

# Integrating HOS Techniques in CQA Assessment

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Biologics Development, Bristol-Myers Squibb

April 5, 2017  
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Gaithersburg Marriott Washingtonian Center, Gaithersburg, Maryland 20878 USA

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# Not all quality attributes are critical

A critical quality attribute: "a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality" - ICH Q8 (R2) ANNEX

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## Abbreviations

- CQA → Critical Quality Attribute
- HOS → Higher Order Structure (2°, 3°, 4° etc)
- PRS → Product Related Substance
- PRI → Product Related Impurity
- Control strategy → Control by Analytical, Process, Formulation

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**Non-CQAs often require robust control**

**e.g. Process consistency**

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**Topics Discussed Today**

- **CQA Assessment**
- Major Quality Attributes: Assessment of Criticality
- Experimental Plan
- Integrating HOS Tools in CQA Assessment
- Case Study – Need for advanced HOS
- Conclusions

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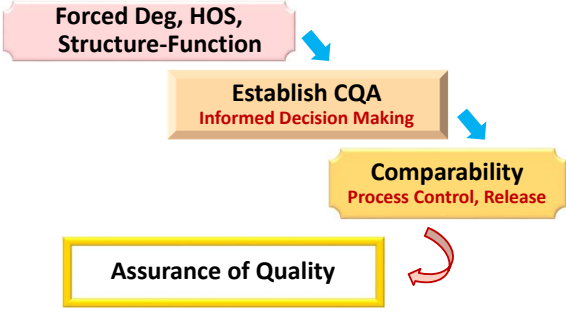
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A good CQA Assessment delivers 'built-in' Quality



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graph TD; A[Forced Deg, HOS, Structure-Function] --> B[Establish CQA  
Informed Decision Making]; B --> C[Comparability  
Process Control, Release]; C --> D[Assurance of Quality];
```

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**Criticality assessment needs a focused experimental plan**

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**2 Key Points**

- **RELEVANT stress studies for criticality assessment**
- **Understanding impact of one event (e.g. oxidation) at a time - on function**

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## Know the Molecular Profile

### Molecule –

- ◆ Physicochemical properties, Isotype for mAbs
- ◆ Structure, Homology model
- ◆ Identified Hot Spots (or predicted hot-spots)
- ◆ Molecule class knowledge

### MoA (Mechanism of Action) –

- ◆ Binding, biological function
- ◆ any Fc-mediated/effector function
- ◆ any known involvement of glycans

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## Quality Attributes & Major Degradation Events

### Protein Property & Degradation

- ◆ Aggregation → %HMW
- ◆ Fragmentation → %LMW
- ◆ Oxidation (Met, Trp) → %Oxd
- ◆ Deamidation → %Deam
- ◆ Isomerization → %Iso
  
- ◆ Glycan → Profile, %critical species
- ◆ Inherent PRS (%)
- ◆ Others

### Solution Properties

- ◆ pH
- ◆ Protein Conc
- ◆ Osmolality
- ◆ others

### Process Impurity

- ◆ Host Cell Protein
- ◆ Residual DNA
- ◆ others

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## Experimental Plan

Quality Attribute	Stress	Analytical Methods	HOS method
Deamidation	High pH	iCIEF, LC, PMAP	CD
Met Oxidation	H2O2, HIL	LC, PMAP	CD
Trp Oxidation	AAPH, HIL	PMAP	CD
Aggregate	Various (Temp, F/T, Agitation, Enrichment etc)	SEC, CE	DLS, AUC
Protein Particle	Various (Temp, F/T, Agitation, Enrichment etc)	Counting, Imaging	Particle characterization, as needed
Asp isomerization	Protein-dependent	LC, PMAP	CD
Fragment	Various (Temp, Low pH, Enrichm)	SEC, CE	MS

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## Acknowledgements

- [BMS Biologics Development](#)
- Hui Wei
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### [Northeastern University](#)

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John Engen

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**Challenge is lack of apriori knowledge which HOS methods to apply**

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- **Large number of samples to run**
- **Selection included:**
  - CD (2° str, 3° str)
  - DLS (large aggs)
  - AUC (as needed)
  - DSC (as needed)
  - Others (HDX-MS, Raman as needed)

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**And ultimately biological function assays (an indirect proof of HOS integrity)**

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**Impact on HOS**

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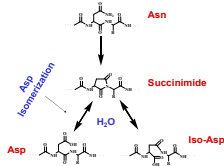
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Low impact on HOS High impact on HOS

Deamidation  
Isomerization  
Succinimide



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Low impact on HOS High impact on HOS

Terminal variants  
De-Glycosylation

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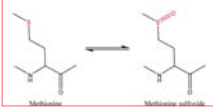
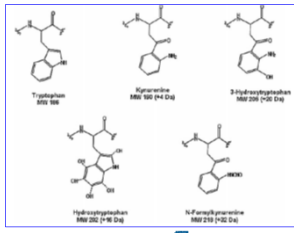
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Low impact on HOS High impact on HOS

**Met Oxidation  
Trp Oxidation**

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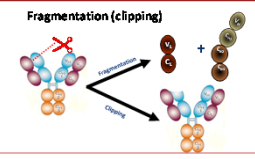

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Low impact on HOS High impact on HOS

Fragmentation Aggregation

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## A complex case of structure-function

Complex Impact of Degradation on Potency



Need to apply advanced HOS techniques

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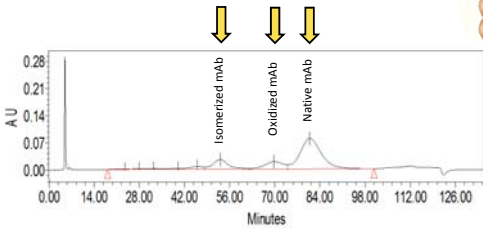
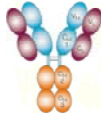
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## mAb2 Isomerization & Oxidation in CDR2

- Complex impact on bioactivity (MetOx & IsoAsp)
- Aged (3+ yrs) samples were used for enrichment



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## Impact on Antigen Binding

Samples	%Relative Binding (to native sample)
Native mAb	100
Iso-mAb	13
Ox-mAb	118

Yan et al, Analytical Chemistry, 2016, Feb 16;88(4):2041-50

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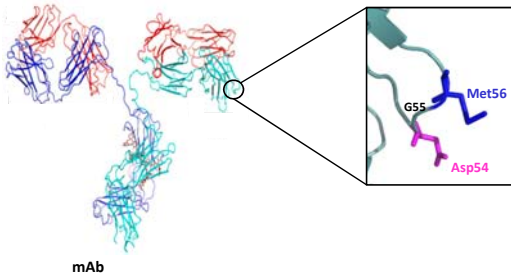
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## Conformational Connectivity of Asp & Met



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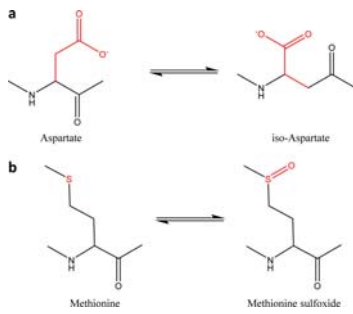
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## Asp Isomerization and Met Oxidation



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## Local Structure

by HDX-MS

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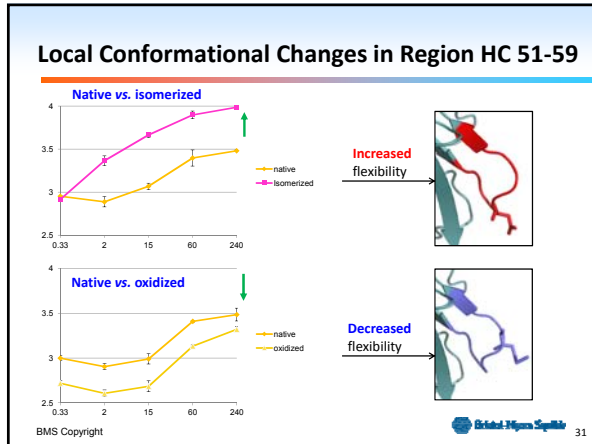
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## Global Structure by HDX-MS

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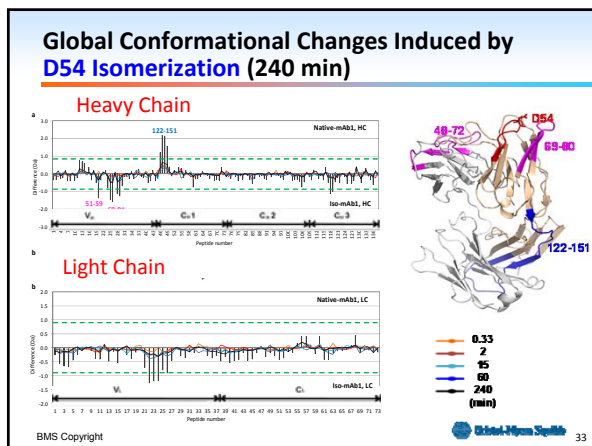
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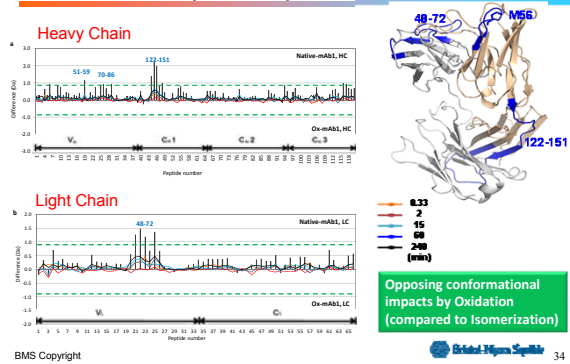
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## Global Conformational Changes Induced by M56 Oxidation (240 min)




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## Impact on Antigen Binding attributed to Increased Disorder in CDR by isoAsp

Samples	%Relative Binding (to native sample)
Native mAb	100
Iso-mAb	13
Ox-mAb	118

- Is Asp isomerization a CQA?
- Establish control

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### Integrating HOS in CQA Assessment

- ❑ Selection of HOS not straightforward
- ❑ Impact on bioactivity provides direct hint
- ❑ Complex structure-function may require advanced HOS tools such as HDX-MS

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THANK YOU

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