

**Electronic Supplementary Information (ESI)
for**

**A Highly Selective and Sensitive Fluorescence Turn-on Sensor for Hg²⁺ and Its
Application in Live Cell Imaging**

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Table of Contents

I. Determination of the Stability Constants

II. Supplementary data

II.1 Figure S1 (1D structure of **8H-BDP**)

II.2 Figure S2 (Analyze of Ks)

II.3 Figure S3 (Time response of **8H-BDP** to Hg²⁺)

II.4 Figure S4 (Confocal fluorescence and brightfield images)

II.5 Figure S5 (Luminescence intensity profile)

II.6 Figure S6 (Z-scan images)

III. ¹H NMR spectra

I. Determination of the Stability Constants¹⁻²

The following considerations apply to complexes of stoichiometry 1:2 with a stability constant defined as

$$K_S = \frac{[ML_2]}{[M][L]^2} \quad (1)$$

Fluorescence intensity of the free ligand F_0 is proportional to the concentration C_0

$$F_0 = aC_0 \quad (2)$$

After addition of a given amount of salt at a concentration C_M , the fluorescence intensity becomes

$$F = a[L] + b[ML_2] \quad (3)$$

In addition to this relation, we have

$$C_0 = [L] + 2[ML_2] \quad (4)$$

$$C_M = [M] + [ML_2] \quad (5)$$

In equation (3) (4) (5), $[ML]$ was omitted because the efficiency of 1:2 complexation of Hg^{2+} with the compound **8H-BDP** is so high and quickly after reaching stoichiometric point, the ligand is fully complexed. F reaches the limiting value.

$$F_{Lim} = b \frac{C_0}{2} \quad (6)$$

From equations 1-6, it is easy to derive the relation

$$x = \frac{F - F_0}{2(F_{Lim} - F_0)} \left\{ \frac{1}{K_S} \left[\frac{F_{Lim} - F_0}{C_0 (F_{Lim} - F)} \right]^2 + 1 \right\} \quad (7)$$

Where $x = [Hg^{2+}]/[8H-BDP]$, F is the fluorescence intensity for the considered x value, F_0 and C_0 are the initial fluorescence intensity and concentration of **8H-BDP**, respectively, F_{Lim} is the limiting value corresponding to the full complexation. K_S is the stability constant of the $[(Hg^{2+})(8H-BDP)_2]$ complex.

(1) J. Bourson, J. Pouget and B. Valeur, *J. phys. Chem.*, 1993, **97**, 4552;

(2) M. N. Berberan-Santos, P. Choppinet, A. Fedorov, L. Jullien and B. Valeur, *J. Am. Chem. Soc.*, 2000, **122**, 11876.

II. Supplementary data

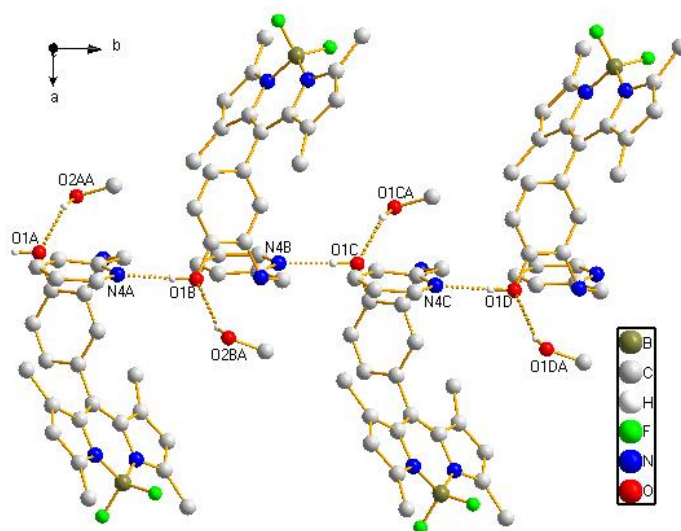


Fig S1. View of the one-dimensional structure formed through the intermolecular hydrogen bonds along *b* direction.

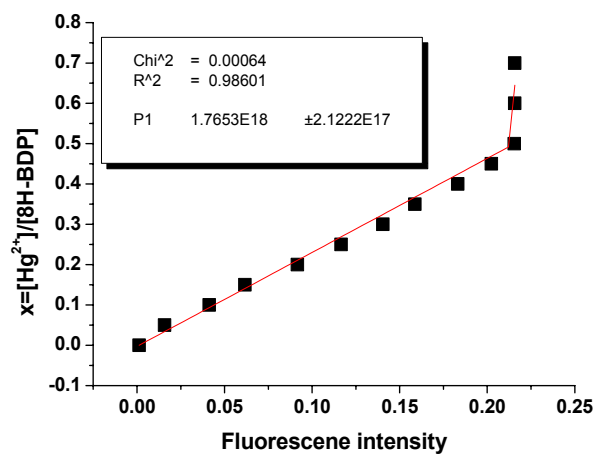


Fig S2. Variations of the ratio $x = [\text{Hg}^{2+}]/[\text{8H-BDP}]$ as a function of the fluorescence intensity at 509 nm, the red line corresponds to the best fit with equation 7

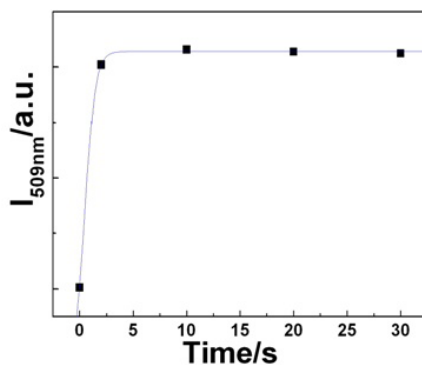


Fig S3. Time course of the fluorescence response of **8H-BDP** (5 μM) to 1 equiv. of Hg^{2+} at 509 nm in DMSO/HEPES buffer (50 mM HEPES, 50mM KNO_3 , 1:99, v/v, PH=7.2) solution.

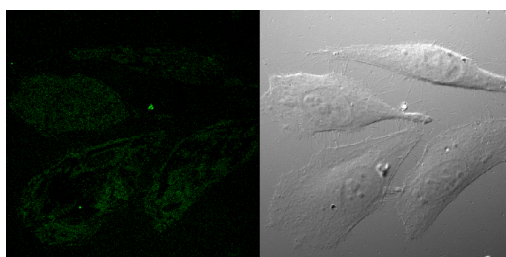


Fig S4. Confocal fluorescence (left) and brightfield (right) images of HeLa cells supplemented with 50 μM Hg^{2+} in the growth media for 1 h at 37°C.

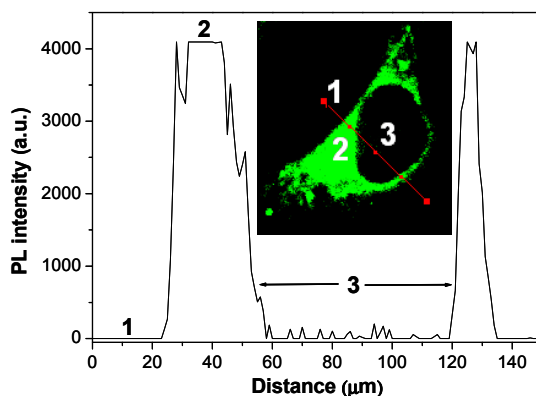


Fig S5. Luminescence intensity profile across the lines shown in confocal luminescence image (inset) of HeLa cells supplemented with 50 μM Hg^{2+} in the growth media for 1 h at 37°C and then stained with 50 μM **8H-BDP** for 10 min at 25 °C. (λ_{ex} = 488 nm).

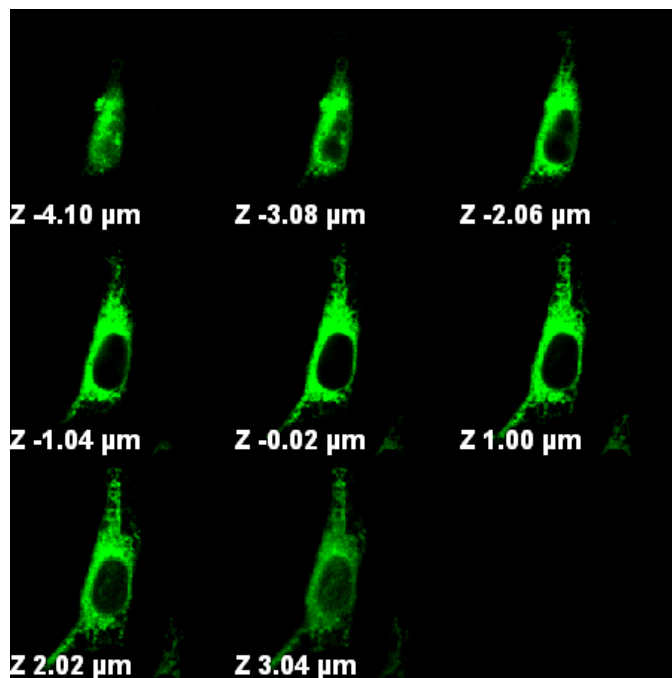


Figure S6. Z-scan confocal fluorescence images of HeLa cells supplemented with 50 μM Hg^{2+} in the growth media for 1 h at 37 $^{\circ}\text{C}$ and then stained with 50 μM **8H-BDP** for 10 min at 25 $^{\circ}\text{C}$.

III. ^1H NMR spectra

