**Electronic Supplementary Information for**

# Development of a Mitochondria Targetable Ratiometric Time-Gated Luminescence Probe for Biothiols Based on Lanthanide Complexes

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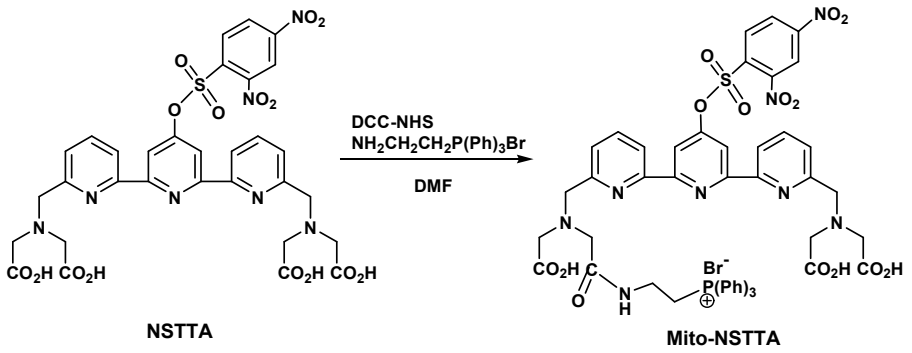


**Scheme S1.** Reaction pathway for the synthesis of the ligand Mito-NSTTA.

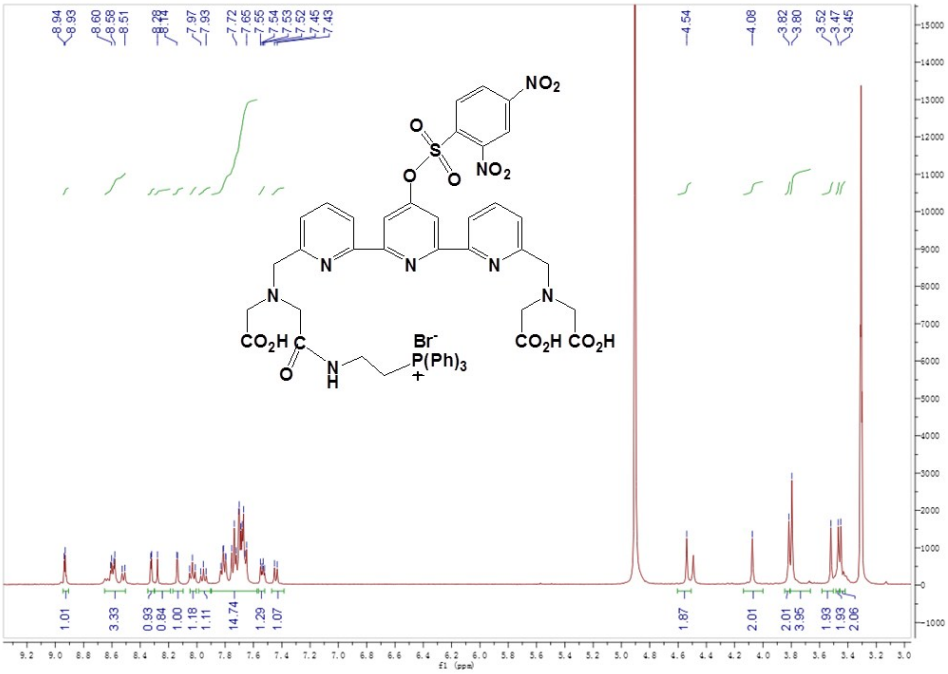


**Fig. S1.** $^{1}$H NMR spectrum of Mito-NSTTA.

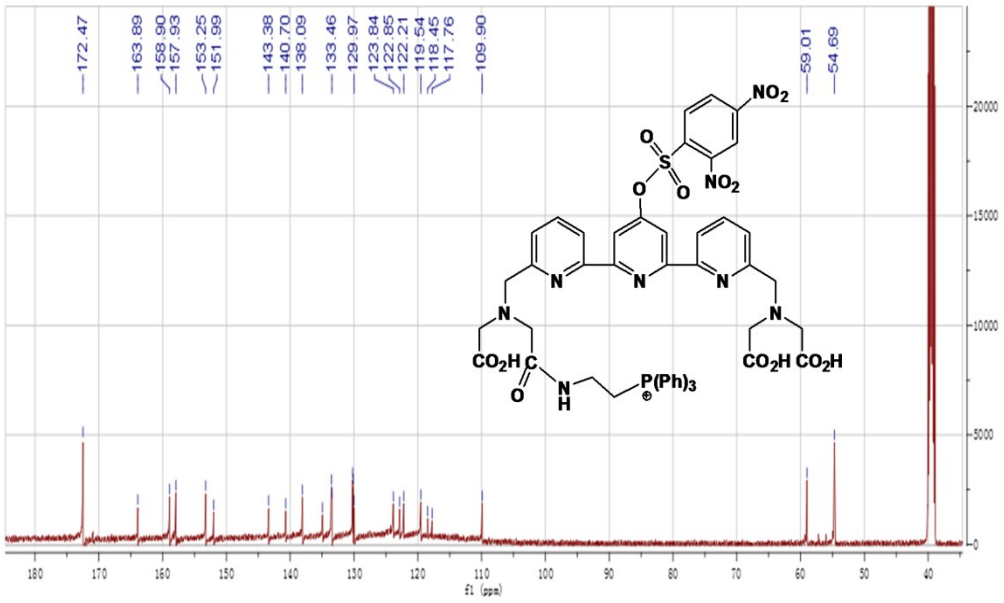


**Fig. S2.** $^{13}C$ NMR spectrum of Mito-NSTTA.

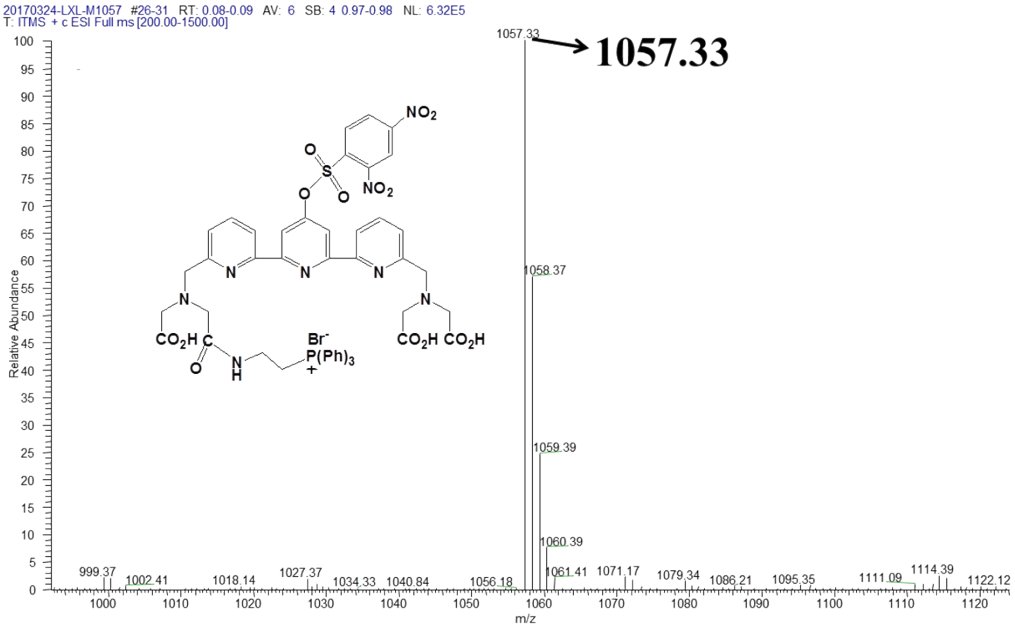


**Fig. S3.** ESI-MS of Mito-NSTTA.

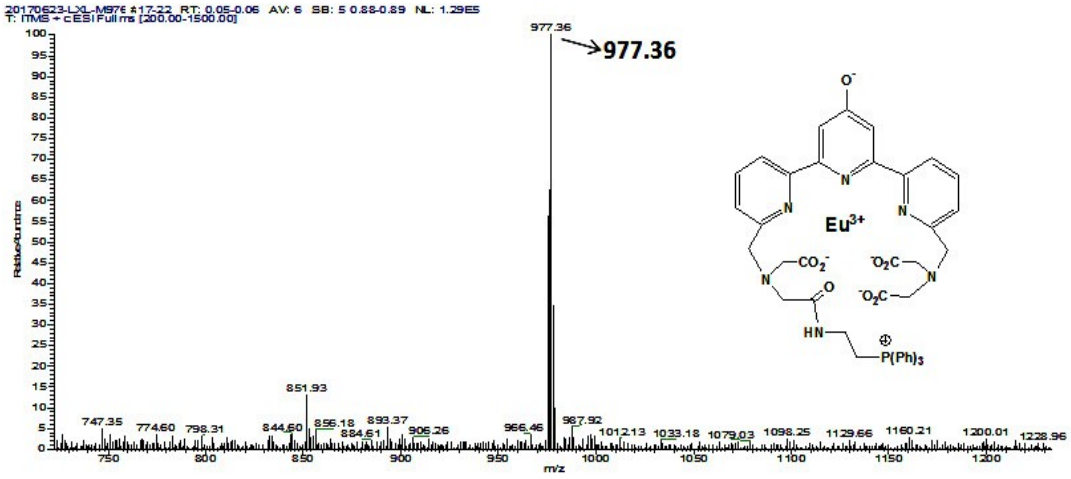


**Fig. S4.** ESI-MS of the product of Mito-NSTTA-$Eu^{3+}$ reacted with GSH in 50 mM PBS of pH 7.4.

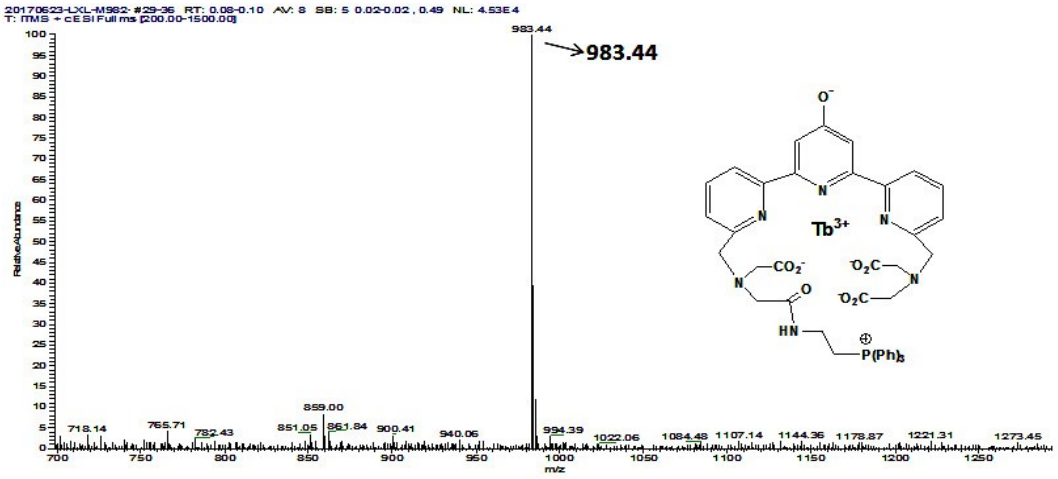


**Fig. S5.** ESI-MS of the product of Mito-NSTTA-$Tb^{3+}$ reacted with GSH in 50 mM PBS of pH 7.4.

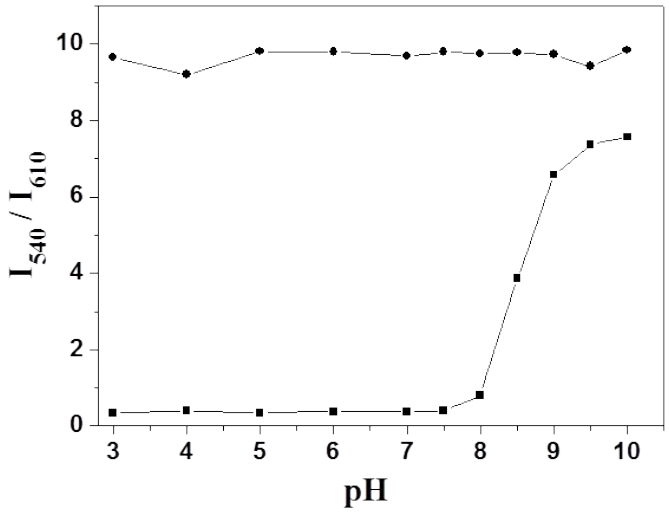


**Figure S6.** The effects of pH on the ratiometric time-gated luminescence signals ($I_{540}/I_{610}$) of Mito-NSTTA-$Eu^{3+}$/$Tb^{3+}$ ($C_{total}$ = 6.0 μM) in the absence (■) and presence (●) of 20 μM GSH in 0.05 M PBS buffers with different pH values.

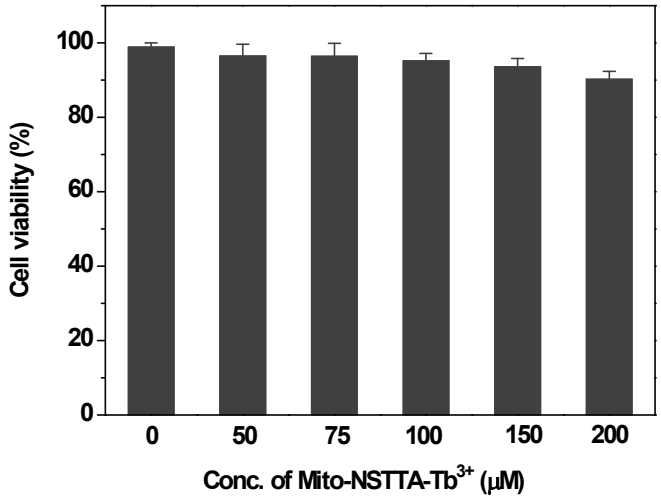


**Fig. S7.** Viabilities of HeLa cells incubated with different concentrations of Mito-NSTTA-$Tb^{3+}$ for 12 h.

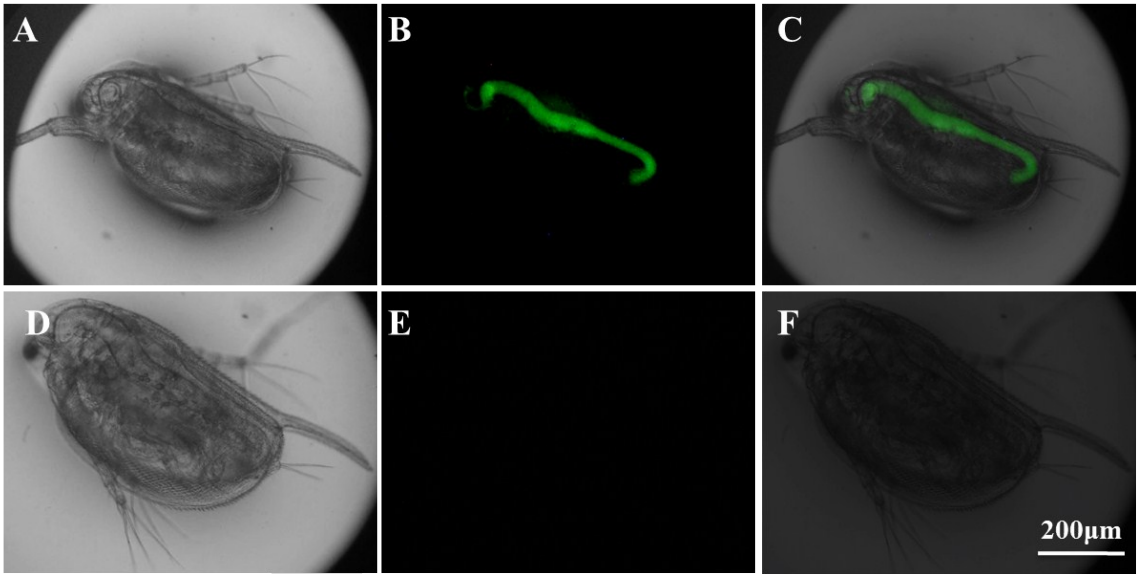


**Fig. S8.** Time-gated luminescence images of endogenous biothiols in *Daphnia magna* using Mito-NSTTA-$Tb^{3+}$ as a probe. A and D: bright-field image; B and E: time-gated luminescence image; C and F: merged images of bright-field with time-gated luminescence. Top (A to C): *Daphnia magna* were incubated with Mito-NSTTA-$Tb^{3+}$; bottom (D to F): *Daphnia magna* were pre-treated with NEM and then incubated with Mito-NSTTA-$Tb^{3+}$.