Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A highly sensitive and genetically encoded fluorescent reporter for ratiometric monitoring of quinones in living cells

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Supplementary Materials and Methods:

Materials:

DNA sequences were purchased from Integrated DNA Technologies (USA). All the chemicals were purchased from Sigma (USA) except indicated. Ampicillin and kanamycin were purchased from Bio Basic Inc (USA). When necessary, antibiotics were added at the following concentrations: ampicillin, 100 μg/ml; kanamycin 50 μg/ml.

Construction of various quinone-sensor constructs:

The genes encoding $QsrR_{1-102}$ and $QsrR_{3-112}$ were PCR amplified from *Staphylococcus aureus* Newman genomic DNA with the primers QSC1F/R, QSC3F/R, respectively (*Table S1*). The cpYFP gene was directly amplified from OHSer[1] with the primers QSC2F/R. The DNA fragments of cpYFP and $QsrR_{3-112}$ were linked together via overlapping PCR. The resulting DNA was digested with *EcoR*I and *Xho*I and cloned into the *EcoR*I-*Xho*I site of pET-28a vector. The recombinant plasmid was amplified in *E. coli* DH10b and further digested with *BamH*I and *EcoR*I. The $QsrR_{1-102}$ was also digested with *BamH*I and *EcoR*I and cloned into the *BamH*I-*EcoR*I digested plasmid to form a final quinone-sensor expressing plasmid pET-28a_$QsrR_{1-102}$-cpYFP-$QsrR_{3-112}$. The various Quinone-sensor constructs were achieved by utilizing site-directed mutagenesis (Stratagene) to change the linkers between $QsrR_{1-102}$ and cpYFP.

To construct Hela-cell expressing plasmid, we digested pET-28a_$QsrR_{1-102}$-cpYFP-$QsrR_{3-112}$ plasmid with *BamH*I and *Xho*I to achieve the *BamH*I-*Xho*I digested $QsrR_{1-102}$-cpYFP-$QsrR_{3-112}$ fragment, which was

further cloned into the *BamH*I-*Xho*I site of pcDNA3 to form a final Hela cell quinone-sensor expressing plasmid pcDNA3_QsrR$_{1-102}$-cpYFP-QsrR$_{3-112}$ of Hela cells. For the control experiments, the sole cpYFP was PCR amplified with the primers cpYFPF/R (*Table S1*), digested with *EcoR*I and *Kpn*I, and finally cloned into the *EcoR*I-*Kpn*I site of pcDNA3.

Protein expression and purification

The plasmids carrying quinone-sensor expressing fragments were transformed into BL-21 (DE3). The cells were grown in LB to OD_{600}= 0.6 at 37 °C and then cell cultures were cooled down to 22 °C followed by the addition of 1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG) for induction overnight. The cells were harvested and frozen at -80 °C. The expressed protein was purified from the frozen cells with a Ni-NTA column (Qiagen) operated with FPLC system (GE) according to standard protocol. Finally the fractions were exchanged into a buffer containing 50 mM Tris.HCl (pH 7.4), 200 mM NaCl with the utilization of a 30 kDa filter (Qiagen).

Fluorescent measurements

All the fluorescence measurements were performed at room temperature on a Varian Cary Eclipse Fluorescence Spectrophotometer. For the construct screening experiments, the samples were excited at 496 nm and the emission spectrum was scanned from 505 nm to 600 nm with the excitation and emission slit widths of 5 nm. The protein samples (10 μM) in the buffer containing 50 mM Tris.HCl (pH 7.4) and 200 mM NaCl were incubated with 20 μM 1, 4-benzoquinone for 10 min at room temperature before data collection. For other fluorescent measurements, excitation spectra were recorded with fixation of the emission wavelength of 515 nm and the excitation and emission slit widths of 5 nm. The protein samples (10 μM) in the buffer containing 50 mM Tris.HCl (pH 7.4) and 200 mM NaCl were incubated with various chemicals at different concentrations at room temperature for 10 min before data collection. For the response measurement of CoQ_{10} and menaquinone (Vitamin K_2), 0.1% triton X-100 was added in order to mimic the lipid environment where they are associated. All the experiments were performed in triplicate.

For the GSH treatment experiments, QSer-quinone complex was mixed at room temperature for 5 min before addition of GSH, which was further incubated for 30 min before data collection.

Live cell fluorescence imaging

HeLa cells were plated onto Lab-Tek™ 8-well-chambered cover glasses in DMEM containing 10% fetal bovine serum. 14 hours after plating, the cells were transfected with pcDNA3-QSer or pcDNA3-cpYFP using Lipofectamine2000 transfection reagent according to the manufacturer's protocol. 24 hours after transfection, the chambers were washed and refilled with PBS, followed by the addition of various reagents. Then confocal fluorescence imaging was performed under Leica TCS SP2 AOBS Laser Scanning Confocal Microscope with 40×oil-immersion objective lens. The acquisition of image data and synchronization of the illumination were performed with sequential excitation at 405 nm and 488 nm, and a fixed emission range from 500 nm to 560 nm.The analysis and calculation of imaging data was performed using ImageJ. Background was subtracted and the fluorescence intensity of same cells was used to calculate the change in the emission ratio with dual excitations. The pseudocolor image of the same cell was generated by calculating the pixel-by-pixel ratio of the 488 nm excitation image by the 405 nm excitation image and converting the images into HSV color space. All the experiments were performed in triplicate.

References

1. Zhao, B. S.; Liang, Y. J.; Song, Y. Q.; Zheng, C. H.; Hao, Z. Y.; Chen, P. R. *J. Am. Chem. Soc.* 2010, **132**, 17065.

Table S1. Primers used in this study.

Description	Sequence (5'-3')
QSC1F	TGTACAGGATCCATGATGGAAGTATGTCCGTATCTCG
QSC1R	TGTACAGAATTCATATGATTGCGCCCATGCTT
QSC2F	TGTACAGAATTCTACAACAGCGACAACGTCTATATCAT
QSC2R	CGGACATACTTCGTTGTACTCCAGCTTGTGCCC
QSC3F	CTGGAGTACAACGAAGTATGTCCGTATCTCGAAGAAAC
QSC3R	TGTACACTCGAGTTATTTAGCAGTACGTTGATCTGTTAAATCG
QS1F	TCCAATTGAAGCATGGGCGCAATCATACAACAGCGACAACGTCTATATCA
QS1R	TGATATAGACGTTGTCGCTGTTGTATGATTGCGCCCATGCTTCAATTGGA
QS2F	TTGAAGCATGGGCGCAATCATATTACAACAGCGACAACGTCTATATC
QS2R	GATATAGACGTTGTCGCTGTTGTAATATGATTGCGCCCATGCTTCAA
QS3F	CAATTGAAGCATGGGCGCAATCATATGTCTACAACAGCGACAACGTCTATATCA
QS3R	TGATATAGACGTTGTCGCTGTTGTAGACATATGATTGCGCCCATGCTTCAATTG
QS4F	CAATTGAAGCATGGGCGCAATCATATGTCGATTACAACAGCGACAACGTCTATATCA
QS4R	TGATATAGACGTTGTCGCTGTTGTAATCGACATATGATTGCGCCCATGCTTCAATTG
QS5F	CCAATTGAAGCATGGGCGCAAGAATTCTACAACAGCGACAAC
QS5R	GTTGTCGCTGTTGTAGAATTCTTGCGCCCATGCTTCAATTGG
QS6F	TTGAAGCATGGGCGCAATCAGAATTCTACAACAGCGACAAC
QS6R	GTTGTCGCTGTTGTAGAATTCTGATTGCGCCCATGCTTCAA
QS8F	AAGCATGGGCGCAATCATATGTCGAATTCTACAACAGCGACAAC
QS8R	GTTGTCGCTGTTGTAGAATTCGACATATGATTGCGCCCATGCTT
QS9F	AAGCATGGGCGCAATCATATGTCGATGAATTCTACAACAGCGACAAC
QS9R	GTTGTCGCTGTTGTAGAATTCATCGACATATGATTGCGCCCATGCTT
cpYFPF	TCGGGTACCGTCGACATGTACAACAGCGACAACGTCTATATCATG
cpYFPR	TCG GAATTC TTAGTTGTACTCCAGCTTGTGCCCCAG

Underscores refer to restriction sites.

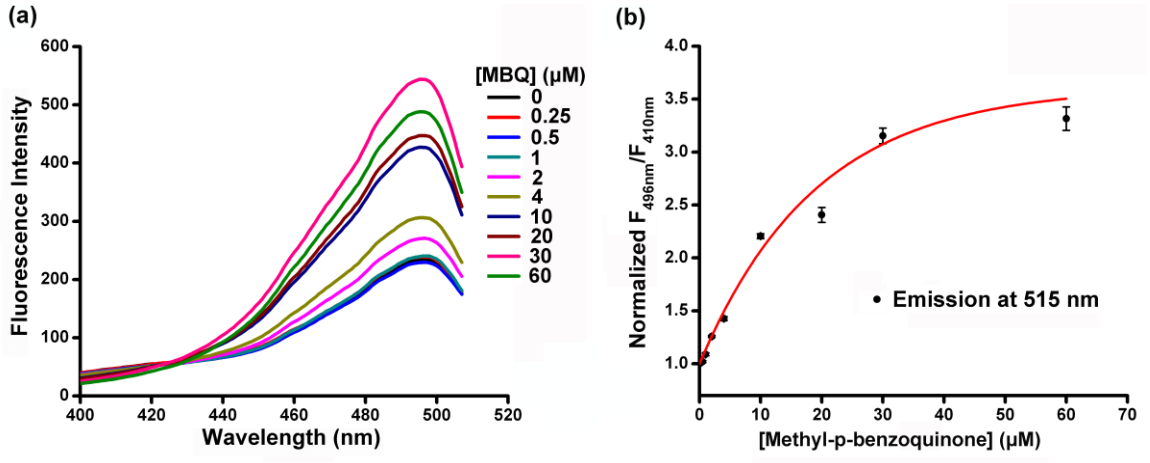

Figure S1. QSer sensitively responds to methyl-p-benzoquinone. (a) Fluorescence intensities of QSer under the induction of various concentrations of methyl-p-benzoquinone with the emission at 515 nm. MBQ represents methyl-p-benzoquinone. (b) The normalized ratios of fluorescence intensities with the emission at 515 nm. The values were generated from the fluorescence intensities at 496 nm divided by that at 410 nm and normalized. Error bars represent SEM.

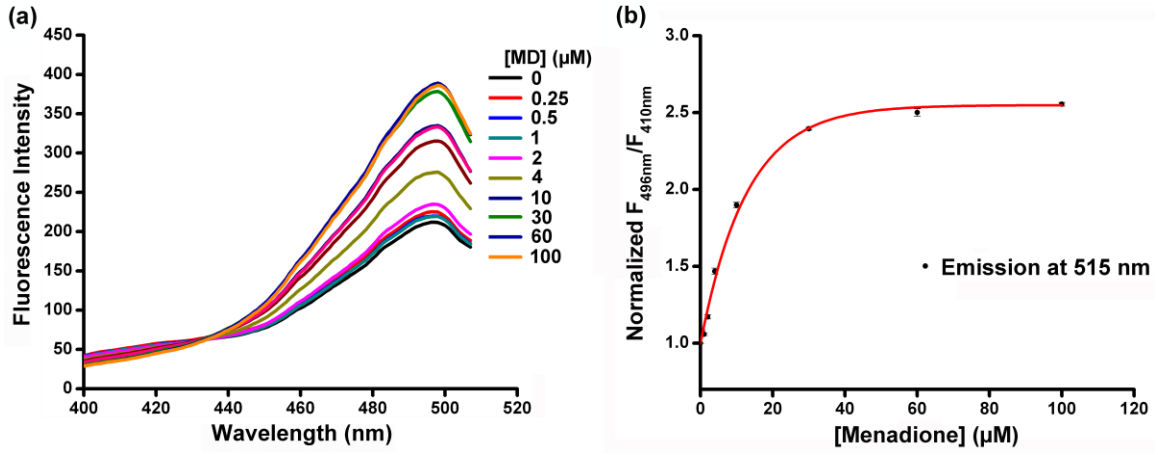

Figure S2. QSer sensitively responds to menadione. (a) Fluorescence intensities of QSer under the induction of various concentrations of menadione with the emission at 515 nm. MD represents menadione. (b) The normalized ratios of fluorescence intensities with the emission at 515 nm. The values were generated from the fluorescence intensities at 496 nm divided by that at 410 nm and normalized. Error bars represent SEM.

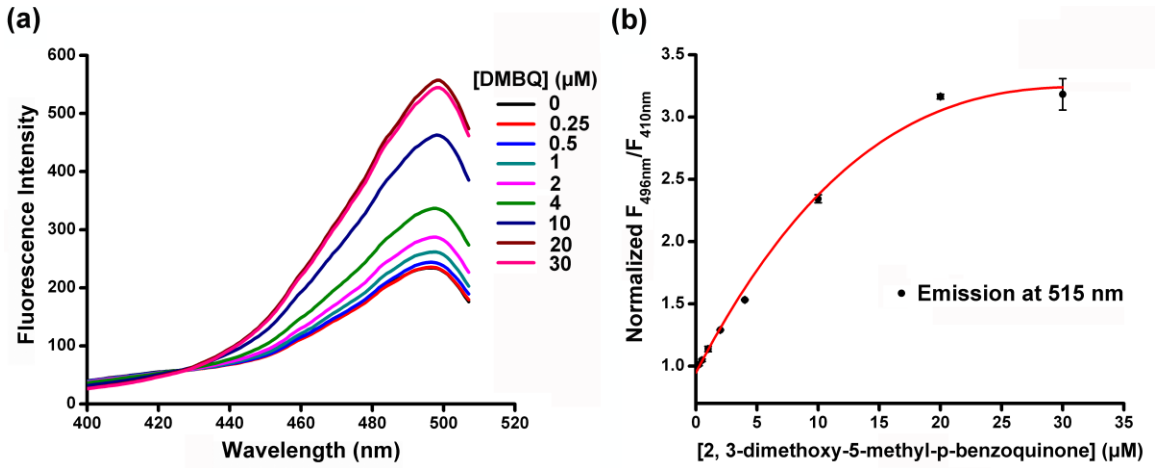

Figure S3. QSer sensitively responds to 2, 3-dimethoxy-5-methyl-p-benzoquinone. (a) Fluorescence intensities of QSer under the induction of various concentrations of 2, 3-dimethoxy-5-methyl-p-benzoquinone with the emission at 515 nm. DMBQ represents 2, 3-dimethoxy-5-methyl-p-benzoquinone. (b) The normalized ratios of fluorescence intensities with the emission at 515 nm. The values were generated from the fluorescence intensities at 496 nm divided by that at 410 nm and normalized. Error bars represent SEM.

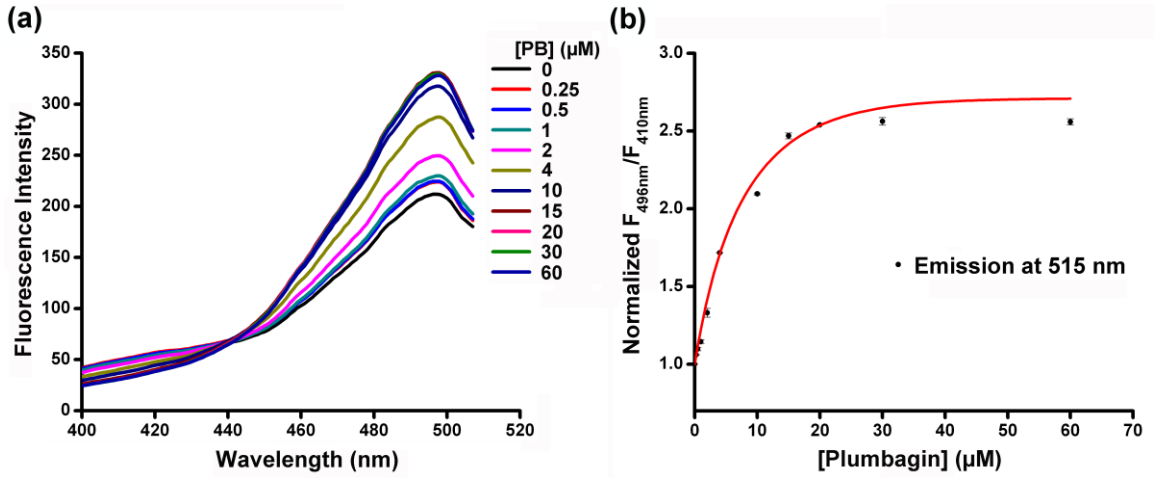

Figure S4. QSer sensitively responds to plumbagin. (a) Fluorescence intensities of QSer under the induction of various concentrations of plumbagin with the emission at 515 nm. PB represents plumbagin. (b) The normalized ratios of fluorescence intensities with the emission at 515 nm. The values were generated from the fluorescence intensities at 496 nm divided by that at 410 nm and normalized. Error bars represent SEM.

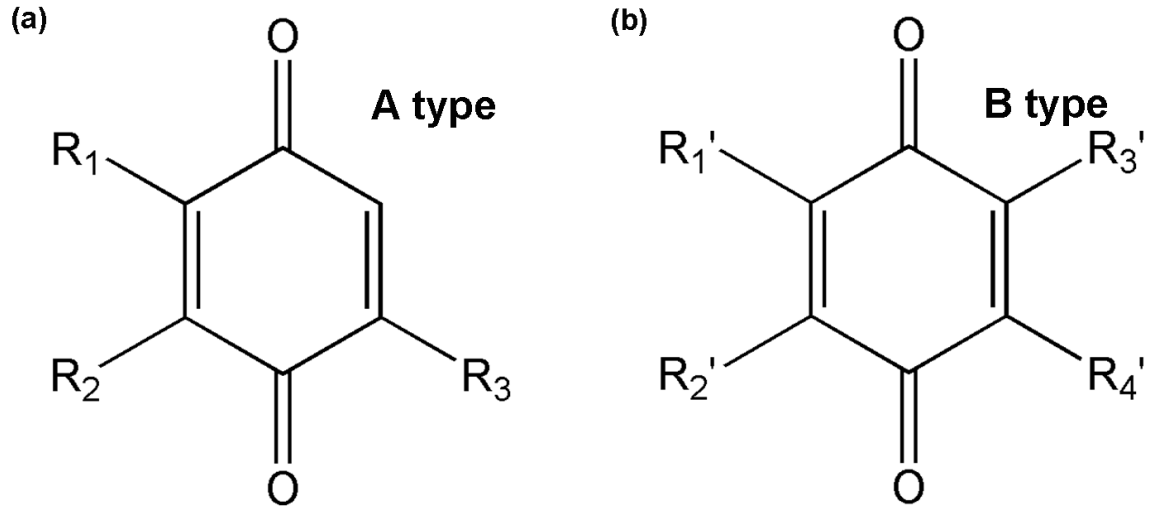

Figure S5. The chemical structures of A-type and B-type quinones. (a) The chemical structure of A-type quinone. R_1, R_2 and R_3 can be any group. (b) The chemical structure of B-type quinone. R_1', R_2', R_3' and R_4' can be any group except –H.

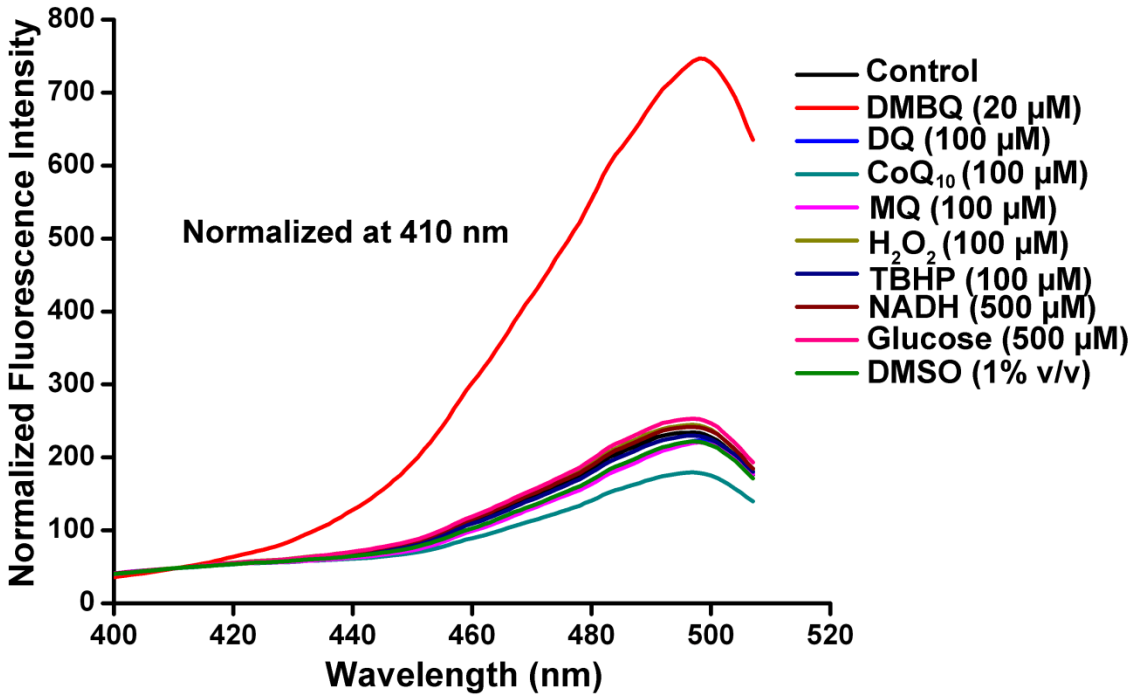

Figure S6. QSer exhibits excellent selectivity towards various kinds of small molecules. Fluorescence intensities of QSer under the induction of various small molecules were monitored with the emission at 515 nm and normalized at 410 nm. DMBQ, DQ and MQ represent 2, 3-dimethoxy-5-methyl-p-benzoquinone, duroquinone and menaquinone (vitamin K_2), respectively.

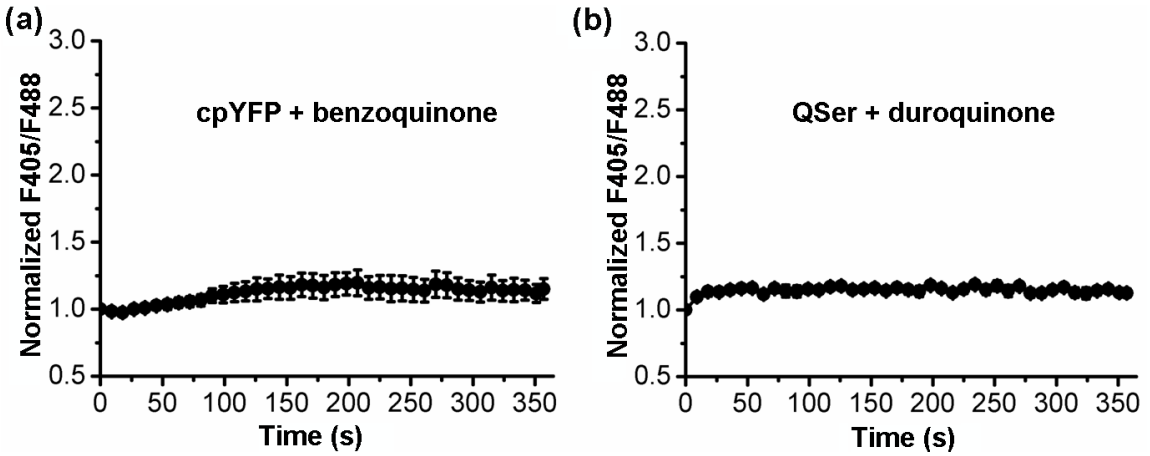

Figure S7. The control experiments for quinone imaging in HeLa cells. (a) The sole cpYFP protein cannot respond to benzoquinone induction in living HeLa cells. 20 μM 1, 4-benzoquinone was used in the assay. (b) The engineered quinone sensor, QSer, cannot respond to duroquinone induction in HeLa cells. 50 μM duroquinone was used in the assay. Error bars represent SEM.

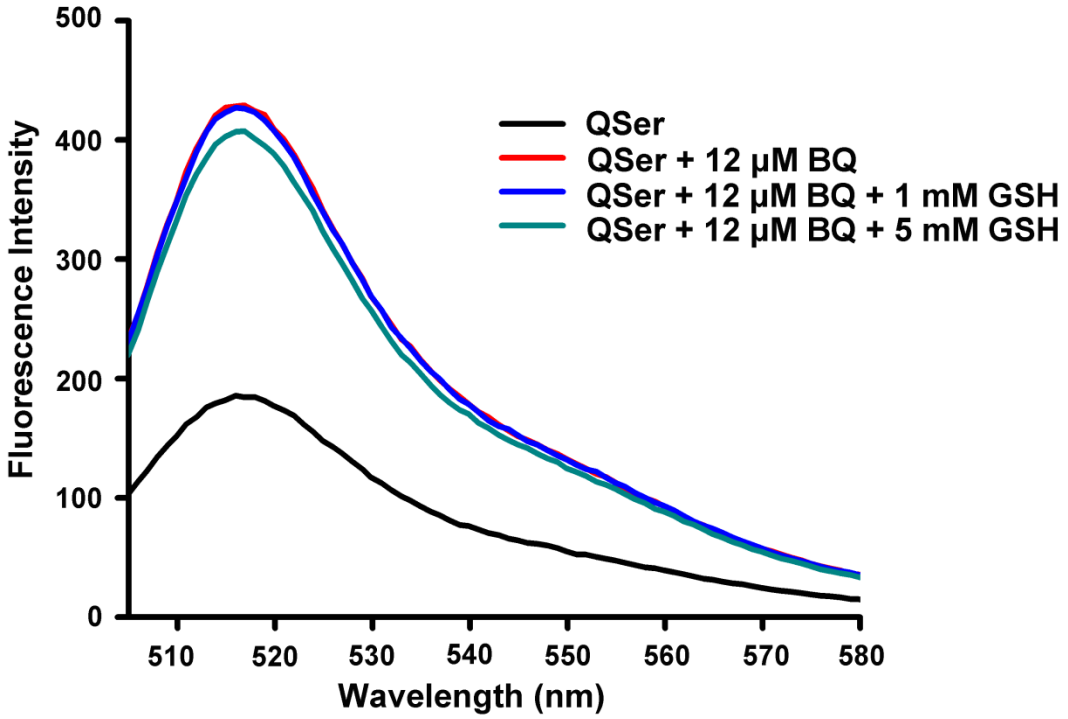

Figure S8. GSH is not able to reverse S-quinonization of QSer. 10 µM QSer (in 100 mM Tris.HCl, pH 7.4, 200 mM NaCl) was incubated with 12 µM BQ at room temperature for 5 min before the addition of 1 or 5 mM GSH. The reaction mixture was further incubated at room temperature for 30 min before data collection.