Forced degradation and MAM workflows in Lead Optimization/Early Development

Alayna George Thompson, PhD

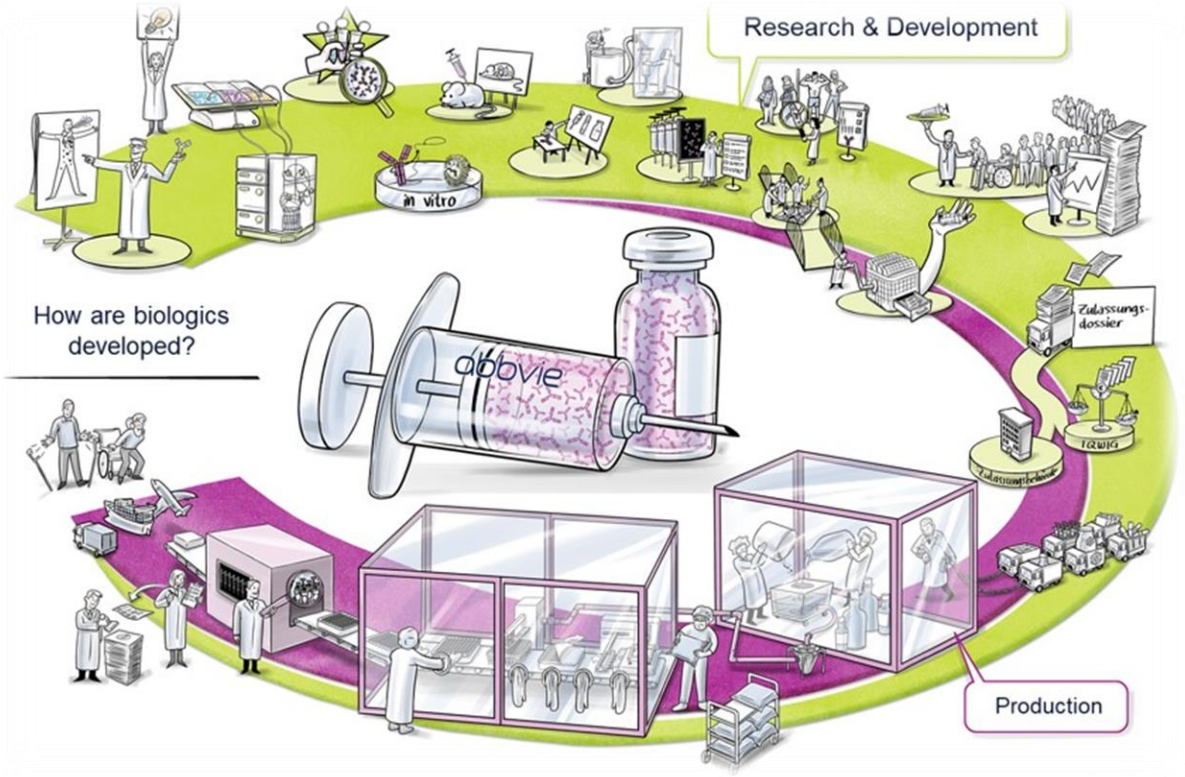
Research and Early Development

ARD, Development Sciences

Sr Research Scientist II

AbbVie, Inc

CASSS MWRF November 4, 2021



abbvie

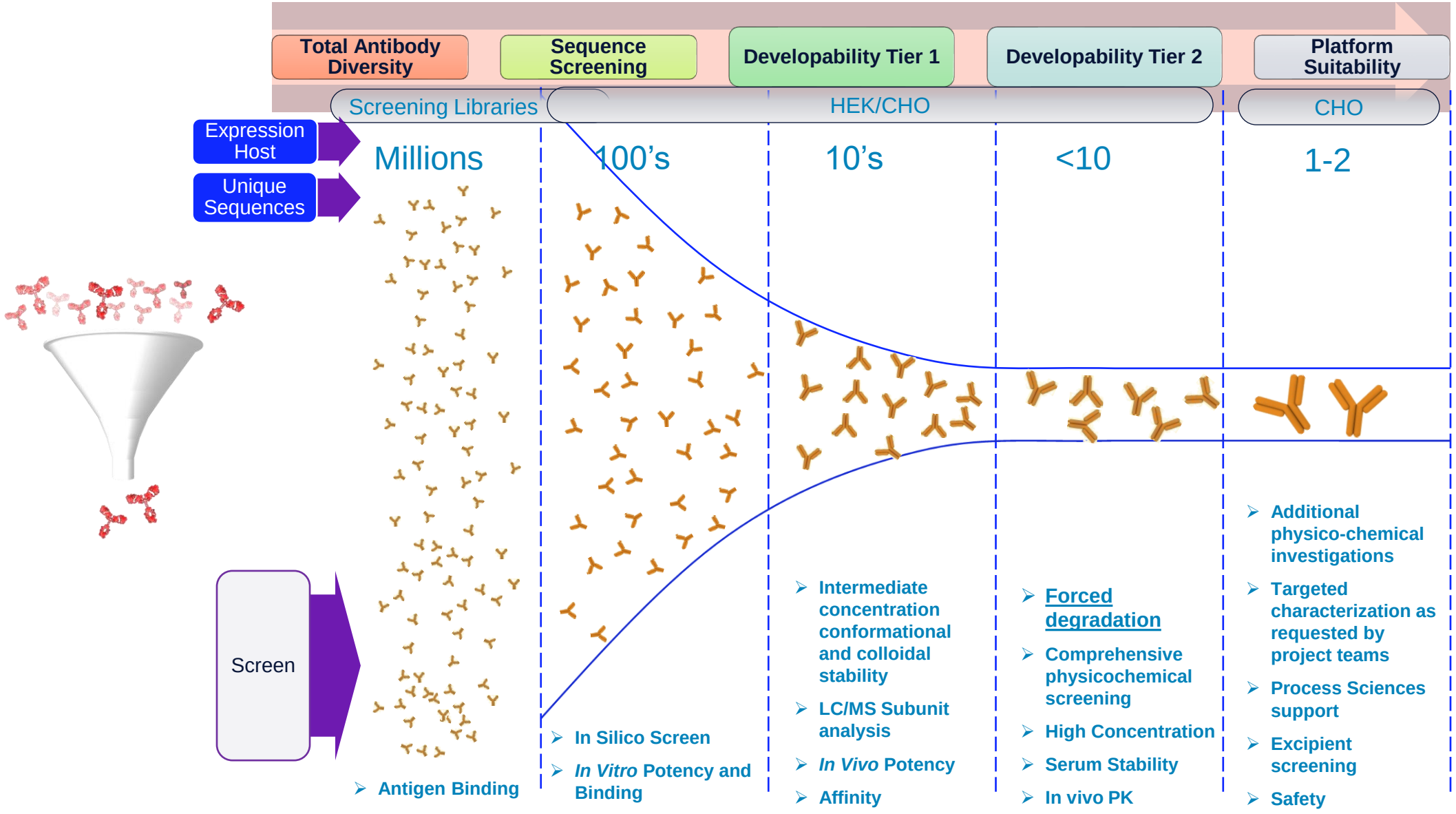
Disclosure

The author, Alayna M. George Thompson is a paid employee of AbbVie Inc.

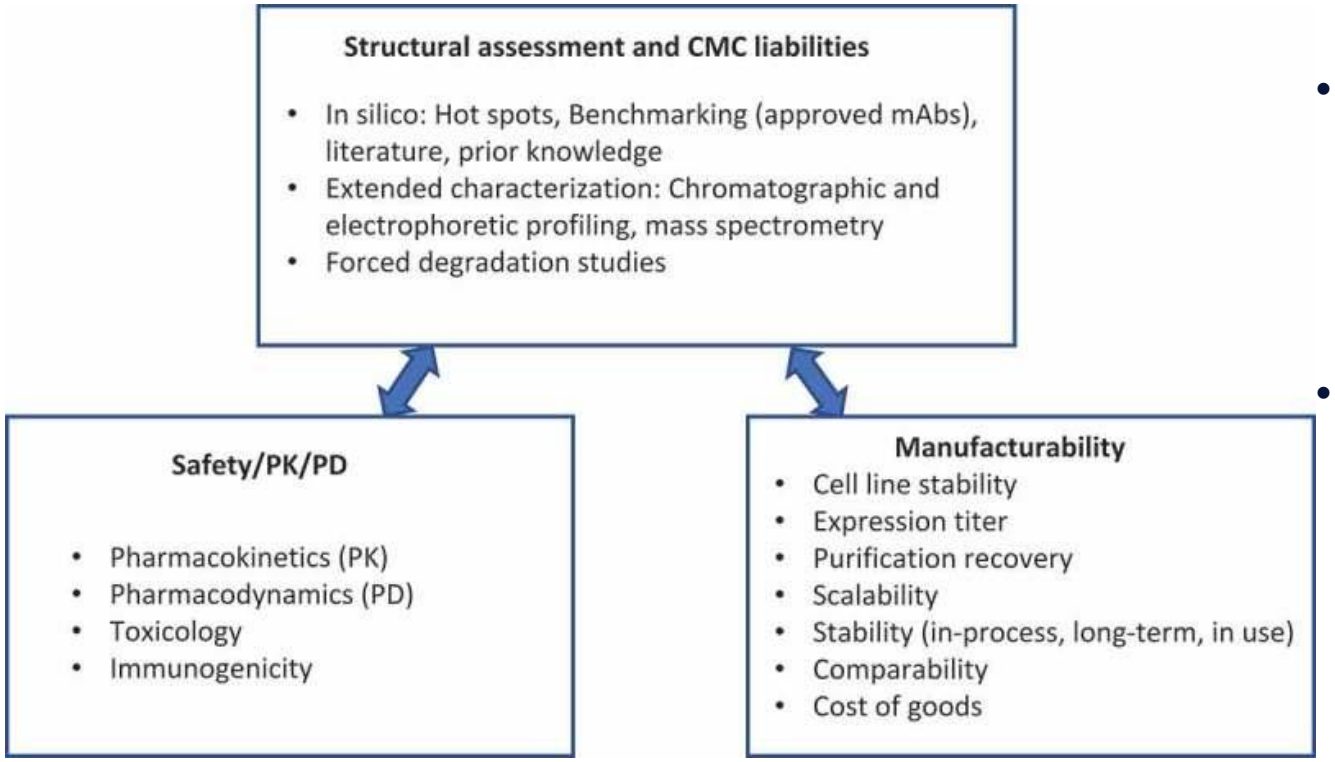
All study design, research, analysis, data collection, and interpretation of data in this presentation, as well as the writing, reviewing, and approval of this presentation was conducted at AbbVie Inc. All materials and equipment used in the study described in this presentation, as well as all funding for the study and the presentation, were provided by AbbVie Inc.

Individuals who have provided medical writing, editorial, or other technical assistance with the study and/or presentation have been acknowledged. These individuals are all paid employees of AbbVie Inc.

Screening Funnel Process for Biologic Candidates: many to few



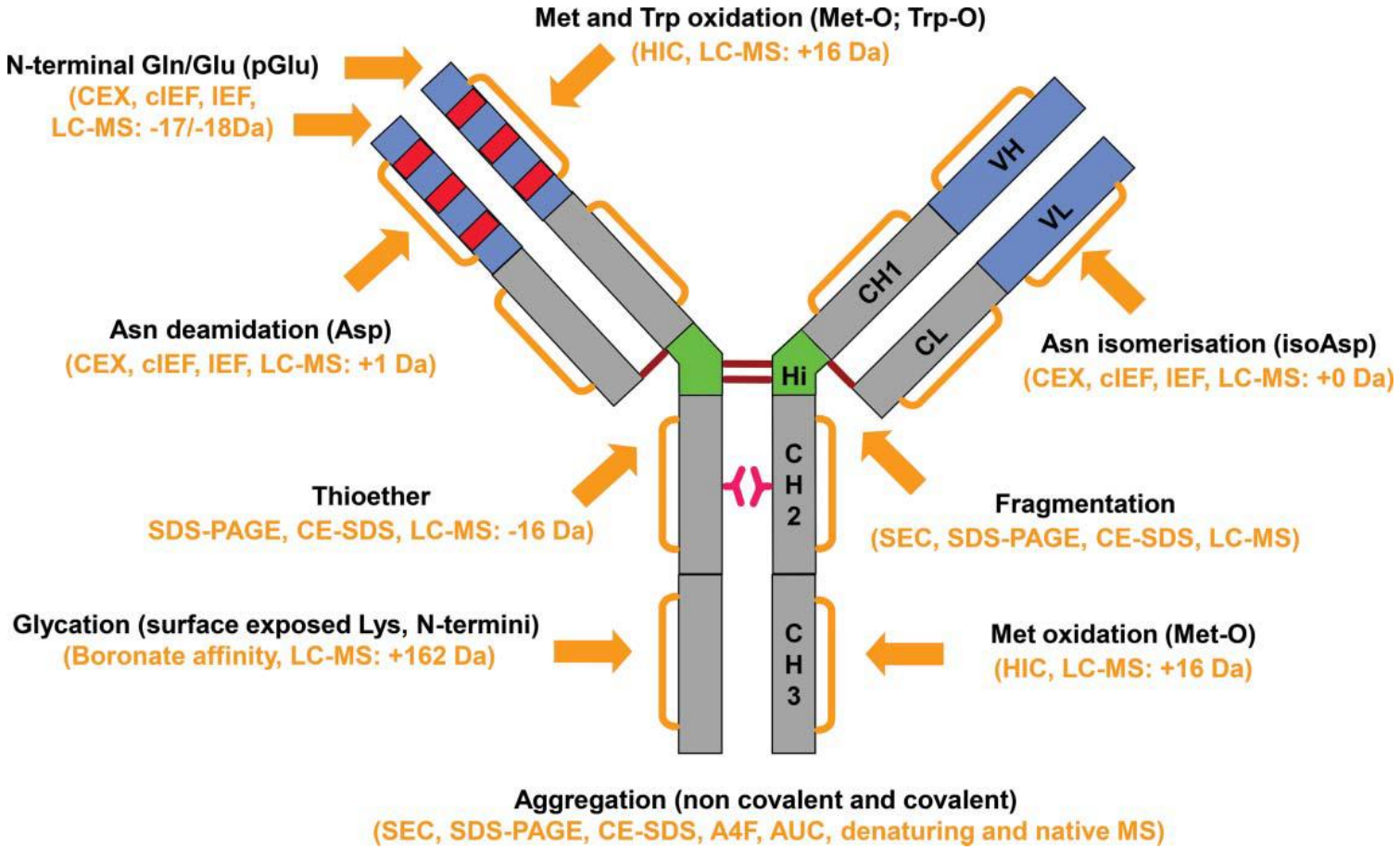
Forced Degradation: What is it?



- In Development: assessment of chemical liabilities early increases likelihood of program success
  - In-depth characterization feasible, expected
- In Discovery: screening data informs parallel and downstream activities
  - In-depth characterization NOT feasible
  - Screening data can assist in refinement of upstream activities (liability engineering/protein design)

Yingda et.al., (2019) Structure, heterogeneity and developability assessment of therapeutic antibodies, mAbs, 11:2, 239-264, DOI: 10.1080/19420862.2018.1553476

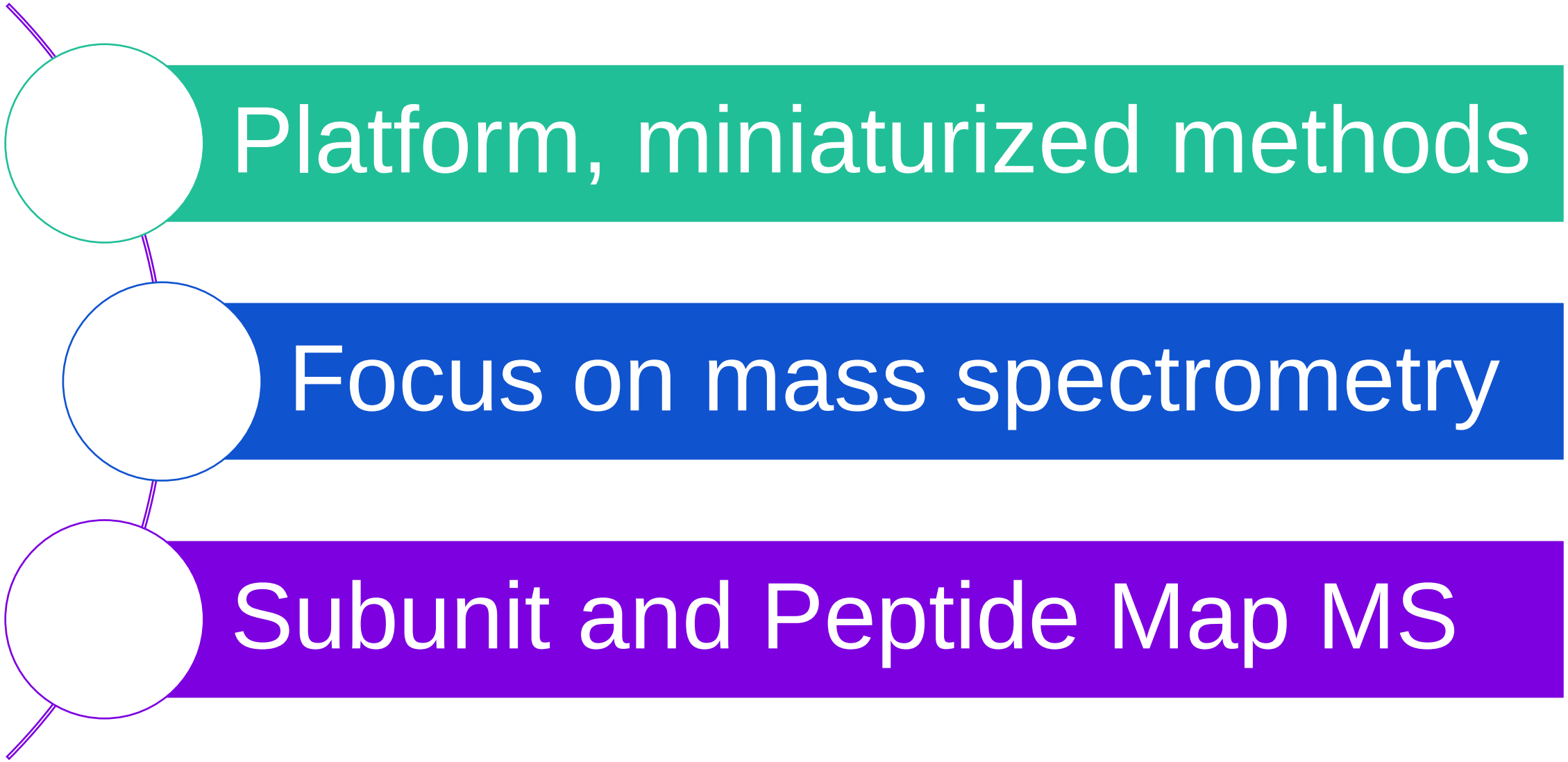
Forced Degradation: What is looked for?



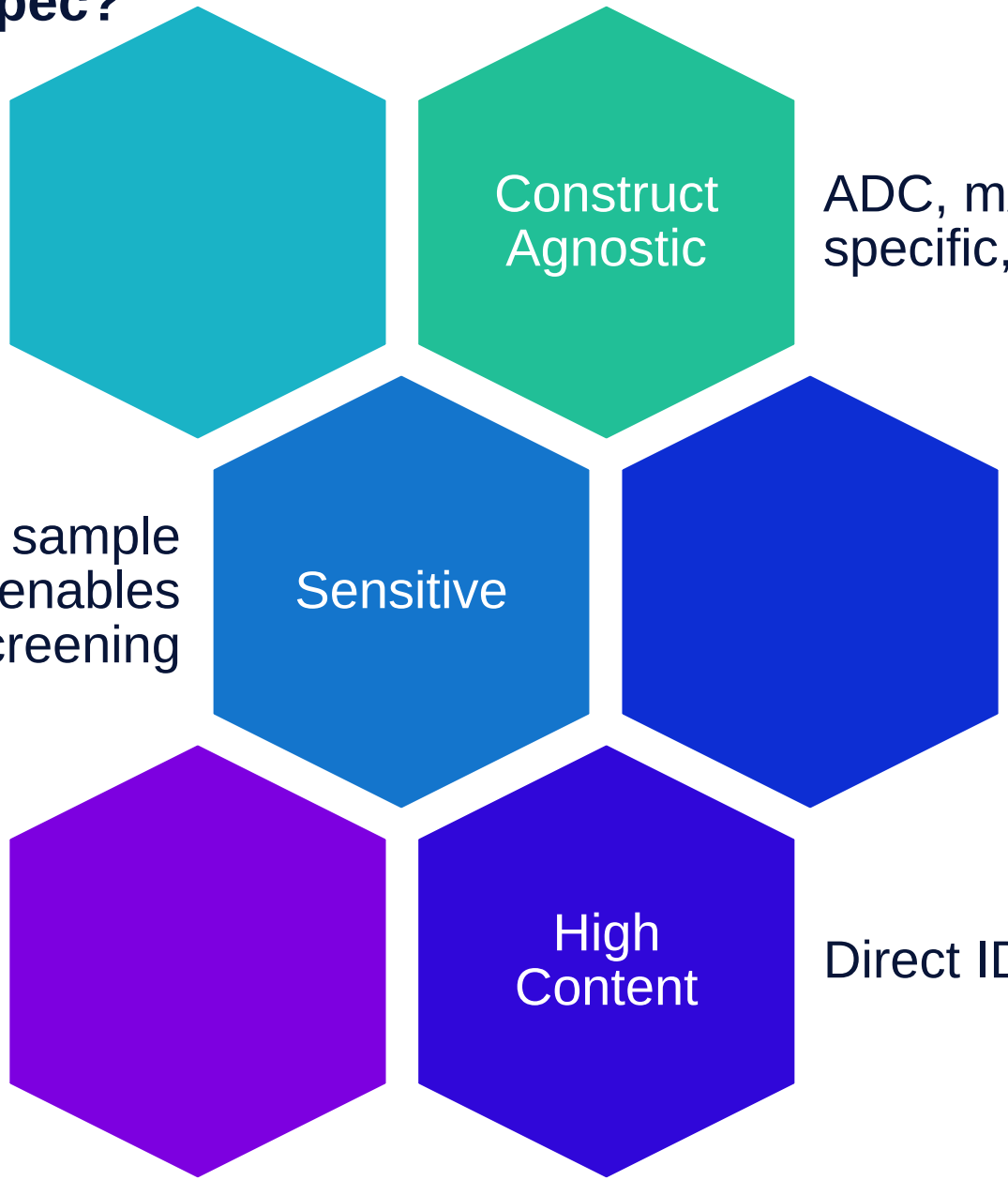
- Many degradations products can occur on antibodies
- Panel of analytical methods is necessary to monitor them all
- In addition to monitoring, functional assessment of degradants is necessary to determine risks to programs and patients

Nowak et.al., (2017) Structure, heterogeneity and developability assessment of therapeutic antibodies, mAbs, 9:8, 1217-1230, DOI: 10.1080/19420862.2017.1368602

Enabling Forced Degradation in Screening with MAM



Why MAM and mass spec?



ADC, mAb, multi-specific, fusion, etc

Small sample input enables screening

Direct ID of PTMs

Integrating Forced Degradation in Candidate Screening has Potential for Impact

Opportunity: Better Drugs

Candidates selected

- Chemical liability risks as screening criteria, can guide towards selection of better candidates

Quality by Design

- CMC risks can be identified before cell line/process development begins

Challenge: Resources

Turnaround time

- Decisions typically monthly
- Stressing takes time
- Method development not an option

Sample Availability

- Limited analytical options
- Most QC friendly methods not applicable

How to apply perform Developability assessments, pre GLP Tox

mAbs/Proteins ☑

Platform/Miniaturize experiments

"Good" is well-defined based on industrial knowledge

ADCs ☑/☒????

Leverage mAbs analytical platforms

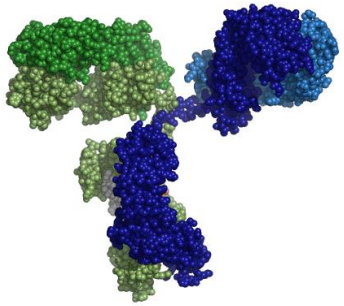
Triage ADCs into good/bad/maybe, but diagnosis difficult/impossible

Decision Making

Focus on key attributes (CDRs, DAR, trend of PTMs)

Characterization for leads only

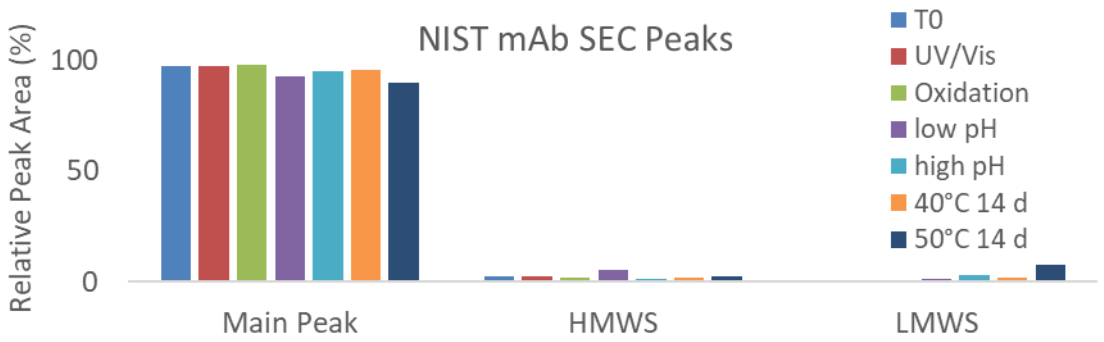
Biologics Screening Workflow



pH Ox

Applying stress

Analysis #1: Size Exclusion Chromatography

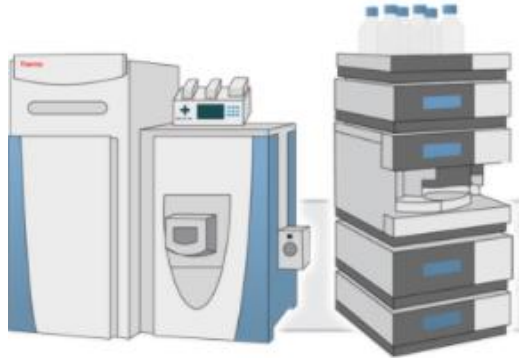


Analysis #2: Intact MS

Monitor gross changes
DAR for ADCs

Analysis #3: Peptide mapping via MS/MS

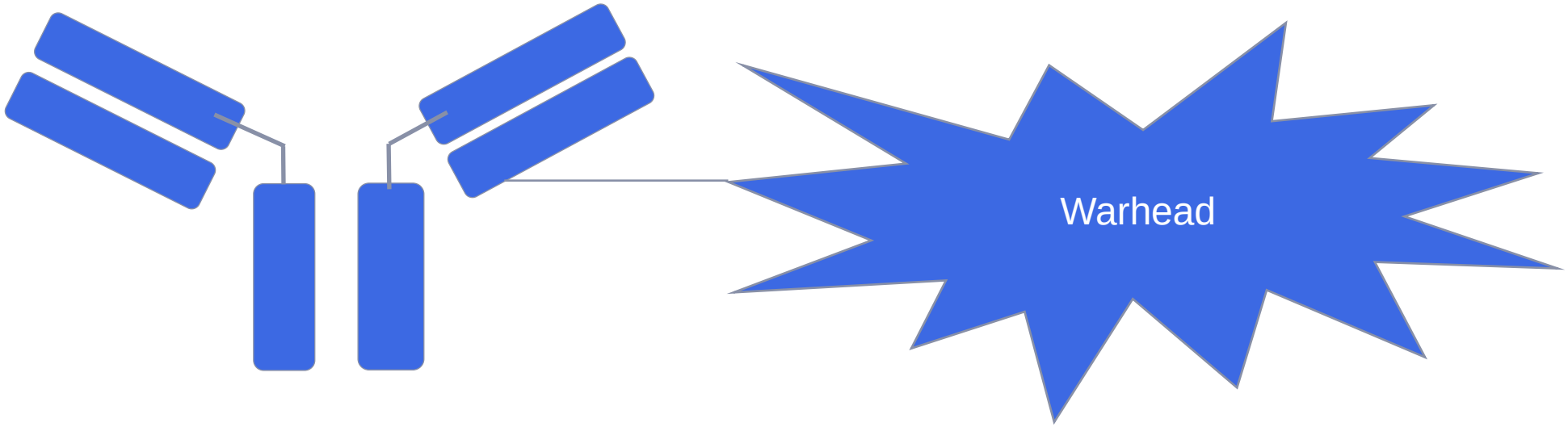
Automated Digest



LC/MS

Peptide level modification report

Antibody Drug Conjugates Forced Degradation: More Complicated than the sum of its parts



Aggregation/fragmentation

Chemical changes: oxidation, hydration, loss of side chains

Deamidations

Structure dependent equilibrium

Chemical changes: oxidation, hydration, bond redox changes

Drug-antibody ratio

On an analyte that's ~150,000 Da

Significant Challenges for Drug-Linker evaluations in the Late Discovery Phase

- Small-scale synthesis required for capped drug linker material
- Material should be of representative quality to accurately predict degradation pathways and identify actual risks

- Low compound solubility in aqueous media
- High molecular weight compound
- Limit material availability
- Potent material handling techniques are required for weighing solid samples

- Intermolecular ADC/drug linker interactions are difficult to characterize
- Drug linker degradation products can be insoluble in ADC formulations and may not detected by free drug linker methods
- Additional method development required to detect real-world drug linker degradation products in stressed ADC samples

Workflows: Drug Linker

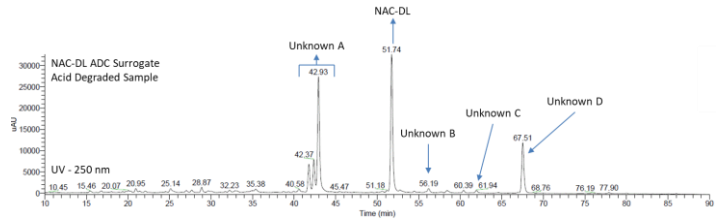
Capped Drug Linker Surrogate synthesis

Development of stability-indicating HPLC method

1. Column and mobile phase screening
2. Method optimization
3. Confirm stability indicating method with degraded sample
4. Additonal optimization (if necessary)

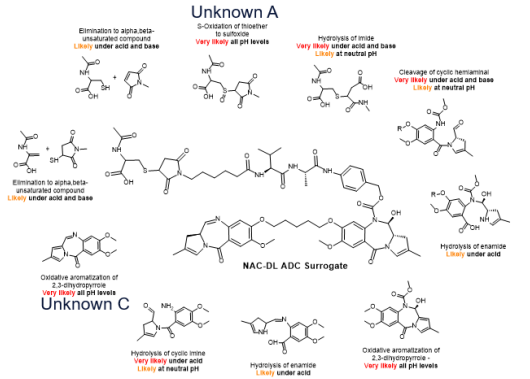
pH Ox

Conditions aligned with biologics workflow



Forced degradation **sample analysis** by HPLC

In silico predictions of degradation pathways
&
Structure elucidation of main degradants by mass spectrometry



Additional forced degradation conditions investigated as needed (i.e. trace metals, more pH studies ...)

What potential CQAs have we seen?

- Thermal instability
  - Variable region deamidation and/or oxidation
- Chemical oxidation induces Met Oxidation

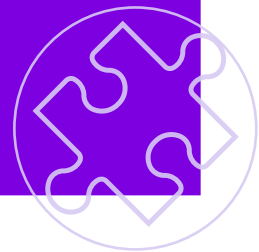
mAbs/Proteins

- Low pH instability:
  - Cysteine conjugations
- UV/Vis light damage:
  - Drug structure
- Thermal stress apparent DAR decrease
  - impurity driven

ADCs

- Thermal and Light stress main drivers of degradants
- Crucial to understand mechanisms of observed ADC instabilities (light damage, impurities)

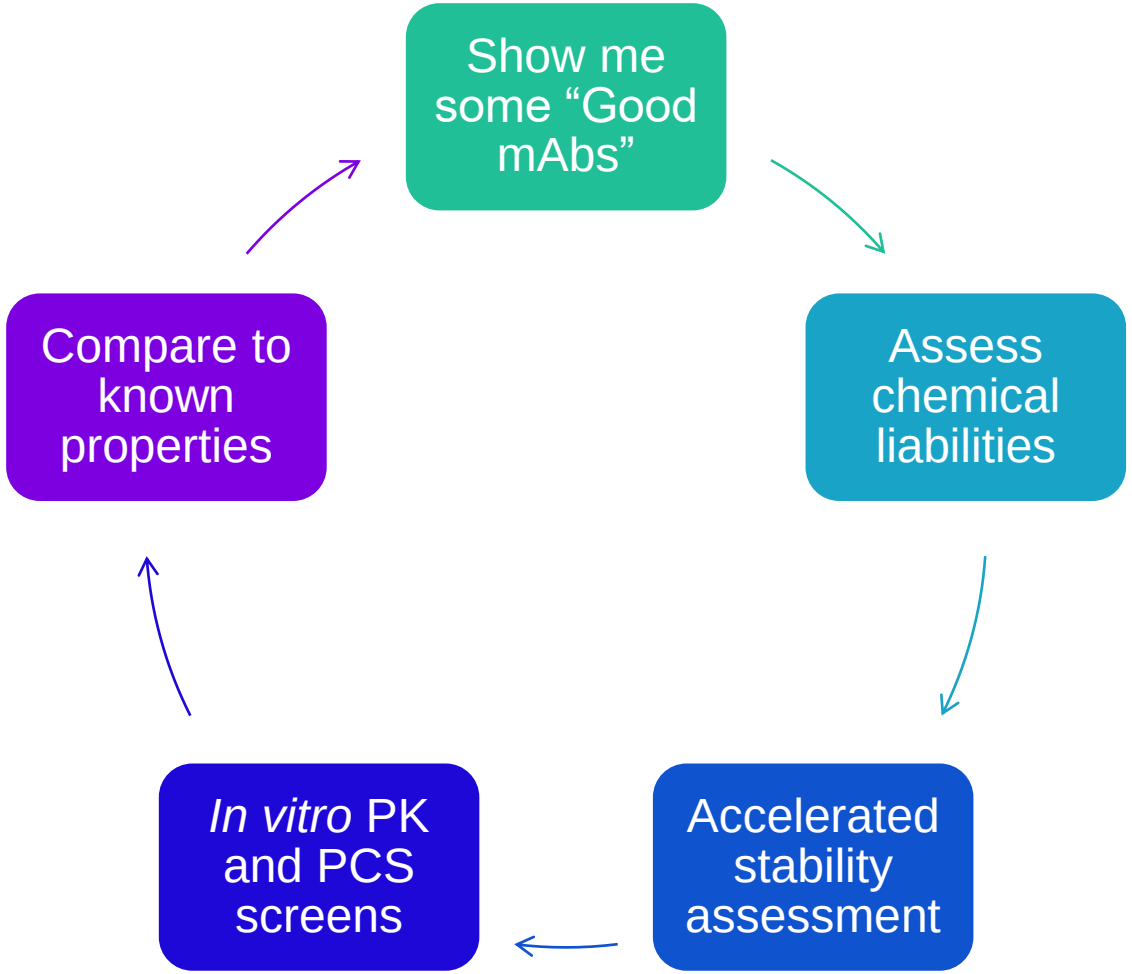
Drug Linker



What have we done with these findings?

- Proteins:
  - Investigate impact to activity, recommended some backup engineering
- ADCs/Drug Linkers:
  - Activity checks of degradants
  - Inform downstream Formulation and Process colleagues of risks, mitigations

“Normal” Biologics Initiative: Chemical Liabilities Lens

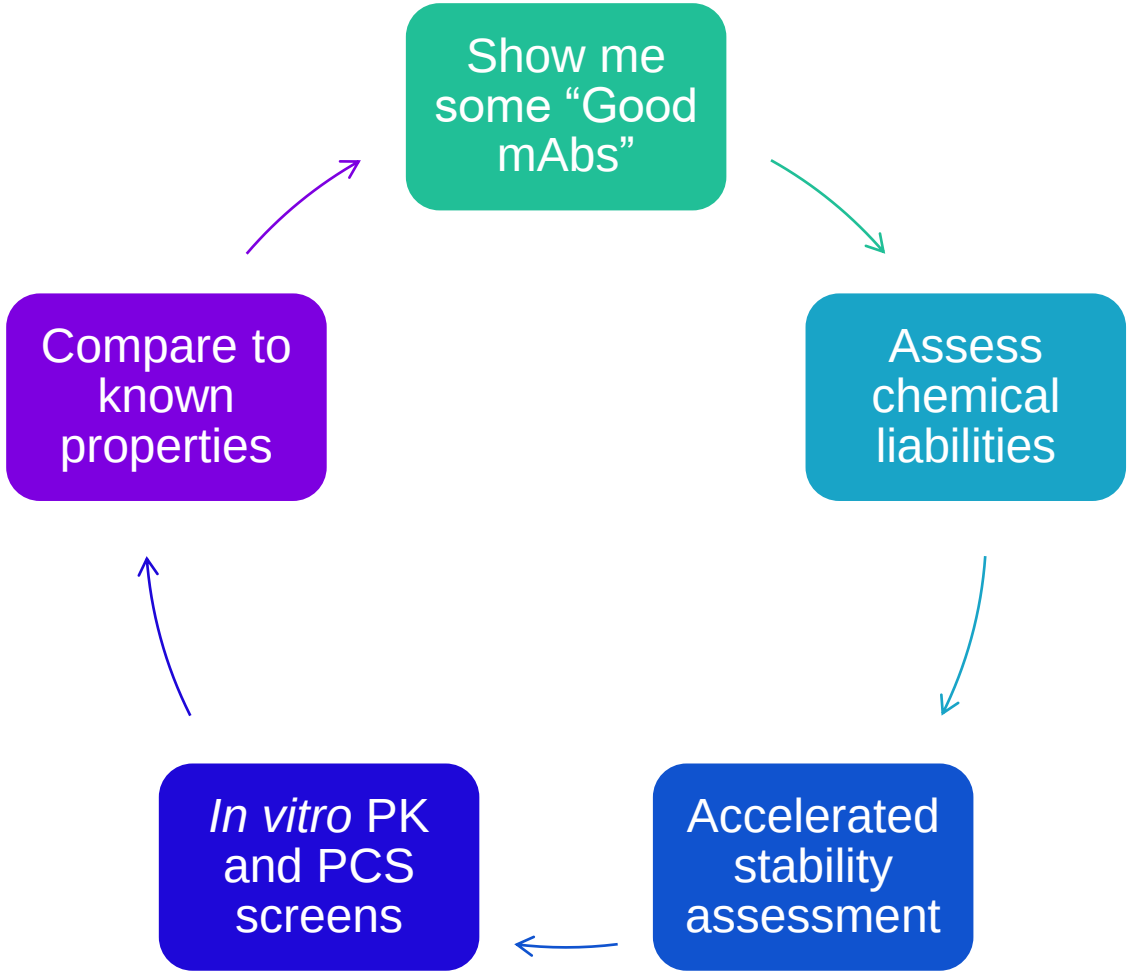


Chemical Liabilities

pH Ox

- SEC: HMWS and LMWS
- Reduced subunit mass spec: gross changes to mass
- Peptide mapping MS/MS: residue level chemical change id and relative quantification

Benchmarking to Improve Analytical Decision Making

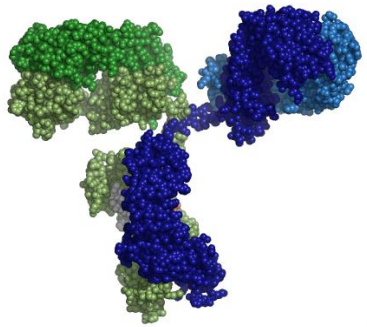


Benchmark “normal” modifications in clinical mAbs

Evaluate methods:

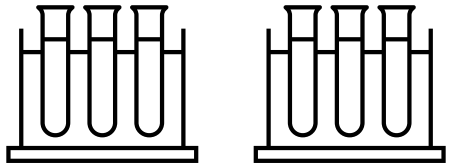
- Are we seeing the necessary liabilities?
- Can we get more information for less work?

In-depth analysis is resource intensive, potential CQAs are rare



pH Ox

Forced Degradation Stresses

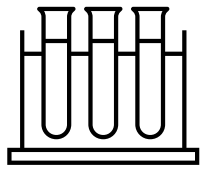


Many stressed samples per tested protein

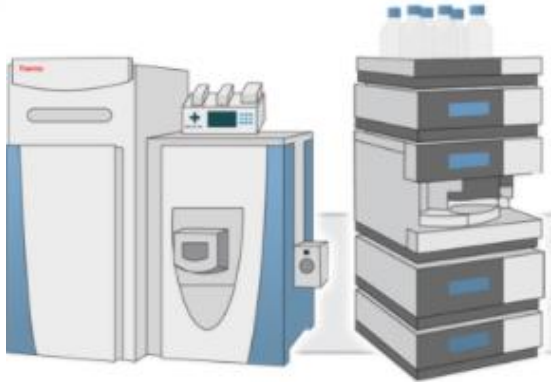
	Simple	In-depth
Sample Consumption (ug)	10	100
Prep Time (min)	20	400
LC-MS run (min)	5-10	35-100
Data Analysis (min)	5-10	120+++

Simple Analysis

In-depth Analysis



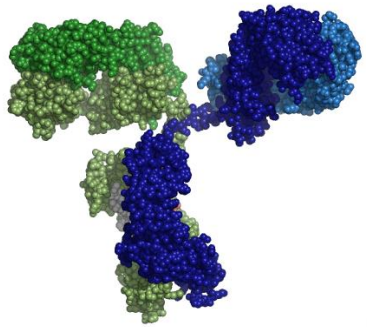
Optional: Reductant



Automated Digest

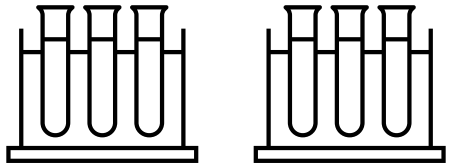
*Most PTMs are found in constant region, and not decision driving

In-depth analysis is resource intensive, potential CQAs are rare



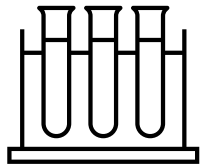
pH Ox

Forced Degradation Stresses



Many stressed samples per tested protein

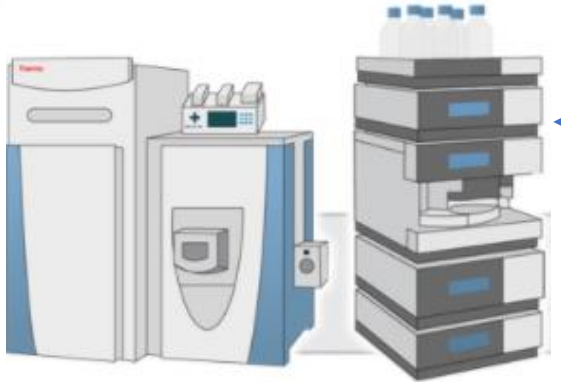
Can we use benchmarking data to develop triage procedures using simple analysis methods for which samples are likely to have potential CQAs?
Benefit: time savings for scientists



Optional: Reductant

Simple Analysis

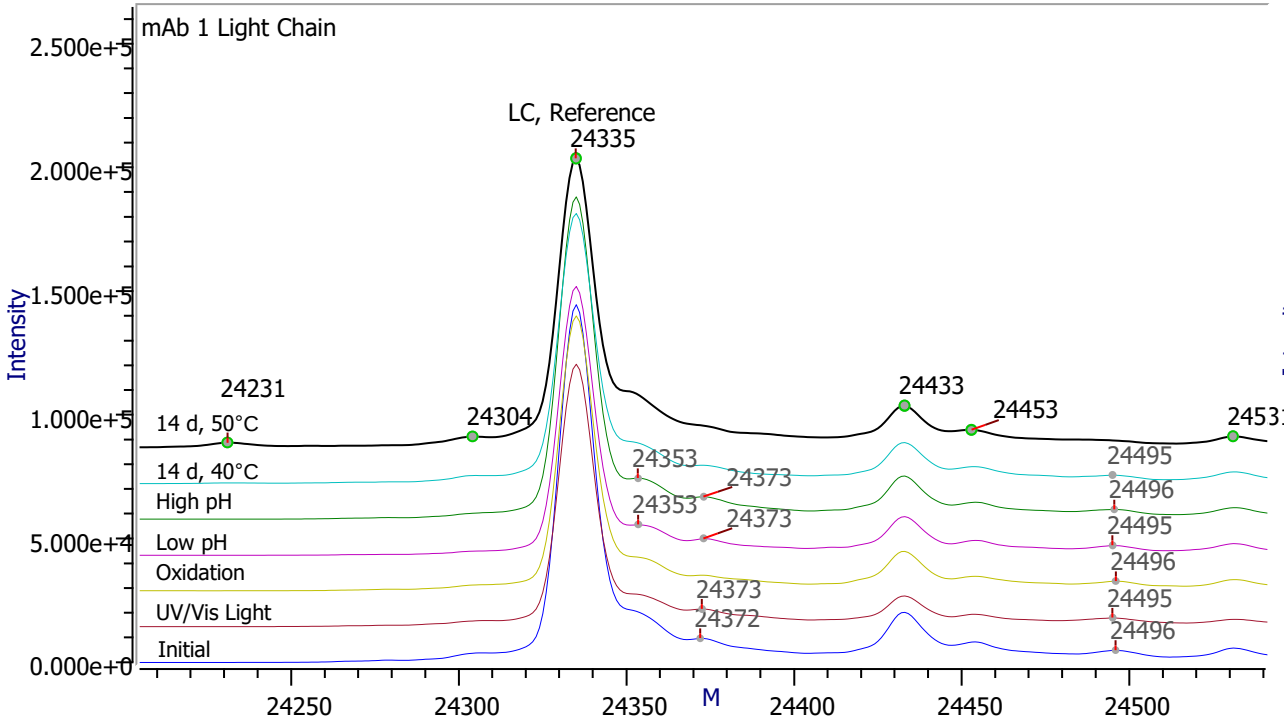
In-depth Analysis

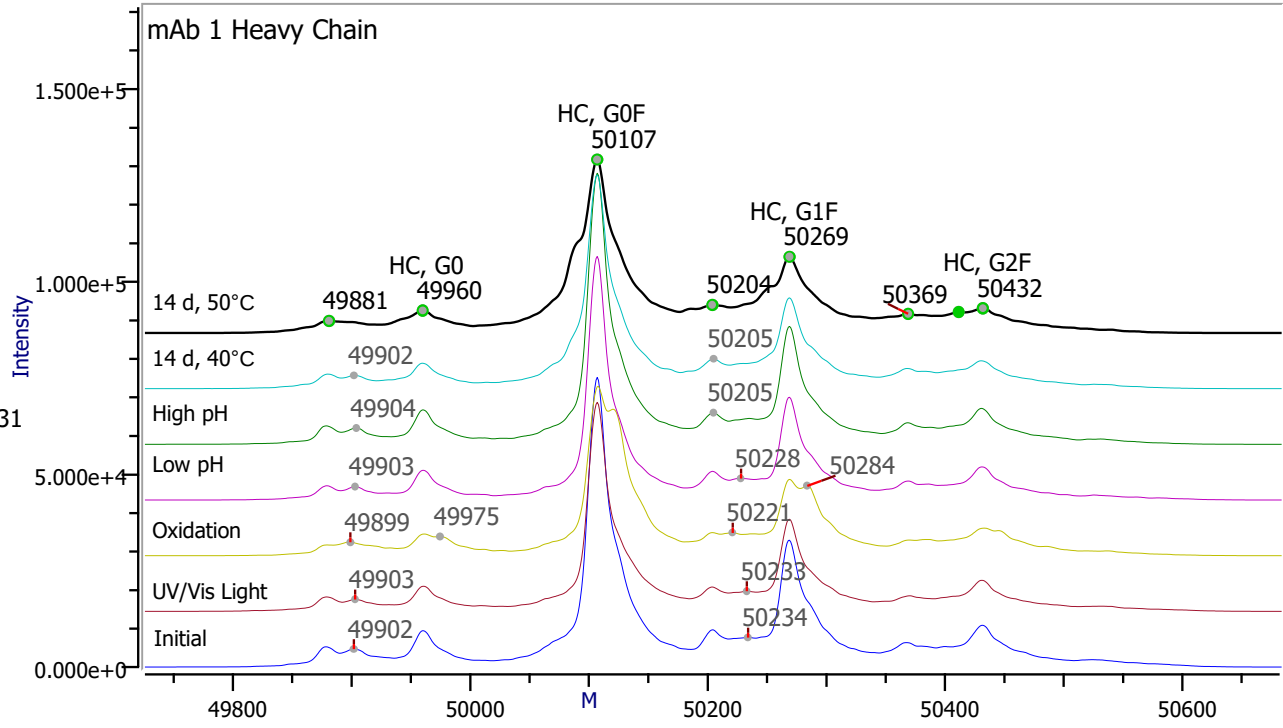


Automated Digest

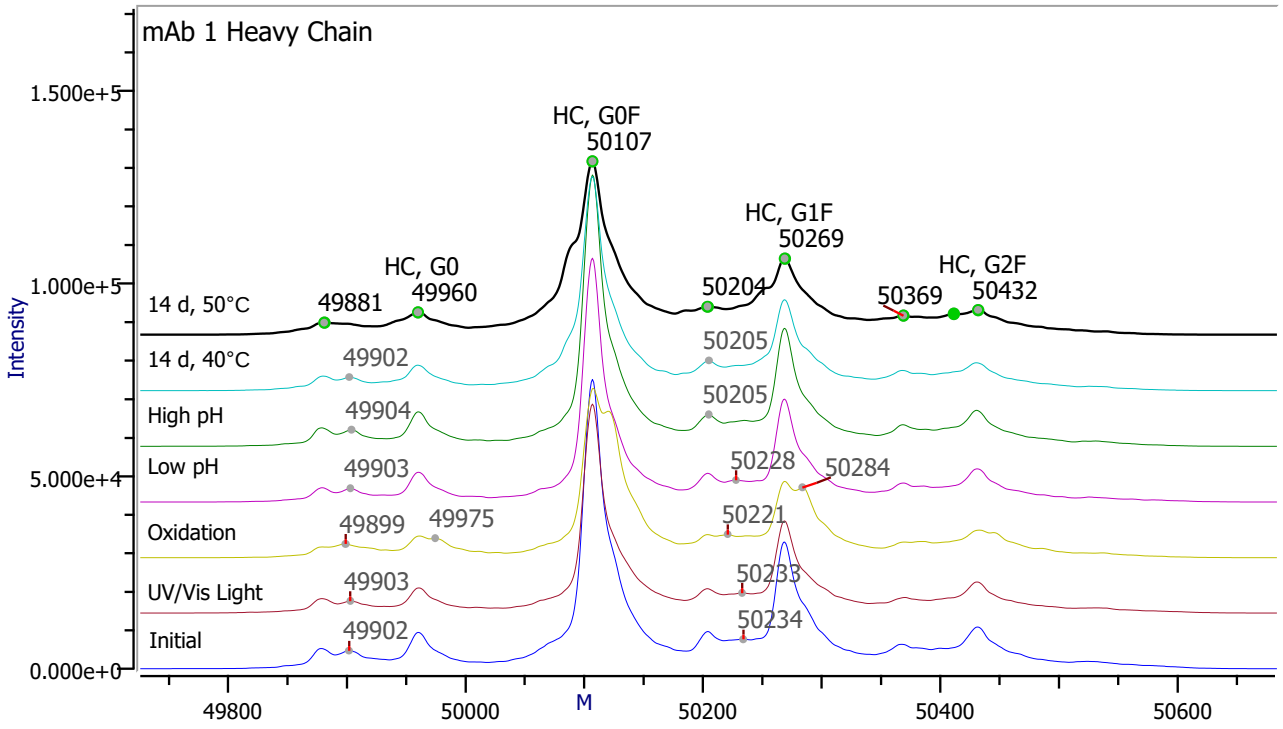
*Most PTMs are found in constant region, and not decision driving

Subunit protein mass spectrometry shows gross changes in structure





PTMs can be reliably tracked at a protein subunit level

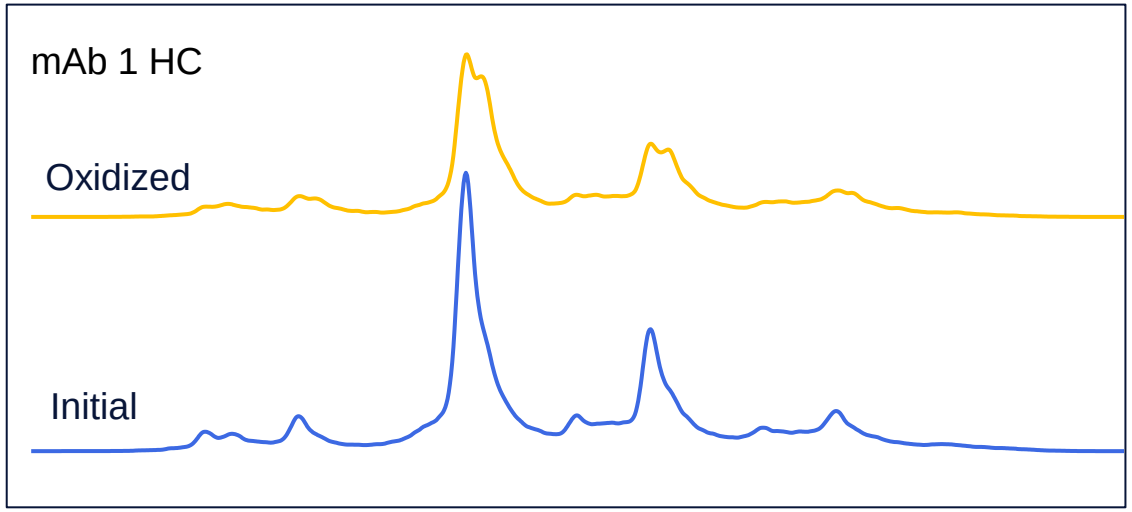


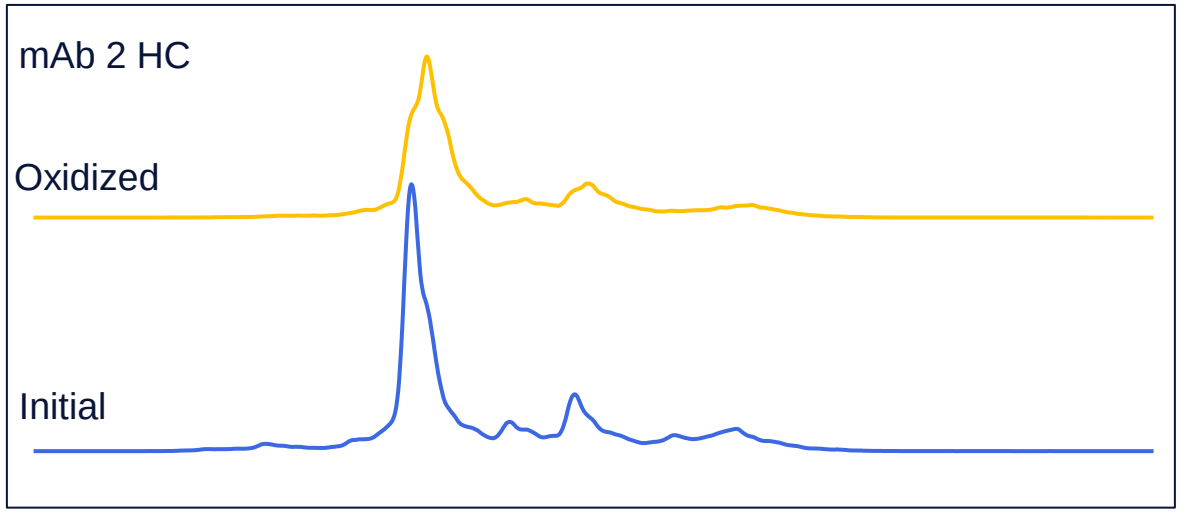
Treatment	Light Chain		Heavy Chain	
	Mass Loss	Mass Gain	Mass Loss	Mass Gain
T0	0	0	0	0
UV/Vis	0	0	0	0
Oxidation	0	0	0	1
Low pH	0	0	0	0
High pH	0	0	0	0
40°C 14 d	0	0	0	0
50°C 14 d	0	0	1	0

All modifications with modifications > 16 Da can be observed on reduced mass spec, if more than ~5% abundance

Triage Example: Oxidation to ID liable Mets in Variable region

Chemical oxidation produces a new peak on mAb heavy chains, and typically produces a bimodal curve, and variable region oxidations produce more complicated peak shapes:





Only constant region methionine residues at risk for oxidation

Variable and constant methionine residues at risk for oxidation

Final Thoughts: Developability is complicated, but important

Mass spectrometry can be applied in a screening paradigm

Protein Drug Conjugates? Must understand each component.

Benchmarking can improve decision making and analytical paradigms

Acknowledgments

Research and Early Development
NCE Early Development Team
Global Protein Science
Biologics Generation
NBE Formulation