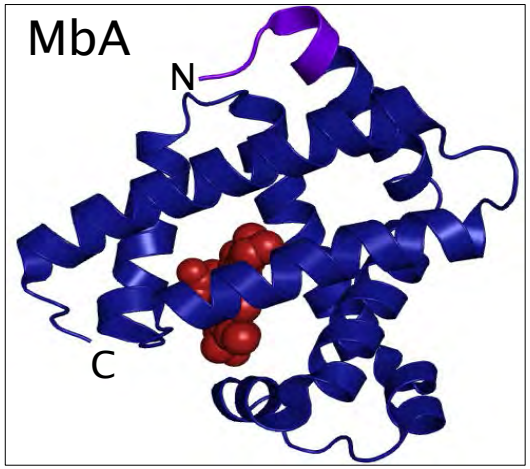
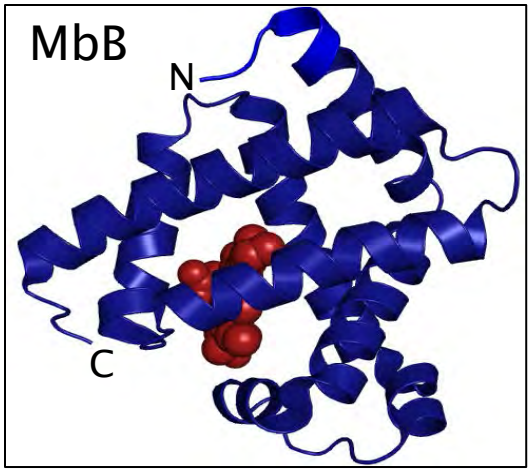
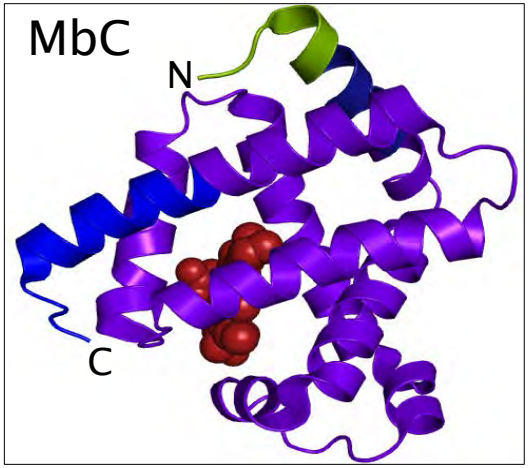
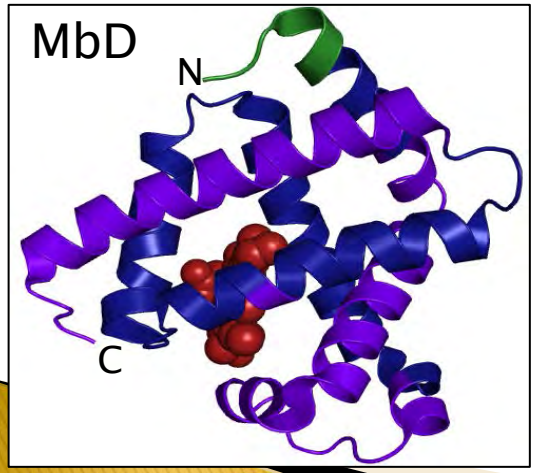
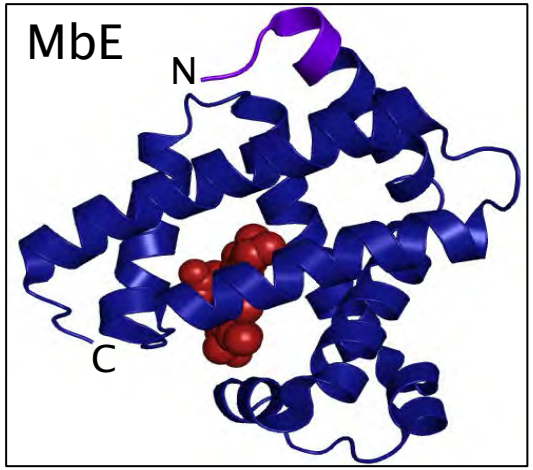
HDX Results Mapped on to the Mb Structure (T=1h)



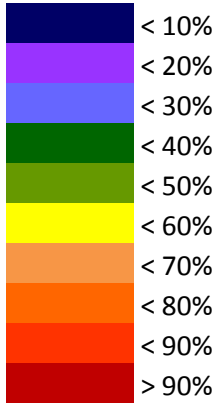




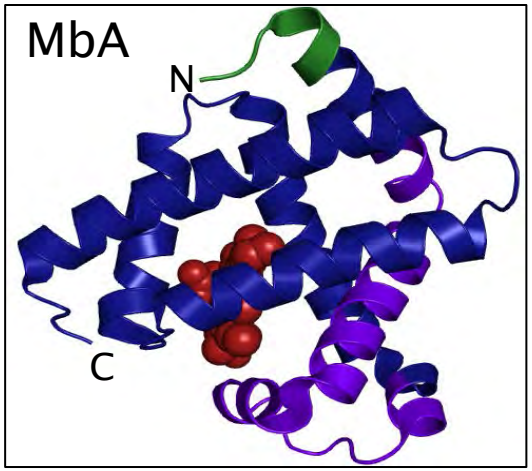


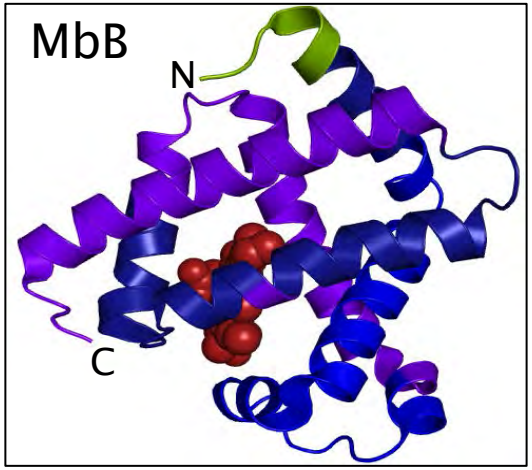


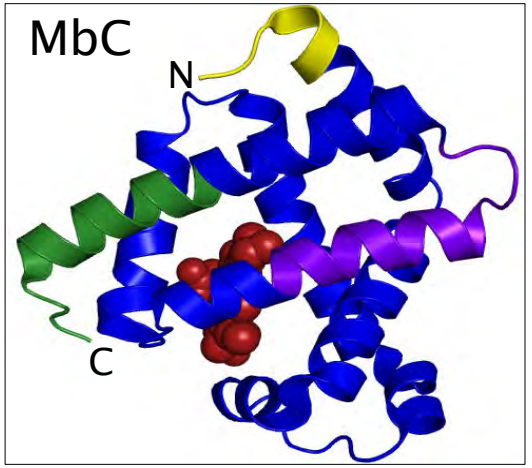
Deuterium Level

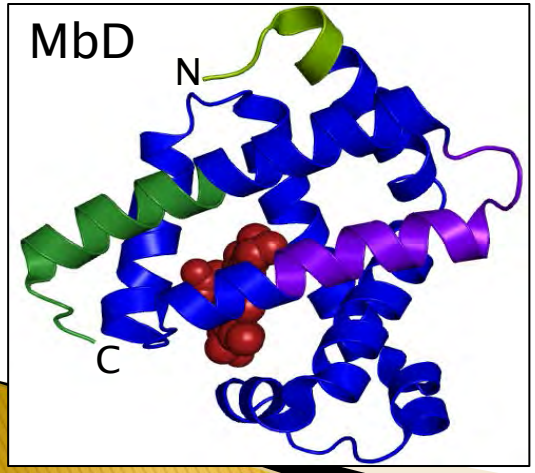


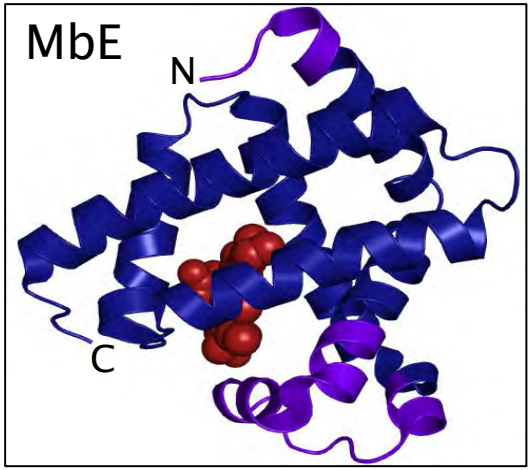
HDX Results Mapped on to the Mb Structure (T=6h)











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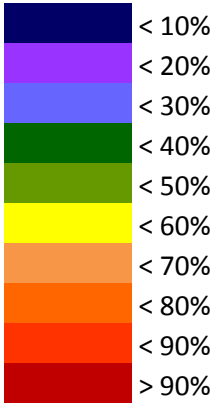
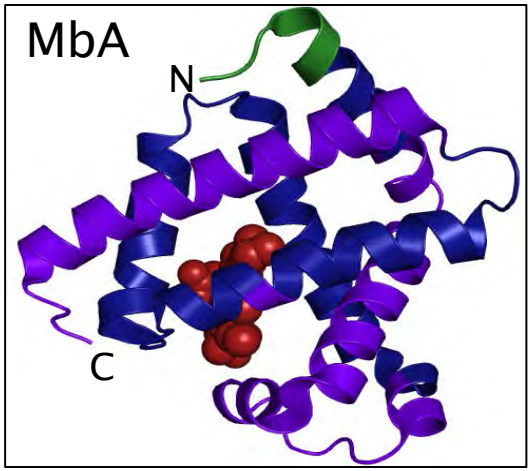
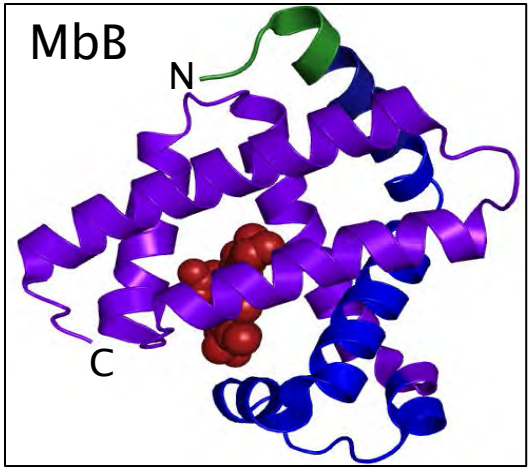
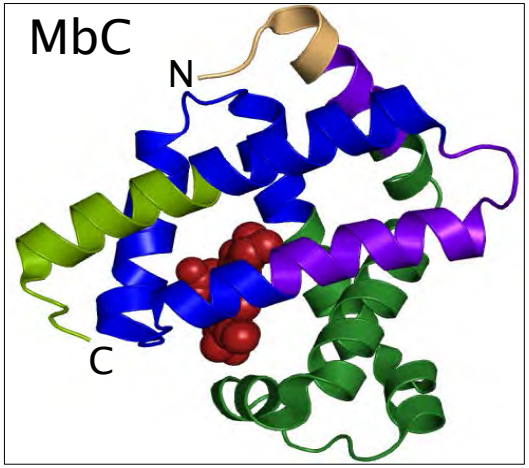
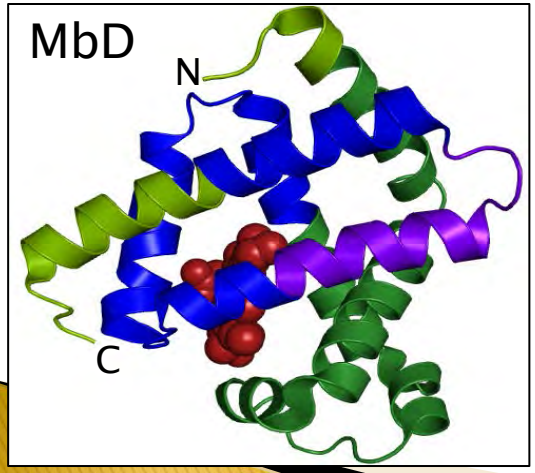


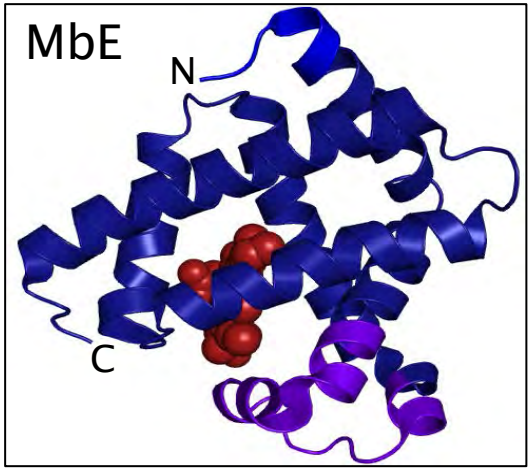
HDX Results Mapped on to the Mb Structure (T=24h)



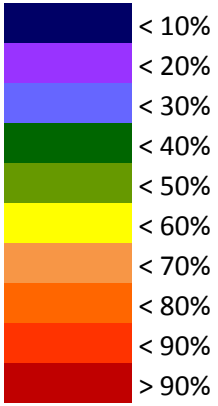




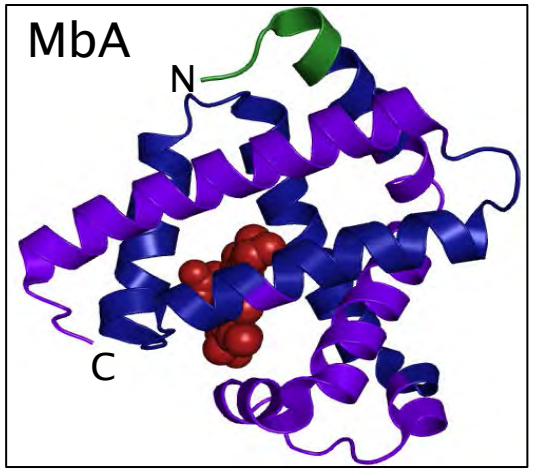


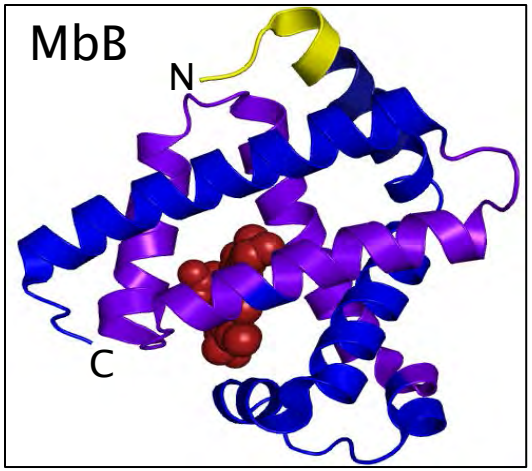


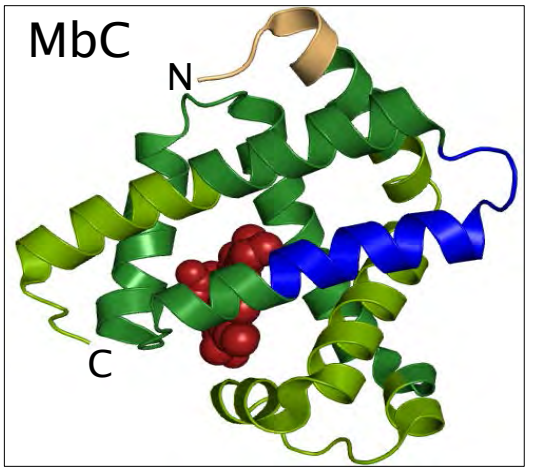
Deuterium Level

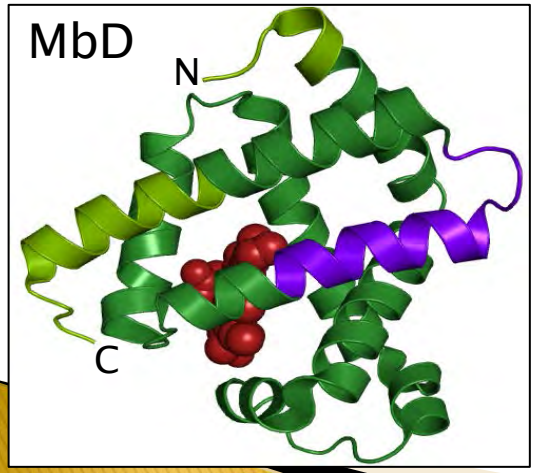


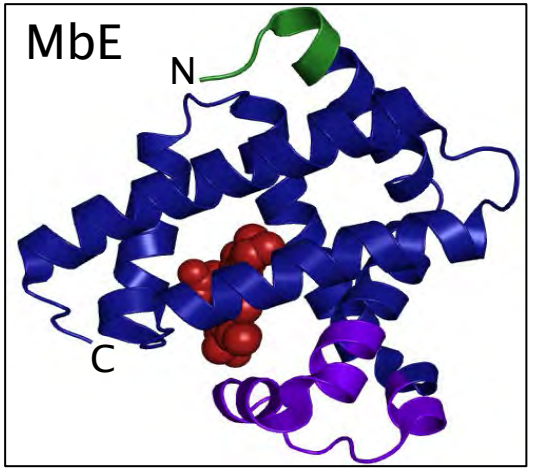
HDX Results Mapped on to the Mb Structure (T=48h)



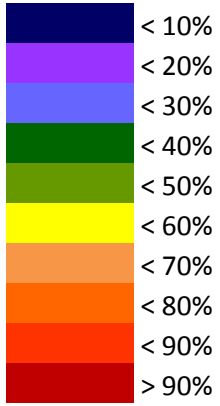




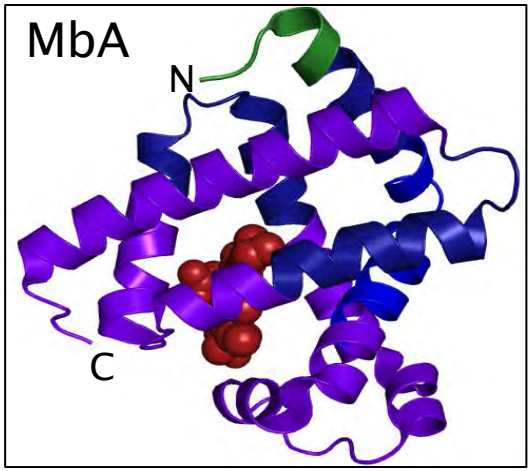


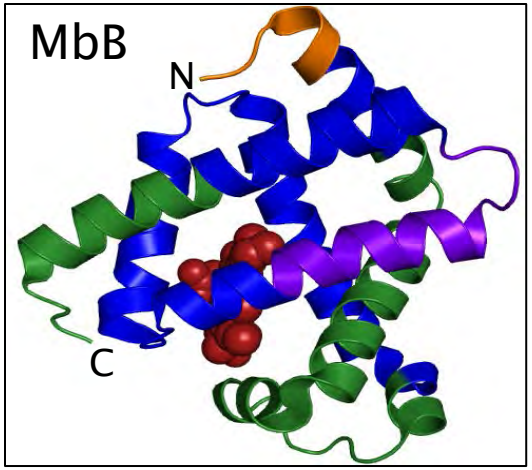


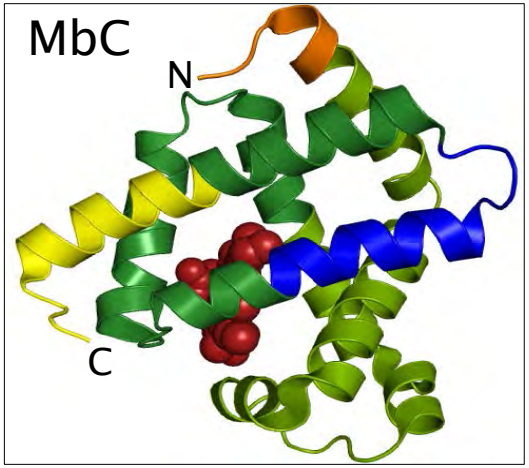
Deuterium Level

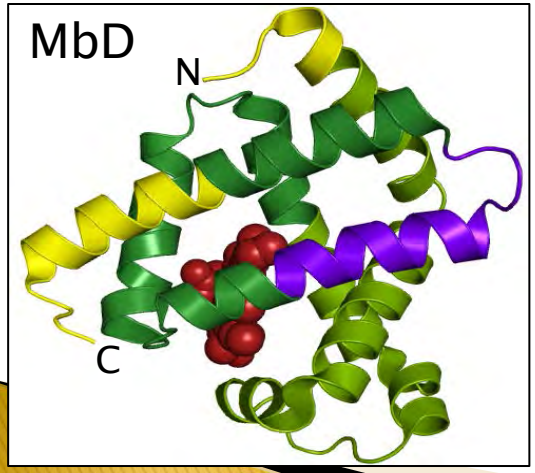


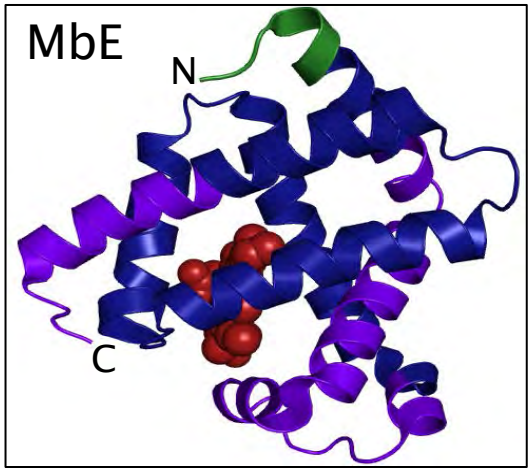
HDX Results Mapped on to the Mb Structure (T=120h)



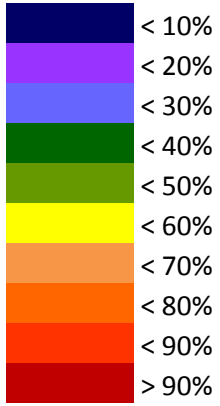




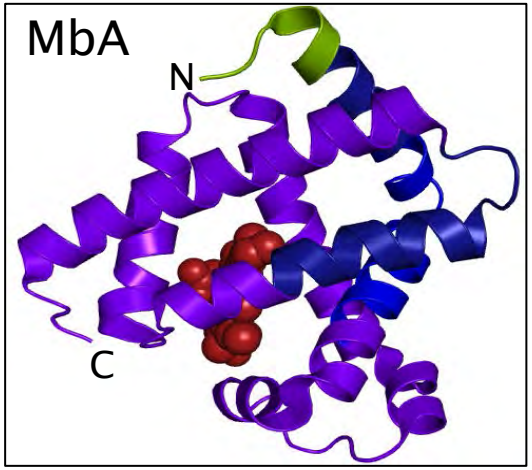


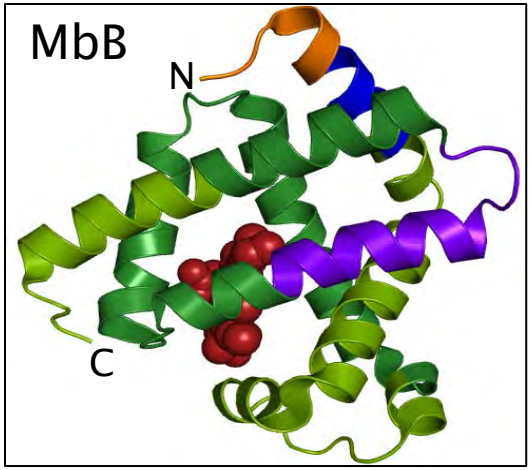


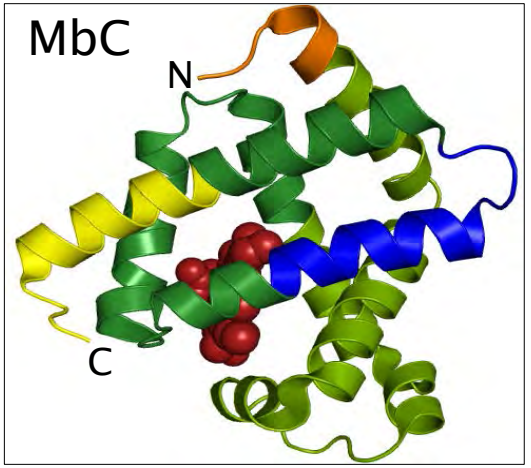
Deuterium Level

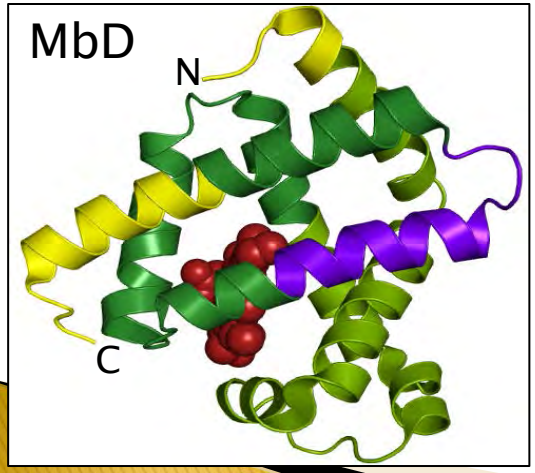


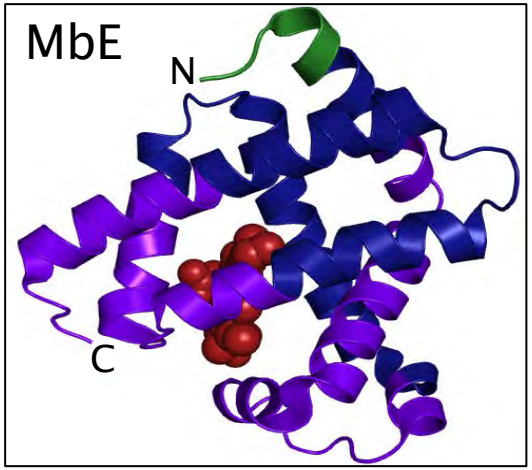
HDX Results Mapped on to the Mb Structure (T=240h)



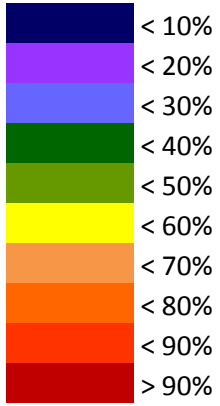






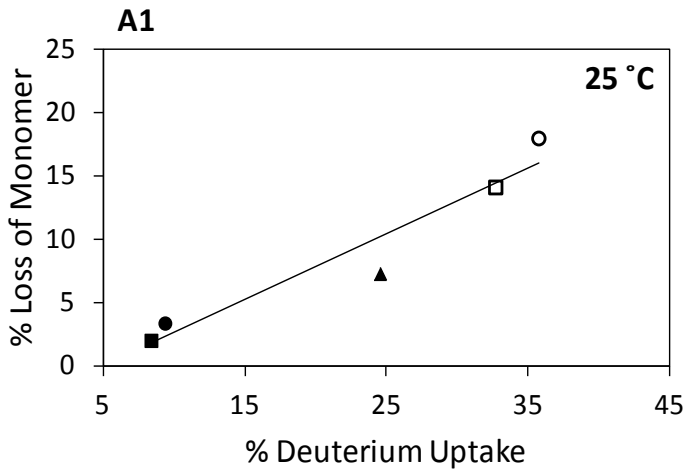


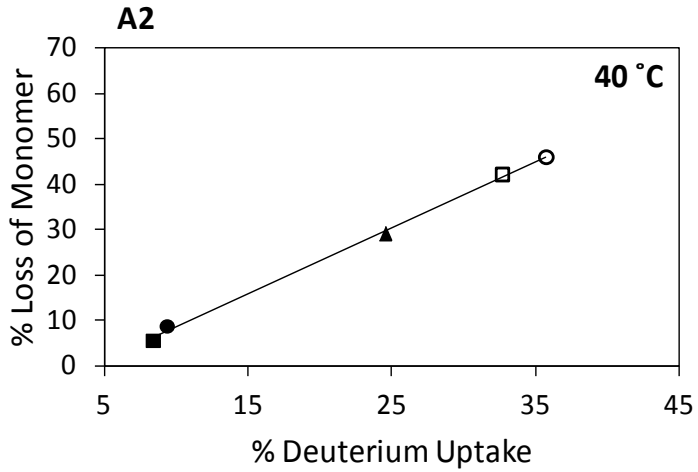
Deuterium Level

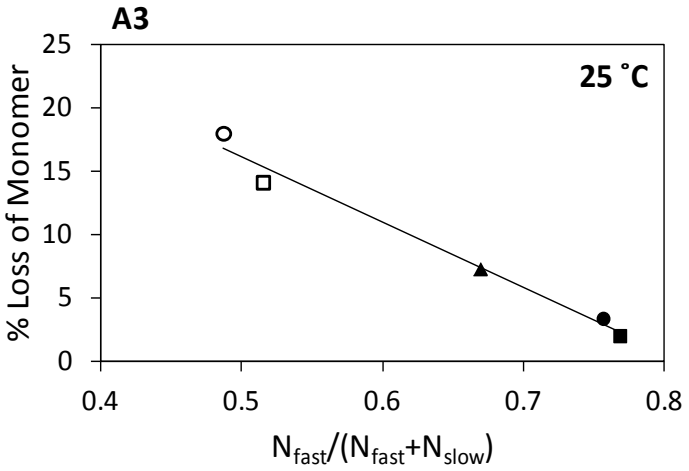


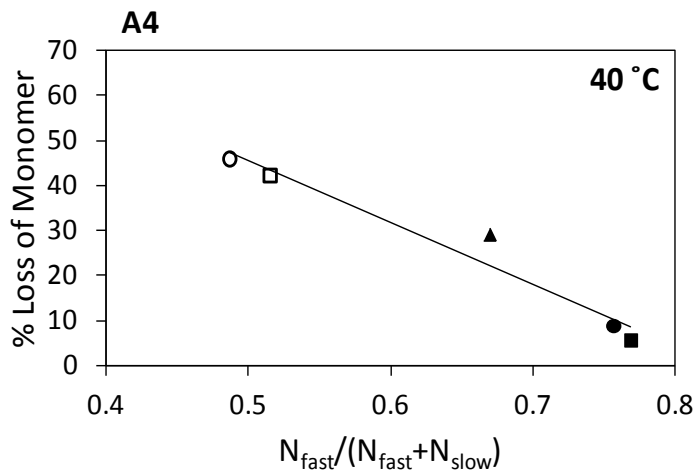
ssHDX and storage stability

Storage for 360 days, 25 and 40 °C; stability by SEC; 48 h ssHDX



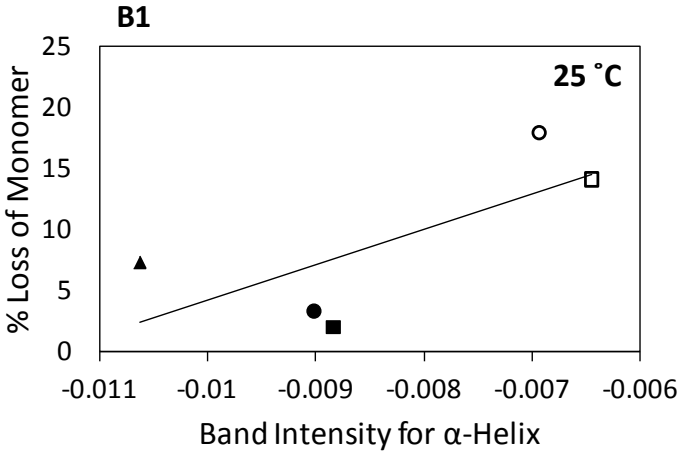


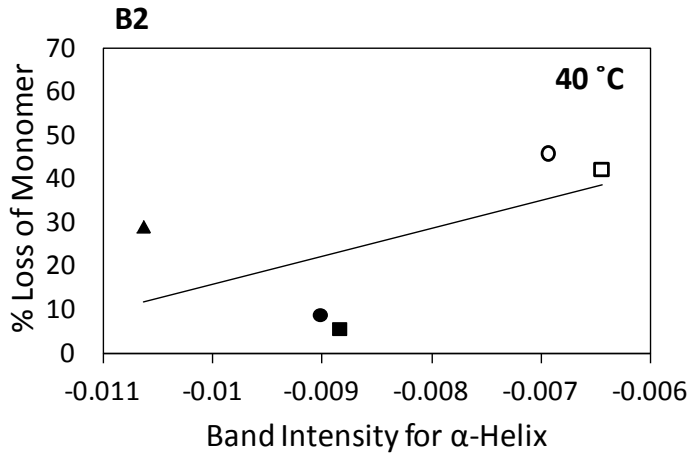


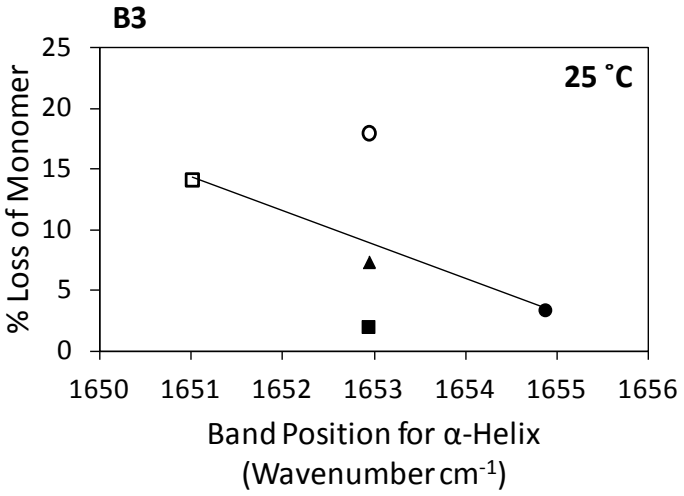


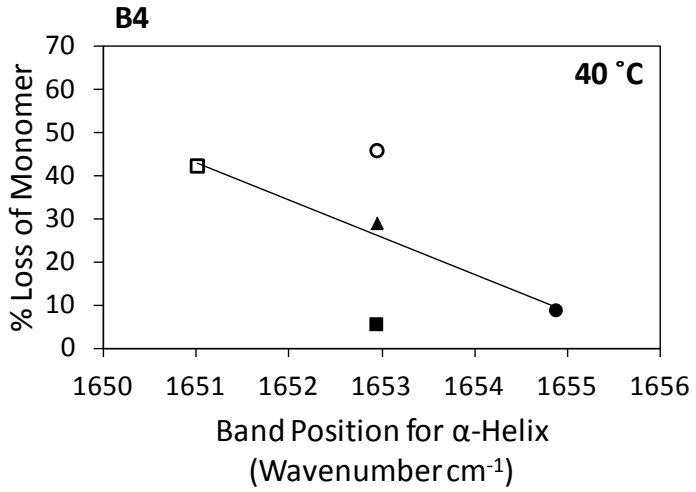
FTIR and storage stability

Storage for 360 days, 25 and 40 °C; stability by SEC









Summary

- ssHDX can be performed in powders using $D_2O(g)$ as a deuterium source.
- ssHDX rates are much slower than vapor sorption.
- Rate and extent of ssHDX are affected by RH, excipient type and amount, temperature.
- For Mb in lyo powders, ssHDX parameters are highly correlated with extent of aggregation on 1-yr storage.

Solid-state photolytic labeling (ssPLL)

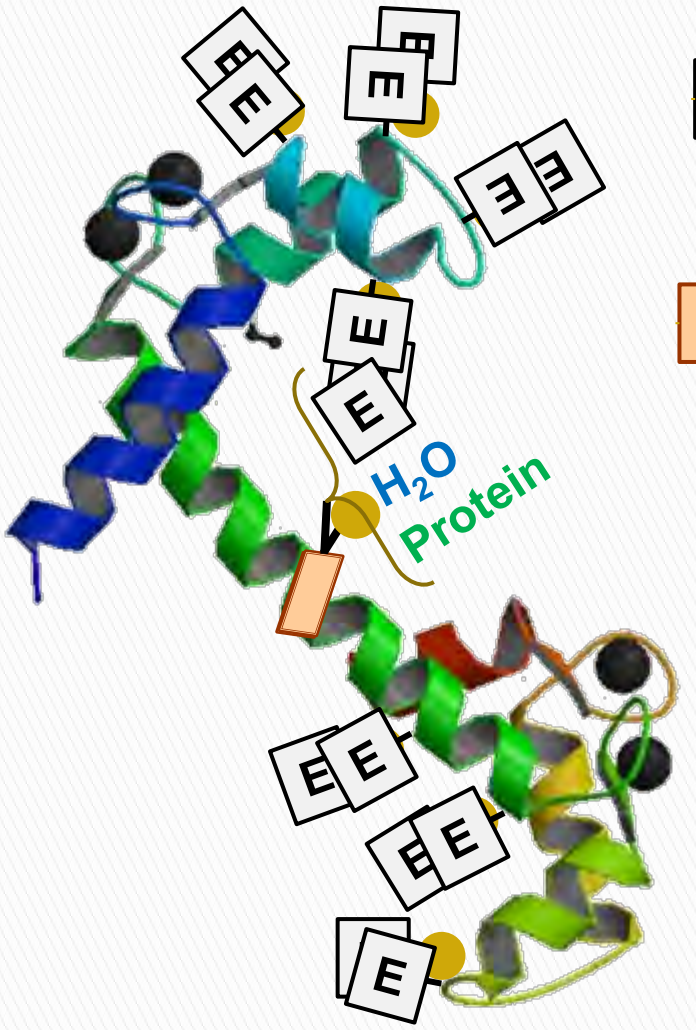
- Mapping protein-protein interactions and the side-chain environment of proteins in amorphous solids

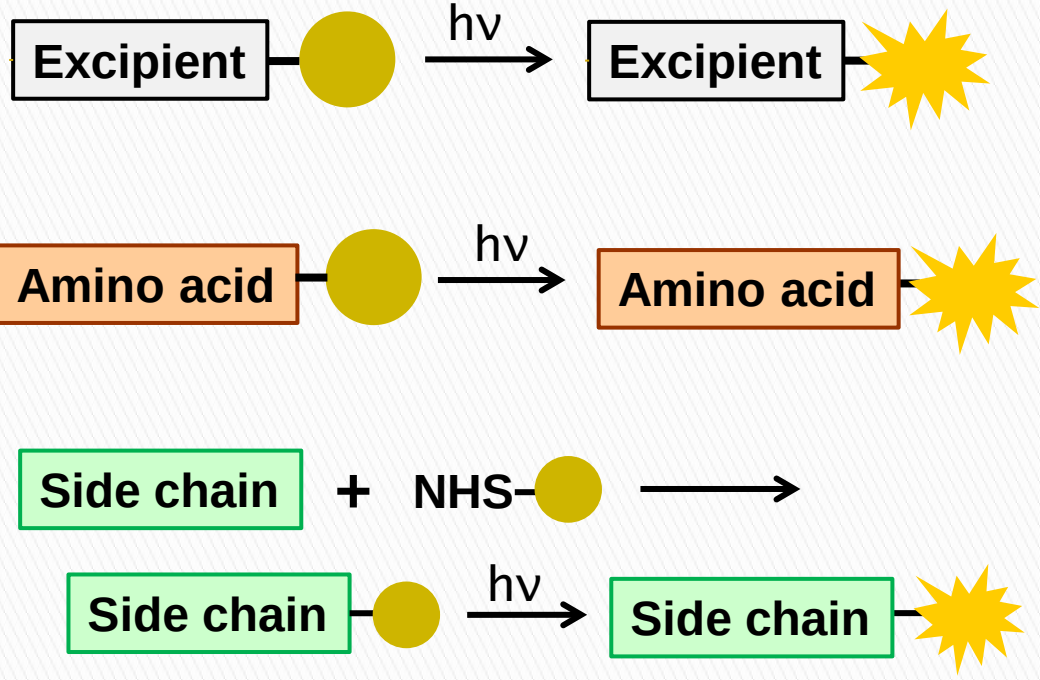
L. Iyer et al., *Mol. Pharm.*, 10: 4629-4639, 2013
B.S. Moorthy et al., *JoVE*, (98), e52503, 2015
L. Iyer et al., *Mol. Pharm.*, 12/9: 3237-3249, 2015

Covalent labeling with MS analysis

- Developed as an alternative and complement to HDX.
- Eliminates back exchange.
- Used to study protein-protein interactions (PPI) in cells.
- A variety of chemical crosslinking strategies, with triggers initiating reaction.
- Here, we evaluate photo-activatable crosslinkers in amorphous lyophilized solids.

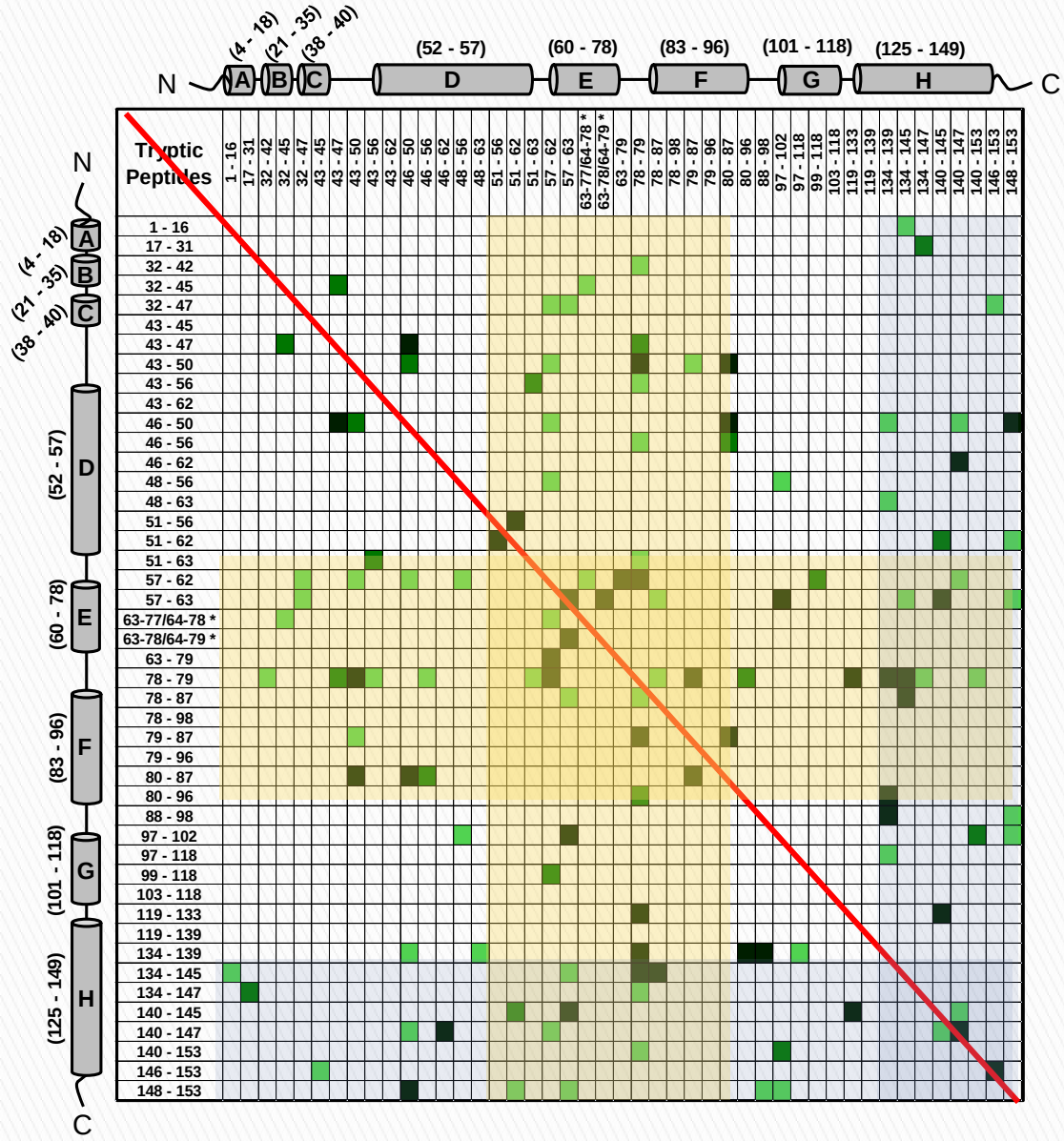
Photolytic labeling





Protein–protein interactions

Lyo Mb, no excipient



- Map shows peptide-peptide adducts detected in 1, 2 or 3 injections
- Symmetric about the diagonal
- Many interactions involving the E and F helices and C-terminus detected

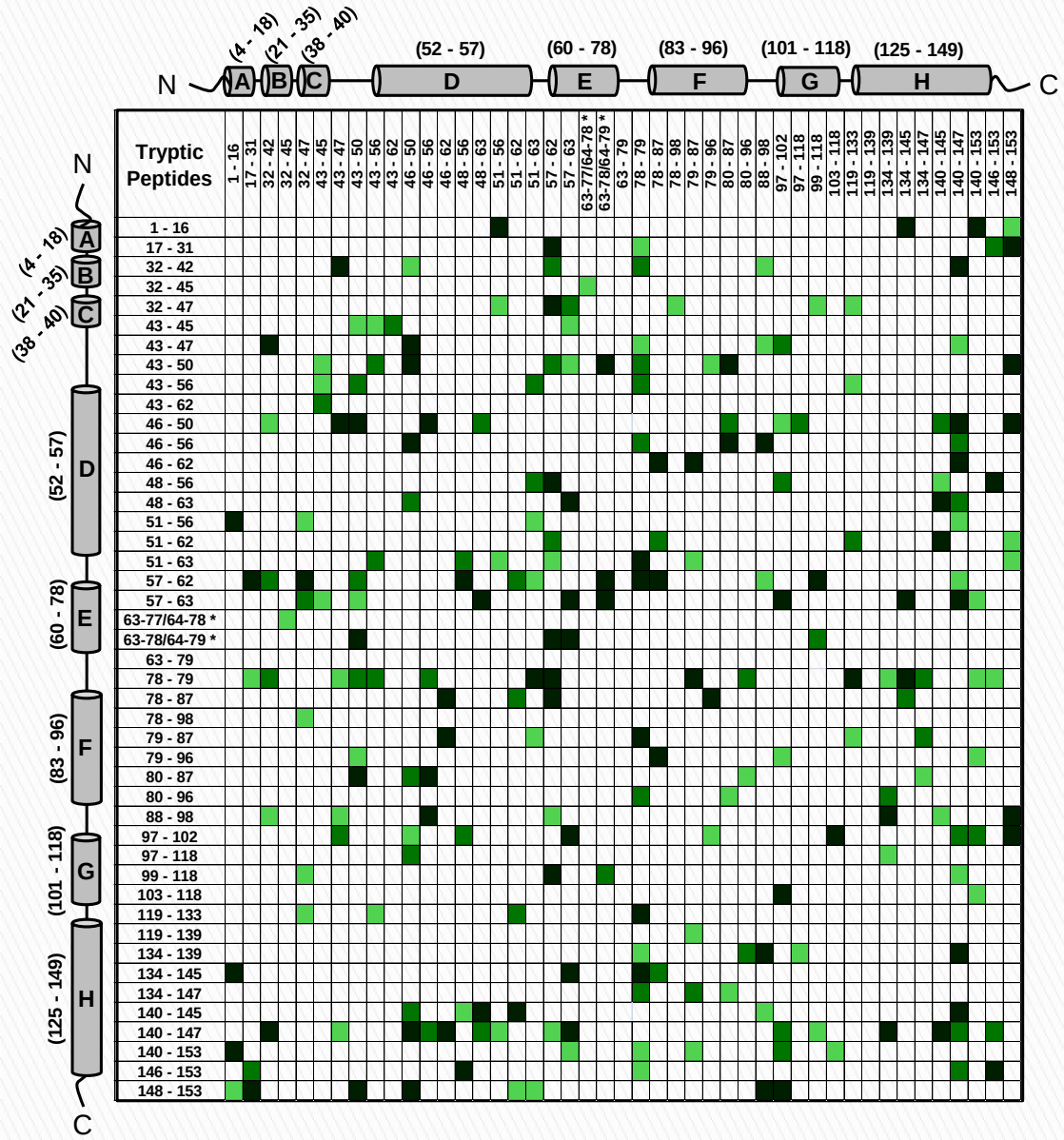
Detected in 1/3 injections

Detected in 2/3 injections

Detected in 3/3 injections

Protein–protein interactions

Lyo Mb, + Gdn HCl



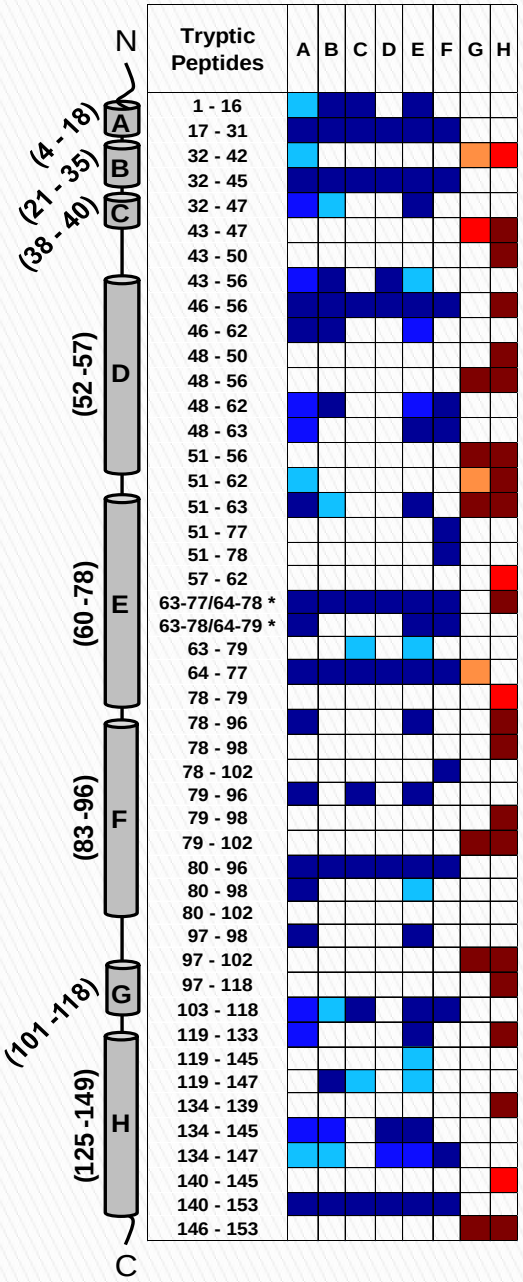
- Many more interactions detected.

Detected in 1/3 injections

Detected in 2/3 injections

Detected in 3/3 injections

Water and excipient interactions



KEY

Peptide/water adducts

A – Mb alone, lyo

B – Mb alone, soln

C – Mb + raffinose, lyo

D – Mb + raffinose, soln

E – Mb + Gdn HCl, lyo

F – Mb + Gdn HCl, soln

Peptide/raffinose adducts

G – Mb + raffinose, lyo

H – Mb + raffinose, soln

Detected in 1/3 injections

Detected in 2/3 injections

Detected in 3/3 injections

Summary

- Photolytic labeling can be performed in solid powders using diazirine chemistry.
- SDA-labeled Mb forms adducts with protein, water and excipient (raffinose) in lyo solids.
- Formulation affects the adducts formed.
- Method allows water replacement hypothesis to be tested.

Acknowledgements

Purdue University (current)

- Dr. Balakrishnan S. Moorthy
- Lavanya Iyer
- Saradha Chandrasekhar
- Jainik Panchal
- Hamed Ghomi (with M. Lill)
- Ehab Moussa
- Anshul Mishra
- Yuan Chen
- Iris Cho
- Reham Nour
- Geoffrey Federspiel
- Dr. Fred Regnier
- Dr. Markus Lill
- Dr. Chiwook Park

Purdue University (past)

- Dr. Andreas Sophocleous
- Dr. Jun Zhang
- Dr. Bo Xie
- Esben Bertelsen
- Jun Xu
- Daniel Epling
- Serene Macaraig
- Jon Oh

University of Kansas

- Dr. Yunsong (Frank) Li
- Dr. Sandipan Sinha
- Dr. Lei Zhang
- Mette Thing
- Steele Reynolds
- Brock Roughton
- Dr. Kyle Camarda
- Dr. Jennifer Laurence
- Dr. David Weis
- Dr. Todd Williams, Director, KU-MSL
- KU Mass Spectrometry Service Laboratory

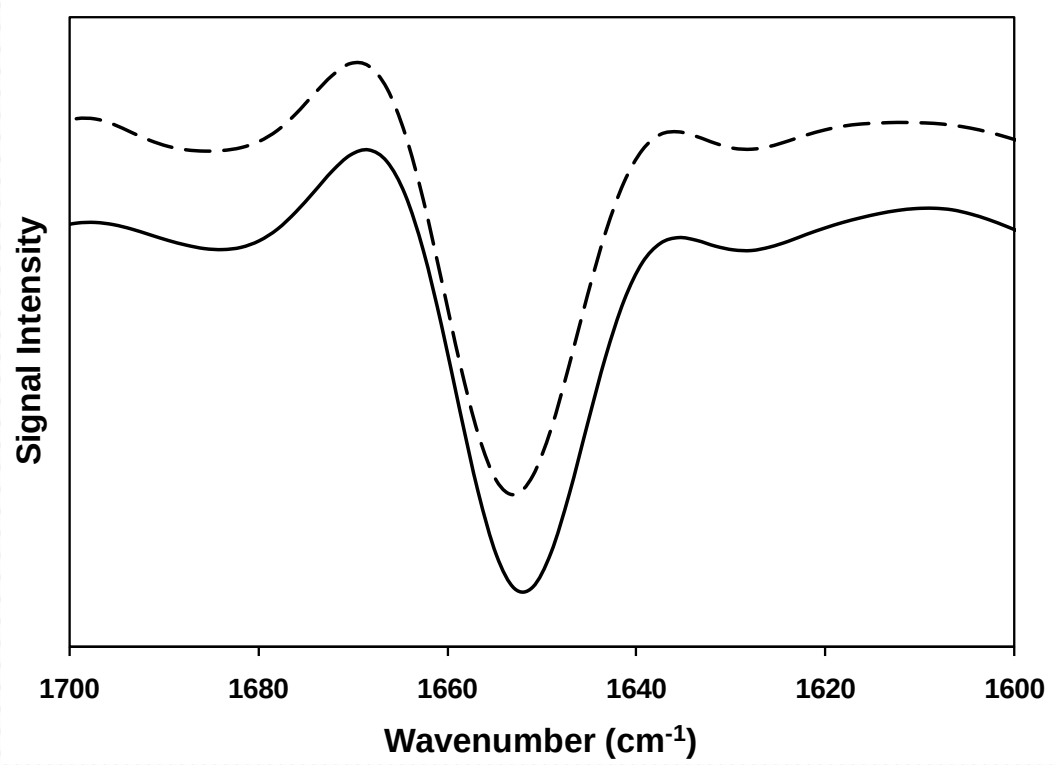
Other

- Dr. Patrick Connelly, Vertex Pharmaceuticals

Financial support: NIH RO1 R01GM085293, PhRMA Foundation Fellowship (AS), FDA HHSF223201310223C, AbbVie Inc., NIPTE Critical Path Manufacturing Sector Initiative U01FD004275, Purdue University, Baxter, Inc., Pfizer, Inc., MedImmune, Inc., Roche/Genentech, Inc., NIST AMTech, Center for Pharmaceutical Processing Research (CPPR)

Supplemental Slides

No detectable secondary structural differences observed post-lyophilization by FTIR

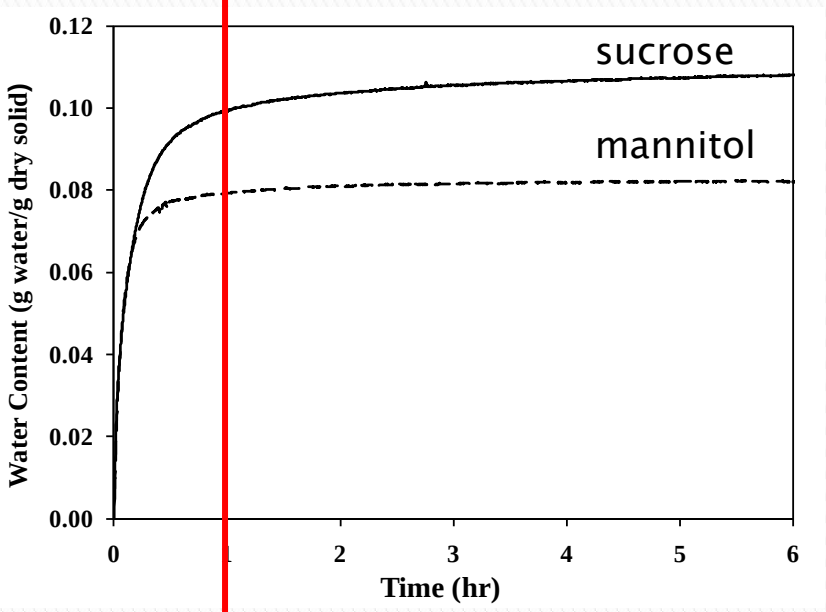


native-like α-helix peak at 1655 cm^{-1}

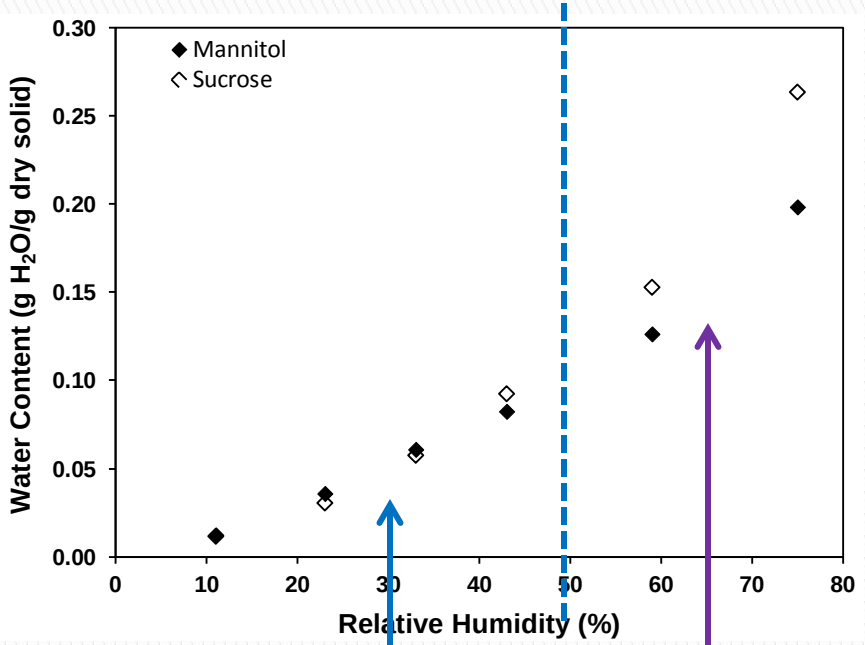
Second-derivative FTIR spectra of Mb in lyophilized solids containing mannitol (solid line) or sucrose (dashed line).

Water sorption and water content

Myoglobin / mannitol or sucrose 1:1, 5 °C



Water vapor sorption is essentially complete in ~ 1 h.



At low RH, water content of mannitol and sucrose formulations are equal.

At high RH, water content in sucrose formulation is greater than in mannitol.