How to achieve a more flexible platform:

Pros & Cons for Current Settings

The estimated labeling time, based on known rate constants, is \( \approx 1 \) µs, a timescale sufficiently short to label only one protein conformation [2].

High speed of FPOP makes feasible an MS-based means of protein footprinting and monitoring on that timescale [3].

Typical, previously established parameters that lead to 1 µs labeling are 15 mM \( \text{H}_2\text{O}_2 \), 20 mM glutamine, 10 µM protein, 40 µJ/pulse laser energy.

Pro & Cons for Current Settings

- Current parameters are suitable for a general protocol, already proven to reveal structural information and conformational change.
- Fixed OH lifetime may afoard too much or too little labeling, depending on sequence of protein under study.

Numerical Simulations

The black curve represents decrease of \( \text{OH} \) from its initial value (0.15 mM) to 10^(-11) M, indicating the lifetime of OH is \( \approx 1 \) µs when there is quencher (20 mM guanidine) in the system.

The green curve represents decrease of \( \text{OH} \) when no quencher is present, the lifetime of \( \text{OH} \) becomes much longer under this condition.

The dashed line describes the reaction between \( \text{OH} \) and a reporter site in this case. The concentration of modified (R-OH) species increases first and then leaves off, indicating the completion of OH labeling.

The reaction between \( \text{OH} \) and reporter is:

\[
R + \text{OH} \rightarrow R-\text{OH}
\]

Simulations predict decrease of \( \text{OH} \) at various \( \text{Glutamine} \) concentration, \( \text{H}_2\text{O}_2 \) concentration, laser power and quencher identity. The A value represents the area under each simulation curve.

Summarize of Experimental Results

We used MathCAD to quantitate the extent of modifications in the spectra. The fraction modified is calculated as (modified/unmodified) for all the spectra.

The extent of modification varies nearly linearly with changing of [glutamine], [\( \text{H}_2\text{O}_2 \)], or laser power, indicating that lifetime of \( \text{OH} \) radicals is controllable.

Reference


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Dosimetry experiments were designed to capture \( \text{OH} \) with Phr and then quantify \( \text{OH} \) produced by laser photolysis by using calibration solutions of Phr and Tyr at various relative concentrations but a total of 2 mM.

A calibration curve is based on ratio of [Tyr + H] to [Phr + H]; intensity for both calibration solutions in which the Tyr fraction increases from 5% to 45%.

Experimental Practice

Intensity of [Tyr + H]
Intensity of [Phr + H]

Dosimetry experiment determined that the initial [OH] formed in each laser pulse is 0.15 µs.

FPOP Experiment

As suggested by numerical simulations, the lifetime of \( \text{OH} \) in FPOP can be varied by adjusting variables in an experiment that should result in different extents of modifications by \( \text{OH} \). This simulation result is consistent with experimental data for ubiquitin, whereby the mass spectra for its +9 charged state for various adjusted experimental conditions show the effects we seek (see below).

Changing scavenger, [Gln], from 20 to 30 mM

Changing Laser Power from 38.5 to 27.3 mJ/pulse

Changing [H_2O_2] from 15 to 5 mM

Summary of Experimental Results

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