Supporting Information to the manuscript:

Mercury(II) Recognition and Fluorescence Imaging *in vitro* through a 3D-Complexation Structure

Mei-Lin Ho,[†,*] *Kew-Yu Chen,*[‡] *Gene-Hsiang Lee,*[‡] Yu-Chun Chen,[‡] *Chih-Chieh Wang,*[†] *Jyh-Fu Lee,*[§]

Wen-Chun Chung[†] *and Pi-Tai Chou*[‡,*]

[†] Department of Chemistry, Soochow University, Taipei, Taiwan.

[‡] Department of Chemistry, National Taiwan University, Taipei, Taiwan.

[§] National Synchrotron Radiation Research Center, Hsinchu 300, Taiwan.

E-mail: meilin_ho@scu.edu.tw, chop@ntu.edu.tw

Supporting Information

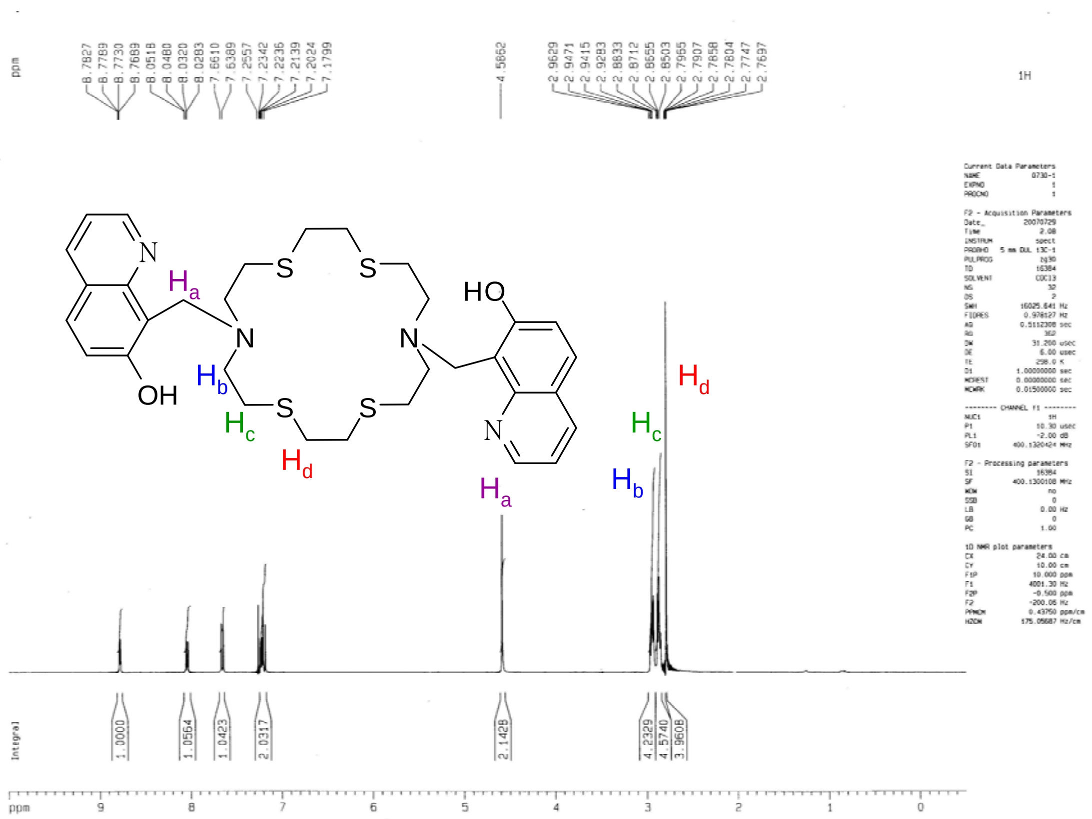

Figure S1. ^{1}H NMR spectra of **TTBQ** in $CDCl_3$.

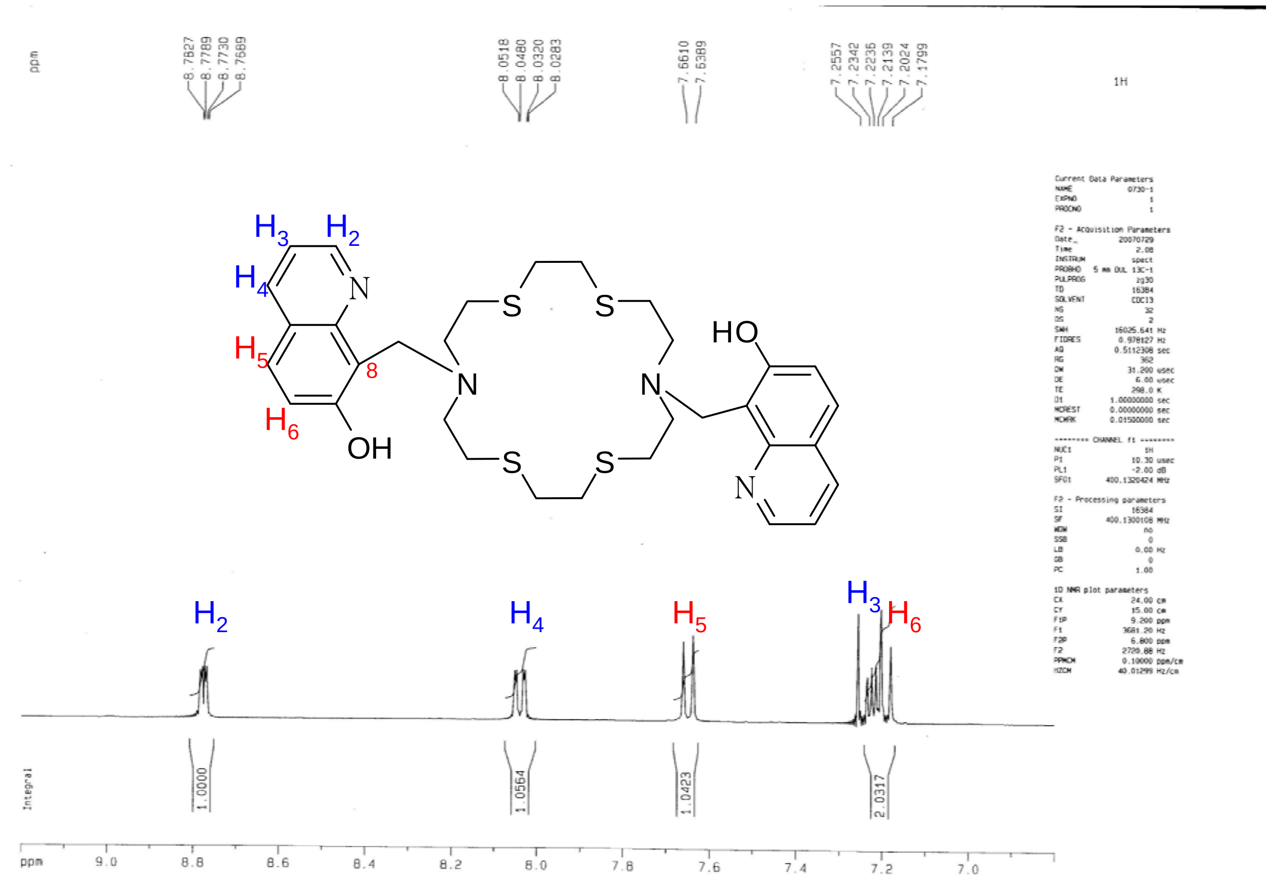

Figure S2. The extension of ^{1}H NMR spectra of **TTBQ** in $CDCl_3$.

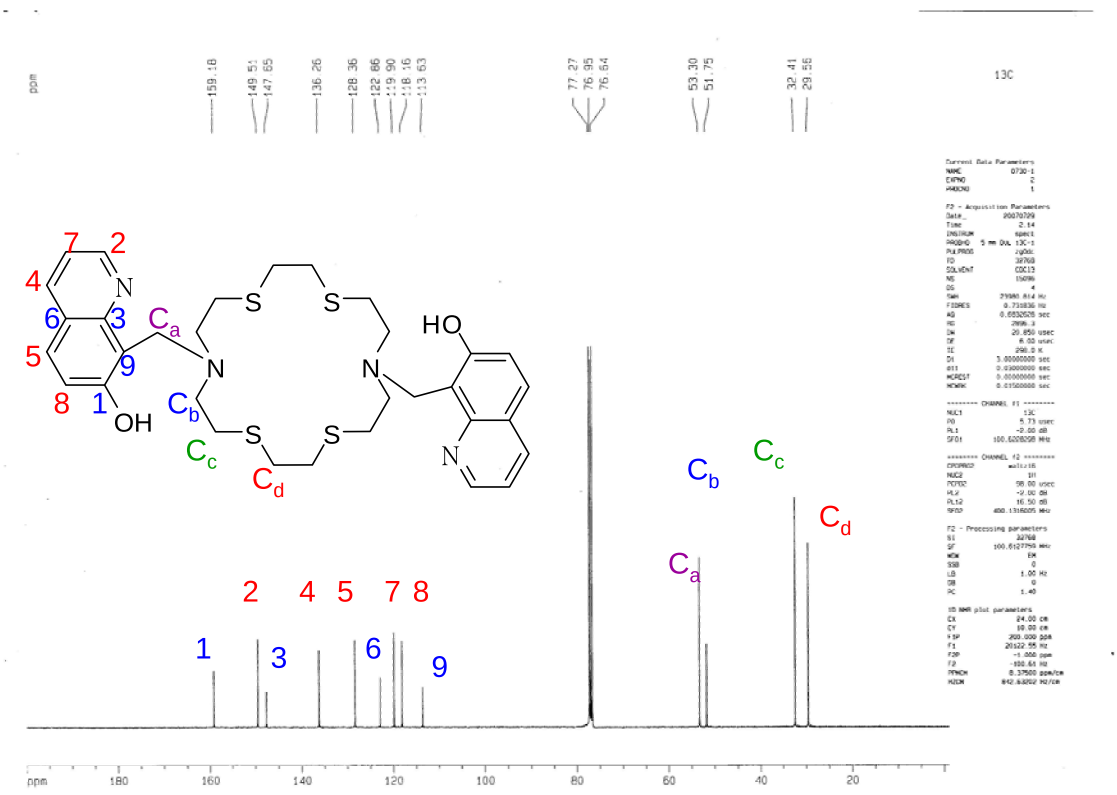

Figure S3. ^{13}C NMR of **TTBQ** in $CDCl_3$. (125MHz)

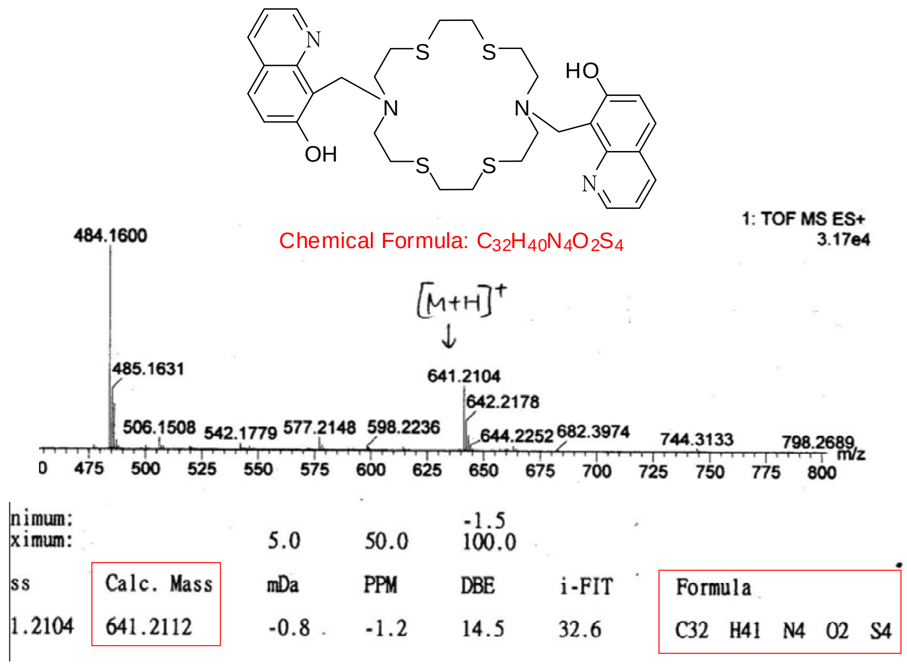

Figure S4. HRMS of **TTBQ** in $CDCl_3$. (125MHz)

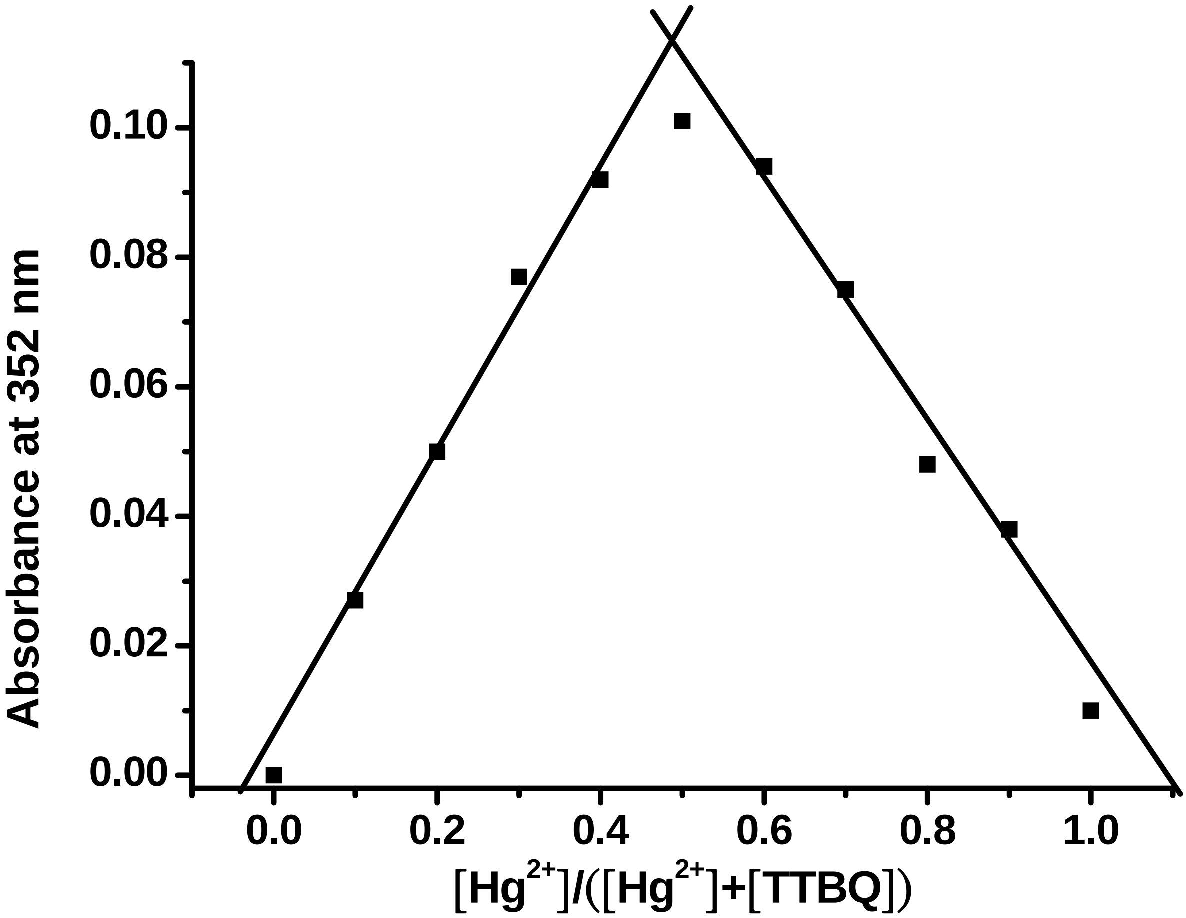

Figure S5. Job's plot of a 1:1 complex of **TTBQ** and Hg^{2+}, where the increase of absorption at 352 nm was plotted against the mole fraction of Hg^{2+}. [**TTBQ**] + [Hg^{2+}] = 1.50 μM.

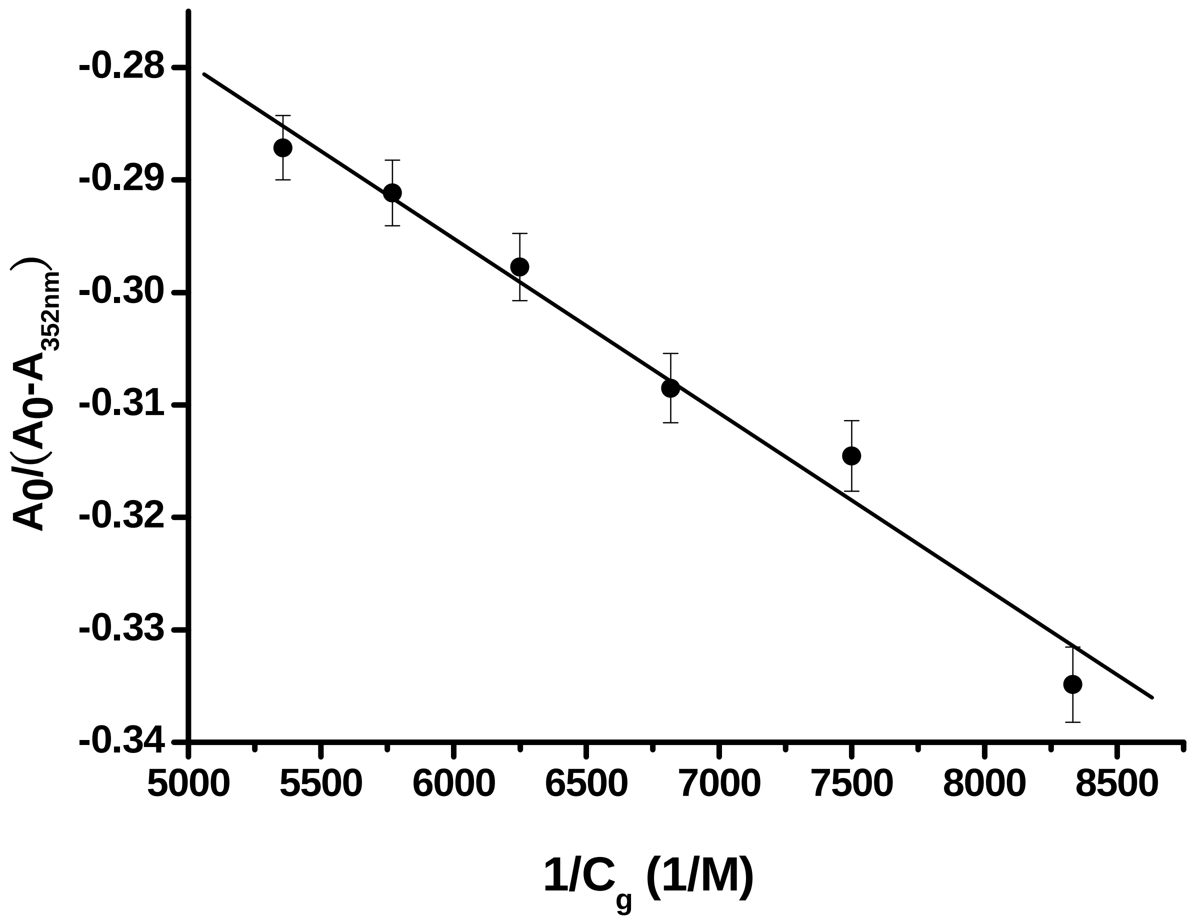

Figure S6. The plot of $A_0/(A_0-A_{352nm})$ against $1/C_g$ at 352 nm. $K_a = 13,020 \pm 520\ M^{-1}$.

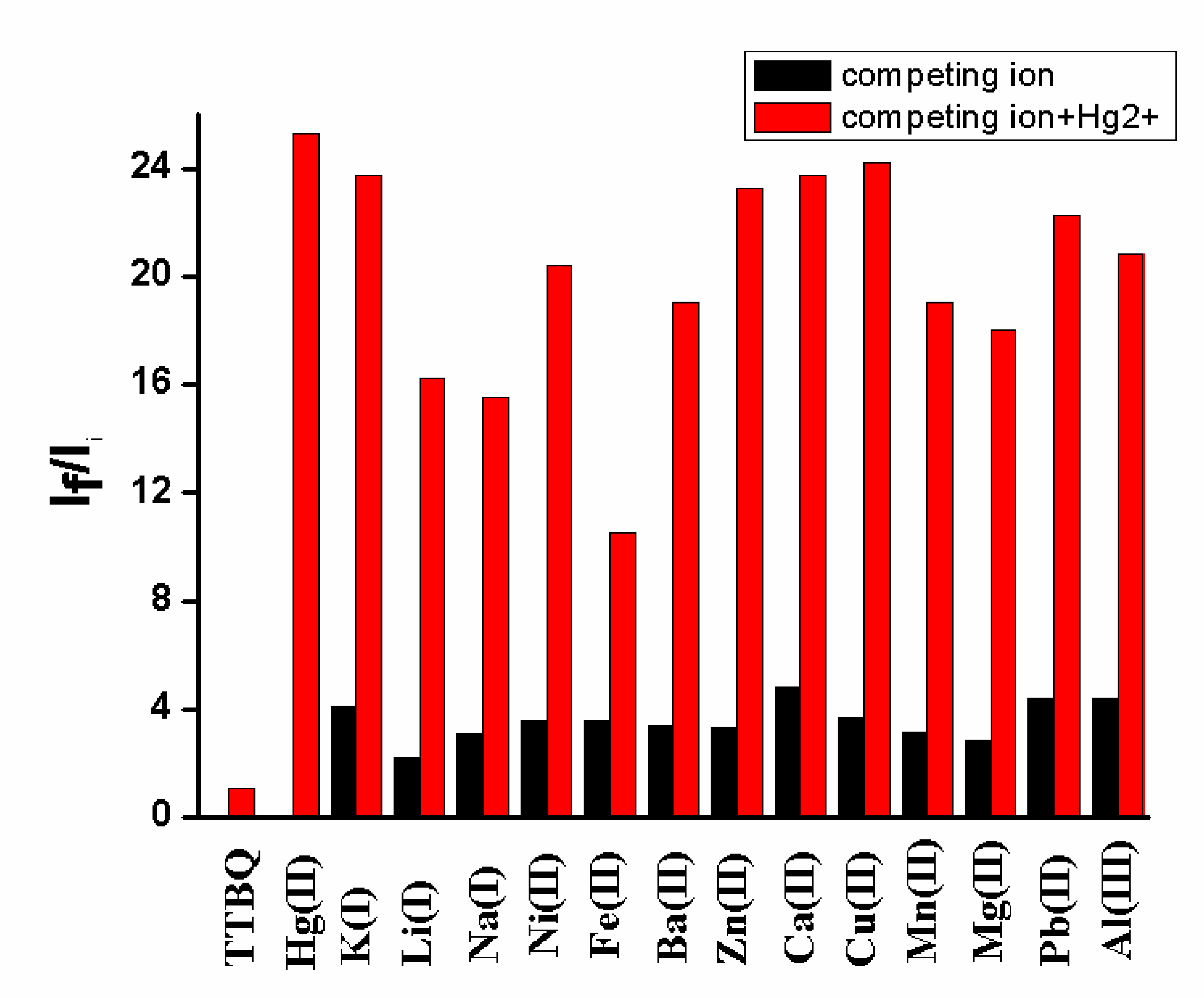

Figure S7. Fluorescence responses of **TTBQ** to various metal ions. Bars represent the final integrated fluorescence response (I_f) over the initial integrated emission (I_i). Initial spectrum was acquired in aerated solution, pH 7. Black bars represent the addition of the appropriate metal ion (1 mM for Li^+, Na^+, K^+, Mg^{2+} and Ca^{2+}, 250 µM for Fe^{2+} and Cu^{2+}, and 300 µM for all other cations) to a 1.5 µM solution of **TTBQ**. Red bars represent the addition of 0.5 mM Hg^{2+} to solutions containing **TTBQ**. Excitation was provided at 352 nm, and the emission was integrated over 400 to 700 nm.

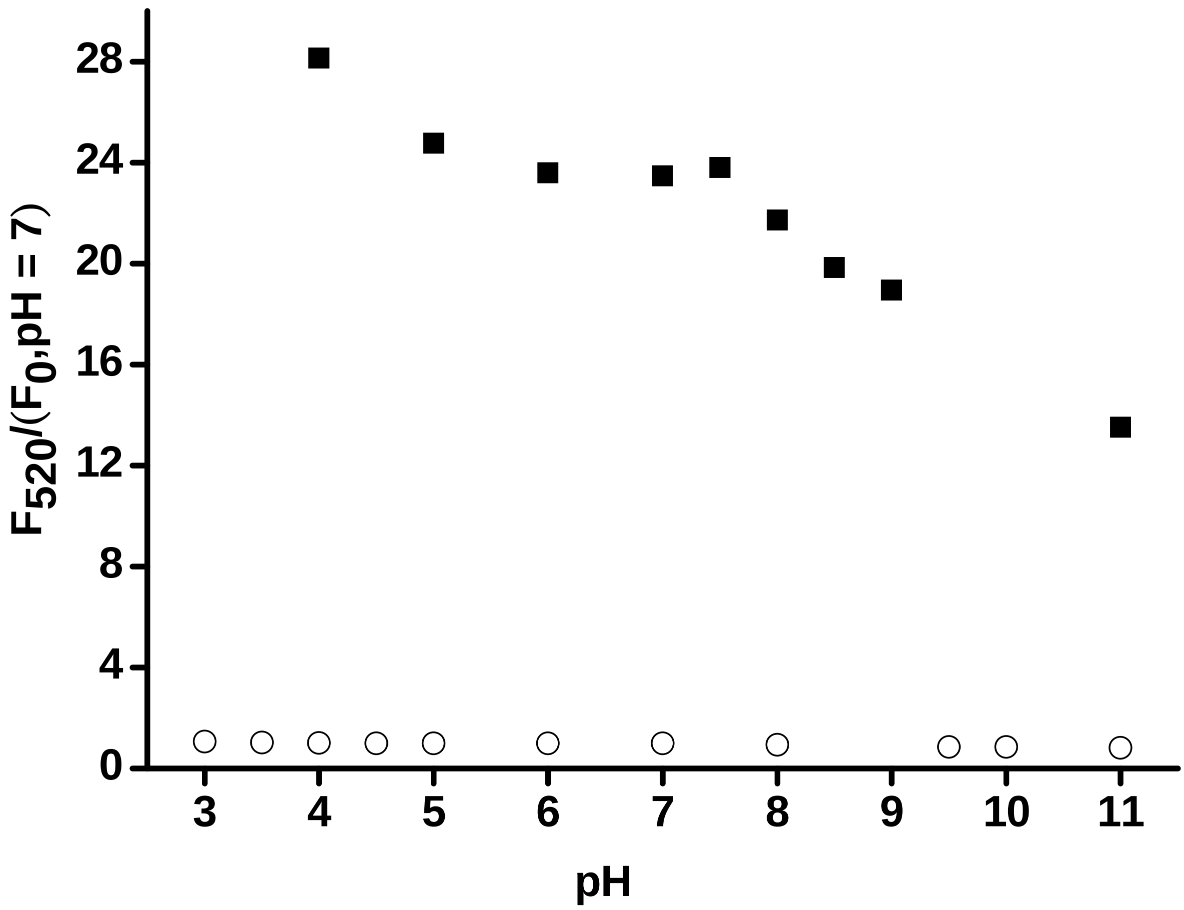

Figure S8. pH-dependent Fluorescence intensity ratio ($F_{520}/(F_0$, pH = 7)) of **TTBQ** (circle, 1.5 μM) plus Hg^{2+} (square, 280 μM) in aerated aqueous solution. λ_{ex} = 355 nm.

Association Constant Derivation from Absorption titration. The association constant K_a of **TTBQ** + Hg^{2+} complex formation calculated by the UV-Vis absorption method can be derived as follows

	TTBQ	+ Hg^{2+}	$\rightleftharpoons$	**TTBQ**/Hg^{2+}
initial	C_0	C_g		0
final	C_M	$\sim C_g$		C_p

On the above expression the association constant is assumed to be not very large so that the concentration of the added Hg^{2+} varies negligibly during the reaction (see text). The absorbance of **TTBQ** at e.g. 352 nm prior to the addition of Hg^{2+} can be expressed by

$$A_0 = C_0 \varepsilon_M \ \therefore\ C_0 = \frac{A_0}{\varepsilon_M}$$

Upon adding the guest molecule C_g

$$C_0 = \frac{A_0}{\varepsilon_M} = C_M + C_p$$

$$\therefore C_p = \frac{A_0}{\varepsilon_M} - C_M \qquad (1) \qquad \text{On the other hand, } K_a = \frac{C_p}{C_M C_g}$$

$$\therefore C_p = K_a C_g C_M \qquad (2)$$

$$(1) = (2) \ \therefore \frac{A_0}{\varepsilon_M} - C_M = K_a C_g C_M \Rightarrow C_M = \frac{A_0}{\varepsilon_M (K_a C_g + 1)}$$

The absorbance of **TTBQ** and **TTBQ**/Hg^{2+} complex at a specific wavelength can be expressed by

$$A = \varepsilon_M C_M + \varepsilon_P C_p = \varepsilon_M C_M + \varepsilon_p \left(\frac{A_0}{\varepsilon_M} - C_M\right) = (\varepsilon_M - \varepsilon_p) C_M + \frac{\varepsilon_p A_0}{\varepsilon_M}$$

$$= \frac{(\varepsilon_M - \varepsilon_p) A_0}{\varepsilon_M (K_a C_g + 1)} + \frac{\varepsilon_p A_0}{\varepsilon_M}$$

$$\therefore \frac{A}{A_0} = \frac{(\varepsilon_M - \varepsilon_p)}{\varepsilon_M (K_a C_g + 1)} + \frac{\varepsilon_p}{\varepsilon_M} = \frac{(\varepsilon_M - \varepsilon_p) + \varepsilon_p (K_a C_g + 1)}{\varepsilon_M (K_a C_g + 1)} \qquad (3)$$

Subtracting (1) from both sides of (3) we obtain

$$\therefore \frac{A}{A_0} - 1 = \frac{(\varepsilon_M - \varepsilon_p) + \varepsilon_p (K_a C_g + 1) - \varepsilon_M (K_a C_g + 1)}{\varepsilon_M (K_a C_g + 1)}$$

$$\therefore \frac{A - A_0}{A_0} = \frac{(\varepsilon_M - \varepsilon_p) + (\varepsilon_p - \varepsilon_M)(K_a C_g + 1)}{\varepsilon_M (K_a C_g + 1)} = \frac{(\varepsilon_p - \varepsilon_M)(K_a C_g)}{\varepsilon_M (K_a C_g + 1)}$$

$$\therefore \frac{A_0}{A_0 - A} = \frac{\varepsilon_M (K_a C_g + 1)}{(\varepsilon_M - \varepsilon_p)(K_a C_g)} = \left(\frac{\varepsilon_M}{\varepsilon_M - \varepsilon_p} \right) \left[\frac{1}{K_a C_g} + 1 \right] \qquad (4)$$