Hydroxyl Radical Protein Footprinting Reveals Buffer Effects in Adalimumab Biosimilar Aggregation and Heat Shock Tolerance

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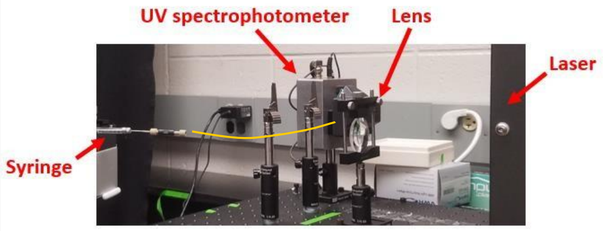
Overview

- **Maintenance of higher-order structure in biotherapeutics necessary for safety and efficacy**
- **Hydroxyl radical protein footprinting (HRPF) is a rapid method for quantitatively comparing protein topography**
- **Two samples with different radical scavenging capacities can yield artifactual differences in HRPF data**
- **Kinetic models and experimental data indicate that differential scavenging effects can be compensated through the use of an internal radical dosimeter standard to equilibrate effective radical exposures**
- **Using dosimetry-based compensation, we identified that citrate buffer resulted in a detectable increase in aggregation of adalimumab biosimilar, confirmed by DLS.**
- **Dosimetry-based compensation HRPF determined that polysorbate-80, which does not alter protein topography alone, completely protects from structural effects due to heat shock at 55°C.**

Introduction

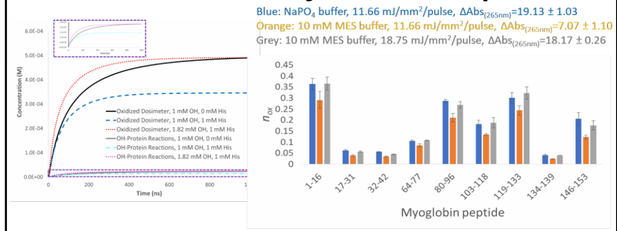
Fast Photochemical Oxidation of Proteins (FPOP) is a fast benchtop method of probing for changes in protein structure, conformation and stability. However, the background scavenging effects of different additives have limited the application of FPOP in several biomedically important areas, including the analysis of formulation on biotherapeutic structure, conformational stability, and aggregation. Here, we present a method for applying FPOP to the analysis of additive effects on the conformation and aggregation of a recombinant biosimilar of the therapeutic mAb adalimumab. Using inline radical dosimetry, we are able to compensate for varying radical scavenging effects of the mAb formulation in real time without compromising the resolution of the FPOP analysis. The resulting data inform a structure-based understanding of the mAb aggregation.

FPOP Optical Bench for Compensated HRPF



A single-lens setup was used with a Compex Pro 102 KrF excimer laser run at a focused fluence of ~10.7 mJ/mm^2/pulse. A Pioneer inline UV spectrophotometer (GenNext Technologies)[1] was used to monitor the oxidation of an internal adenine dosimeter through changes in UV absorbance. Hydroxyl radical dose compensation was performed by altering the hydrogen peroxide concentration until the dosimeter response was equivalent between two samples. For experiments altering radical dose by changing laser fluence, a 1mm aperture is installed just in front of the capillary to maintain a uniform illuminated width.

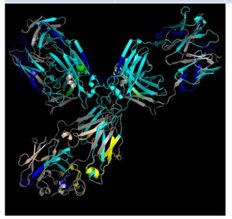
Validation of Dosimetry-Based Compensation



(Left) Four component kinetic simulation of compensation using Tenua. Addition of 1 mM histidine scavenger reduced oxidation of both the protein analyte and the dosimeter. Increasing the amount of hydroxyl radical generated to the point where dosimeter oxidation in the scavenger sample was equivalent to the scavenger-free sample also yielded equivalent protein analyte oxidation. **(Right)** Experimental validation of dosimeter-based compensation examining topography of myoglobin in radical-unreactive sodium phosphate buffer versus scavenging MES buffer. When fluence is increased to compensate for the scavenging capacity of MES, the HRPF results of myoglobin are equivalent to phosphate buffer.[1]

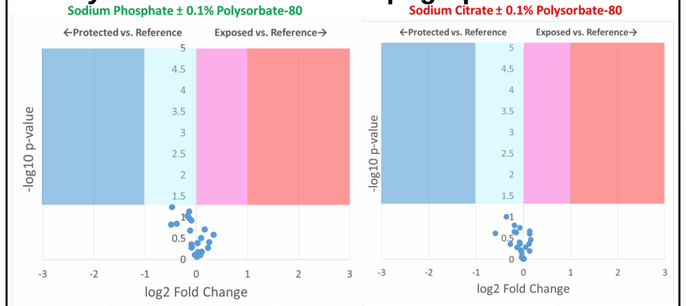
Citrate Buffer Increases Aggregation of Biosimilar

	ΔAbs265 (mAU)	St. Dev
Phosphate	9.767	0.309
Citrate	5.033	0.525
Citrate (Comp)	9.433	1.266



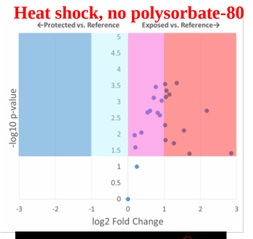
(Left) Volcano plot of compensated FPOP of adalimumab biosimilar in sodium phosphate buffer (reference state) vs. citrate buffer. The X-axis shows fold change in oxidation versus the reference state, while the Y-axis shows the p-value of the result from a triplicate measure, both in log scale. **(Upper right)** Inline adenine dosimetry measurements underlying compensation. **(Bottom right)** Compensated FPOP results plotted against a model of the adalimumab biosimilar structure, with protection/exposure plotted as colored on the volcano plot. Widespread protection was observed, often characteristic of aggregation. DLS data showed both in increase in the average oligomer size, and an increase in the proportion of protein in an oligomeric state (data not shown).

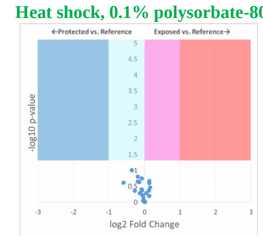
Polysorbate-80 Has No Topographical Effect

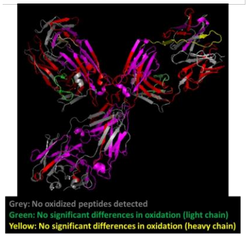


Compensated FPOP of adalimumab biosimilar showed no topographical change after addition of polysorbate-80 in **(left)** sodium phosphate buffer or **(**right) sodium citrate buffer. DLS data similarly showed no change in aggregate size or amount (data not shown).

Polysorbate-80 Protects from Topographical Changes Due to Heat Shock







(Upper Left) Compensated FPOP of adalimumab biosimilar in sodium citrate buffer (reference state) before and after heat shock at 55°C for 1 hour. **(Bottom Left)** Heat shock leads to disaggregation, with the largest changes in the Fab region. **(Right)** Addition of polysorbate-80 completely eliminates the topographical effects of heat shock. While heat shock had a different effect in sodium phosphate buffer, polysorbate-80 similarly relieved all effects (data not shown).

Conclusions:

- Compensated HRPF allows for the comparison of protein higher order structures even in vastly different scavenging backgrounds
- Topographical changes in biosimilar structure identified by compensated HRPF consistent with DLS data, but provides additional structural resolution and molecular detail
- Using real-time compensation, biopharmaceuticals in vastly different formulations can be compared for higher order structure changes

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References

1. Sharp JS; Misra SK, *et al,* (2018) *Anal Chem* 6;90 (21): 12625-12630.