Immuto Scientific

Aggregation of Antibodies and Strategies to Mitigate Risk Early in Development

Why do antibodies aggregate?

Aggregation is a critical issue in the development of antibodies. It can be attributed to partial unfolding events due to stress conditions exposing residues that have a tendency to undergo modification and/or form new interactions leading to aggregation. Protein structure, vs. sequence alone, is critical for understanding aggregation.

How can we mitigate aggregation?

Although aggregation of antibodies can be affected by experimental conditions such as temperature, pH and concentration, they are largely dependent on antibody's intrinsic properties as determined by its structure. Characterizing the higher-order structure (HOS) of protein aggregates and localizing the interfacial domains of protein interactions provides critical information about the molecular mechanism of aggregation and directs sequence modification to change the most aggregation-prone residues.

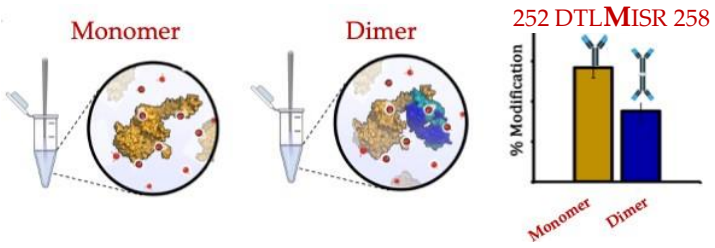
Hydroxyl Radical Footprinting (HRF) for characterizing aggregation

Recently, mass spec-based Hydroxyl Radical Footprinting (HRF) has gained momentum for characterization of protein aggregates. In HRF, hydroxyl radicals covalently modify amino acid side chains of proteins. Once the protein has undergone the rapid labeling process, the sample is enzymatically digested and analyzed according to well established proteomics techniques.

HRF resolves regions involved in protein aggregation, conformational changes, and intermediate structures that form during aggregation at amino acid-level resolution. Furthermore, it delivers results in under a week and is compatible with a 96-well plate high throughput format to characterize and compare aggregation sites of a multitude of samples with different formulations, concentrations, or temperatures.

How does HRF compare with other techniques?

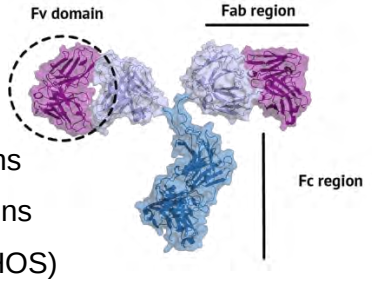
Technique	Cost	Resolution	Throughput
CryoEM	$$$	High	Low
CD / SAXS	$	Low	High
HRF	**$$**	**High**	**High**



HRF can provide actionable insights into the residues involved in aggregation to inform engineering of individual protein subunits to avoid downstream aggregation of the fully formed intact biotherapeutic.

Other HRF Applications:

- Epitope mapping
- Protein-ligand interactions
- Protein-protein interactions
- Higher order structure (HOS)



2401 American Ln, Madison, WI
www.immutoscientific.com
info@immutoscientific.com
(608) 733-2069