

## Supporting Information

# A FRET Based Two-Photon Fluorescent Probe for Visualizing Mitochondrial Thiols of Living Cells and Tissues

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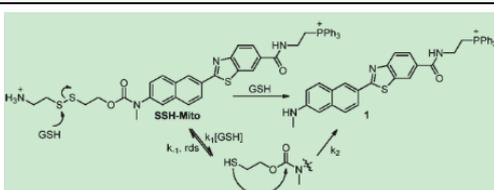
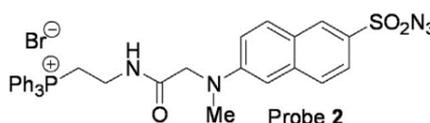
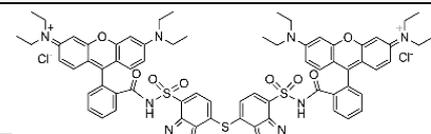
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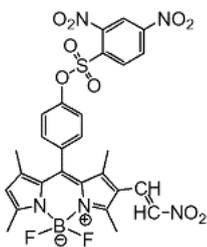
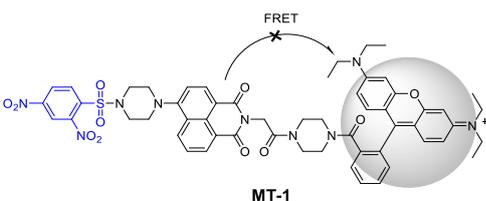
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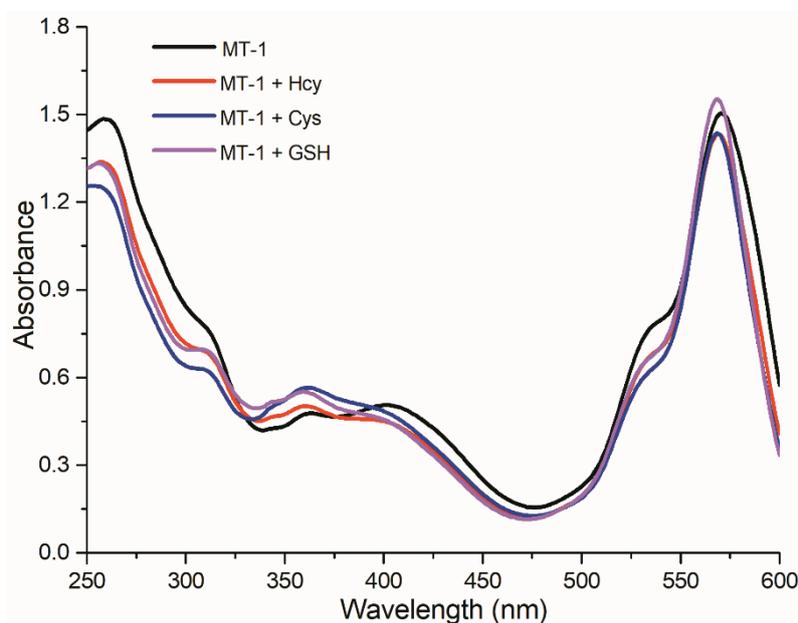
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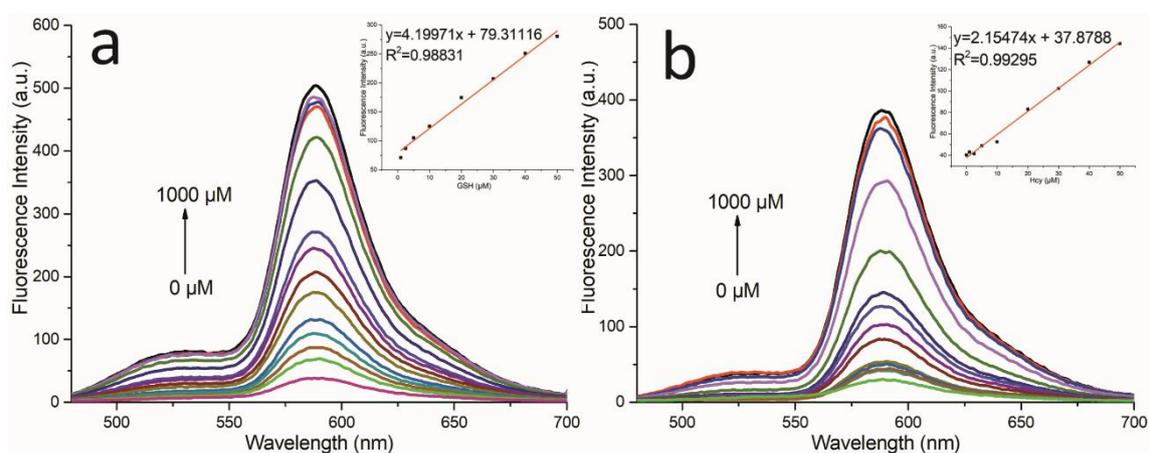
**Table 1.** Probes for mitochondria thiols detection.

Numbers	Probes for mitochondria thiols detection	Journals	Strategies	Mechanism for selectivity mitochondrial thiol detection
1		J. Am. Chem. Soc. 2011.[1]	Two photon $\lambda_{\text{ex}} = 740$ nm $\lambda_{\text{em}} = 545$ nm	not mentioned
2		Dyes and Pigments 2013.[2]	Two photon $\lambda_{\text{ex}} = 750$ nm $\lambda_{\text{em}} = 442$ nm	not mentioned
3		Anal. Chem. 2018[3]	$\lambda_{\text{ex}} = 550$ nm $\lambda_{\text{em}} = 580$ nm	different pH between mitochondria and cytoplasm
4		Sensors and Actuators B	Two photon $\lambda_{\text{ex}} = 730$ nm	not mentioned

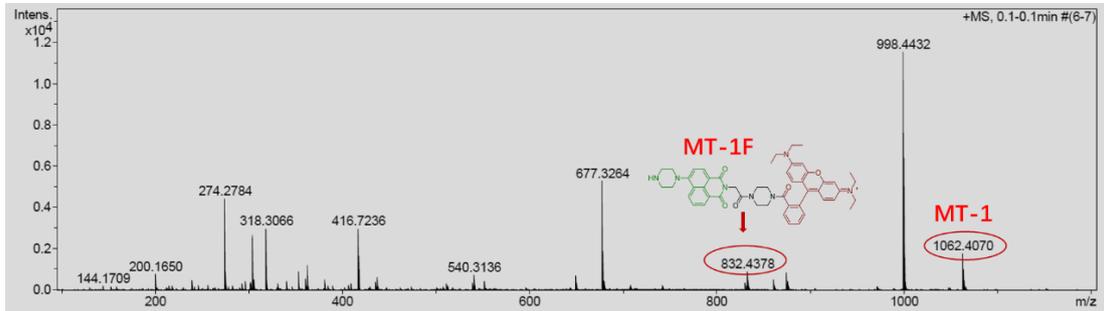
		2017.[4]	$\lambda_{em} = 482$ nm	
5		Dyes and Pigments 152 (2018) 29– 35.[5]	$\lambda_{ex} = 504$ nm $\lambda_{em} = 543$ nm	not mentioned
This work		Our work	Two photon FRET $\lambda_{ex} = 800$ nm $\lambda_{em} = 590$ nm	pH dependent reactivity of probe



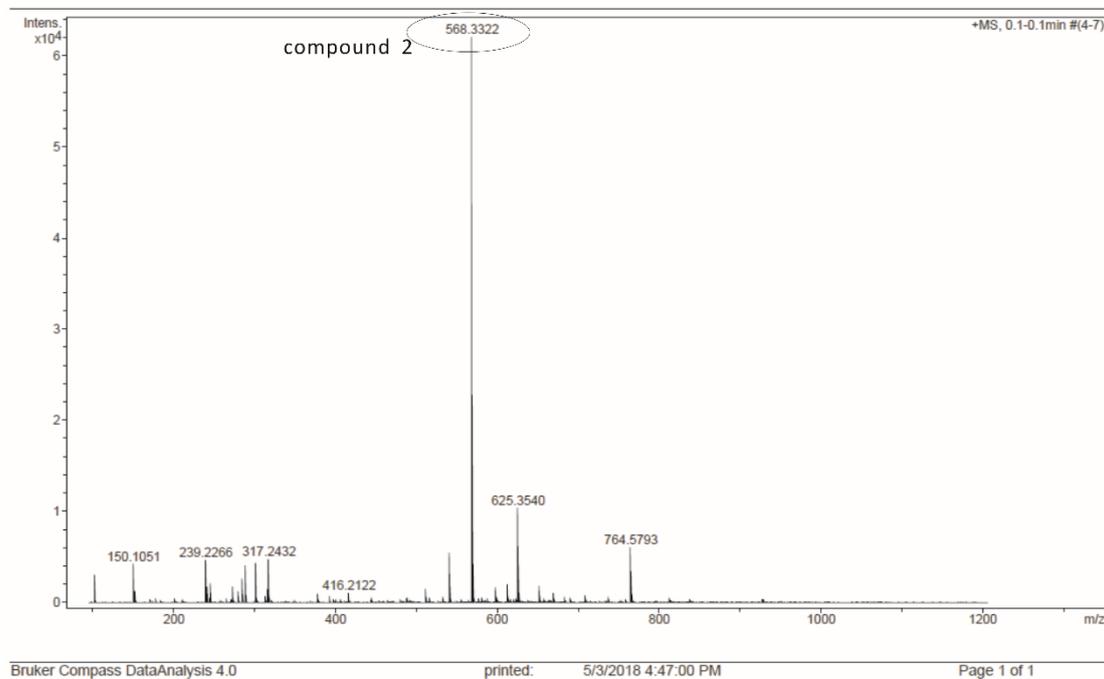
**Figure S1.** Absorption spectra of MT-1 before and after treatments with three biothiols (100  $\mu\text{M}$ , respectively) in 10% (V/V) DMSO/PBS buffer (50 mM, pH = 7.4) at 37  $^{\circ}\text{C}$ .



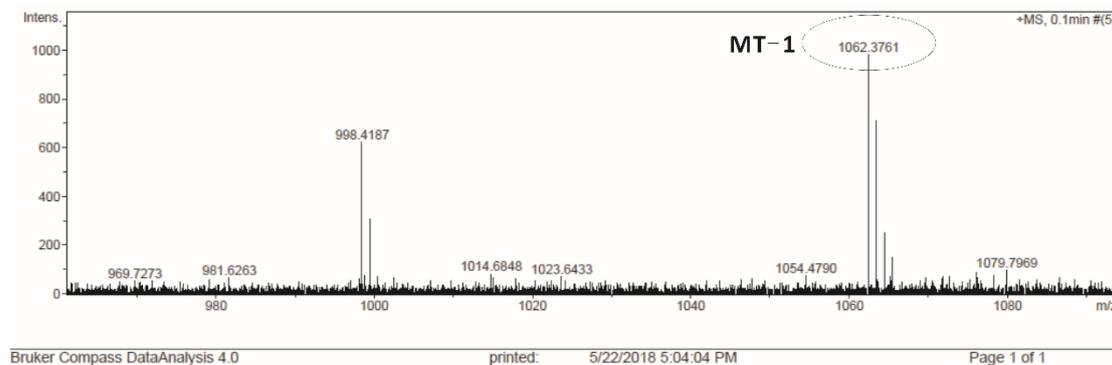
**Figure S2.** Fluorescence intensity and the linear relationship of MT-1 (10  $\mu$ M) with different concentrations of GSH (a) and Hcy (b) for 1 hour in 10% (V/V) DMSO/PBS buffer (50 mM, pH = 7.4) at 37  $^{\circ}$ C.  $\lambda_{\text{ex}}$  = 395 nm,  $\lambda_{\text{em}}$  = 589 nm, slits (10, 10).



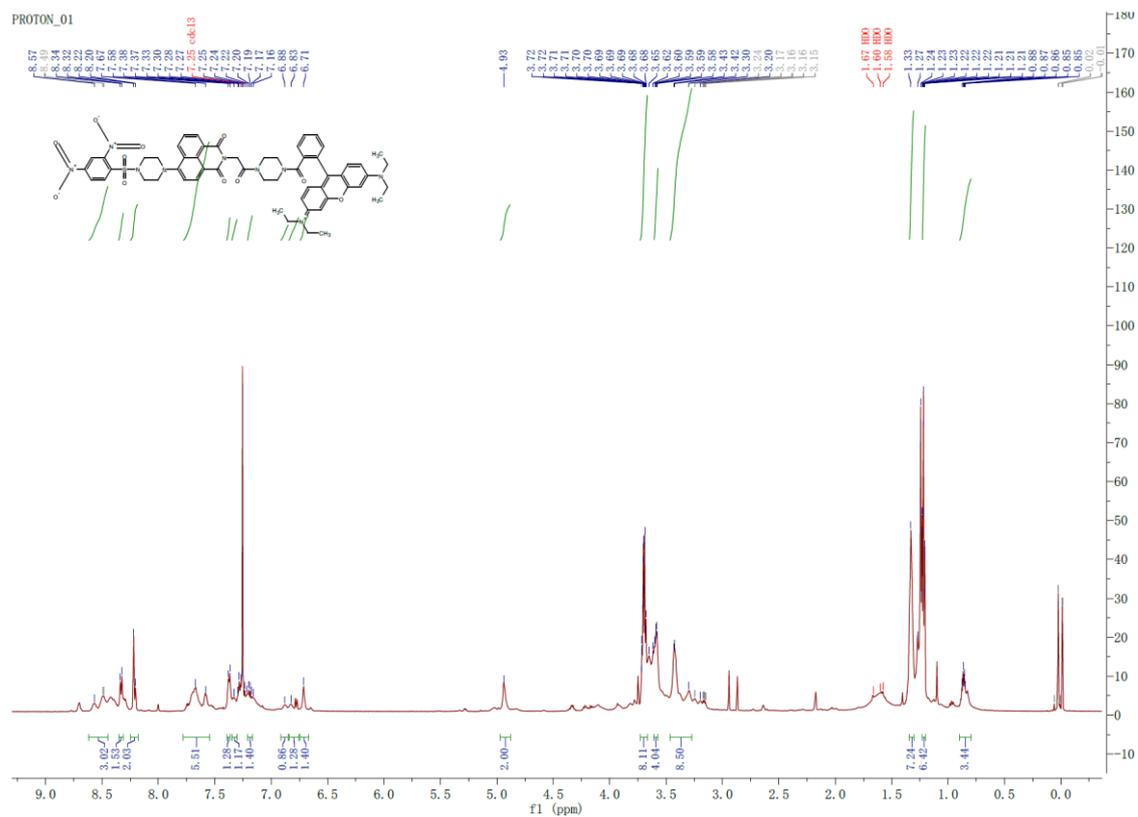
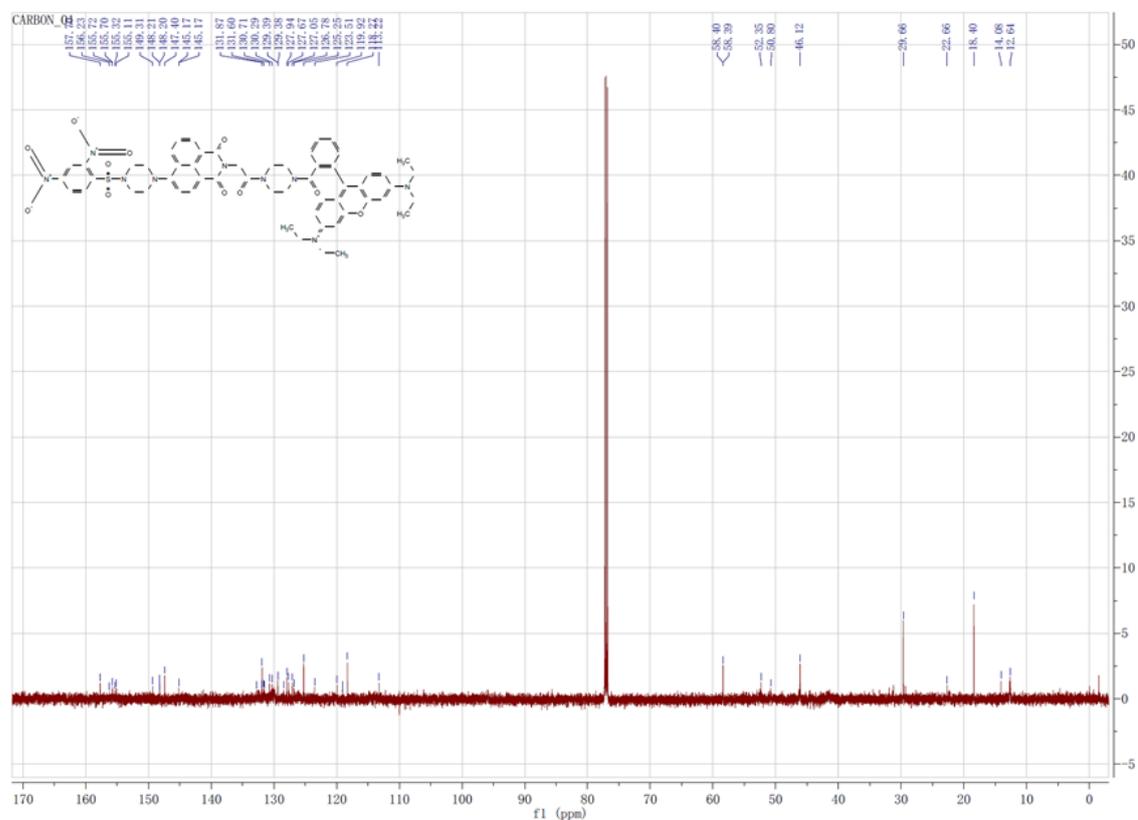
**Figure S3.** ESI-MS spectrometry of MT-1 upon addition of Cys in 10% DMSO/PBS (V/V) buffer (50 mM, pH = 7.4) at 37  $^{\circ}$ C.



**Figure S4.** ESI-MS of 2.



**Figure S5.** ESI-MS of MT-1.

Figure S6.  $^1\text{H}$  NMR of MT-1.Figure S7.  $^{13}\text{C}$  NMR of MT-1.

## References

1. Su, L.C., et al., *Ratiometric detection of mitochondrial thiols with a two-photon fluorescent probe*. Journal of the American Chemical Society, 2011. **133**(29): p. 11132-5.
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