Protein conformation in amorphous solids by hydrogen-deuterium exchange

International Symposium on the Higher Order Structure of Protein Therapeutics

Biological Consequences of HOS Session

April 4, 2022 (virtual)

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Acknowledgements

***Purdue University** (current)*

- Dr. Karthik Chandrababu
- Yuan Chen
- Iris Cho
- Harshil Renawala
- Raj Kammari
- Lia Bersin
- Rishabh Tukra
- Andrew Karaki

***Purdue University** (past)*

- Dr. Andreas Sophocleous
- Dr. Balakrishnan S. Moorthy

Roche / Genentech

- Dr. Andrea Allmendinger
- Dr. Dan Zarraga
- Dr. Ben Walters
- Dr. Lokesh Kumar
- Dr. Kathleen Abadie

AbbVie

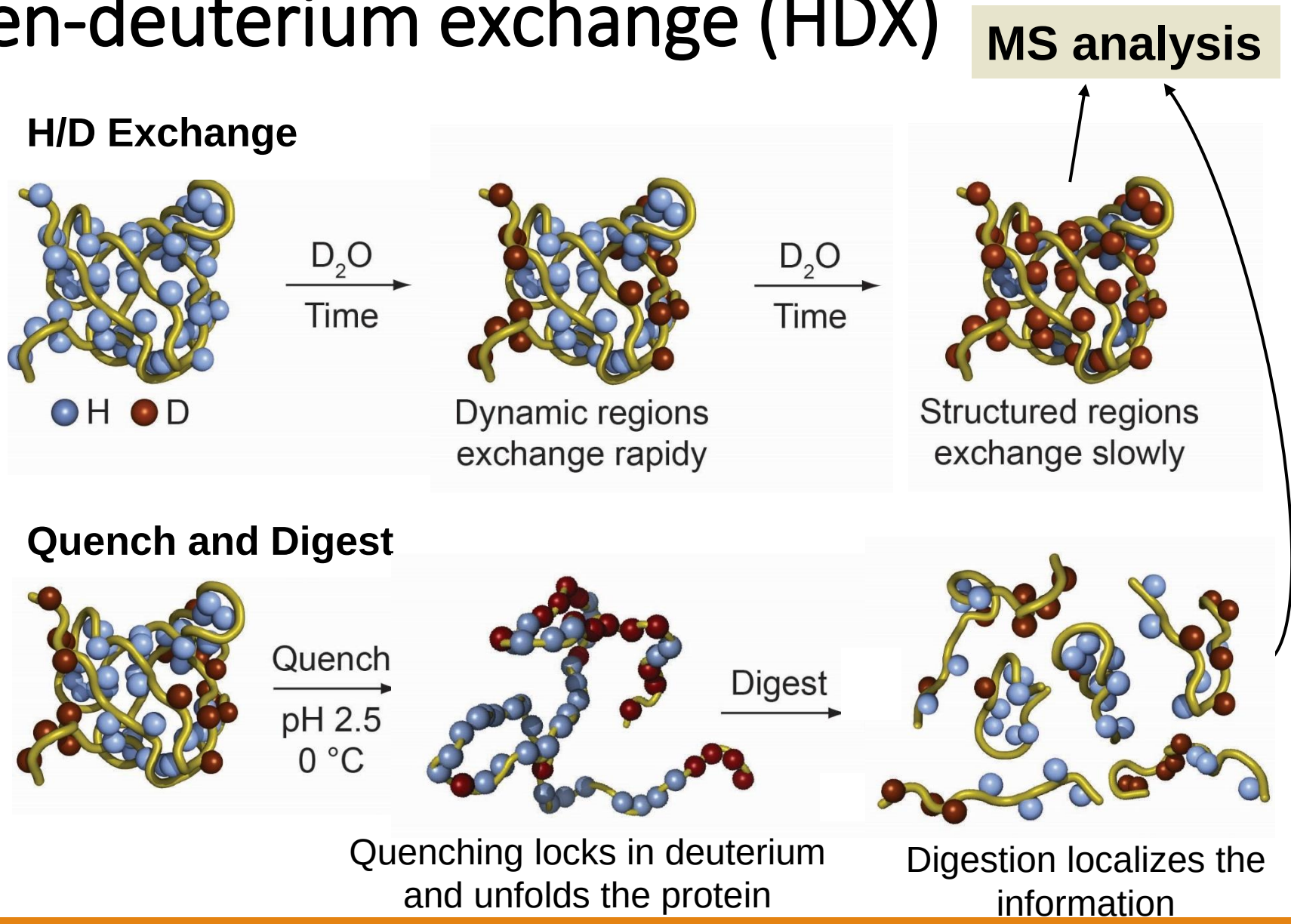
- Dr. Steve Schultz
- Ms. Sherry Kim

Financial support: NIH RO1 R01GM085293, PhRMA Foundation Fellowship (AS), Purdue University, Roche/Genentech, Inc., NIST AMTech, NIIMBL

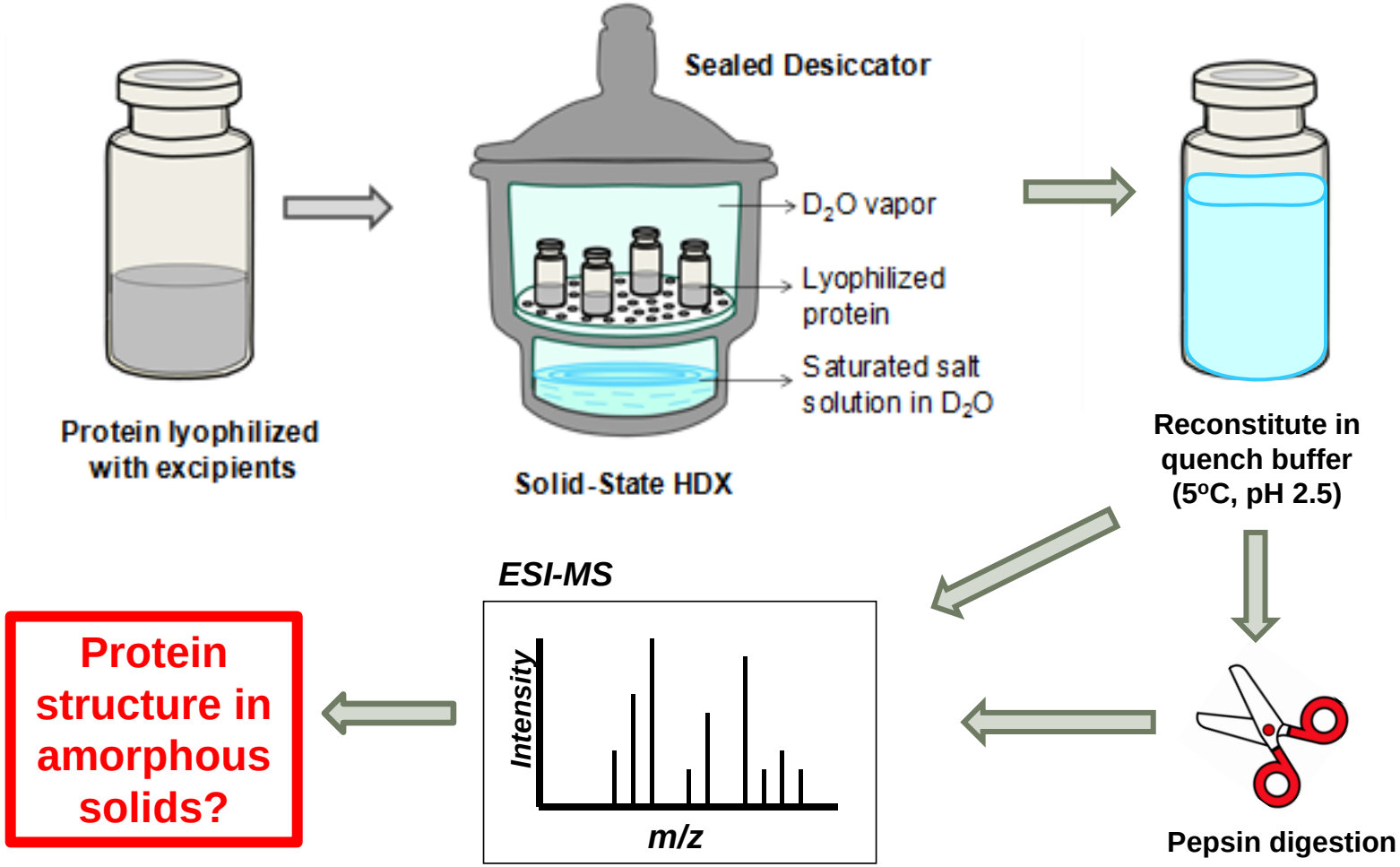
Proteins in the solid state

- Protein drugs are an important part of the biopharmaceutical industry.
- Over the past decade, ~40% of newly approved protein drugs have been marketed as solids.
- Maintaining HOS in the solid state is important for storage stability.
- But methods to measure HOS in solids are few and low-resolution.

Hydrogen-deuterium exchange (HDX)

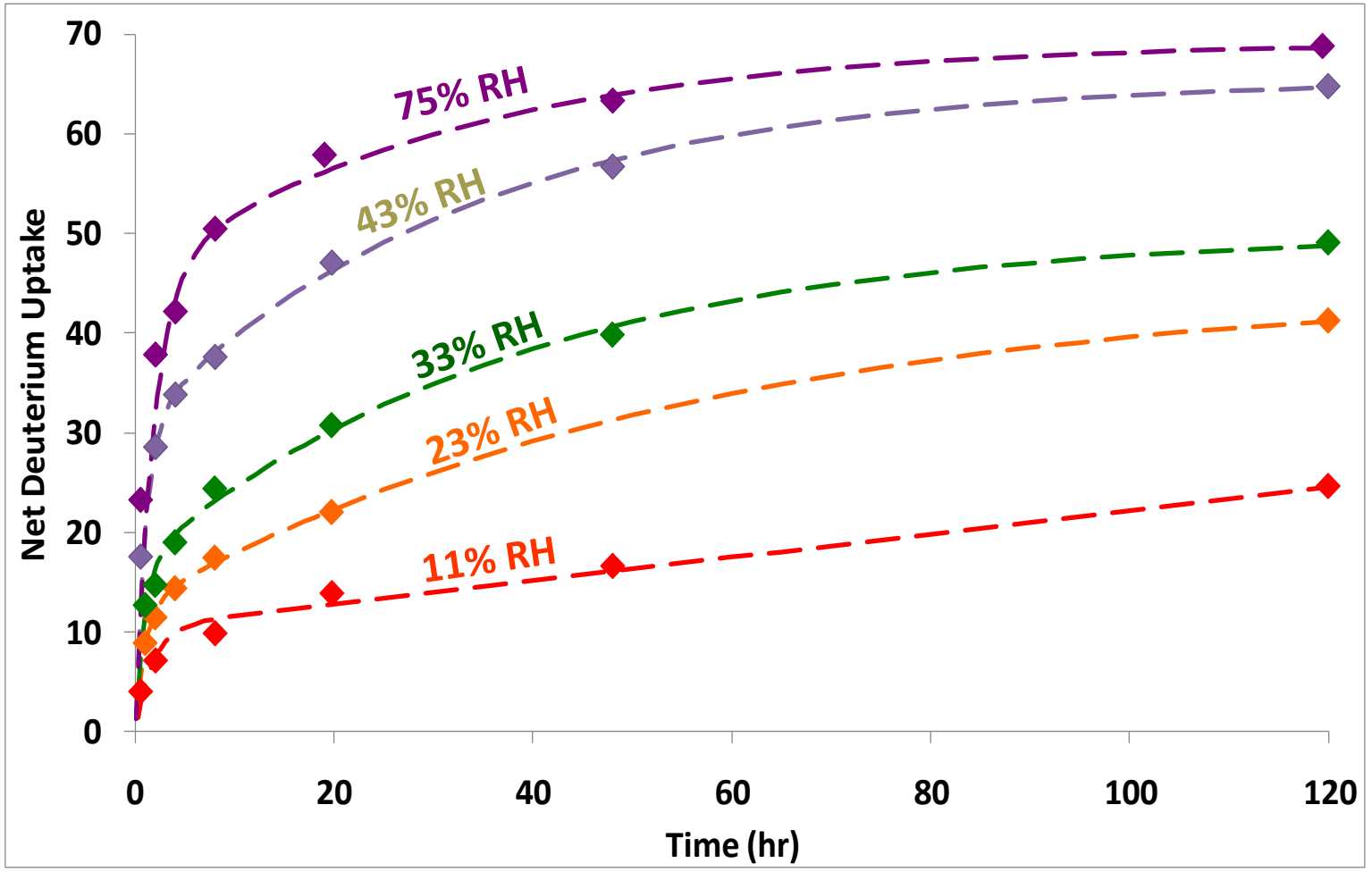


Solid-state HDX (ssHDX-MS)



Effect of RH on deuterium uptake

Intact protein: *Myoglobin / mannitol 1:1, 5 °C*



Effect of RH on deuterium uptake

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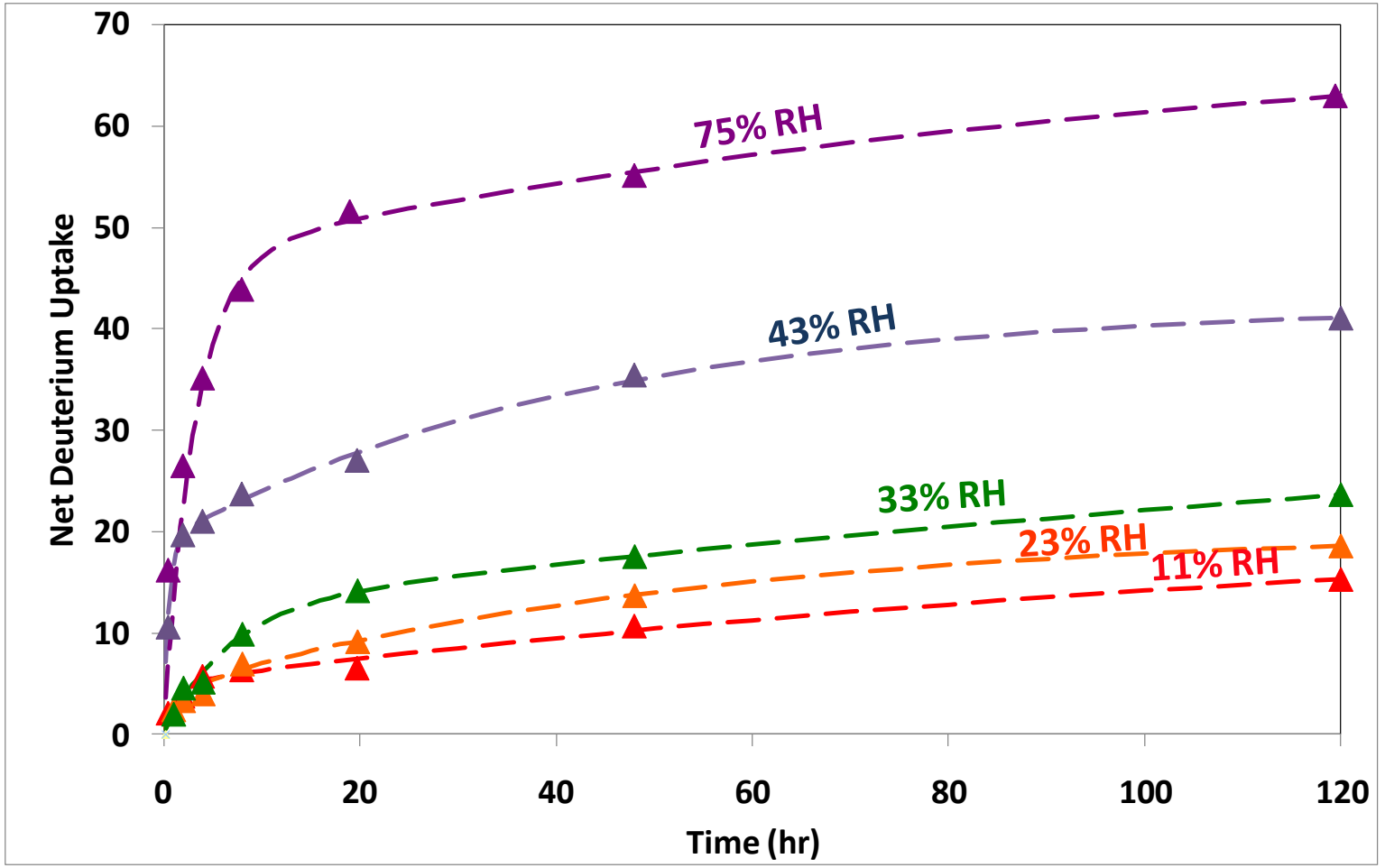
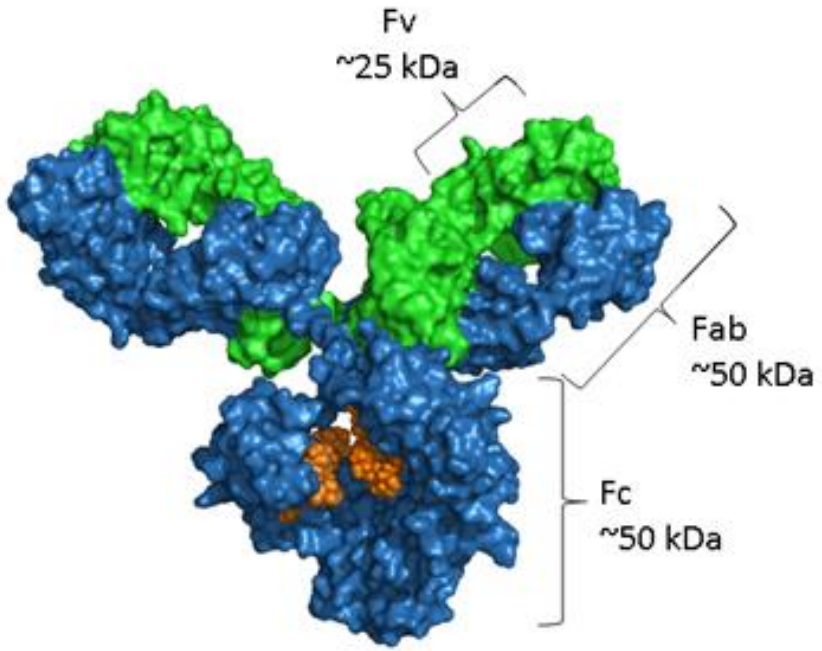


mAb formulations

Can ssHDX be applied to mAbs?

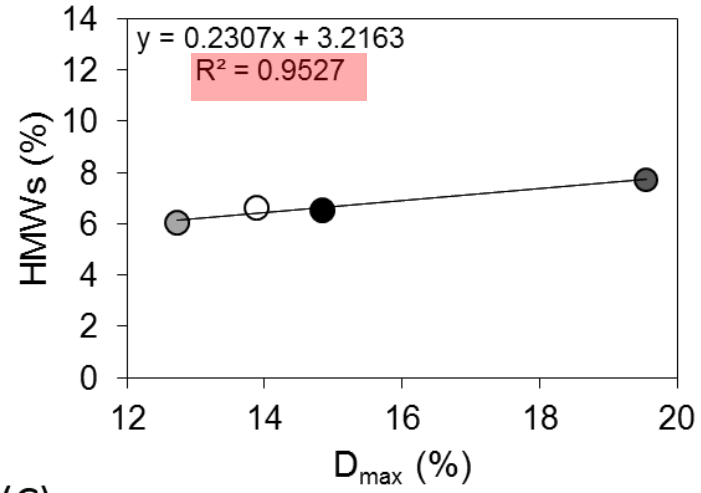


Formulation	Composition		
	Basic composition	Mannitol (mg/mL)	Sucrose (mg/mL)
F1	50 mg/mL mAb1 in buffer at pH 6.0	-	40
F2		-	80
F3		32	8
F4		53	27

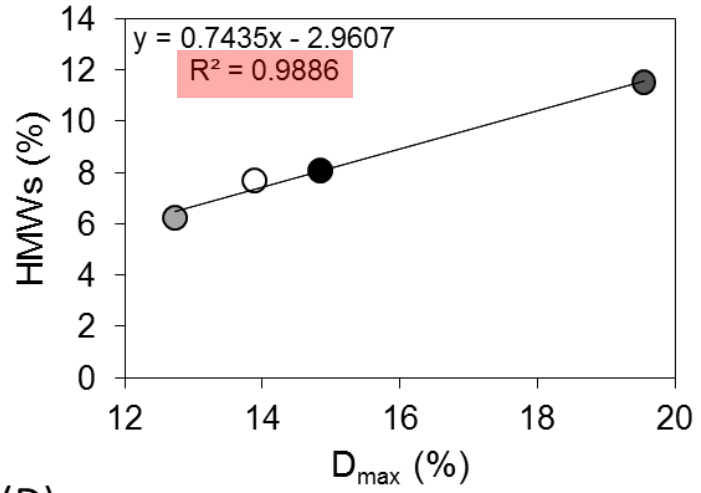
Knowing only this, rank order the storage stability of the four formulations using ssHDX-MS.

mAb stability and ssHDX-MS

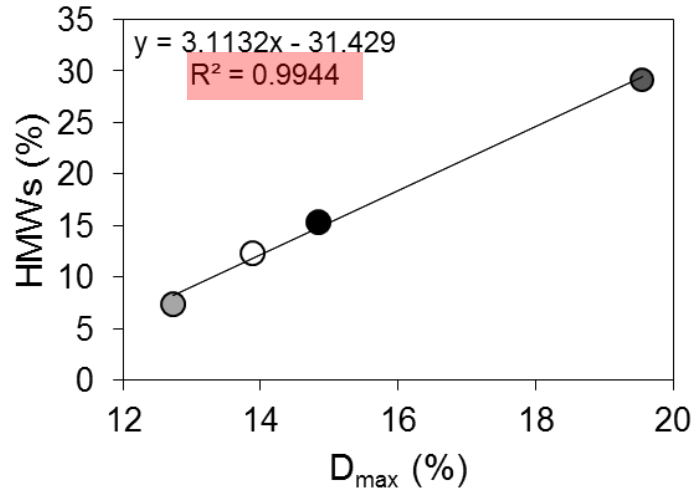
(A) Stored @ 5°C, 960 days



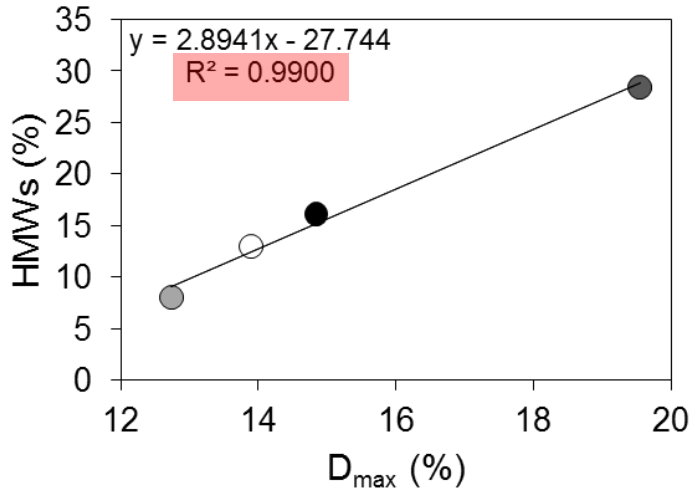
(B) Stored @ 25°C, 960 days



(C) Stored @ 40°C, 960 days

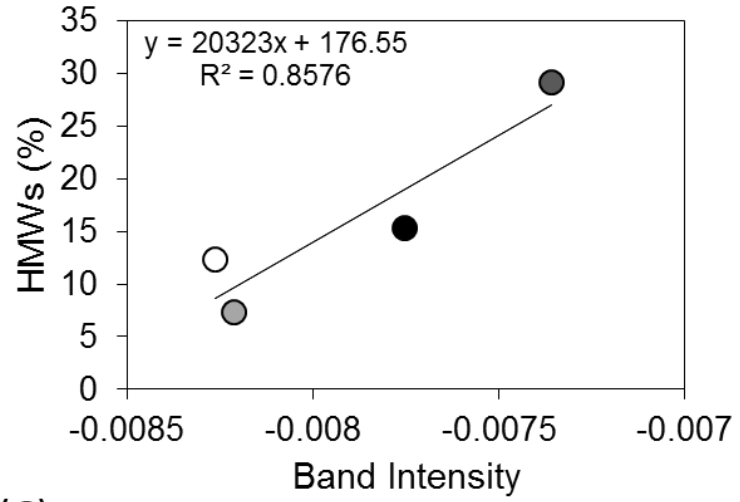


(D) Stored @ 50°C, 180 days

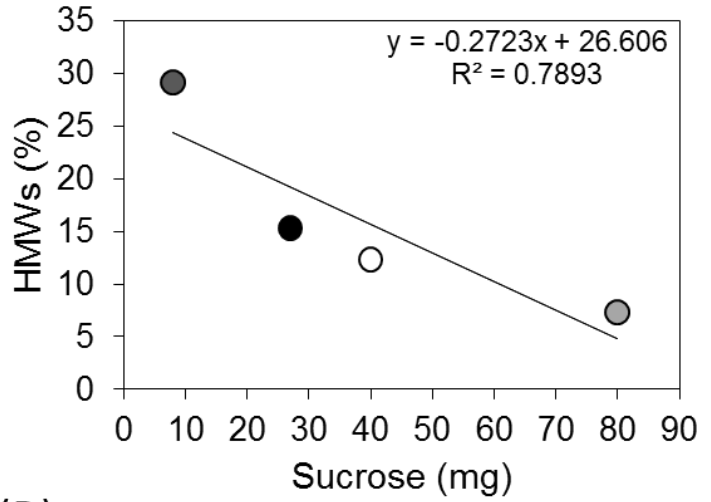


mAb stability and solid properties

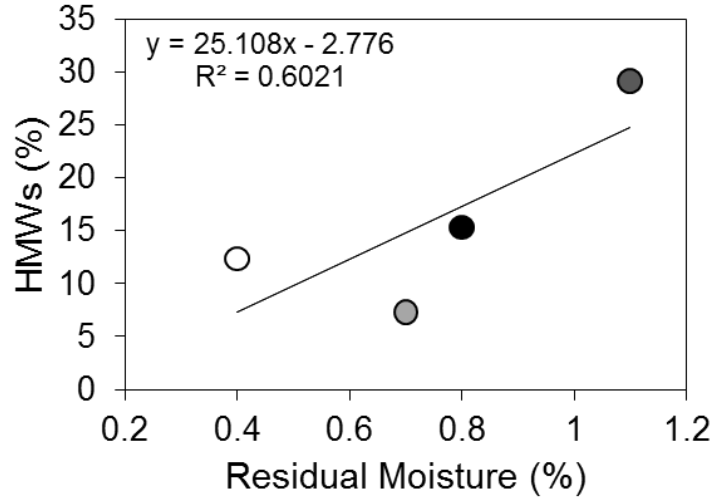
(A) FTIR band intensity



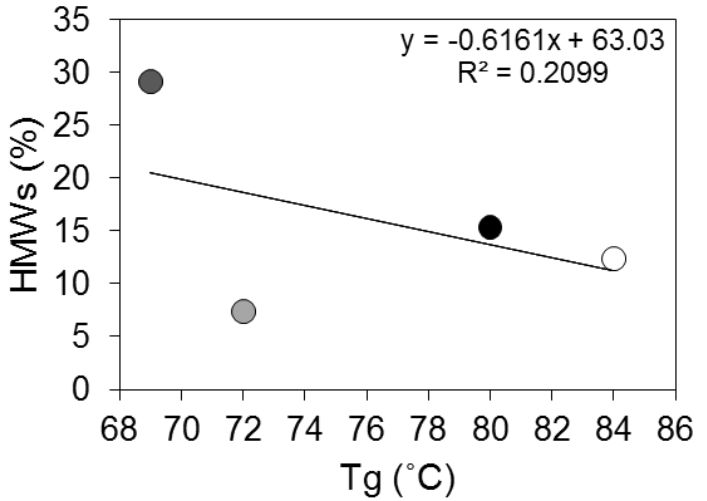
(B) Sucrose content



(C) Moisture content



(D) Glass transition, Tg

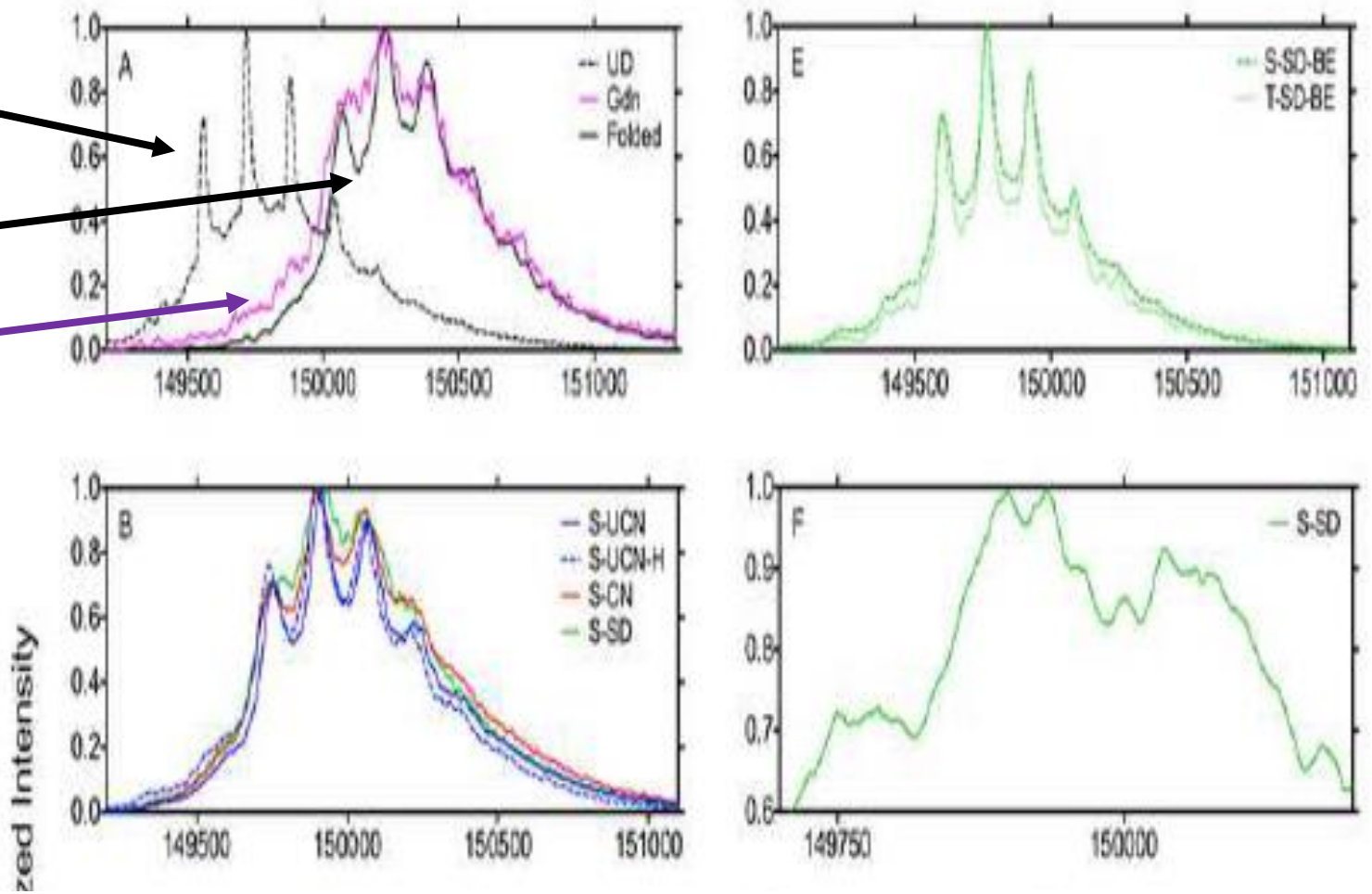


Process effects

Native

Native, deuterated

Unfolded, deuterated



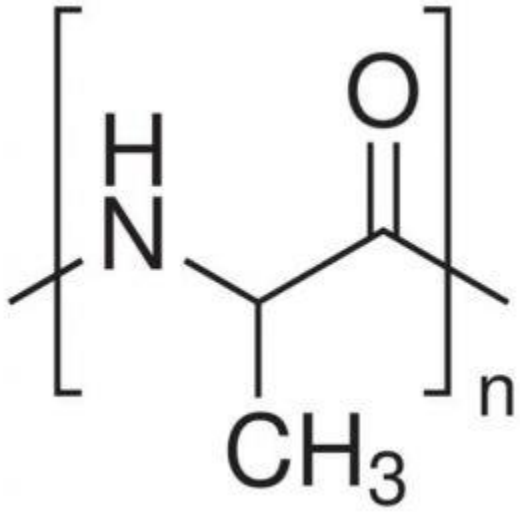
Lyophilized, uncontrolled nucleation

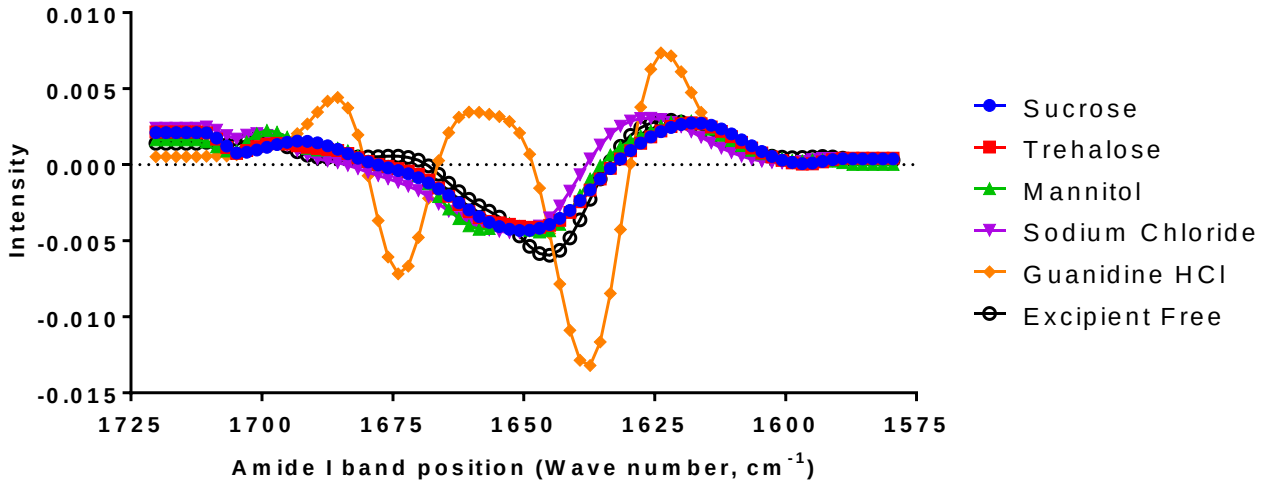
Lyophilized, controlled nucleation

Lyophilized, uncontrolled nucleation with hydration

Spray dried

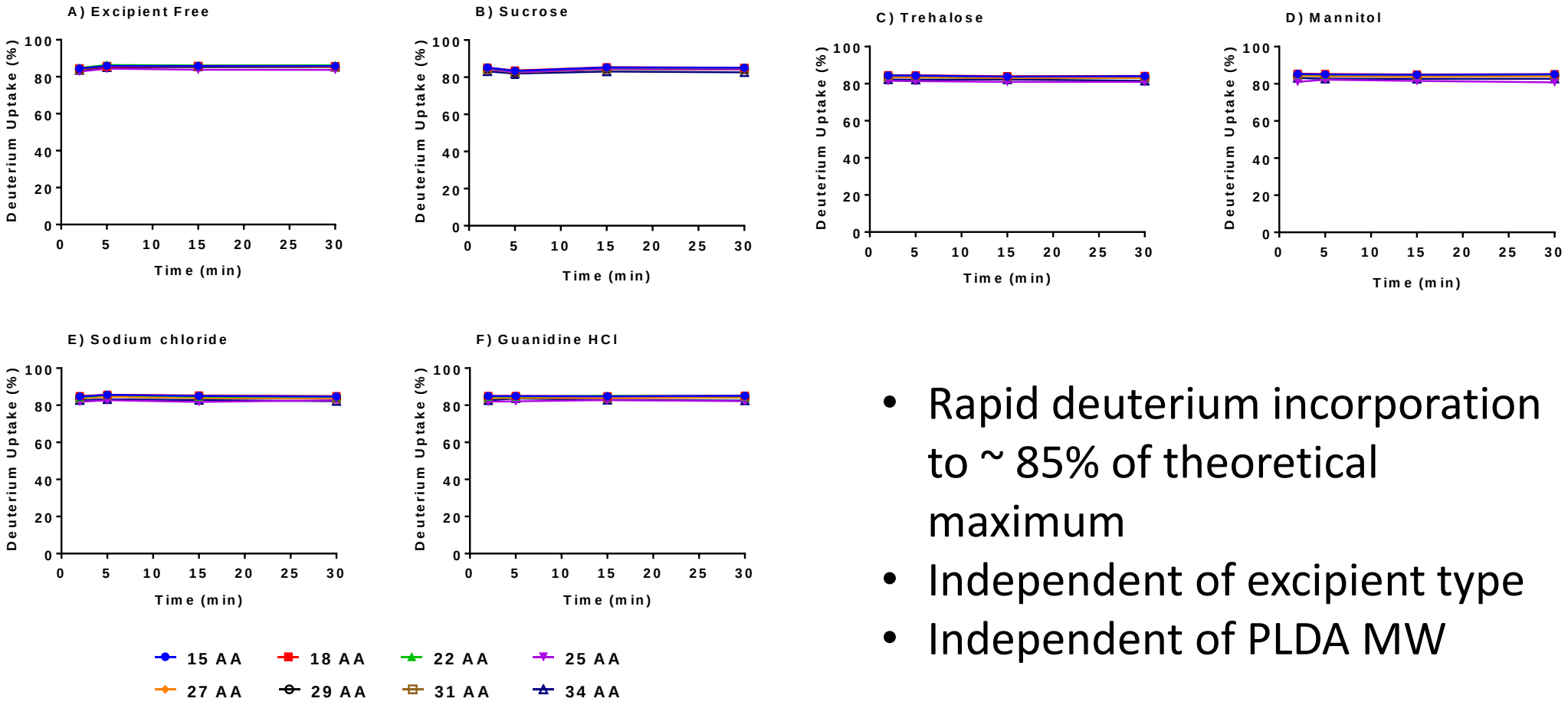
Poly-D,L-alanine (PDLA)





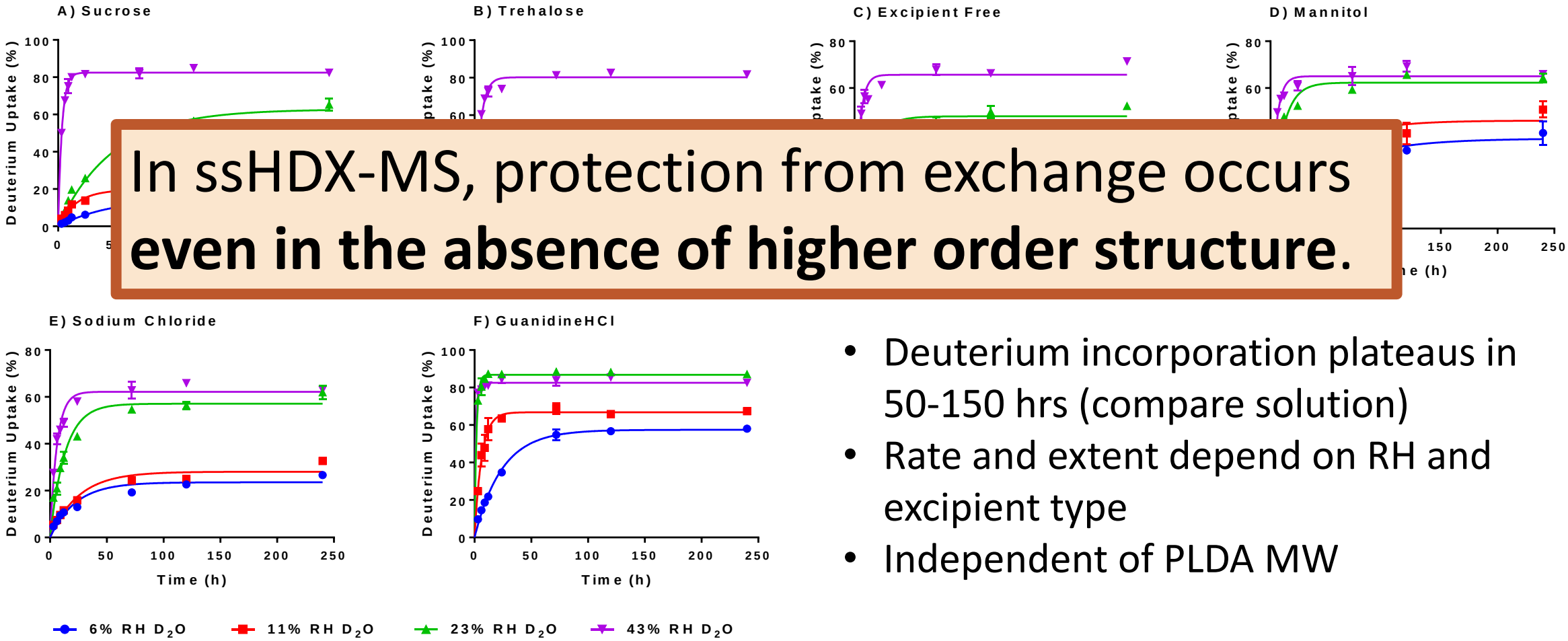
- Unstructured poly-amino acid
- Polydisperse mixture – oligomers with 15-34 aa selected
- Formulated with sucrose, trehalose, no excipient, mannitol, NaCl, Gdn HCl
- Lyophilized solids and solution controls

Solution HDX-MS of PDLA



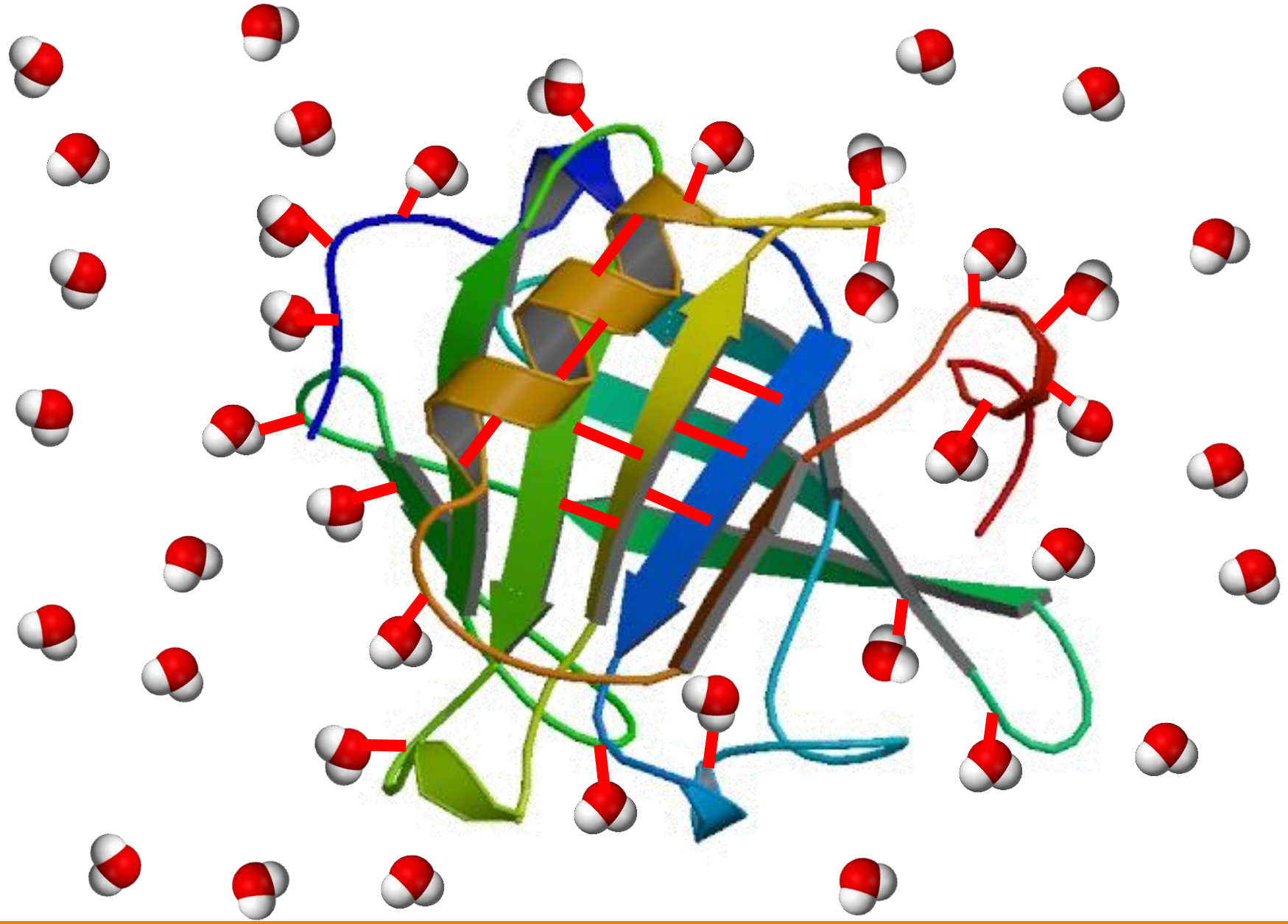
- Rapid deuterium incorporation to ~ 85% of theoretical maximum
- Independent of excipient type
- Independent of PLDA MW

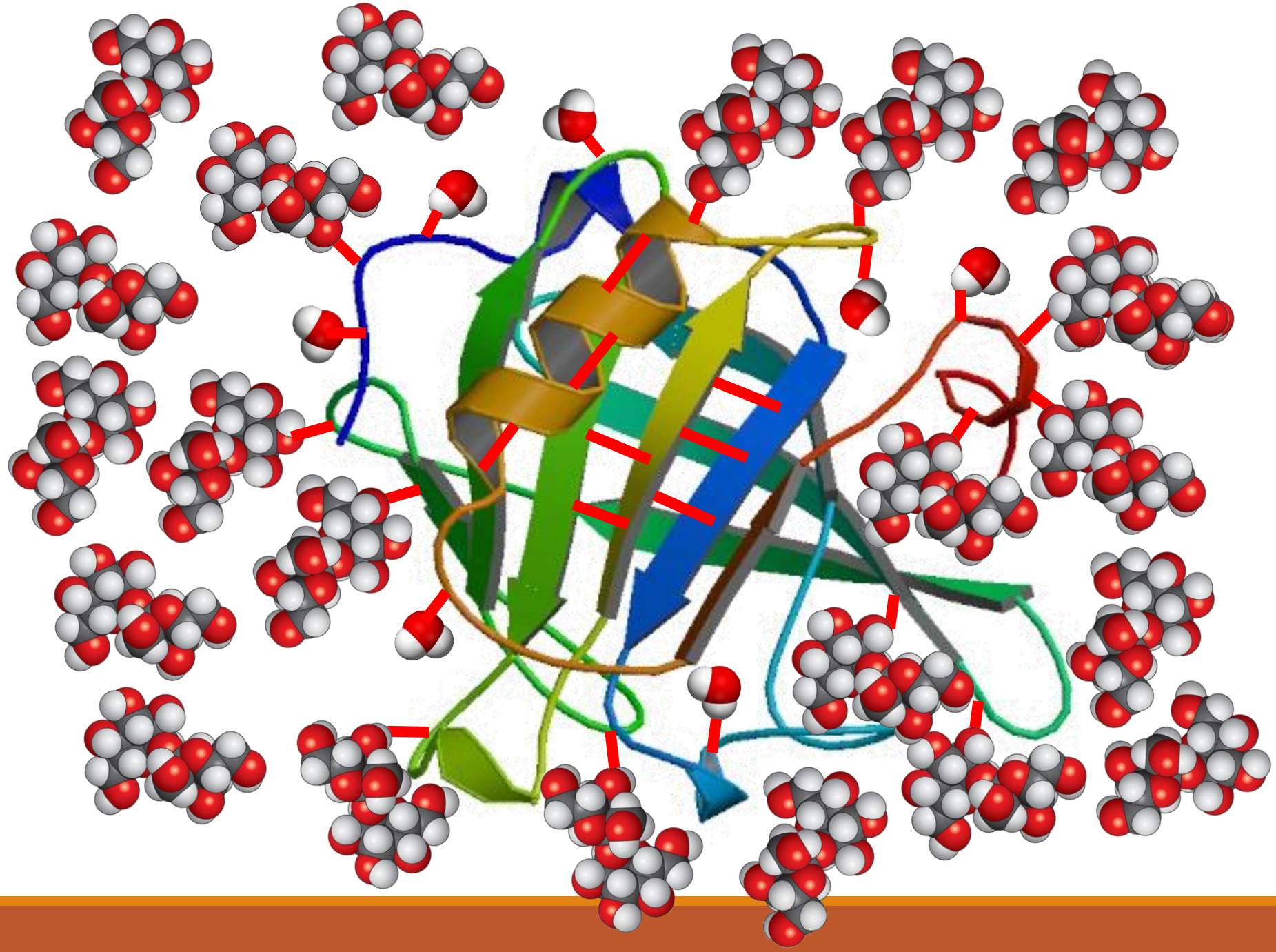
ssHDX-MS of PDLA

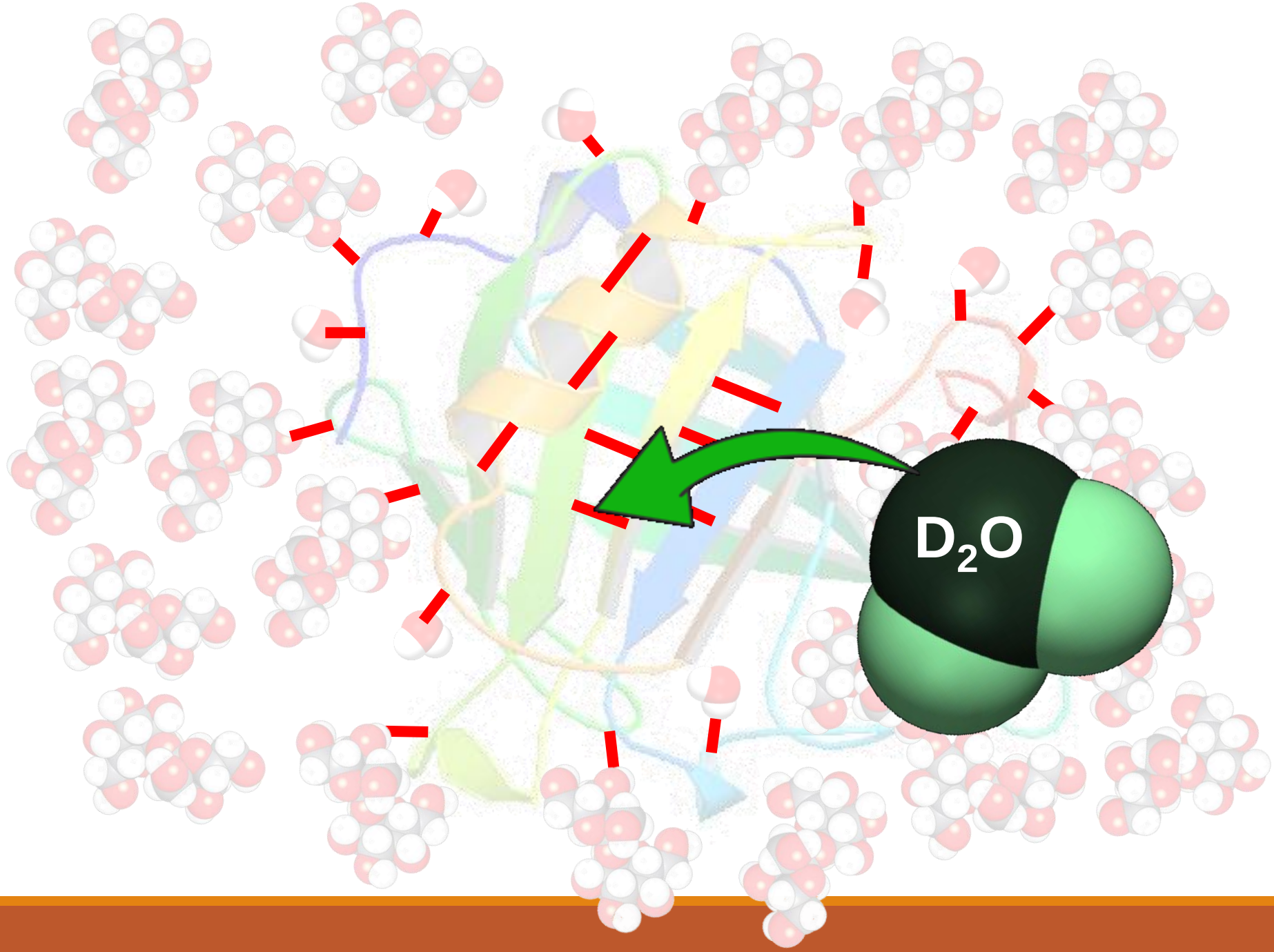


In ssHDX-MS, protection from exchange occurs **even in the absence of higher order structure**.

- Deuterium incorporation plateaus in 50-150 hrs (compare solution)
- Rate and extent depend on RH and excipient type
- Independent of PLDA MW






D_2O

Summary

- ssHDX-MS **interrogates the H-bond network** of proteins in solid powders, providing information on HOS and matrix interactions with peptide-level resolution.
- For a given protein, ssHDX-MS kinetics are affected by excipient type and amount, RH in D_2O, temperature and processing method.
- The extent of deuterium incorporation (D_{max}) is **correlated with aggregation** on storage.
- Studies with unstructured peptides (PDLA) show protection from exchange in ssHDX-MS, in the absence of structure.