



Laser-Free Oxidation (Fox™) Hydroxyl Radical Protein Footprinting (HRPF) for mAb Drug Development

Presented by:

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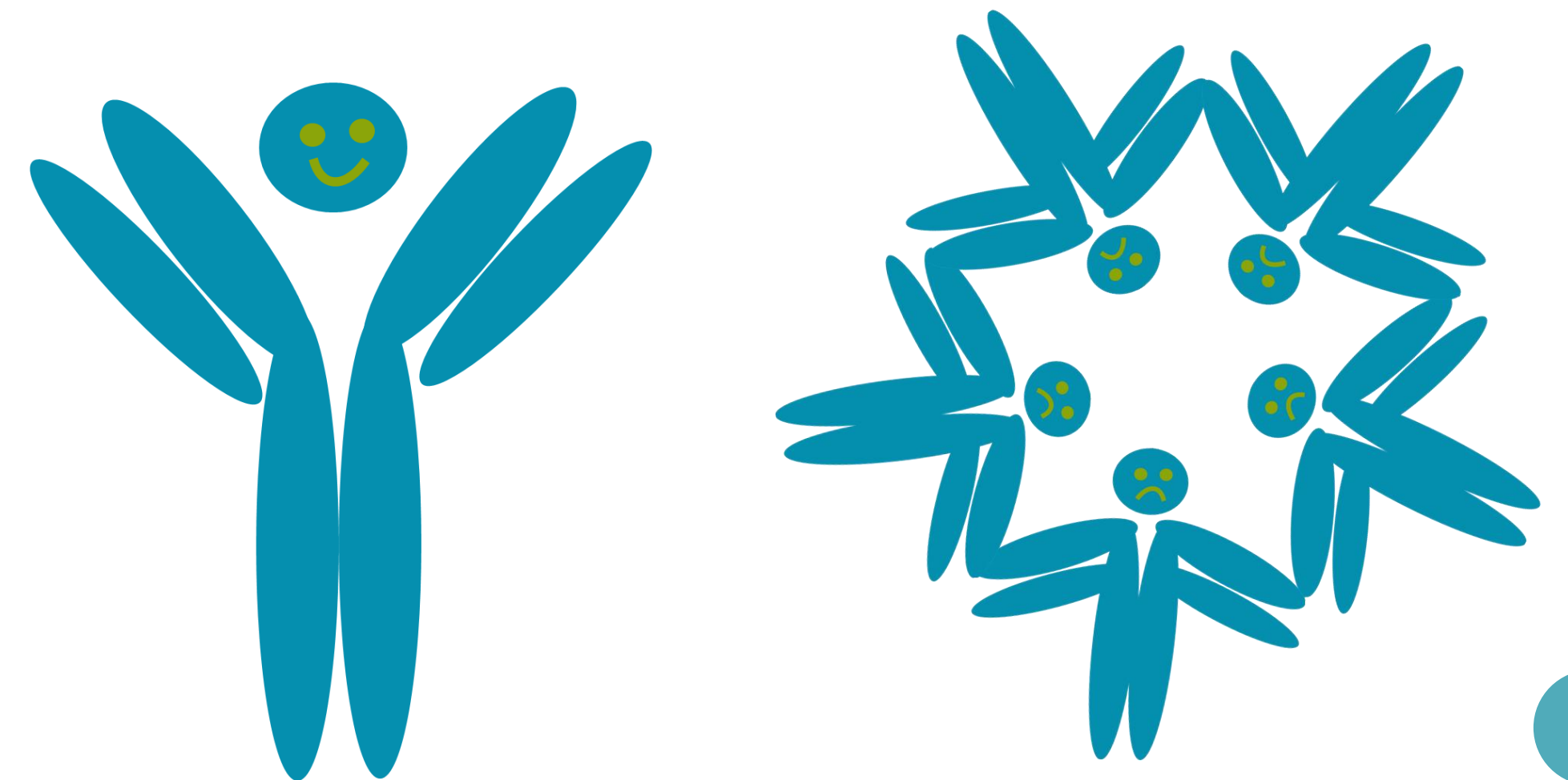
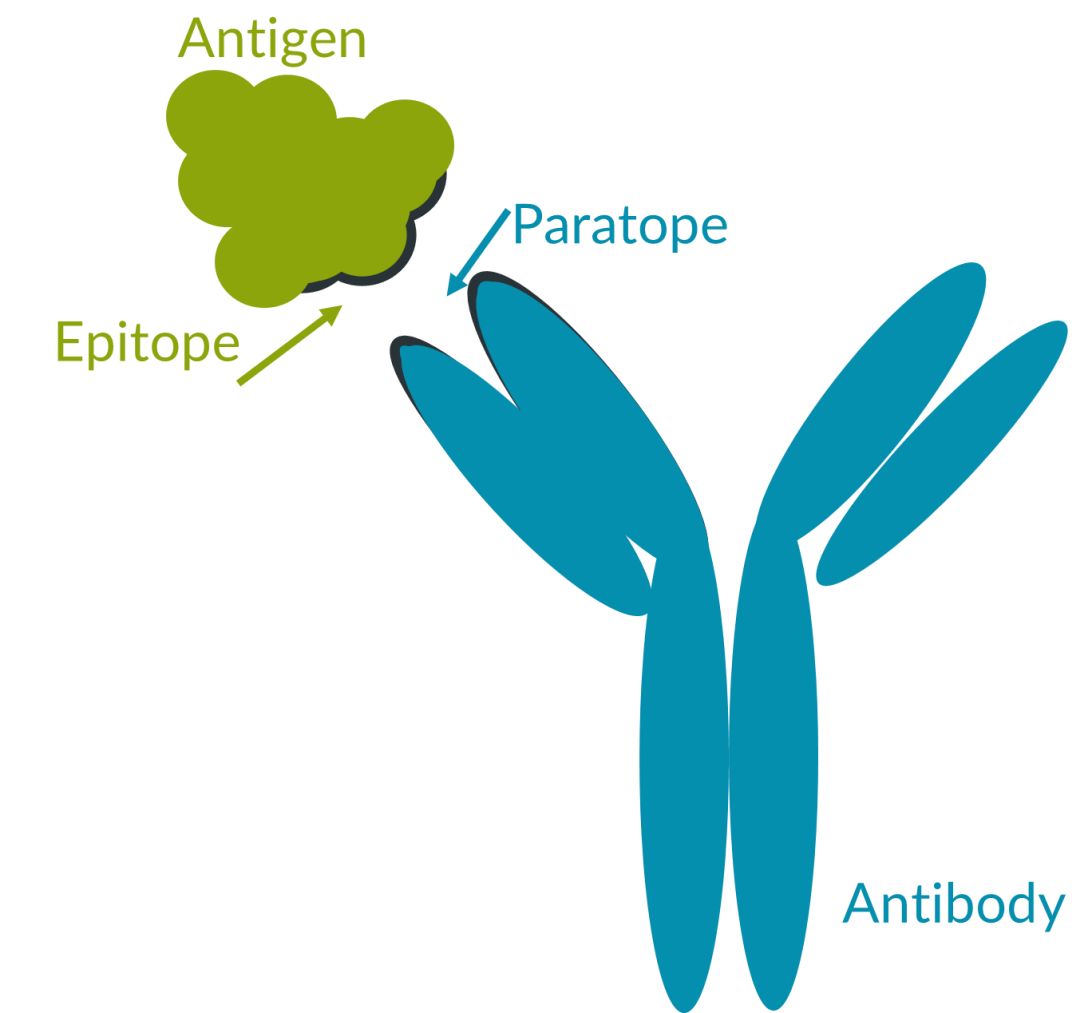


Monoclonal Antibody Therapy

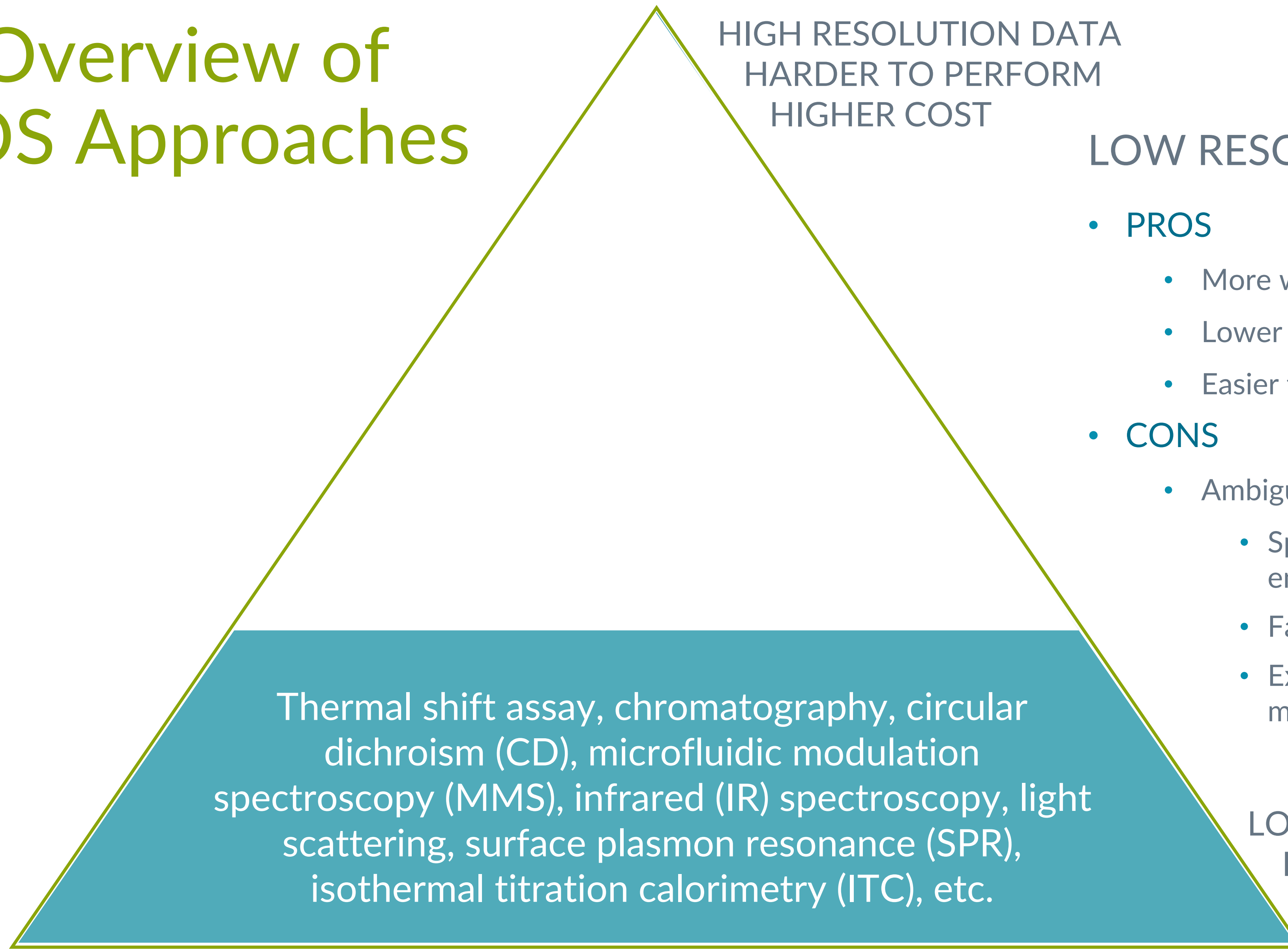
Epitope and Paratope Mapping

- Global mAb therapy market is projected to grow from \$178.50 billion in 2021 to \$451.89 billion in 2028¹
 - Biosimilars
- In-depth analytical studies to understand physicochemical and functional characteristics
 - Epitope and Paratope Characterization
 - Intermolecular interactions/ Aggregation

1. Fortune Business Insights. August 30, 2021



Overview of HOS Approaches



HIGH RESOLUTION DATA
HARDER TO PERFORM
HIGHER COST

LOW RESOLUTION DATA:

- PROS

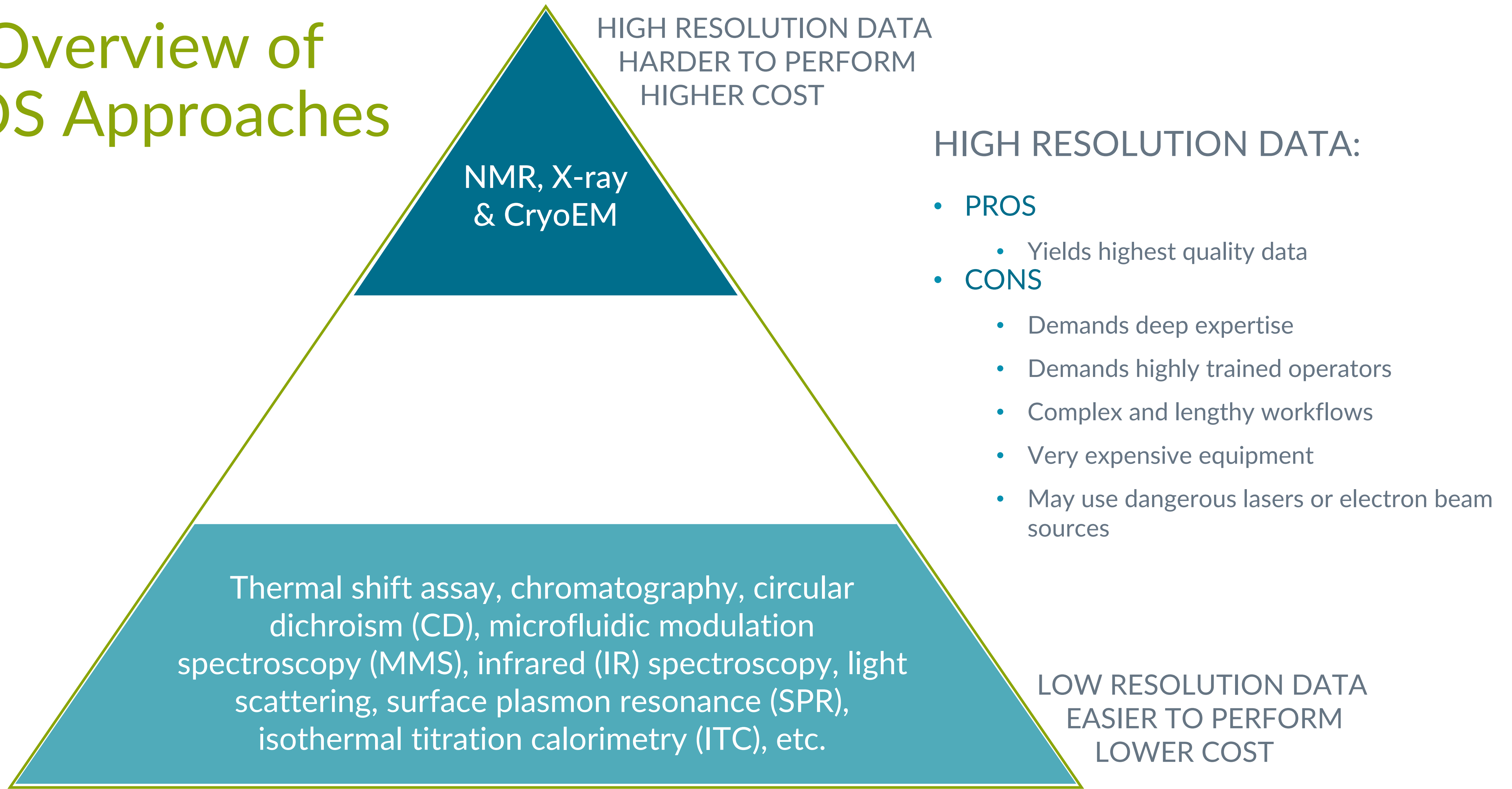
- More widely available
- Lower cost
- Easier to use

- CONS

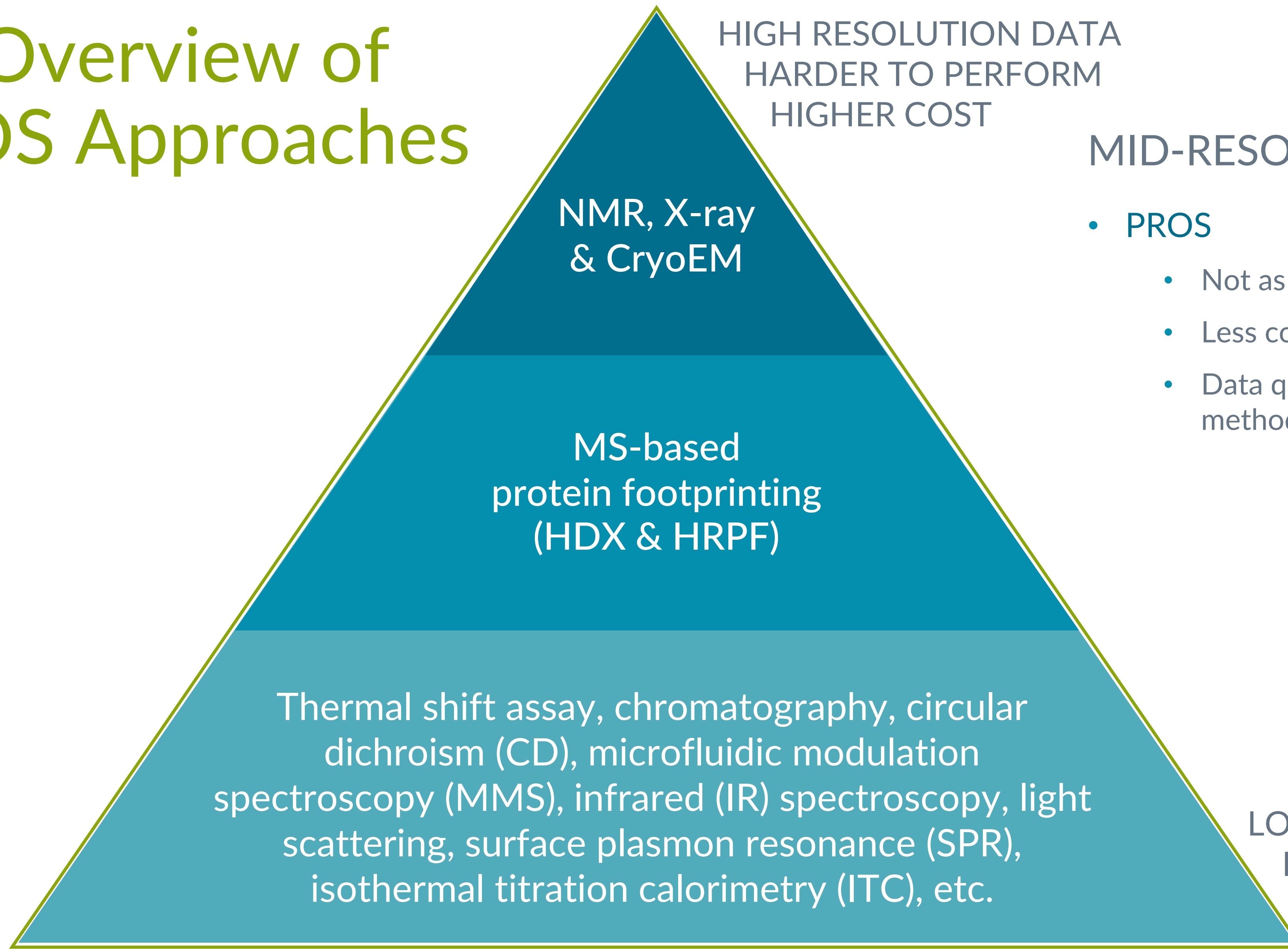
- Ambiguous and marginally actionable HOS data
 - Spatially averaged information over the entire protein population
 - Fails to inform on a residue-level
 - Examines a very limited number of specific moieties in the protein structure

LOW RESOLUTION DATA
EASIER TO PERFORM
LOWER COST

Overview of HOS Approaches



Overview of HOS Approaches



MID-RESOLUTION DATA:

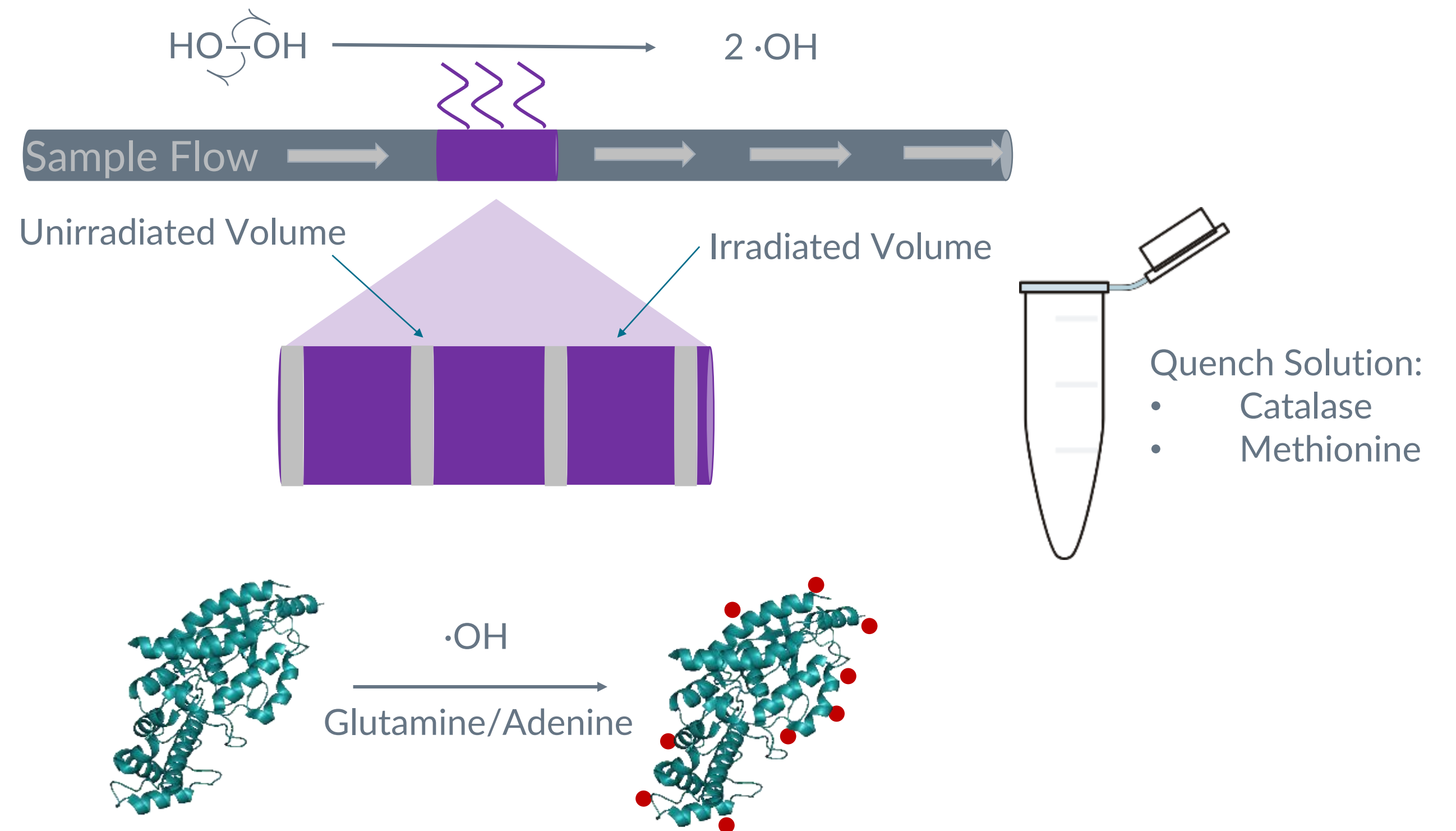
- PROS

- Not as difficult as high-resolution methods
- Less cost than high resolution methods
- Data quality in parity with high resolution methods

Fast Photochemical Oxidation of Protein (FPOP)

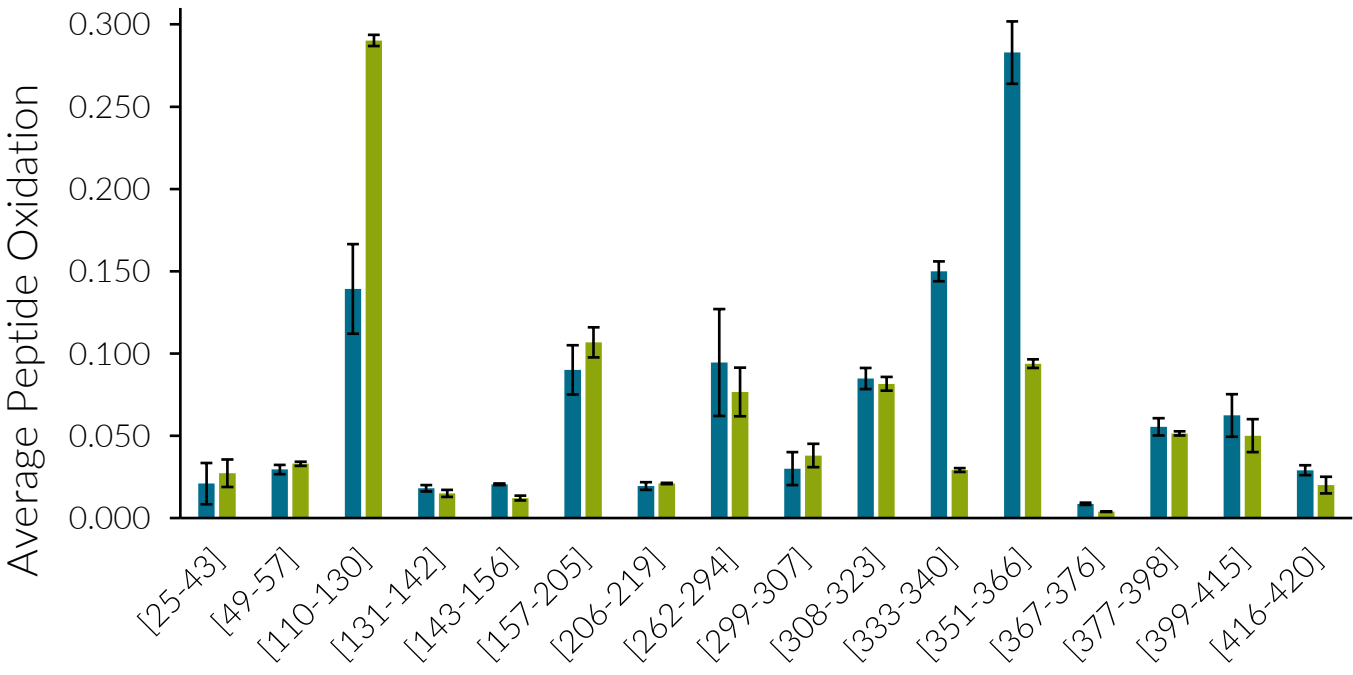
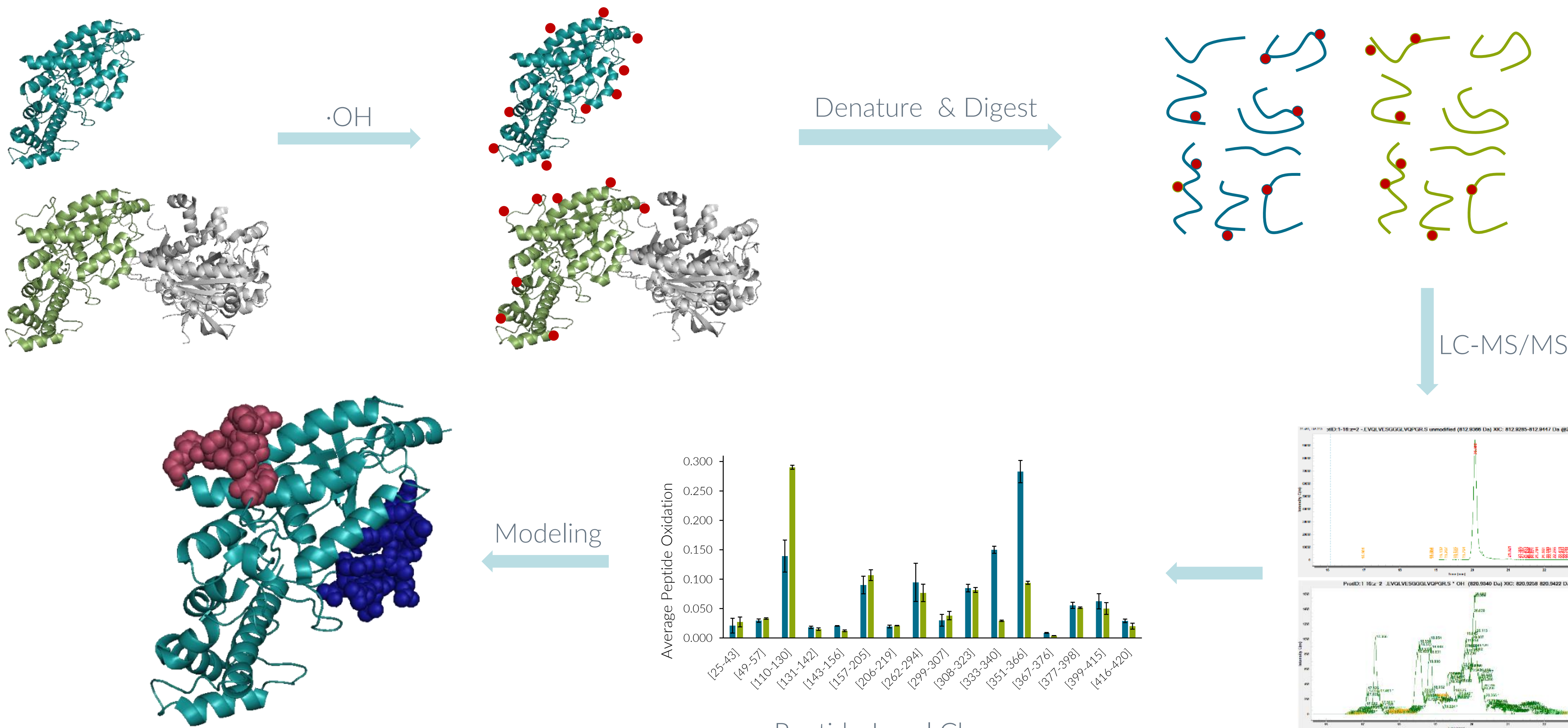
HRPF HOS Analysis

- Proteins exposed to a pulse of diffusing hydroxyl radicals
 - OH generated by flash photolysis of hydrogen peroxide
- Modifies exposed side chains
- Measures protein topography at peptide to amino acid resolution



Hydroxyl Radical Protein Footprinting

Typical Workflow



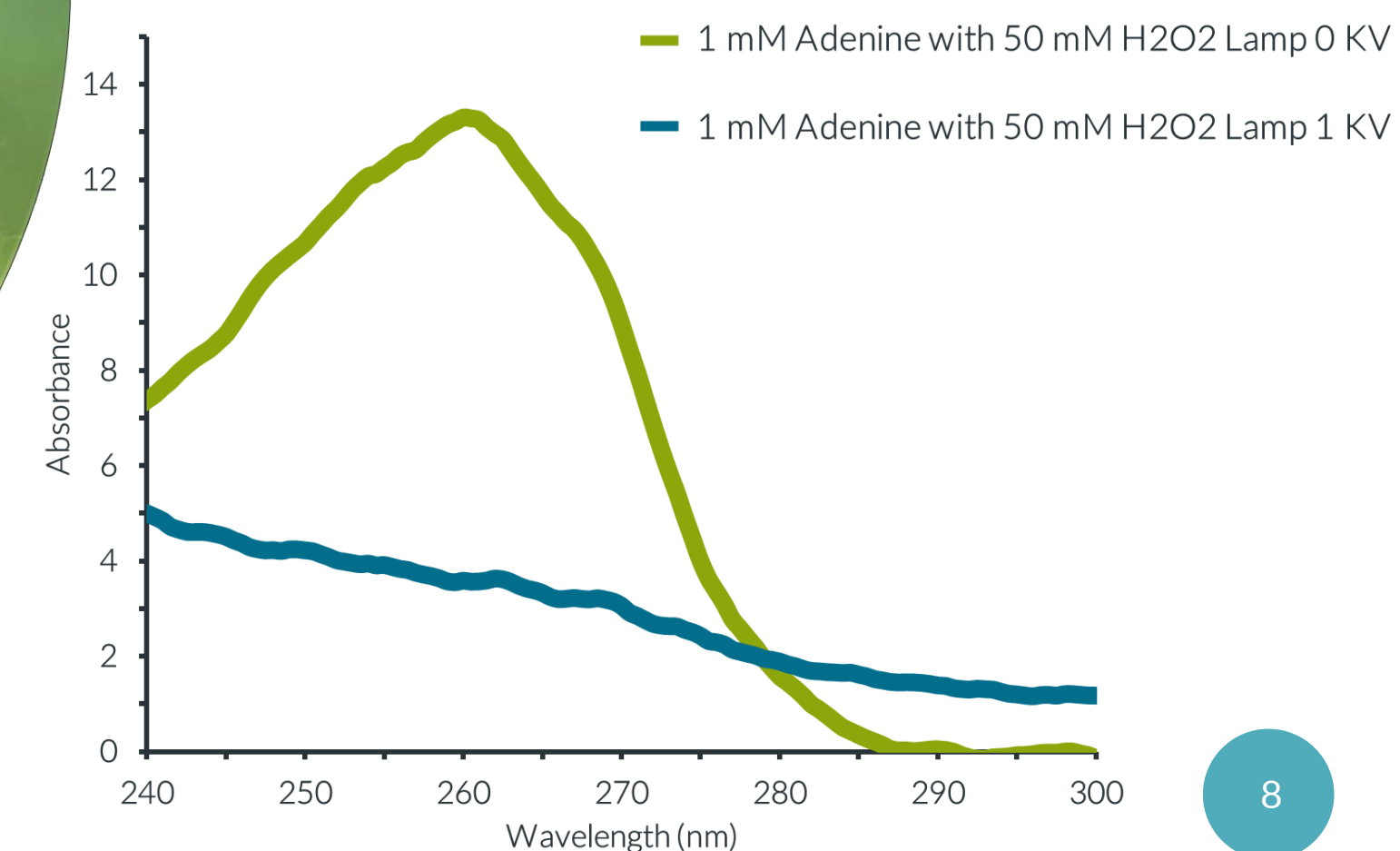
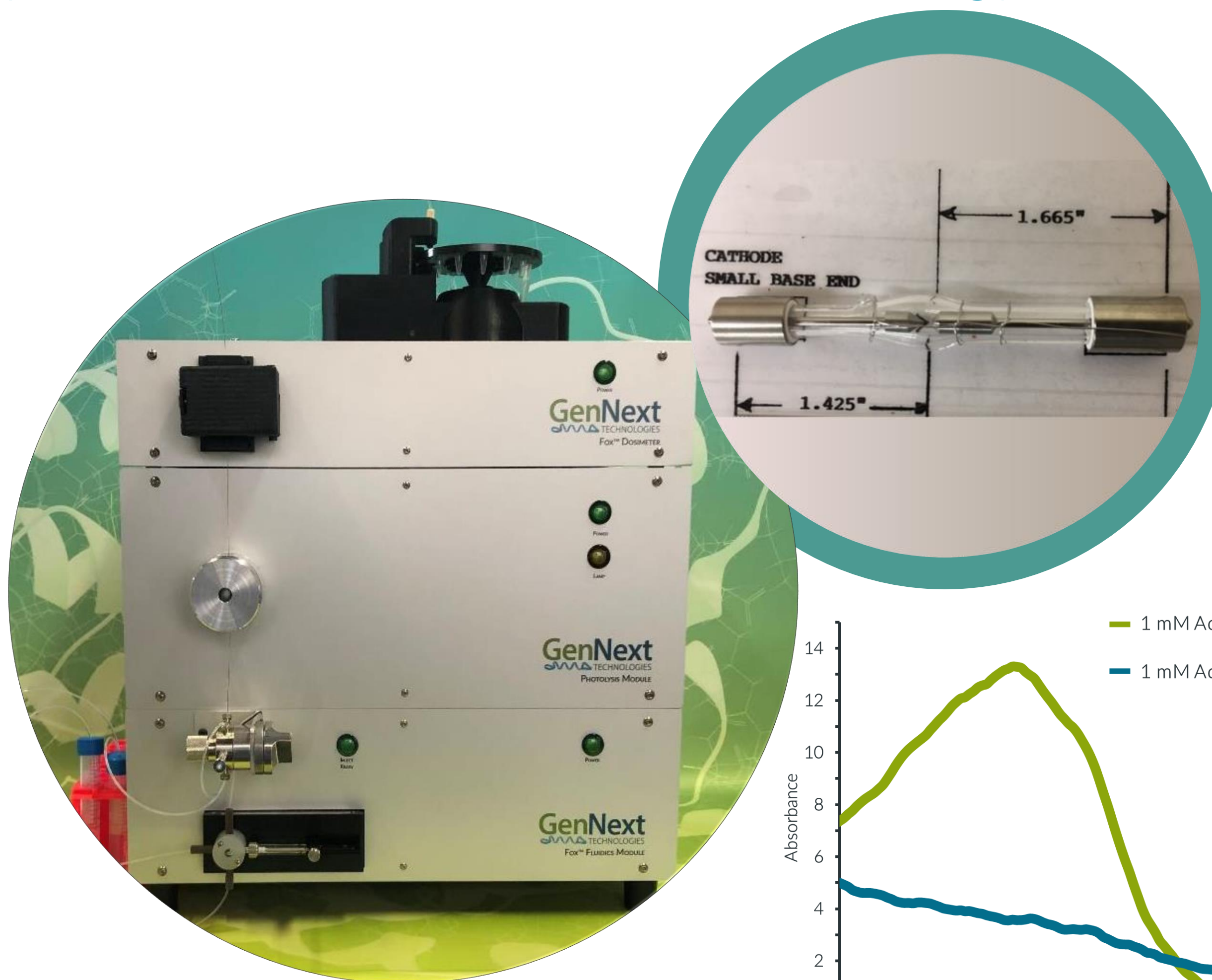
Peptide-Level Changes

Client Confidential

Fox™ Protein Footprinting System

Laser-free Delivery of Reproducible & Reliable Structural Biology Data

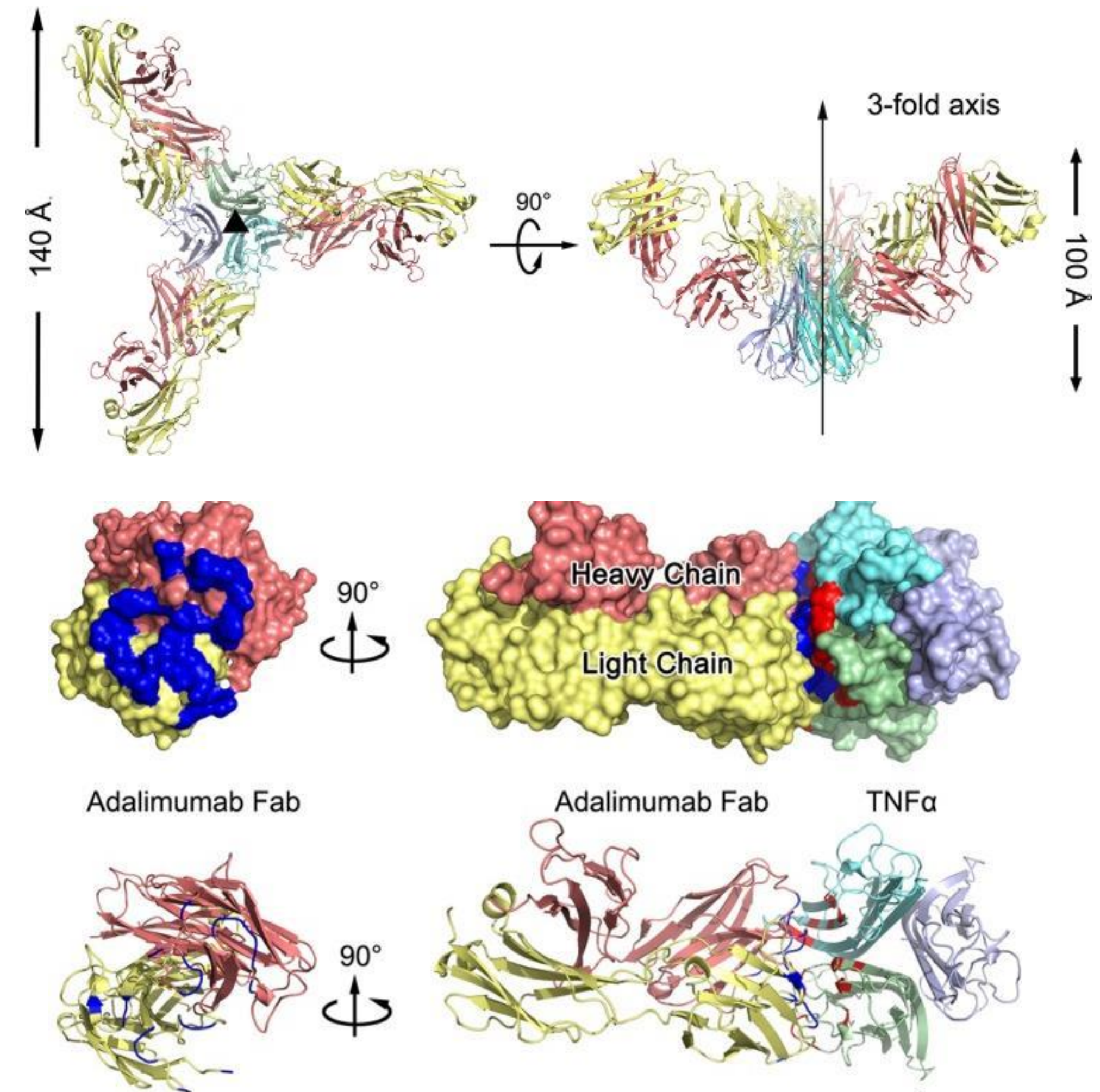
- Fox system replaces hazardous lasers with proprietary plasma lamp technology in a safe and easy-to-use benchtop package
- Dosimeter enables real-time adjustment of scavenging, providing confident and actionable results
- Automated product collector facilitates collection of properly labeled sample while disposing unwanted products and reagents to waste



Epitope and Paratope Mapping

*Fox™ System Case Study:
TNFα:Adalimumab*

- Tumor necrosis factor α (TNFα) is a pro-inflammatory cytokine
- Adalimumab (Humira) is a monoclonal antibody prescribed to treat inflammatory diseases
- TNFα:Adalimumab epitope has been well characterized with an available crystal structure
- Can Fox™ Protein Footprinting accurately detect regions involved in the epitope and paratope?

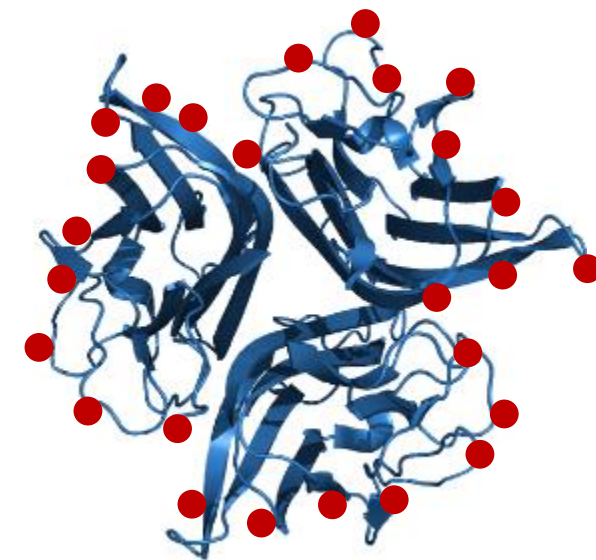


J Biol Chem. 2013 Sep 20; 288(38): 27059–27067.
PDB ID 3WD5

Hydroxyl Radical Protein Footprinting

Amino acids can stabilize proteins, minimizing intermolecular interactions

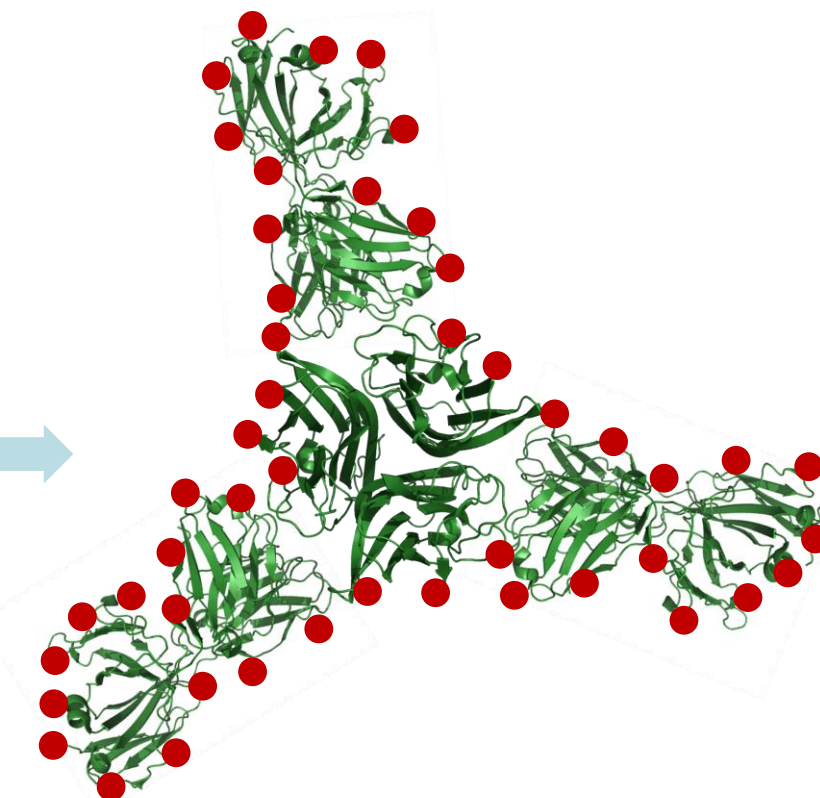
Condition 1:
TNF α alone



Condition 2:
TNF α with
Adalimumab

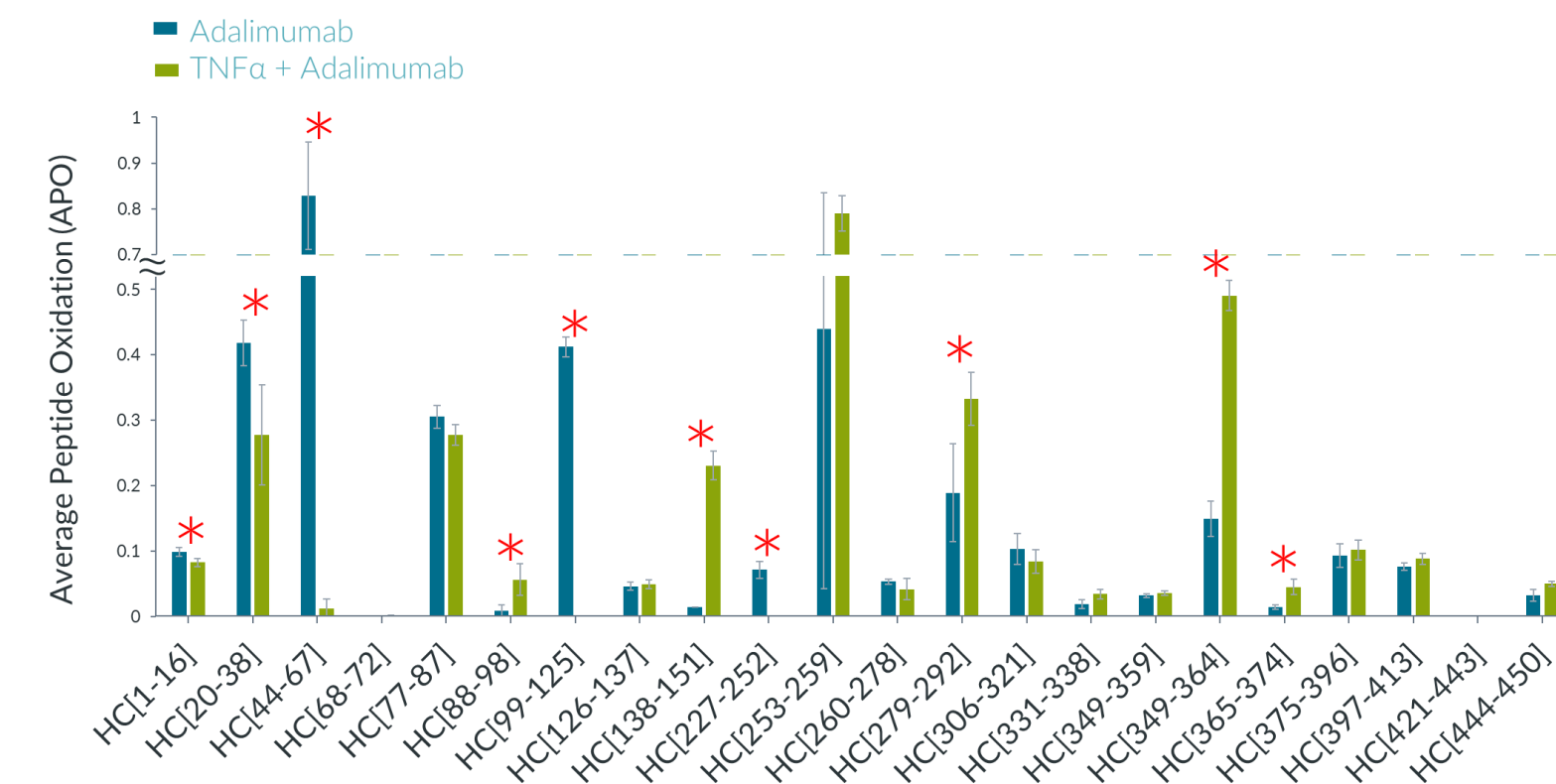
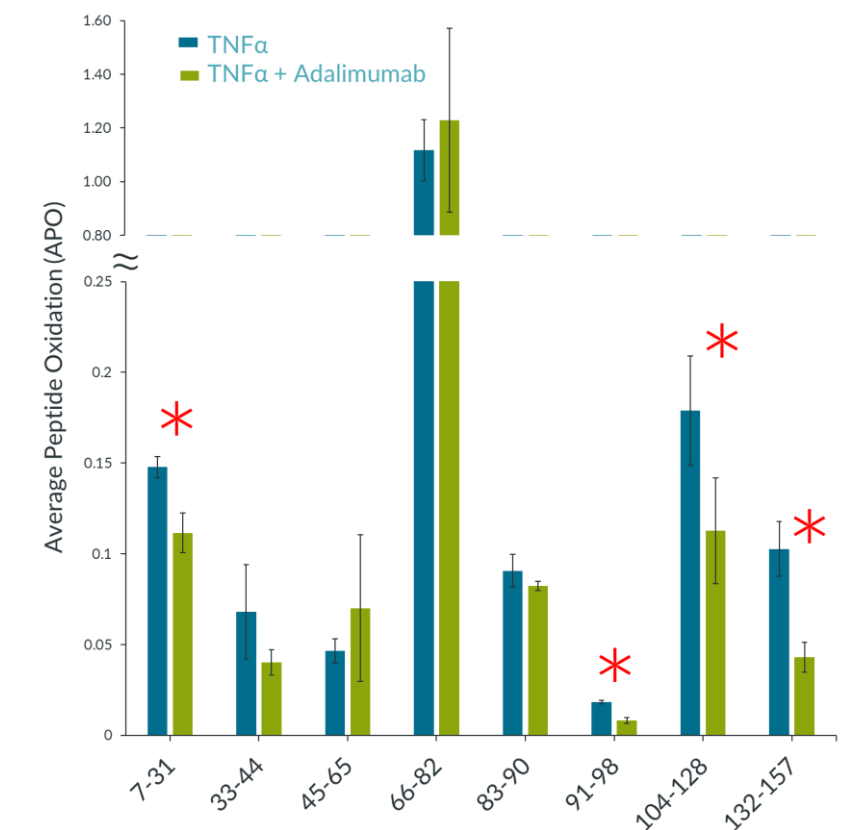
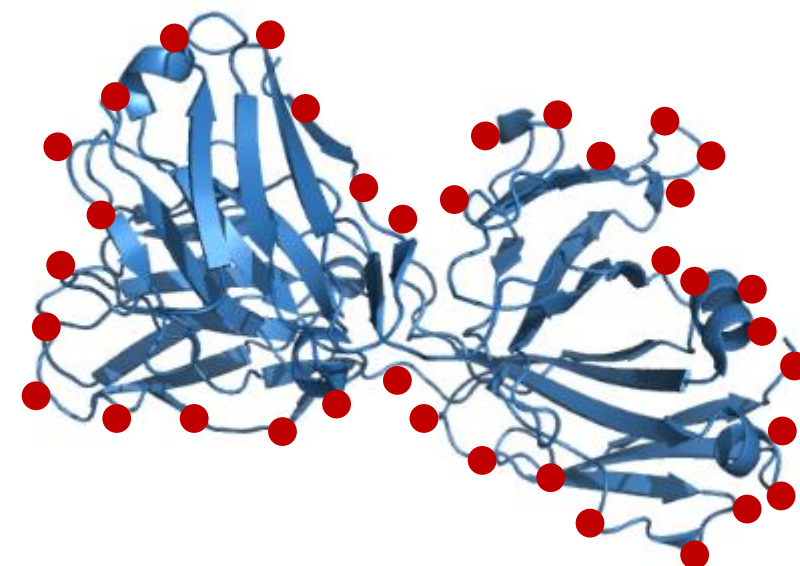
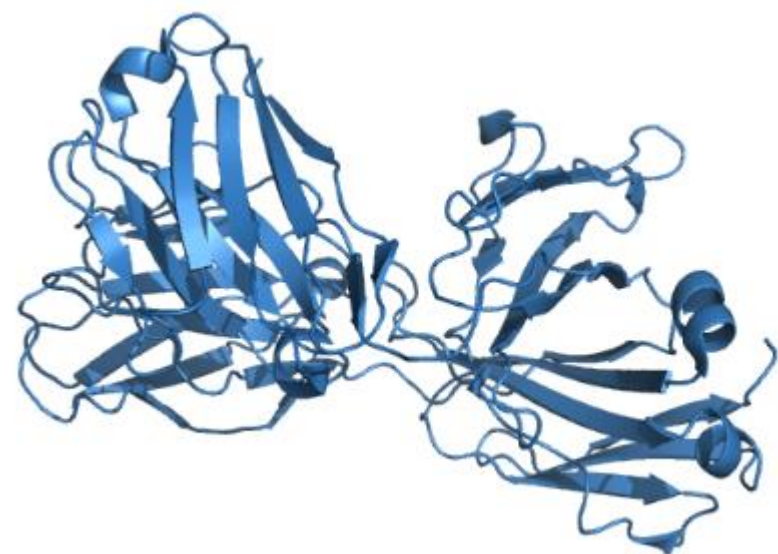


HRPF



Bottom-Up
Proteomics

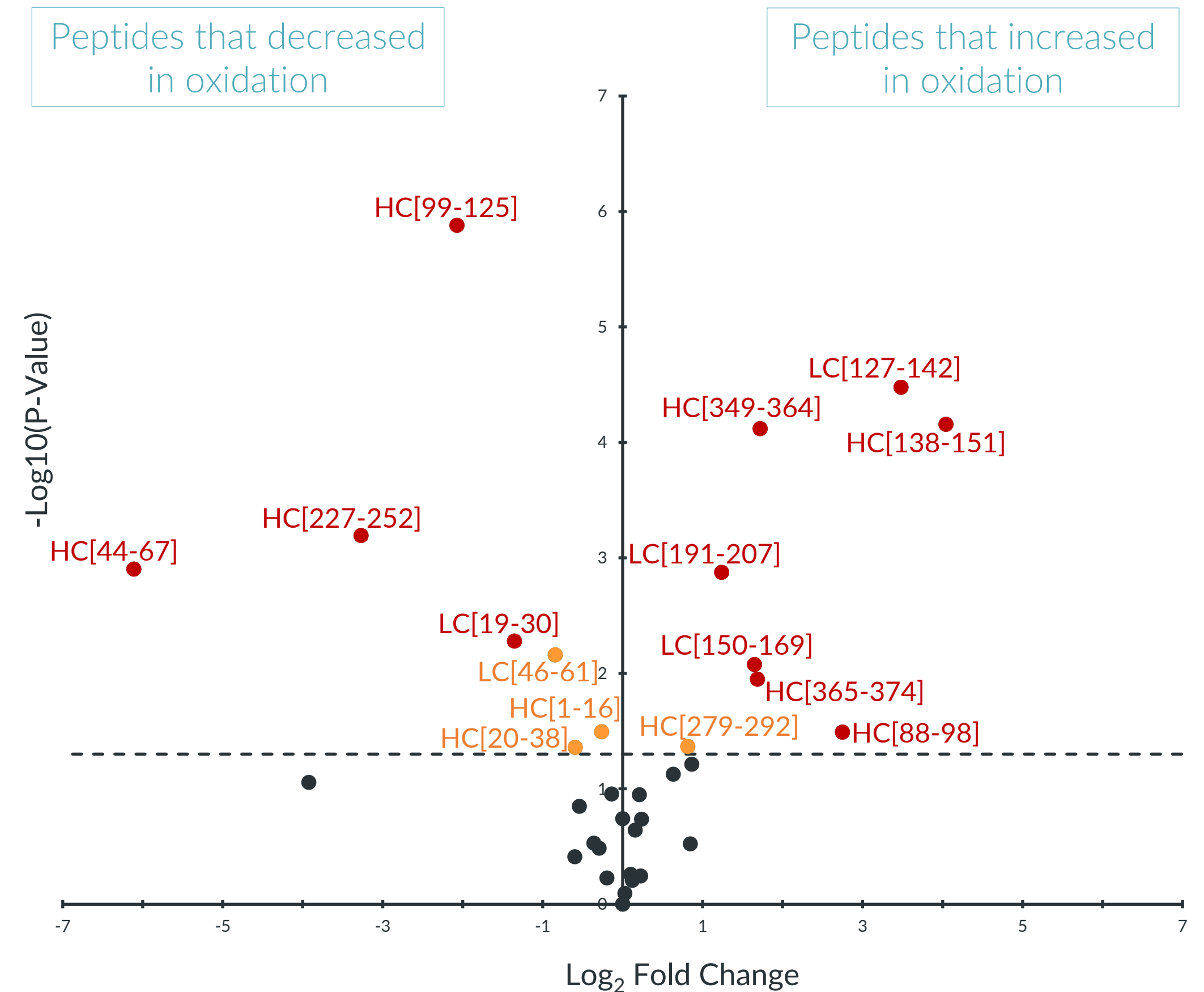
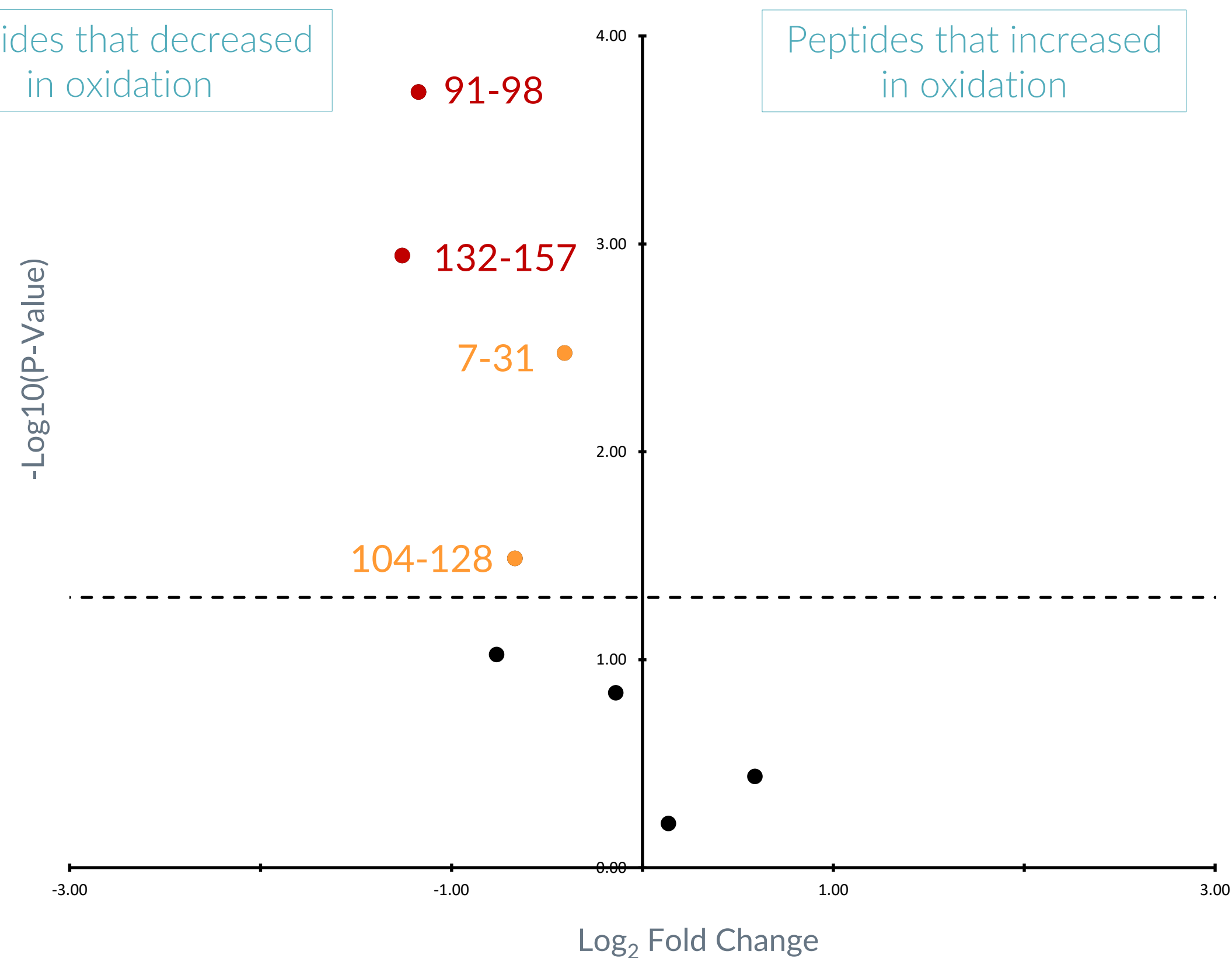
Condition 3:
Adalimumab Alone



Peptide-Level Changes

Epitope Mapping

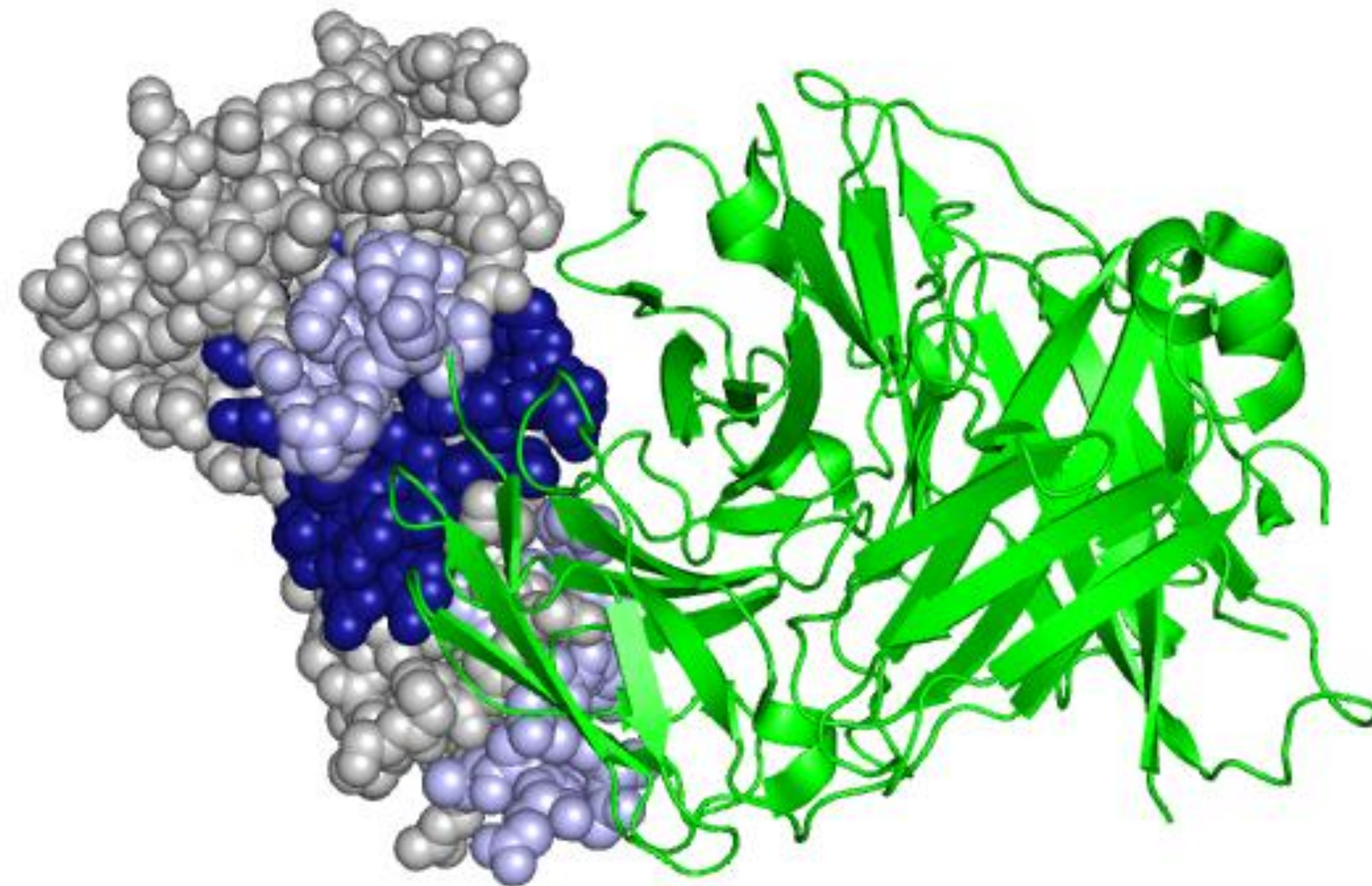
Fox™ Case Study: Histogram & Volcano Plot Pairwise Analysis



TNF α – Adalimumab HRPf Characterized Epitope and Paratope

Fox™ System Case Study: Map Oxidation Changes on Crystal Structure

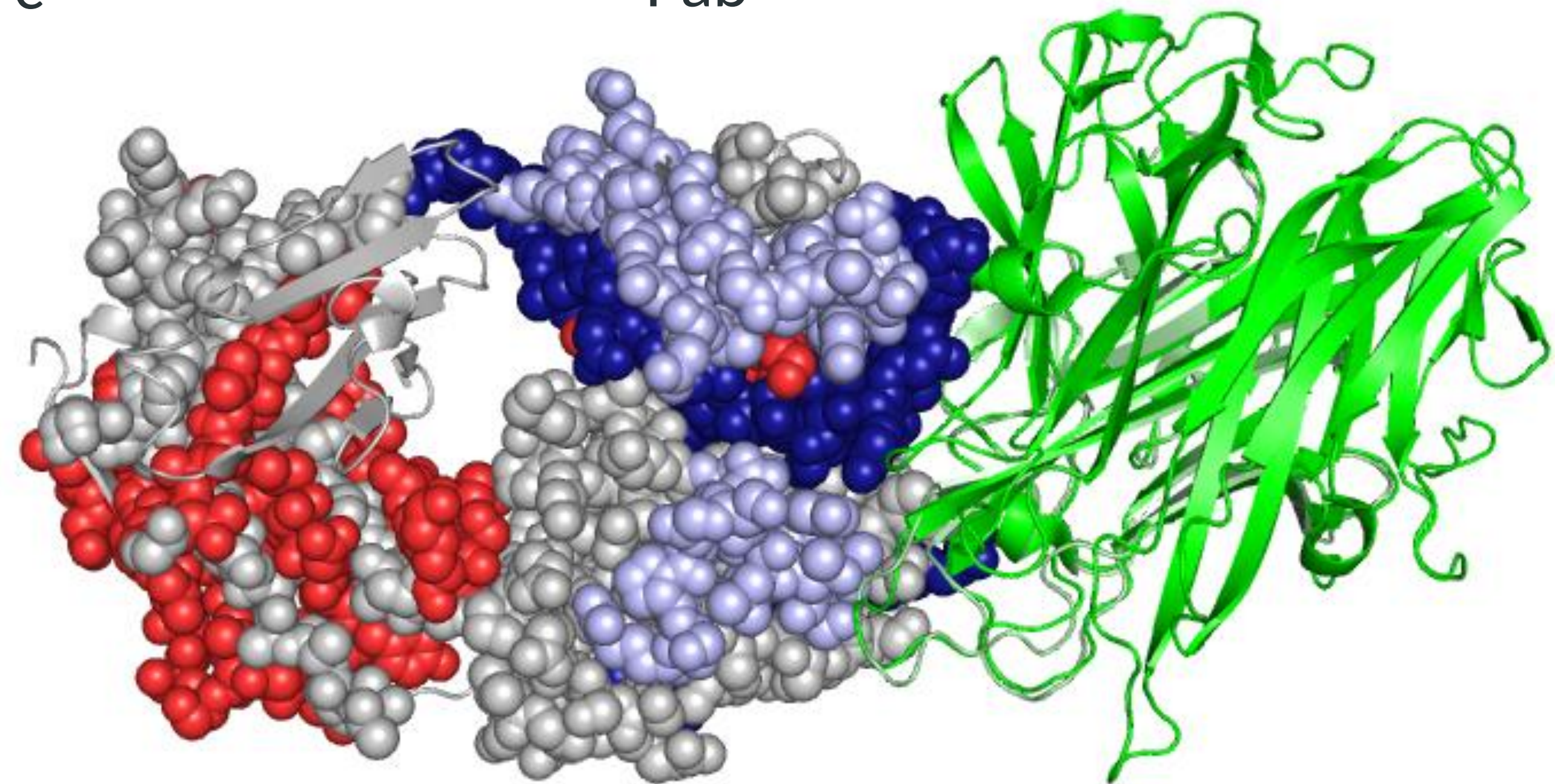
TNF α – Adalimumab HRPf Characterized Epitope



TNF α – Adalimumab HRPf Characterized Paratope

Fc

Fab



Monoclonal Antibody Aggregation

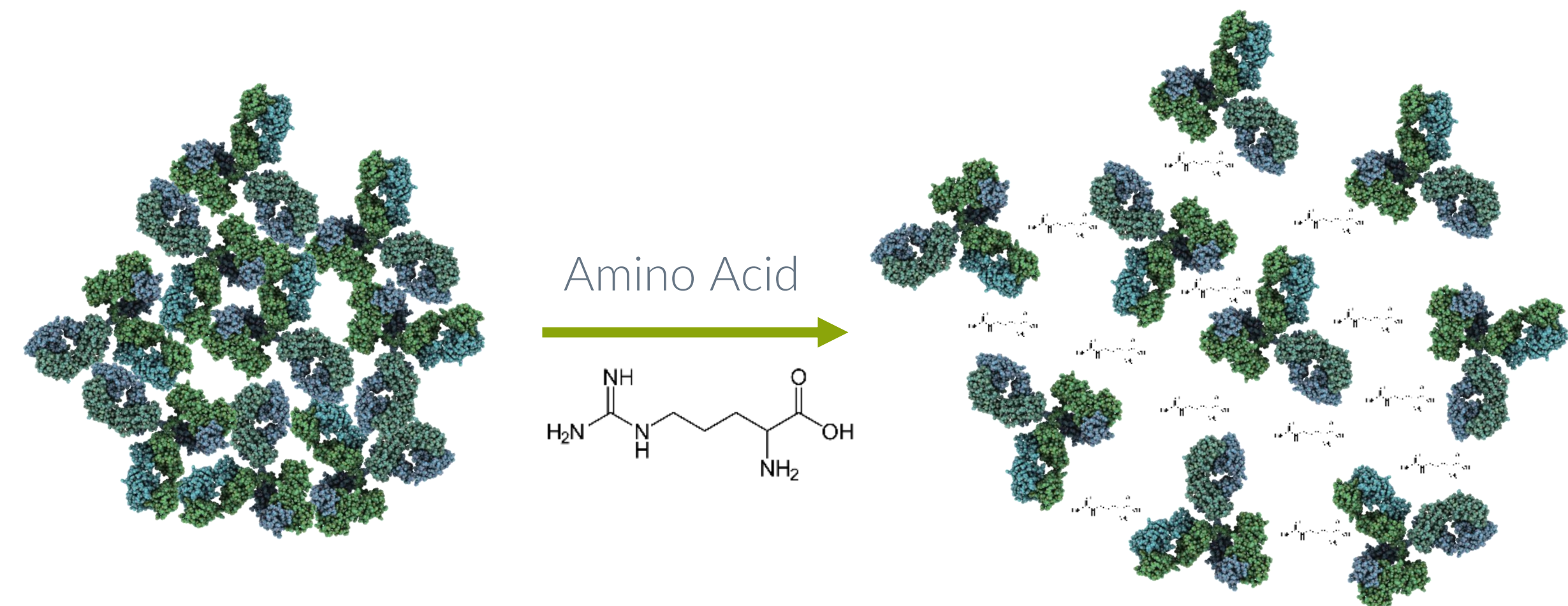
Identification of Aggregate Domains

- **Excipient effects on antibody aggregation**

- Excipients, like amino acids, have been effective in stabilizing proteins that undergo intermolecular interactions at high concentrations

- **Fox™ mAb aggregation domain analysis**

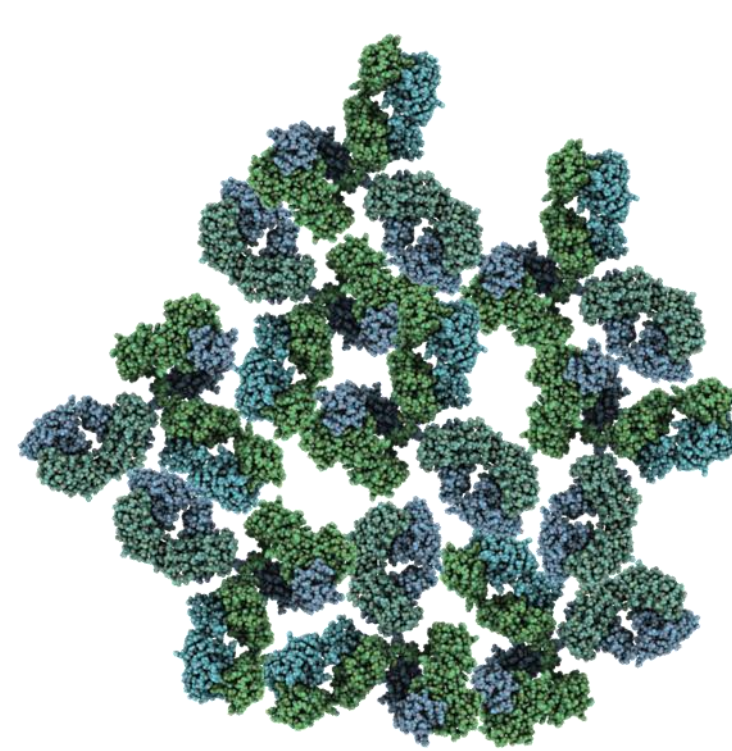
- Pairwise differential labeling experiment of aggregated and nascent Mab
- Aggregated surfaces are protected from labeling
- Labeling differences highlights interfacial domains for aggregation



Hydroxyl Radical Protein Footprinting

Amino acids can stabilize proteins, minimizing intermolecular interactions

Condition 1:
High concentration
antibody without
amino acid



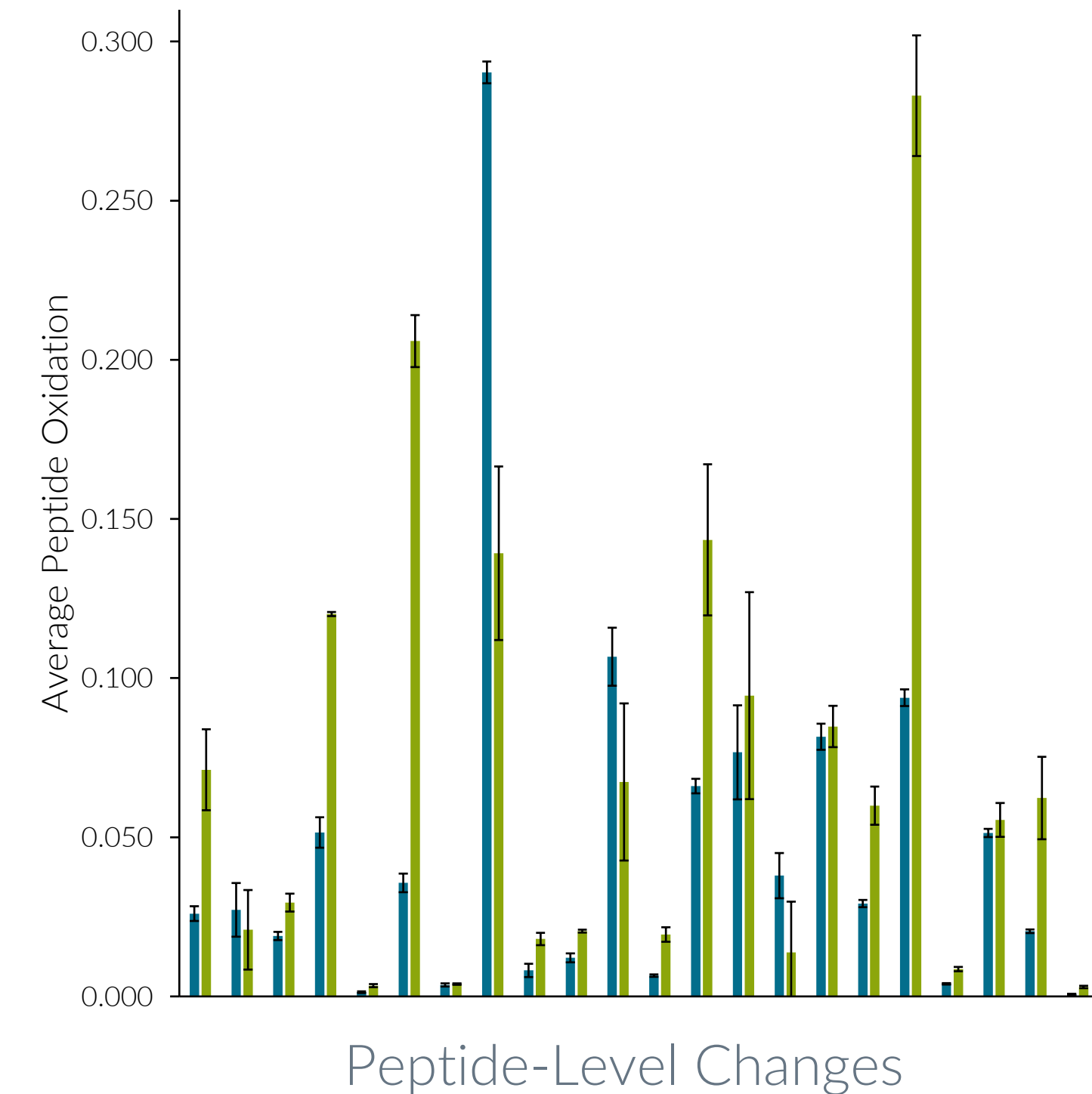
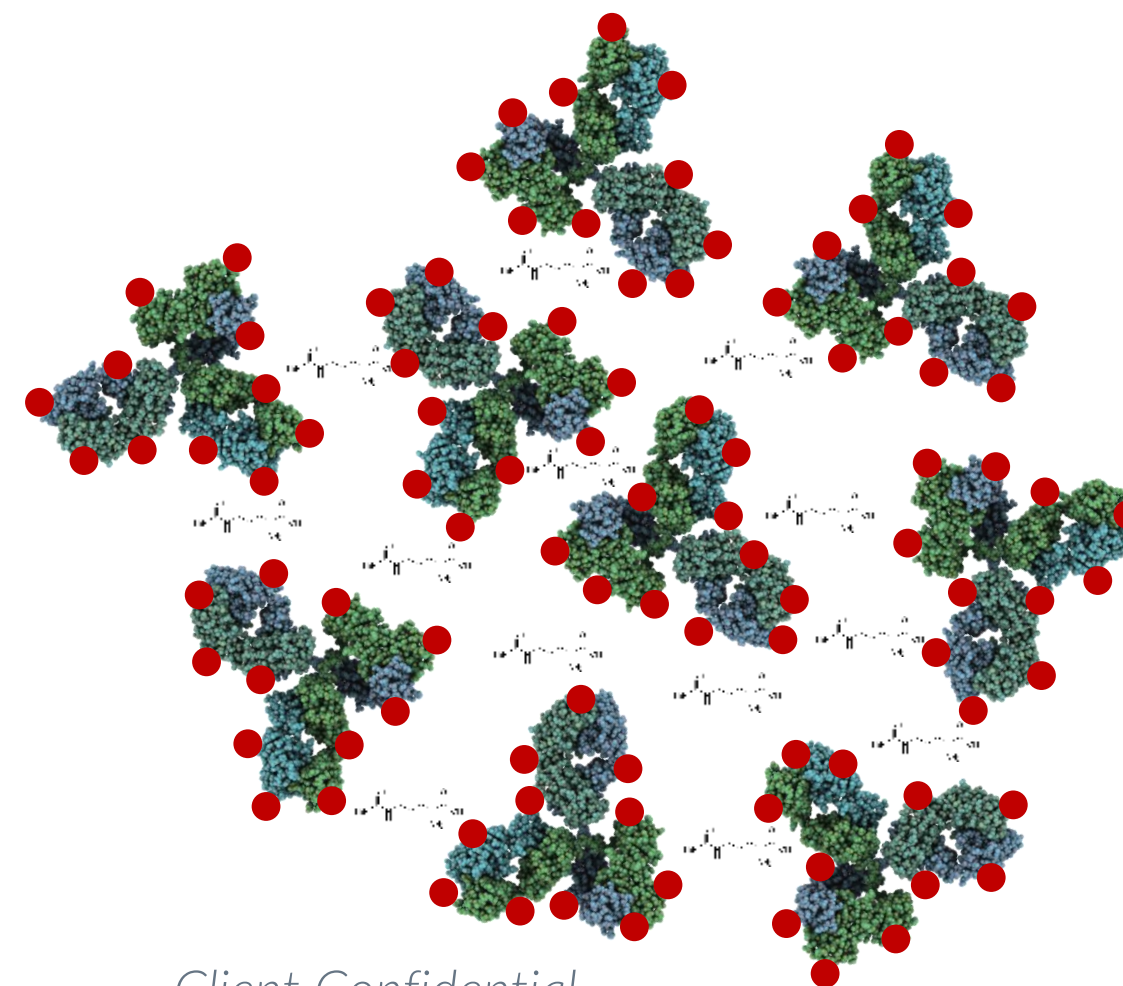
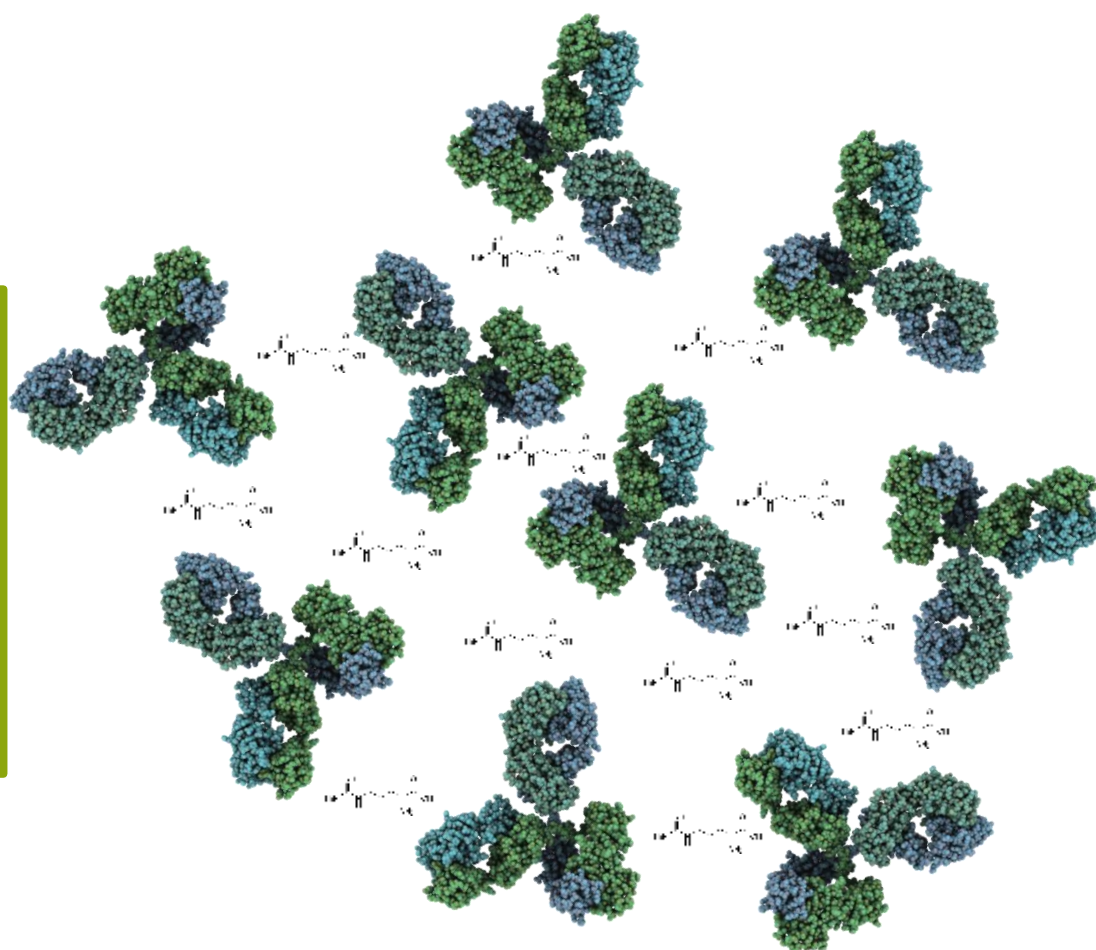
HRPF



Bottom-Up
Proteomics

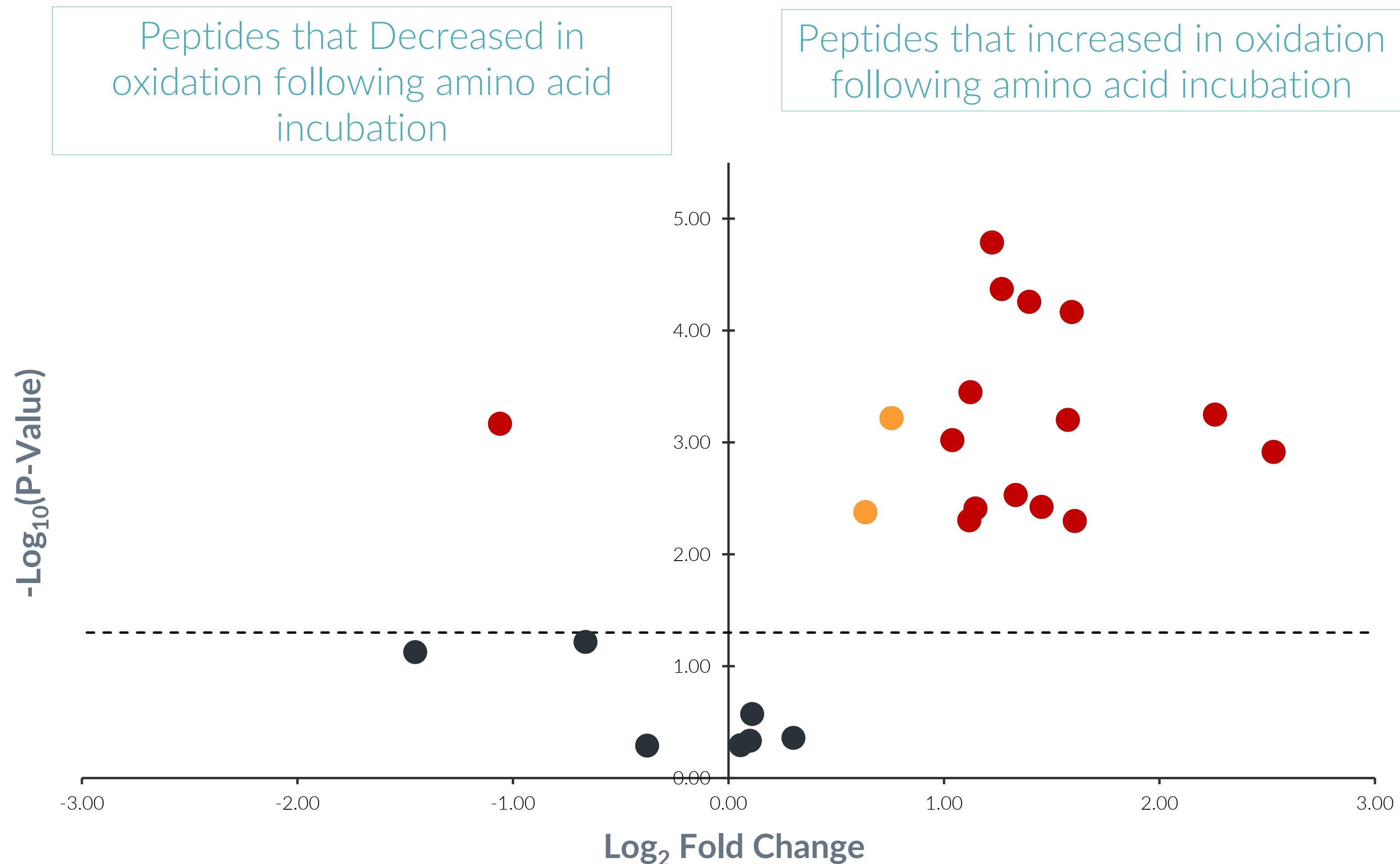


Condition 2:
High concentration
antibody with amino acid



Monoclonal Antibody Aggregation

Identifying peptides involved in intermolecular interactions



Protein Footprinting Webinar Series



All Available
On-Demand at
[www.gnxtech.com/
webinar-series](http://www.gnxtech.com/webinar-series)

Introduction to Protein Footprinting

- Professors Mark Chance of Case Western Reserve University and Joshua Sharp of the University of Mississippi

Protein Footprinting Applications in Structural Biology

- Professor Michael Gross of the Washington University in St. Louis

Fast Photochemical Oxidation of Proteins (FPOP) HRPF

- Professor Joshua Sharp of the University of Mississippi

HRPF Data Processing and Higher Order Structural Analysis

- Professor Joshua Sharp of the University of Mississippi

In-cell and *in vivo* FPOP

- Professor Lisa Jones of the University of Maryland Baltimore



Thank You for Your
Participation!

To learn more, please
visit our virtual booth.

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