Integrating Mass Spectrometry-Based Structural Proteomics and Molecular Docking to Analyze Ebola Protein-Host Binding Interfaces

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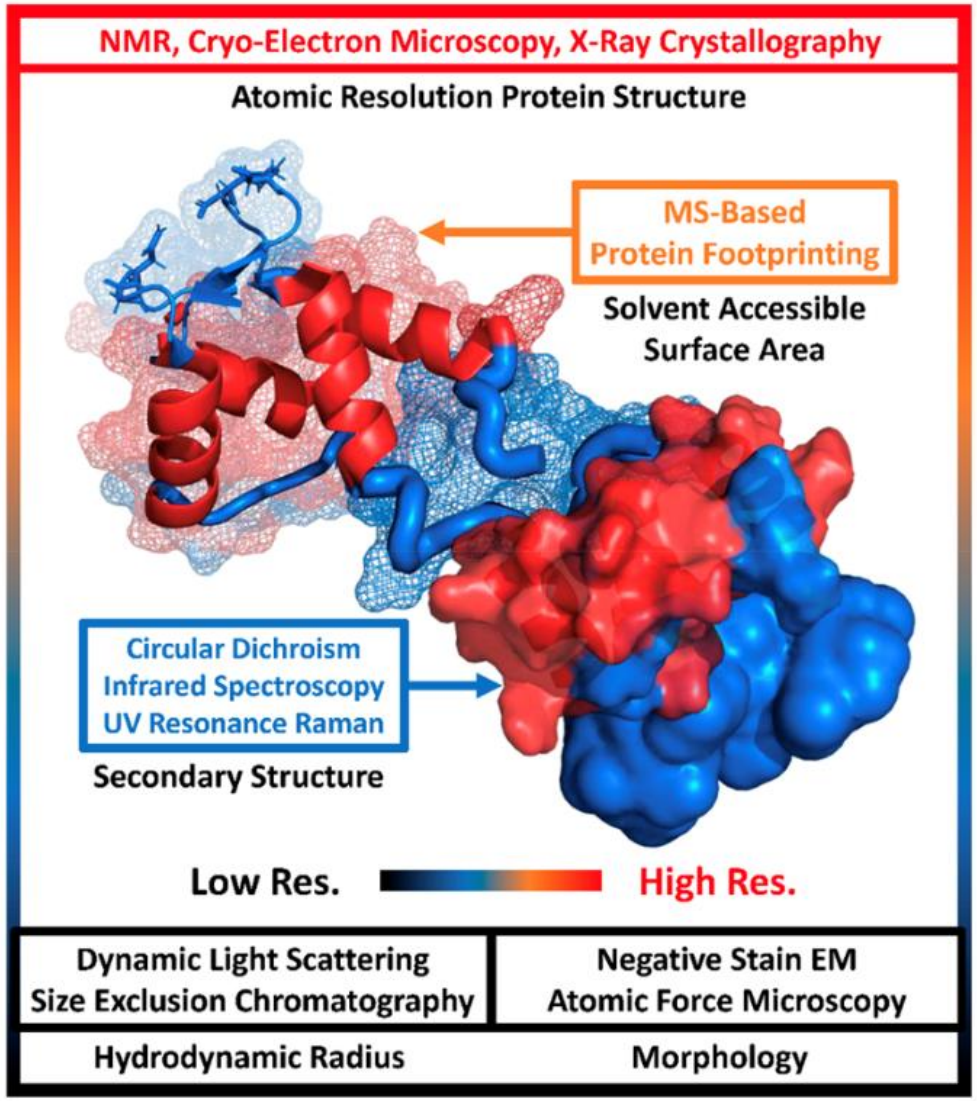
Biophysical Tools in Protein Higher Order Structure (HOS)

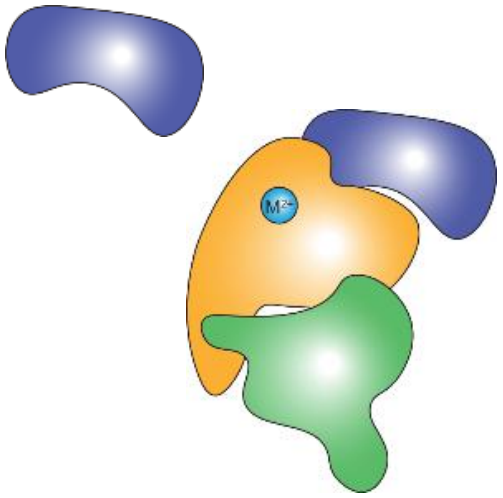
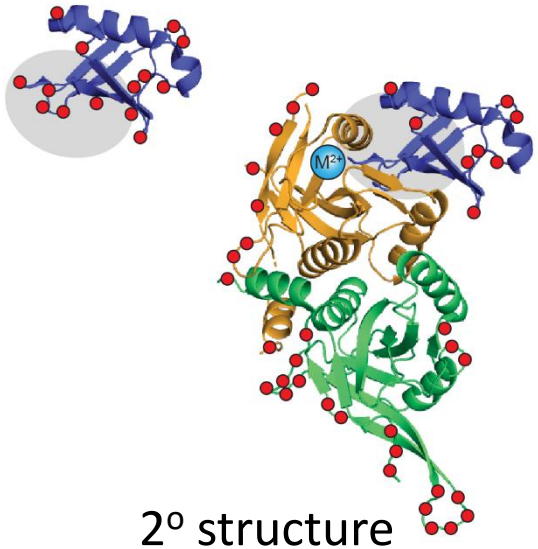
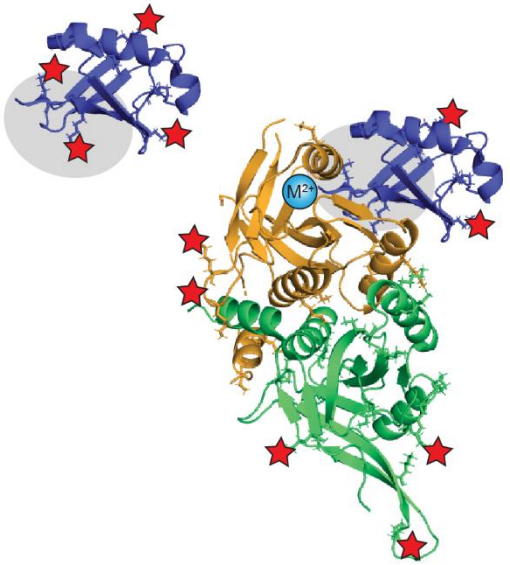
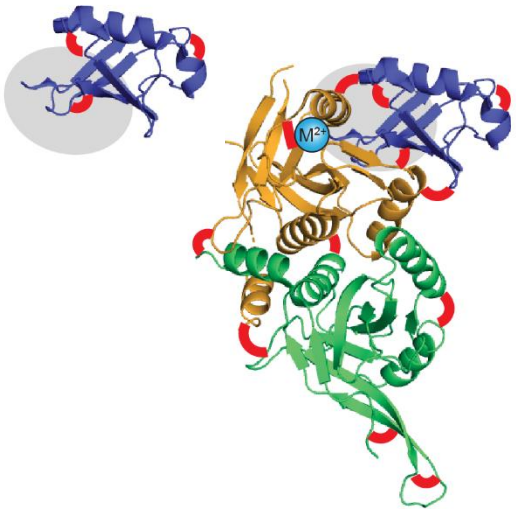


- **Protein Higher Order Structure**
- Secondary to quaternary
- Protein biological function, stability and molecular interactions
- **Mass Spectrometry-Based Approaches**
- Fast and sensitive
- Real-time characterization in solution (under physiological conditions)
- High throughput capability
- Simple sample preparation; requires only ng-μg amounts
- High spatial resolution (peptides & residues)
- …

Liu et al., *Chemical Rev.* **2020**, 120, 4355-4454

Our suite of mass spectrometry approaches for HOS characterization

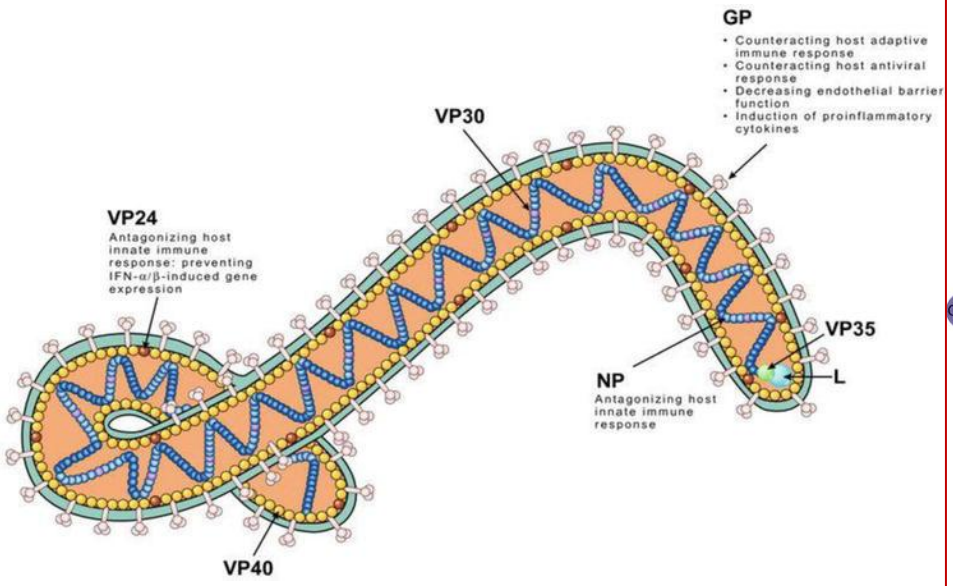
Structural Proteomics– add labels/localize with proteomics

Native MS	HDX	Footprinting	Crosslinking
			
Stoichiometry and assembly	2° structure and solvent accessibility	Side-chain interactions	Distance limited contact
	sec-hr reaction times	ms-min reaction times	min-hr reaction times

A comprehensive characterization platform is necessary for protein HOS analysis

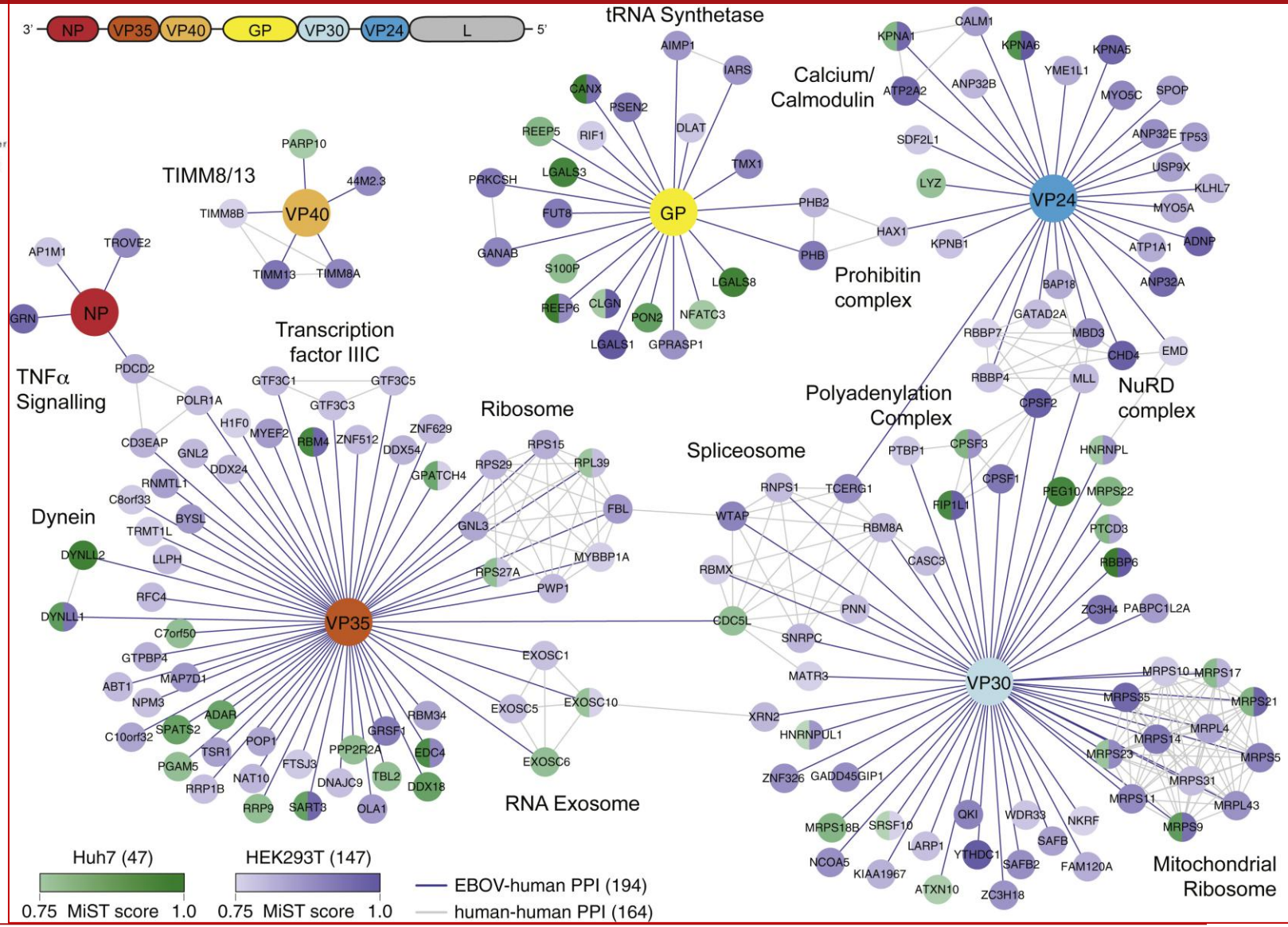
PDB 5u4p

The biological question we are interested: Ebola Protein-Host E3 ligase



Ebola virion

- **eVP35:** multifunctional cofactor essential for viral replication and immune evasion
- **eVP35-E3 ubiquitin ligase MIB2 interaction**

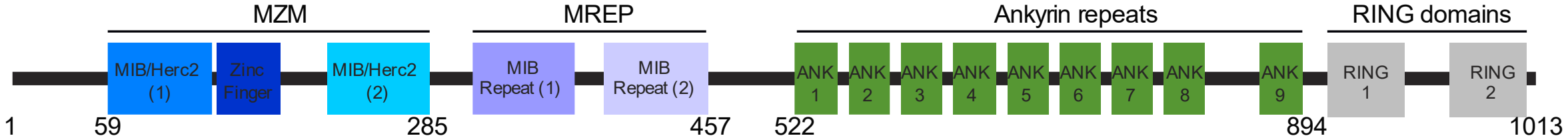


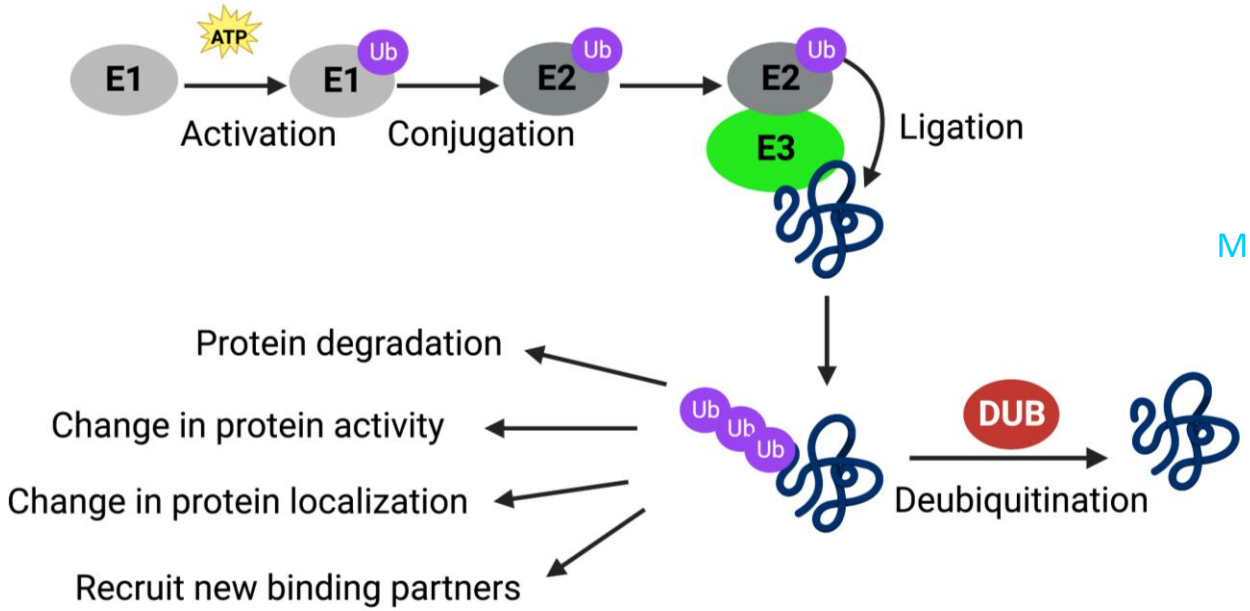
Ebola-Human Interactome

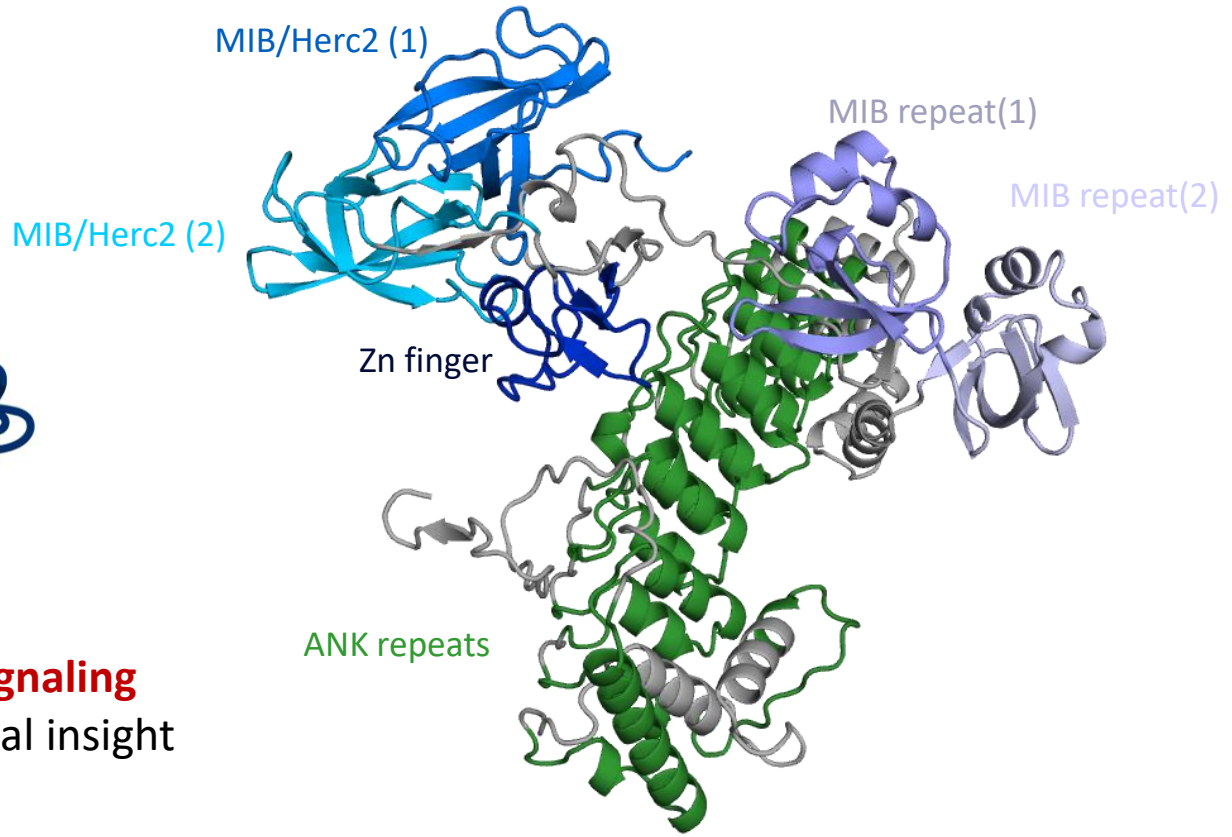
Batra, J. et. Al. *Cell*. **2018.** *175*, 1917-1930.e13

Host E3 ubiquitin ligase: MIB2 (Mindbomb protein 2)

MIB2: Mindbomb protein 2, E3 ubiquitin ligase







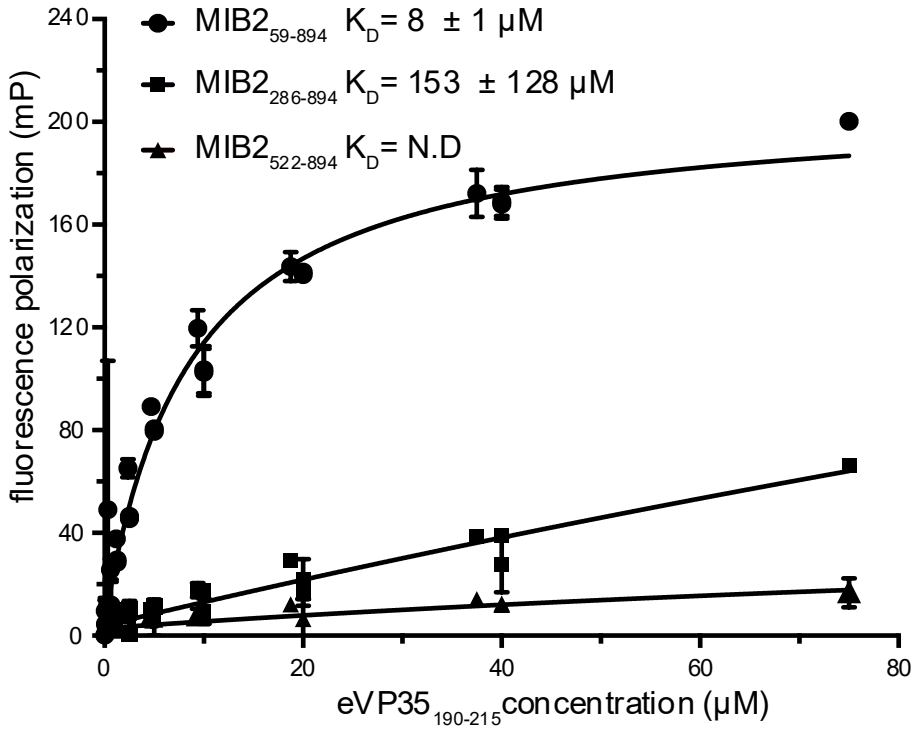
- **eVP35 binding inhibits the function of MIB2 in Type I IFN signaling**
- Understanding MIB2-eVP35 interaction will provide additional insight into EBOV viral pathogenesis

Batra, J. et. Al. *Cell*. **2018.** *175*, 1917-1930.e13

MIB2 AlphaFold structure

Platform: Binding interaction between eVP35-MIB2

	MIB2 construct	Binds eVP35?
1-1013	MZM MREP ANK RING	+++
59-894		+++
286-894		+
522-894		-



- Truncation experiments characterized MIB2 **MZM and MREP** domains are critical for VP35 binding
- MIB2 $_{59-894}$ construct was selected

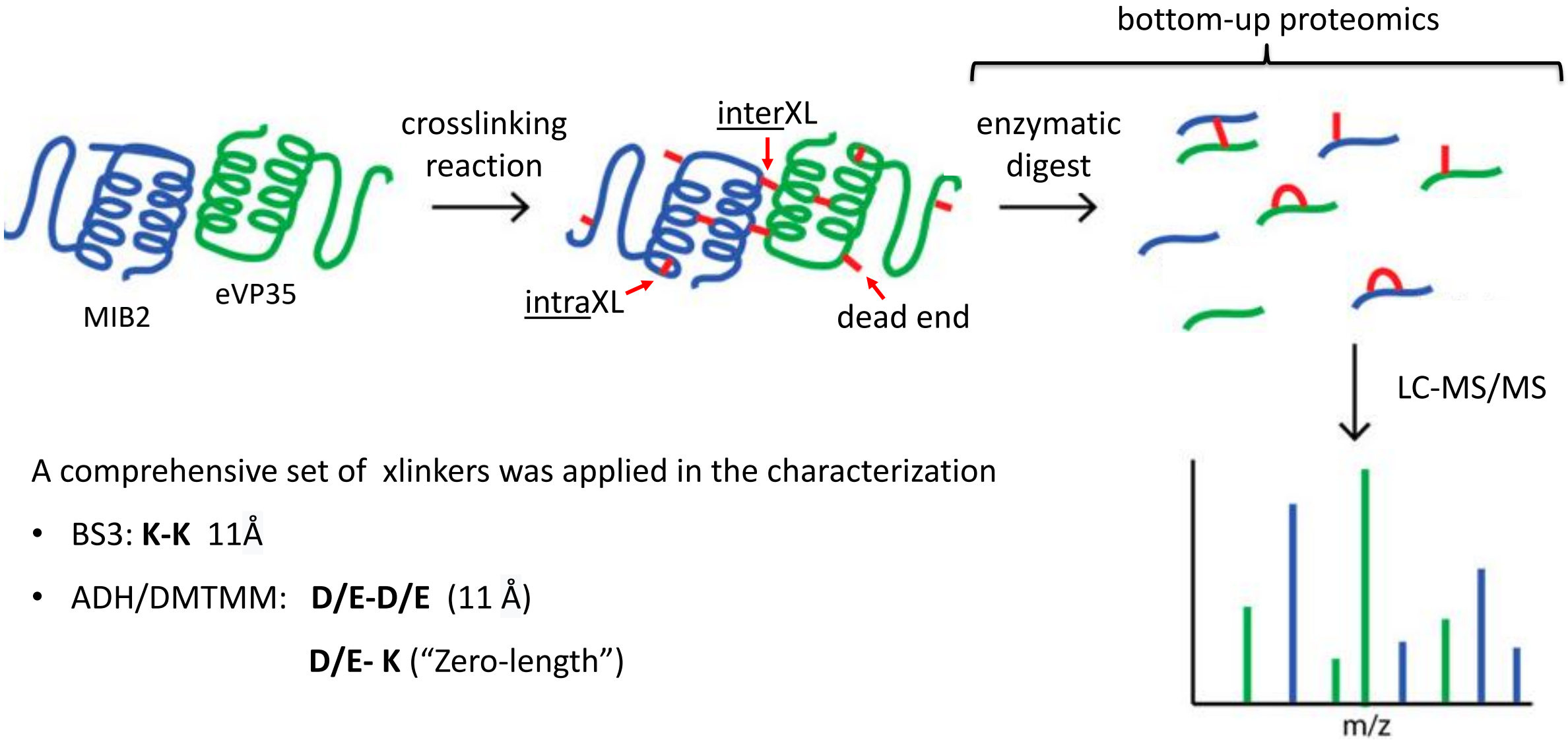
Conformational binding interfaces? Binding induced structural changes?

Proposed experiment

HDX-MS and XL- MS to localize the eVP35 binding sites on MIB2

Uwase, G. et. Al. *bioRxiv*. **2025.**

Crosslinking (XL)-MS identifies distance-limited proximity

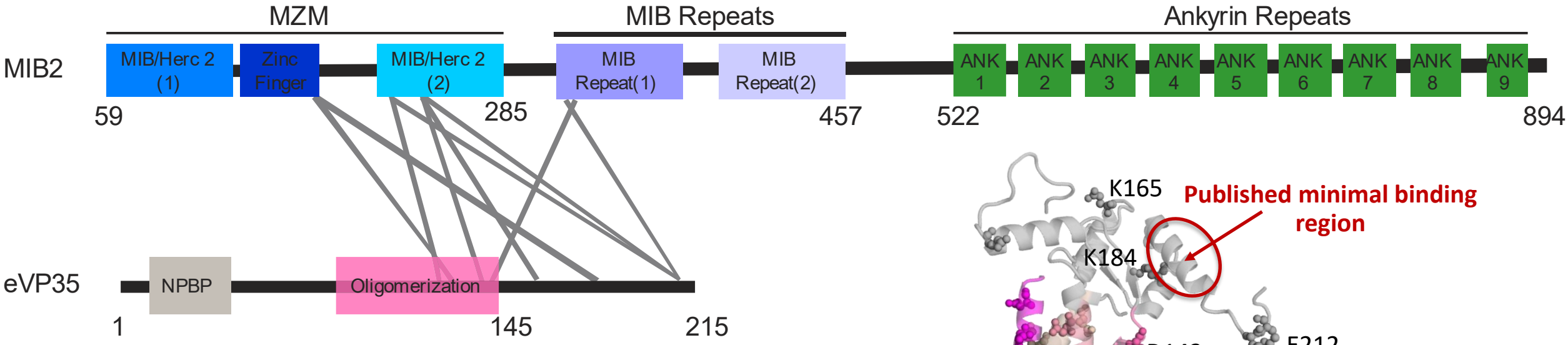


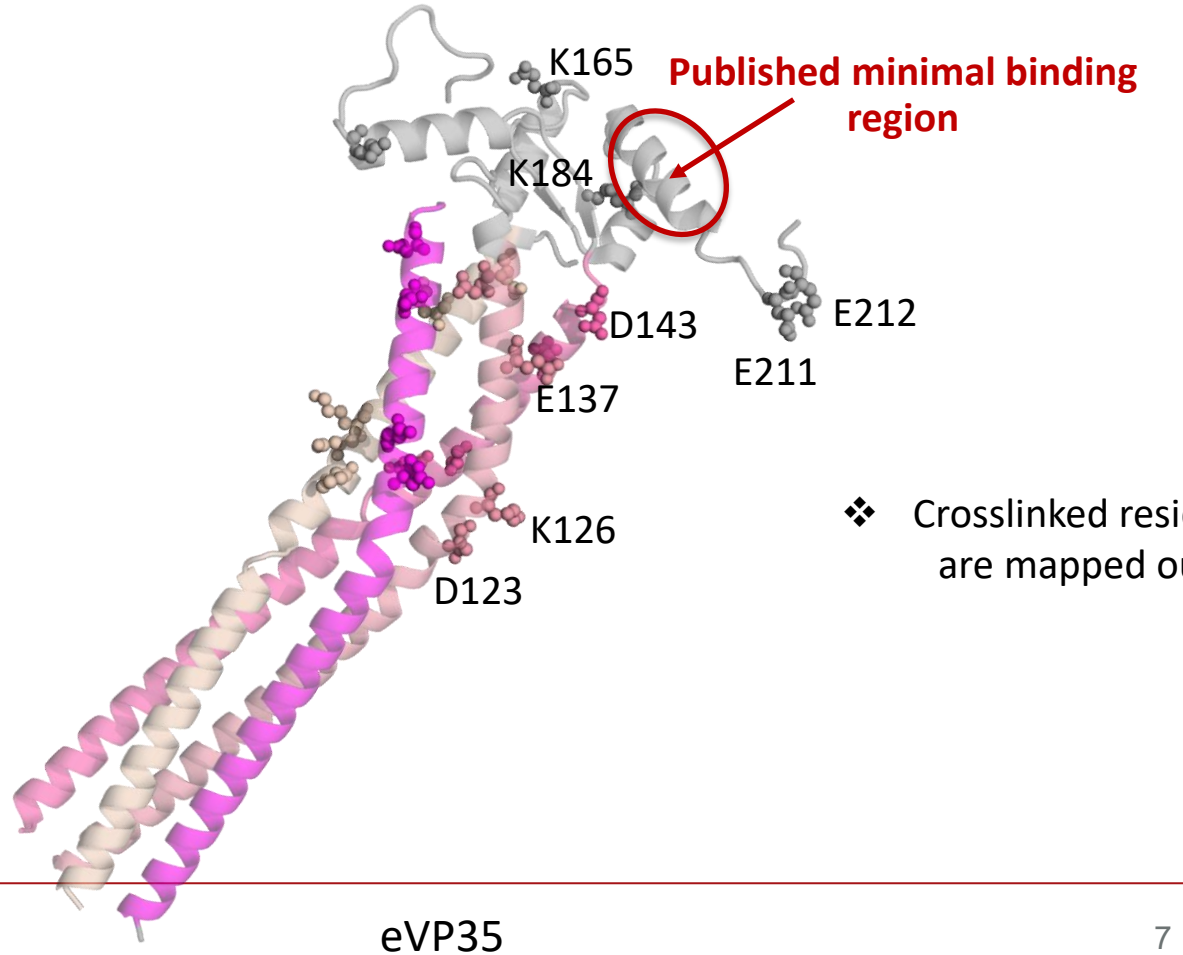
A comprehensive set of xlinkers was applied in the characterization

- BS3: **K-K** 11Å
- ADH/DMTMM: **D/E-D/E** (11 Å)

  D/E- K ("Zero-length")

MIB2 MZM and MREP domains crosslink to eVP35 near the identified minimal binding site

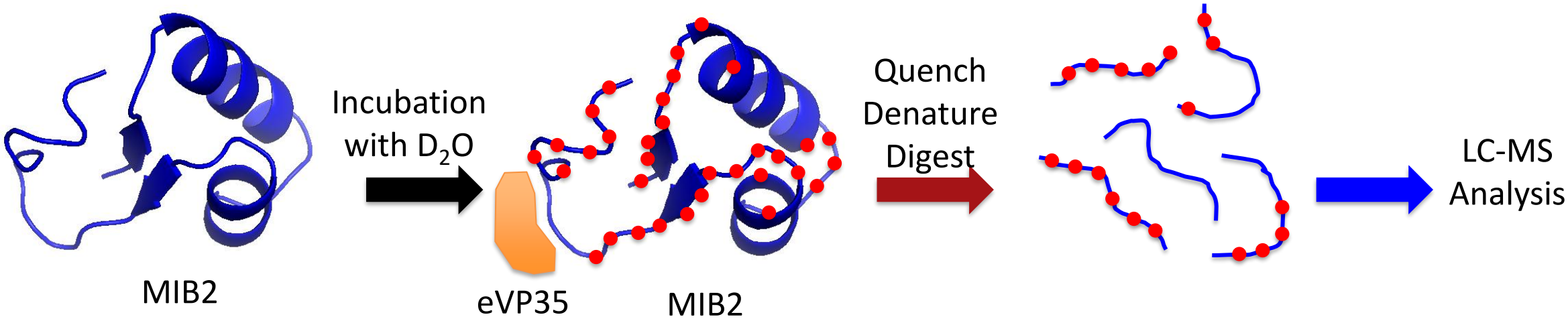




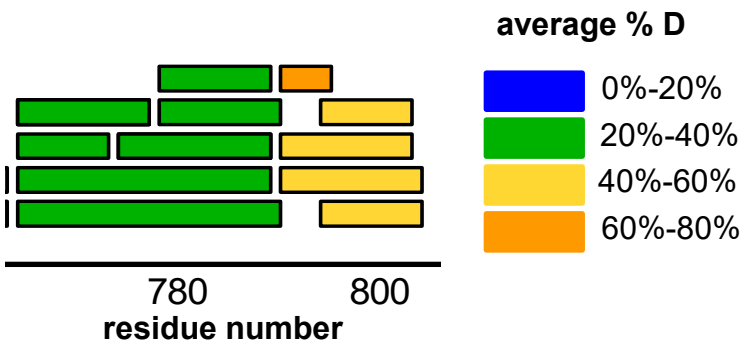
- eVP35: high consistency between MS and published results
- MIB2: **MZM** and **MREP** are close to the binding interface

❖ Crosslinked resides are mapped out

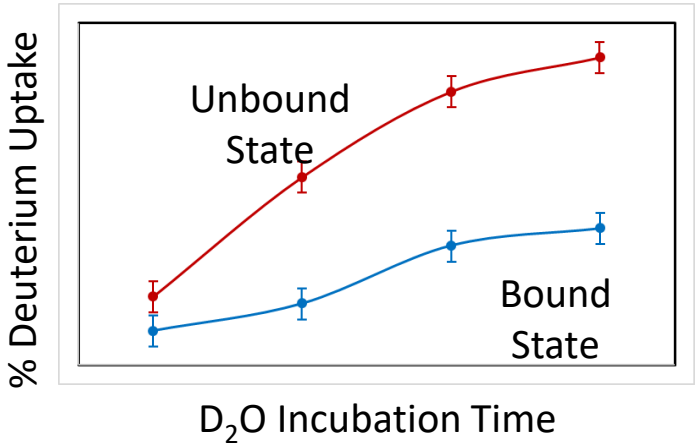
HDX-MS provides insight into second structure and solvent accessiblity

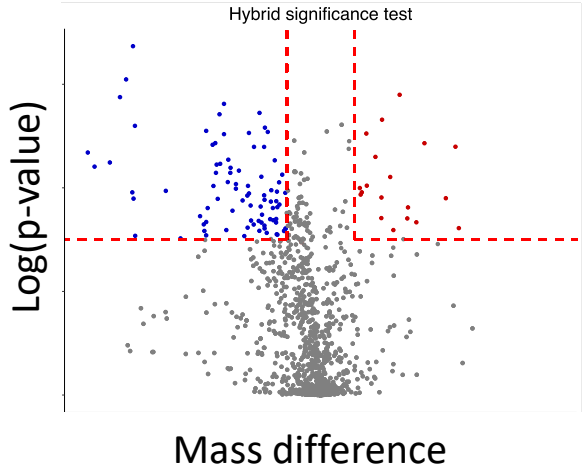


Reports on regions of flexibility/dynamics

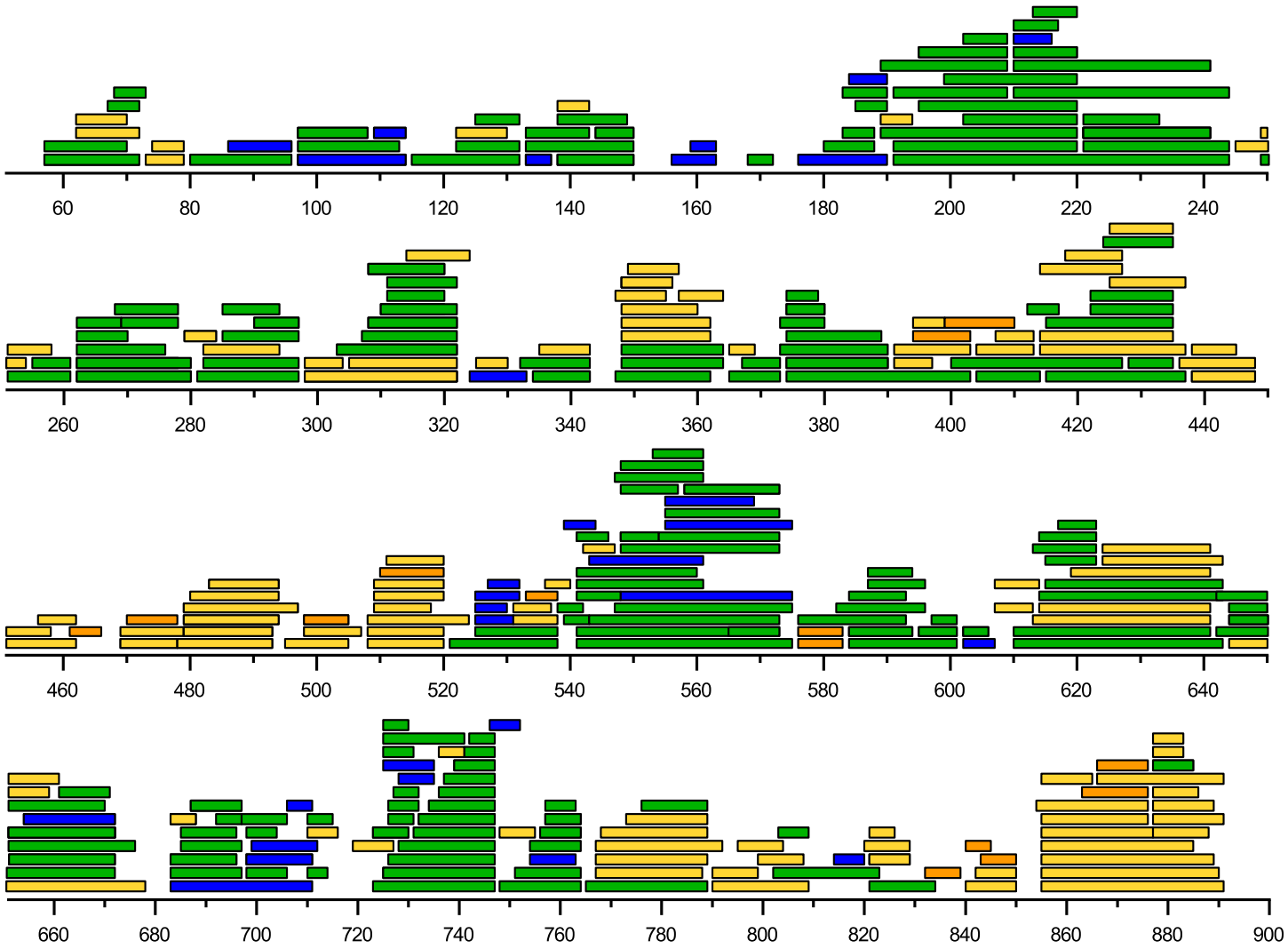


Identifies statistically significant differences





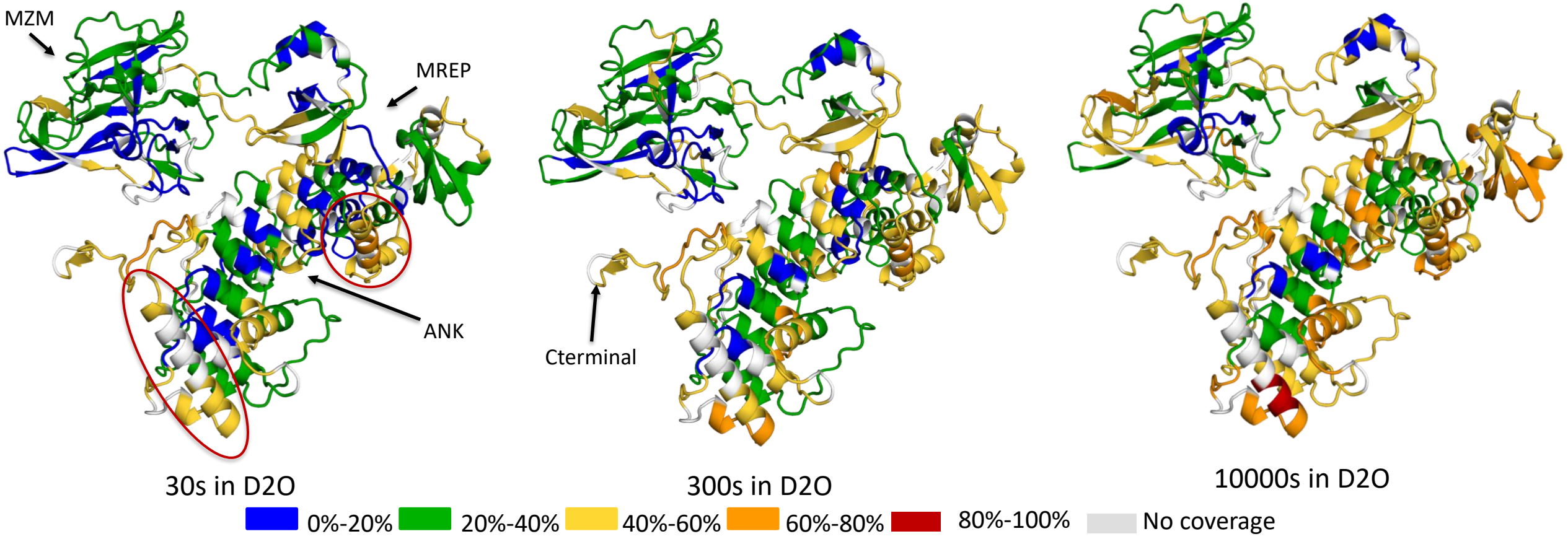
HDX-MS reveals regions of stability and flexibility within MIB2



- Digestion: Fungal XIII-pepsin at 4C
- 376 peptides
- 96% coverage
- Redundancy: 6
- Avg peptide length: 12.5

average % D for all time points 0%-20% 20%-40% 40%-60% 60%-80% No coverage

HDX-MS reveals MIB2 regions of stability and flexibility within MIB2

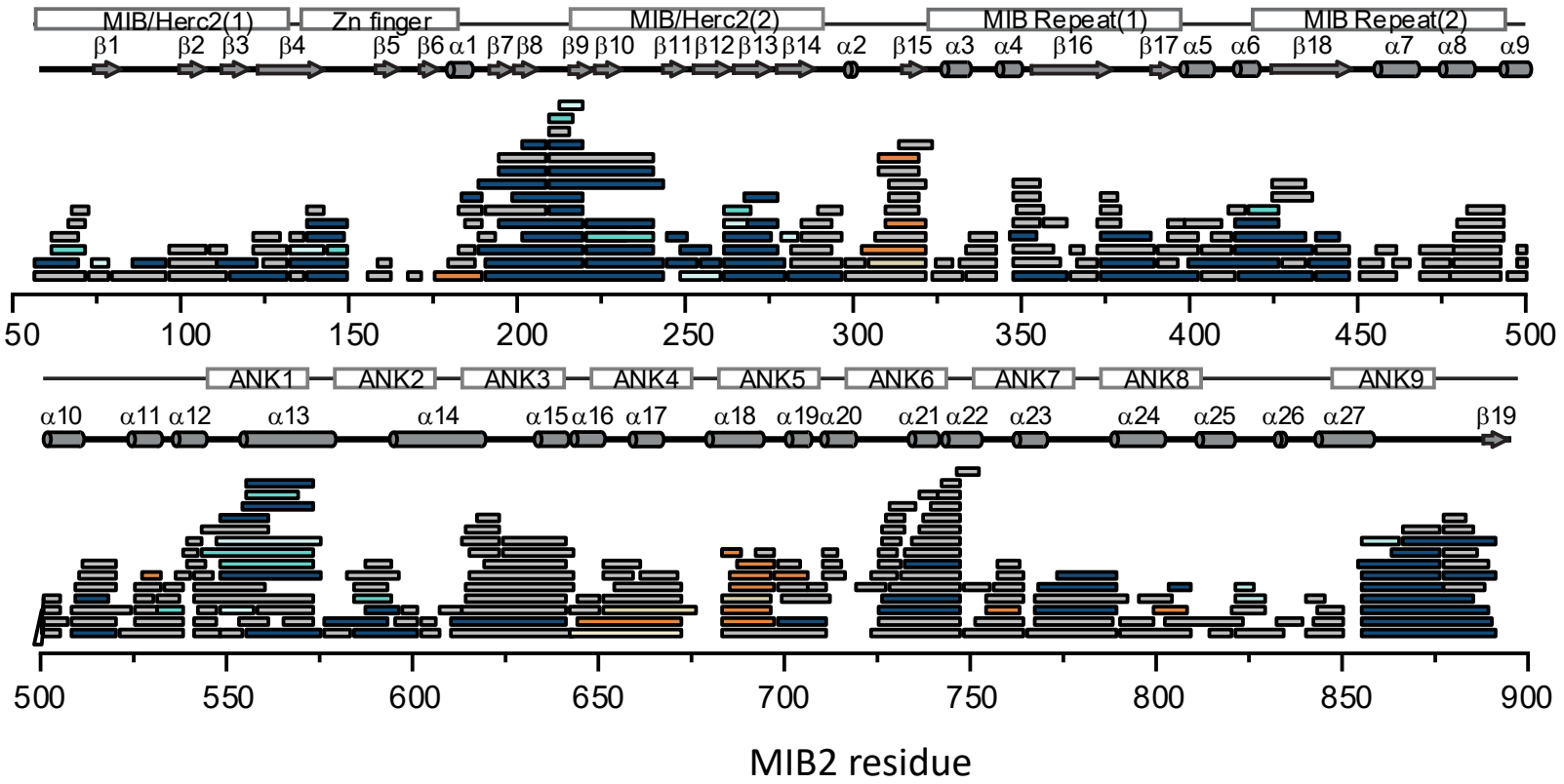


- MZM exhibits relative stability
- Some of the long helices predicted by AlphaFold show > 40% deuterium uptake even at 30 sec of HDX
- HDX results complement the AlphaFold structure by revealing dynamic features of MIB2.

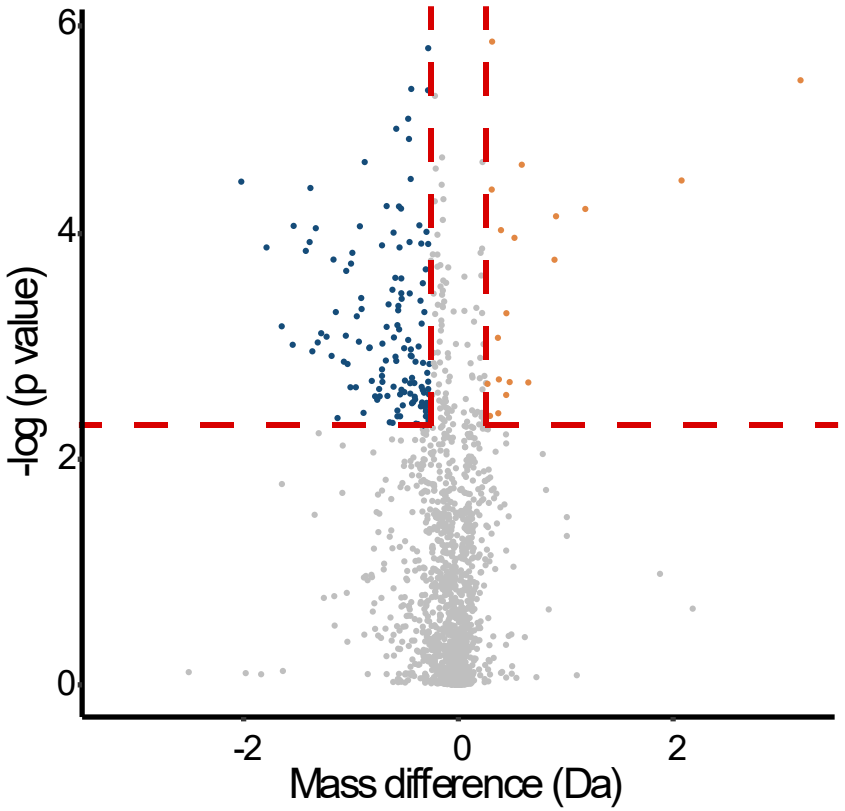
Differential HDX reveals potential eVP35 binding sites and remote conformational changes of MIB2

Differential HDX between MIB2 -/+ eVP35

Differential HDX peptide map



Volcano plot



decreased HDX: P <0.005, P <0.01, P <0.05
increased HDX: P <0.005, P <0.01, P <0.05
non-significant

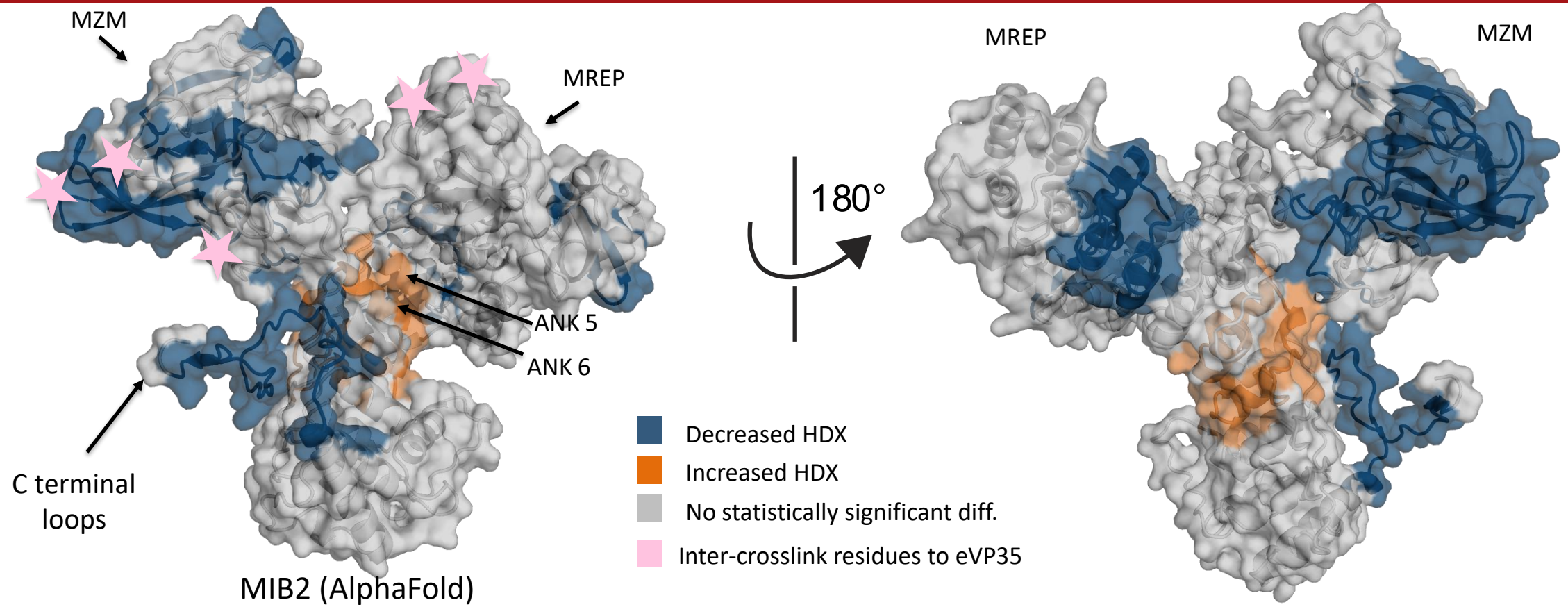
- Decreased HDX: binding sites or more hydrogen bonds
- Increased HDX: gain flexibility or dynamics

P-value cutoff: 0.005

Global significance limit: 0.25 Da

DOI: 10.1002/pro.70096

Differential HDX mapped onto an AlphaFold structure reveals discontinuous interaction surface



- Protected regions form a **discontinuous surface** (consistent with interXL)

Possibilities:

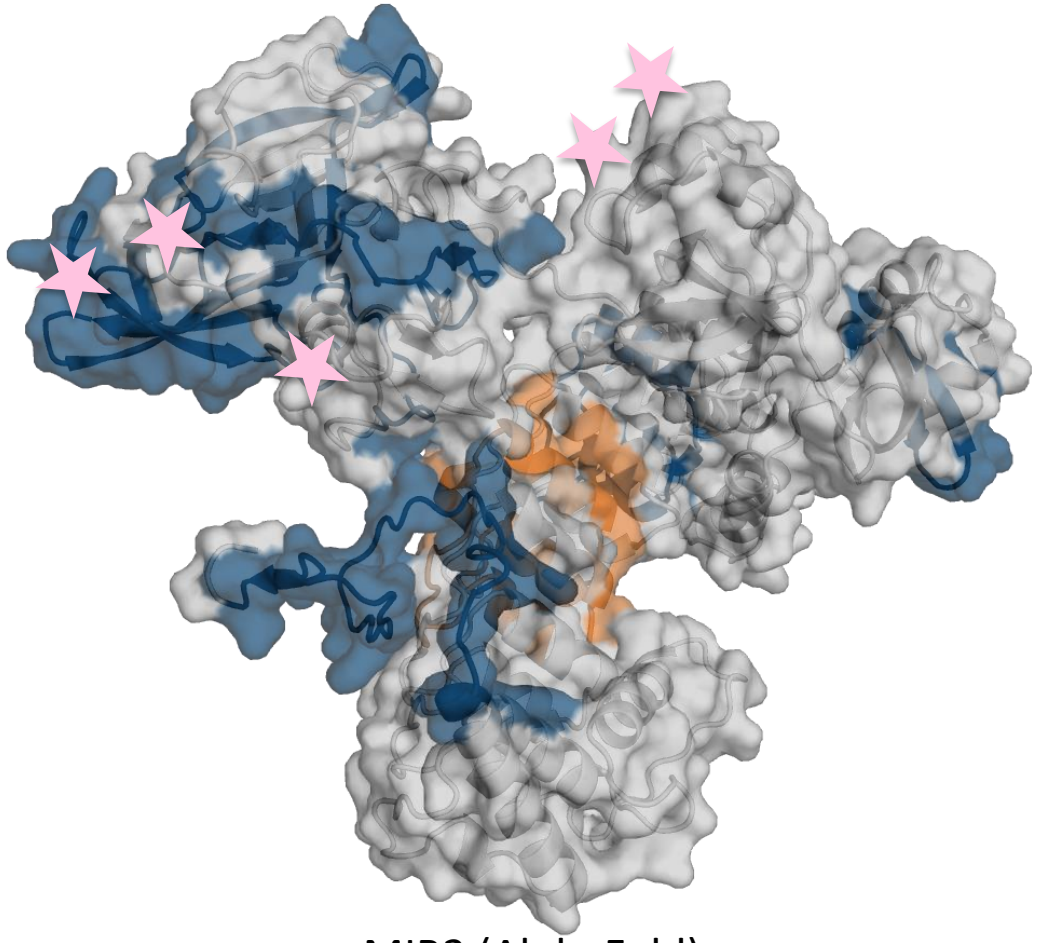
(i) binding induced conformational changes?

(ii) Or this structure is inconsistent in this context (especially domain-orientation)? (consistent with interXL)

(iii) MZM and MREP appear reoriented upon eVP35 binding?

- ANK 5-6 shows increased flexibility or dynamic motion upon binding

Conclusions and Questions remained



MIB2 (AlphaFold)

Decreased HDX

Increased HDX

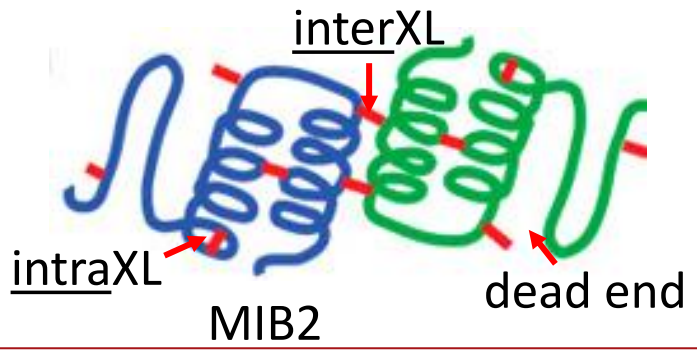
No statistically significant diff.

Inter-crosslink residues to eVP35

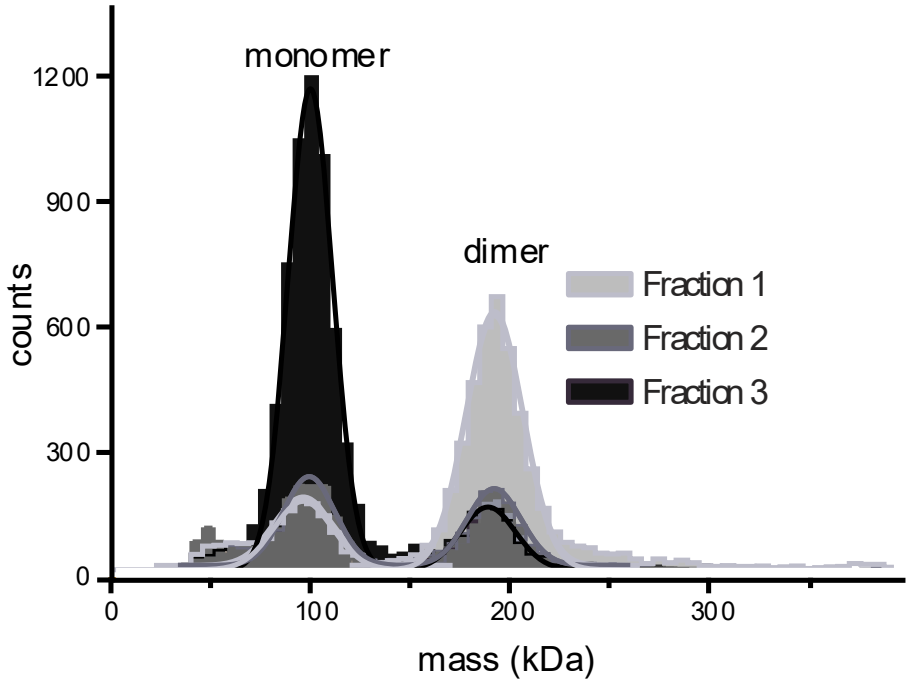
- **HDX-MS and XL-MS results report a potential binding site but also additional structural perturbations**
- eVP35 binds MIB2 MZM and MREP domains
- **MS results complements AlphaFold, revealing dynamics and alternative domain orientations.**

- Inspiration: MIB2 domains orientation?

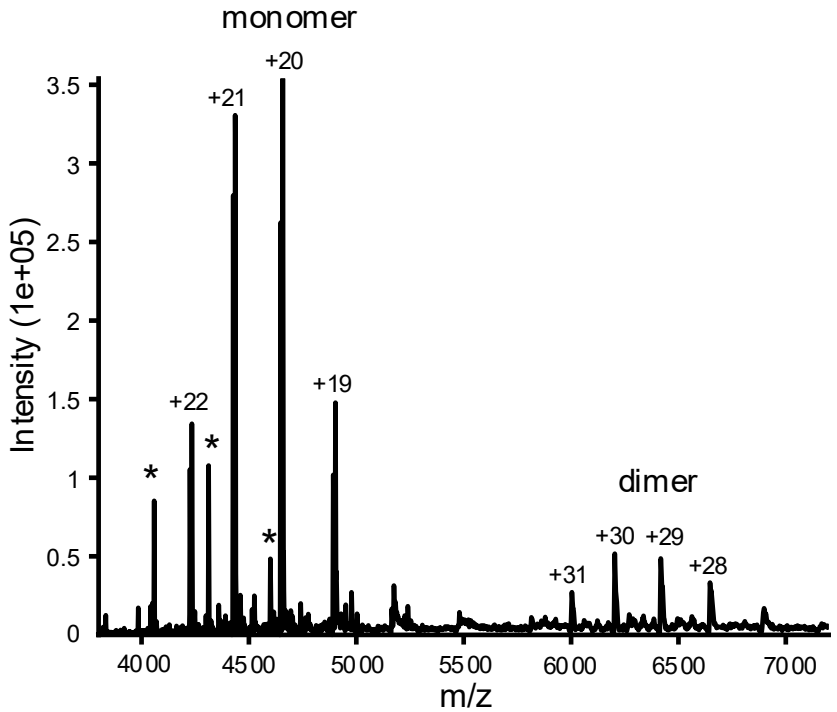
intramolecular XLs within MIB2 inform on domains proximity



Challenge: MIB2 adopts monomeric and dimeric forms



Mass photometry



Native MS

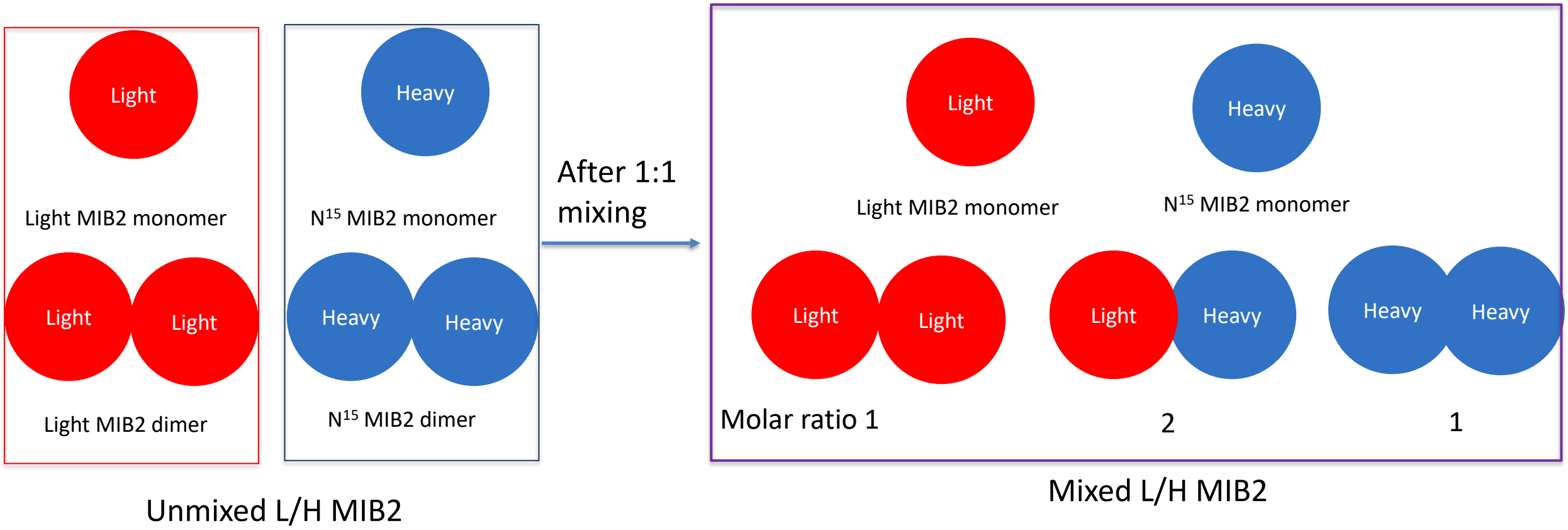
Crosslink types:

1. intramolecular xlink (within monomer or one subunit of dimer)
2. oligomeric xlink (between two subunits of dimer)
3. mixture of two

How to differentiate them?

Mass photometry data was acquired by Grace Uwase

Solution: XL-MS of light/heavy mixtures of MIB2 enables differentiation

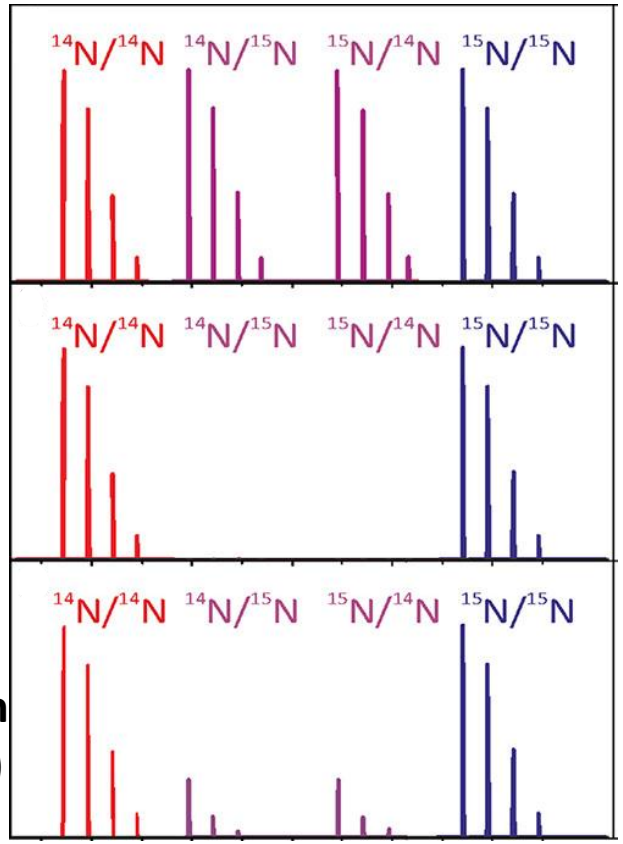


XL-MS of light/heavy mixtures of MIB2 enables differentiation

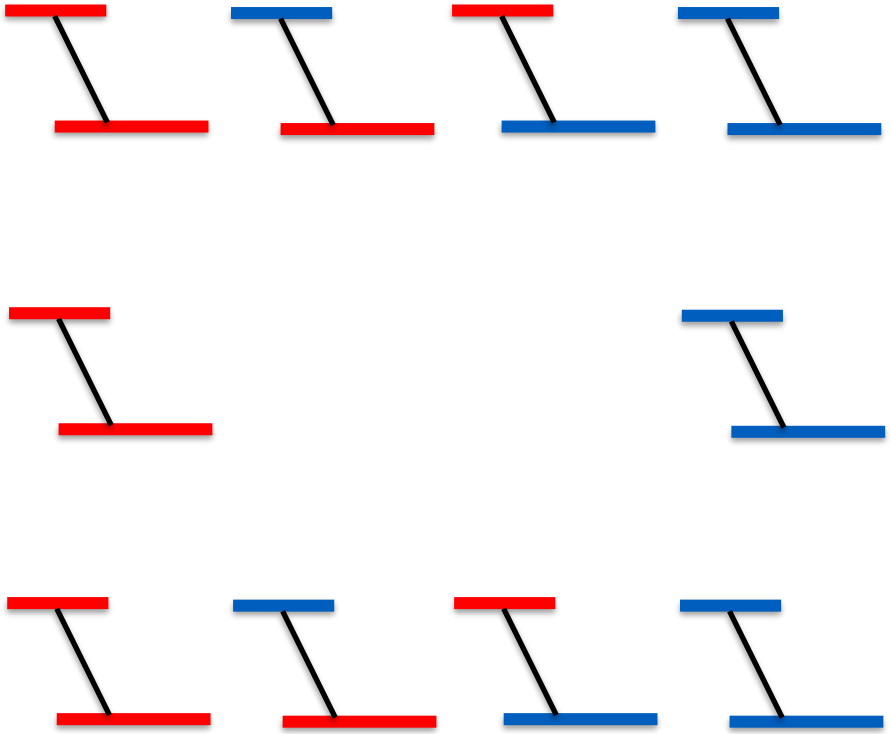
Oligomeric XL

intraXL

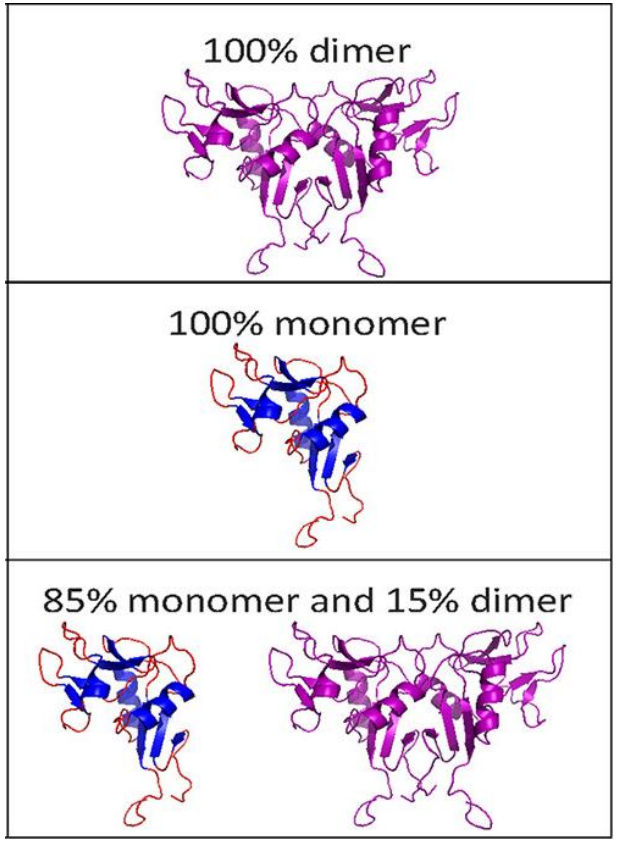
Mixture (if dimer has much lower abundance)

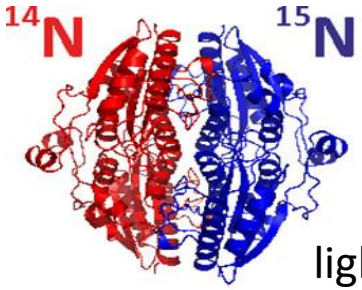


m/z



Crosslinked peptides

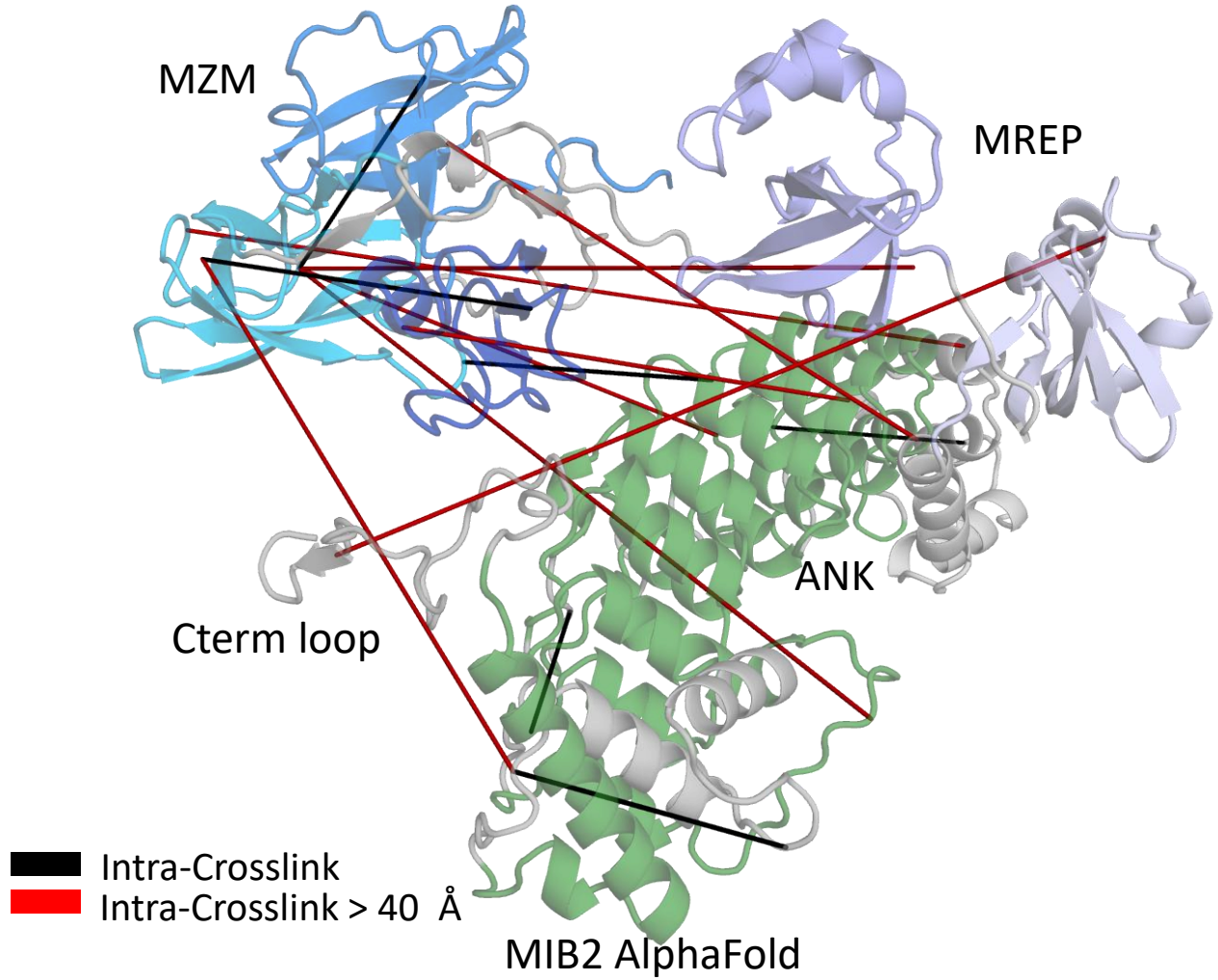




light-heavy MIB2 dimer

MIB2 Intra-xlinks contradict AlphaFold predicted structure

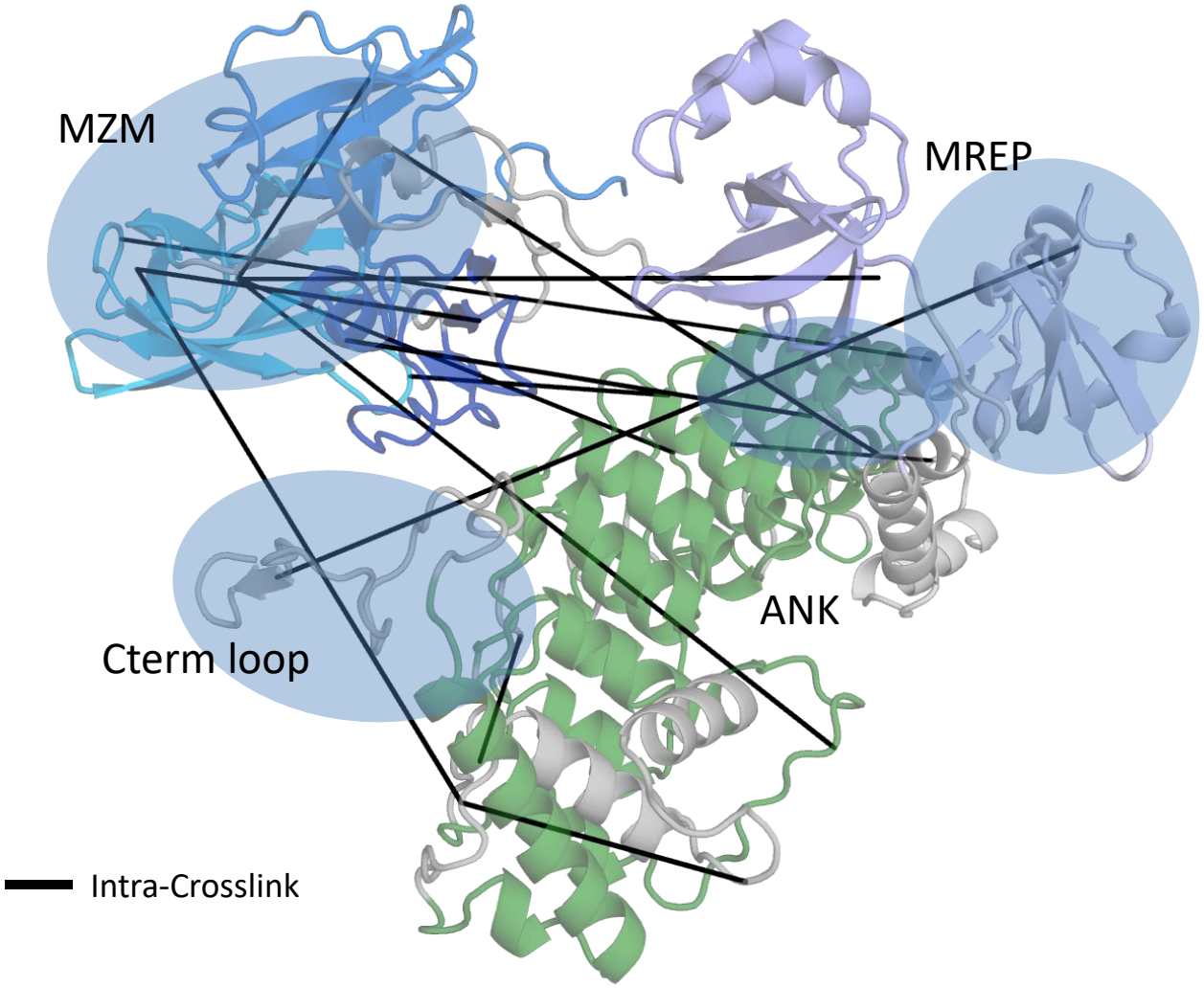
Crosslink sites		Cα-Cα distance on alphafold (Å)	Theoretical range of Cα-Cα distance (Å)
XL site 1	XL site2		
D131	E228	29.9	9-30
K163	K228	34.5	9-30
E228	K320	69.8	6-16
D293	E457	34.5	9-30
K181	E603	47.5	6-16
K220	D820	61.7	6-16
E228	D583	44.9	9-30
E228	D778	80.4	9-30
E236	E508	82.5	9-30
E251	E645	25.1	9-30
D489	E550	25.8	9-30
K402	E889	76.5	6-16
K689	K725	13.2	9-30
E803	D820	23.8	9-30



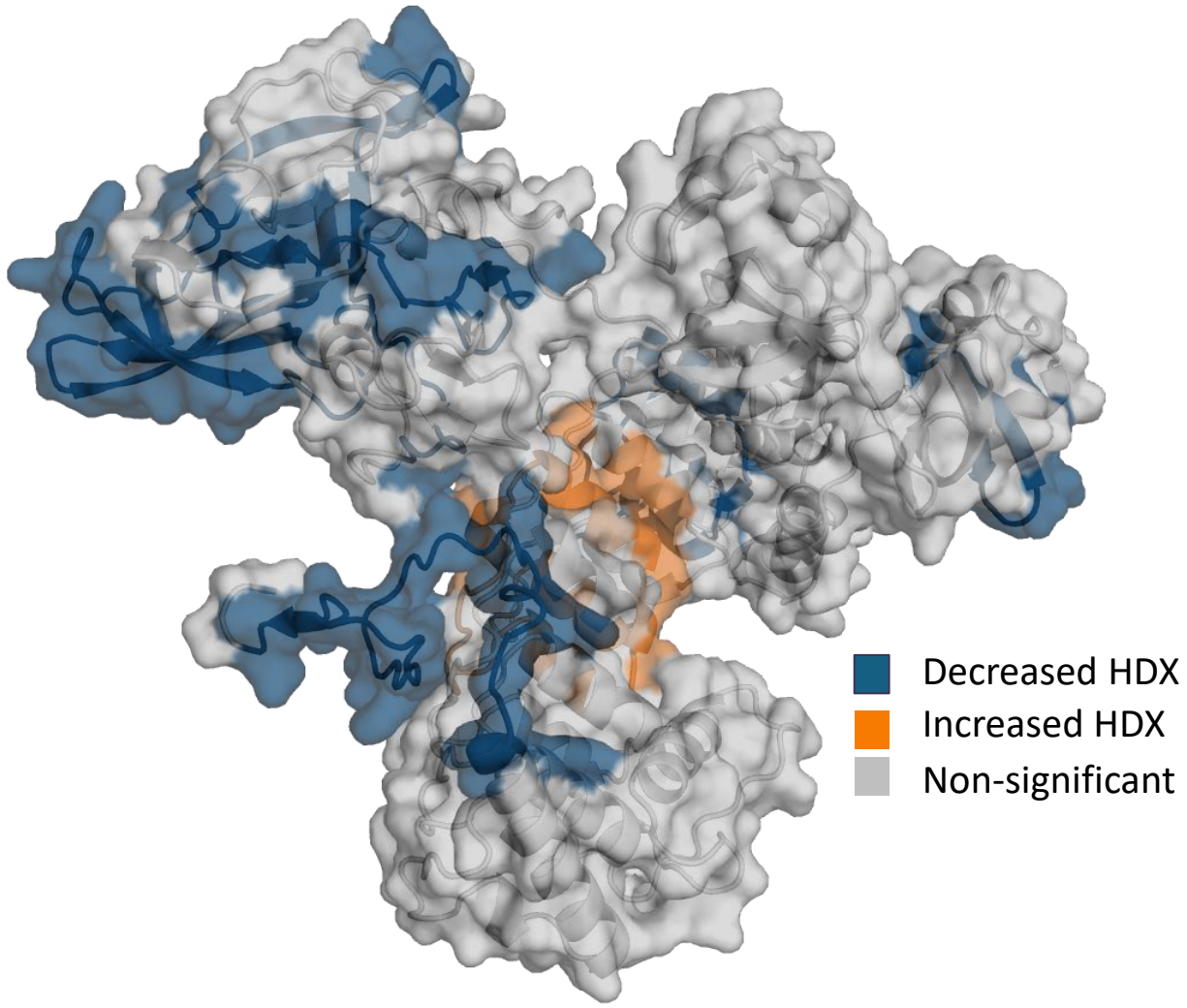
- Most of the intra-xlinks observed are not reasonable for the alphafold structure
- Close proximity between MZM-MREP, MZM-ANK, and MREP - Cterm loop

Intra-xlinks indicate the HDX “protected” regions are in close proximity

Summary of intraXL

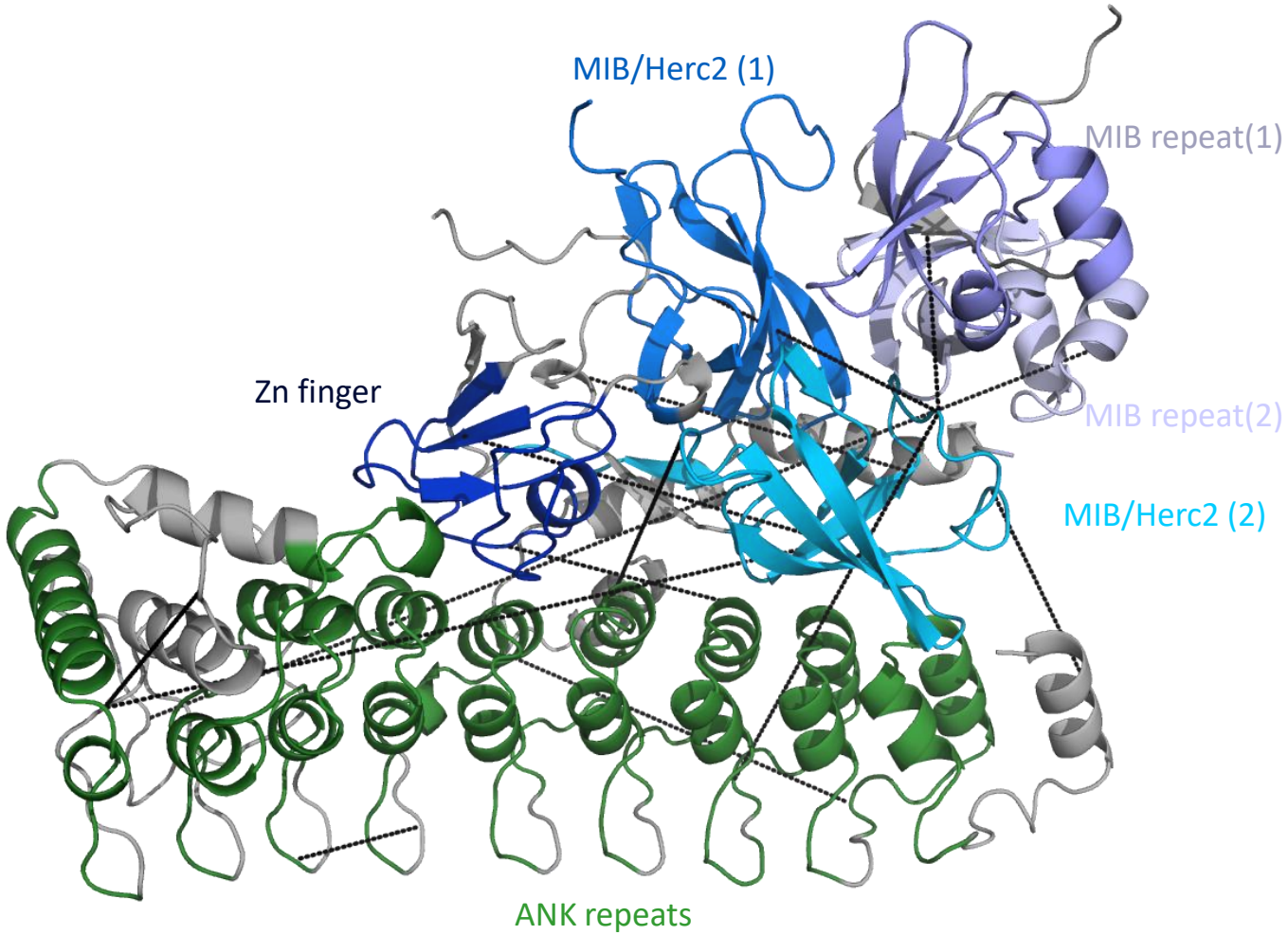


Summary of HDX



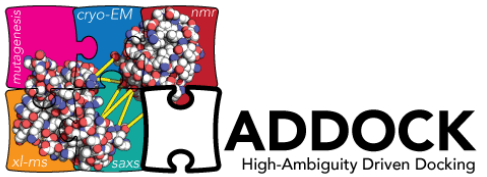
❖ **Decreased HDX regions seem in close proximity**

Experimentally derived MIB2 structure



MIB2 docking representative model

- HADDOCK was used for docking and **intra-crosslink were imported as distance restraints**
- Ambiguous interaction restraints were applied
- Model suggests a more **compact structure** --- contrasting AlphaFold extended structure
- **MZM and MREP domains are positioned closely together and adjacent to the ANK**
- Docking does not account for loop flexibility or consider conformational changes, potentially introducing artifacts due to enforced rigidity.

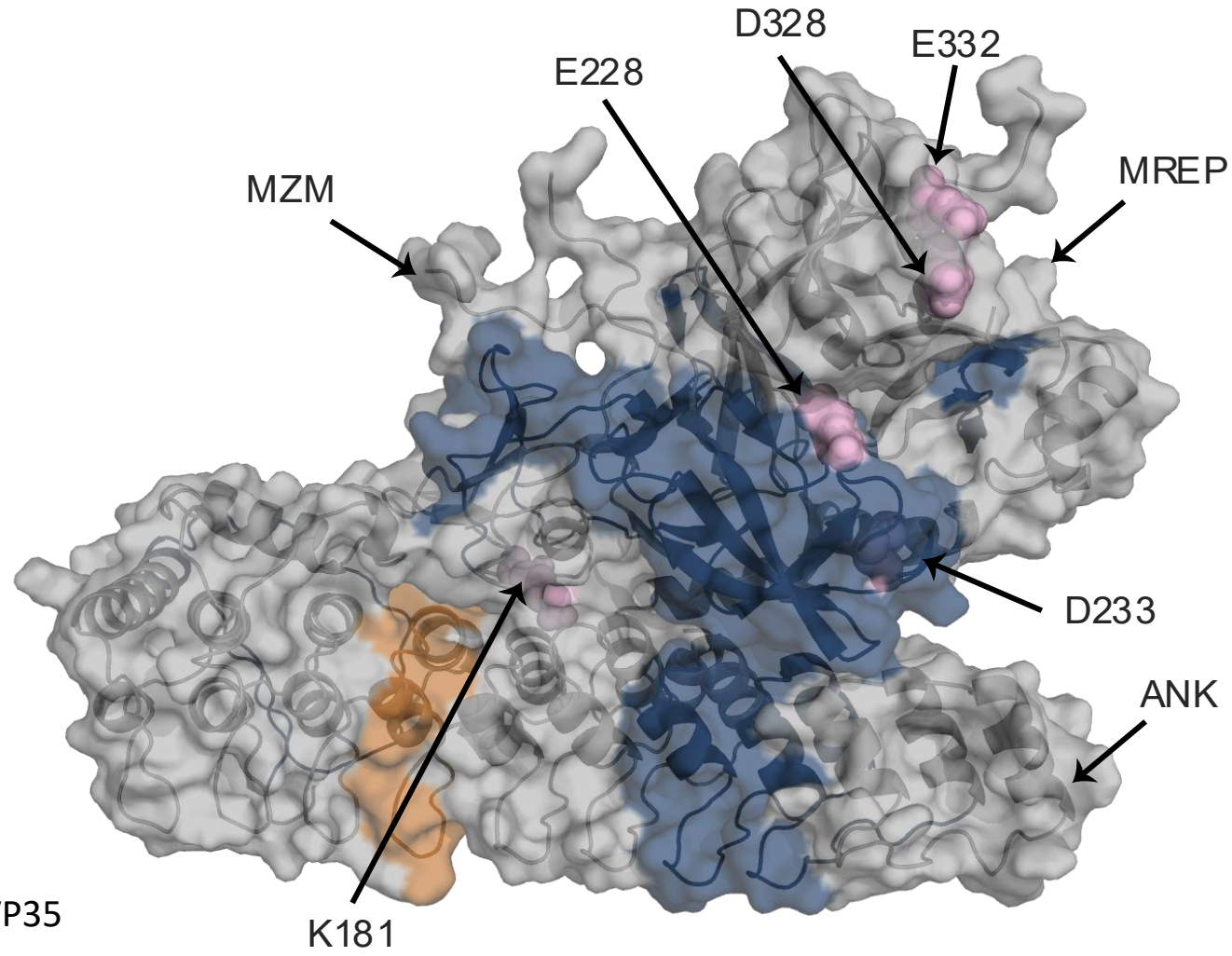


Honorato et al., *Nature Prot.* **2024**, 19, 3219-3241

■ Intra-Crosslink

Experimentally derived MIB2 structure

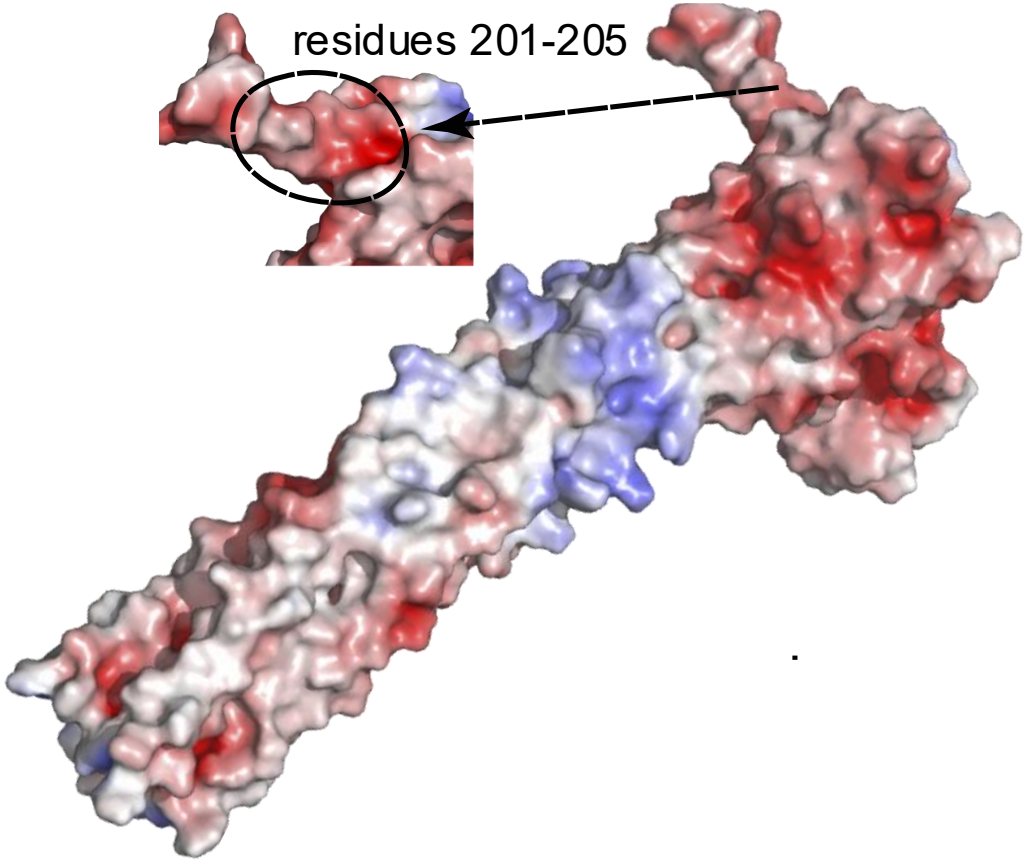
- **The representative model aligns well with HDX and inter-crosslinks results**
- HDX protected regions form a continuous surface (primary protection region)
- MIB2 residues inter-crosslinked to eVP35 are spatially proximal and overlap with the primary protection region



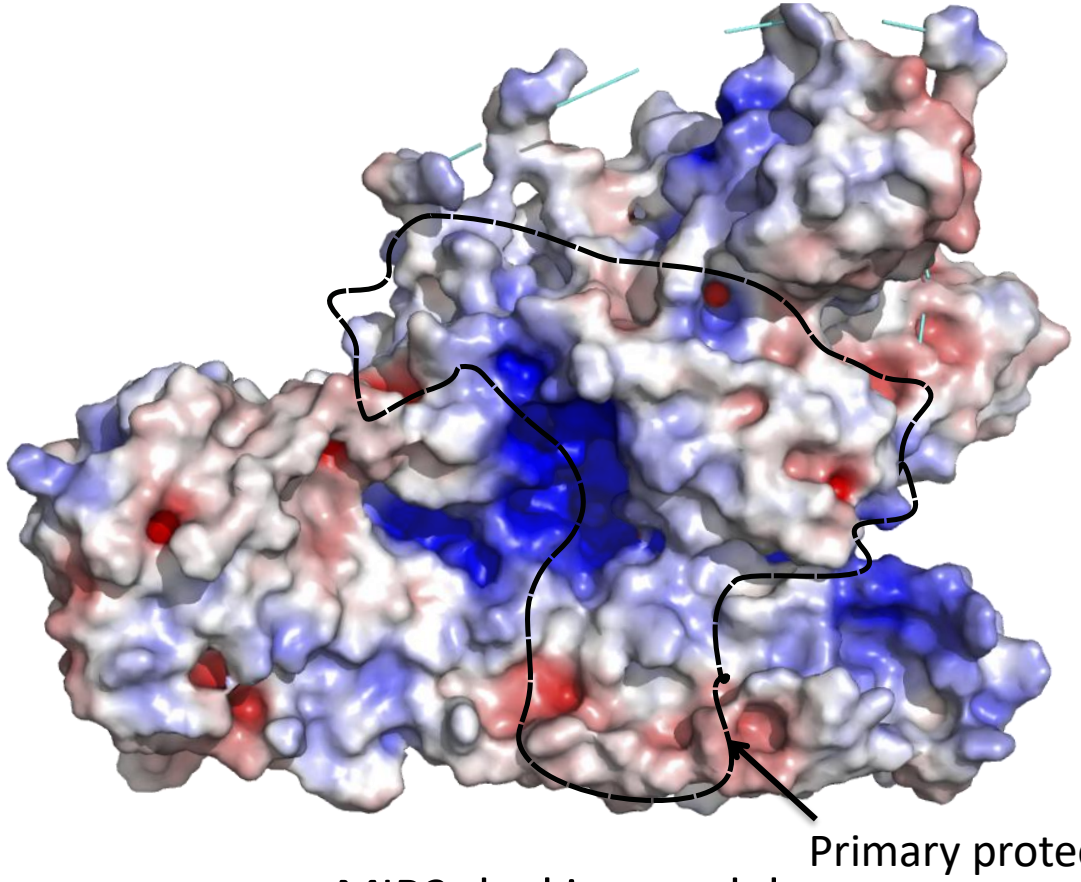
MIB2 docking representative model

Honorato et al., *Nature Prot.* **2024**, 19, 3219-3241

How to validate the docking model?



eVP35



MIB2 docking model

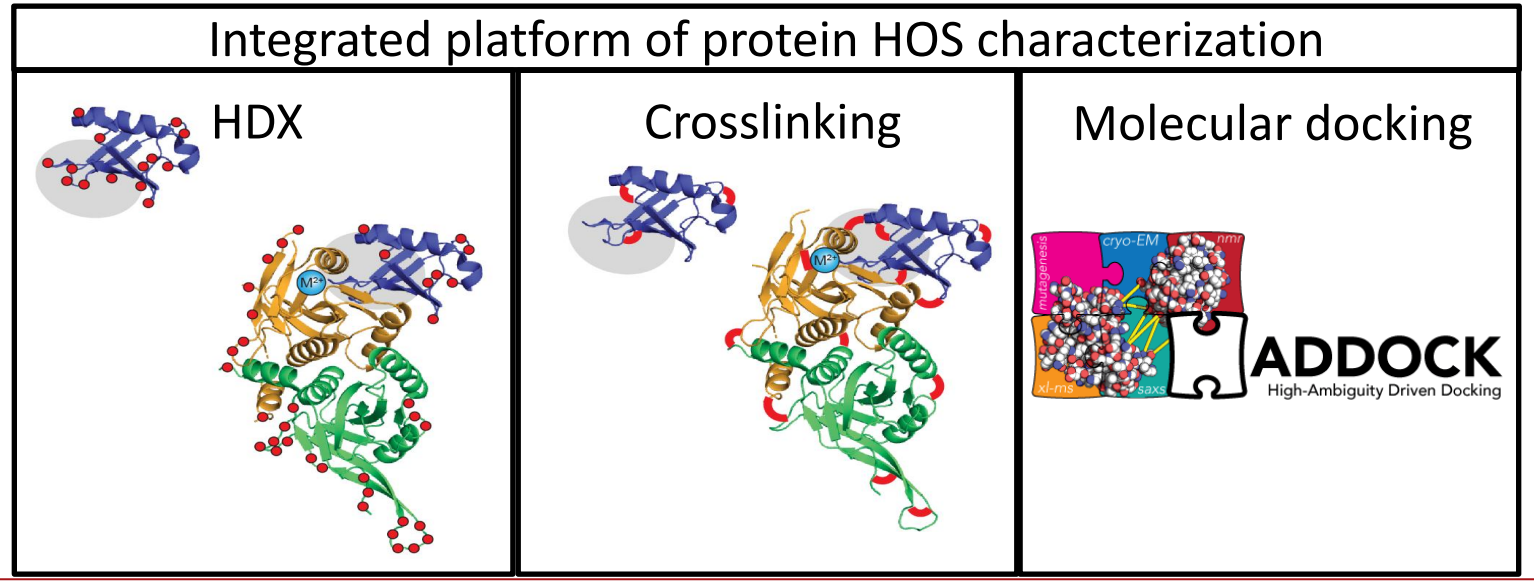
This electrostatic complementarity at the interface supports the plausibility of the proposed domains orientation.

The model is not expected to represent MIB2 definitive conformations.

Negatively charged — Positively charged

Conclusions and Perspectives

1. Characterized successfully the MIB2-eVP35 binding interface and achieved an experimentally-derived MIB2 structure.
2. Allowed additional structural understanding of Ebola viral pathogenesis.
3. Established an integrated platform for protein HOS and protein-protein interface characterization, particularly for multi-domain, flexible protein like MIB2 that eludes AI-based docking alone.
4. Provided approaches that would aid the design of protein therapeutics



Manuscript in preparation

PDB 5u4p

Honorato et al., *Nature Prot.* **2024**, 19, 3219-3241

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- Hsin-Chieh Yang
- Nolan McLaughlin
- Cole Hediger
- Xinzhu Li
- Polen Yunus

Amarasinghe & Leung Lab

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- **Daisy W. Leung (PI)**
- **Grace Uwase**

Washington University in St. Louis
Arts & Sciences

Protein Metrics

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Thank You for your attention!