GenNext TECHNOLOGIES

Revolutionizing Protein Higher Order Structural Analysis

Offering products & services to pharmaceutical researchers investigating biopharmaceutical structure, interactions, folding, aggregation, formulation, and delivery.

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Laser-Free Flash Oxidation (Fox™) Protein Footprinting System Elucidates Changes in Peptide to Amino Acid Solvent Accessibility

HOS 2022 Technical Talk April 4th

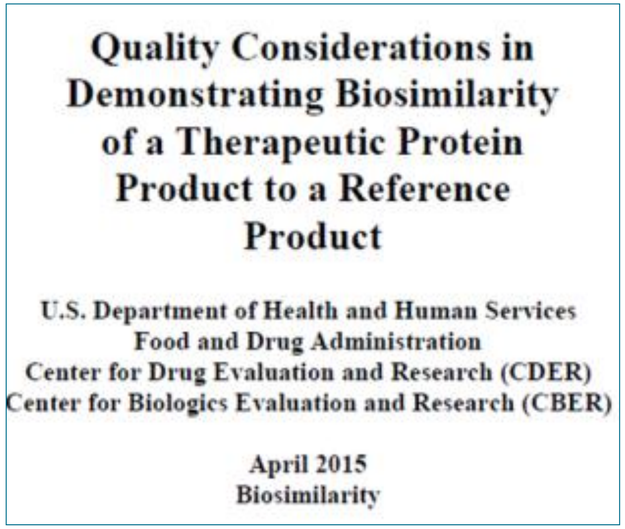
Presented by:

Emily E. Chea

Higher Order Structure Analysis

Enabling Biopharmaceutical Development

- HOS analysis is important for the design and characterization of biopharmaceuticals
- FDA, CDER, CBER, EDA all recognize the need for new and improved HOS analytics
    - Accelerate development and minimize adverse drug reactions
- By leveraging the power of Fox™ technology, GenNext's mission is to revolutionize the practice of HOS structural biology research



Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

April 2015
Biosimilarity

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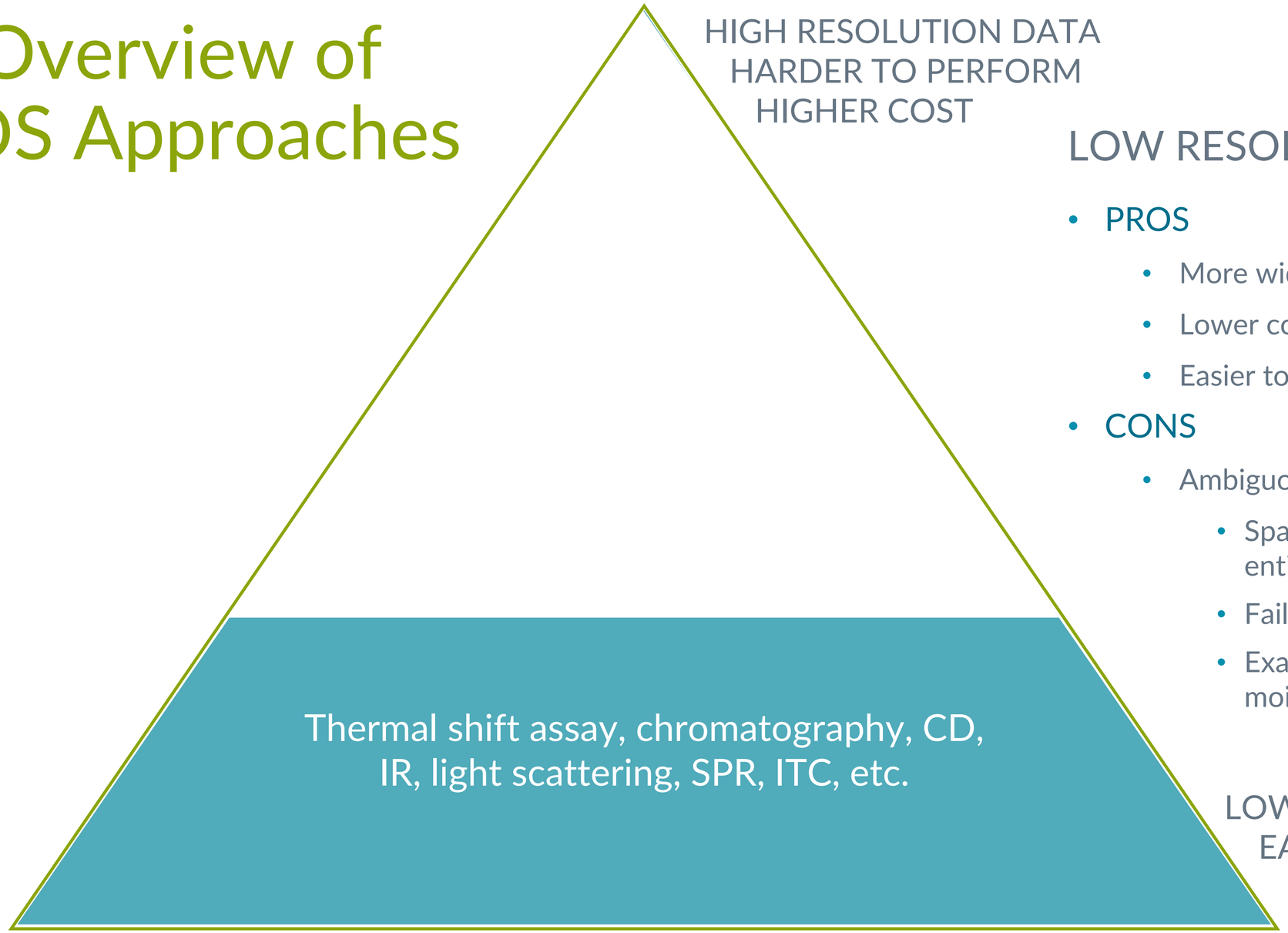
Overview of HOS Approaches



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

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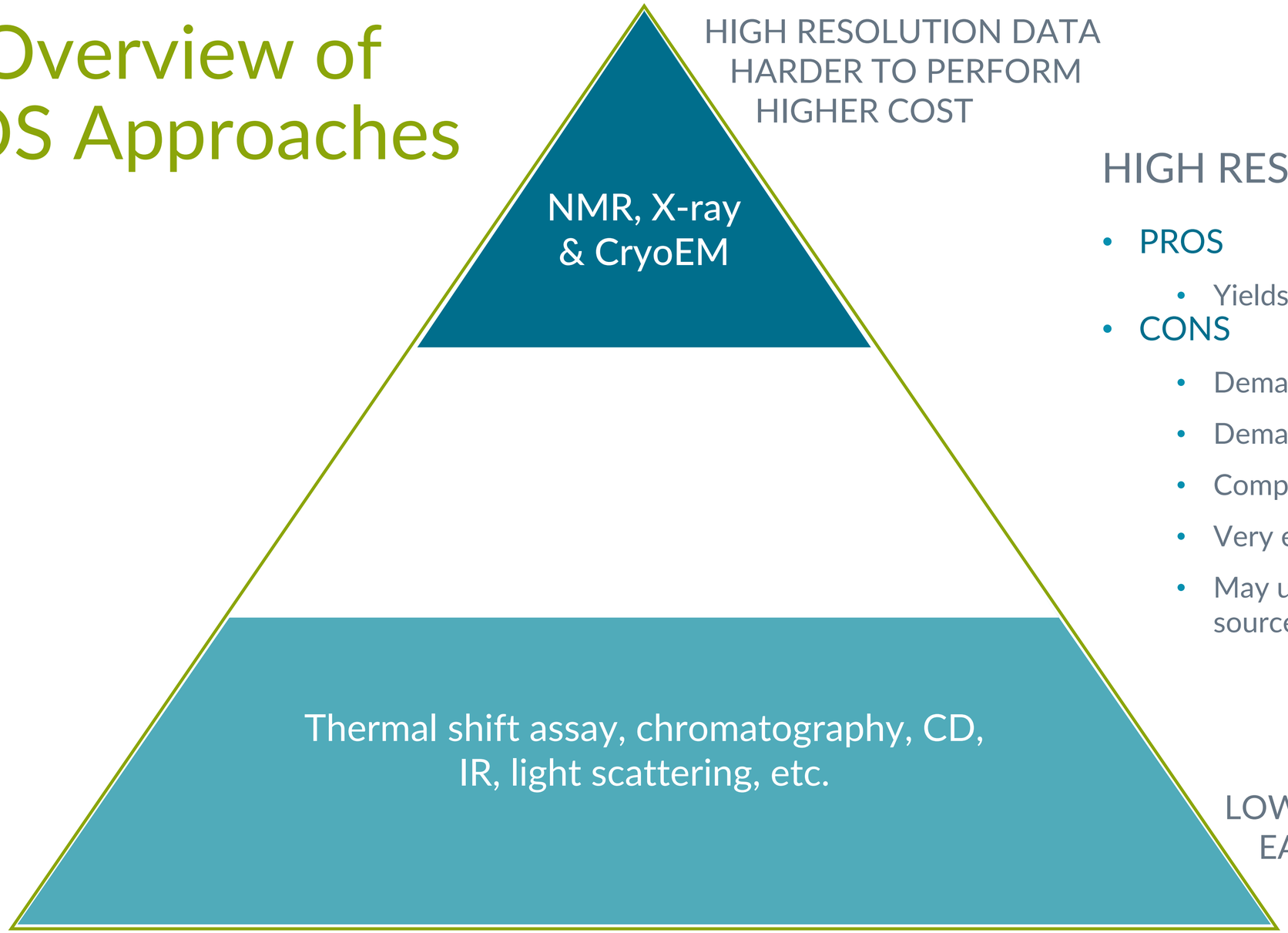
Slide courtesy of Professor Michael Gross, WUSTL

Overview of HOS Approaches



HIGH RESOLUTION DATA:

- PROS
  - Yields highest quality data
- CONS
  - Demands deep expertise
  - Demands highly trained operators
  - Complex and lengthy workflows
  - Very expensive equipment
  - May use dangerous lasers or electron beam sources

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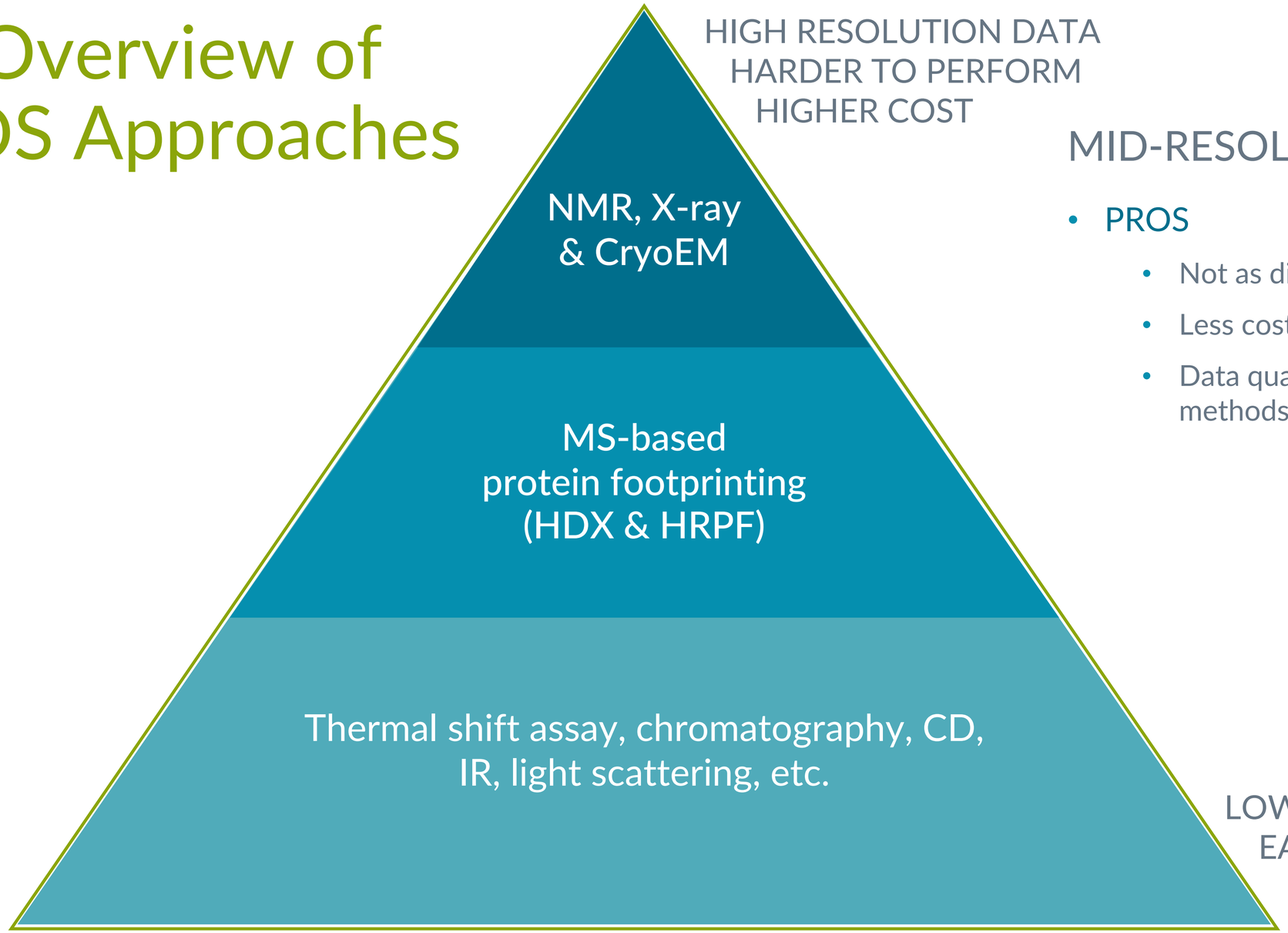
Slide courtesy of Professor Michael Gross, WUSTL

Overview of HOS Approaches



MID-RESOLUTION DATA:

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

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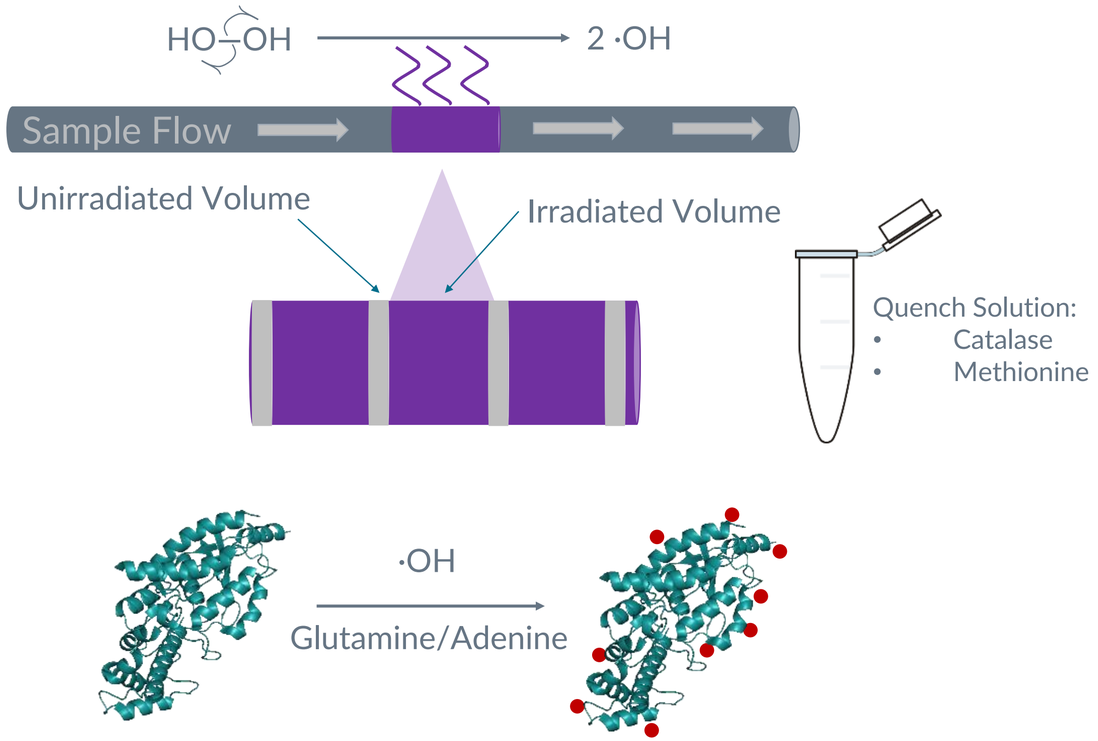
Slide courtesy of Professor Michael Gross, WUSTL

Hydroxyl Radical Protein Footprinting

Enabling 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



Amino Acid	Rate Constant ($L*mol^{-1}*s^{-1}$)
Cysteine	3.40E+10
Tryptophan	1.30E+10
Tyrosine	1.30E+10
Methionine	8.30E+09
Phenylalanine	6.50E+09
Histidine	5.00E+09
Arginine	3.50E+09
Isoleucine	1.80E+09
Leucine	1.70E+09
Valine	7.60E+08
Glutamine	5.40E+08
Threonine	5.10E+08
Proline	4.80E+08
Lysine	3.50E+08
Serine	3.20E+08
Glutamic Acid	1.60E+08
Alanine	7.70E+07
Aspartic Acid	7.50E+07
Asparagine	4.90E+07
Glycine	1.70E+07
Reference: Buxton et al, J. Phys. Chem. Ref. Data, Vol. 17, No. 2, 1988	

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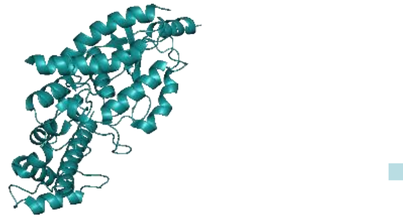
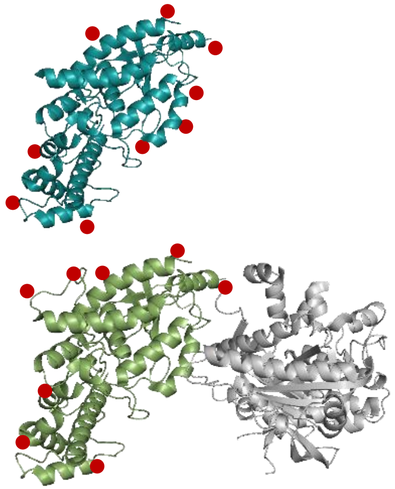
Hydroxyl Radical Protein Footprinting

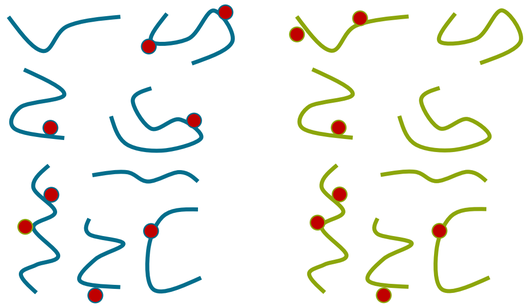
Typical Workflow



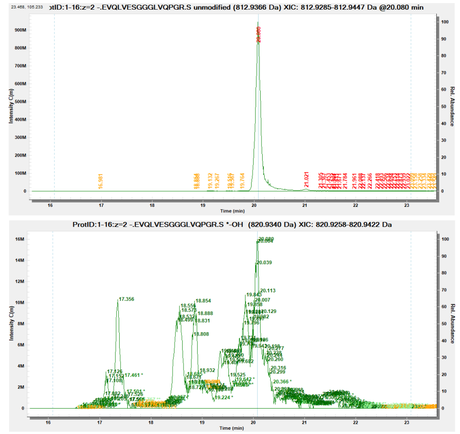
·OH

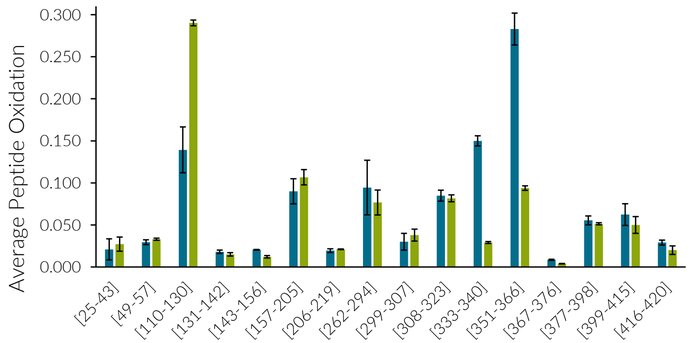


Denature & Digest



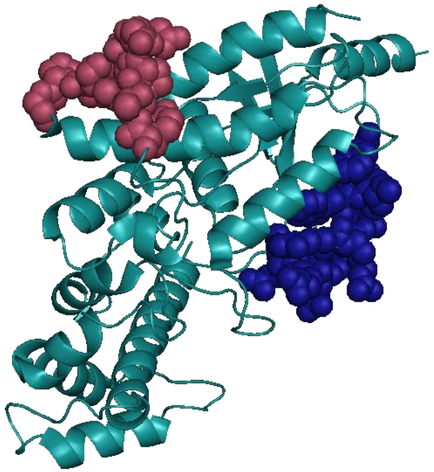
LC-MS/MS





Peptide-Level Changes

Modeling



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"I invented HRPF because HDX was too hard!"
-Mark Chance

HDX Versus HRPF

Well-Characterized Techniques for Protein Footprinting

	HDX	HRPF
Amino Acid Resolution	No	Yes
Glycosylated Proteins	No	Yes
Time Sensitive Conformations	No	Yes
Complex Samples	No	Yes

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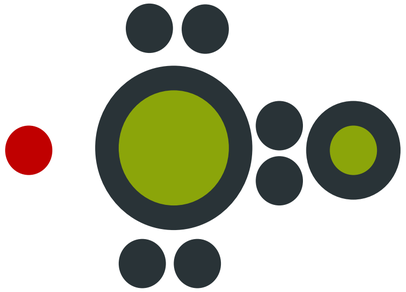
Barriers to BioPharma Adoption

Dangerous Lasers, Poor Reproducibility & Arduous Data Processing

Expensive and hazardous lasers create undue risk that limits HRPF adoption and perceived value

Compromised robustness and reproducibility from background scavenging erodes confidence

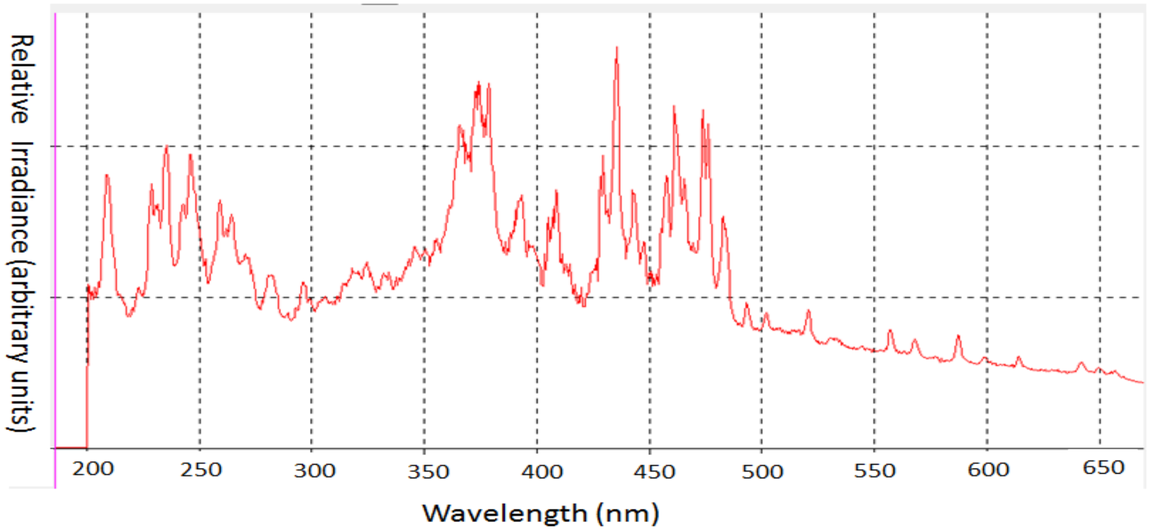


Data processing burden takes days to overcome slows productivity and tarnishes appeal

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**



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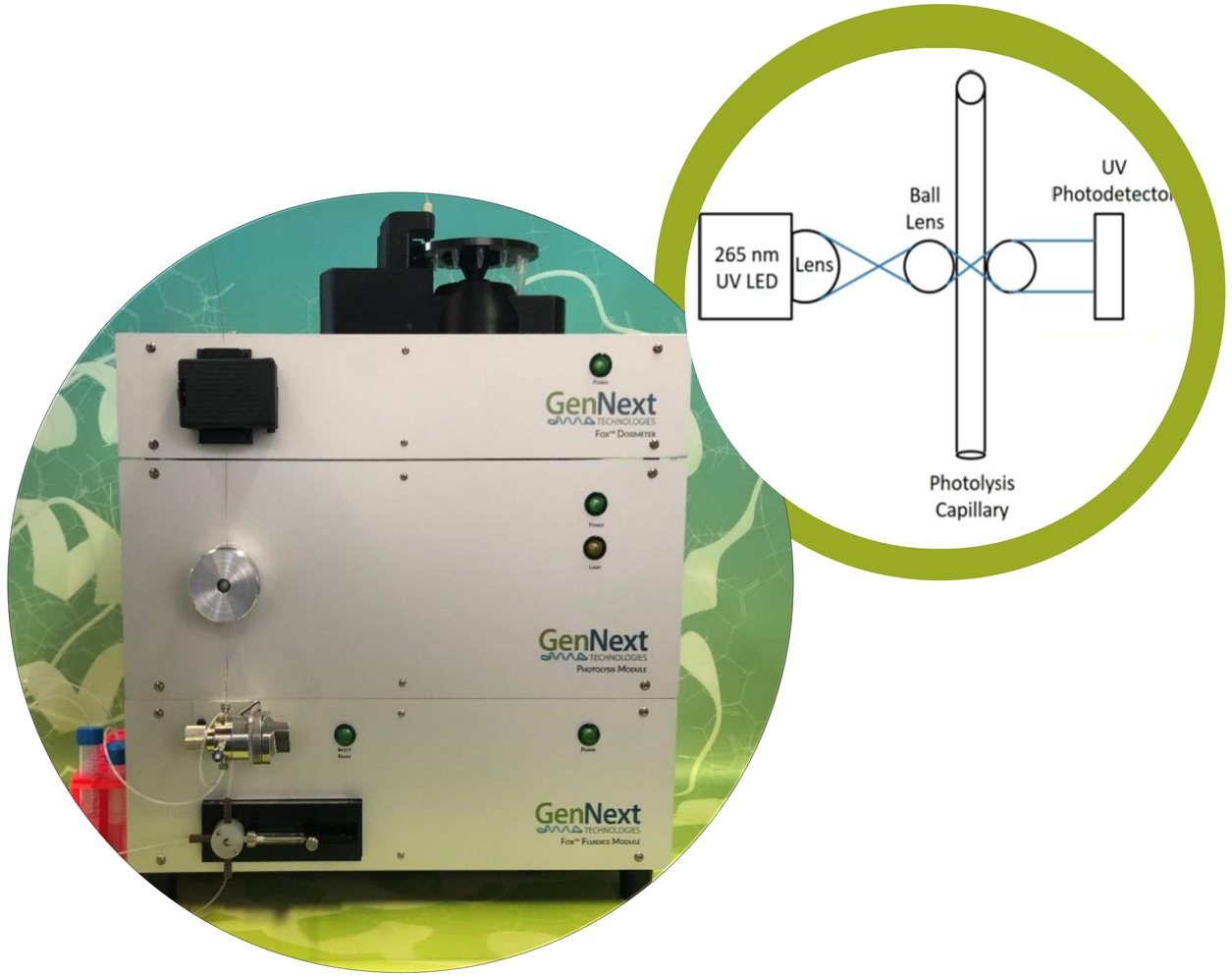
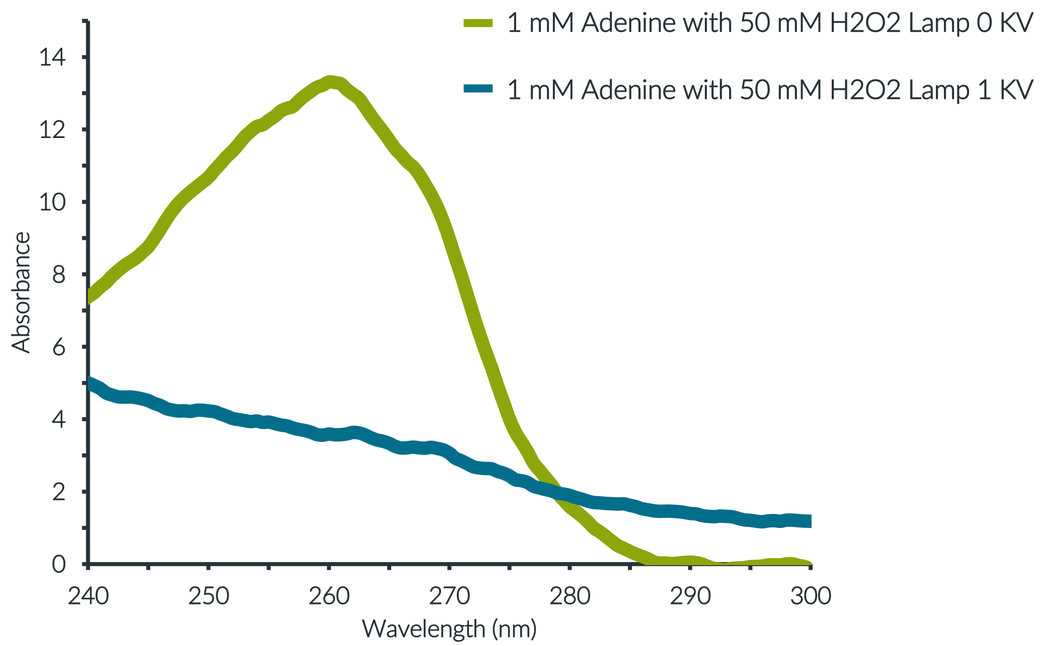
- **Dosimeter enables real-time adjustment of scavenging, providing confident and actionable results**
- Automated product collector facilitates collection of properly labeled sample while disposing unwanted products and reagents to waste



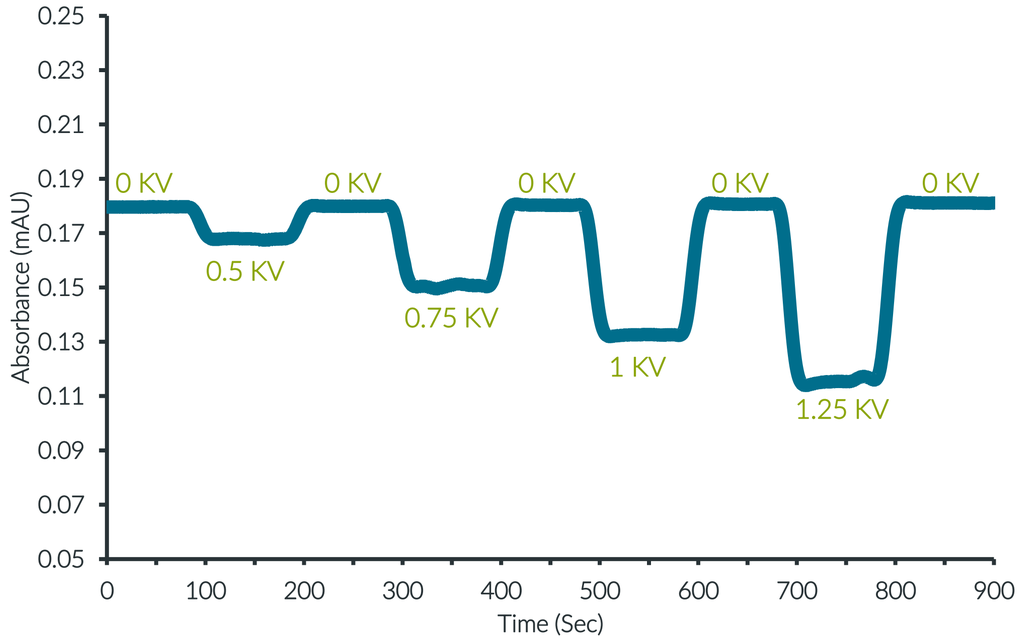
Real Time Radical Dosimetry for FPOP HRPF

Real-time OH Radical Load Measurement

Adenine UV Absorbance



Absorbance change with increasing lamp voltage



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FOX™ Protein Footprinting Workflow

Real-time correction for background scavenging

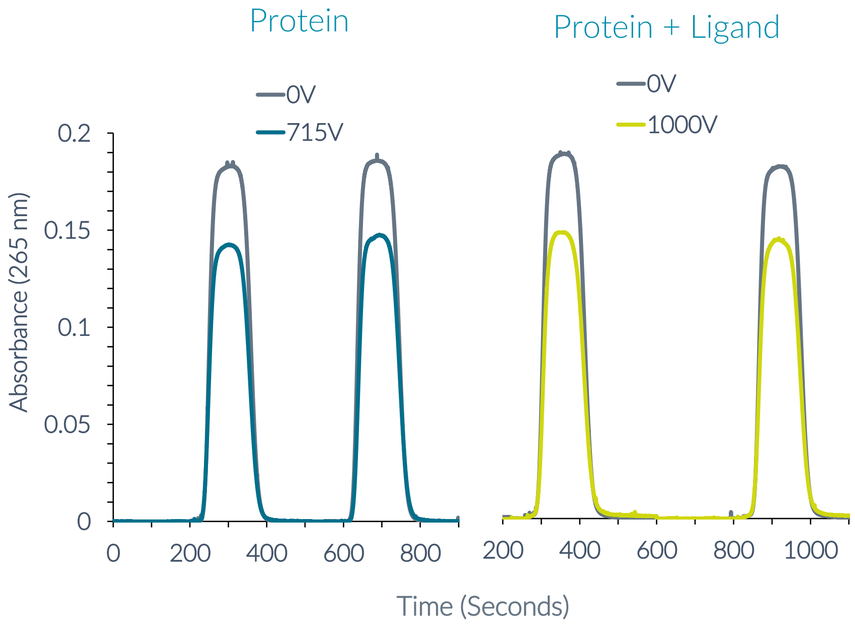
Automatic product collection

Real-time dosimetry

Flash lamp energy control

Photolysis

Inject protein, peroxide and dosimeter



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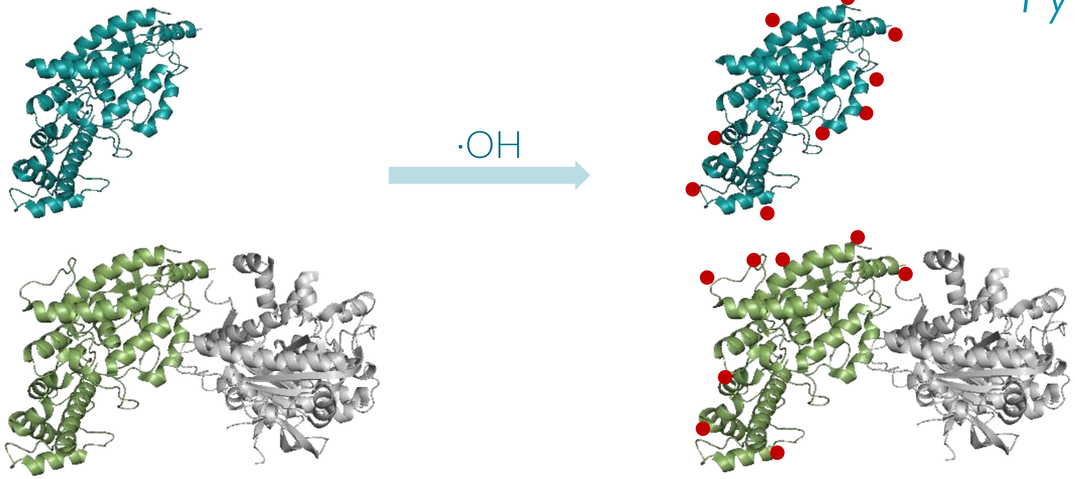
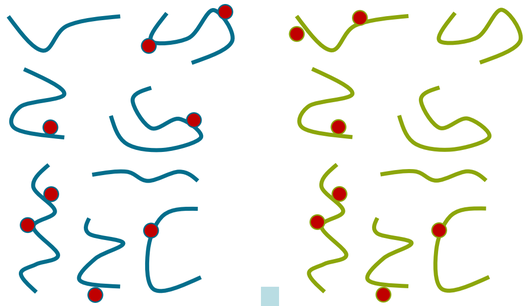
Hydroxyl Radical Protein Footprinting

Typical Workflow



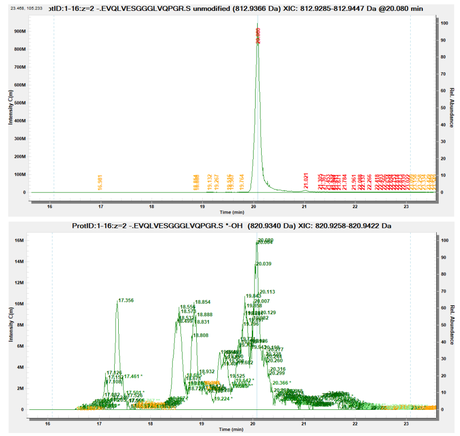
·OH

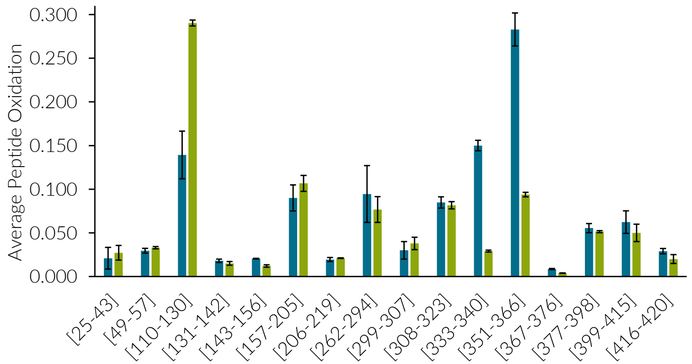
Denature & Digest



LC-MS/MS

FoxWare™ Protein Footprinting Data Processing Software





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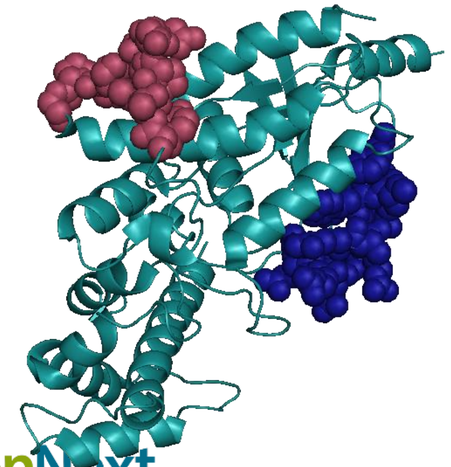

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FoxWare™ Protein Footprinting Data Processing Software

FoxWare Pre-Scan Program

- Quick test to confirm good protein coverage and consistent retention times across replicates

FoxWare Data Processing Software

- Searches for desired FPOP modifications
- Generates XICs for all unmodified and modified peptides
- Calculates the average peptide oxidation (APO)

FoxWare Radical Dosimetry Software

- Compare APO across varying levels of hydroxyl radical yield
- Sets up method and builds confidence the protein is labeled in its native conformation

FoxWare Data Analysis Software

- Subtracts background oxidation
- Easily compare APO for multiple conditions
- Identify peptides with a significant change in APO
- Map changes to available crystal structure

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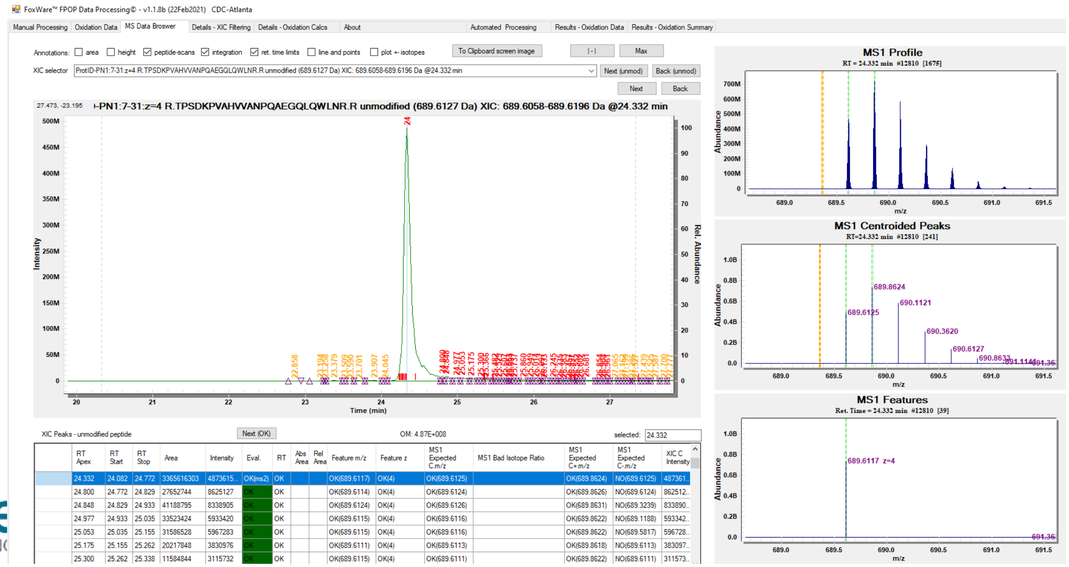
FoxWare™ Data Processing Software

Manual and Automated Data Processing

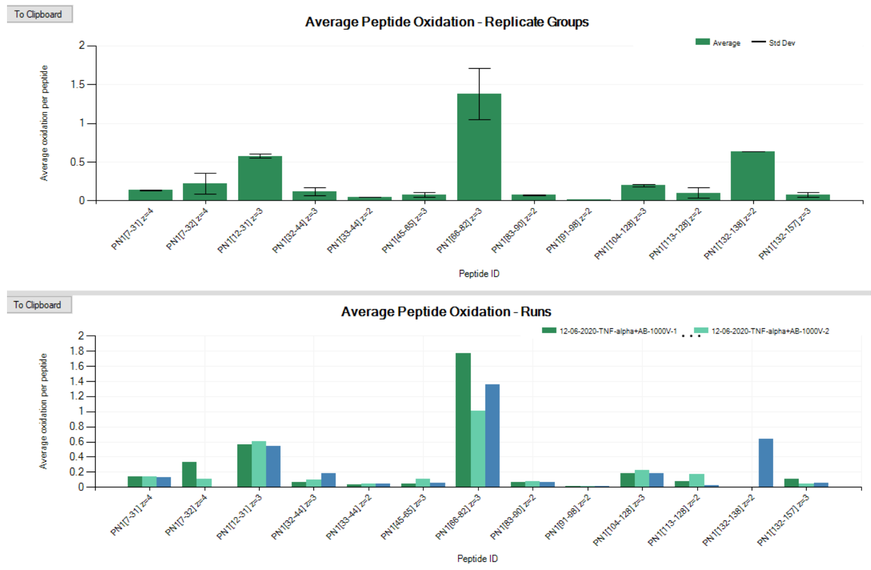
1) Searches for desired FPOP modifications

	Ox. Class	Select	Residue	Modification	Code	ΔMass(Da)	Max Allowed	Occurance
▶	Default	☑	All except Gly, G	hydroxy	*-OH	+15.9949	3	Common
	Default	☐	All except Gly, G	carbonyl	*=O	+13.9793	1	Rare
	Residue Specific	☐	Ser, S	carbonyl	S=O	-2.0156	1	Rare
	Residue Specific	☐	Thr, T	carbonyl	T=O	-2.0156	1	Rare
	Residue Specific	☐	Cys, C	hydroxy	C-OH	-15.9772	1	Rare
	Residue Specific	☐	Asp, D	decarboxylation	D-DCB	-30.0106	1	Rare
	Residue Specific	☐	Glu, E	decarboxylation	E-DCB	-30.0106	1	Rare
	Residue Specific	☐	His, H	ring-open	H-RO1	-22.0320	1	Rare
	Residue Specific	☐	His, H	ring-open	H-RO2	-10.0320	1	Rare
	Residue Specific	☐	His, H	ring-open	H-RO3	+4.9789	1	Rare
	Residue Specific	☐	Arg, R	deguanidination	R-DG	-43.0534	1	Common
	Residue Specific	☐	Arg, R	deguanidination	R-DG	-43.0534	1	Rare

2) Generates XICs for all unmodified and modified peptides

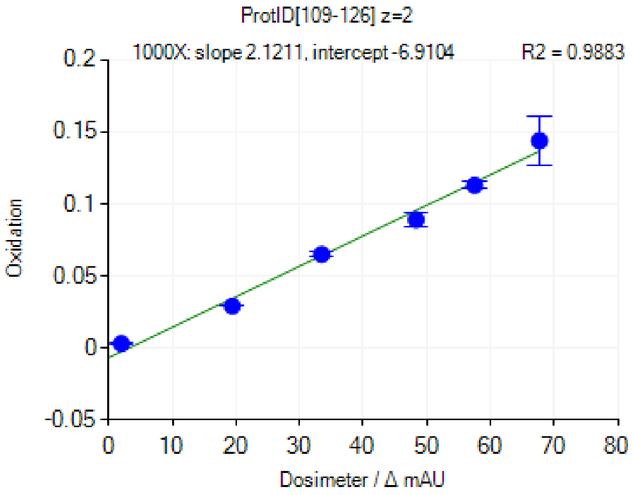


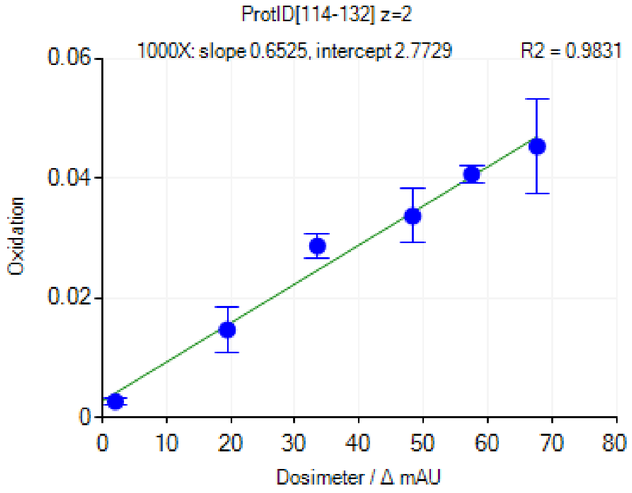
3) Calculates the average peptide oxidation (APO)

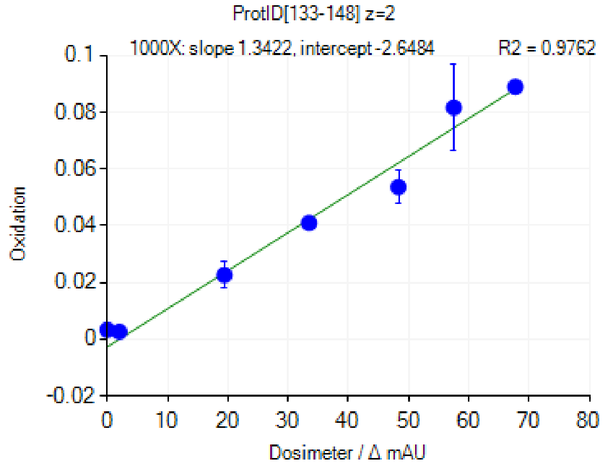


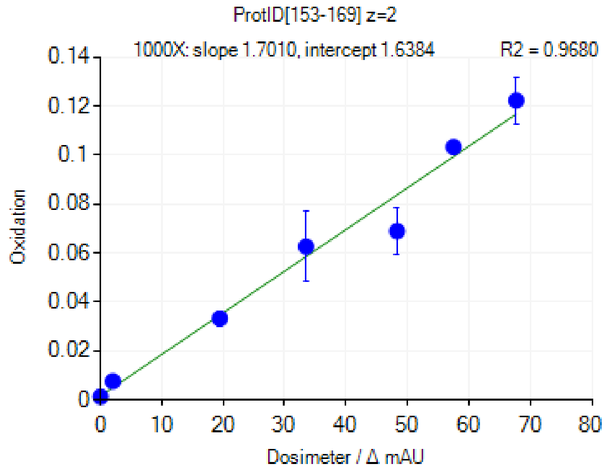
Hydroxyl Radical Protein Footprinting

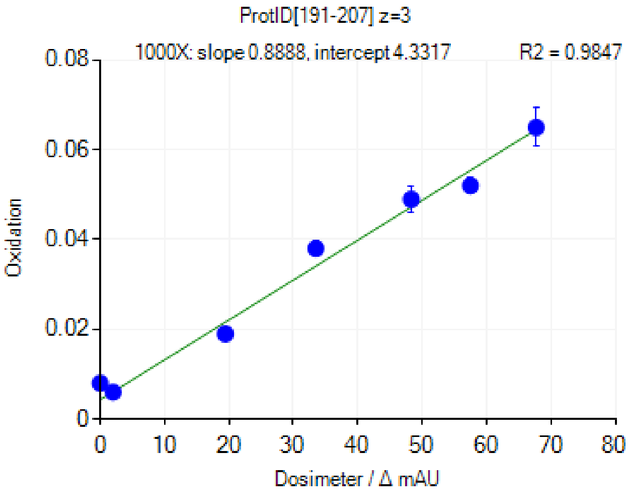
Protein Radical Dosimetry

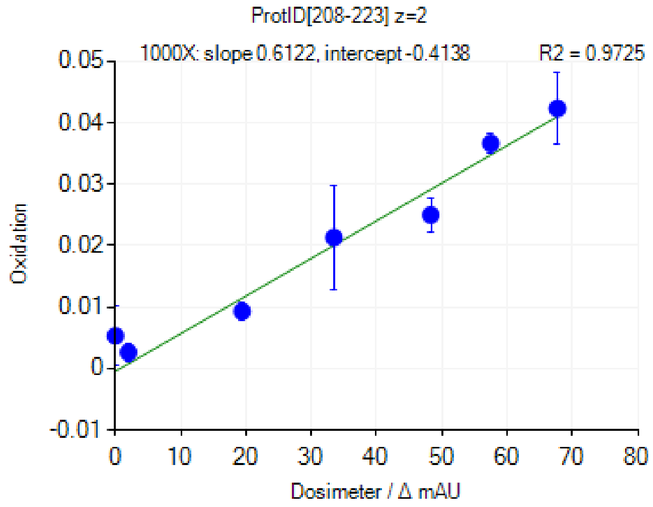


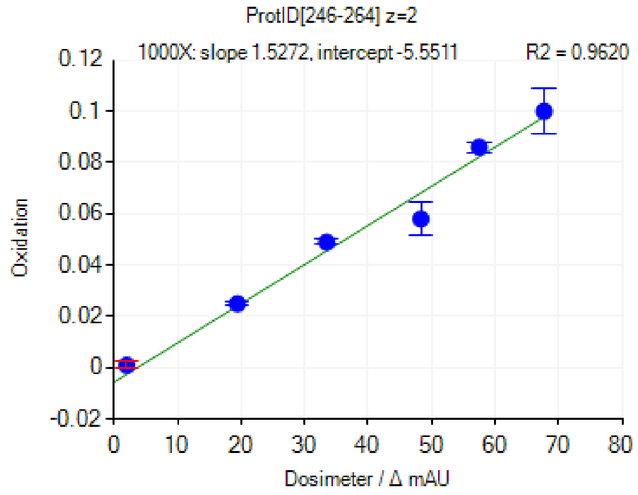


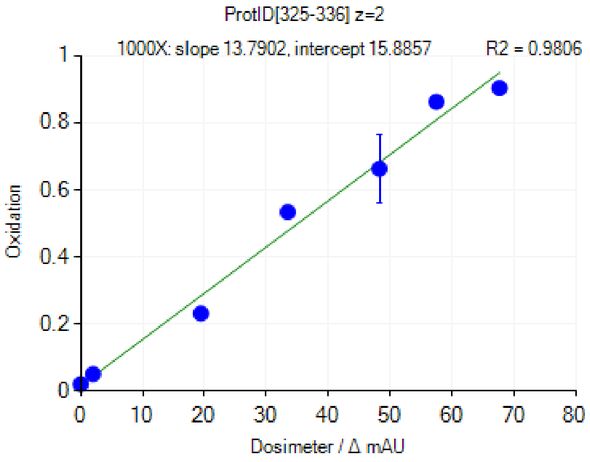












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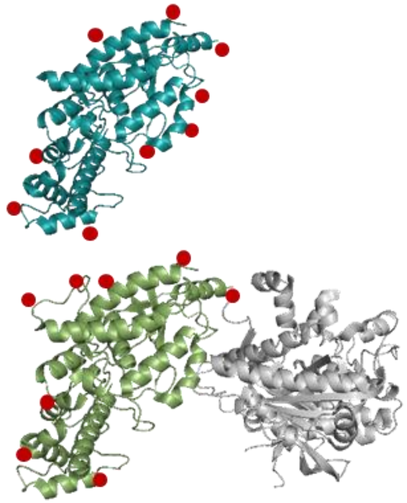
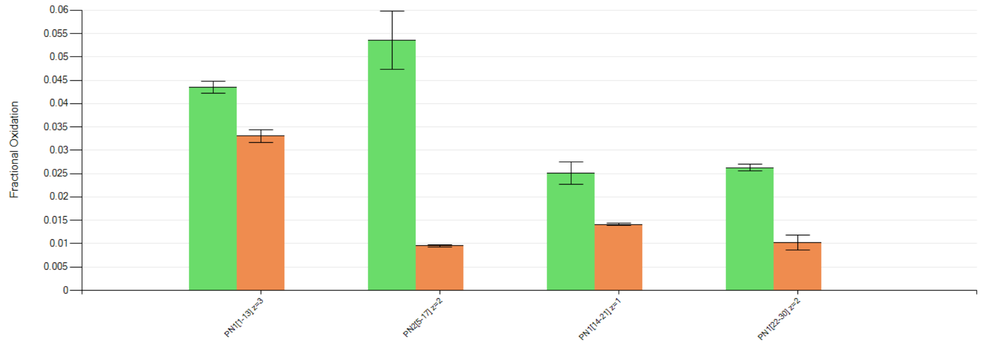
FoxWare™ Data Analysis Software

FPOP Oxidation for Differential Studies

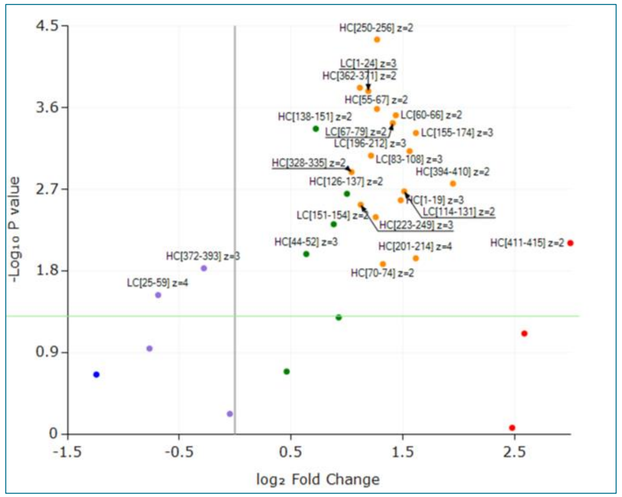
1) Combine and organize data across a full differential experiment



2) H_2O_2 induced modification subtraction



3) Advanced analytics and data presentations



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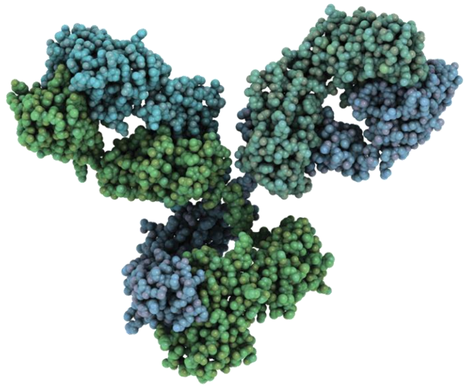
Protein Footprinting Applications

Biopharmaceutical Drug Discovery & Development



- Monoclonal Antibodies
  - Epitope Mapping
  - Paratope Mapping
  - Aggregation
  - Formulation Studies
- Druggable Target Discovery
  - Drug Binding Site
  - Allostery
- Biosimilar Development
  - Originator Comparison
- Biomolecular Interactions
  - Protein-Ligand
  - Protein-Protein
- Expression Platform Studies
- Excipient Effects
- Structure
- Stability

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Protein Footprinting Webinar Series

All Available On-Demand at
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

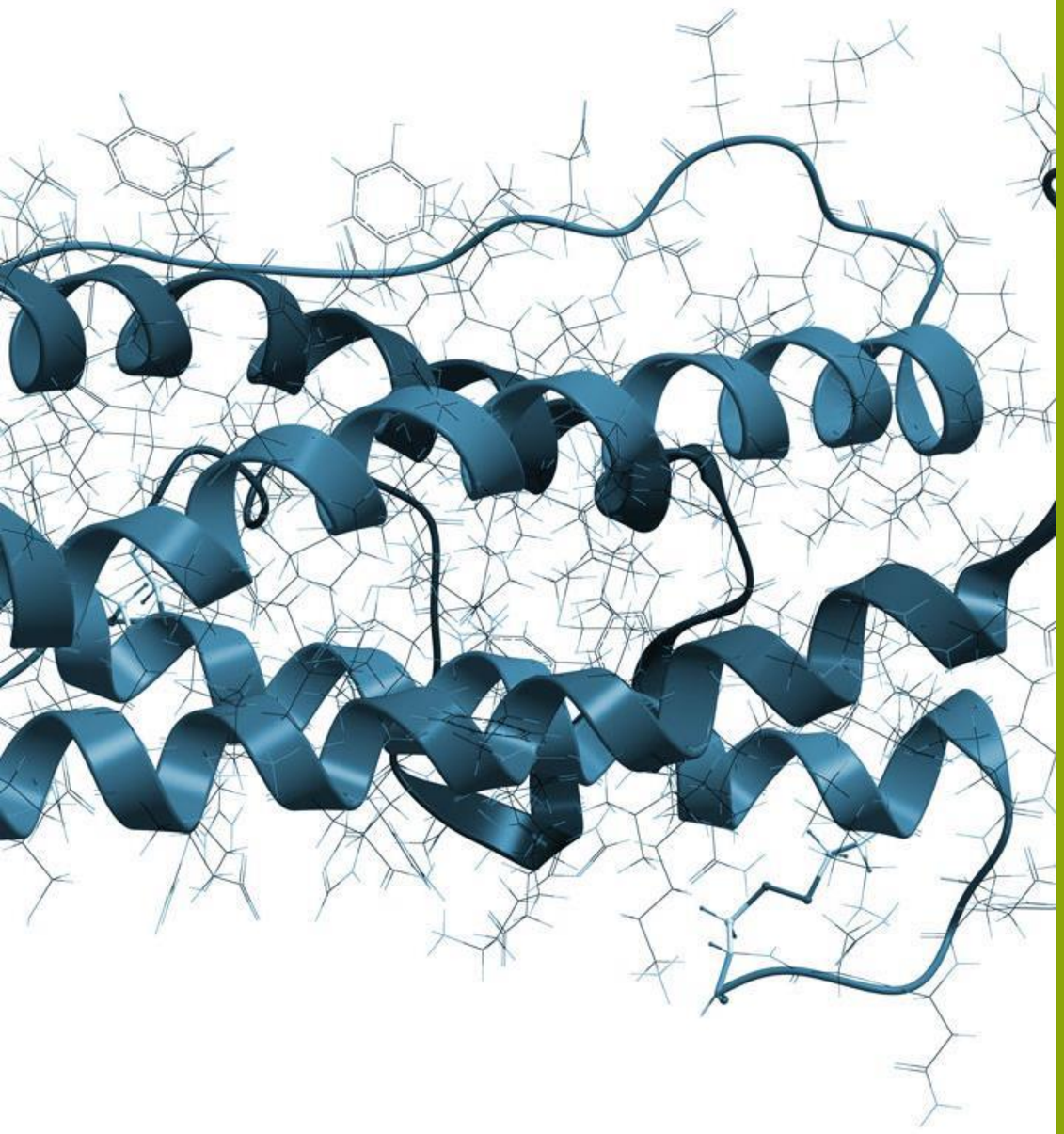
- Professor Joshua Sharp of the University of Mississippi

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



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

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Thank You for Your Participation!

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