cysTMTRAQ – an integrative method for unbiased thiol-based redox proteomics

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Supplementary information is available in the online version of the paper.

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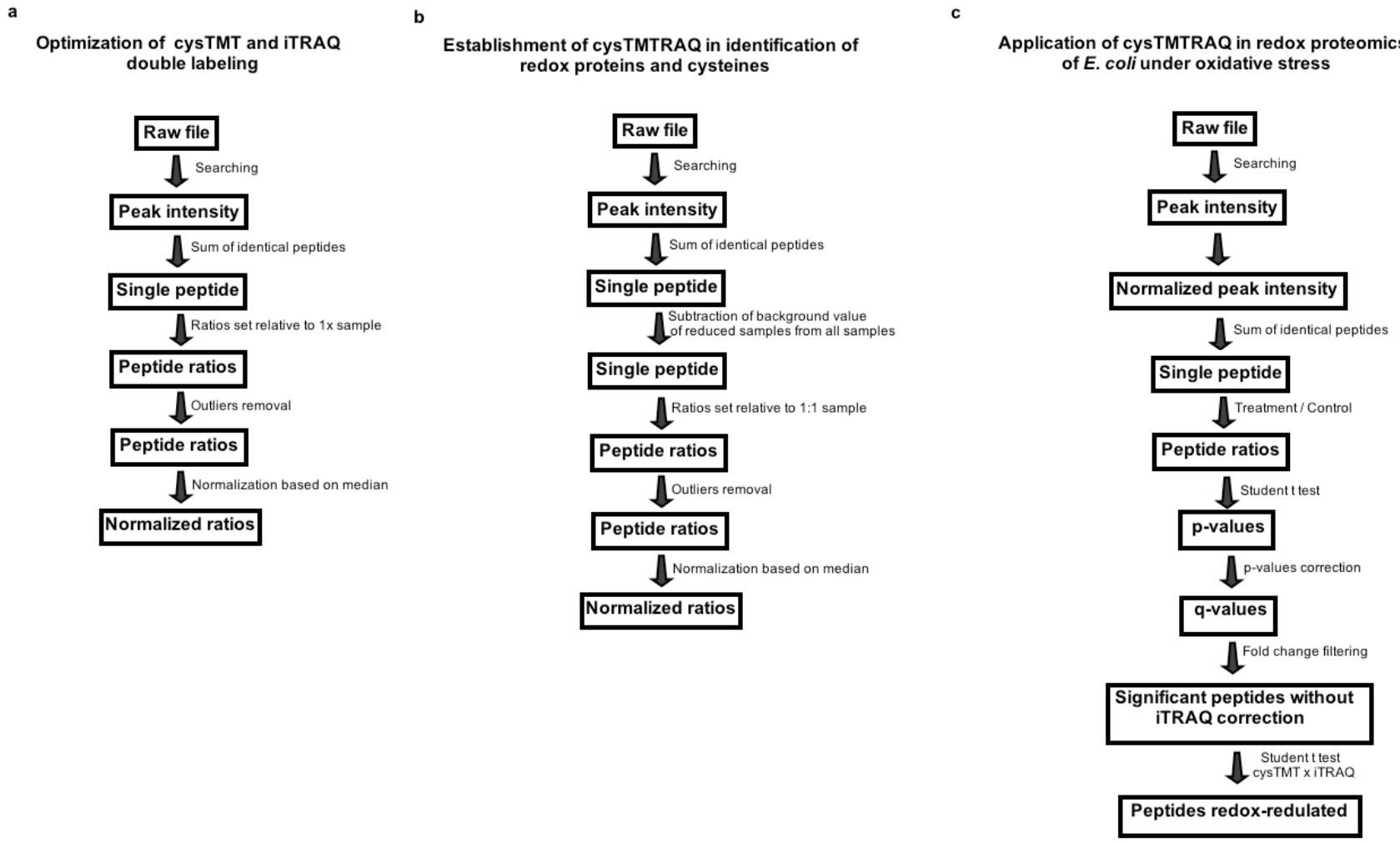

Figure S1. cysTMTRAQ data analysis workflow.

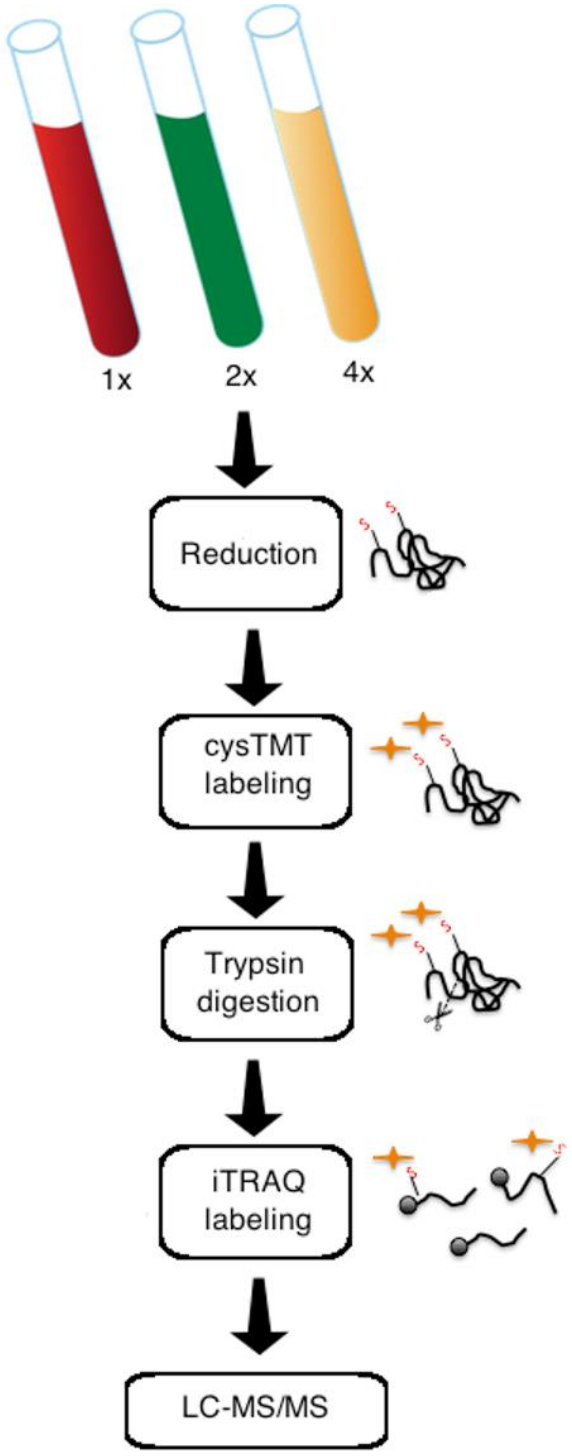

Figure S2. Experimental workflow of the cysTMTRAQ labeling procedure. A six protein mixture (bovine serum albumin, α-lactabumin, β- galactosidase, lysozyme, apotransferrin, and β-lactoglobulin) from the AB Sciex iTRAQ labeling kit was prepared at 10 μg, 20 μg, and 40 μg in order to examine 1:2:4 fold change ratios. Two independent replicates of each concentration were prepared. The scheme shows only one representative tag for cysTMT and for iTRAQ, though 6 cysTMT tags (*m/z* 126, 127, 128, 129, 130 and 131) and 6 iTRAQ tags (*m/z* 114, 115, 116, 117, 119 and 121) were multiplexed.

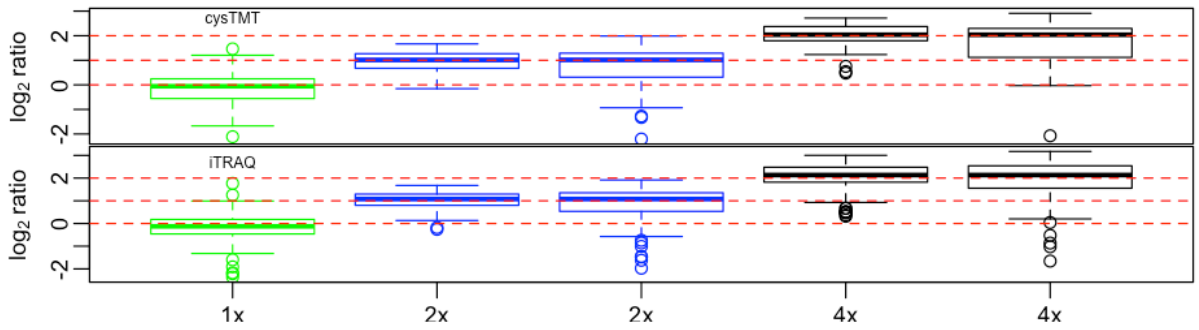

Figure S3. Performance of the cysTMTRAQ labeling at peptide level. Box plots showing the measured (box and whiskers) and expected values (dashed red lines – 0,1, and 2) of peptide ratios for cysTMT and iTRAQ quantification at 1:2:4 ratios in two independent replicates. Boxplot illustrates the median (stripe), the 25th to 75th percentile (interquartile range), 1.5 times the interquartile range (whiskers), and outliers (open circles).

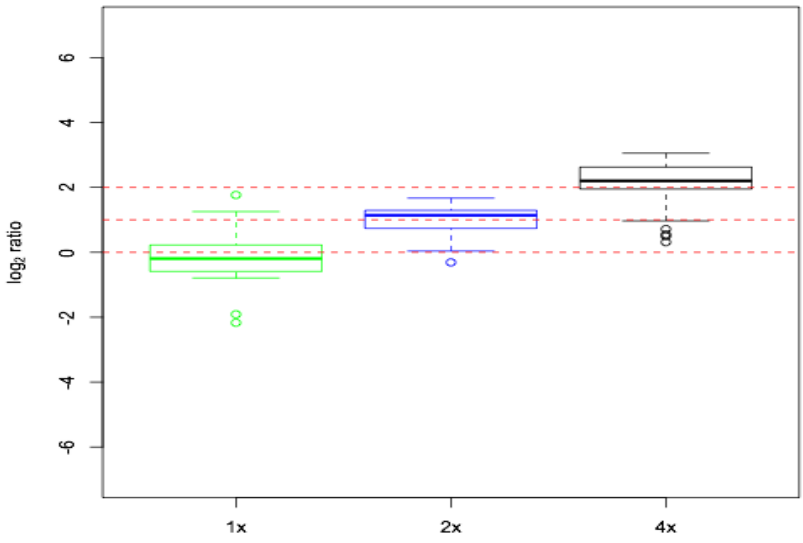

Figure S4. Performance of the cysTMTRAQ labeling at peptide level-iTRAQ quantification of cysteine-containing peptides. Box plots showing the measured (box and whiskers) and expected values (dashed red lines – 0,1, and 2) of averaged peptide ratios for cysTMT and iTRAQ quantification at 1:2:4 ratios. Boxplot illustrates the median (stripe), the 25th to 75th percentile (interquartile range), 1.5 times the interquartile range (whiskers), and outliers (open circles).

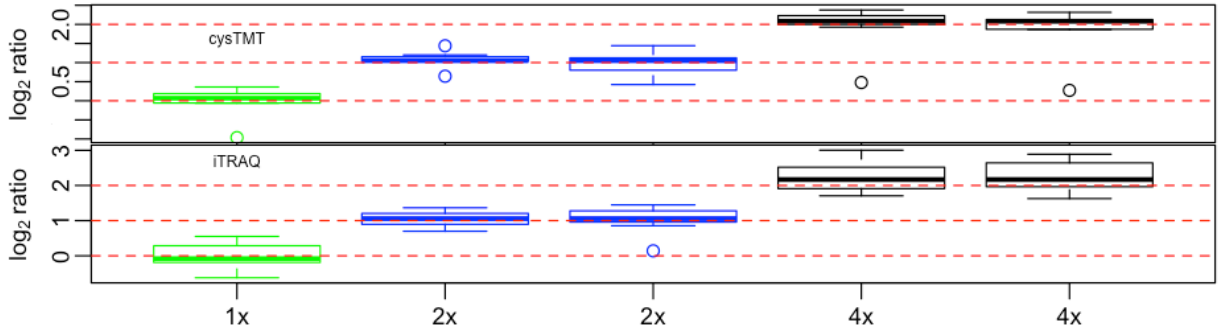

Figure S5. Performance of the cysTMTRAQ labeling at protein level. Box plots showing the measured (box and whiskers) and expected values (dashed red lines – 0,1, and 2) of protein ratios for cysTMT and iTRAQ quantification at 1:2:4 ratios in two independent replicates. Boxplot illustrates the median (stripe), the 25th to 75th percentile (interquartile range), 1.5 times the interquartile range (whiskers), and outliers (open circles).

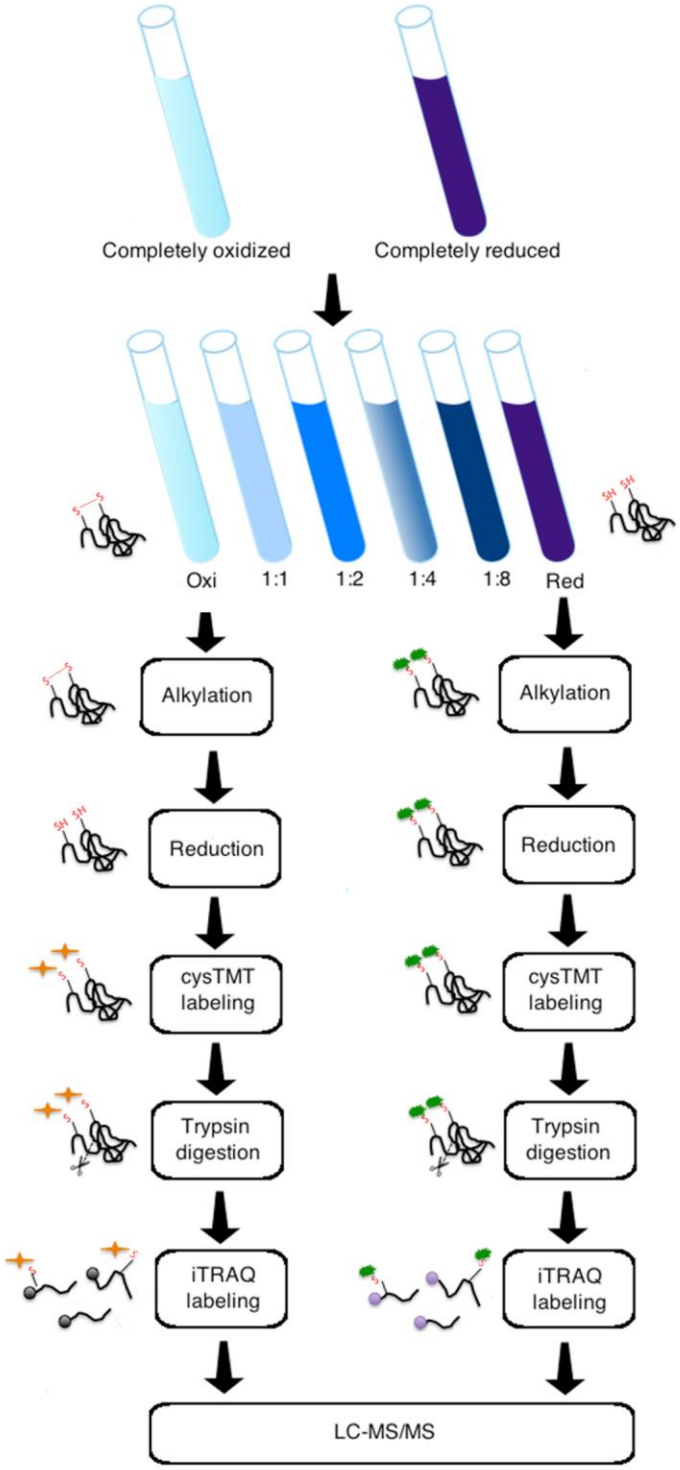

Figure S6. Experimental workflow of the identification of redox cysteines using cysTMTRAQ. The six protein mixture from the AB Sciex iTRAQ kit was completely oxidized and reduced, respectively. The six samples (each of 20 µg) represented completely oxidized, mixed with completely reduced at ratios of 1:1, 1:2, 1:4, and 1:8, and completely reduced sample. Two independent replicates of each sample were prepared. Scheme shows only one representative tag for cysTMT and for iTRAQ, though 6 cysTMT tags (*m/z* 126, 127, 128, 129, 130 and 131) and 6 iTRAQ tags (*m/z* 114, 115, 116, 117, 119 and 121) were multiplexed.

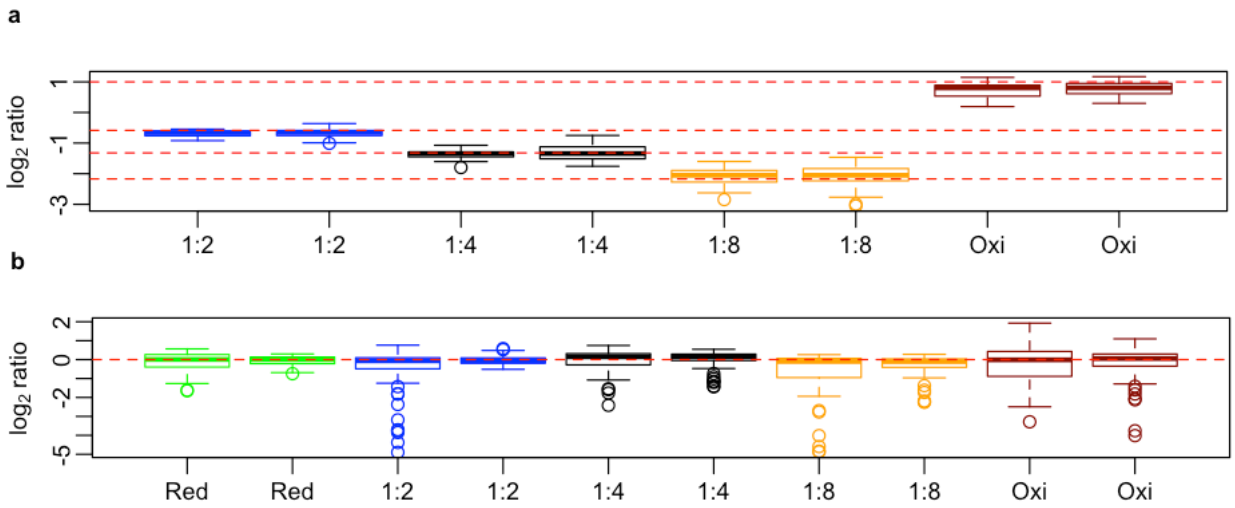

Figure S7. Identification and quantification of cysteine redox changes by cysTMT peptides and total peptide level changes by iTRAQ peptides. Box plot showing the measured (box and whiskers) and expected values (dashed red lines) of peptide ratios for (**a**) cysTMT (expected values: -0.58, -1.32, -2.17, and 1) and (**b**) iTRAQ (expected value: 0) quantification in samples ranged from oxidized to reduced at ratios of 1:2, 1:4, and 1:8 in two independent replicates. Boxplot illustrates the median (stripe), the 25th to 75th percentile (interquartile range), 1.5 times the interquartile range (whiskers), and outliers (open circles).

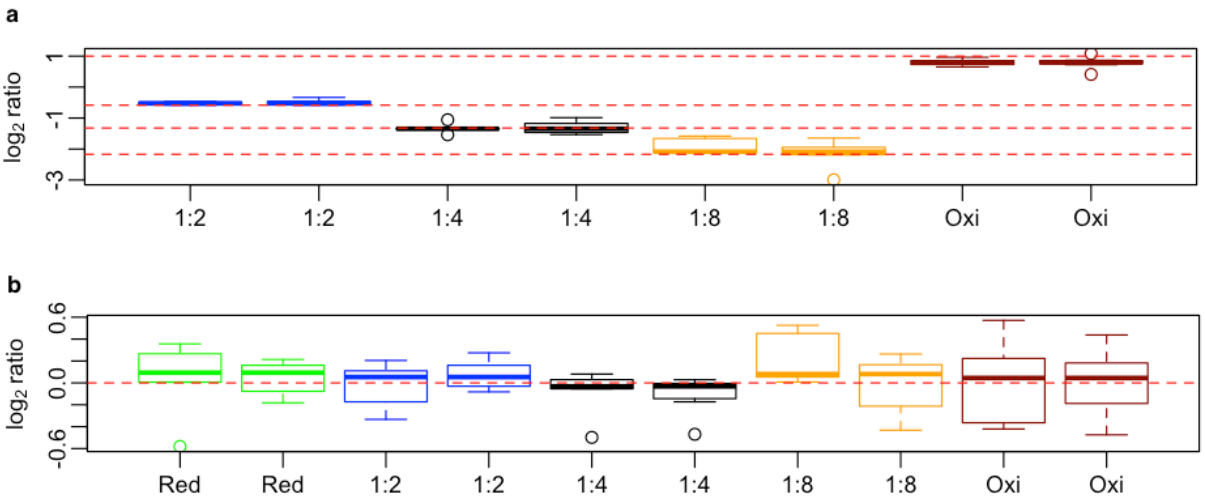

Figure S8. Identification and quantification of thiol redox proteins by cysTMT peptides and total protein level changes by iTRAQ peptides. Box plot showing the measured (box and whiskers) and expected values (dashed red lines) of peptide ratios for (**a**) cysTMT and (**b**) iTRAQ quantification in samples ranged from oxidized to reduced at ratios of 1:2, 1:4, and 1:8 in two independent replicates. . Boxplot illustrates the median (stripe), the 25th to 75th percentile (interquartile range), 1.5 times the interquartile range (whiskers), and outliers (open circles).

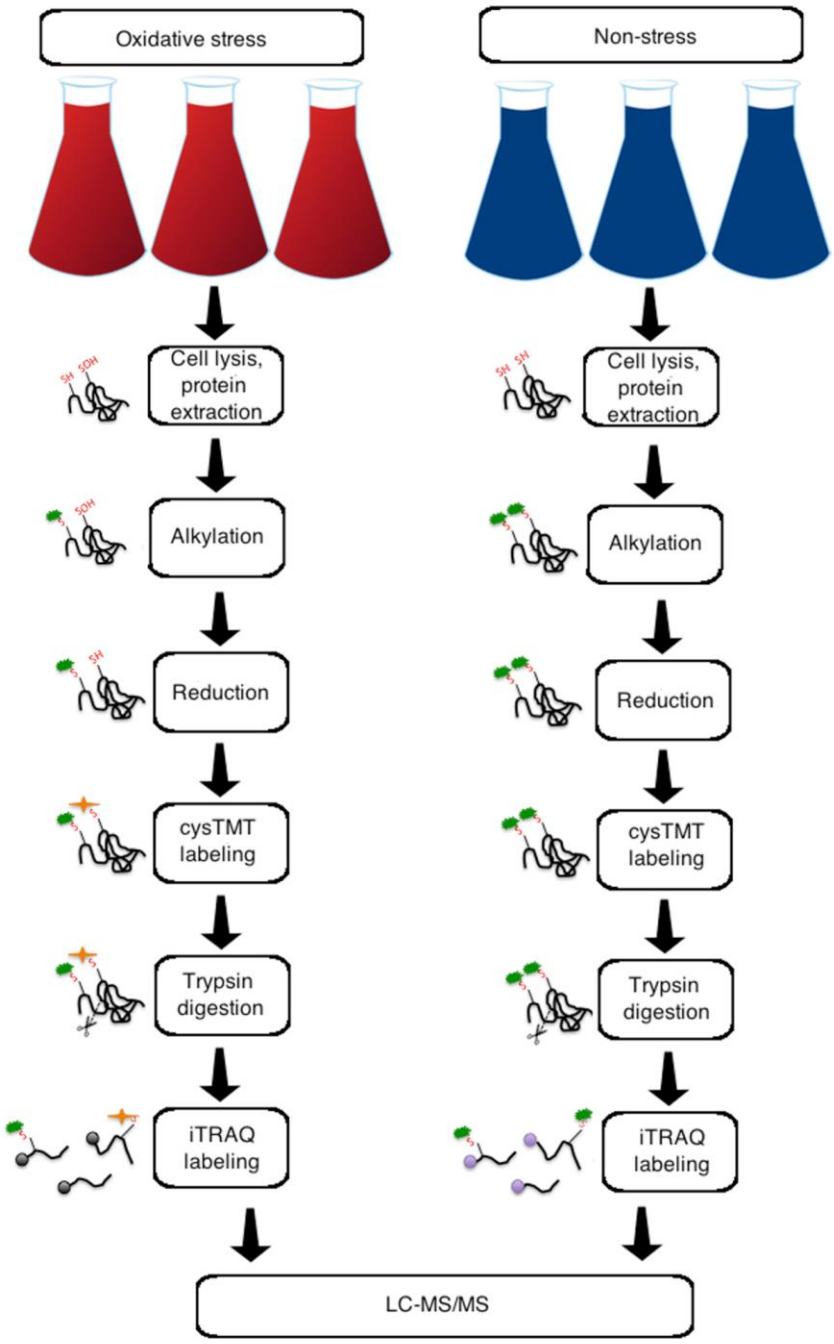

Figure S9. Experimental workflow of a biological application of cysTMTRAQ in identification of thiol redox proteins in *Escherichia coli* after oxidative stress treatment. *E. coli* culture was divided in two samples (treatment and control), and treated sample was submitted to NaOCl oxidative stress. Total protein was extracted from both control and treated sample for cysTMTRAQ double labeling in order to identify redox-regulated proteins in response to NaOCl. Three independent replicates of each sample were prepared. Scheme shows only one representative tag for iTRAQ and cysTMT, though 6 cysTMT tags (*m/z* 126, 127, 128, 129, 130 and 131) and 6 iTRAQ tags (*m/z* 113, 114, 116, 117, 119 and 121) were multiplexed.

Table S1. Redox regulated cysteine containing peptides before and after correction of protein level changes.

Peptide	Protein	q-value	FC (cysTMT)	FC (iTRAQ)	Corrected FC	Remarks
AALIDCLAPDR	Outer membrane protein A	0.048	1.3	-		Dsba substrate[1]
GMGESNPVTGNTCDNVK		0.028	1.5	-		
AALQISQSGQTCALLSK	Succinate dehydrogenase, flavoprotein	0.046	0.6	-		
RGFEYSDCWVDDAR	Glycerol-3-phosphate dehydrogenase	0.046	0.8	1		H_2O_2-mediated thiol modification[2]
ACEEAGISAEAIDPQQAR		0.049	0.8	1		
AIAVQAYQTLGCAGMAR	D-ala ligase N-terminal domain protein	0.049	0.7	1		
ALSVPCSDSK	Chaperonin GroL	0.046	1.2	1		Protein up-regulated in *dsba* muatnt[3]
AMASGVSACLATPFK	Beta-ketoacyl-acyl-carrier-protein synthase I	0.046	0.8	1		
APSLLQLSPDWTSNSCR	Colicin I receptor	0.049	2.4	1.5	1	
AVAEACGSQAVIVR	Phosphoenolpyruvate-protein phosphotransferase	0.046	0.6	-		S-nitrosylation[4]
AVQINSLSGFCLTK	Adenylosuccinate synthase	0.047	1.2	1		
CFEDNGLLYDLLEQNGR	Regulator of ribonuclease activity A	0.046	1.4	1.1	1.2	
ECITSMVSR	Protein-export chaperone SecB	0.046	1.2	1.1		
ELASGLSCPVGFK	DAHP synthase	0.046	1.2	1		Thioredoxin A substrate[5]
FCQFYQQDPLQR	Uncharacterized protein conserved in bacteria	0.046	0.7	-		
GCGALDWGMQSR	Fructose-bisphosphate aldolase	0.046	0.8	0.83	1	
GDLGLVIACLPYA	Ribosomal protein L35	0.049	1.3	-		S-nitrosylation[4]
GGGLCLGGK	Bifunctional aconitate hydratase	0.046	1.3	1.1		
GSLAYAEDPCGAEQGFSGR	Glucarate dehydratase	0.05	0.8	0.9		
IATTNSGELLSLTPVEHVCR	Hypothetical protein HMPREF9536	0.046	0.4	0.8		
IIGIDLGTTNSCVAIMDGTTPR	Chaperone protein DnaK	0.046	1.2	1.2		
LCDLWLAPK	Nitrate reductase, alpha subunit	0.038	1.6	1		
LLLECVVK	TraM protein	0.046	0.7	0.8	0.9	

Table S1. *Continued*

Peptide	Protein	q-value	FC (cysTMT)	FC (iTRAQ)	Corrected FC	Remarks
NPNDIELYMFAQANSEHCR	Phosphoribosylformylglycinamidine synthase	0.046	0.7	0.9		
NVGSFDNNDENVGSGMVGAPACGDVMK	FeS cluster assembly scaffold IscU	0.046	2.6	1.1	2.3	
QGIPLDAGSWQAICDAAR	Malate dehydrogenase	0.047	1.4	0.9		H2O2-mediated thiol modification[2]
RLFVVDAFCGANPDTR	Phosphoenolpyruvate carboxykinase (ATP)	0.046	0.6	0.9		S-nitrosylation[6]
SQGLDDYICK	CTP synthase	0.046	1.2	1		S-nitrosylation[7]
SQQVTDACK	Fumarate hydratase class I	0.049	1.3	1		
SVESEPCK	Hypothetical protein HMPREF9536	0.04	1.2	-		
VLGLGNCTIWQTSLAGK	Phenazine biosynthesis protein, PhzF family	0.05	0.7	1		
VSVGQEPACVK	Formate dehydrogenase N beta subunit	0.049	1.2	-		
VVVGQEPACVK	Formate dehydrogenase, beta subunit, partial	0.049	1.3	1.1		

References for Table S1

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