SUPPORTING INFORMATION: The Sulfate Radical Anion is a New Reagent for Fast Photochemical Oxidation of Proteins (FPOP) †

Global mass spectrometry of FPOP-labeled β-lactoglobulin. The capacity of the 0.6 μL bed $Ziptip_{C4}$ was approximately 3.3 μg, so that 180-230 pmol samples of BLG were infused at a flow rate adjusted to ensure accuracy in the time-to-digital conversion of the QTOF multi-channel plate detector, requiring 80-180 ion counts/scan base peak. Scans spanning the entire chromatogram were summed to improve the signal to noise, typically 60-150 scans depending on the flow rate.

β-lactoglobulin FPOP global product distribution analysis. A 1216-1245 *m/z* spectrum window centered about the 15th charge state of BLG was fit with a model FPOP product distribution described previously[1] for each BLG duplicate. The window range encompassed all detected product peaks, and a 10 *m/z* region lower than the unmodified peak average *m/z* was used for baseline estimation. A Mathcad 14 Minimize algorithm was used to fit a Poisson distribution to the resulting set of 0, +16, +32… state abundances.

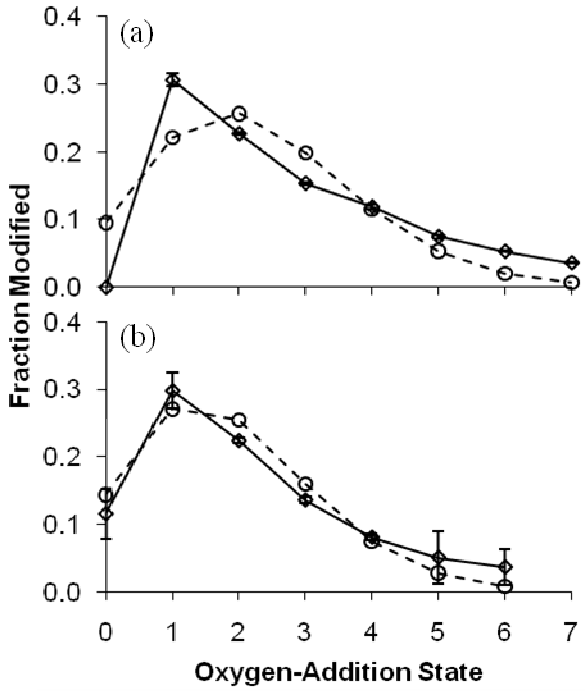
Optimal Sodium Persulfate FPOP Conditions. Tuning the level of radical exposure controls the kind and extent of modifications. Early footprinting work used Fenton-generated •OH radicals to cleave DNA and protein backbones; absence of a cleavage product was indicative of protection due to biomolecular interaction.[2] The ESI mass spectrum of BLG labeled by FPOP with 15 mM $Na_2S_2O_8$ and without Gln radical scavenger shows a background in each BLG charge state m/z region ≥ 20% the charge state's base peak (data not shown). We attribute this high baseline to the mass-spectral convolution of many protein fragments generated by radical-induced cleavage along the protein backbone. Under these conditions, too much radical labeling agent has persisted for too long an exposure. The reactivity of protein side

chains is, in general, not high at α-carbons because steric hindrance is protective under typical low-exposure conditions.[3, 4] Thus, the identification and quantitation of side chain-modified residues for *scavenger-free* $Na_2S_2O_8$ FPOP-labeled protein would not measure the solvent accessibility at these sites in the protein's native state.

A more difficult problem is that this excessive labeling may also be the case with insufficient scavenger or too high a concentration of the radical precursor; that is, the quenching is not fast enough to stop labeling before side chain modifications cause protein conformational change leading to misleading labeling. Therefore, we undertook an analysis on the underlying protein product distributions of the Figure 1 spectra to support the hypothesis that 5 mM $Na_2S_2O_8$, 20 mM Gln (Figure 1c) is sufficient to label the native protein state without sampling partially unfolded products.

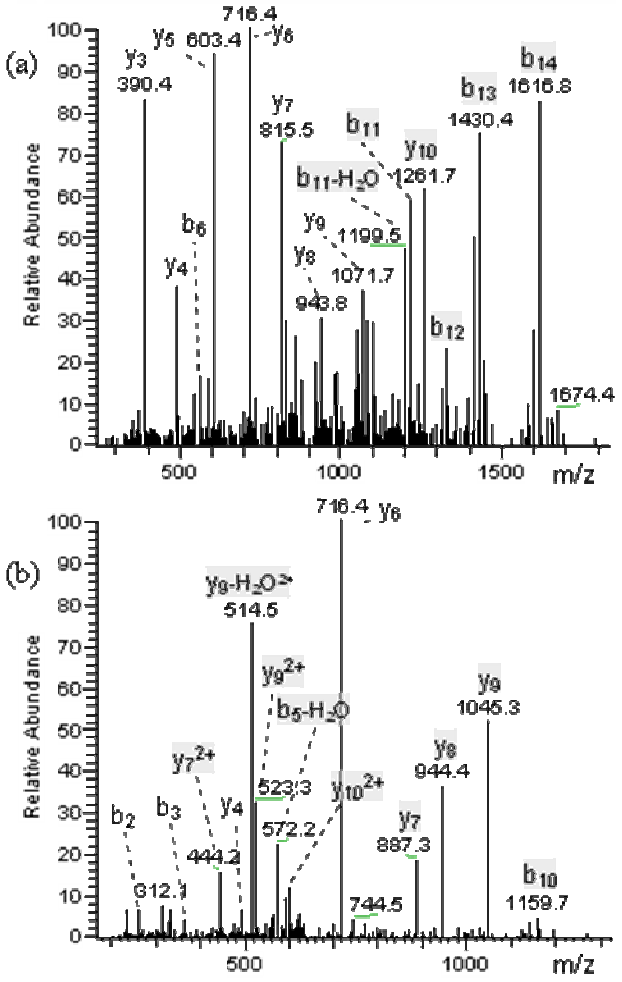
In a previous study, we provided empirical evidence that FPOP with H_2O_2 labels proteins faster than any conformational response to •OH modifications.[1] This result was based on the analysis of the mass spectra of the modified proteins, where a model was used to digitize signals for FPOP-labeled proteins into bins of primary modification of 0, +16, +32, … Da. Simplification of the modification spectrum to a 0, +16, +32, … distribution allowed for a comparison to a Poisson distribution. For BLG, CaM, and lysozyme, a good match occurred only when radical scavenger was present. Testing for a Poisson distribution, which should apply for proteins having an invariant single conformation during labeling and for which there are many sites available to •OH modification, is an appropriate means of establishing the "snapshot" nature of a footprinting method . We employed the same analysis here, using BLG as the test protein because it is highly sensitive to conformational changes due to oxidation.[5] At 5 mM $Na_2S_2O_8$, the modification distribution is approximately Poisson (Supplemental Figure 1), but

when a larger number of sulfate anion radicals are produced with 15 mM $Na_2S_2O_8$, FPOP labeling gives a distribution that clearly fails this test. Moreover, modeling the latter spectrum failed to meet a requirement that at least 20% of protein signal should contribute to the unmodified, 0^{th} state. This requirement stems from matching the laser pulse frequency, irradiation volume, and sample flow rate to ensure that all labeled protein, and an un-reacted volume that is 20% of the irradiation volume, vacates the flow cell before the next laser shot. At 5 mM $Na_2S_2O_8$, the 20% exclusion fraction can be properly accounted for by the modeling, and the 0, +16, +32, … distribution is consistent with a single BLG conformation during labeling. Thus, the appropriate persulfate level for FPOP labeling is $\leq$ 5 mM $Na_2S_2O_8$, with 20 mM constituent Gln and laser and optics parameters set as described in the Experimental Procedures section.



Supporting Information Figure 1. The 0, +16, +32, ... Da ion counts are modeled for the 15th charge state QTOF *m/z* spectrum of β-lactoglobulin. Solid line-connected diamonds with standard error bars plot the average of the normalized ion counts for duplicate FPOP treatments. Dashed line-connected circles show the non-linear regression, best-fitting Poisson distribution. (a) is for FPOP with 15 mM $Na_2S_2O_8$. (b) is for FPOP with 5 mM $Na_2S_2O_8$. The modeling was constrained to subtract 20% of total protein signal from the 0th state because this signal is attributed to the irradiation-masked FPOP volume. In case (a), all 0th state signal is attributed to this non-reaction fraction but is modeled as only 7.4% of total protein signal. The number of states per sample distribution fit to a Poisson was chosen to account for at least 98% of protein signal.

Chemistry of $Na_2S_2O_8$ FPOP. Comparing the product-formation reactivities of SO_4^{-}• and •OH requires quantitation with good precision of every detectable modification. Figure 3 shows the product-ion mass spectra for two unusual modifications. Kynurenination (Figure 3a, +3.9949) of tryptophan is not a major oxidation product in water radiolysis labeling of Trp-NH_2[6], but it is a common metal-catalyzed protein oxidation product.[7-9] This modification pathway significantly contributes to the total Trp modification with both H_2O_2 and persulfate FPOP methods. A novel His and Tyr modification only seen with persulfate FPOP, however, is of +33.974 Da (Figure 3b), which comprises 50-90% of the total modification reactions of His but is of trivial abundance for Tyr. The nature of this modification has not yet been determined.



Supporting Information Figure 2. The LTQ product-ion spectra of myoglobin peptides showing uncommon •OH modifications. (a): $[M + 2H]^{2+}$ of *m/z* 910.4569. The annotation is for peaks matching the theoretical y- and b- fragment ions of GLSDGEW*QQVLNVWGK, with W7 modified by a net +4 Da (kynurenine) mass shift. Ions labeled in gray boxes carry this modification. These spectra were produced in both peroxide and persulfate FPOP replicates. (b): $[M +2 H]^{2+}$ of *m/z* 653.3199. The annotation is for LFTGH*PETLEK with H36 modified by a net

+34 Da mass shift. Ions labeled in gray boxes carry this modification. This spectrum was only observed for persulfate FPOP replicates.

Supporting Information Table 1: Calcium-free Calmodulin Fraction Modified per Residue[a]

residue	SASA ($Å^2$)[a]	$k_{\bullet OH}$ (M^{-1} sec^{-1})[b]	native	peroxide	persulfate	persulfate control
T5	55.63	5.1E+08	0.16 +/- 0.01%	1.25 +/- 0.05%	0.22 +/- 0.01%	0.15 +/- 0.02%
I9	58.52	1.8E+09	0.026 +/- 0.005%	0.159 +/- 0.008%	0.022 +/- 0.007%	0.016 +/- 0.004%
F12	7.58	6.9E+09	0.76 +/- 0.03%	2.9 +/- 0.2%	1.19 +/- 0.04%	0.67 +/- 0.01%
K13	112.84	3.5E+08	0.20 +/- 0.01%	0.224 +/- 0.004%	0.17 +/- 0.01%	0.17 +/- 0.02%
T28	47.02	5.1E+08	0.5 +/- 0.2%	2.6 +/- 0.4%	0.13 +/- 0.08%	0.06 +/- 0.04%
E31	46.21	2.3E+08	2.6 +/- 0.4%	9.4 +/- 0.8%	7.8 +/- 0.4%	4 +/- 1%
M36	2.44	8.5E+09	13 +/- 2%	35 +/- 2%	33 +/- 1%	15 +/- 4%
M76	39.87	8.5E+09	24 +/- 1%	49 +/- 3%	62 +/- 2%	26 +/- 2%
S81	46.75	3.2E+08	1.5 +/- 0.2%	4.3 +/- 0.3%	3.4 +/- 0.4%	2.3 +/- 0.5%
Y99	101.55	1.3E+10	0.33 +/- 0.02%	2.0 +/- 0.2%	2.2 +/- 0.1%	0.22 +/- 0.02%
M109	0	8.5E+09	17 +/- 2%	46 +/- 5%	45 +/- 3%	25 +/- 5%
M124	15.55	8.5E+09	43 +/- 4%	79 +/- 9%	64 +/- 4%	46 +/- 6%
M144	43.19	8.5E+09	37 +/- 2%	59 +/- 2%	58 +/- 3%	44 +/- 4%
M145	10.97	8.5E+09	11 +/- 1%	49 +/- 7%	48 +/- 4%	19 +/- 2%

[a]All Table 1 footnotes apply except that 1CFC.pdb was used for the calmodulin SASA calculation (51).

Supporting Information Table 2: Peptide Mixture Fraction Modified per Residue

residue	$k_{\cdot OH}$ (M^{-1} sec^{-1})[a]	native	peroxide	persulfate	persulfate control
Y4_Angiotensin II	1.3×10^{10}	0.027 +/- 0.006%	11 +/- 4%	17.8 +/- 0.9%	0.8 +/- 0.1%
P7_Angiotensin II	6.5×10^{8}	0	1.7 +/- 0.4%	0.020 +/- 0.008%	0
F8_Angiotensin II	6.9×10^{9}	0.0010 +/- 0.0005%	6 +/- 1%	1.1 +/- 0.2%	0.0011 +/- 0.0002%
P2_Bradykinin	6.5×10^{8}	0.000154 +/- 0.000002%	0.4 +/- 0.2%	0.07 +/- 0.01%	0.00007 +/- 0.00003%
F5_Bradykinin	6.9×10^{9}	0.0016 +/- 0.0004%	7 +/- 2%	1.66 +/- 0.05%	0.05 +/- 0.04%
S6_Bradykinin	3.2×10^{8}	0.013 +/- 0.002%	1.9 +/- 0.7%	0.6 +/- 0.3%	0.3 +/- 0.1%
P7_Bradykinin	6.5×10^{8}	0.0008 +/- 0.0003%	3 +/- 2%	1.2 +/- 0.3%	0.000400 +/- 0.000009%
F8_Bradykinin	6.9×10^{9}	0.0008 +/- 0.0004%	3 +/- 1%	1.11 +/- 0.03%	0.0007 +/- 0.0004%

[a] http://allen.rad.nd.edu/browse compil.html.

(1) Gau, B. C.; Sharp, J. S.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **2009**, *81*, 6563-6571.
(2) Heyduk, E.; Heyduk, T. *Biochemistry* **1994**, *33*, 9643-9650.
(3) Hawkins, C. L.; Davies, M. J. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2001**, *1504*, 196-219.
(4) Hawkins, C. L.; Davies, M. J. *J. Chem. Soc., Perkin Trans.* **1998**, *2*, 2617-2622.
(5) Venkatesh, S.; Tomer, K. B.; Sharp, J. S. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 3927-3936.
(6) Xu, G.; Chance, M. R. *Anal. Chem.* **2005**, *77*, 4549-4555.
(7) Stadtman, E. R.; Judith, P. K. In *Methods in Enzymology*; Academic Press, 1995; Vol. Volume 258, pp 379-393.
(8) Finley, E. L.; Dillon, J.; Crouch, R. K.; Schey, K. L. *Protein Sci.* **1998**, *7*, 2391-2397.
(9) Sofia, G.; Rui, V.; Rosário, D.; Francisco, A.; Pedro, D. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2307-2315.