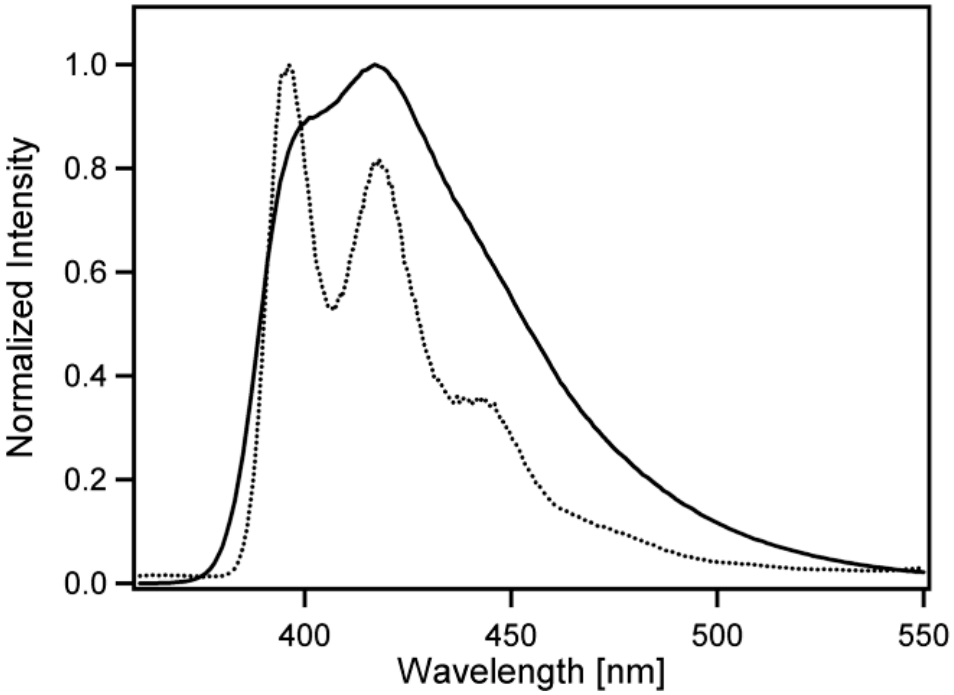


Figure S7. Emission spectra of compound **2** in water (solid) and MeOH/EtOH (1:4) at 77 K (dotted), λ_{exc} = 312 nm.

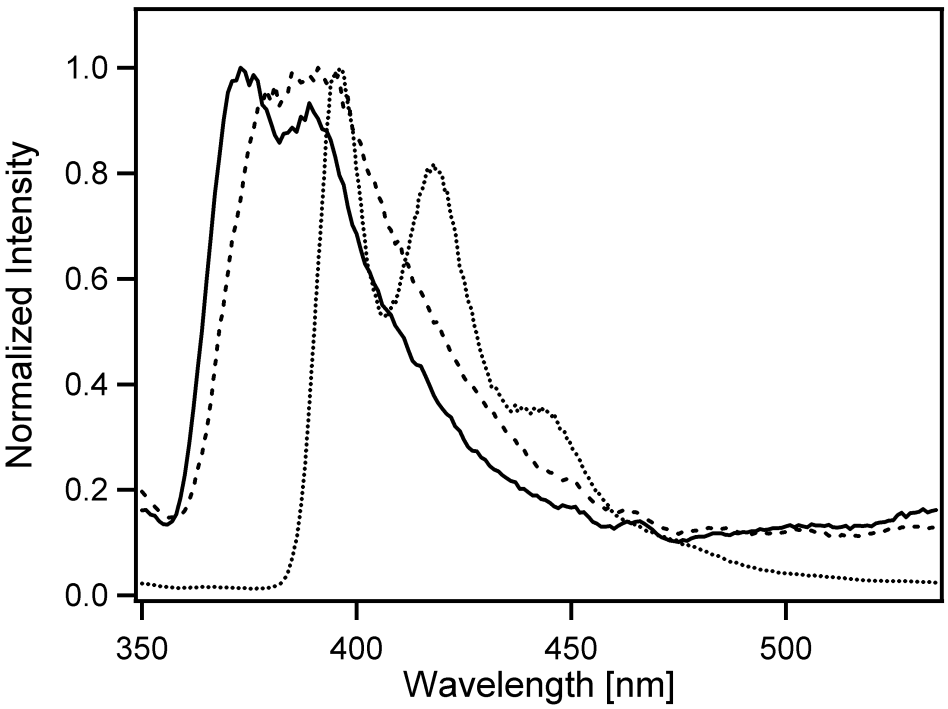


Figure S8. Emission spectra of compounds **1** (solid), **2** (dotted), and **3** (dashed), in MeOH/EtOH (1:4) at 77 K, λ_{exc} = 312 nm.

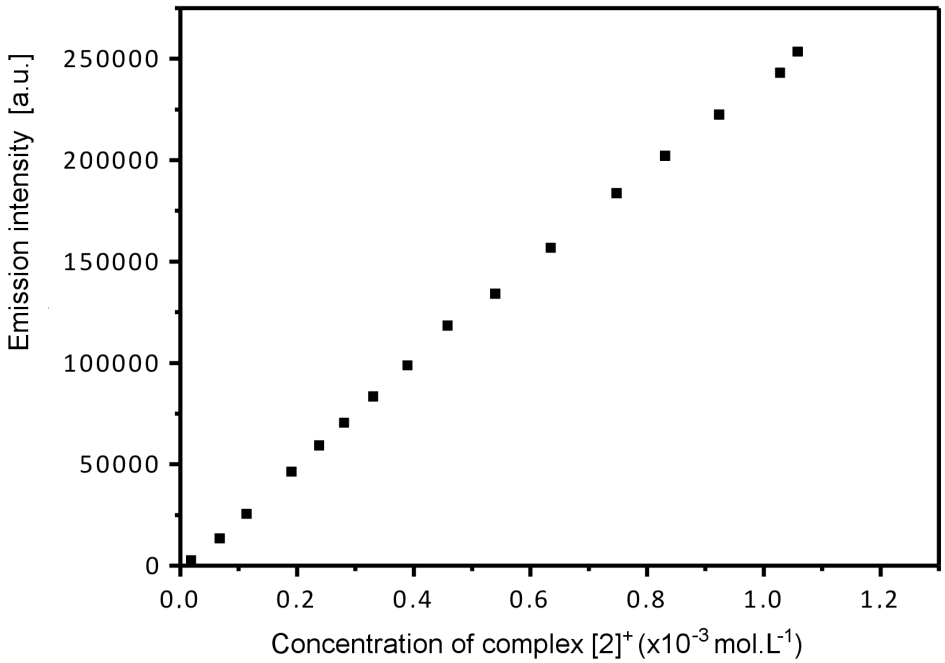


Figure S9. Plot of the emission intensity at 440 nm *vs.* concentration for **2** in water. Excitation: 404 nm.

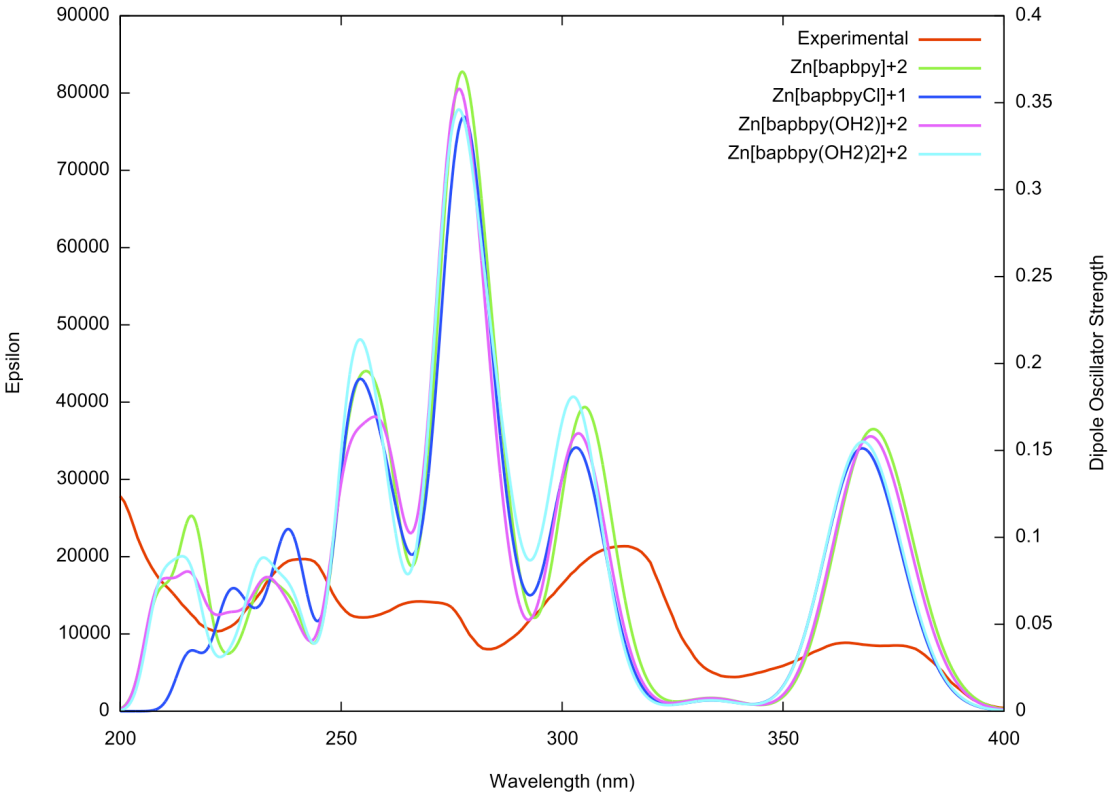


Figure S10. Simulated UV-vis spectra (right axis) for $[Zn(bapbpy)Cl]^{+}$, $[Zn(bapbpy)]^{2+}$, $[Zn(bapbpy)(OH_2)]^{2+}$ and $[Zn(bapbpy)(OH_2)_2]^{2+}$ as calculated with TD-DFT/B3LYP in water using PCM (singlet-to-singlet transition only are reported). The final spectra were convoluted using Gaussian broadening of the calculated spectral lines. The experimental spectrum of compound **2** is given (in red, left axis) for comparison.

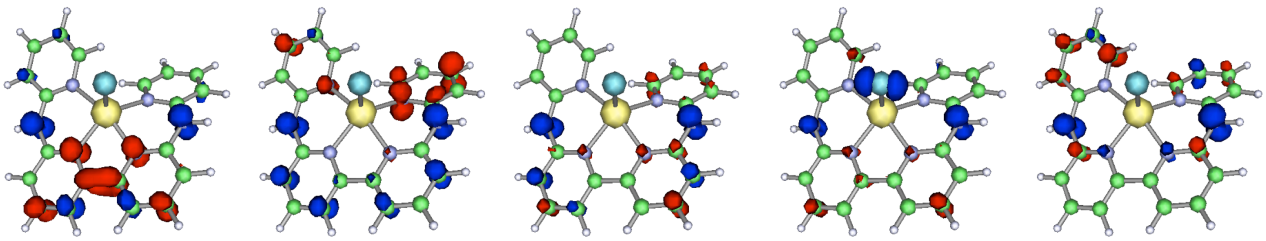


Figure S11. Electron density difference plot and calculated wavelengths for the 5 first most intense ($f > 0.1$) singlet-to-singlet electronic transitions of $[Zn(bapbpy)Cl]^{+}$, according to TD-DFT/B3LYP calculations in COSMO(water) as implemented in NWCHEM. Blue lobes correspond to region of depleted electron density in the excited state, red lobes to increased electron density in the excited state.

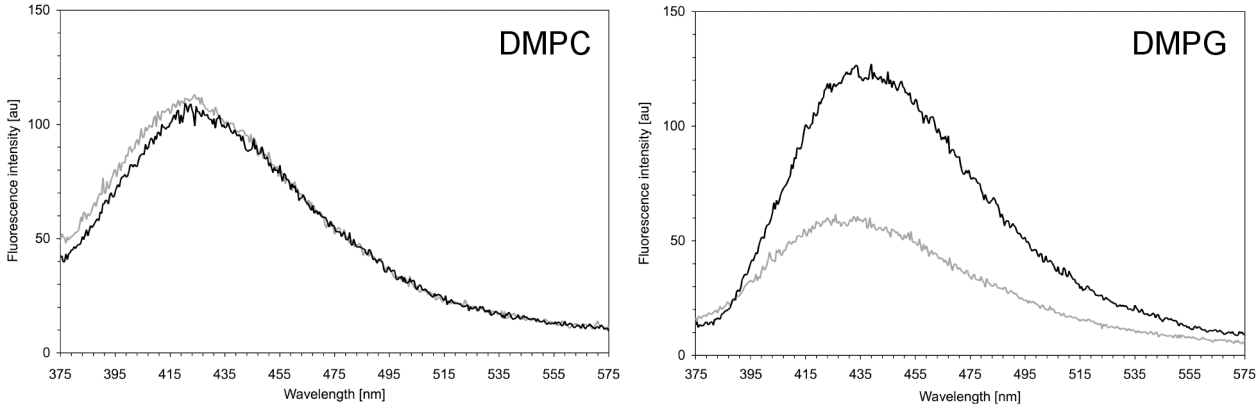


Figure S12. Effect of the addition of Zn^{2+} (1 eq.) on DMPC (left) and DMPG (right) vesicles containing 1 mol% of ligand **3**. The grey curve corresponds to the fluorescence spectrum before zinc addition, the black curve after zinc addition. Conditions: LUV (1.67 µmol.L^{-1}), 25 °C, 10 mM phosphate buffer pH = 7.0, total ionic strength 50 mM. [au] = arbitrary units.

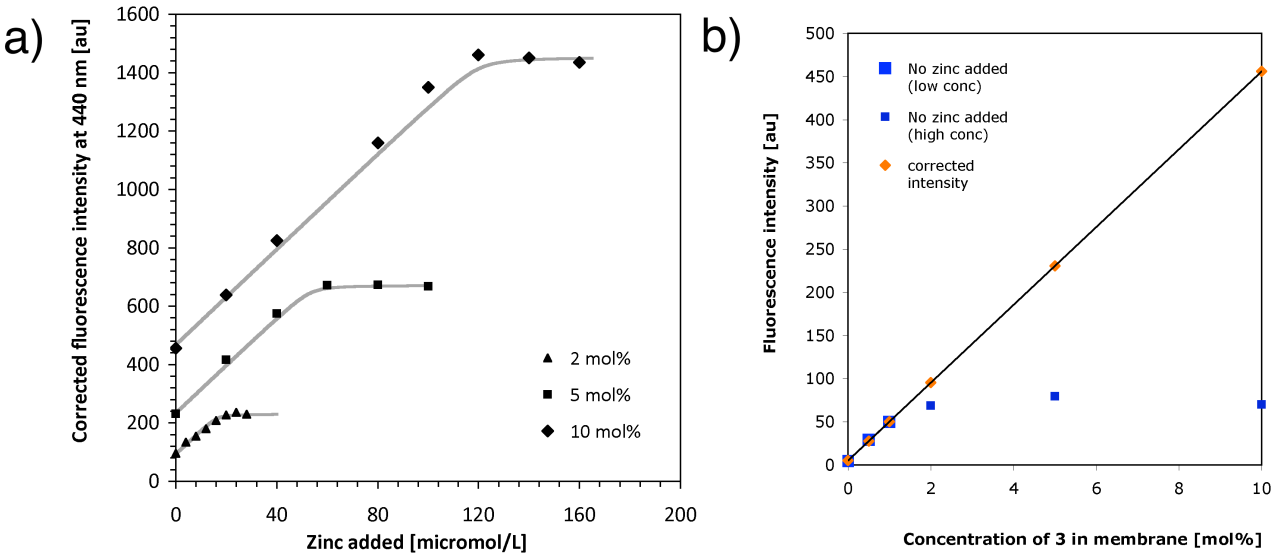


Figure S13. a) Evolution of the fluorescence intensity at 440 nm upon addition of zinc dichloride, with 2, 5, and 10 mol% of ligand **3** in DMPG membranes. Solid curves show modeling with a binding constant of 10^{-7} M (see below). Conditions: LUV, lipid concentration 1.7 mM, 25 °C, 10 mM phosphate buffer pH = 7.0, total ionic strength 50 mM. [au] = arbitrary units. The experimental fluorescence intensities were multiplied by a factor of 1.39, 2.90, and 6.54, for membrane concentrations of 2, 5, and 10 mol%, respectively, in order to take into account the non-negligible light absorption by the concentrated solutions. b) Non-linear fluorescence intensity (at zero added zinc) due to self-filtering effect (absorption of emitted light by the solution at high concentrations in ligand **3**, *i.e.*, at 2, 5, and 10 mol%).

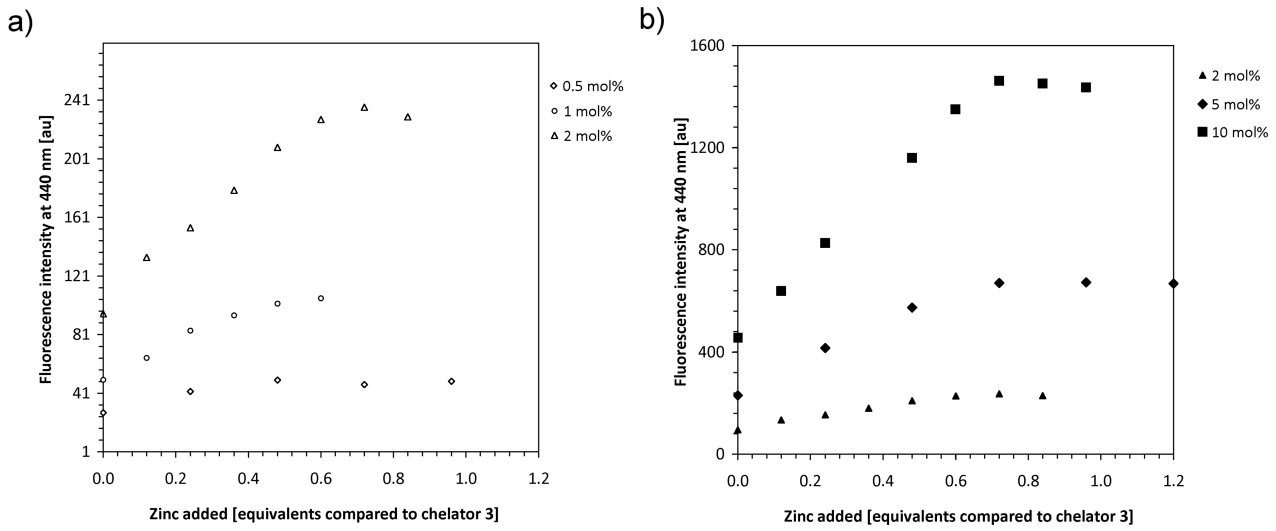


Figure S14. Evolution of the fluorescence intensity at 440 nm *vs*. Zn^{2+} concentrations expressed in equivalents of ligand **3**, a) with 0.5, 1, or 2 mol% and b) with 2, 5, or 10 mol% of ligand **3** in DMPG membranes. Conditions: LUV, lipid concentration 1.7 mM, 25 °C, 10 mM phosphate buffer pH = 7.0, total ionic strength 50 mM. [au] = arbitrary units. The experimental fluorescence intensities were multiplied by a factor of 1.39, 2.90, and 6.54, for membrane concentrations of 2, 5, and 10 mol%, respectively, in order to take into account the non-negligible light absorption by the concentrated solutions (see Figure S13).

Model for the binding of free zinc(II) at membrane-embedded ligand 3, and equilibrium constant determination

We split the ligand embedded in the membrane between one fraction $(1-f)$ that is not available for zinc coordination (*e.g.* because it is inside the membrane and zinc ions cannot cross the bilayer), and one fraction f that is available for zinc coordination (*e.g.* because it is ouside the membrane). The concentration that should appear in the thermodynamic formation constant K_a is the part available for coordination only, whereas the contribution of the ligand L to the fluorescence of the sample includes both available and non-available fractions of free ligand, *i.e.*, $[L]_0$.

If ξ is the advancement of the coordination reaction:

	L +	Zn^{2+}	⇔	ZnL^{2+}
Concentrations before reaction	$f[L]_0$	$[Zn]_{added}$		0
Concentrations after reaction	$f[L]_0(1-\xi)$	$[Zn]_{added} - f[L]_0\xi$		$f[L]_0\xi$

At low concentration, i.e. by approximating activities by the concentrations, the complex formation thermodynamic constant K_a of this reaction can be expressed as:

$$K_a \approx \frac{[ZnL]}{[Zn]_{free}\cdot[L]_{available}} = \frac{f[L]_0\xi}{([Zn]_{added} - f[L]_0\xi)\cdot f[L]_0(1-\xi)}$$

$$K_a = \frac{\xi}{(1-\xi)([Zn]_{added} - f[L]_0\xi)} = \frac{1}{K_d}$$

where K_d is the thermodynamic dissociation constant.

By rearranging and if $x = f\cdot[L_0]\cdot\xi$ is the concentration of complex ZnL^{2+} we can write:

$$x^2 - x\cdot\left(K_d + [Zn]_{added} + f[L]_0\right) + [Zn]_{added}\cdot f[L]_0 = 0$$

The solution of which is

$$x = f\cdot[L_0]\cdot\xi = \frac{K_d + [Zn]_{added} + f[L]_0 - \sqrt{\left(K_d + [Zn]_{added} + f[L]_0\right)^2 - 4[Zn]_{added}\cdot f[L]_0}}{2}$$

The total concentration in ligand L is expressed by:

$$[L]_{tot} = [L]_{available} + [L]_{nonavailable} = f[L]_0(1-\xi) + (1-f)[L]_0$$

The total fluorescence intensity of the sample, I , is given by:

$$I = I_L + I_{ZnL} = \alpha[L]_{tot} + \beta[ZnL]$$

$$I = \alpha[L]_0\left(f(1-\xi) + (1-f)\right) + \beta f[L]_0\xi = \alpha[L]_0 + x\cdot(\beta-\alpha)$$

The coefficients α and β are the response coefficient of ligand L and its zinc complex, respectively, regarding fluorescence. They can be derived from the fluorescence intensity before zinc addition ($I_0, \xi = 0$) and at saturation (I_{sat}) of the available coordination sites ($\xi_{sat} \approx 1$) as follows:

$$I_0 = \alpha[L]_0$$

$$I_{sat} = \alpha[L]_0(1-f) + \beta f[L]_0 \rightarrow \beta = \frac{I_{sat} - I_0(1-f)}{f[L]_0}$$

Finally, at any point the advancement ξ can be calculated as a function of the fluorescence intensity according to the relation:

$$\xi = \frac{I - I_0}{f[L]_0(\beta-\alpha)}$$

And the final fluorescence intensity is given by equation (1):

$$I = \alpha[L]_0 + (\beta-\alpha)\cdot\frac{K_d + [Zn]_{added} + f[L]_0 - \sqrt{\left(K_d + [Zn]_{added} + f[L]_0\right)^2 - 4[Zn]_{added}\cdot f[L]_0}}{2} \quad (1)$$

In this model, the raw emission data were first corrected from filtering effects as shown in Figure S14, and the fluorescence count at 440 nm was taken as intensity I. The values used for $[L]_0$ were 8.35×10^{-6}, 1.67×10^{-5}, 3.34×10^{-5}, 8.35×10^{-5}, and 1.67×10^{-4} $mol.L^{-1}$ for 0.5, 1, 2, 5, and 10 mol% of ligand **3** in the DMPG membrane, respectively (DMPG concentration: 1.67 mM). A satisfactory fit for the five data sets $I = f([Zn]_{added})$ altogether was obtained for values of α, β, and K_d, of 2.8×10^6 $counts.M^{-1}$, 1.1×10^7 $counts.M^{-1}$, and 2×10^{-7} M, respectively, and for different values of f: 0.42, 0.47, 0.50, 0.64, and 0.72, respectively. Thus, the binding constant K_a of free zinc at DMPG bilayers is 5×10^6 M^{-1}.