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Detecting Protein Sulfenylation in Cells Exposed to a Toxicant

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Abstract

Protein sulfenylation is a posttranslational modification that is linked to many cell signaling networks and specific protein functions, thus the detection of any sulfenylated protein after a toxicological exposure is of importance. Specifically, the detection of protein sulfenylation can provide multiple levels of mechanistic insight towards understanding the impact of a toxicological exposure. For instance, sulfenylation is caused by only a handful of reactive chemical species, any altered sulfenylation suggests a change in cellular health, and the elucidation of the specific protein target that undergoes sulfenylation can help ascertain downstream targets and associated adverse outcomes. This document describes straightforward approaches to detect protein sulfenylation of total protein as well as individual proteins of interest with a focus on immunoblotting approaches.

Keywords

Oxidative Stress; Sulfenylation; Hydrogen Peroxide; Posttranslational Modification; Immunoblotting

INTRODUCTION

Oxidative stress is a frequently cited mechanism in the toxicity of many chemical and environmental exposures (Cross et al., 2002). Despite the widespread acknowledgment of oxidative stress as a toxicological feature through which adverse biological effects could occur, the specific mechanisms have been largely undefined. For instance the fluorogenic compound dichlorofluorescein (DCF) can be used to provide evidence of chemical induced oxidative stress; however, the results obtained from these DCF-based assays lack the specificity to provide mechanistic insights (Chen et al., 2010; Winterbourn, 2014). Only recently have methodologies become available to detail the mechanistic contribution of oxidative stress in toxicological exposures.

Conflict of Interest

The authors declare no conflict of interest.

The protocols described in this unit provide a methodological approach to detect protein sulfenylation, a posttranslational modification associated with oxidative stress-induced signaling. Detailed are immunoblotting methods to detect the occurrence of protein sulfenylation after a toxicological exposure using both antibody- and biotin/avidin-based approaches (Basic Protocol 1 and Basic Protocol 2, respectively). Also highlighted is a method (Alternate Protocol 2) that permits the detection of a specific protein of interest.

BASIC PROTOCOL 1

Detection of Protein Sulfenylation in Cells

Protein sulfenylation can be stabilized utilizing the small, cell permeable molecule dimedone. Dimedone has high selectivity and affinity to irreversibly label sulfenic acids making it a useful tool to detect protein sulfenylation (Burgoyne and Eaton, 2011). Immunoblotting of sulfenylated proteins using antibodies raised against the dimedone-protein adduct is a relatively straightforward semi-quantitative approach to determine whether a toxicological exposure can induce protein sulfenylation (Maller et al., 2011; Seo and Carroll, 2009). The following protocol describes a method to detect protein sulfenylation in cells grown in 100-mm plates using hydrogen peroxide (H_2O_2) as a model toxicant.

Materials

Cell cultures in 100-mm tissue culture dishes Serum-free cell culture media

1X Phosphate Buffered Saline (PBS)

Deionized, distilled water (H_2O)

10 mM H_2O_2 in H_2O

Dimethyl Sulfoxide (DMSO)

1 M Dimedone in DMSO

100 mM Dithiothreitol (DTT) in H_2O

Strong Lysis Buffer (See Recipe), chilled on ice

1.5 mL Microcentrifuge tubes

4X Laemmli sample buffer

Precast SDS-PAGE gel

Nitrocellulose membrane

Tris-Buffered Saline w/Tween 20 (TBST, See Recipe) Dry Milk

Anti-dimedone antibody (EMD Millipore 07-2139 or EMD Millipore ABS30)

Secondary antibody against rabbit IgG

Cell culture incubator Microcentrifuge, precooled to 4° C

Protocol Steps

Label Sulfenic Acids

- 1) Culture $>5 \times 10^6$ cells (~80% confluent) in two dishes.
- 2) Replace growth media with 10 mL serum-free media at least 24 hours before exposure.

Appropriate cell culture density and growth conditions may vary based on cell type.

- 3) Wash each dish with 5 mL PBS, repeat for a total of two times.
- 4) Expose both dishes of cells to 100 μM H_2O_2 (1 μL of 10 mM H_2O_2 diluted in 10 mL of serum-free media) for 5 minutes.

Time and concentration of exposure will need to be optimized for each chemical of interest.

- 5) Wash each dish with 5 mL PBS, repeat for a total of two times.
- 6) Label one dish with 5 mM dimedone (50 μL of 1 M dimedone diluted in 10 mL of serum-free media) and label the other with media not containing dimedone (50 μL of DMSO in 10 mL of serum-free media) for one hour at 37° C, 5% CO_2 .
- 7) Wash each dish with 5 mL PBS, repeat for a total of two times.

Lyse Cells

- 8) Add 1 mM DTT to strong lysis buffer (10 μL of 100 mM DTT to 1 mL of strong lysis buffer).
- 9) Lyse each dish in 1 mL ice-cold strong lysis buffer for 5 minutes.

If needed additional lysing may be required including sonication or mechanical disruption.

- 10) Transfer strong lysis buffer from each dish to a separate tube.
- 11) Centrifuge 10 minutes at 12,000 $\times g$, 4° C.
- 12) Transfer supernatant to clean tube.
- 13) Determine protein concentration of supernatant and normalize protein concentrations.

Multiple kits are available to determine protein concentration of cell lysates. Do take note that unless the kit or in-house assay is detergent compatible then the lysate will need to be diluted to an extent that provides reliable results.

- 14) Aliquot portion of sample to tube with 4X Laemmli sample buffer.
For example 75 μL of sample to 25 μL of 4X Laemmli sample buffer.
- 15) Boil samples for 10 minutes.

Preparation and Implementation of Immunoblotting

- 16) Add appropriate volume to precast SDS-PAGE gel and apply voltage for size separation.

Each SDS-PAGE gel, or other appropriate gel for electrophoresis, requires different specifications for working volume and mass of sample added, running buffer conditions, and applied voltage. This should be optimized for each particular case.

- 17) Transfer protein to nitrocellulose membrane.

Methods for transferring proteins on a SDS-PAGE gel to a nitrocellulose membrane (or PVDF membrane if preferred) are frequently lab preferred. Both wet-based and semi-dry-based transfer methods exist and have their advantages and disadvantages.

- 18) Incubate membrane in 5% (w/v) milk in TBST for 1 hour at room temperature.
- 19) Incubate membrane in anti-dimmedone antibody overnight at 4° C.
- 20) Wash membrane with TBST for 5 minutes, repeat twice for a total of three times.
- 21) Incubate membrane in secondary antibody for 1 hour at room temperature.
- 22) Wash membrane with TBST for 5 minutes, repeat twice for a total of three times.
- 23) Analyze membrane with appropriate detection method.

Method for detection should be based on the secondary antibody used. Most secondary antibodies are conjugated to either horse radish peroxidase (hrp) or a fluorescent group, and as such each should be detected by the manufacturer's recommended suggestions.

BASIC PROTOCOL 2

Avidin-Blotting for Protein Sulfenylation using Copper-Catalyzed Alkyne-Azide Cycloaddition

Although using immunoblotting to detect protein sulfenylation by an antibody is straightforward, it does not lead to identification of the proteins modified. To enhance selectivity as well as sensitivity, analogs of dimedone that contain an alkyne or azide have been synthesized in order to exploit the strong biotin-avidin interaction (Paulsen et al., 2012). The most successful dimedone analogs, which contain an alkyne group, are commercially available (DYn-2 from Cayman Chemical; DCP-alkyne from Kerafast). Once the dimedone analog labels the sulfenic acids the labeled proteins undergo Copper-Catalyzed Alkyne-Azide Cycloaddition (CuAAC, also referred to as "click chemistry") in order to introduce a biotin group that can permit biotin-avidin based detection methods. Specifically, this biotin group can then be used to detect protein sulfenylation exploiting the high affinity of the biotin-avidin interaction. The following protocol describes a method to detect protein sulfenylation by avidin-blotting in cells grown in 100-mm plates after a toxicological exposure using H₂O₂ as a model toxicant.

Materials

Cell cultures in 100-mm tissue culture dishes
Serum-free cell culture media
1X PBS

10 mM H₂O₂ in H₂O

1 M DYn-2 in DMSO

100 mM DTT in H₂O

Weak Lysis Buffer (See Recipe), chilled on ice

5 mM biotin-azide in DMSO (ThermoFisher B10184)

50 mM Tris(benzyltriazolymethyl)amine (TBTA) in DMSO

100 mM Copper Sulfate in H₂O, prepare within one hour of use
100 mM Sodium Ascorbate in H₂O, prepare within one hour of use

1.5 mL Microcentrifuge tubes

4X Laemmli sample buffer Precast SDS-PAGE gel Nitrocellulose membrane

Tris-Buffered Saline w/ Tween 20 (TBST, See Recipe) Biotin-free Blocking Buffer

Pierce™ High Sensitivity NeutrAvidin™-HRP (31030)

ECL Western Blotting Substrate

Cell culture incubator Microcentrifuge, precooled to 4° C

Protocol Steps

Label Sulfenic Acids

- 1) Culture >5x10⁶ cells (~80% confluent) in two dishes.
- 2) Replace growth media with 10 mL serum-free media at least 24 hours before exposure.

Appropriate cell culture density and growth conditions may vary based on cell type.

- 3) Wash each dish with 5 mL PBS, repeat for a total of two times.
- 4) Expose both dishes of cells to 100 uM H₂O₂ (1 uL of 10 mM H₂O₂ diluted in 10 mL of serum-free media) for 5 minutes.

Time and concentration of exposure will need to be optimized for each chemical of interest.

- 5) Wash each dish with 5 mL PBS, repeat for a total of two times.
- 6) Label one dish with 5 mM DYn-2 (50 µL of 1 M DYn-2 diluted in 10 mL of serum-free media) and label the other with media not containing DYn-2 (50 µL of DMSO in 10 mL of serum-free media) for one hour at 37° C, 5% CO₂.

DCP-alkyne can be used instead of DYn-2.

- 7) Wash each dish with 5 mL PBS, repeat for a total of two times.

Lyse Cells

- 8) Add 1 mM DTT to weak lysis buffer (10 µL of 100 mM DTT to 1 mL of weak lysis buffer).

- 9) Lyse each dish in 1 mL ice-cold weak lysis buffer for 20 minutes.

If needed additional lysing may be required including sonication or mechanical disruption.

- 10) Transfer weak lysis buffer from each dish to a separate tube.
- 11) Centrifuge for 10 minutes at 12,000×g, 4° C.
- 12) Transfer supernatant to clean tubes.
- 13) Determine protein concentration of supernatant and normalize protein concentrations (1.0-2.0 mg/mL protein) using weak lysis buffer as diluent.

Multiple kits are available to determine protein concentration of cell lysates. Do take note that unless the kit or in-house assay is detergent compatible then the lysate will need to be diluted to an extent that provides reliable results.

- 14) Preclear lysates using streptavidin beads (Optional, see Support Protocol 1).

Add Biotin to Labeled Samples by Click Chemistry

- 15) Transfer 486.5 µL supernatant from each sample to a clean tube.
- 16) Add 2.5 µL 40 mM biotin-azide to each tube for a final concentration of 0.2 mM. Gently mix.

Do not vortex.

- 17) Add 1.0 µL 50 mM TBTA to each tube for a final concentration of 0.1 mM. Gently mix.

Do not vortex.

- 18) Add 5 µL copper sulfate solution to each tube for a final concentration of 1 mM. Gently mix.

Do not vortex. Prepare copper sulfate solution within one hour of use.

- 19) Add 5.0 µL 100 mM sodium ascorbate to each tube for a final concentration of 1 mM. Gently mix.

Do not vortex. Prepare sodium ascorbate solution within one hour of use.

- 20) Mix samples by inversion for 1 hour at room temperature.
- 21) Aliquot portion of sample to tube with 4X Laemmli sample buffer.

For example 75 uL of saple to 25 uL of 4X Laemmli sample buffer.

- 22) Boil for 10 minutes.

Preparation and Implementation of Immunoblotting

- 23) Add appropriate volume to precast SDS-PAGE gel and apply voltage for size separation.

Each SDS-PAGE gel, or other appropriate gel for electrophoresis, requires different specifications for working volume and mass of sample added, running buffer conditions, and applied voltage. This should be optimized for each particular case.

- 24) Transfer protein to nitrocellulose membrane.

Methods for transferring proteins on a SDS-PAGE gel to a nitrocellulose membrane (or PVDF membrane if preferred) are frequently lab preferred. Both wet-based and semi-dry-based transfer methods exist and have their advantages and disadvantages.

- 25) Incubate membrane in biotin-free blocking buffer for 1 hour at room temperature.
- 26) Incubate membrane in NeutrAvidin™-HRP, diluted in TBST for 1 hour at room temperature.

Based on HRP color development solution the concentration should be appropriately determined, usual dilution is 1:10,000 to 1:50,000. Other avidin-based detection probes can be utilized if needed.

- 27) Wash membrane with TBST for 5 minutes, repeat twice for a total of three times.
- 28) Detect reactive NeutrAvidin™ using ECL Western Blotting Substrate manufacturer's recommended specifications.

ALTERNATE PROTOCOL 2

Immunoprecipitation of Specific Sulfenylated Proteins using Copper-Catalyzed Alkyne-Azide Cycloaddition

The identification of specific cellular proteins sulfenylated in response to a toxicological exposure is often the ultimate goal. The addition of immunoprecipitation steps after the click chemistry reaction permits the detection of specific proteins of interest with cysteines that are oxidatively modified to the sulfenic acid. The following protocol describes a method to detect sulfenylation of specific cellular proteins using H₂O₂ as a model toxicant.

Additional Materials

- Protein A Agarose
- Primary Antibody for Protein of Interest
- Secondary Antibody appropriate for primary antibody used

Protocol Steps

- 1) Repeat steps 1-20 from Basic Protocol 2.

Immunoprecipitate Protein Target

- 2) Aliquot portion of sample to a clean tube and bring to 500 µL using PBS.

Different masses of total protein will be needed based on the relative concentration of the protein of interest in the cell lysate, i.e. low abundant proteins will require more total protein mass than more common proteins such as GAPDH or Actin.

- 3) Add 10 μ L of primary antibody to tube.
- 4) Mix by inversion overnight at 4° C.
- 5) Using a wide orifice tip, add 50-100 μ L of Protein A Agarose slurry.
- 6) Mix by inversion for 2 hours at 4° C.
- 7) Centrifuge for 2 minutes at 2,300 $\times$ g, 4° C.
- 8) Discard supernatant.

Supernatant can be transferred to a clean tube for subsequent immunoprecipitations.

- 9) Add 1 mL of PBS, then centrifuge sample for 2 minutes at 2,300 $\times$ g, 4° C and discard supernatant. Repeat twice for a total of three times.

PBS can be replaced with stronger washing solutions such as the weak lysis buffer or strong lysis buffer.

- 10) Add 25-100 μ L of 1X Laemmli sample buffer.
- 11) Boil for ten minutes.

Preparation and Implementation of Immunoblotting

- 12) Add appropriate volume of sample to precast SDS-PAGE gel and apply voltage for size separation. Do not add any of the agarose beads to the gel.

Each SDS-PAGE gel, or other appropriate gel for electrophoresis, maintains different specifications for working volume and mass of sample added, running buffer conditions, and applied voltage. This should be optimized for each particular case.

- 13) Transfer protein to nitrocellulose membrane.

Methods for transferring proteins on a SDS-PAGE gel to a nitrocellulose membrane (or PVDF membrane if preferred) are frequently lab preferred. Both wet-based and semi-dry-based transfer methods exist and have their advantages and disadvantages.

- 14) Incubate membrane in biotin-free blocking buffer for 1 hour at room temperature.
- 15) Incubate membrane in NeutrAvidin™-HRP, diluted in TBST for 1 hour at room temperature.

Based on HRP color development solution the concentration should be appropriately determined, usual dilution is 1:10,000 to 1:50,000. Other avidin-based detection probes can be utilized if needed.

- 16) Wash membrane with TBST for 5 mins, repeat twice for a total of three times.
- 17) Detect reactive NeutrAvidin™ using ECL Western Blotting Substrate manufacturer's specifications.

Immunoblotting for Total Protein Control

- 18) Repeat steps 12-14.
- 19) Incubate membrane in primary antibody diluted in biotin-free blocking buffer for 1 hour at room temperature.
- 20) Incubate membrane in primary antibody overnight at 4° C.
- 21) Wash membrane with TBST for 5 minutes, repeat twice for a total of three times.
- 22) Incubate membrane in secondary antibody for 1 hour at room temperature.
- 23) Wash membrane with TBST for 5 minutes, repeat twice for a total of three times.
- 24) Analyze membrane with appropriate detection method.

Method for detection should be based on the secondary antibody used. Most secondary antibodies are conjugated to either hrp or a fluorescent group, and as such each should be detected by the manufacturer's recommended suggestions.

SUPPORT PROTOCOL 1

Preclearing lysates of endogenous biotin

Certain cell types, such as hepatocytes, naturally have substantial amounts of biotin. In order to ultimately reduce background from endogenous biotin and biotinylated proteins when utilizing avidin-based probes, it is strongly suggested that all lysates undergo a preclear step.

Materials

- Protein lysate
- Weak Lysis Buffer (see recipe)
- Streptavidin-conjugated resin
- Microcentrifuge Tubes
- Microcentrifuge, precooled to 4° C

Protocol Steps

- 1) Add resin (50 µL/100 µg of protein) to microcentrifuge tube.
- 2) Add 500 µL of weak lysis buffer to resin, centrifuge for 2 minutes at 2,300×g, 4° C, then discard supernatant. Repeat twice for a total of three times.
- 3) Load a known amount of protein lysate to resin.
- 4) Bring volume up to 500 µL with weak lysis buffer.
- 5) Mix by inversion for 30-60 minutes at 4° C.
- 6) Centrifuge sample at 2,300g, 2 minutes, 4° C.

- 7) Transfer supernatant to a clean tube, or alternatively proceed to appropriate step of protocol.

REAGENTS and SOLUTIONS

TBST

150 mM NaCl

20 mM Tris base

0.1% (v/v) Tween-20

Adjust to final pH of 7.5

Store for 6 months at room temperature.

Weak Lysis Buffer

150 mM NaCl

150 mM Tris-HCl (pH 7.4)

1% (v/v) NP40

1×Complete Protease Inhibitor Cocktail Set III (Calbiochem)

Lysis buffer without added protease inhibitors can be stored at 4° C or room temperature for 6 months. Once the protease inhibitors are added the lysis buffer should be kept on ice or at 4° C and used within 24 hours.

Strong Lysis Buffer

150 mM NaCl

150 mM Tris-HCl (pH 7.4)

1% (v/v) NP40

0.1% SDS

0.5% Sodium Deoxycholate

1×Complete Protease Inhibitor Cocktail Set III (Calbiochem)

Lysis buffer without added protease inhibitors can be stored at 4° C or room temperature for 6 months. Once the protease inhibitors are added the lysis buffer should be kept on ice or at 4° C and used within 24 hours.

COMMENTARY

Background Information

Protein sulfenylation is an oxidative posttranslational modification involving the oxidation of a cysteine thiol to the sulfenic acid; under most biological conditions this oxidation is done by H_2O_2 (Lee et al., 1998; Poole et al., 2004). However, theoretically there are other reactive chemical species that could cause protein sulfenylation (Trujillo et al., 2016). Based on these chemical limitations it is likely that any toxicological exposure that changes endogenous H_2O_2 levels could impact protein sulfenylation. In fact the environmentally relevant chemical 1,2-naphthoquinone, which is known to elevate intracellular H_2O_2 levels, was recently demonstrated to induce protein sulfenylation (Wages et al., 2015).

It should also be noted that the power of assaying for protein sulfenylation in toxicological studies lies in its capacity to provide evidence for a potential cause-and-effect mechanism and its respective mediator. Protein sulfenylation is similar to other posttranslational modifications such as phosphorylation, as the modification can either enhance or inhibit the activity of a protein essentially serving as an on-off switch for proteins (Crump et al., 2012; Kulathu et al., 2013; Lee et al., 1998; Paulsen et al., 2012). Additionally, proteins that undergo sulfenylation have also been reported to change their function in cellular processes altogether (Barrett et al., 2012; Klomsiri et al., 2011). Thus, when specific protein targets are sulfenylated as a result of a toxicological exposure it can allow for the development of hypotheses of impacted cell signaling networks, which is difficult to do when only global oxidant stress is measured.

Finally, using variations on the small molecule dimedone additional modifications to the above protocols can be implemented to further explore the role of sulfenylation in toxicological exposures. There are dimedone analogs that contain a biotin group (Klomsiri et al., 2010), which allows for biotin-streptavidin purification without using click chemistry, which could be especially beneficial for precious samples. However, this particular analog is not cell permeable and, therefore, the tissue must be lysed before the dimedone labeling can occur, which may lead to unwanted oxidation of proteins.

There are also exist cleavable versions of biotin-azide (Yang et al., 2015) and isotopic variations of dimedone (Seo and Carroll, 2011; Yang et al., 2014) which allow for proteomic analysis of the targets using mass spectrometry methods (Yang et al., 2016). Additionally, there are fluorescent based probes that utilize the dimedone-based chemical motif, some of which use a biotin-like labeling strategy, which can be utilized for intracellular localization studies microscopy (Nelson et al., 2010).

Critical Parameters

It is critical that the intended exposure be characterized for optimal concentration and time. This is in part due to the reversible, unstable nature of endogenous protein sulfenylation. It is good practice to utilize both dose- and time-dependent exposures to determine the optimal exposure before expanding to more time and resource expensive experiments. Based on previous experience the temporal parameter of exposure-induced sulfenylation is of

particular importance as the results can also provide adaptive processes to ameliorate the toxicity.

Troubleshooting

Click Reaction—The success of the protocol using DYn-2 or DCP-alkyne is largely founded on the efficiency of the click chemistry reaction. The theory and basis of this reaction is explained in excellent detail elsewhere (Chen et al., 2011; Martell and Weerapana, 2014). Some basic troubleshooting parameters for the success of click chemistry include having sufficient protein substrate (1.0-2.0 mg/mL total protein) and minimizing the concentration of detergent present in the reaction.

The presence of detergents is likely the most concerning issue for molecular biologists, as many lysis buffers use sodium deoxycholate, SDS, NP-40 and/or TritonX. The weak lysis buffer detailed in this protocol has been used successfully. An alternate lysis buffer that uses 0.1% SDS and 0.1% TritonX instead of 1% NP-40 has reported equal success. Thus detergent conditions for the lysis buffer could be modified within reason to obtain a successful result; however, it should be noted that to date the use of sodium deoxycholate appears to dramatically impair the efficiency of the click reaction. It should also be mentioned that the methods and conditions of click chemistry with protein as a substrate are continually being improved. For instance recently the use of a water-soluble ligand THPTA, instead of TBTA has been shown to improve the overall efficiency of the reaction (Hong et al., 2010).

Immunoblotting—The general process of immunoblotting requires optimization which have been highlighted elsewhere (Janes, 2015). However, for this particular set of protocols it is important to include a reducing agent in the lysis buffer to reduce the background caused by oxidation of proteins where dimedone and dimedone-analogs may still be present. The inclusion of a reducing agent, such as DTT or TCEP, in the lysis buffer can halt this process and restrict the signal to experimentally relevant sulfenylation.

Additionally, it should be noted that many practitioners of immunoblotting strip their membrane in order to reprobe for other proteins. Due to the strong affinity of biotin to avidin-based probes, normal stripping practices will not be successful. Thus, probing with avidin should be conducted last in the sequence of immunoblotting.

Anticipated Results

The use of the dimedone analogs containing the alkyne tail should provide very specific and sensitive results, especially if the lysates were precleared of endogenous biotinylated proteins. If the anti-cysteine dimedone antibody is used, there is a high likelihood of streaking bands and non-specific binding. For total protein analysis multiple protein bands should be present upon detection in the dimedone or dimedone-analog labeled cell samples. If immunoprecipitation is used (Alternate Protocol 2) then one protein band should be present in the dimedone or dimedone-analog labeled cell samples.

Based on the chemical exposure used, the results may differ than if a pro-oxidant such as H₂O₂ or 1,2-naphthoquinone is utilized. For instance, potent electrophiles such as O₃ or

electrophiles that are formed after a toxicological exposure, including 4- hydroxynonenal, could lead to decreased protein sulfenylation as the cysteine group is no longer available to be oxidized. It should also be noted that prolonged exposure to pro-oxidants could hyperoxidize the protein sulfenic acid, which would also be observed as decreased sulfenylation.

Time Considerations

The majority of the time necessary for these protocols involves the SDS-PAGE gel electrophoresis, transfer, and immunoblotting which will take roughly two full days to complete. The exposure and labeling of sulfenic acids is largely dictated by the time of exposure, which should be determined in advance of pursuing these protocols. However, the labeling strategy with dimedone or dimedone analogs should take 1 to 2 hours based on whether click chemistry is needed.

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SIGNIFICANCE STATEMENT

Oxidative stress is a commonly cited mechanism through which a toxicological exposure leads to adverse outcomes. One oxidative stress measurement that is particularly informative is protein sulfenylation. Protein sulfenylation is a posttranslational modification that can modulate protein function, making it a regulatory control for a variety of cell signaling networks. Elucidating the role of protein sulfenylation in toxicological exposures provide multiple mechanistic insights, as opposed to just monitoring the reactive chemical species formed as a result of said exposure.