FPOP Labels Proteins Faster than They Unfold.

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1) Hambly, D.M., Gross, M.L. Laser Flash Photolysis of Hydrogen Peroxide to Oxidize Protein

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Introduction

- Fast photochemical oxidation of proteins (FPOP) is a chemical footprint method.
- Exposed hydrophobic residues are labeled by hydroxyl radical giving a +16 Da oxygen addition component, which is determined by mass spectrometry.

Methods

General FPOP Scheme

Apo-Calmodulin

- START 150 μM protein, 20 mM GSH scavenger
- Add H2O2 just prior to titration
- Collect in single tube, 20 mM PBS, 20 mM EGTA as a scavenger of extraneous calcium
- All proteins run in 5% SDS, power 47 nJ/pulse, 10 mM Met
- "Normal" samples as described
- "Absent Scavenger" samples, no GSH
- "Absent" samples, no GSH, EGTA, or Met
- "20% Exclusion Volume" samples: flow rate & pulse frequency, adjust to establish target exclusion volume

Mass Spectrometry

- Lactoglobulin

- Protein is denatured in 8 M urea prior to spectrum acquisition. Carbamylation side-reactions prevented satisfactory fitting of spectra of fully denatured samples (data not shown).

Deducing the Oxygen Product State Distribution

- Model

- The oxygen state amplitude IC’s were determined by a Non-linear quasi-Newton regression fitting of BH to IC’s (x) in the spectrum with MathCAD 14 on a dedicated 8 Xeon core server with a tolerance of 10^-13.

Conclusions

- FPOP with radical and peroxide control provides a means of footprinting proteins without perturbing their conformation during labeling. Although some proteins underwent several oxidations at longer times (by time of DISMAL), the unifying feature was that FPOP, as indicated by the fit of product distributions to a Poisson distribution (e.g., higher oxygen states of Lactoglobulin peak higher charge states than lower oxidized forms, indicating more sites were exposed). Moreover, this fit was not ad-hoc as the RMSD for three proteins is significant. It may play a valuable role in any oxidation footprinting.