

## CELL BIOLOGY

# Evidence that the ProPerDP method is inadequate for protein persulfidation detection due to lack of specificity

Kaili Fan, Zhigang Chen, Huaiwei Liu\*

Protein persulfidation (protein-SSH) is a previously unidentified type of modification found in both eukaryotic and prokaryotic cells in recent years. Although a few persulfidated proteins have been identified, analyzing protein persulfidation from a proteomic level is still a big challenge. ProPerDP is a persulfidation detection method recently reported in *Science Advances*. The authors claimed that this method could specifically detect persulfidated proteins of cell lysate with minor false-positive hits; hence, it could be used for proteomic-level analysis of protein persulfidation. However, when using this method for *Escherichia coli* cell lysate analysis, we found that the percentage of false-positive hit was >90%. We performed a systematic study on this method and discovered that iodoacetyl-PEG2-biotin tag mislabeling is the reason causing this low specificity. We concluded that the ProPerDP method is completely inadequate for persulfidation analysis. The previous findings based on the ProPerDP method need to be reinvestigated.

## INTRODUCTION

Hydrogen sulfide (H<sub>2</sub>S) is proposed as the third gasotransmitter after nitric oxide (NO) and carbon monoxide (CO) (1). Over two decades, studies have demonstrated that H<sub>2</sub>S plays a myriad of physiological roles such as neuromodulation, vascular tone regulation, cytoprotection, oxygen sensing, inflammatory regulation, and cell growth control (2). One of the modes by which H<sub>2</sub>S functions is through modification of target proteins, known as sulfhydration or persulfidation. In this process, a free Cys residue (–SH) of a target protein is modified to –SSH (3). This modification happens both peri- and posttranslation, and polysulfides, the oxidized species of H<sub>2</sub>S, are proposed to be an important modifier (4). Persulfidation increases the reactivity of the cysteine residue by lowering its pK<sub>a</sub> (where K<sub>a</sub> is the acid dissociation constant) and/or changes conformation of the modified protein. It has been reported that many proteins critical for glucose metabolism, stress signaling, and autophagy in mammalian cells undergo persulfidation modification, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the transcriptional factor p65 (5, 6). Current studies indicated that persulfidation is not only restricted to the animal kingdom but also present in prokaryotes and plants (7, 8).

A few methods have been developed for the proteomic-level analysis of persulfidation, including biotin-switch assay, tag-switch assay, cysteinyl-labeling assay, and maleimide assay. To minimize nonspecific detections, there is still room for improvement of these methods (9). In 2016, Dóka *et al.* (10) published a paper in *Science Advances*, reporting that they had developed an easy, convenient, and reliable protein persulfide detection protocol (ProPerDP) with high specificity. The authors tested ProPerDP in both human and yeast cells and concluded that it specifically detected protein persulfide/polysulfide species with minor false-positive hits. They claimed that ProPerDP had advantages over other previously reported methods. On the basis of the data of ProPerDP analysis, Dóka *et al.* (10) concluded that the protein persulfidation level was positively correlated

with polysulfide concentration in the cell. Here, we show evidence that the specificity of ProPerDP is very low with >90% hits false positive; hence, this method is inadequate for persulfidation analysis.

## RESULTS

### Using ProPerDP to evaluate the protein persulfidation level of *Escherichia coli*

Previously, we discovered that deleting OxyR, the redox-sensitive transcriptional factor, in *E. coli* BL21 leads to a notable increase in its intracellular polysulfide level (11). On the basis of the conclusion of the report (10), we speculated that the protein persulfidation level should also be higher in *E. coli* BL21ΔoxyR than in *E. coli* BL21 wild type (wt). To test this, we used ProPerDP to analyze the protein persulfidation level in both strains. Persulfidated proteins were separated from the total proteins of cell lysate, and the percentage (milligram of persulfidated protein/milligram of total protein) was calculated. The experimental protocol was from (10) (workflow 1, Fig. 1). Our tests showed that 3.86% of the total protein in ΔoxyR had persulfidation. As the control, the percentage in wt was 9.71% (Fig. 2A). These results suggested that, opposite to our speculation, high polysulfide concentration did not lead to high protein persulfidation level in *E. coli*.

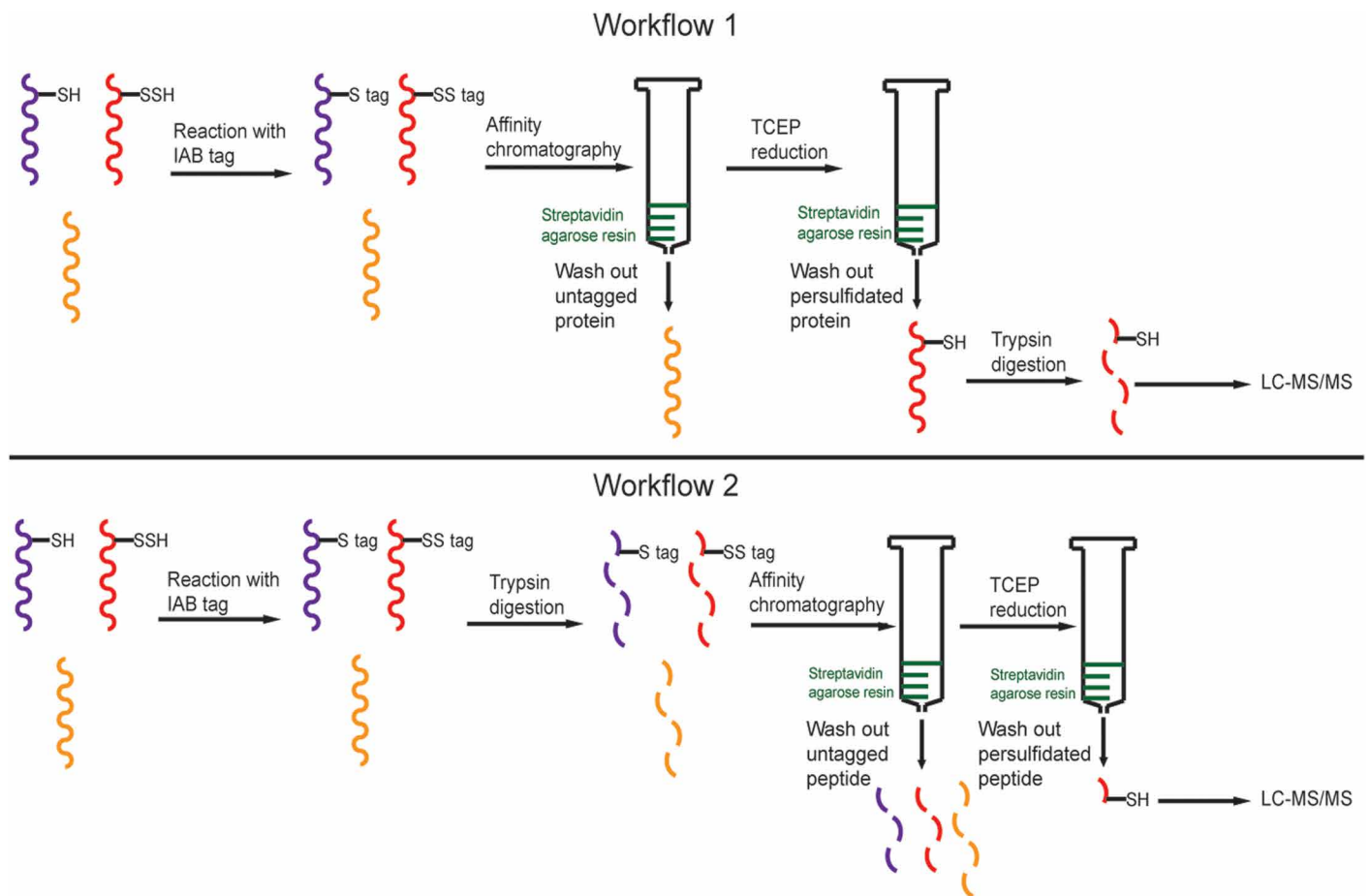
### Liquid chromatography–tandem mass spectrometry analysis of the persulfidated proteins

To further examine the species of persulfidated proteins, we used trypsin to digest them and then used liquid chromatography–tandem mass spectrometry (LC-MS/MS) to analyze the produced peptides (workflow 1, Fig. 1). Results showed that 66 and 118 protein species were identified from ΔoxyR and wt samples, respectively. Among them, 51 were overlaps presenting in both samples (Fig. 2B and data file S1). However, when we inspected the details of the LC-MS/MS data, we found that most peptides identified by LC-MS/MS contained no cysteine residue (Fig. 2C). This was quite unexpected because ProPerDP is based on the principle that only Cys-containing protein has the chance to be labeled by iodoacetyl-PEG2-biotin (IAB) tag, thereby being separated from untagged proteins. Theoretically,

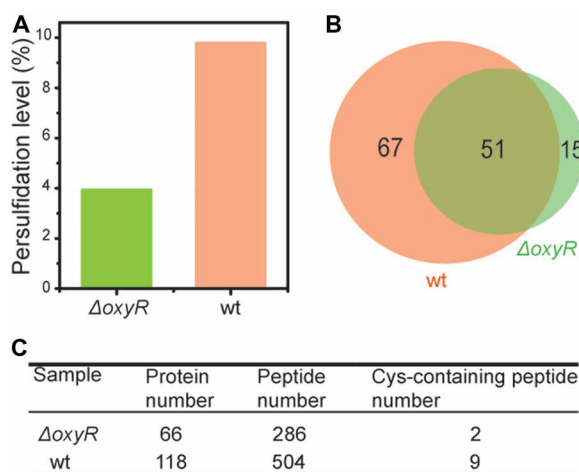
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State Key Laboratory of Microbial Technology, Shandong University, 72 Binhai Road, Qingdao 266237, People's Republic of China.

\*Corresponding author. Email: liuhuaiwei@sdu.edu.cn



**Fig. 1. A schematic presentation of the ProPerDP method.** Workflow 1 was from (10) and was used to separate the IAB-labeled proteins. Workflow 2 was used to separate IAB-labeled peptides.



**Fig. 2. Using the ProPerDP method to analyze protein samples from *E. coli* strains.** (A) Detected persulfidation levels of proteome samples from  $\Delta oxyR$  and wt strains. (B) Distribution of the identified protein species in these two proteome samples. (C) Numbers of proteins and peptides detected by LC-MS/MS.

when the final obtained proteins are digested by trypsin and the produced peptides are identified by LC-MS/MS, Cys-containing peptides should be detected.

#### Finding the reason resulting in low specificity of ProPerDP

Two possible reasons may lead to the phenomenon that less Cys-containing peptides were detected: Most MS signals corresponding to Cys-containing peptides were lost/misinterpreted during LC-MS/MS data processing, or other amino acid residues were also labeled by the IAB tag. In the case of the first reason, ProPerDP results are still reliable because it does not affect the persulfidated protein separation step and, hence, causes no false-positive hit. It only impairs the accuracy of the protein identification data. Considering in the LC-MS/MS data processing step, each protein ID (identification) is verified by more than one corresponding peptides, the first reason is not a big caveat for the ProPerDP method. However, in the case of the second reason, results obtained from this method are not reliable because IAB lacking specificity on Cys residue is a critical defect and causes false-positive hits.

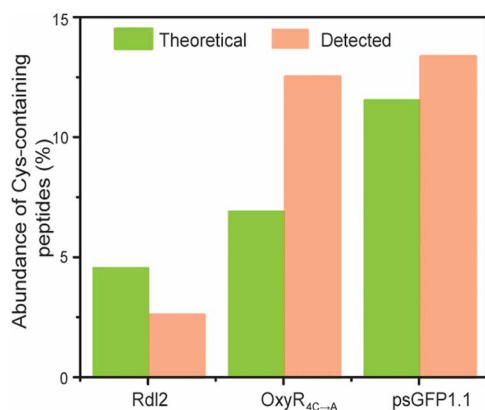
To find out which is the main reason causing the phenomenon in our results, we chose three proteins: Rdl2 (GenBank: KZV08173.1),

OxyR<sub>4C→A</sub>, and psGFP1.1 as standards, which contains one, two, and three Cys residues, respectively. These proteins were expressed in *E. coli* and purified with His tags. After trypsin digestion, they were subjected to LC-MS/MS analysis. Results showed that Cys-containing peptides were detected from each protein sample, and their relative abundance was close to theoretical levels (Fig. 3). These results indicated that the MS signals corresponding to Cys-containing peptides were correctly recognized during the LC-MS/MS data processing step. Thus, the phenomenon in our results is not ascribed to the first reason.

We then analyzed the same  $\Delta$ oxyR and wt samples using a changed protocol. After the total proteins were reacted with the IAB tag, they were digested by trypsin. The produced peptides were sequentially subjected to streptavidin agarose resin separation and LC-MS/MS analysis (workflow 2, Fig. 1). Theoretically, the final obtained peptides should be all Cys-containing ones. However, our results showed that, again, most identified peptides contained no Cys residue (Table 1 and data file S2). For confirmation, we conducted this experiment five times using samples from different *E. coli* strains (BL21 and MG1655), and the peptides lastly obtained were analyzed by LC-MS/MS. Results showed that no more than 10% of Cys-containing peptides were detected in any sample. We also analyzed the sample from *Saccharomyces cerevisiae* BY4742 and obtained similar result (Table 1 and data file S2). These experiments indicated that peptides containing no Cys were also labeled by IAB tags, which resulted in the high percentage of false-positive hit.

### Testing the characteristics of IAB labeling

IAB mislabeling may happen in two ways: It randomly labels proteins of the sample or it has certain selections including Cys-containing ones. To find out which is the actual way happening in our experiment, we performed independent ProPerDP analysis (workflow 1) on three parallel samples, the mid-log phase cells of *E. coli* MG1655 that have the same proteome background (Fig. 4). The hypothesis is that if IAB randomly labels proteins, then the distribution of “persulfidated protein” species identified from the three samples should



**Fig. 3. Using LC-MS/MS to analyze Rdl2, OxyR<sub>4C→A</sub>, and psGFP 1.1.** We used the Protein Digestor tool of <http://db.systemsbiology.net> to perform the in silico digestion, and results showed that after trypsin digestion, Rdl2 can release 4.5%, OxyR<sub>4C→A</sub> can release 6.9%, and psGFP 1.1 can release 11.5% of Cys-containing peptides (theoretical, green bars). When using LC-MS to analyze the digested proteins, we experimentally detected 2.6, 12.5, and 13.4% of Cys-containing peptides, respectively (detected, orange bars). The percentage was calculated from the signal intensity of the peptide precursor.

be divergent. Whereas, if IAB has certain selections, then the distribution should be convergent. To guarantee the data accuracy, we sent the final obtained persulfidated proteins to Applied Protein Technology Company (Shanghai) for the LC-MS/MS analysis. Results showed that a total of 519 different protein species were identified from the three samples, and among them, 23.31, 12.14, and 16.96% protein species were exclusively present in samples 1, 2, and 3, respectively. Only 22.54% protein species were present in all three samples, indicating the divergent distribution. In addition, a low percentage of Cys-containing peptides was observed again (Table 2 and data file S3). These results suggested that IAB tends to randomly label proteins in the samples.

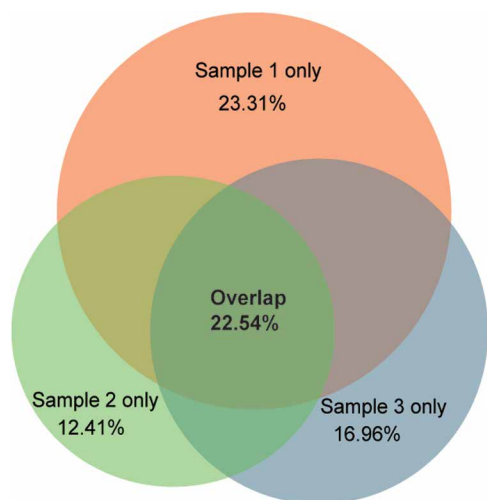
### DISCUSSION

ProPerDP uses the percentage index (milligram of persulfidated protein/milligram of total protein) to represent the persulfidation level. After a comprehensive investigation on the persulfidated protein species obtained from ProPerDP with LC-MS/MS, we concluded that the percentage index is not reliable. Using peptides as references, we found that peptides containing no Cys residue also can pass through the streptavidin agarose resin separation step, which results in a >90% false-positive hit. IAB mislabeling is the reason causing this phenomenon. It seems that IAB randomly reacts with proteins of the cell lysate other than targeting proteins that contain thiol (–SH) or hydrogen persulfide (–SSH) groups.

IAB labels proteins with its iodoacetamide (IAA) group. IAA has long been used as a thiol blocking reagent in the field of MS-based proteomics. It was proposed that the nucleophilicity of thiols exceeds that of all other amino acid residues; hence, electrophilic IAA intends to stably block thiols and even sulfenic acid groups through covalent modification (12). However, this reaction mechanism has been challenged since the last century because more and more unspecific side reactions were observed. Other amino acid residues bearing peripheral polar functional groups such as lysine, histidine, methionine, and tyrosine were found to react with IAA too (13). In addition, the N-terminal NH<sub>2</sub> and C-terminal carboxylic acid groups of proteins/peptides also can be alkylated by IAA (14). Unspecific side reactions are not a serious problem for MS-based protein identification because it can be overcome by updating algorithms of the data processing software. However, for IAA labeling-based protein separation, they become a critical defect that cannot be compensated in silico.

**Table 1. LC-MS/MS analysis of peptides detected from *E. coli* and yeast samples using workflow 2.**

Sample	Peptide number	Cys-containing peptide number	Percentage
$\Delta$ OxyR	155	0	0%
wt	130	2	1.54%
MG1655 <sup>a</sup>	51	2	3.92%
MG1655 <sup>b</sup>	58	1	1.72%
MG1655 <sup>c</sup>	44	4	9.09%
BY4742	172	8	4.65%



**Fig. 4.** The distribution of proteins detected from three parallel samples using workflow 1. Protein numbers are shown in Table 2.

In conclusion, the key design of ProPerDP is using IAB to label proteins containing thiol (—SH) or hydrogen persulfide (—SSH) groups and then using streptavidin agarose resin to screen them out from unlabeled proteins. However, unspecific side reactions of IAB lead to failure of this design. Dóka *et al.* (10) ignored the disturbance of IAB side reactions on ProPerDP, which failed to design experiments for testing to what extent the side reactions can affect the accuracy of the final data. Our evidences indicated that the false-positive hits of ProPerDP can be higher than 90%. Thus, this method is inadequate for protein persulfidation analysis.

## MATERIALS AND METHODS

### Reagents, strains, and culture conditions

IAB tag and streptavidin agarose resin were purchased from Thermo Fisher Scientific. TCEP (tris(2-carboxyethyl)phosphine) was purchased from Sigma-Aldrich. Nickel–nitrilotriacetic acid (Ni-NTA) agarose resin was purchased from Invitrogen. Other chemicals were purchased from Shanghai Sangon Biotech. *E. coli* strains were cultured in Lysogeny broth (LB) medium at 37°C unless indicated otherwise. *S. cerevisiae* BY4742 was cultured in synthetic complete medium containing 6.7 g liter<sup>-1</sup> yeast nitrogen bases without amino acids, 1.89 g liter<sup>-1</sup> of dropout mix with all the amino acids and supplements, and 20 g/liter of dextrose at 30°C.

### Protein expression in *E. coli* and purification

Genes encoding Rdl2, OxyR<sub>4A→C</sub>, and psGFP1.1 (polysulfides sensitive green fluorescent protein) were cloned into pET30a vector with an N-terminal His tag. Recombinant plasmids were transformed into *E. coli* BL21 (DE3) for expression. *E. coli* was grown in LB at 30°C with shaking until OD<sub>600</sub> (optical density at 600 nm) reached about 0.6. 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added, and the cells were further cultivated at 16°C for 20 hours. Cells were collected via centrifugation, washed twice with ice-cold lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl and 20 mM imidazole, pH 8.0), and broken through the high-pressure crusher SPCH-18 (Stansted). Cell debris was removed via centrifugation, and the supernatant was loaded onto the Ni-NTA agarose resin. The resin

**Table 2.** LC-MS/MS analysis of proteins obtained from three parallel samples using workflow 1.

Sample	Protein number	Peptide number	Cys-containing peptide number
1	361	1259	16
2	243	940	9
3	280	835	40

was washed with five column volumes of the lysis buffer, followed by elution of the His-tagged protein with an elution buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, and 250 mM imidazole, pH 8.0). The eluted protein was loaded onto PD-10 desalting column (GE Healthcare) for buffer exchange. The final obtained protein was dissolved in Hepes buffer (25 mM Hepes, 300 mM NaCl, and 10% glycerol, pH 8.0) containing 1 mM dithiothreitol. Purity of the protein was analyzed by SDS–polyacrylamide gel electrophoresis.

### The ProPerDP method for cell lysate analysis

The ProPerDP experimental protocol (workflow 1) was from (10). For workflow 2, we first digested IAB-labeled proteins (1 mg ml<sup>-1</sup>) with trypsin (0.5 mg ml<sup>-1</sup>) in 10 ml of Hepes buffer (50 mM, pH 8.0) at 37°C for 12 hours and then desalted the generated peptides with a C18 column and eluted the peptides in Hepes buffer (50 mM, pH 7.4). The obtained peptide samples were subjected to streptavidin separation and LC-MS/MS analysis the same as protein samples.

### LC-MS/MS analysis

For protein samples, 1 ml of purified proteins or persulfidated protein mixtures (1 mg ml<sup>-1</sup>) was mixed with 50 μl of IAA solution (1 M). After a reaction at room temperature for 30 min, the mixture was filtrated with a Microcon YM-3k filter. The filter was washed four times with 360 μl of 25 mM NH<sub>4</sub>HCO<sub>3</sub>. The filtrated protein sample was digested by trypsin (0.5 mg ml<sup>-1</sup>) in 10 ml of Hepes buffer (50 mM, pH 8.0) at 37°C for 12 hours. The generated peptides were desalted by using a C18 column, eluted in 70% acetonitrile and 0.1% trifluoroacetic acid, and freeze dried. The final obtained product was resuspended in high-performance LC–grade water. For peptide samples obtained from workflow 2, they were also blocked using IAA and freeze dried.

The Prominence nano-LC System (Shimadzu) equipped with a custom-made silica column (75 μm × 15 cm) packed with 3-μm ReproSil-Pur 120 C18-AQ was used. For the elution process, a 100-min gradient from 0 to 100% of solvent B (0.1% formic acid in 98% acetonitrile) at 300 nl min<sup>-1</sup> was used; solvent A was 0.1% formic acid in 2% acetonitrile. The eluent was ionized and electrosprayed via LTP (linear trap quadropole) Orbitrap Velos Pro CID (collision-induced dissociation) mass spectrometer (Thermo Scientific), which was run in data-dependent acquisition mode with Xcalibur 2.2.0 software (Thermo Scientific). Full-scan MS spectra [from 400 to 1800 mass/charge ratio (*m/z*)] were detected in the Orbitrap with a resolution of 60,000 at 400 *m/z*. The peptide false discovery rate (FDR) was set to 0.01.

For confirmation, the persulfidated protein samples obtained from ProPerDP were also sent to Applied Protein Technology



Company (Shanghai) for the proteomic analysis. The materials and methods used by the company are described below.

Each fraction was injected for nano-LC-MS/MS analysis. The peptide mixture was loaded onto a reverse phase trap column (Thermo Scientific Acclaim PepMap 100, 100  $\mu\text{m}^2$  cm, nanoViper C18) connected to the C18 reversed-phase analytical column (Thermo Scientific Easy Column, 10-cm length, 75- $\mu\text{m}$  inner diameter, 3- $\mu\text{m}$  resin) in buffer A (0.1% formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% formic acid) at a flow rate of 300 nl/min controlled by IntelliFlow technology. The linear gradient was determined by the project proposal: 1 hour gradient, 0 to 35% buffer B for 50 min, 35 to 100% buffer B for 5 min, hold in 100% buffer B for 5 min. LC-MS/MS analysis was performed on a Q Exactive mass spectrometer (Thermo Scientific) that was coupled to Easy-nLC (Proxeon Biosystems, now Thermo Fisher Scientific) for 60 min. The mass spectrometer was operated in positive ion mode. MS data were acquired using a data-dependent top 10 method dynamically choosing the most abundant precursor ions from the survey scan (300 to 1800  $m/z$ ) for HCD (higher energy collisional dissociation) fragmentation. Automatic gain control target was set to  $3 \times 10^6$ , and maximum inject time to 10 ms. Dynamic exclusion duration was 40.0 s. Survey scans were acquired at a resolution of 70,000 at  $m/z$  200, resolution for HCD spectra was set to 17,500 at  $m/z$  200, and isolation width was 2  $m/z$ . Normalized collision energy was 30 eV, and the underfill ratio, which specifies the minimum percentage of the target value likely to be reached at maximum fill time, was defined as 0.1%. The instrument was run with peptide recognition mode enabled. Peptide mass tolerance was set to 20 parts per million, fragment mass tolerance was set to 0.1 Da, protein FDR was set to 0.01, peptide FDR was set to 0.01, and iBAQ (intensity based absolute quantification) was set to true.

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/6/33/eabb6477/DC1>

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## REFERENCES AND NOTES

- B. D. Paul, S. H. Snyder, H<sub>2</sub>S: a novel Gasotransmitter that signals by sulfhydration. *Trends Biochem. Sci.* **40**, 687–700 (2015).
- H. Kimura, Hydrogen sulfide and polysulfide signaling. *Antioxid. Redox Signal.* **27**, 619–621 (2017).
- N. Sen, Functional and molecular insights of hydrogen sulfide signaling and protein sulfhydration. *J. Mol. Biol.* **429**, 543–561 (2017).
- T. Akaike, T. Ida, F. Y. Wei, M. Nishida, Y. Kumagai, M. M. Alam, H. Ihara, T. Sawa, T. Matsunaga, S. Kasamatsu, A. Nishimura, M. Morita, K. Tomizawa, A. Nishimura, S. Watanabe, K. Inaba, H. Shima, N. Tanuma, M. Jung, S. Fujii, Y. Watanabe, M. Ohmuraya, P. Nagy, M. Feelisch, J. M. Fukuto, H. Motohashi, Cysteinylyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat. Commun.* **8**, 1177 (2017).
- S. Mir, T. Sen, N. Sen, Cytokine-induced GAPDH sulfhydration affects PSD95 degradation and memory. *Mol. Cell* **56**, 786–795 (2014).
- H. F. Langer, T. Chavakis, Leukocyte-endothelial interactions in inflammation. *J. Cell. Mol. Med.* **13**, 1211–1220 (2009).
- Á. Aroca, A. Serna, C. Gotor, L. C. Romero, S-Sulfhydration: a new post-translational modification in plant systems. *Plant Physiol.* **168**, 334–342 (2015).
- D. P. Giedroc, A new player in bacterial sulfide-inducible transcriptional regulation. *Mol. Microbiol.* **105**, 347–352 (2017).
- D. Zhang, J. Du, C. Tang, Y. Huang, H. Jin, H<sub>2</sub>S-induced sulfhydration: biological function and detection methodology. *Front. Pharmacol.* **8**, 608 (2017).
- É. Dóka, I. Pader, A. Biró, K. Johansson, Q. Cheng, K. Ballagó, J. R. Prigge, D. Pastor-Flores, T. P. Dick, E. E. Schmidt, E. S. Arnér, P. Nagy, A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems. *Sci. Adv.* **2**, e1500968 (2016).
- N. Hou, Z. Yan, K. Fan, H. Li, R. Zhao, Y. Xia, L. Xun, H. Liu, OxyR senses sulfane sulfur and activates the genes for its removal in *Escherichia coli*. *Redox Biol.* **26**, 101293 (2019).
- J. A. Reisz, E. Bechtold, S. B. King, L. B. Poole, C. M. Furdui, Thiol-blocking electrophiles interfere with labeling and detection of protein sulfenic acids. *FEBS J.* **280**, 6150–6161 (2013).
- E. S. Boja, H. M. Fales, Overalkylation of a protein digest with iodoacetamide. *Anal. Chem.* **73**, 3576–3582 (2001).
- Z. Yang, A. B. Attygalle, LC/MS characterization of undesired products formed during iodoacetamide derivatization of sulphydryl groups of peptides. *J. Mass Spectrom.* **42**, 233–243 (2007).

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**Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

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