Chemical Penetration Enhancers Increase Hydrogen Peroxide Uptake in *C. elegans* for *In Vivo* Fast Photochemical Oxidation of Proteins

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**ABSTRACT:** Fast photochemical oxidation of proteins (FPOP) is a hydroxyl radical protein footprinting method that covalently labels solvent-accessible amino acids by photolysis of hydrogen peroxide. Recently, we expanded the use of FPOP for *in vivo* (IV-FPOP) covalent labeling in *C. elegans*. In initial IV-FPOP studies, 545 proteins were oxidatively modified in all body systems within the worm. Here, with the use of chemical penetration enhancers (CPEs), we increased the number of modified proteins as well as the number of modifications per protein to gain more structural information. CPEs aid in the delivery of hydrogen peroxide inside *C. elegans* by disturbing the highly ordered lipid bilayer of the worm cuticle without affecting worm viability. IV-FPOP experiments performed using the CPE azone showed an increase in oxidatively modified proteins and peptides. This increase correlated with greater hydrogen peroxide uptake by *C. elegans* quantified using a chemical fluorophore demonstrating the efficacy of using CPEs with IV-FPOP. Mass spectrometry proteomics data are available via ProteomeXchange with identifier PXD019290.

**KEYWORDS:** *in vivo*, protein footprinting, FPOP, *C. elegans*, chemical penetration enhancers

**INTRODUCTION**

Mass spectrometry (MS) has emerged in recent years as a powerful method for analyzing protein structures. Specifically, MS-based protein footprinting methods have been used to study protein conformational changes as well as protein–protein and protein–ligand interactions. These methods utilize a chemical label to monitor changes in solvent accessibility in response to binding events or conformational changes. Depending on the chemical label used, these interactions can monitor changes in solvent accessibility at various time scales extending from nanoseconds to minutes. Hydroxyl radical protein footprinting (HRPF) is a labeling method that utilizes hydroxyl (OH) radicals to label solvent-accessible side chains in proteins on the nanosecond to second time scale. In HRPF, OH radicals are generated by several means including water radiolysis, fenton chemistry, or laser photolysis of hydrogen peroxide. These OH radicals irreversibly label solvent-accessible amino acid side chains. Subsequent downstream proteomic analysis by liquid chromatography tandem MS is then used to identify labeled amino acids and quantify the extent of labeling.

Fast photochemical oxidation of proteins (FPOP) is an HRPF method that utilizes a 248 nm KrF excimer laser for photolysis of hydrogen peroxide to label proteins on the microsecond time scale in the absence of secondary radicals. *In vitro* applications of FPOP include the study of protein conformers, conformational changes, and protein–ligand interactions. Recently, we expanded the use of FPOP for the study of protein structure *in vivo* in *Caenorhabditis elegans* (*C. elegans*), in a new method entitled *in vivo* FPOP (IV-FPOP). *C. elegans*, who share a common ancestor with humans, have been used as a model system for the study of many human diseases. While we were able to show the capabilities of IV-FPOP for *in vivo* protein structural analysis, protein oxidative coverage and the total number of peptides per single protein modified were less than desirable. In total, 545 proteins were oxidatively modified within the worm with an average of one peptide modified per protein. An increase in both the number of modified proteins and the number of modifications per protein would increase the structural information obtained by IV-FPOP.

HRPF methods are susceptible to radical scavenging by different buffer compositions and chemical additives. This scavenging can have a significant impact on the extent of oxidative modifications during *in vitro* FPOP experiments by suppressing the final radical concentration available in the
solution after hydrogen peroxide photolysis. Significant advancements have been made on utilizing dosimeters in FPOP experiments to account for these scavenging effects and to compensate for them. In the case of in vitro FPOP, radical scavenging and quenching effects can be overcome by increasing the hydrogen peroxide concentration to increase the final concentration of hydroxyl radicals. However, for in vivo FPOP, increasing the final hydrogen peroxide concentration prior to photolysis is less desirable since it can have negative effects on sample viability as well as unwanted background oxidative stress in the worms. For IV-FPOP, hydrogen peroxide enters C. elegans both by ingestion and penetration through the skin. Skin penetration is limited by the worm cuticle, a highly structured extracellular matrix that protects C. elegans from the environment. The method also relies on diffusion of H$_2$O$_2$ throughout the worm body. Rather than increasing the concentration of hydrogen peroxide mixed with the worms, we aim to increase the uptake of hydrogen peroxide by the worms.

Here, we report the use of chemical penetration enhancers (CPEs) to increase hydrogen peroxide uptake by C. elegans. The use of CPEs has long demonstrated the ability to increase the permeability of drugs and other chemical compounds in humans and animals by disrupting the highly ordered lipid bilayer of the skin. The CPEs used should be nontoxic to the organism of interest and prove to be effective within the necessary experimental time scale. In the case of IV-FPOP, the CPE used should also not scavenge the OH radicals. We tested five different CPEs of varying chemistries to determine their efficacy in increasing peroxide uptake while not scavenging the OH radicals. These five CPEs, azone (AZ), dimethylacetamide (DMAC), dimethylformamide (DMF), oleic acid (OA), and propylene glycol (PG) (Figure 1), were selected based on literature reports describing their penetration-enhancing activity. These CPEs are from different chemical classifications including amides, fatty acids, glycols, and sulfoxide-like molecules. We chose to test CPEs with varying chemistries because studies have demonstrated that CPE potency is difficult to predict and appears to be drug-specific. We demonstrate that CPEs can increase hydrogen peroxide uptake by C. elegans, which leads to an increase in the number of oxidatively modified proteins by IV-FPOP.

**EXPERIMENTAL SECTION**

**Materials**

Unless otherwise noted, all materials were purchased from Thermo Fisher Scientific and used without any further purification.

**In Vitro FPOP**

The protocol was performed as described by Hambly and Gross with minor modifications. Ubiquitin from bovine erythrocytes (Sigma Aldrich) was prepared in 10 mM phosphate-buffered saline (PBS; Sigma Aldrich) at a final concentration of 0.18 mg/mL in a 50 μL final volume. L-glutamine was added to the protein sample as the hydroxyl radical scavenger at a final concentration of 40 mM. The effect of five different CPEs (azone (AZ), dimethylacetamide (DMAC), dimethylformamide (DMF), oleic acid (OA), or propylene glycol (PG); Sigma Aldrich) on the extent of FPOP modification was tested at various concentrations (0, 0.1, 0.5, 1, and 2%). Immediately prior to FPOP, hydrogen peroxide was added to a final concentration of 7.5 mM. The 50 μL sample was infused using a syringe pump through a 150 μm inner diameter (i.d.) fused silica capillary (Polymer MicroTechnologies) using a 34.19 μL/min flow rate, 20% exclusion fraction, 2.58 mm spot width, and 10 Hz laser frequency. A 248 nm KrF excimer laser (GAM Laser, Inc.) was used to irradiate the sample and photolyze hydrogen peroxide at 161 mJ/pulse. Samples were collected in a vial containing a final concentration of 30 mM methionine and 500 nM catalase to quench excess OH radicals and hydrogen peroxide, respectively. A total of three laser-irradiated samples and three controls (no laser irradiation) were prepared for each condition.

**Global MS Analysis**

Intact mass spectra analysis was completed using a nano-Acquity UPLC in line with a Synapt G2S mass spectrometer (Waters) operating in resolution mode. Each sample was loaded onto a MassPREP Micro Desalting column (Waters), washed for 10 min with 0.1% formic acid (FA) in water (Solvent A), and eluted with 60% acetonitrile (ACN) in 0.1% FA for 10 min at a rate of 100 μL/min. The mass spectrometer electrospray ionization (ESI) source capillary voltage was set at +3.0 kV and 100 °C temperature. The acquisition mass range was 500–2000 m/z. Mass spectra were deconvoluted, and the area of the first +16 modification peak was calculated using UniDec.

**Nematode and Bacterial Culture**

C. elegans strain BY250 (Pdat-1::GFP) was maintained following standard methods on either 8P plates with NA22 Escherichia coli or nematode growth media (NGM) plates with OP50 E. coli as a food source at 20 °C.

**Viability Assay**

Worm viability assays were performed on synchronized L4 larvae in a 96-well plate as previously described with minor modifications. Hydrogen peroxide at a final concentration of 200 mM with the indicated CPE was added to

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**Figure 1.** Chemical structures of the five CPEs tested. Each CPE has varying chemical properties.
approximately 1000 worms. After 30 s, the reaction was quenched using the IV-FPOP quench solution at a final concentration of 20 mM N,N’-dimethylthiourea (DMTU; Acros Organics) and 20 mM N-tert-butyl-α-phenylnitrone (PBN; Sigma Aldrich). Worms were pelleted by centrifugation at 2000 rpm and washed with M9 buffer (0.02 M KH₂PO₄, 0.08 M Na₂HPO₄, 0.08 M NaCl, 1 mM MgSO₄). Worms were suspended in M9 with 40 μM propidium iodide (PI; Sigma Aldrich) and incubated at room temperature for 15 min before fluorescence imaging using a Nikon Eclipse TI microscope.

Hydrogen Peroxide Uptake

Hydrogen peroxide uptake was quantified as described by Fu et al. with some modifications. Peroxy Orange 1 (PO1; Tocris Bioscience) was dissolved in DMSO at a stock concentration of 5 mM and then freshly diluted in M9 buffer to prepare 50 μM PO1 working solution. L4 larvae were incubated with 200 mM hydrogen peroxide mixed with the indicated CPE in M9 buffer at room temperature for 30 s. Immediately after, the reaction was quenched using the IV-FPOP quench solution. Worms were collected by centrifugation at 2000 rpm and washed twice with M9 buffer and re-suspended in 0.1% FA in MPO1 working solution for 2 h at room temperature with mild shaking in the dark. Afterwards, worms were washed twice with M9 buffer, placed in glass slides with coverslips, and subjected to fluorescence imaging. The images were collected using a Nikon Eclipse TI microscope (10 × 0.3 air Nikon objective) with a Texas Red filter (600 nm excitation and emission in the range of 604–644 nm). Imaging data was processed and analyzed using ImageJ (NIH).

IV-FPOP, Protein Extraction, and Proteolysis

The procedure was performed with minor modifications as previously described. Prior to IV-FPOP, worms were kept separate from 200 mM hydrogen peroxide and the indicated CPE, mixed using a homemade flow system, and incubated for approximately 30 s just prior to IV-FPOP. Samples were flowed through a 250 μm i.d. capillary at a final flow rate of 379 μL/min, an irradiation window of 2.58 mm, and 50 Hz laser frequency and the emission in the range of 604–644 nm. Imaging data was processed and analyzed using ImageJ (NIH).

RESULTS AND DISCUSSION

Effect of CPEs on In Vitro FPOP Oxidation

A first step in demonstrating the potential of the five CPEs in increasing hydrogen peroxide uptake for IV-FPOP is determining whether they scavenge hydroxyl radicals. To test the effect of CPEs on the oxidation of the pattern of FPOP, in vitro FPOP of ubiquitin was performed in the presence and absence of five different CPEs. The modification distribution and the peak area for the +16 modification for each condition were compared (Figure 2).

Under standard FPOP conditions, the +16 modification peak was 48% of the unmodified peak of ubiquitin (Figure 2). This is typical of FPOP on ubiquitin and indicates a quantifiable level of modification without over labeling the protein. The +16 modification for DMAC, DMF, and PG showed a significant decrease when compared to the FPOP control (no CPE added). The use of 0.1% DMAC showed a...
contrast, the +16 modifications from our group have shown < 2% loss in viability when incubated in 200 mM hydrogen peroxide for up to 30 s.\textsuperscript{15} For IV-FPOP, an important parameter to consider is the presence and absence of either AZ or OA in combination with hydrogen peroxide should also demonstrate the ability to increase hydrogen peroxide uptake in C. elegans. To quantify hydrogen peroxide uptake in the presence and absence of either AZ or OA in vivo, the fluorophore PO1 was used (Figure 3). PO1 has been shown to linearly quantify in vivo hydrogen peroxide uptake and diffusion in cells and C. elegans.\textsuperscript{25,32} For AZ, no statistically significant changes were observed in the uptake of 200 mM hydrogen peroxide in the presence of 0.1−0.5% AZ. Only 1% AZ showed a significant increase in hydrogen peroxide uptake quantified by PO1 fluorescence (Figure 3A, right). The uptake of hydrogen peroxide by C. elegans using 1% AZ was 1.24-fold higher compared to the samples containing 200 mM hydrogen peroxide in the absence of 1% AZ. Fluorescence imaging of hydrogen peroxide-treated worms in the presence and absence of 1% AZ shows the difference in uptake in the presence of AZ (Figure 3B). These imaging studies also demonstrate diffusion of hydrogen peroxide throughout the full body length of the worm enabling the modification of proteins across all body systems within the worm. Unforeseen, for OA, none of the concentrations tested, 0.1−1%, showed an increase in hydrogen peroxide uptake (Figure 3C, right). These results indicated that only 1% AZ can have advantageous effects in

21.2% decrease for the +16 oxidation peak, while a decrease in 23.2% for the +16 oxidation peak was observed for both 0.1% DMF and 0.1% PG (Figure 2). This was not unexpected for DMAC and DMF, which are similar to sulfoxides, known scavengers of hydroxyl radicals, and are used as CPEs in place of dimethylsulfoxide (DMSO), a potent but problematic CPE. For PG, some glycols, specifically ethylene glycol, have also been shown to be efficient OH radical scavengers.\textsuperscript{29} In contrast, the +16 modification peaks of ubiquitin modified in the presence of AZ or OA did not show any statistical significance decrease at concentrations between 0.5 and 1% (Figure 2 and Supporting Information, Figure S1) when compared to the FPOP control. This suggests that for in vivo FPOP 0.5−1% of either AZ or OA can be used as possible CPEs without observed radical scavenging properties.

**C. elegans Viability in the Combined Presence of Hydrogen Peroxide with CPEs and Hydrogen Peroxide Uptake**

The use of certain CPEs, such as sulfoxides, at low concentrations (0.5−2%) has shown to increase the lifespan of C. elegans by up to 50%, but worm death has been observed at higher concentrations (>6%).\textsuperscript{30,31} Previous viability experiments from our group have shown < 2% loss in C. elegans viability when incubated in 200 mM hydrogen peroxide for up to 30 s.\textsuperscript{15} For IV-FPOP, an important parameter to consider is the additive toxic effects of the combined presence of hydrogen peroxide and a CPE could have on C. elegans viability. Since only two CPEs, AZ and OA, did not show significant radical scavenging properties from the initial five candidates, viability studies using 200 mM hydrogen peroxide were performed in the presence and absence of AZ and OA at varying concentrations (0.5−1%) (Figure 3).

The presence of 0.1−1% AZ plus 200 mM hydrogen peroxide did not show any statistically significant loss in C. elegans viability when compared to the 200 mM hydrogen peroxide control after a 30 s incubation (Figure 3A, left). Additionally, neither the 200 mM control nor the 0.1−1% AZ plus 200 mM hydrogen peroxide showed a loss in viability in comparison to the positive control samples composed of C. elegans in the IV-FPOP quench solution. Similarly, 0.1−1% OA plus 200 mM hydrogen peroxide did not show significant changes in viability when compared to samples either in the presence of 200 mM or the absence of hydrogen peroxide controls (Figure 3C, left).

In addition to minor changes in viability, the presence of AZ or OA in combination with hydrogen peroxide should also demonstrate the ability to increase hydrogen peroxide uptake in C. elegans. To quantitatively quantify in vivo hydrogen peroxide uptake and diffusion in cells and C. elegans, PO1 was used.\textsuperscript{25,32} For AZ, no statistically significant changes were observed in the uptake of 200 mM hydrogen peroxide in the presence of 0.1−0.5% AZ. Only 1% AZ showed a significant increase in hydrogen peroxide uptake quantified by PO1 fluorescence (Figure 3A, right). The uptake of hydrogen peroxide by C. elegans using 1% AZ was 1.24-fold higher compared to the samples containing 200 mM hydrogen peroxide in the absence of 1% AZ. Fluorescence imaging of hydrogen peroxide-treated worms in the presence and absence of 1% AZ shows the difference in uptake in the presence of AZ (Figure 3B). These imaging studies also demonstrate diffusion of hydrogen peroxide throughout the full body length of the worm enabling the modification of proteins across all body systems within the worm. Unforeseen, for OA, none of the concentrations tested, 0.1−1%, showed an increase in hydrogen peroxide uptake (Figure 3C, right). These results indicated that only 1% AZ can have advantageous effects in
increasing hydrogen peroxide uptake, thus increasing the number of hydroxyl radicals generated inside the worm and ultimately increasing the number of oxidatively modified proteins and peptides by IV-FPOP. For subsequent IV-FPOP experiments, 200 mM hydrogen peroxide in the presence and absence of 1% AZ was used.

**IV-FPOP in the Presence of the CPE Azone**

For AZ to be considered a good CPE, the number of oxidatively modified proteins and peptides by IV-FPOP should be greater than the hydrogen peroxide only control. LC-MS/MS analysis of oxidatively modified proteins by IV-FPOP in the absence of AZ showed 1084 ± 81 total modified proteins, while 1140 ± 207 total modified proteins were observed in the presence of AZ (Figure 4A and Tables S1 and S2).

Across two biological replicates, the use of hydrogen peroxide in combination with AZ increased the total number of oxidatively modified proteins by 1.05-fold. Likewise, an increase in the total number of oxidatively modified peptides was observed in the presence of AZ across two biological replicates (Figure 4B and Table S1). In the IV-FPOP control group, only 1755 ± 291 peptides were oxidatively modified, while 1987 ± 556 oxidatively modified peptides were quantified in the presence of AZ (Tables S1 and S2), equal to a 1.13-fold increase in modified peptides across two biological replicates. While the final number of oxidatively modified proteins and peptides did not increase dramatically, the increase fold differences are consistent with the hydrogen peroxide uptake quantified above (Figure 3A, right). The increase is also seen in the total number of oxidatively modified proteins in the background oxidation control samples in the presence and absence of 1% AZ (Supporting Information, Figure S2).

To further demonstrate the advantage of using 1% AZ as a CPE against the IV-FPOP control group, the extent of IV-
FPOP oxidation at the peptide level for the protein myosin was calculated (Supporting Information, Figure S3). Myosin is a highly abundant protein in *C. elegans* and responsible for muscle contraction and intracellular transport. In the presence of 1% AZ, one additional peptide was detected in comparison to the IV-FPOP control group without AZ. This increase in the total number of oxidatively modified peptides per single protein was observed in 203 (28%) proteins when AZ was used (Figure 4C). Still, 399 (54%) proteins showed an equal number of oxidatively modified peptides per single protein in the presence and absence of AZ, while only 132 (18%) proteins showed more modified peptides in the absence of AZ (Figure 4C).

**IV-FPOP in the Presence of Two CPEs**

The use of single CPEs has been shown to offer limited permeability in drug delivery. As a result, a common practice is to employ a mixture of CPEs to enhance drug delivery.33 To test if these synergistic interactions can increase the uptake of hydrogen peroxide in IV-FPOP, the combined use of two CPEs was implemented. Since the initial screening demonstrated 1% AZ and 1% OA did not affect the labeling pattern of in vitro FPOP, two different combinations of AZ in addition to OA were tested (Figure 5).

When comparing the peak area for the +16 modification of in vitro FPOP on ubiquitin, a 2% decrease in oxidation was observed when using 0.5% AZ plus 0.5% OA (0.5% 2CPEs), while a 4.7% decrease in oxidation was observed when using 1% AZ plus 1% OA (1% 2CPEs) (Figure 5A). However, the decrease in oxidation was not statistically significant, indicating that either combination of both CPEs is applicable for IV-FPOP.

Viability experiments in the presence of either 0.5% 2CPEs or 1% 2CPEs showed no statistically significant decrease in *C. elegans* viability when exposed to 200 mM hydrogen peroxide in comparison to the hydrogen peroxide control (Figure 5B). Interestingly, hydrogen peroxide uptake quantitation showed only 0.5% 2CPEs to significantly increase the absorption of hydrogen peroxide by *C. elegans*, while 1% 2CPEs did not show an increase (Figure 5C). Across two biological replicates, IV-FPOP experiments using 0.5% 2CPEs showed 685 ± 70 oxidatively modified proteins (Table S3), while only 607 ± 13 proteins were modified in the absence of 0.5% 2CPEs (Table S4), equivalent to a 1.13-fold increase in oxidatively modified proteins (Figure 5D and Tables S1 and S2). Unexpectedly, the fold increase in oxidatively modified proteins by 0.5% 2CPEs is equal to the increase in modified proteins when only 1% AZ is present. Although both conditions show an increase in hydrogen peroxide uptake when compared to hydrogen peroxide control (Figure 5C), 0.5% 2CPEs and 1% AZ are not statistically significant when compared to each other. These results suggest that AZ is majorly responsible for the increase in oxidatively modified proteins by IV-FPOP, and the combined use of AZ and OA is not additive.

**CONCLUSIONS**

Here, we report the use of CPEs to increase the total number of oxidatively modified proteins and peptides by IV-FPOP. Through in vitro FPOP, viability, and hydrogen peroxide uptake quantitation, only one CPE, AZ, showed to be advantageous to increase oxidatively modified proteins by IV-FPOP. This amide molecule did not scavenge OH radicals but did increase the uptake of hydrogen peroxide through the skin leading to a higher number of oxidatively modified proteins. This first attempt at using CPEs indicates the effectiveness of using this as an approach to increase the number of oxidatively modified proteins and modified peptides by IV-FPOP. This will increase the structural information obtained by IV-FPOP, thus increasing its utility as a method for structural biology. Further optimizations such as increased incubation time with the CPE may be useful to further increase the number of modified proteins. However, this must be carefully controlled as to not structurally damage the cuticle of the worms. There are strains of *C. elegans* that have disruptions in the low permeable cuticle, but these strains have other phenotypes that alter the biological function and may not be desirable when studying certain disease states.34,35 Perhaps the most useful approach would be to test more CPEs to determine which works best with hydrogen peroxide. Since it has been demonstrated that CPE efficacy is drug- or chemical compound-specific, a trial and error study of multiple CPEs would be useful in identifying the best CPE for hydrogen peroxide uptake. Our data demonstrates this with the OA CPE, which was not compatible with *H. O₂* even though its CPE activity for other compounds is well documented. Studies have shown that the mechanisms of enhancement between OA and AZ are different. The cis double bond in OA contributes to the mechanism of enhancement of this molecule and differentiates OA from AZ.36 It is highly probable that *H₂O₂* is reducing this double bond lowering the efficacy of this molecule as a penetration enhancer.37,38 Literature reports show that the cis double bond in OA is oxidizable by reactive oxygen species (ROS) to synthesize azelaic and pelargonic acid.39,40 AZ, which worked well as an *H₂O₂* enhancer, does not have this double bond. Here, we have tested CPEs classified as sulfoxide-like, fatty acids, amides, and glycols. Other chemistries available include alcohols, esters, glycol esters, pyrrolidones, surfactants, and terpenes. Undoubtedly, some of the molecules in these categories will be radical scavengers but others may be useful as CPEs for hydrogen peroxide uptake.

The IV-FPOP study testing the 2CPE combination had significantly fewer proteins modified than the original AZ study. Across two biological replicates in the AZ study, 1084 proteins were modified in the FPOP control sample, while only 607 were modified in the FPOP control for the 2CPE study. This difference in modification could be due to unforeseen scavenging and underscores the need for dosimetry in IV-FPOP to detect and compensate for scavenging. The increase in oxidatively modified proteins shown in the AZ study was concurrent with the quantified increase in hydrogen peroxide uptake using the PO1 fluorophore. This demonstrates the utility of PO1 in quantifying hydrogen peroxide uptake and the potential to use this molecule to compare hydrogen peroxide conditions across replicate worm samples. PO1 is highly specific for *H₂O₂* over other reactive oxygen species,32 so it is sensitive to hydrogen peroxide quantification though is short of a true dosimeter for IV-FPOP studies, where quantification of OH radicals is most desirable. It is unclear whether PO1 would be a good dosimeter for IV-FPOP because radical generation would lead to a decrease in fluorescence potentially leading to a loss in sensitivity of the measurement and because the molecule is not a direct measure of radical generation. However, the usefulness of PO1 quantifying hydrogen peroxide uptake does suggest a fluorescence-based dosimeter may be successful for IV-FPOP.
This initial CPE study does demonstrate the effectiveness that these molecules have in increasing the hydrogen peroxide uptake of C. elegans. IV-FPOP in the presence of 1% AZ showed an increase in the final number of oxidatively modified peptides per single protein, thus demonstrating the ability to gain higher structural information in vivo. Nonetheless, a higher increase in oxidatively modified proteins and peptides is still desired, requiring further screening of compatibility of other CPE categories with IV-FPOP, like alcohols, amides, or esters.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.0c00245.

Intact mass analysis of ubiquitin (Figure S1); background oxidation (Figure S2); C. elegans myosin extent of oxidation (Figure S3).

IV-FPOP oxidatively modified proteins using 200 mM hydrogen peroxide plus 1% AZ (Table S1); IV-FPOP oxidatively modified proteins using 200 mM hydrogen peroxide only (Table S2); IV-FPOP oxidatively modified proteins using 200 mM hydrogen peroxide plus 2CPEs (Table S3); IV-FPOP oxidatively modified proteins using 200 mM hydrogen peroxide (2CPE FPOP Control) (Table S4) (XLSX)

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**Author Contributions**

L.M.J. devised the project, Z.Z. performed hydrogen peroxide absorption imaging, and J.A.E. performed viability, IV-FPOP experiments, and data analysis. L.M.J. and J.A.E. wrote the manuscript.

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