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Biomedical Mass Spectrometry Resource

Integrating Mass Spectrometry-Based Footprinting Approaches and Molecular Docking for Protein Binding Interfaces Analysis

Mengru Mira Zhang[1,Δ], Brett R. Beno[2], Richard Y.-C. Huang[3], Ekaterina G. Deyanova[3], Jing Li[3], Guodong Chen[3,*], Michael L. Gross[1,*]

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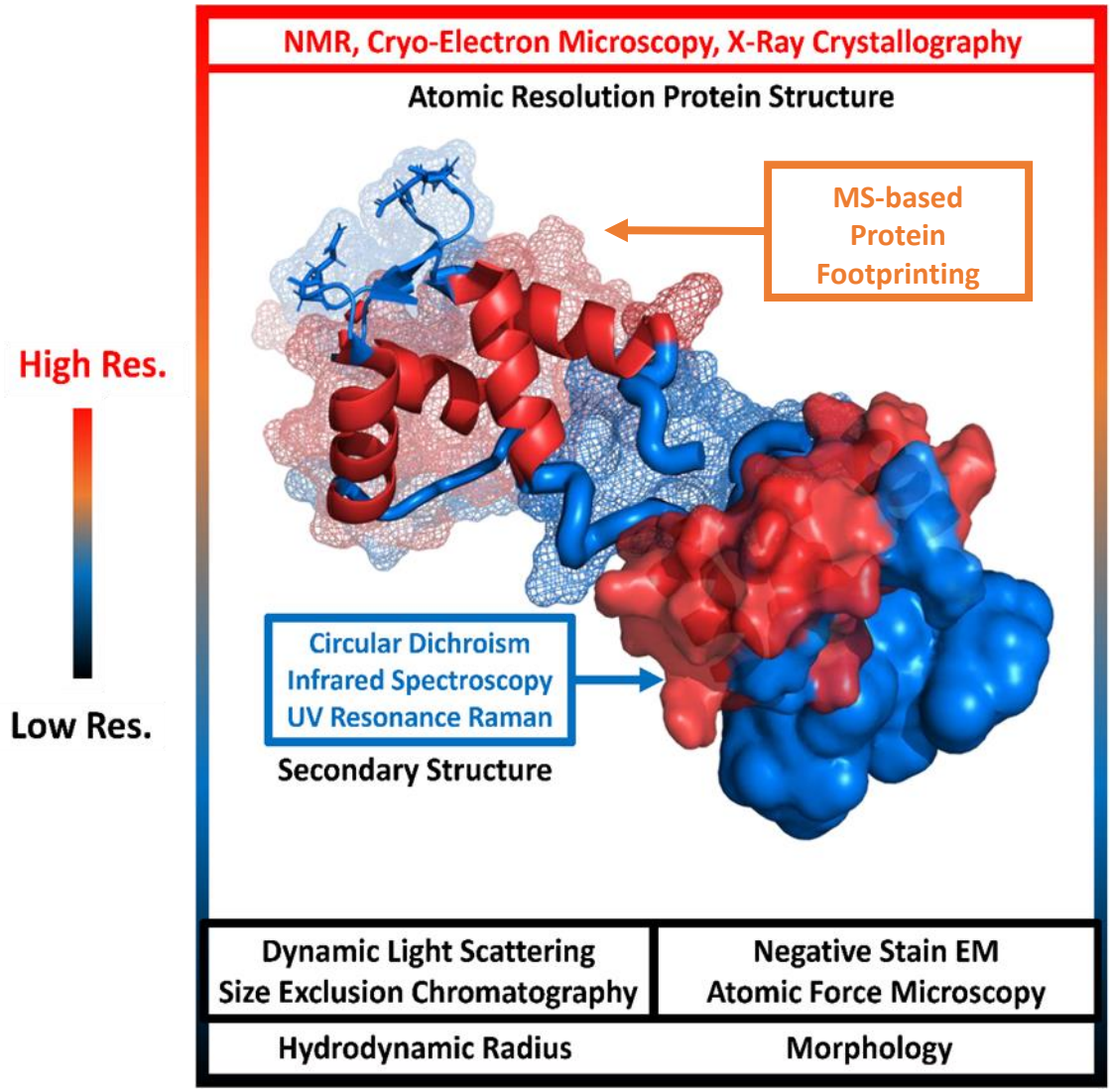
[2] Molecular Structure & Design, Bristol-Myers Squibb
[3] Pharmaceutical Candidate Optimization, Bristol-Myers Squibb

2020.08.11

[Δ] Current Address: Analytical Sciences, Seattle Genetics
[*] Corresponding authors

Biophysical Approaches in Structural Proteomics



❑ Protein Higher Order Structure

- Secondary to quaternary structure
- Diverse biological functionalities

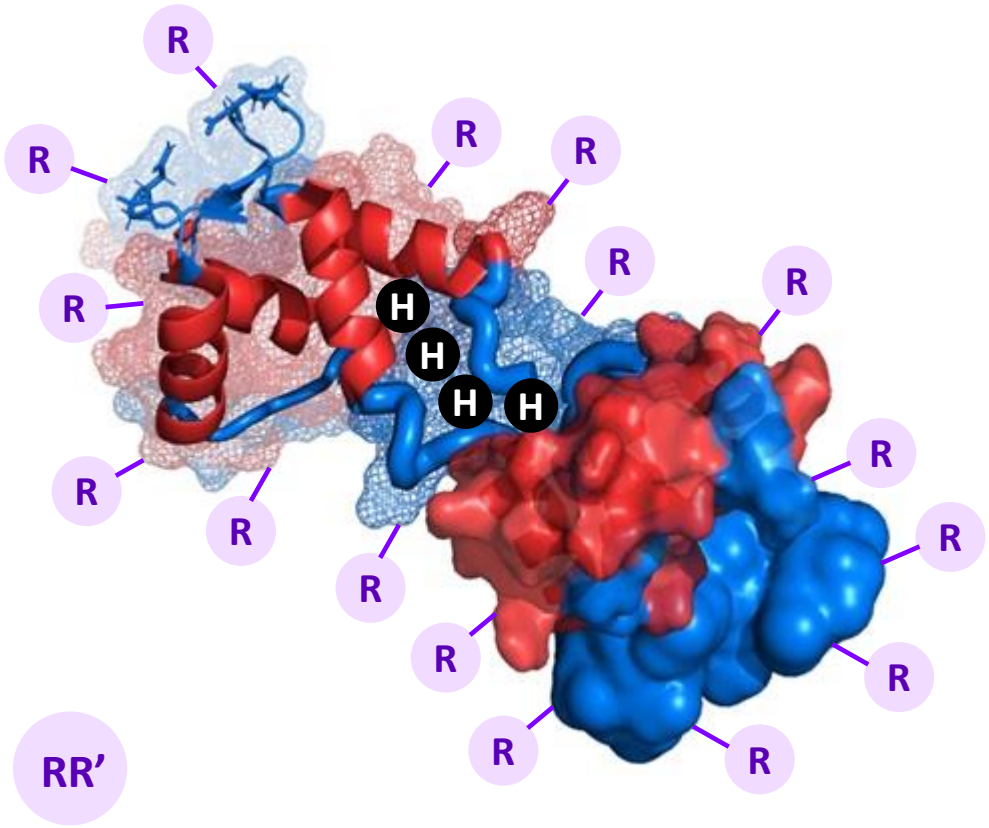
❑ Advantages of Mass Spectrometry-Based Approaches

- In-solution characterization
- Small amount of sample (ng - µg)
- Sensitive detection with low detection limit
- Fast throughput

Liu, X. R.§; **Zhang, M. M.**§; Gross, M. L. *Chem. Rev.* **2020,** 120, 4355-4454.

Biophysical Approaches in Structural Proteomics

NIGMS MS Resource



Solvent Accessible Surface Area (SASA)

❑ Protein Higher Order Structure

- Secondary to quaternary structure
- Diverse biological functionalities

❑ Advantages of Mass Spectrometry-Based Approaches

- In-solution characterization
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- Fast throughput

❑ Mass Spectrometry–Based Footprinting

- Reversible footprinting
  - Hydrogen deuterium exchange MS (HDX-MS)
- Irreversible footprinting
  - Radical labeling
    e.g., $OH^{\bullet}$ and RC:
  - Targeted labeling
    e.g., carboxyl group footprinting and chemical cross-linking

Liu, X. R.§; **Zhang, M. M.**§; Gross, M. L. *Chem. Rev.* **2020,** 120, 4355-4454.

Protein Binding Interfaces Analysis by HDX

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❑ Mass Spectrometry–Based Footprinting

- Reversible footprinting
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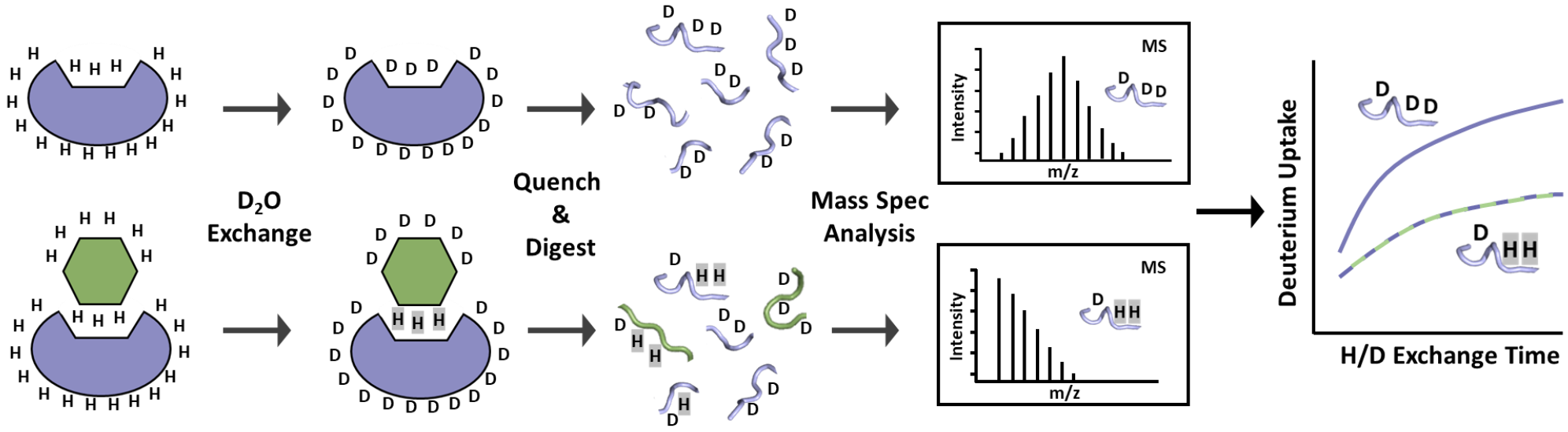
❑ Limitations of Stand-Alone HDX:

- Spatial resolution limited by proteolysis
- Non-discriminating readout between the direct binding interaction and remote conformational changes

❑ Advantages of HDX:

- Minimal perturbation on protein structure
- Universal labeling of AAs besides Proline
- Robust and commercialized setup
- Well developed data processing software



Platform for Protein Binding Interfaces Analysis

NIGMS MS Resource

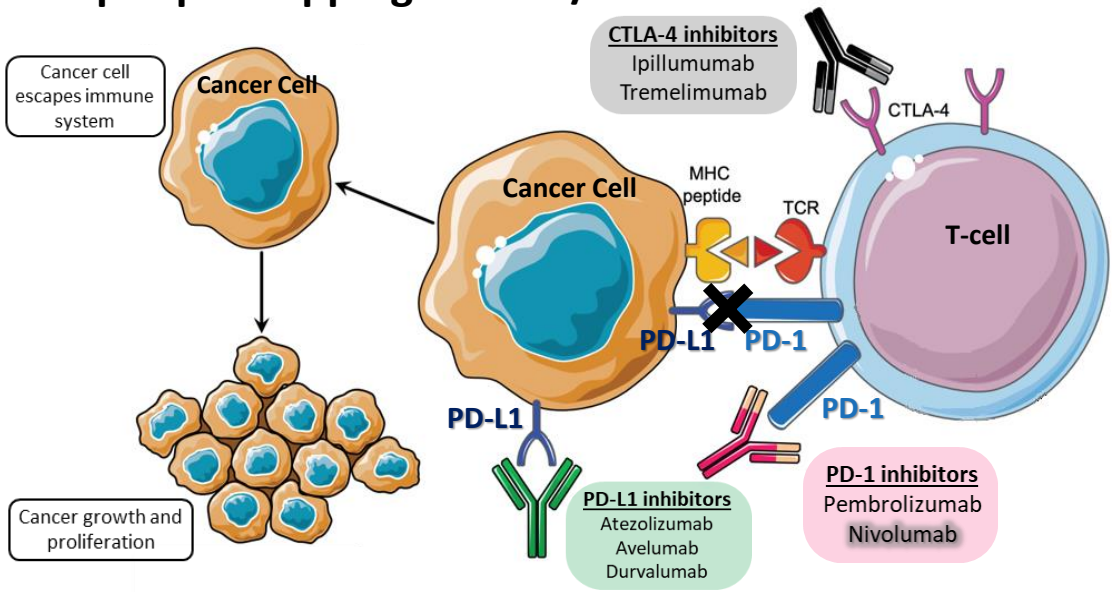
❑ **Limitations of Stand-Alone HDX:**

- Spatial resolution limited by proteolysis
- Non-discriminating readout between the direct binding interaction and remote conformational changes

➢ **Aim to Establish a Comprehensive Characterization Platform for Protein Binding Interface Analysis**

- HDX-MS, reversible footprinting
- Electron transfer dissociation (ETD)
- Chemical cross-linking (XL-MS), irreversible footprinting
- Molecular docking

❑ **Epitope Mapping of PD-1/Nivolumab**



- Programmed cell death-1 (PD-1), an antigen-independent co-receptor on cell surface
- Nivolumab, one of the FDA-approved immuno-checkpoint inhibitors
- Blockage of PD-1/PD-L1 pathway and restore cell-mediated immunity
- Available crystal structure for final evaluation

Ayoub, N. M. et al. *Breast Cancer* **2019**, *11*, 53-69.

Epitope Mapping of PD-1

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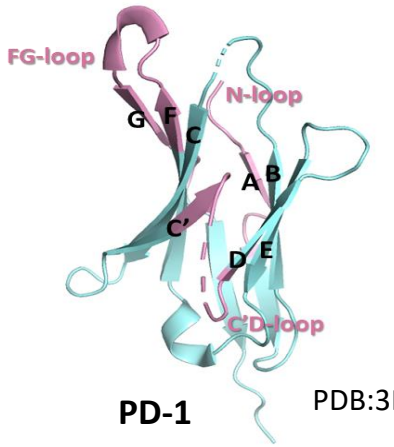
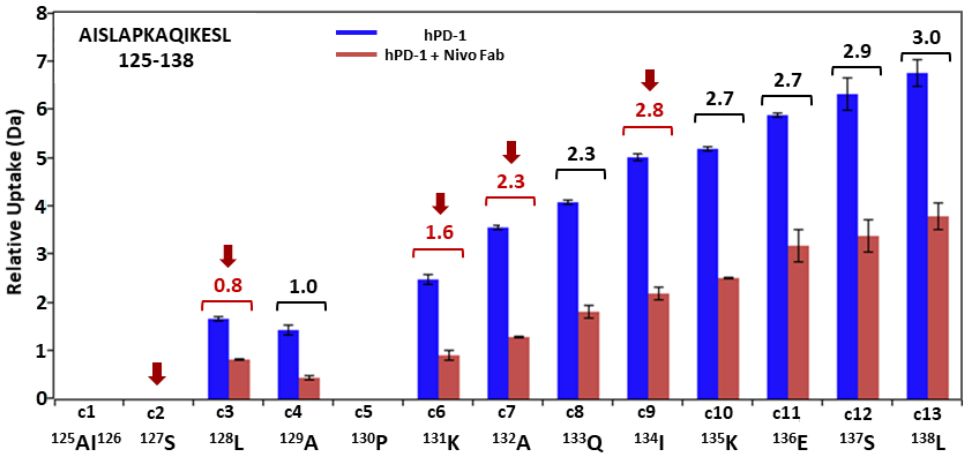
❑ HDX of PD-1

- N-loop: ^{25}LDSPDRPWNPPTFSPALL^{42}
- C'D-loop: ^{80}AAFPEDRSQPGQDCRF^{95}
- ***FG-loop: ^{125}AISLAPKAQIKESL^{138}***



PD-1 PDB:3RRQ

❑ HDX-ETD of PD-1



❑ Summarized Epitopes Regions Identified by HDX, HDX-ETD

N-loop C'D-loop

^{1}MQIPQAPWPV VWAVLQLGWR PGWFLDSPDR PWNPPTFSPA LLVVTEGDNA TFTCSFSNTS ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG^{90}

^{91}QDCRFRVTQL PNGRDFHMSV VRARRNDSGT YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQ

FG-loop

Characterize PD-1 epitope with an orthogonal approach: chemical cross-linking (XL-MS)

Zhang, M. M.;§ Huang, R. Y-C.;§ Beno, B. R. Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2020.** 92, 9086-9094.

Irreversible Footprinting Approach: XL-MS

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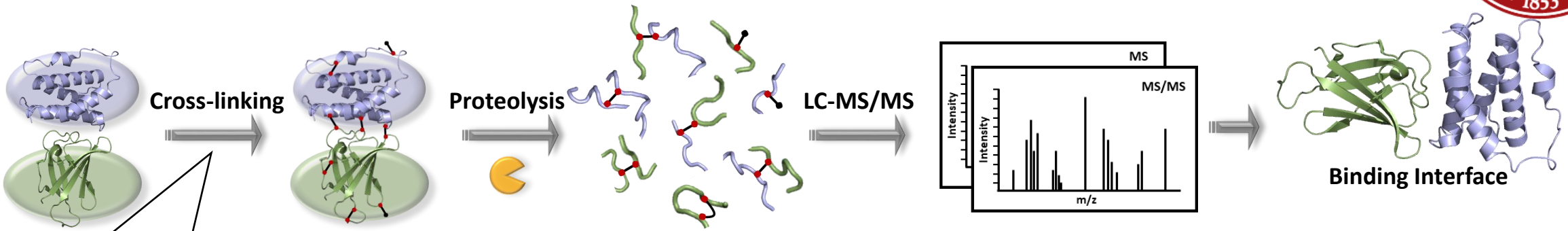
❑ **XL-MS:**



❑ **Multi-Crosslinker Strategy**

- **BS^3 and BS^2G :** cross-links on side chain of **K – K**
- **EDC :** cross-links on side chain of **D&E – K**
  - ✓ Larger dynamic range: 5-30 Å
  - ✓ Isotope-encoded feature (1:1 of heavy : light)

	PD1	Nivo	Cross-linker	Epitope	Paratope
1	S27 –	K57(H)	BS^2G /BS^3	N-Loop	CDR-H2
2	D26 –	K57(H)	EDC		
3	S27 –	Y35(L)	BS^3		CDR-L1
4	S62–	N-term (H)	BS^3	BC-Loop	N-terminus (H)
5	E61 –	N-term (H)	EDC		
6	K135 –	K57(H)	BS^3	FG-Loop	CDR-H2
7	K135 –	Y35(L)	BS^3		CDR-L1
8	K135 –	N-term (H)	BS^3		N-terminal (H)

Zhang, M. M.;[§] Huang, R. Y-C.;[§] Beno, B. R. Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2020.** 92, 9086-9094.

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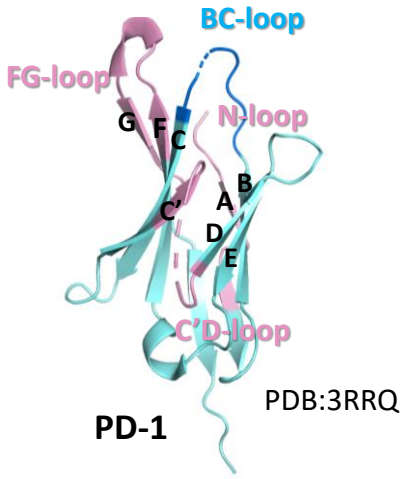
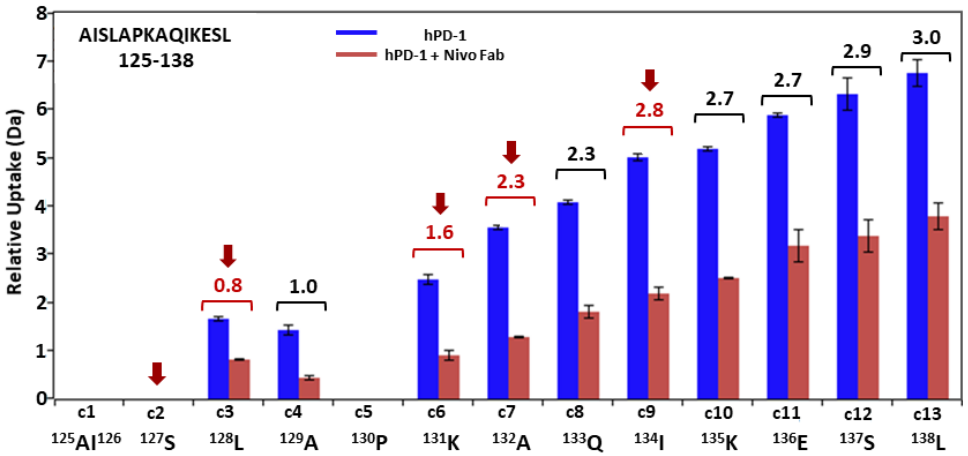
- ***FG-loop: ^{125}AISLAPKAQIKESL^{138}***
- BC-loop: ^{56}FSNTSESF^{63}



❑ HDX-ETD of PD-1

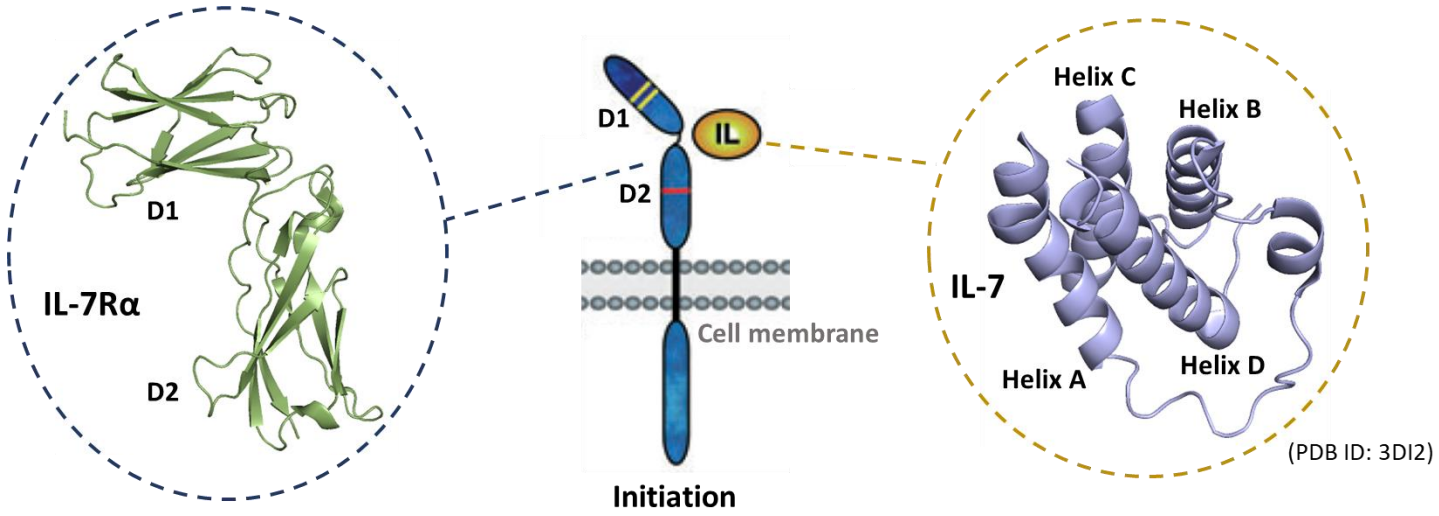


❑ Summarized Epitopes Regions Identified by HDX, HDX-ETD and XL-MS (X-ray Structure)

Integration with XL-MS and HDX-ETD increase the spatial resolution of stand-alone HDX

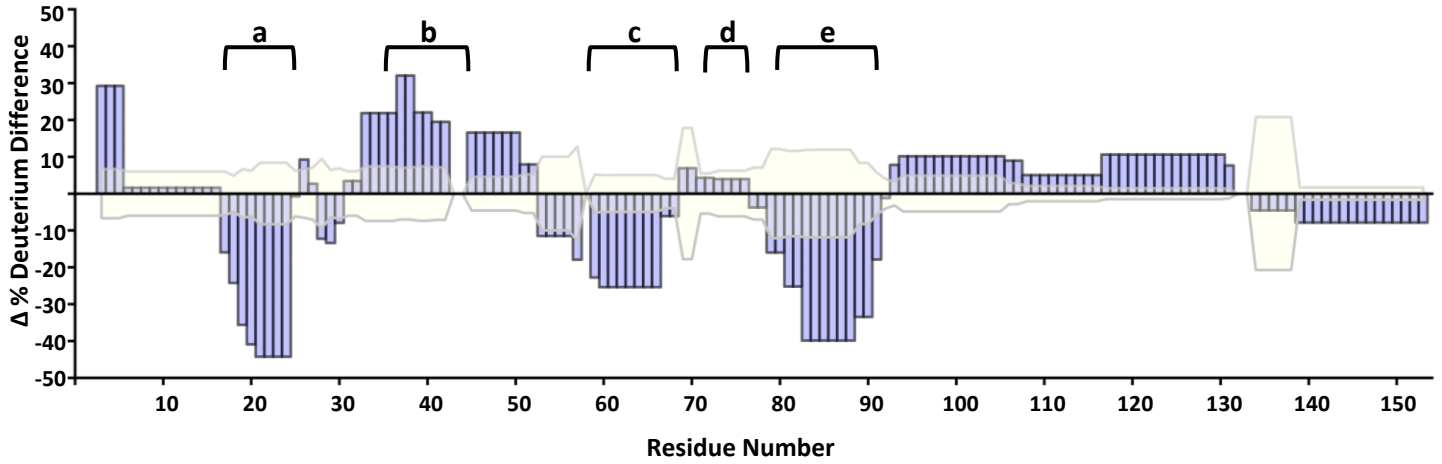
Zhang, M. M.;§ Huang, R. Y-C.;§ Beno, B. R. Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2020.** 92, 9086-9094.

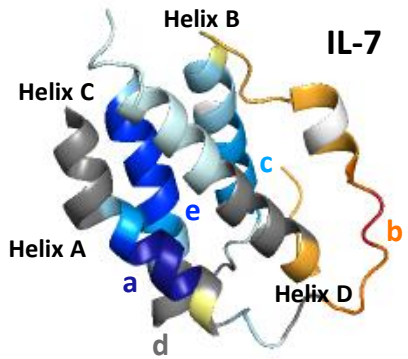
Binding Interface Analysis of IL-7 / IL-7Rα

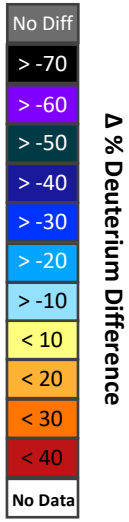


- Interleukins (IL), a group of cytokines
- Bind with their matching receptors, induce the formation of heterotrimer with γ_c
- Trigger the JAK/STAT signaling pathway and modulate the development, proliferation and homeostasis of B and T cells

❑ Cumulative Deuterium Uptake of IL-7 HDX Kinetics







Walsh. S. T. R. *Immunol. Rev.* **2012**, *250*, 303-316.

Walsh, S. T. R. et al. *Structure* **2009**, *17*, 54-65.

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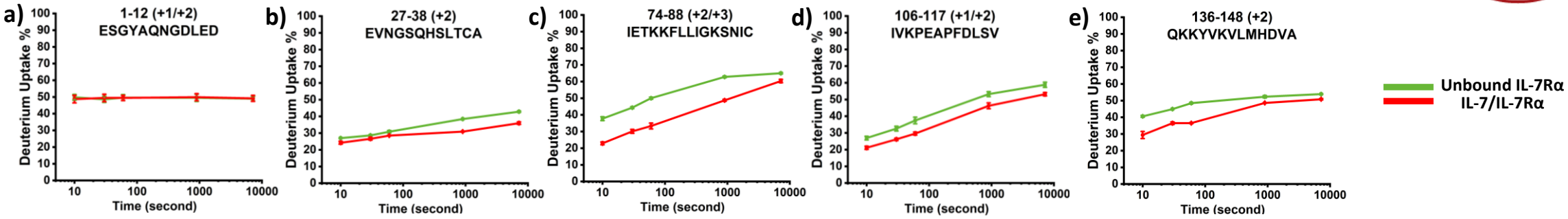
Binding Interface Analysis of IL-7 / IL-7Rα

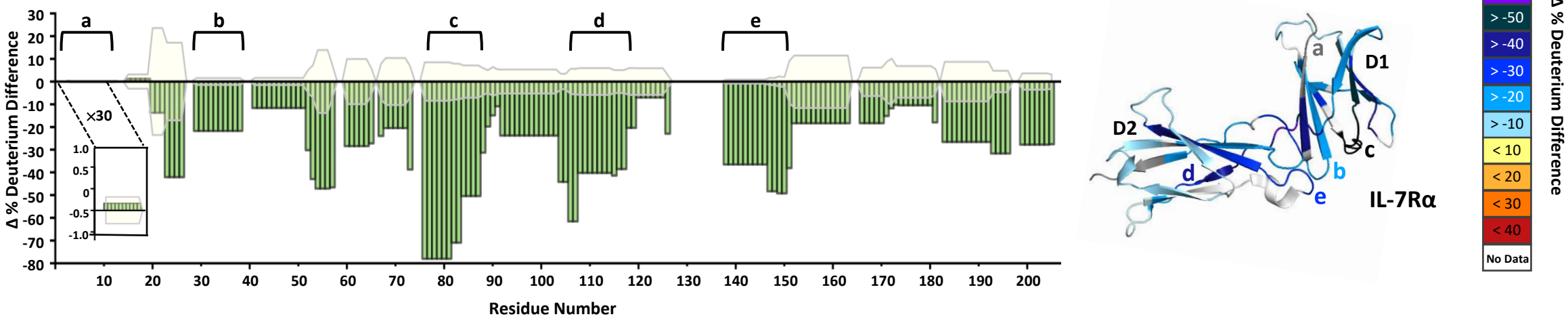
NIGMS MS Resource

❑ Representative HDX Kinetics:



No distinct evidence of the binding interface or the remote conformational change

❑ Cumulative Deuterium Uptake of IL-7Rα HDX Kinetics



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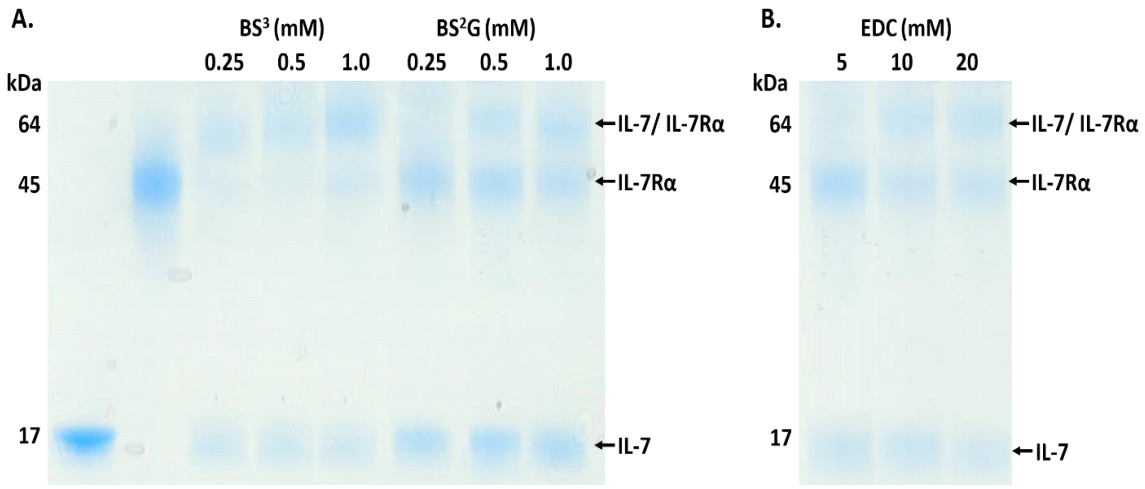
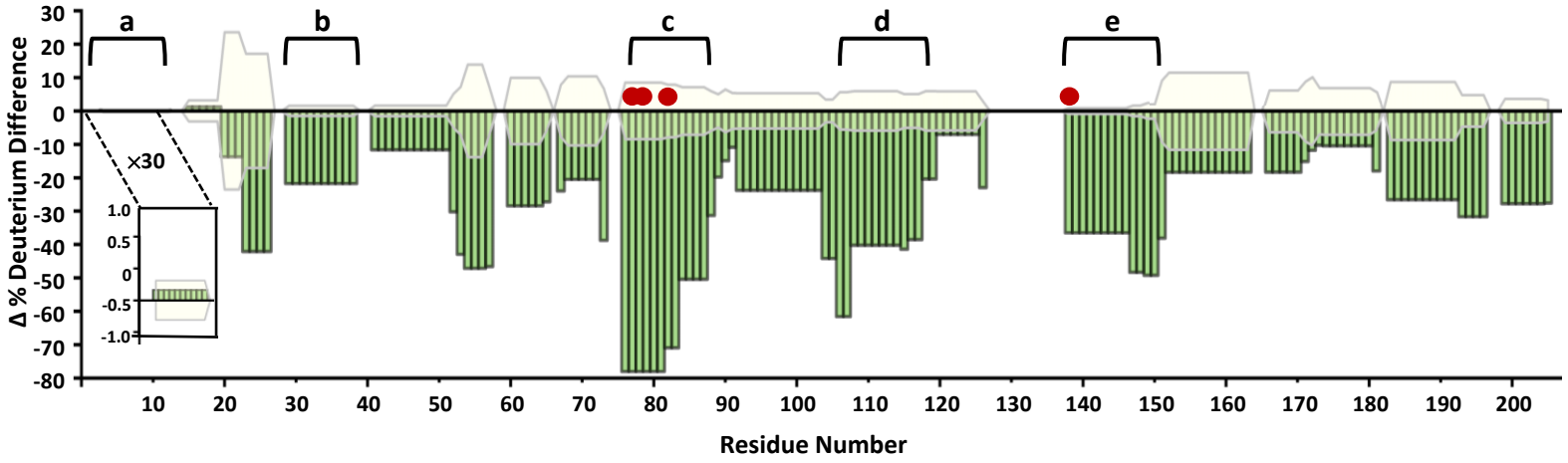
XL-MS of IL-7 / IL-7Rα

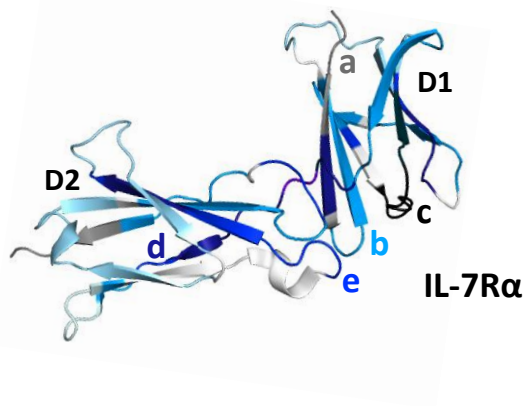


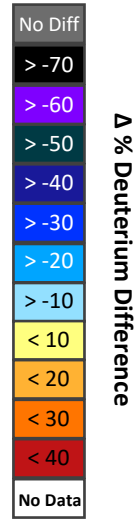
	IL-7	IL-7Rα	Cross-linker
1	K11	K84	BS^3
2	K11	K141	BS^3
3	D75	K77	EDC
4	K69	K78	BS^3
5	K152	K141	BS^3
6	K8	K84	BS^2G / BS^3
7	N-term	K84	BS^2G / BS^3
8	D2	K84	EDC
9	D4	K84	EDC
10	E6	K84	EDC

- **IL-7Rα:** Region c and e are the binding interfaces

❑ Cumulative Deuterium Uptake of IL-7Rα HDX Kinetics







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Restraints-Based Protein-Protein Docking

NIGMS MS Resource

	IL-7	IL-7Rα	Cross-linker
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2	K11 – K141	BS^3
3	D75 – K77	EDC
4	K69 – K78	BS^3
5	K152 – K141	BS^3
6[a]	K8 – K84	BS^2G / BS^3
7[a]	N-term – K84	BS^2G / BS^3
8[a]	D2 – K84	EDC
9[a]	D4 – K84	EDC
10[a]	E6 – K84	EDC

[a] These cross-links locate on the unresolved domain on IL-7

Rosetta Commons
The hub for Rosetta modeling software

Protein-Protein Docking

- BS^3 Linked Cα-Cα Distance: 9 to 30.0 Å
- EDC Linked Cα-Cα Distance : 6 to 16.0 Å

How many cross-links are needed to generate a high-confidence model?

Baker, D. J. et al. *J. Mol. Biol.* **2003**, *331*, 281-299.

Gray, J. J. et al. *PLoS One* **2011, *6*,** e22477.

Zhang, M. M. Beno, B. R.; Huang, R. Y-C; Adhikari, J.; Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 15709-15717.

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[a] These cross-links locate on the unresolved domain on IL-7

Rosetta Commons
The hub for Rosetta modeling software

Protein-Protein Docking

Each Docking Job
(using # of restraints)

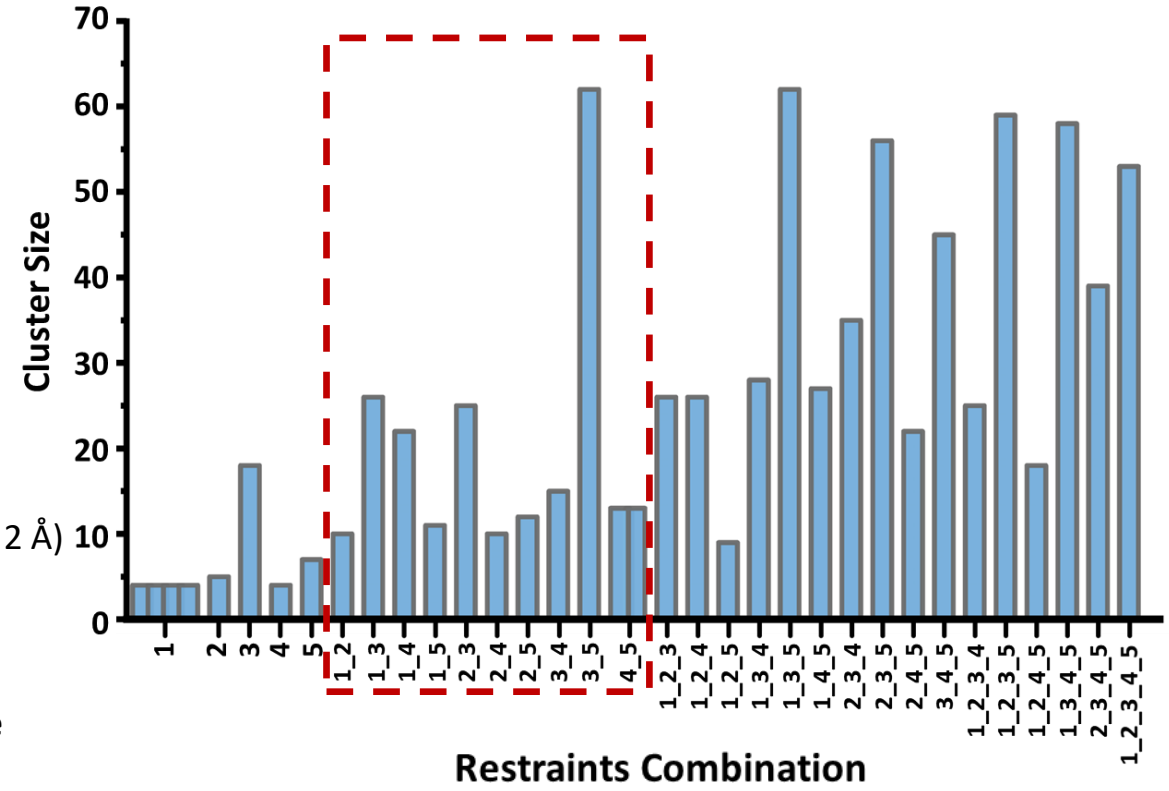
Choose Top 100
(Total Score)

Clustering
(Based on structure < 2 Å)

Find the Largest Cluster

Compare the model with crystal structure

Good Model (≤ 2Å) ?



Can any two of the cross-links lead to a high-confidence model with info. from HDX?

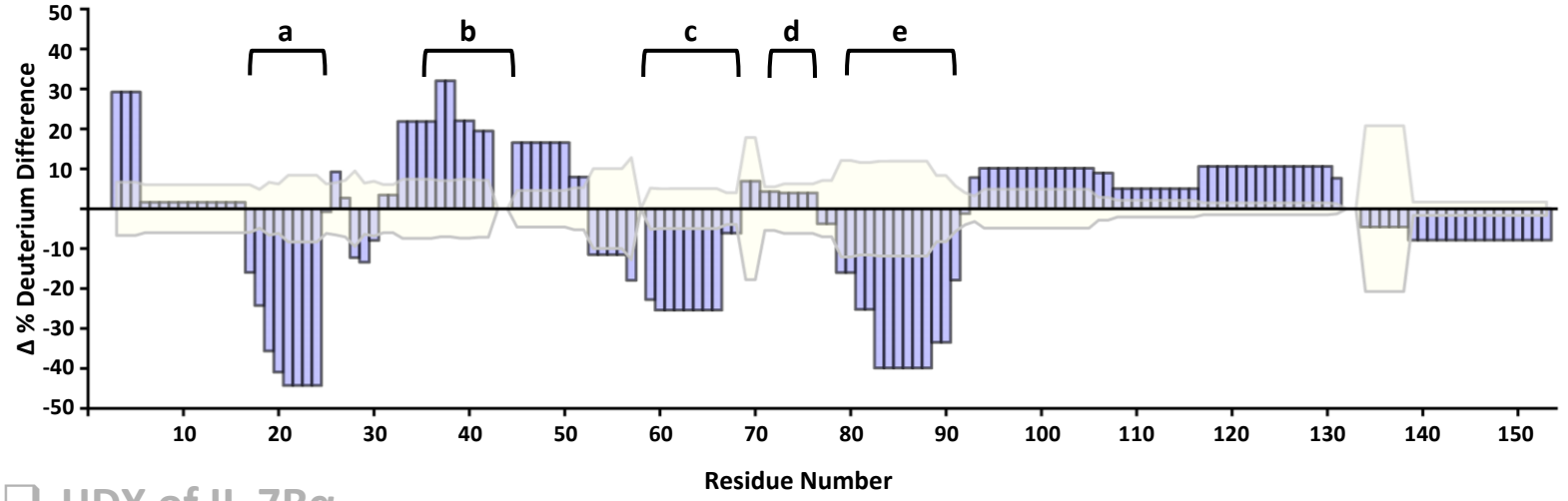
Zhang, M. M. Beno, B. R.; Huang, R. Y-C; Adhikari, J.; Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 15709-15717.

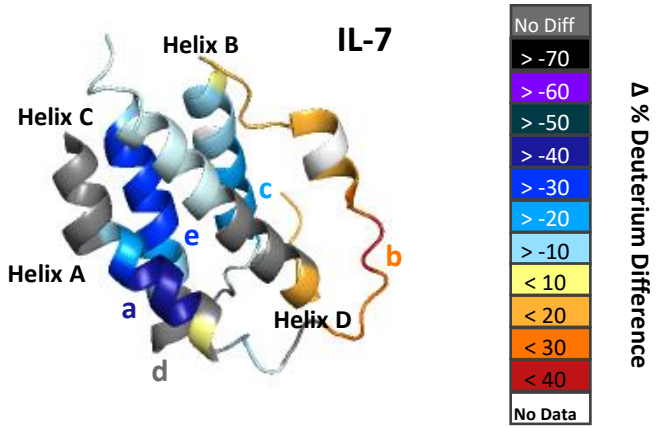
* Docking exercises were performed by Brett from BMS team.

HDX Adjudication of Docking Models

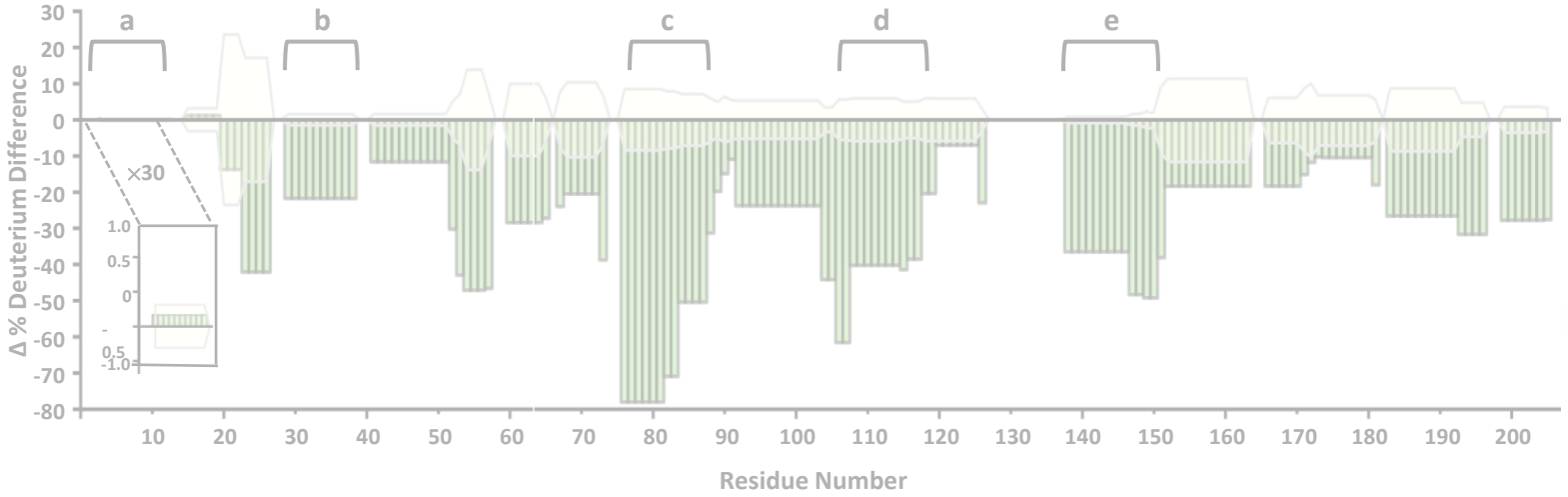
NIGMS MS Resource

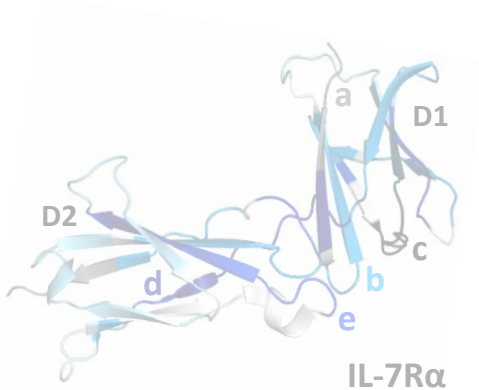
❑ HDX of IL-7





❑ HDX of IL-7Rα





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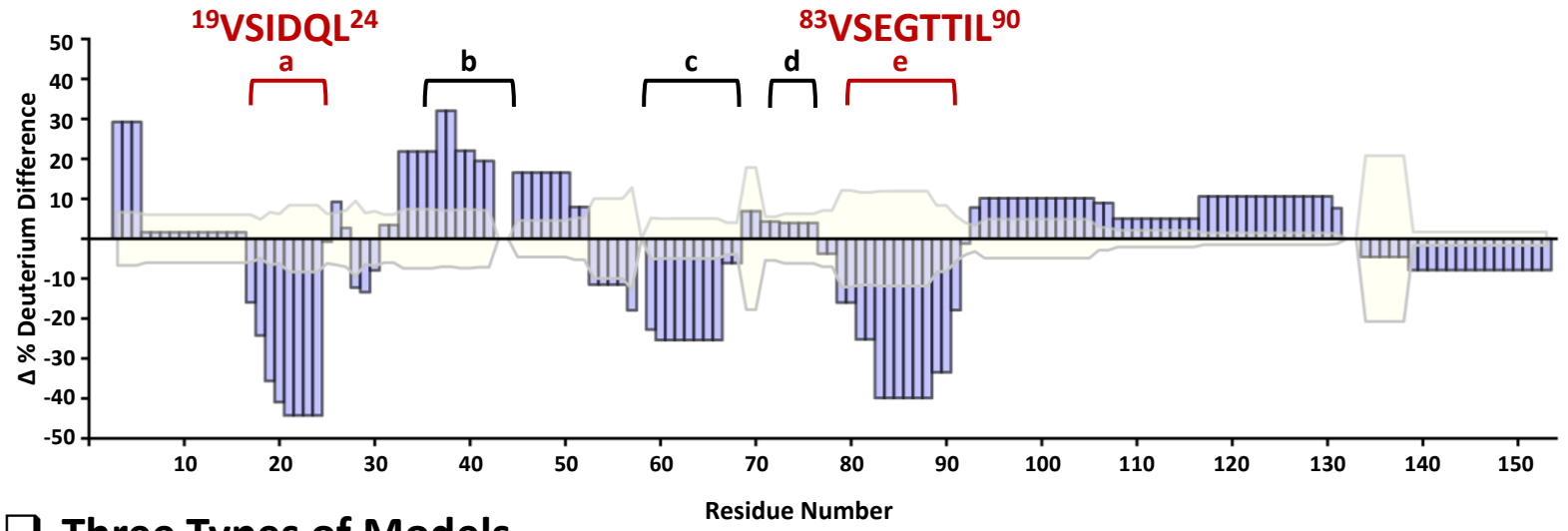
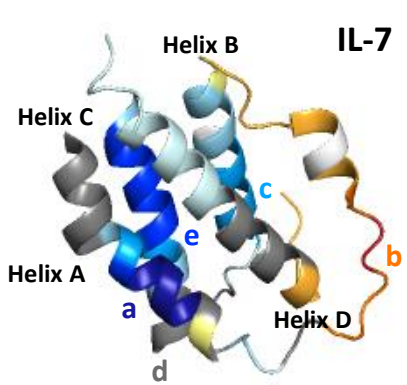
HDX Adjudication of Docking Models (2XLs)

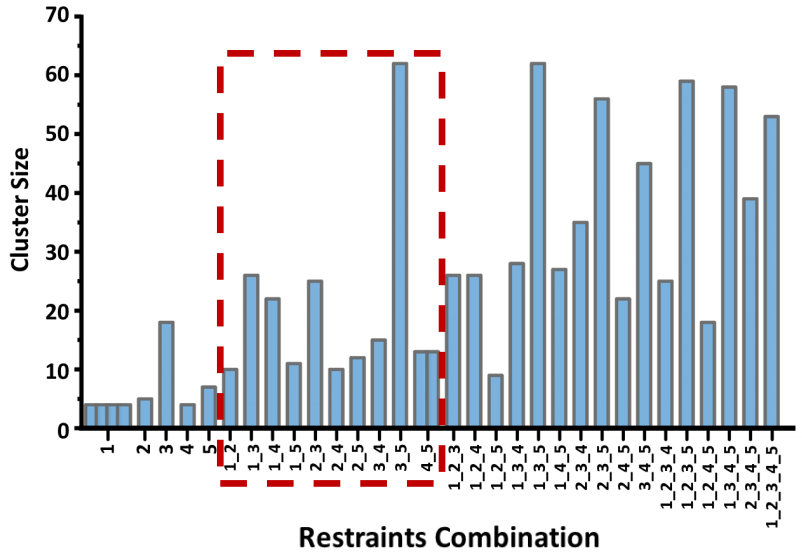
NIGMS MS Resource

❑ HDX of IL-7





❑ Three Types of Models



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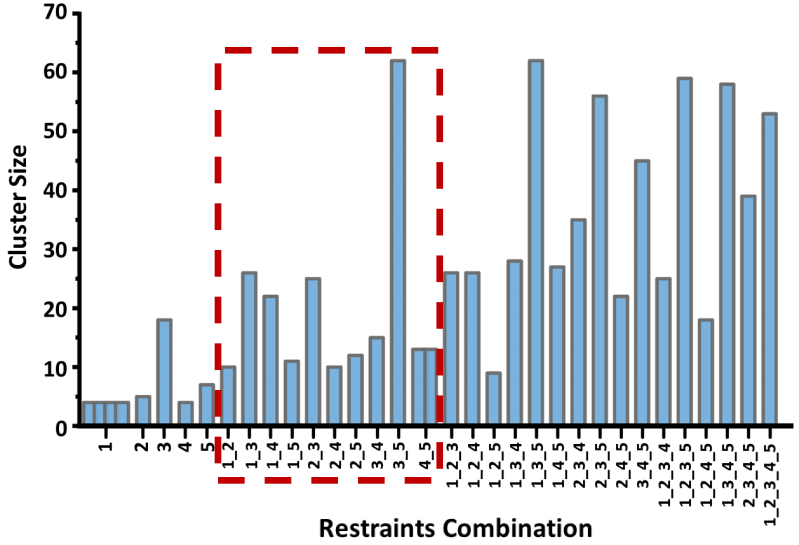
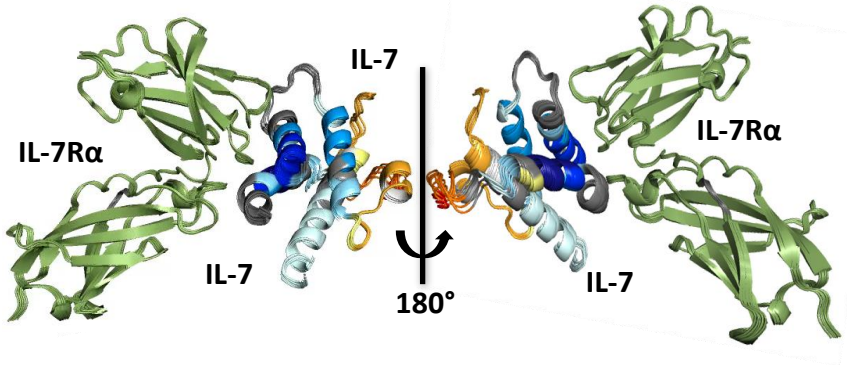
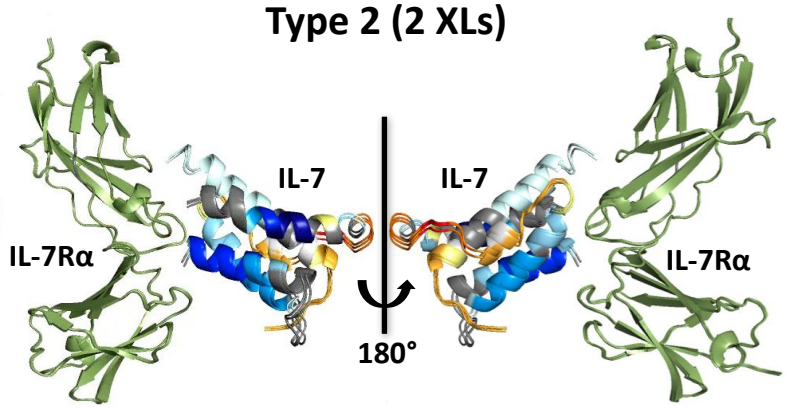


Crosslink-Based Constraints		Cluster Size	Category
1_2		10	Type 3
1_3		26	Type 1
1_4		22	Type 2
1_5		11	Type 1
2_3		25	Type 1
2_4		10	Type 2
2_5		12	Type 1
3_4		15	Type 1
3_5		62	Type 1
4_5	4_5.1	13	Type 1
	4_5.2	13	Type 2

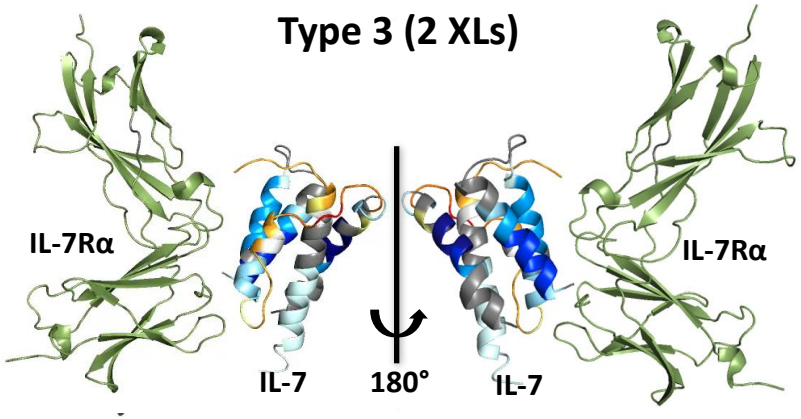
Type 1 (2 XLs)



Type 2 (2 XLs)



Type 3 (2 XLs)



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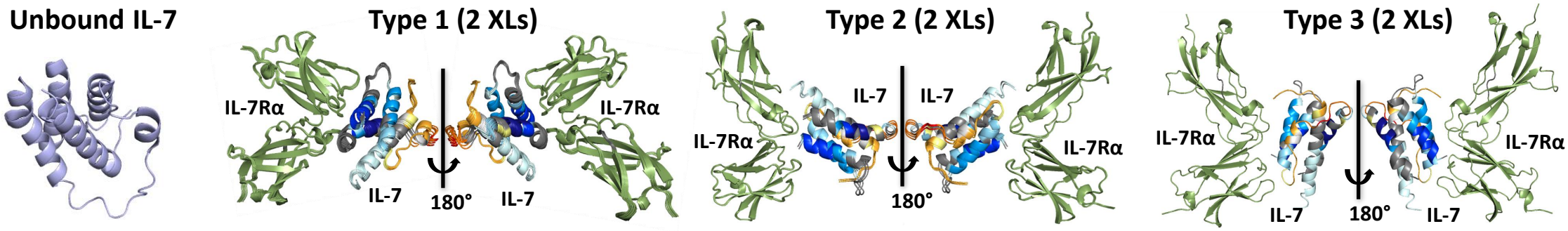
HDX Adjudication of Docking Models (2XLs)

NIGMS MS Resource

❑ **SASA of Two Protected Regions in Each Model**



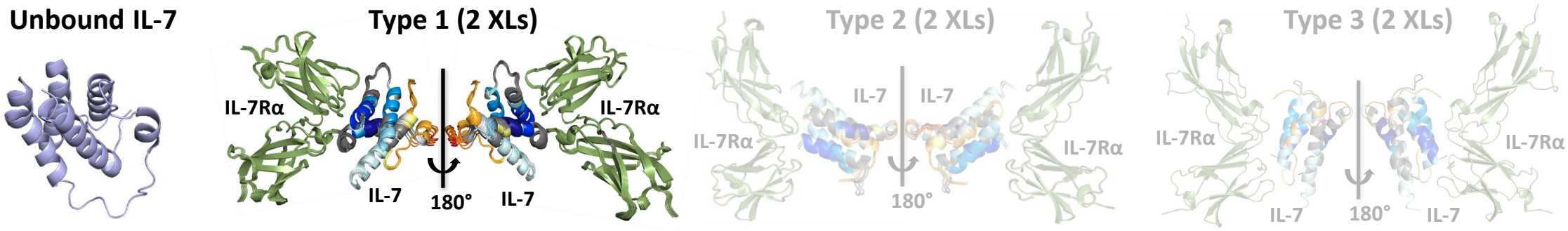
^{19}VSIDQL^{24}	230 Å	143 ± 12 Å	230 ± 1 Å	230 Å
^{83}VSEGTTIL^{90}	319 Å	212 ± 6 Å	256 ± 9 Å	268 Å

Zhang, M. M. Beno, B. R.; Huang, R. Y-C; Adhikari, J.; Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2019**, *91*, 15709-15717.

HDX Adjudication of Docking Models (2XLs)

NIGMS MS Resource

❑ SASA of Two Protected Regions in Each Model



^{19}VSIDQL^{24}	230 Å	143 ± 12 Å	230 ± 1 Å	230 Å
^{83}VSEGTTIL^{90}	319 Å	212 ± 6 Å	256 ± 9 Å	268 Å
Matching with HDX		Y	N	N
Comparison with X-ray Structure		1.6 ±0.2 Å	10.9 ± 0.05 Å	12.3 Å

- ✓ Guidance from HDX kinetics allows ruling out dubious docking models
- ✓ Restraints of two intermolecular cross-links are adequate to identify an accurate quaternary model

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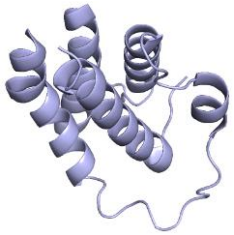
HDX Adjudication of Docking Models (3-4XLs)

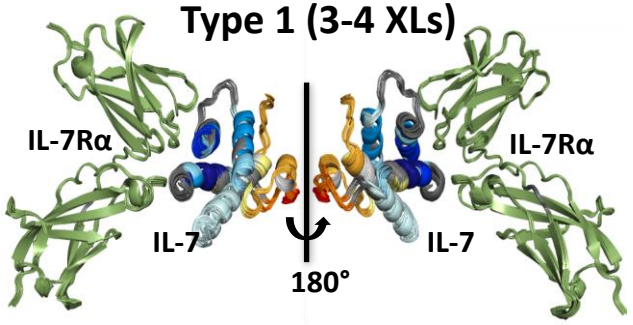
NIGMS MS Resource

❑ SASA of Two Protected Regions in Each Model

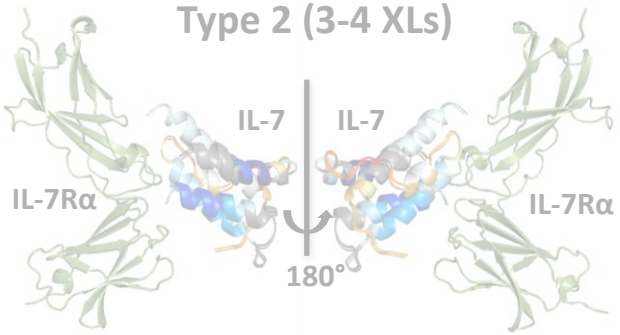
Unbound IL-7



Type 1 (3-4 XLs)



Type 2 (3-4 XLs)



^{19}VSIDQL^{24}	230 Å	138 ± 7 Å	230 ± 0 Å
^{83}VSEGTTIL^{90}	319 Å	208 ± 8 Å	249 ± 2 Å
Matching with HDX		Y	N
Comparison with X-ray Structure		1.6 ± 0.1 Å	11 ± 0 Å

Crosslink-Based Constraints	Cluster Size	Category
1_2_3	26	Type 1
1_2_4	26	Type 2
1_2_5	9	Type 1
1_3_4	28	Type 1
1_3_5	62	Type 1
1_4_5	27	Type 2
2_3_4	35	Type 1
2_3_5	56	Type 1
2_4_5	22	Type 1
3_4_5	45	Type 1
1_2_3_4	25	Type 1
1_2_3_5	59	Type 1
1_2_4_5	18	Type 2
1_3_4_5	58	Type 1
2_3_4_5	39	Type 1

- ✓ More cross-links leads to better populated model types
- ✓ Can identify a high-confidence model with HDX adjudication

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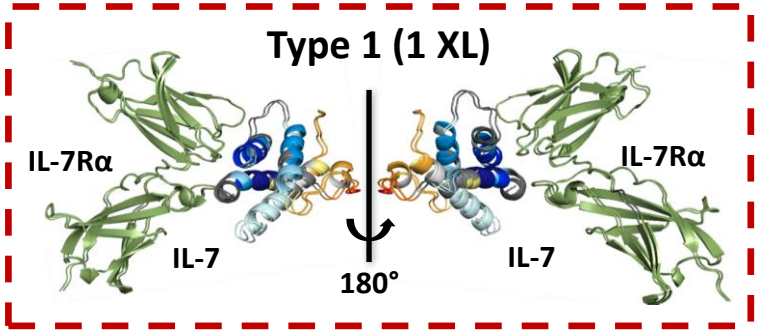
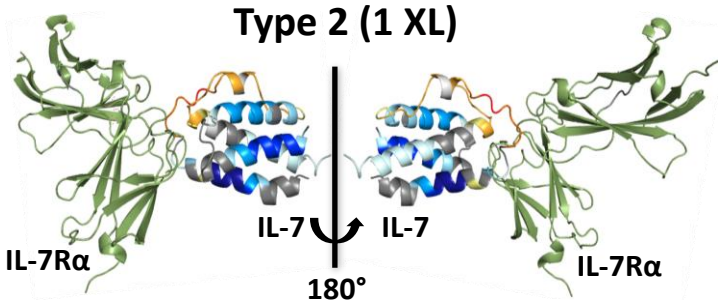
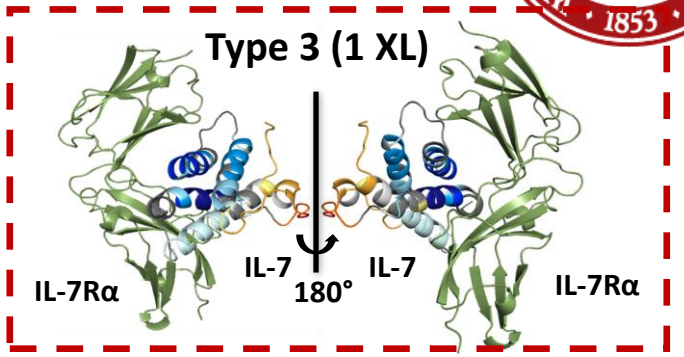
HDX Adjudication of Docking Models (1XLs)

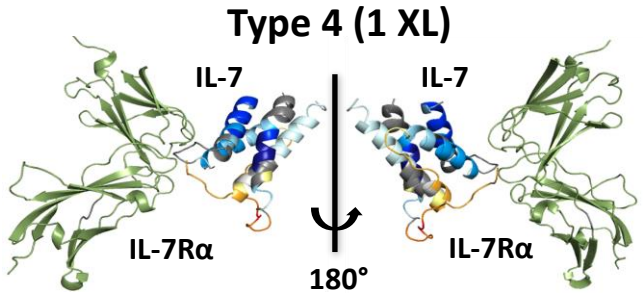
NIGMS MS Resource

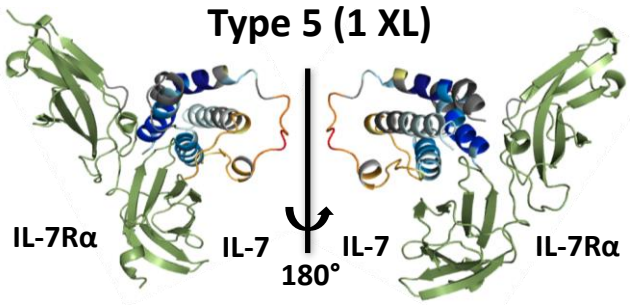
❑ Seven Types of Models using Only One Cross-link

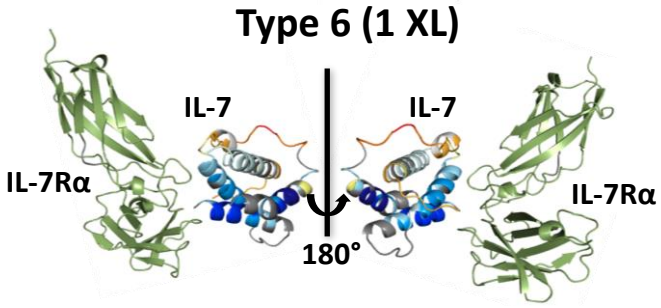


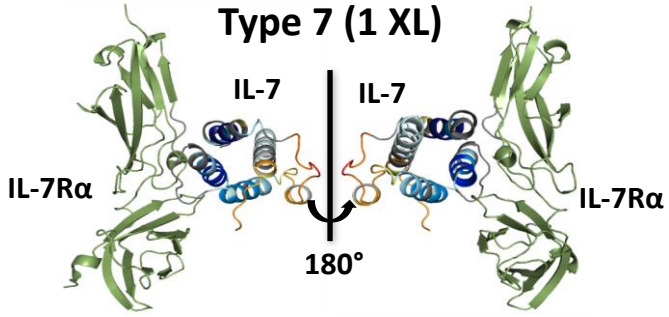












Crosslink-Based Constraints		Cluster Size	Category
1	1.1	4	Type 1
	1.2	4	Type 2
	1.3	4	Type 3
	1.4	4	Type 4
2		5	Type 5
3		18	Type 1
4		4	Type 6
5		7	Type 7

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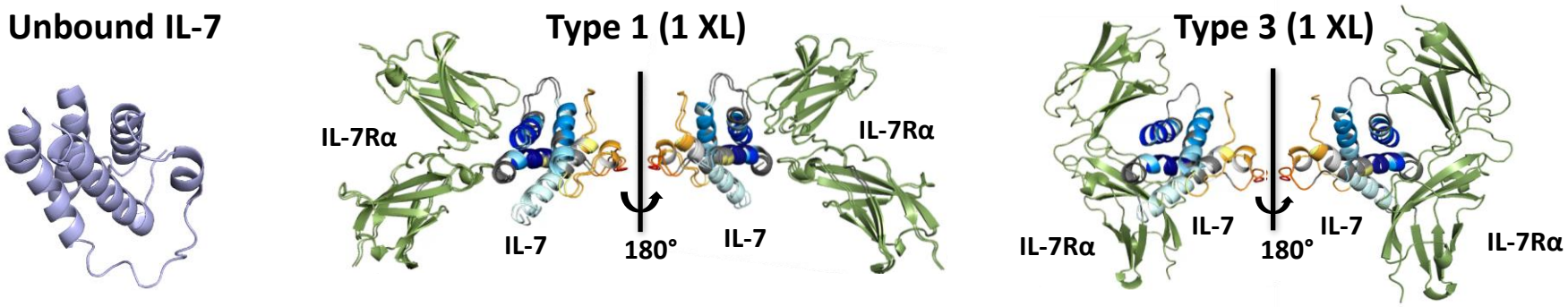
HDX Adjudication of Docking Models (1XLs)

NIGMS MS Resource

❑ **SASA of Two Protected Regions in Each Model**



^{19}VSIDQL^{24}	230 Å	138 ± 1 Å	65 Å
^{83}VSEGTTIL^{90}	319 Å	213 ± 6 Å	182 Å
Matching with HDX		Y	Y
Comparison with X-ray Structure		1.6 ± 0.1 Å	14.8 Å

- ✓ One cross-link is insufficient to assign an accurate quaternary structure
- ✓ The minimal number of cross-links to fulfill this goal is two

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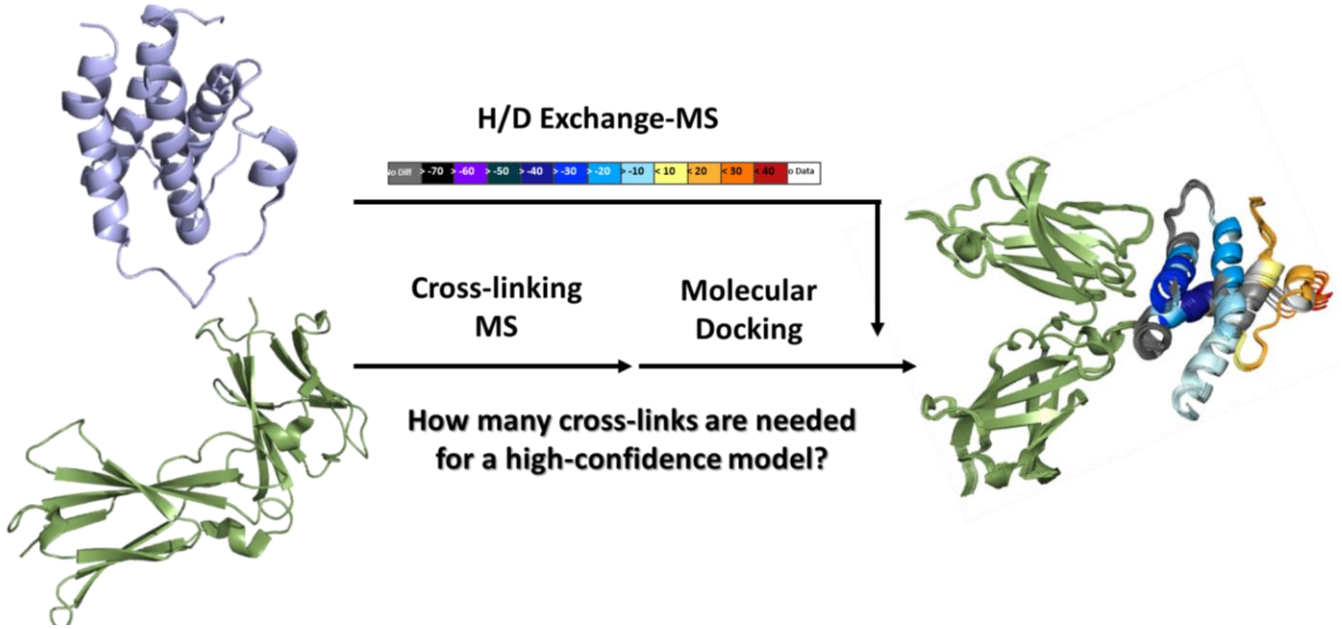
Conclusion and Perspective



- Establish an efficient integrated platform for protein-protein binding interface determination
- Characterize successfully the IL-7/IL-7Rα binding interface and PD-1/Nivolumab interaction
- Enable structural information from tertiary to quaternary
- Allow comprehensive understanding of protein interaction events
- Provide an intriguing alternative that would aid the design of protein therapeutics in multiple states

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Zhang, M. M.;§ Huang, R. Y-C.;§ Beno, B. R. Deyanova, E. G.; Li, J.; Chen, G.; Gross, M. L. *Anal. Chem.* **2020.** 92, 9086-9094.