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)

H/D Exchange

Dynamic regions exchange rapidly

Structured regions exchange slowly

Quench and Digest

Quenching locks in deuterium and unfolds the protein

Digestion localizes the information

MS analysis

Quench pH 2.5 0 °C

http://mvsc.ku.edu/content/hydrogen-deuterium-exchange-mass-spectrometry
Solid-state HDX (ssHDX-MS)

Protein lyophilized with excipients

Solid-State HDX

Reconstitute in quench buffer (5°C, pH 2.5)

Pepsin digestion

Protein structure in amorphous solids?

ESI-MS

Intensity

m/z
Effect of RH on deuterium uptake

Intact protein: *Myoglobin/mannitol 1:1, 5 °C*
Effect of RH on deuterium uptake

Intact protein: Myoglobin / sucrose 1:1, 5 °C
mAb formulations
Can ssHDX be applied to mAbs?

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Basic composition</th>
<th>Mannitol (mg/mL)</th>
<th>Sucrose (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>-</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td>F2</td>
<td>50 mg/mL mAb1 in buffer at pH 6.0</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>F3</td>
<td>32</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>53</td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

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

\[
y = 20323x + 176.55 \\
R^2 = 0.8576
\]

(B) Sucrose content

\[
y = -0.2723x + 26.606 \\
R^2 = 0.7893
\]

(C) Moisture content

\[
y = 25.108x - 2.776 \\
R^2 = 0.6021
\]

(D) Glass transition, Tg

\[
y = -0.6161x + 63.03 \\
R^2 = 0.2099
\]
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

https://www.scbt.com/scbt/product/poly-dl-alanine-25281-63-4
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
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 D₂O, 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.