Cysteine Thiol Sulfinic Acid in Plant Stress Signaling

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ABSTRACT

Cysteine thiols are susceptible to various oxidative post-translational modifications (PTMs) due to their high chemical reactivity. Thiol-based PTMs play a crucial role in regulating protein functions and are key contributors to cellular redox signaling. Although reversible thiol-based PTMs, such as disulfide bond formation, S-nitrosylation, and S-glutathionylation, have been extensively studied for their roles in redox regulation, thiol sulfinic acid ($–SO_2H$) modification is often perceived as irreversible and of marginal significance in redox signaling. Here, we revisit this narrow perspective and shed light on the redox regulatory roles of $-SO_2H$ in plant stress signaling. We provide an overview of protein sulfinylation in plants, delving into the roles of hydrogen peroxide-mediated and plant cysteine oxidase-catalyzed formation of $-SO_2H$, highlighting the involvement of $–SO_2H$ in specific regulatory signaling pathways. Additionally, we compile the existing knowledge on the $–SO_2H$ reducing enzyme, sulfiredoxin, offering insights into its molecular mechanisms and biological relevance. We further summarize current proteomic techniques for detecting $–SO_2H$ and furnish a list of experimentally validated cysteine $–SO_2H$ sites across various species, discussing their functional consequences. This review aims to spark new insights and discussions that lead to further investigations into the functional significance of protein $–SO_2H$-based redox signaling in plants.

KEYWORDs

Thiols, redox regulation, post-translational modifications, sulfinic acid, peroxiredoxin, sulfiredoxin

Introduction

Protein cysteine thiols (-SH) are pivotal in redox biology, which is intricately intertwined with life and essential to a multitude of biological processes. Due to their chemical versatility, thiols are highly susceptible to oxidative post-translational modifications (PTMs), which can regulate protein functions. Cysteine, despite being one of the least abundant essential amino acids in organisms, is frequently conserved as functional sites involved in protein structure, catalysis, regulation, and binding (Marino and Gladyshev, 2010; Bak et al., 2019). Cysteine thiol-based PTMs distinguish from enzyme-driven PTMs such as phosphorylation, ubiquitination, methylation, and acetylation, which have well-defined regulatory 'on' and 'off' mechanisms (Ueda and Seki, 2020). They also stand out from non-enzymatically triggered PTMs like protein carbonylation, which occurs spontaneously and irreversibly across multiple amino acid residues and is commonly considered as an indicator of protein oxidation (Suzuki et al., 2010). Thiol-based PTMs can occur either through enzymatic processes involving enzymes such as disulfide isomerases, thioredoxins (Trx), peroxidases, plant cysteine oxidases (PCO), and sulfurtransferases, or non-enzymatically through reactive molecular species including reactive oxygen, nitrogen, and sulfur species (ROS, RNS, and RSS). This leads to highly dynamic and complex networks of thiol-based PTMs that are essential for cellular redox regulation.

Thiols are known to react with hydrogen peroxide (H_2O_2), the most stable ROS, leading to the formation of sulfenic acid (–SOH) intermediates (Figure 1). These intermediates can subsequently react with various RSS, including glutathione (GSH), protein-free thiols, and hydrogen sulfide (H_2S), to form disulfide bond-based PTMs including S-glutathione adducts (–S–SG), inter- or intra- disulfide bonds (–S–S–), and persulfides (–S–SH), respectively. These PTMs are predominantly reversible and have potential to regulate protein functions, thus contributing to cellular redox homeostasis. Under particular conditions, –SOH can undergo further oxidation by H_2O_2, leading to higher oxidation states of the sulfur atom and the formation of sulfinic acid ($–SO_2H$) and sulfonic acid ($–SO_3H$), which are typically considered as overoxidation. While $–SO_3H$ is irreversible, the $–SO_2H$ modification on specific set of proteins can be reversed on by sulfiredoxin (Srx) through an ATP-dependent reaction (Biteau et al., 2003, Iglesias-Baena et al., 2011). Notable examples of such reversibility include 2-cysteine peroxiredoxins (2-CysPrx) found across various species (Biteau et al., 2003, Chang et al., 2004, Liu et al., 2006, Lowther and Haynes, 2011), as well as recently identified human proteins such as protein tyrosine phosphatase nonreceptor type 12 and Parkinson protein 7 (PARK7 or DJ-1) (Akter et al., 2018). Additionally, the formation of $–SO_2H$ can occur

enzymatically through the action of protein cysteine oxidase (PCO) on N-terminal cysteine thiols in specific cases (Weits et al., 2014).

Generally, while the regulatory roles of reversible post-translational modifications (PTMs), especially those involving the formation of disulfide bonds, are well appreciated and extensively reviewed, protein $–SO_2H$ formation is often considered to lead the protein instability and degradation. Consequently, it is frequently perceived as a dead-end for protein function (Alcock et al 2018; Jacob et al 2004; Knoke and Leichert, 2023; Lee et al 2013), leading to this aspect being often disregarded. However, several compelling examples demonstrate that protein $–SO_2H$ modification actively participates in redox regulation. For instance, protein $–SO_2H$ can confer chaperone activity on 2-CysPrxs in human, yeast, and plant (Jang et al., 2004, König et al., 2013, Moon et al., 2005). Additionally, the $–SO_2H$ modification plays a crucial role in redirecting the localization of the Parkinson's disease-associated protein DJ-1 to the mitochondria, thereby enabling its neuroprotective function (Canet-Avilés et al., 2004).

In this review, we present an overview of the current knowledge on protein cysteine thiol $–SO_2H$ modification in plants. It covers the mechanisms of $–SO_2H$ formation and reduction, outlines methodologies for $–SO_2H$ detection, and presents a summary of known cysteine $–SO_2H$ sites in various species. We hope this review will be instrumental in guiding future research into $–SO_2H$-based redox signaling within the plant kingdom.

Protein sulfinic acid formation in plant cells

H_2O_2-induced $–SO_2H$ formation

The principal pathway responsible for the formation of $–SO_2H$ in plant cells involves the reaction between protein thiols and H_2O_2. Given that H_2O_2 is ubiquitously produced in various plant cell compartments (Figure 1), this reaction is prevalent and widespread. The reactivity of thiols primarily depends on their pKa, which typically stands at 8.6, although it can vary depending on the specific subcellular microenvironment (Chung et al., 2013). Nucleophilic thiols exhibit high reactivity and readily undergo deprotonation to form thiolate anions under physiological pH conditions (pH 7.4). Consequently, they readily engage in reactions with proximal H_2O_2, resulting in their oxidation and the formation of diverse PTMs. The microenvironment of protein cysteine is influenced by various factors, including pH (Tsai and Schmidt, 2021), H_2O_2 concentrations (Foyer and Noctor, 2016, Mittler et al., 2022, Niemeyer

et al., 2021), and redox equivalents such as GSH redox potential (Schwarzländer et al., 2008, Smirnoff and Arnaud, 2019) within the subcellular compartments of plant cells (Figure 1). These factors provide a degree of specificity to the oxidation of cysteine thiols. However, accurately assessing the diverse reactivity of protein thiols and their potential to form $–SO_2H$ across various subcellular compartments remains challenging, especially in the light of environmental changes that can disrupt cellular redox homeostasis in plants.

The ATP-dependent reduction of $–SO_2H$ by Srx has been demonstrated for chloroplast 2-CysPrxs and mitochondrial peroxiredoxin IIF (PRXIIF) in plants (Iglesias-Baena et al., 2011) (Figure 1), a topic that will be further discussed in this review. However, a recent study in mammals, using advanced chemoproteomics, has uncovered novel Srx substrates, extending our understanding beyond the previously recognized 2-CysPrx isoforms (Akter et al., 2018). This discovery raises an intriguing question: a*re there additional Srx substrates in plants?* The search for other possible substrates of plant Srx remains uncharted territory. Furthermore, due to the historical disregard of a regulatory role of $–SO_2H$, alternative biological pathways for the reduction of $–SO_2H$ have been largely overlooked (Akter et al., 2018, Depuydt et al., 2009, Gupta and Carroll, 2014). Currently, the mechanisms involved in reducing $–SO_2H$ in subcellular compartments other than plastids and mitochondria, such as cytosol and nucleus, remains unclear (Figure 1).

PCO catalyzed -SO_2H formation

The formation of $–SO_2H$ on protein N-terminal cysteines is enzymatically catalyzed by specific iron-dependent thiol dioxygenases, known as N-terminal cysteine oxidases, which use oxygen (O_2) as a co-substrate. These oxidases were initially identified in plants and referred to PCOs (Weits et al., 2014; White et al., 2017), and have subsequently been discovered in animals (Masson et al., 2019). The discovery of N-terminal cysteine oxidases represented a significant advance in the field of O_2 sensing signaling. Notably, this is one of the rare instances where our understanding of plant processes has outpaced that of animals, highlighting the importance of plant-focused research. Arabidopsis features five PCOs, designated as PCO1 through PCO5, which can be categorized into A and B-types. A-type PCOs are conserved across all plant species and their mRNAs are generally unaffected at the mRNA level by changes to variations in O_2 levels. In contrast, and B-type PCOs emerged in spermatophytes and acquired transcriptional regulation in response to hypoxic conditions. Among the PCOs, A-type PCO3,

PCO4, and PCO5 are localized in both the nucleus and cytosol, while B-type PCO1 and PCO2 are predominantly restricted to the nucleus (Weits et al., 2023, Figure 1).

Protein sulfinic acid-induced signaling: insights from 2-CysPrx and N-end rule substrates

2-CysPrx and redox signaling

The role of plant 2-CysPrxs in H_2O_2-mediated signaling has been extensively studied (Kim et al., 2009, König et al., 2002, König et al., 2013, Liebthal et al., 2021, Liebthal et al., 2020, Vaseghi et al., 2018) and reviewed (Dietz, 2011, Dietz et al., 2006, Vogelsang and Dietz, 2022). These studies have highlighted the structural flexibility of 2-CysPrxs and their ability to undergo conformational changes depending on their redox states, which are modulated by H_2O_2-mediated thiol PTMs, thereby expanding their regulatory functions (Figure 2A). In Arabidopsis, there are two isoforms of 2-CysPrxs, 2-CysPrx A and 2-CysPrx B, both locate in chloroplast. The reduced form of 2-CysPrx functions as a homodimer and can further assemble into a decamer comprising five homodimers, actively serving as peroxidase to scavenge H_2O_2 within the cells (Figure 2A). Upon H_2O_2-mediated oxidation, the reduced 2-CysPrxs form disulfide bond-linked dimers, leading to the loss of their peroxidase activity (Figure 2A). Notably, the oxidized dimers of 2-CysPrxs can indirectly oxidize the thiols of specific target proteins through disulfide exchange by oxidizing Trx. This mechanism has been demonstrated in the case of fructose-1,6-bisphosphatase and NADPH-dependent malate dehydrogenase, which can be inactivated by oxidized 2-CysPrx dimers in a Trx-dependent process. Such inactivation disrupts assimilation pathways coupled with the photosynthetic electron transport chain, particularly during the light-dark transition (Vaseghi et al., 2018). These findings align with evidence showing that redox-regulated proteins in chloroplast undergo oxidation through a Trx-like 2/2-CysPrxs redox cascade in darkness (Yoshida et al., 2018). Furthermore, the involvement of the oxidized dimer form of 2-CysPrx in the oxidative activation of glucose-6-phosphate dehydrogenase further highlights the significant role of its Trx oxidase function (Yoshida et al., 2019).

The formation of $–SO_2H$ in 2-CysPrx is triggered by the process of overoxidation, which is mediated by an excess of H_2O_2. This overoxidation event leads to the loss of peroxidase activity and the formation of an overoxidized decamer of 2-CysPrx (Figure 2A). The overoxidized decamer of 2-CysPrx can further assemble into high molecular weight (HMW) aggregates, actively functioning as chaperones (Kim et al., 2009) (Figure 2A). The ability to form oligomers

is a distinctive feature of 2-CysPrxs, whereas other plant Prxs predominantly exist as monomers or dimers (Liebthal et al., 2021). Although the chaperone function of overoxidized 2-CysPrx is well-recognized, its precise involvement in redox signaling and plant physiological processes remains largely unexplored. A recent study has shed light on this matter by revealing a conformation-specific interactome for 2-CysPrx (Liebthal et al., 2020), providing valuable insights into the specific physiological functions associated with the five distinct redox-dependent conformations of chloroplast 2-CysPrx (Figure 2A).

N-end rule substrates

In Arabidopsis, the N-terminal cysteines known to be subject to the O_2-mediatd N-degron pathway are currently limited to a small group of transcription factors. These transcription factors are stabilized under hypoxic conditions, thereby initiating particular downstream gene expression. These transcription factors include VII Ethylene Response Factors (ERF-VIIs) such as Hypoxia Responsive 2 (HRE2) and Related to AP2 (RAP2.2) (Gibbs et al., 2011; Licausi et al., 2011), Vernalisation 2 (VRN2) (Gibbs et al., 2018), and Little Zipper Protein 2 (ZPR2) (Weits et al., 2019) (Figure 2B). These N-degron pathway substrates are typically degraded under normoxic conditions. The degradation process involves the removal of the N-terminal methionine by the METHIONINE AMINOPEPTIDASE (MetAP) (Bradshaw et al., 1998; Meinnel et al., 2006), after which the neo-N-terminal cysteine residue is oxidized by PCOs in an O_2-dependent manner, creating a secondary destabilizing residue (Weits et al., 2014) (Figure 2B). Arginyl-tRNA protein transferases (ATEs) recognize these N-terminal cysteine–SO_2H and add an arginine residue to the N-terminus of the protein, creating a primary destabilizing residue (Graciet and Wellmer, 2010), which is recognized by a specific E3 ligase, PROTEOLYSIS6 (PRT6) (Figure 2B). This E3 ligase then ubiquitinates the target protein, leading it to the 26S proteasome for proteasomal degradation (Graciet and Wellmer, 2010; Tasaki et al., 2012). While RAP2.2 has been confirmed as a substrate of Arabidopsis PCO1 and PCO2 *in planta* (Weits et al., 2014), the evidence for VRN2 and ZPR2 as PCO substrates is only based on *in vitro* biochemical analysis and awaits confirmation *in planta* (Taylor-Kearney et al., 2022, Figure 2B).

Sulfiredoxin-mediated turnover of sulfinic acid

Srx, a protein of relatively small size with a conserved active cysteine residue, is found exclusively in cyanobacteria and eukaryotes (Biteau et al., 2003; Boileau et al., 2011; Liu et al., 2006). Its occurrence in these organisms suggests a potential connection between its evolution and the emergence of oxygenic photosynthesis in cyanobacteria. Srx and 2-CysPrx are believed to have coevolved through an ancient gene transfer from cyanobacteria to the ancestors of eukaryotes, and later to plants via chloroplastic endosymbiosis (Boileau et al., 2011). The absence of Srx in most prokaryotes is likely due to its essential role in reducing overoxidized eukaryotic 2-CysPrxs, which are significantly more sensitive to overoxidation than those found in prokaryotes. Structural analyses have shown that this increased sensitivity of eukaryotic 2-CysPrxs is tightly associated with several specific structural and sequence motifs, such as GGLG and the C-terminal YF motifs (Bolduc et al., 2018; Wood et al., 2003).

In the initial discovery of Srx by Biteau et al. (2003), a hypothetical model of the molecular mechanism was proposed for the reduction of protein cysteine $–SO_2H$ for yeast peroxiredoxin Tsa1 by Srx, involving an ATP-dependent reaction. According to this model (Figure 3), Srx acts as both a phosphotransferase and a thioltransferase in a two-step reaction to reduce $Prx–SO_2H$. In the first step, Srx utilizes ATP as a cofactor to phosphorylate $Prx–SO_2H$, resulting in the formation of a phosphoryl ester ($Prx{-}SO_2{-}PO_3^{2-}$). The phosphoryl ester then reacts with Srx via its active cysteine residue, forming a thiosulfinate ($Prx{-}SO{-}S{-}Srx$) that is highly unstable and readily reducible by other thiol-containing molecules, such as GSH and Trxs (Biteau et al., 2003). The reduction of $Prx{-}SO{-}S{-}Srx$ leads to the formation of Prx–SOH and the release of Srx, which can participate in another catalytic cycle to reduce other $Prx–SO_2H$. A different perspective on the first step reaction of $Prx{-}SO_2{-}PO_3^{2-}$ formation was proposed in a study of human Srx (HsSrx), suggesting that Srx may attack the phosphate of ATP via its active cysteine, generating a thiophosphate ($Srx{-}S–PO_3$) (Jeong et al., 2006). Nevertheless, this conundrum was rapidly resolved through subsequent investigations employing structural, kinetic, and mass spectrometry analysis. These studies conclusively excluded the possibility of $Srx–S–PO_3$ formation and provided support for the originally proposed molecular mechanism of yeast Srx (Jönsson et al., 2008b and 2008a). However, there is currently a lack of in-depth and focused biochemical analysis on the molecular mechanism of $–SO_2H$ reduction specifically for plant Srx. As a result, it is generally believed that plant Srx shares the same molecular mechanism as HsSrx and yeast Srx (Figure 3).

To date, the crystal structures of HsSrx (Jönsson et al., 2008a, Jönsson et al., 2005) and Arabidopsis Srx (AtSrx) have been elucidated (Liu et al., 2019). These structural analyses reveal overall structural similarities between AtSrx and HsSrx, with both sharing some

resemblance to the ParB domain fold, a DNA-binding protein involved in chromosome partitioning in bacteria (Basu and Koonin, 2005). Notably, AtSrx exhibits nuclease activity independent of its active cysteine (Chi et al., 2012). Structural analysis shows the ADP binding pocket and the putative interaction surface of AtSrx with 2-CysPrx are more positively charged compared to those of HsSrx, suggesting a different mechanism for -SO_2H reduction by AtSrx (Liu et al., 2019).

In Arabidopsis, a single gene encodes Srx, which produces five splice variants at the mRNA level (The Arabidopsis Information Resource, TAIR, available at www.arabidopsis.org). AtSrx functions as an antioxidant enzyme, similar to other redoxins involved in redox regulation and the protection from overoxidation (Liu et al., 2006; Galvez-Valdivieso et al., 2009). However, the functional characterization of Srx in plants has received limited attention, possibly because no distinguishable phenotype has been observed between the loss-of-function *Srx* mutants (T-DNA insertion transgenic plants) and wild-type plants under standard growth conditions in Arabidopsis (Liu et al., 2006; Rey et al., 2007). Nevertheless, Srx is suggested to play a protective role during oxidative stress, as evidenced by the increased sensitivity of *Srx* mutant compared to wild-type plants when treated with 10 µM of the herbicide paraquat (Liu et al., 2006) or 50 mM of H_2O_2 (Iglesias-Baena et al., 2010). In contrast, *Srx* mutant plants have displayed increased tolerance compared to wild-type plants under combined low temperature and high light stress, a scenario typically inducing photooxidative stress (Rey et al., 2007). Such disparate results may not only be a consequence of differing stress treatments but also suggest that Srx's biological role in plants may be more intricate than initially anticipated. This complexity of Srx is further supported by its role in deglutathionylation, with documented cases for protein tyrosine phosphatase 1B (PTP1B), actin, and 2-CysPrx in human (Findlay et al., 2006; Park et al., 2009), as well as 2-CysPrx in pea (Calderón et al., 2017). Unlike the ATP-required reduction of –SO_2H, *in vitro* biochemical analyses have shown that Srx can facilitate protein deglutathionylation independently of cofactors (Findlay et al., 2006; Calderón et al., 2017). However, the precise molecular mechanisms and physiological significance of Srx-mediated deglutathionylation at the cellular level are yet to be fully elucidated. Moreover, a recent discovery has unveiled an Srx-driven denitrosylation pathway for human Prxs that involves N-phosphorylation (Sunico et al., 2016). Taken together, these findings illuminate the sophisticated biological functions of Srx and its crucial role in the regulation of redox processes associated with thiol-based PTMs.

Detection approaches for Protein Sulfinic Acid

Accurate detection of $–SO_2H$ modifications on proteins through biochemical and proteomic approaches is a crucial first step in studying their biological functions. Traditionally, protein $–SO_2H$ modifications can be indirectly detected through a gel-based method in conjunction with thiol-labeling agents such as maleimide chemicals, including 4-acetamido-40-maleimidylstilbene-2,20-disulfonic acid (Biteau et al., 2003; Rey et al., 2007) and methoxy PEG maleimide (Bi et al., 2022; Muthuramalingam et al.; 2010, Telman et al., 2020). These maleimide componds typically induce a molecular size shift, distinguishable on SDS-PAGE gels (Figure 4A). In this method, the reduced form of the protein, which contains free thiols, exhibits the highest occupancy of maleimide labeling. On the other hand, the oxidized form of the protein can either be occupied by disulfide bonds or form $–SO_{2/3}H$ modifications, resulting in fewer free thiols available for reaction with maleimide. To specifically detect $–SO_2H$ modification, reversible thiol PTMs are first reduced using dithiothreitol (DTT). Subsequently, after alkylation with maleimide compounds, the protein samples can be analyzed using non-reducing SDS-PAGE gels or Western blot, employing specific antibodies for the targeted protein. The presence of $–SO_2H$ modification can be determined by comparing the position of the protein bands on the SDS-PAGE gel (Figure 4A). While this approach offers a convenient biochemical assay to detect thiol redox status for a target protein, it can pose challenges when the target protein is of low endogenous abundance or contains an excessive number of reactive thiols. As an alternative, specific antibodies have been developed for the detection of protein-specific $–SO_{2/3}H$ modifications. Commercially available antibodies are now accessible for proteins such as 2-CysPrx–$SO_{2/3}H$ and overoxidized PARK7/DJ1.

From a proteomic perspective, $–SO_2H$ modifications can be directly detected by mass spectrometry (MS), as they lead to a distinguishable mass increase of 32 Da. However, it's crucial to note that $–SO_2H$ modifications can also be formed during sample preparation through spontaneous oxidation. Therefore, it is vital to employ a well-controlled oxidation-free extraction method. For instance, proteome extraction using trichloroacetic acid (TCA) creates a strong acidic environment in which all free thiols are rapidly protonated. This protonation helps protect them from overoxidation during the extraction process. Our recent study has demonstrated significant differences in oxidation levels when comparing samples with and without TCA extraction (Huang et al., 2023).

Chemical probes are valuable tools for the direct and specific labeling of thiol PTMs, significantly advancing the field of cysteine redoxome profiling in redox biology (Willems et al., 2021). Initially, an aryl-nitrosothiol probe coupled to biotin (NO-Bio) was developed to label protein $–SO_2H$ modifications and utilized in human cell lysates (Lo Conte et al., 2015), In

this method, the cell lysates are first isolated in the presence of DTT, then, free thiols are blocked using 4,4'-dipyridyl disulfide (4-DPS). The protein samples are subsequently incubated with NO-Bio for $–SO_2H$ labeling, and the signal can be directly detected by Western blot using a streptavidin-horseradish peroxidase (HRP) conjugate (Figure 4B). Similarly, a biotinylated *S*-nitrosothiol-linked probe (GSNO-Biotin) was developed based on the cross-reactivity between $–SO_2H$ and S-nitrosothiol (Majmudar et al., 2016). The procedure of this method is similar to that for NO-Bio, except that free thiols are blocked using iodoacetamide (IAM) instead of 4-DPS. Following the blocking step, the protein samples are incubated with GSNO-Biotin for specific labeling of $–SO_2H$. Upon labeling, the signal can again be directly detected using Western blot, employing a streptavidin-HRP conjugate. The labeled proteome can be further enriched by utilizing streptavidin beads. The enriched proteins are then subjected to trypsin digestion and subsequent MS analysis (Figure 4C). This approach has enabled the identification of hundreds of proteins and numerous cysteine sites that form $–SO_2H$ in human cells. A maleimide-linked probe coupled to biotin was further developed for specific labeling of $–SO_2H$ under acidic conditions (Kuo et al., 2017, Figure 4D). However, these biotin-linked chemical probes are not cell-permentrated and they generate complex fragmentation patterns in MS analysis, making them unsuitable for *in situ* large-scale MS-based proteomic analysis in cells. A recent significant advancement in the field involved the development of an electrophilic diazene alkyne (DiaAlk) probe with remarkable sensitivity for the mapping of the S-sulfinome in mouse and human cells (Akter et al., 2018). In this method, after blocking thiols with 4-DPS, DiaAlk was employed to label $–SO_2H$ in protein lysates. Subsequently, UV cleavable biotin-azide (Az-UV-biotin) was conjugated to the labeled peptides via Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC). The DiaAlk-modified peptides are then enriched and quantified by MS analysis (Figure 4E). The developement of DiaAlk represents a significant advancement in redox field, offering a powerful tool for large-scale proteomic analysis of $–SO_2H$ modifications. To date, no specific proteomics analyses targeting $–SO_2H$ on a global scale have been reported in plant research, which significantly hinders our understanding of the functional roles of $–SO_2H$ in plant biology. Nevertheless, in our recent study monitoring cysteine oxidation degree in response to an excess of light in Arabidopsis, we successfully identified over one hundred $–SO_2H$ (+32 Da) sites by designing them as variable modifications in our analysis (Huang et al., 2023). Such instances of overoxidation are commonly observed in shotgun proteomic data, suggesting that similar searches for $–SO_2H$ modifications could be performed using public plant proteomic datasets. However, caution must be exercised when using different datasets, as $–SO_2H$ could also arise from artefactual overoxidation during sample preparation. This issue can

be mitigated by promptly neutralizing free thiols through TCA extraction (Huang et al., 2023). We believe that extracting proteomic information could open avenues for gaining valuable insights into $–SO_2H$ modifications in plants.

Proteins undergo sulfinic acid modification: exploring biological significance

To gain a deeper understanding of the biological function of $–SO_2H$ modification, it is essential to investigate the downstream signaling mediated by this PTM. This section provides a summary of the current knowledge regarding experimentally validated $–SO_2H$ modification on specific cysteine sites and their effects on protein functions (Table 1). The summary reveals the widespread $–SO_2H$ formation across different species. Notably, most of the listed proteins (17 out of 37) undergo $–SO_2H$-mediated enzyme inactivation. For instance, peroxiredoxins, such as 2-CysPrxs from various organisms (Iglesias-Baena et al., 2010, 2011; Jang et al., 2004; Yang et al., 2002; Wang et al., 2012), PrxIIF from pea (Iglesias-Baena et al., 2011), and PrxQ from *Xanthomonas campestris* (Perkins et al., 2016). Additionally, the formation of $–SO_2H$ on Cys-111 of human superoxide dismutase 1 (SOD1) not only inhibits its enzymatic activity (Xie et al., 2021), but also facilitates subunit exchange between oxidized and unoxidized homodimers, leading to the formation of heterodimers (Zhang et al., 2023). Another well-known example of enzymatic inactivation is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key protein in glycolysis, which is affected by $–SO_2H$ modification in both animal and plant systems (Barinova et al., 2023; Bedhomme et al., 2012).

It is worth noting that $–SO_2H$ formation regulates several key proteins, each contributing to distinct aspects of cellular function. For instance, $–SO_2H$ modification triggers the translocation of the PARK7/DJ-1 to the mitochondria, enabling its neuroprotective function in human (Canet-Avilés et al., 2004). In addition, $–SO_2H$ modification on Cys113 of the proline isomerase Pin1 has observed to significantly increase in human brains with Alzheimer's disease, leading to the inhibition of its downstream signaling activity (Chen et al., 2015). Furthermore, the formation of $–SO_2H$ on Cys108 of yeast D-amino acid oxidase has been found to reduce its specific activity while potentially enhancing its stability through the induction of a conformational change (Slavica et al., 2005). In contrast to $–SO_2H$-mediated enzymatic inactivation observed in many enzymes, it has been shown to be essential for the catalytic activity of bacterial nitrile hydratase (Murakami et al., 2000; Miyanaga et al., 2001). Taken together, the evidence highlights the broad impact of $–SO_2H$ modification on protein functions, spanning various aspects and participating in numerous biological processes.

Conclusion and perspectives

Despite extensive studies highlighting the critical role of thiol-based PTMs in plant growth, development, and response to environmental stimuli (Chae et al., 2023, Corpas et al., 2022, Dietz and Vogelsang, 2023, Zhou et al., 2023), the regulatory role of $–SO_2H$ modification has been underestimated. Consequently, our current understanding of the molecular mechanisms underlying the signaling function of protein $–SO_2H$ in plants remains limited. From a future perspective, we believe that directing proteomics analysis towards the specific targeting of cysteine $–SO_2H$ in plants, within specific biological contexts, will not only enhance our knowledge of the biological functions associated with this modification but also contribute significantly to a more comprehensive understanding of thiol-based redox regulation. In this regard, we have outlined some outstanding questions: 1) What are the functional consequences of $–SO_2H$ modification on protein structure, stability, and activity? How should we interpret the effect of $–SO_2H$ modification: as oxidative damage, as part of redox regulation, or as a regulatory signal? 2) Can $–SO_2H$ be recycled by pathways independent of Srx? If so, under which conditions does this recycling occur? 3) Is there any overlap between H_2O_2-mediated and PCO-catalyzed $–SO_2H$ pathways? Are there additional PCO substrates? Can they be reduced? If so, what are the potential mechanisms of reduction? 4) Are there specific environmental cues or stimuli that induce the formation of $–SO_2H$ modifications? How do these modifications contribute to plant adaptation and response to stress conditions?

Addressing these questions will contribute significantly to advancing our knowledge of the complexities and functional implications of cysteine $–SO_2H$ modifications in plant systems.

Authors' Contributions

J.H. conceived the article, J.H., F.V.B. and L.D.V wrote the article.

Author Disclosure Statement

Authors declare that they have no conflict of interest.

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Table 1: Biochemically characterized cysteine sulfinylation across various species and its impact on protein functionality

Protein name	Cys–SO_2H site	Effect	Reference
H. sapiens			
Prx1	51	Enzyme inactivation	(Yang et al., 2002)
Prx4	87	Enzyme inactivation	(Wang et al., 2012)
Cdc25b	377	Enzyme inactivation	(Sohn and Rudolph, 2003)
Cdc25c	473	Enzyme inactivation	(Sohn and Rudolph, 2003)
MMP-7	70	Enzyme inactivation	(Fu et al., 2001)
PTP1B	215	Enzyme inactivation	(Wang et al., 2004)
GAPDH	152	Enzyme inactivation	(Barinova et al., 2023)
SOD1	111	Enzyme inactivation	(Xie et al., 2021)
		Accelerating SOD1 heterodimerization between reduced and oxidized homodimers	(Zhang et al., 2023)
RGS4	2	Activation of N-degron pathway	(Lee et al., 2005)
RGS5	2	Activation of N-degron pathway	(Lee et al., 2005)
ACAD10	2	Activation of N-degron pathway	(Shim et al., 2022)
IL-32	2	Activation of N-degron pathway	(Masson et al., 2019)
PARK7/DJ-1	106	Leading to the translocation to mitochondria and enabling its protective function in Parkinson disease	(Canet-Avilés et al., 2004)
TPx-B	51	Forming stable decamer	(Schröder et al., 2000)
Pin1	113	Inactivation	(Chen et al., 2015)
Orai1	195	Leading to reduced subunit interaction	(Alansary et al., 2016)
L-PGDS	65	Protecting cell from apoptosis without losing ligand-binding function	(Fukuhara et al., 2012)
HSA	34	Unknown	(Turell et al., 2008)
Oryctolagus cuniculus			
GAPDH	149	Enzyme inactivation	(Souza and Radi, 1998)
A. thaliana			

2-CysPrx	119	Enzyme inactivation	(Iglesias-Baena et al., 2010)
GapC1	149	Enzyme inactivation	(Bedhomme et al., 2012)
HRE2	2	Activation of N-end rule pathway	(Weits et al., 2014)
RAP2.12	2	Activation of N-end rule pathway	(Licausi et al., 2011)
VRN2	2	Activation of N-end rule pathway	(Gibbs et al., 2018)
ZPR2	2	Activation of N-end rule pathway	(Weits et al., 2019)
Pisum sativum			
PRXIIF	89	Enzyme inactivation	(Iglesias-Baena et al., 2011)
Brassica campestris L. ssp. pekinensis			
2-CysPrx1	124	Inducing oligomerization and chaperone activity	(Kim et al., 2009)
Saccharomyces cerevisiae			
cPrxI	47	Inducing oligomerization and chaperone activity	(Jang et al., 2004)
Trigonopsis variabilis			
D-amino acid oxidase	108	Reducing specific activity, while potentially stabilizing the protein	(Slavica et al., 2005)
Schizosaccharomyces pombe			
Tpx1	48	Enzyme inactivation, thereby inhibiting the redox signal transfer to downstream Pap1 transcription factor	(Vivancos et al., 2005)
E. coli			
DnaK	15	Enzyme inactivation	(Winter et al., 2005)
Tpx	61	Enzyme inactivation	(Baker and Poole, 2003)
YajL	106	Unknown	(Wilson et al., 2005)
Xanthomonas campestris			
PrxQ	48	Enzyme inactivation	(Perkins et al., 2016)
Mycobacterium tuberculosis			
AhpE	45	Enzyme inactivation	(Hugo et al., 2009)
Pseudonocardia thermophila JCM 3095			
Nitrile hydratase	111	Essential for the catalytic activity	(Miyanaga et al., 2001)
Rhodococcus sp. N-771			
Nitrile hydratase	112	Essential for the catalytic activity	(Murakami et al., 2000)
Thiobacillus thioparus			
SCNase	133	Enzyme inactivation	(Arakawa et al., 2009)

Abbreviations: 2CysPrx, 2-Cys peroxiredoxin; ACAD10, acyl-CoA dehydrogenase family member 10; AhpE, alkyl hydroperoxide reductase E; Cdc25c, Cell division cycle 25 c; GapC1, cytosolic glyceraldehyde-3-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HRE2, Hypoxia Responsive2; HSA, human serum albumin; Hsc70, heat shock cognate protein 70; IL-32, interleukin-32; L-PGDS, lipocalin-type prostaglandin D synthase; MMP-7, MATRIX METALLOPROTEINASE7; Nm23-H1, nucleoside diphosphate kinase Nm23-H1; PARK7/DJ1, Parkinson's disease protein 7; Pin1, peptidyl-prolyl cis/trans isomerase; PRX/Prx, peroxiredoxin; PTP1B, protein tyrosine phosphatase 1B; RGS, regulator of G protein signaling; SCNase, thiocyanate hydrolase; SOD1, superoxide dismutase 1; Tpx/TPx, thiol peroxidase; VRN2, Vernalization2; ZPR2, Little Zipper 2.

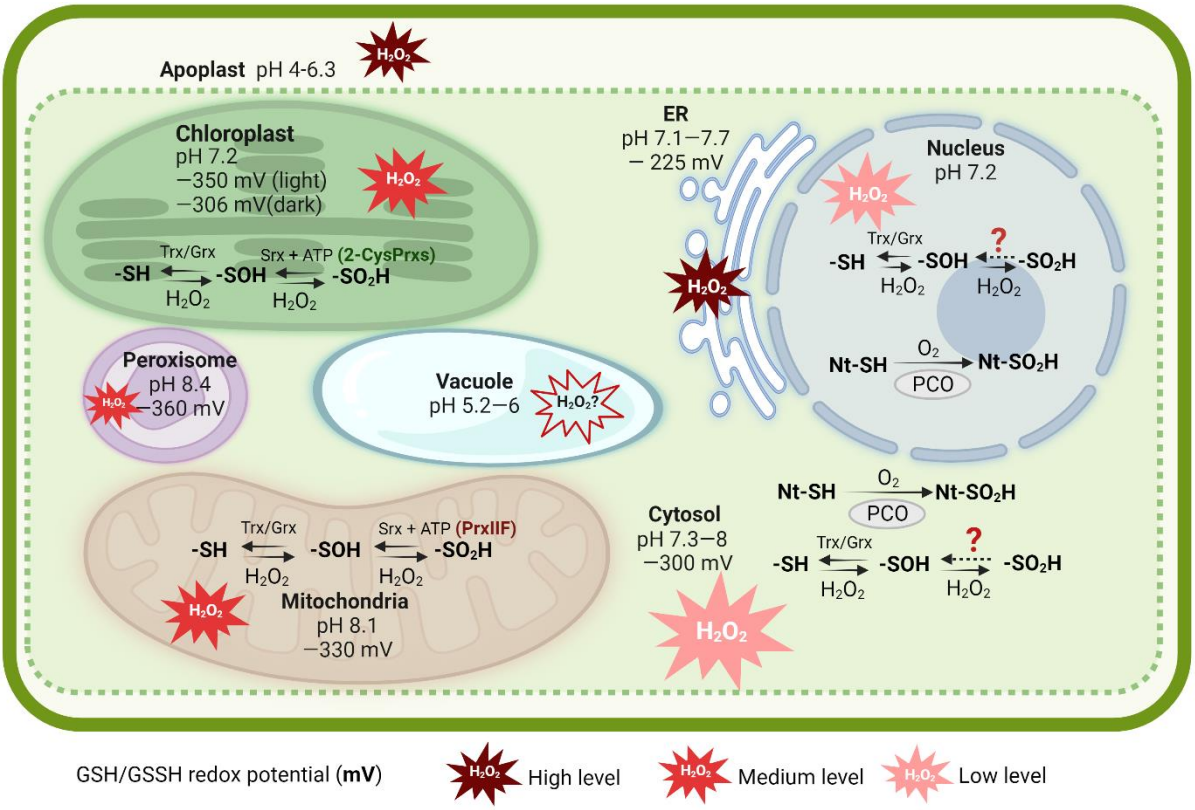

Figure 1. Formation of protein $–SO_2H$ and the parameters influencing thiol reactivity in different subcellular organelles in plant cells. Protein $–SO_2Hs$ are generated in various subcellular organelles within plant cells through two distinct mechanisms. They can be formed by the reaction of protein –SHs with H_2O_2, or can be catalyzed by PCOs that specifically target Nt-SH. The protein $–SO_2H$ formation of chloroplastic 2-CysPrxs and mitochondrial PrxIIF can be reversed by Srx via ATP-dependent reaction. However, the exact mechanism by which protein $–SO_2H$ is reduced in other organelles remains unknown. The formation of Nt-SO_2H, mediated by PCOs, triggers N-end rule degradation in the cytosol and nucleus. Several factors have been identified that influence the reactivity of -SH groups, including H_2O_2 levels, pH, and the redox potential (mV) of GSH/GSSG. 2-CysPrxs, chloroplastic 2-Cys peroxiredoxins; ER, endoplasmic reticulum; Grx, glutaredoxin; GSH/GSSG, reduced/oxidized glutathione; H_2O_2, hydrogen peroxide; Nt-SH, N-terminal cysteine thiol; Nt-SO_2H, N-terminal sulfinic acid; PCOs, plant cysteine oxidases; –SH, protein cysteine thiols; $–SO_2H$, protein cysteine sulfinic acid; Srx, sulfiredoxin; Trx, thioredoxin.

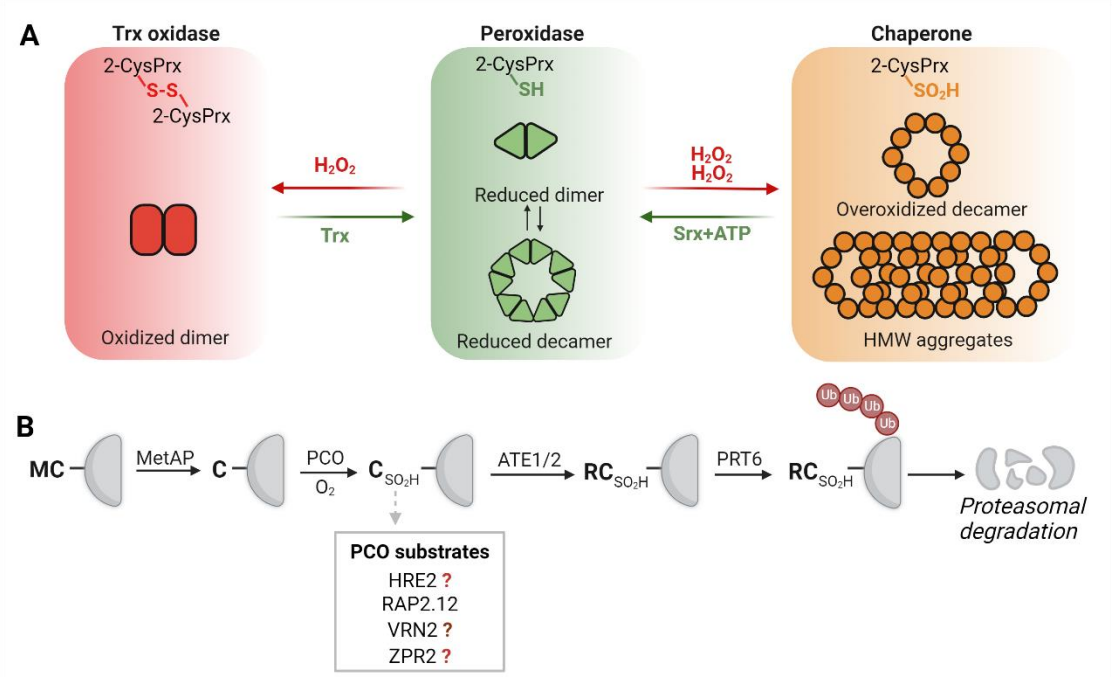

Figure 2. Protein $–SO_2H$ triggered signaling for 2-CysPrx and N-end rule substrates. (A) The formation of $–SO_2H$ on 2-CysPrx has both structural and functional implications. In its reduced state, 2-CysPrx functions as a peroxidase and exists as a homodimer or a decamer. Upon exposure to H_2O_2, 2-CysPrx forms an inter-disulfide bond, resulting in the formation of an oxidized dimer. This transition enables its function as a Trx oxidase. An excess of H_2O_2 induces the $–SO_2H$ modification on 2-CysPrx, leading to the formation of overoxidized decamers and the aggregation of 2-CysPrx into HMW complexes, which function as a chaperone. (B) Proteins starting with amino acid sequence with MC are targeted by MetAP to remove the N-terminal methionine. In the presence of O_2, PCOs oxidize the N-terminal cysteine to $–SO_2H$, creating a secondary destabilizing residue. Subsequently, arginine (R) is added to the N-terminus by ATEs. This results in a primary destabilizing residue that is recognized by the specific E3 ligase PRT6 for proteasomal degradation. Within the N-end rule pathway, four substrates have been identified and validated: HRE2, RAP2.2, VRN2, and ZPR2. Among them, RAP2.2 has been confirmed as the *in vivo* substrate for PCOs, while confirmation for the others is still pending. ATEs, arginine-tRNA protein transferases; HMW, high molecular weight; HRE2, Hypoxia Responsive 2; MC, methionine and cysteine; MetAP, Methionine AminoPeptidase; O_2, oxygen; R, arginine; PRT6, Proteolysis 6; RAP2.2, Related to AP2; VRN2, Vernalisation 2; ZPR2, Little Zipper Protein 2

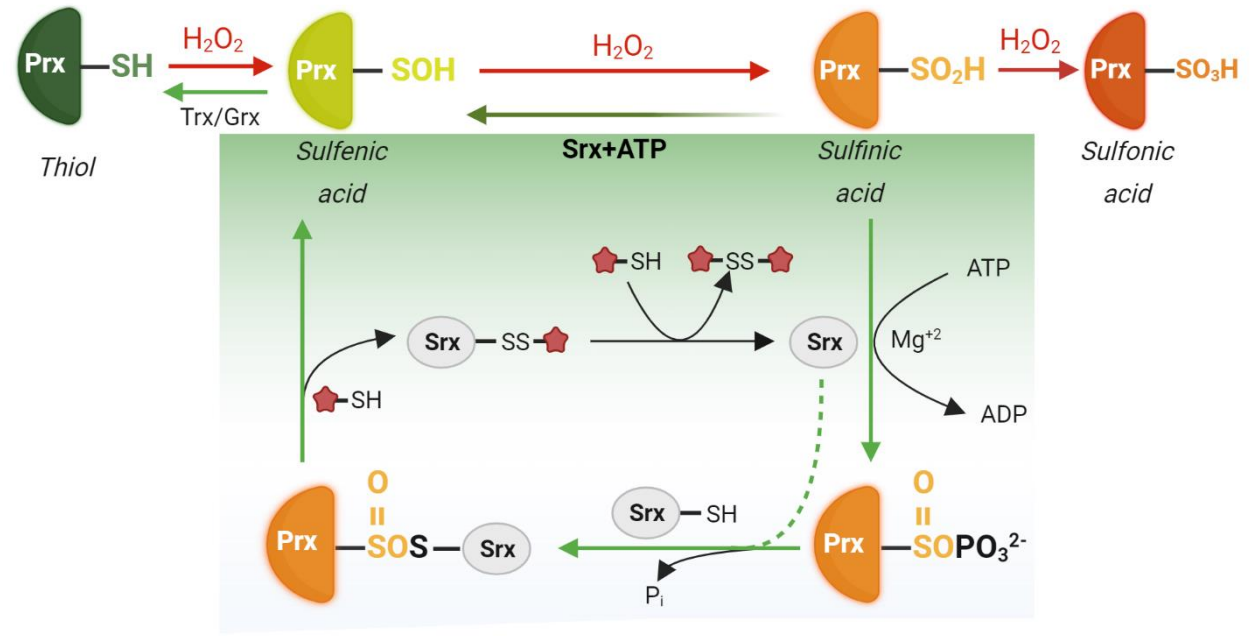

Figure 3. The molecular mechanism of Srx-based reduction of $–SO_2H$ in Prx. The cysteine -SH group of Prx reacts with H_2O_2 to form Prx–SOH, which can be further oxidized to Prx–SO_2H and Prx-SO_3H. Srx employs ATP as a cofactor to phosphorylate Prx–SO_2H, resulting in a phosphoryl ester, Prx−SO_2−PO_3^{2-}. This ester then reacts with Srx through its active cysteine residue, forming a thiosulfinate form, Prx−SO−S−Srx, which can subsequently be reduced by other thiol-containing molecules. The reduction of Prx−SO−S−Srx results in the formation of Prx-SOH and releases Srx, enabling it to reenter the catalytic cycle. The red star indicates that a thiol-containing protein functions as reducing power in this reduction mechanism. Prx–SOH, sulfenic acid form of Prx; Prx–SO_2H, sulfinic acid form of Prx; Prx-SO_3H, sulfonic acid form of Prx.

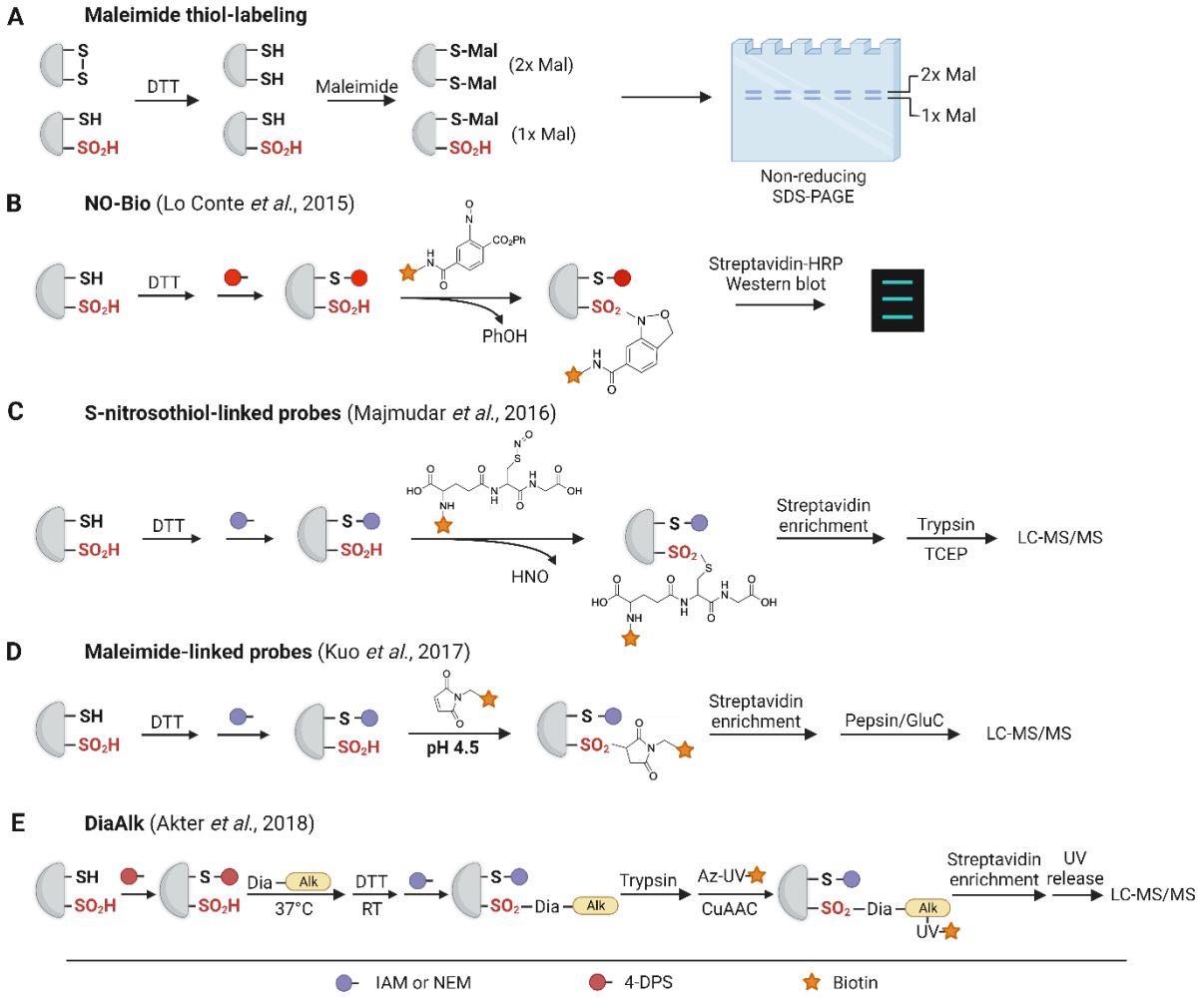

Figure 4. Methods for protein –SO_2H detection. (A) Maleimide thiol-labeling method. Protein –SH groups can be labeled by maleimide-based chemicals, leading to a molecular size shift in SDS-PAGE gels. The reduced protein, containing more –SH groups, will be labeled by multiple maleimide molecules and exhibit a higher molecular weight band on SDS-PAGE compared to the oxidized protein. (B) NO-Bio chemical probe. Cell lysates are isolated in the presence of DTT, and –SH groups are blocked by 4-DPS. The extracted proteome is then incubated with NO-Bio for –SO_2H labeling. The signals can be directly detected by Western blot using a streptavidin-HRP conjugate. (C) GSNO-Biotin chemical probe. Following proteome isolation in the presence of DTT and –SH blocking with IAM, proteins are incubated with GSNO-Biotin for –SO_2H labeling. Detection is via Western blot with a streptavidin-HRP conjugate. Alternatively, the labeled proteome can be enriched with streptavidin beads, followed by trypsin digestion and subsequent MS analysis. (D) Maleimide-biotin probe. This probe specifically labels protein –SO_2H under acidic conditions (pH 4.5). The labeled proteome is enriched by streptavidin beads, then subjected to pepsin/GluC digestion, and analyzed by MS. (E) DiaAlk-

based chemoproteomics. The proteome is isolated and incubated with 4-DPS to block –SH grops, DiaAlk is then employed to label $–SO_2H$ in protein lysates. The labeled proteome is digested by trypsin, and Az-UV-biotin is then conjugated to the labeled peptides via CuAAC. The peptides are enriched by straptatidin beads, and biotin is released upon UV exposure. Finally, DiaAlk-modified peptides are enriched and quantified by MS analysis. Az-UV-biotin, UV Cleavable Biotin-Azide; CuAAC, Cu(I)-catalyzed azide–alkyne cycloaddition; DiaAlk, diazene alkyne; 4-DPS, 4,4'-dipyridyl disulfide; HRP, horseradish peroxidase; IAM, iodoacetamide; Mal, maleimide; MS, mass spectometry.