CONSEQUENCES OF SAMPLE AGE ON BIOOTHERAPEUTIC HIGHER ORDER STRUCTURE: INSIGHTS FROM NATIVE ION MOBILITY-MASS SPECTROMETRY METHODS

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Identifying the Consequences of Biotherapeutic Age on Higher Order Structure

• Monoclonal Antibodies (mAb’s) are inherently complex.
• Raises concerns regarding the effect of lot-to-lot variations on their long term thermal stability.
Identifying the Consequences of Biotherapeutic Age on Higher Order Structure

- Monoclonal Antibodies (mAb’s) are inherently complex.
- Raises concerns regarding the effect of lot-to-lot variations on their long term thermal stability.
- ICH Q1A, 2.1.7: Assessment of product thermal stability, to aid determination of shelf-life.
- Conditions used to evaluate product shelf life (ICH Q1A), and patterns of degradation (ICH Q5C).
- ICH Accelerated aging conditions:
  - 25±2°C, 60±5% Relative Humidity.
  - 6 month minimum incubation.
- Controls: 0 and 6 months at 4°C.
Accelerated Aging: Sample Selection

• Three commercially available IgG1 mAbs:
  – mAb 1a, 10 mg/ml, Exp: Aug 2017.
  – mAb 1b, 10 mg/ml, Exp: Jun 2014.
  – NIST RM8671, 10 mg/ml, Exp: Apr 2021.

• **Question:** how do we plan on analyzing these samples to identify their age dependent products of degradation?

• **Answer:** Native Ion Mobility-Mass Spectrometry (IM-MS) methods.
Native Ion Mobility-Mass Spectrometry

- Mass analysis of structurally separated native-like, gas phase, biological structures and complexes.
- Native-like buffers preserve the solution-phase structures, and covalent interactions.
- Energy minimized instrumental parameters prevent gas phase unfolding, and dissociation.
Ion-Mobility Spectrometry

- Gas phase electrophoresis method.
Ion-Mobility Spectrometry

- Gas phase electrophoresis method.
- Drift time can be used to calculate collision cross sections (CCS), providing an accurate estimate of an ions rotationally averaged size.
- Allows conformational, and oligomeric families with overlapping $m/z$ to be structurally separated.
Native Ion Mobility-Mass Spectrometry
Native Ion Mobility-Mass Spectrometry

- Three dimensions of data: mass, size and intensity.
- Native IM-MS permits the mass analysis of structurally separated biological structures and complexes.
Native IM-MS Analysis: mAbs

- Classical MS: native structure and non-covalent interactions lost, with broad charge state distribution at low m/z values.
- Native MS: native structure and non-covalent interactions preserved, with narrow charge state distribution at high m/z values.
Native IM-MS Analysis: mAbs

• Classical MS: native structure and non-covalent interactions lost, with broad charge state distribution at low m/z values.

• Native MS: native structure and non-covalent interactions preserved, with narrow charge state distribution at high m/z values.

• Native IM-MS: Extracted drift times (tD) used to calculate biological CCS values.

• **Question**: Can we identify age dependent products of degradation based on shifts in CCS?
• No difference in CCS between controls.
• No observed change in CCS after incubation.
• **Question**: What do these data tell us?
• A) No change in structure occurs after 6 months.
• B) Changes in structure may be too subtle to detect.

**CCS values for +22 charge State Shown.**
• mAbs are relatively big biotherapeutic complexes:
  – ~ 145-180 kDa.
  – ~ 7600 Å².
• Proteins are not rigid structures, they’re flexible.
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  – ~ 145-180 kDa.
  – ~ 7600 Å².
• Proteins are not rigid structures, they’re flexible.
• **Answer**: B) Changes in structure may be too subtle to detect.
• **Question**: How else can we use native IM-MS to identify these subtle differences?
• **Answer**: Test the effect of these modifications on mAb structural stability.
Collision Induced Unfolding (CIU)

- Gas phase unfolding strategy to assess the unfolding pathway of an analyte by collisionally activating its precursor ions prior to IM-MS.
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- Gas phase unfolding strategy to assess the unfolding pathway of an analyte by collisionally activating its precursor ions prior to IM-MS.
- Analyte ions subjected to sequentially higher collisional activation.
- Spectra combined to produce a heatmap known as a CIU fingerprint.
Collision Induced Unfolding (CIU)

\[ SDS_i = \sum_{j=0}^{j=m} \frac{(X_{ij} - A_{ij}) \cdot A_{ij}}{S_{ij}} \]

\( X = \) Experimental data  
\( A = \) Reference data  
\( S = \) standard deviation  
\( i = \) given collision voltage (\( \Delta E \))  
\( j = \) given drift time  
\( m = \) sum of all drift times
Monoclonal Antibody CIU: Controls

- **mAb 1a** (Exp: Aug ‘17)
  - RMSD: 3.67%

- **mAb 1b** (Exp: Jun ‘14)
  - RMSD: 3.82%

- **mAb 2** (Exp: May ‘17)
  - RMSD: 4.57%

- **NIST** (Exp: Apr ‘21)
  - RMSD: 3.66%
6 Months: 25±2°C, 60±5% RH

**mAb 1a**  
(Exp: Aug '17)  
RMSD: 5.48%

**mAb 1b**  
(Exp: Jun '14)  
RMSD: 4.76%

**mAb 2**  
(Exp: May '17)  
RMSD: 5.05%

**NIST**  
(Exp: Apr '21)  
RMSD: 4.54%

Control (0 Months)
6 Months: 25±2°C, 60±5% RH

mAb 1a  
(Exp: Aug ‘17)

mAb 1b  
(Exp: Jun ‘14)

mAb 2  
(Exp: May ‘17)

NIST  
(Exp: Apr ‘21)

Scaled Deviation Score

Drift Time (ms)

Collision Voltage (V)

RMSD: 7.20%

RMSD: 3.06%

RMSD: 10.17%

RMSD: 6.34%

6 Months at 25°C/60% RH  
Control (0 Months)
6 Months: 25±2°C, 60±5% RH

mAb 1a
(Exp: Aug ‘17)

mAb 1b
(Exp: Jun ‘14)

mAb 2
(Exp: May ‘17)

NIST
(Exp: Apr ‘21)
mAb 2: 25±2°C, 60±5% RH
6 Months: 25±2°C, 60±5% RH

mAb 1a
(Exp: Aug ‘17)

mAb 1b
(Exp: Jun ‘14)

mAb 2
(Exp: May ‘17)

NIST
(Exp: Apr ‘21)
Question: While ‘x’ appears indicative of sample aging, how do we account for the observed differences between mAbs?

<table>
<thead>
<tr>
<th>mAb 1a</th>
<th>mAb 1b</th>
<th>mAb 2</th>
<th>NIST</th>
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<tbody>
<tr>
<td><img src="mAb_1a.png" alt="Image" /></td>
<td><img src="mAb_1b.png" alt="Image" /></td>
<td><img src="mAb_2.png" alt="Image" /></td>
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<table>
<thead>
<tr>
<th>Expiration Date</th>
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<tr>
<td>Exp: Aug 2017</td>
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<td>Exp: Jun 2014</td>
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<td>Exp: Apr 2021</td>
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<tr>
<th>Formulation: Concentration</th>
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<tr>
<td>10 mg/ml</td>
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<tr>
<td>10 mg/ml</td>
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<tr>
<td>25 mg/ml</td>
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<td>10 mg/ml</td>
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<tr>
<th>Formulation: Excipients</th>
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<td>0.7 mg/ml Polysorbate 80</td>
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<tr>
<td>0.7 mg/ml Polysorbate 80</td>
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<tr>
<td>0.4 mg/ml Polysorbate 20</td>
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<tr>
<td>No Polysorbate</td>
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Question: While ‘x’ appears indicative of sample aging, how do we account for the observed differences between mAbs?

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<thead>
<tr>
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<th>Heavy Chain</th>
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<th>Light Chain</th>
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<tr>
<td></td>
<td>mAb 1</td>
<td>mAb 2</td>
<td>NIST</td>
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<tr>
<td>mAb 1</td>
<td>x</td>
<td>87%</td>
<td>81%</td>
</tr>
<tr>
<td>mAb 2</td>
<td>87%</td>
<td>x</td>
<td>84%</td>
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<td>x</td>
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• **Question**: Can we correlate these age dependent stability differences with further changes in higher order structure?

• Hydrogen-Deuterium Exchange IM-MS (HDX-IM-MS):
  – Non-covalent labelling approach that identifies differences in structure based on mass shifts associated with solvent accessible labile Hydrogen-Deuterium exchange events.
HDX: Controls vs 6 Months (25°C)
Most significant changes in Deuterium uptake are clustered near the disulfide bond rich hinge region and C₁/C₅ domains.
Speculated that these differences may be correlated with changes in CIU.
So what do these data teach us?

• IM-MS CCS: high degree of structural freedom exhibited by larger biotherapeutics may mask subtle CCS changes.

• IM-MS CIU: consequences of product aging identified as a function of their effect on structural stability.

• HDX-IM-MS: most pronounced changes in mAb structure are clustered within the hinge and C₁/C₂ domains.

• These complementary data reveal the impact of sample age on the higher order structure and stability of a chosen biotherapeutic, which may impact its safety and/or efficacy.

• Several sample variables are likely contribute to the observed differences between the mAb products studied, including:
  – Primary amino acid sequence.
  – Formulation: concentration and/or excipients.
Future Directions

• Assess the application of other mass spectrometric methods to identify further changes in mAb stability and higher order structure, consistent with the observed differences:

  – Disulfide bond mapping: assess the impact of sample age on bond scrambling/rearrangement events.

  – Size Exclusion Chromatography (SEC): assess the impact of sample age on the distribution of all measurable low order oligomers.

• Identify sample and formulation dependent variables that contribute to the observed differences in stability and higher order structure when comparing biotherapeutic products.
Acknowledgements

FDA Division of Pharmaceutical Analysis:
  • Hongping Ye
  • David Keire

Brandon Ruotolo Research Group
(University of Michigan)

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