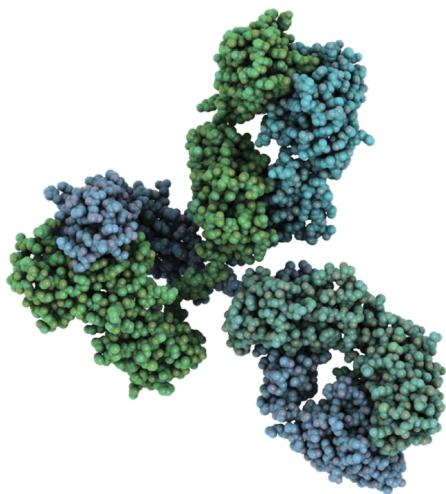


# Revealing Antibody Aggregation with Fox™ Protein Footprinting Technology

Understanding protein-protein interactions so researchers can develop high concentration biotherapeutics.



## Facing the Noncompliance Challenge

Therapeutics which require protracted and often painful intravenous administration can result in medication noncompliance by patients. When the drug delivery experience is unpleasant, avoidance is common, especially among patients with chronic disease.

In response to this challenge, Biopharmaceutical companies strive to develop antibodies and other protein therapeutics that increase the concentration of the compound so that a single, small intramuscular or subcutaneous injection is efficacious.

However, high antibody concentration pushes the molecules closer together causing molecular crowding. In turn, crowding increases the propensity for protein-protein interactions that can disrupt the hydrogen bonding matrix, induce protein misfolding, and can eventually manifest as antibody aggregation.<sup>1</sup>

When developing high concentrations of antibodies, it is essential to pinpoint the interfacial domains of protein interaction by conducting protein aggregation studies. By understanding antibody Higher Order Structure (HOS), the antibody formulation can be optimized to limit aggregation.

Available approaches for conducting protein aggregation studies range from high-resolution but very difficult to perform techniques such as NMR, X-Ray Crystallography, and CryoEm to low resolution, more rudimentary approaches like CD spectroscopy, light scattering, and UV-Vis absorbance. Nestled right in the middle is Hydroxyl Radical Protein Footprinting (HRPF), a robust technology that produces high quality data with a relatively easy workflow.

## HRPF for HOS Analysis

The Flash Oxidation (Fox™) Protein Footprinting System (the only commercially available HRPF platform) is gaining momentum in protein interaction studies because of its relatively simple workflow that produces high-resolution, reproducible results.<sup>2</sup>

This method utilizes hydroxyl radicals ( $\cdot\text{OH}$ ) to covalently modify solvent exposed side chains of amino acids. Because  $\cdot\text{OH}$  modification is very stable, there is ample time to denature and digest the protein for subsequent bottom-up proteomics. The average peptide and residue oxidation is calculated by examining the chromatographic peak areas of the unmodified and modified peptides. When solvent accessibility has changed due to alterations in protein structure or interactions,  $\cdot\text{OH}$  modification concordantly changes.



## Identifying Interfacial Domains of mAb Aggregation Using Fox™-Based HRPF

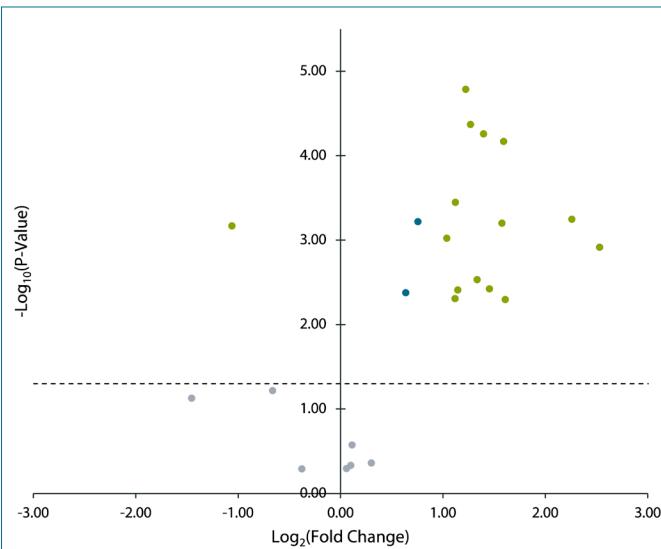
Excipients such as amino acids can stabilize high concentrations of antibodies by limiting protein intermolecular interactions.<sup>3</sup>

A differential Fox-based HRPF experiment compared the change in oxidation between an aggregated antibody formulation and a non-aggregated formulation that contained an amino acid excipient. The results showed that peptides involved in the intermolecular interactions for the aggregated samples were protected from •OH modification, whereas with the addition of the amino acid, many of those same peptides were exposed to •OH attack.

Employing a volcano plot, the peptides with significant change in oxidation were easily identified (Figure 1). Several peptides significantly increased in oxidation following amino acid addition. Figure 1 highlights the substantial intermolecular interactions that predominated and were ultimately disrupted by the addition of the amino acid. Armed with this actionable data, drug researchers can make further improvements to antibody design and formulation.

## Take the Next Step

Start doing structural biology the easy and robust way. Learn how GenNext Technologies products can fit smoothly into your lab's workflow or test-drive the Fox System on an outsourced project basis.



**Figure 1: Volcano plot comparing the change in oxidation before and after the addition of an amino acid.** Peptides with a p-value of  $\leq 0.05$  are plotted above the dotted line. Peptides highlighted in blue have a fold change of less than two and peptides highlighted in green have a fold change equal to or greater than two. Peptides with a positive fold change increased in oxidation following the amino acid addition while a negative fold change indicates a decrease in oxidation.

## References

- 1) Shire SJ, Shahrokh Z, Liu J. [Challenges in the development of high protein concentration formulations](#). J Pharm Sci. 2004 Jun.
- 2) Sharp JS, Chea EE, Misra SK, Orlando R, Popov M, Egan RW, Holman D, Weinberger SR. [Flash Oxidation \(FOX\) System: A Novel Laser-Free Fast Photochemical Oxidation Protein Footprinting Platform](#). J Am Soc Mass
- 3) Kemter K, Altrichter J, Derwand R, Kriehuber T, Reinauer E, Scholz M. [Amino Acid-Based Advanced Liquid Formulation Development for Highly Concentrated Therapeutic Antibodies Balances Physical and Chemical Stability and Low Viscosity](#). Biotechnol J. 2018 Jul.

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## Discover the Benefits of Protein Footprinting

Contact us for products and services to investigate biopharmaceutical structure, interactions, folding, aggregation, formulation, and delivery.