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## A Mitochondria-targeted Ratiometric Two-photon Fluorescent Probe for Detecting Intracellular Cysteine and Homocysteine

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**Abstract:** A novel mitochondria-targeted ratiometric two-photon fluorescent probe (**Mito-MQ**) for detecting intracellular cysteine (Cys) and homocysteine (Hcy) has been designed. **Mito-MQ** showed the ratiometric fluorescent detection signal (the green-to-blue emission from 517 nm to 460 nm) to cysteine (Cys) and homocysteine (Hcy) over glutathione (GSH), along with the fast response rate (10 min). The detection mechanism was illustrated by <sup>1</sup>H-NMR, ESI-MS and theoretical calculation. The co-localization coefficient of 0.87 between **Mito-MQ** and MitoTracker Red revealed that the probe was predominantly present in mitochondria, therefore, **Mito-MQ** was successfully applied to detect mitochondrial oxidative stress by detecting the change of Cys/Hcy. Moreover, imaging in fresh tissue slices indicated that **Mito-MQ** could work in deep tissue (ca. 130 μm) under two-photon excitation. Furthermore, the measurement of Cys/Hcy detection in zebrafish showed that probe can be used in determination of biothiols *in vivo*.

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