Antibody Drug Conjugates: Process Development and Analytical Considerations

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Bristol-Myers Squibb

Chemical and Synthetic Development

World ADC

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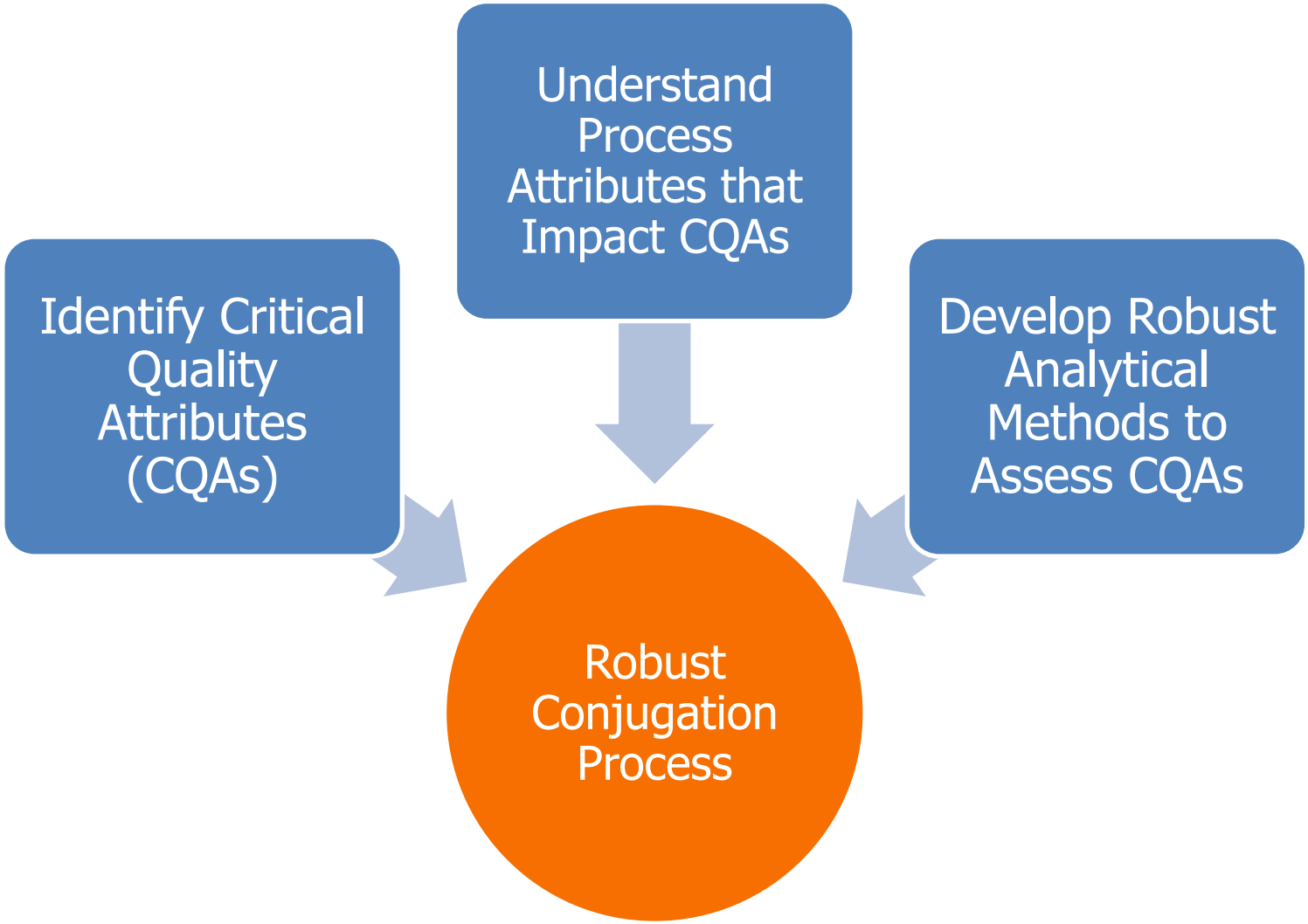
Defining a Robust Conjugation Process



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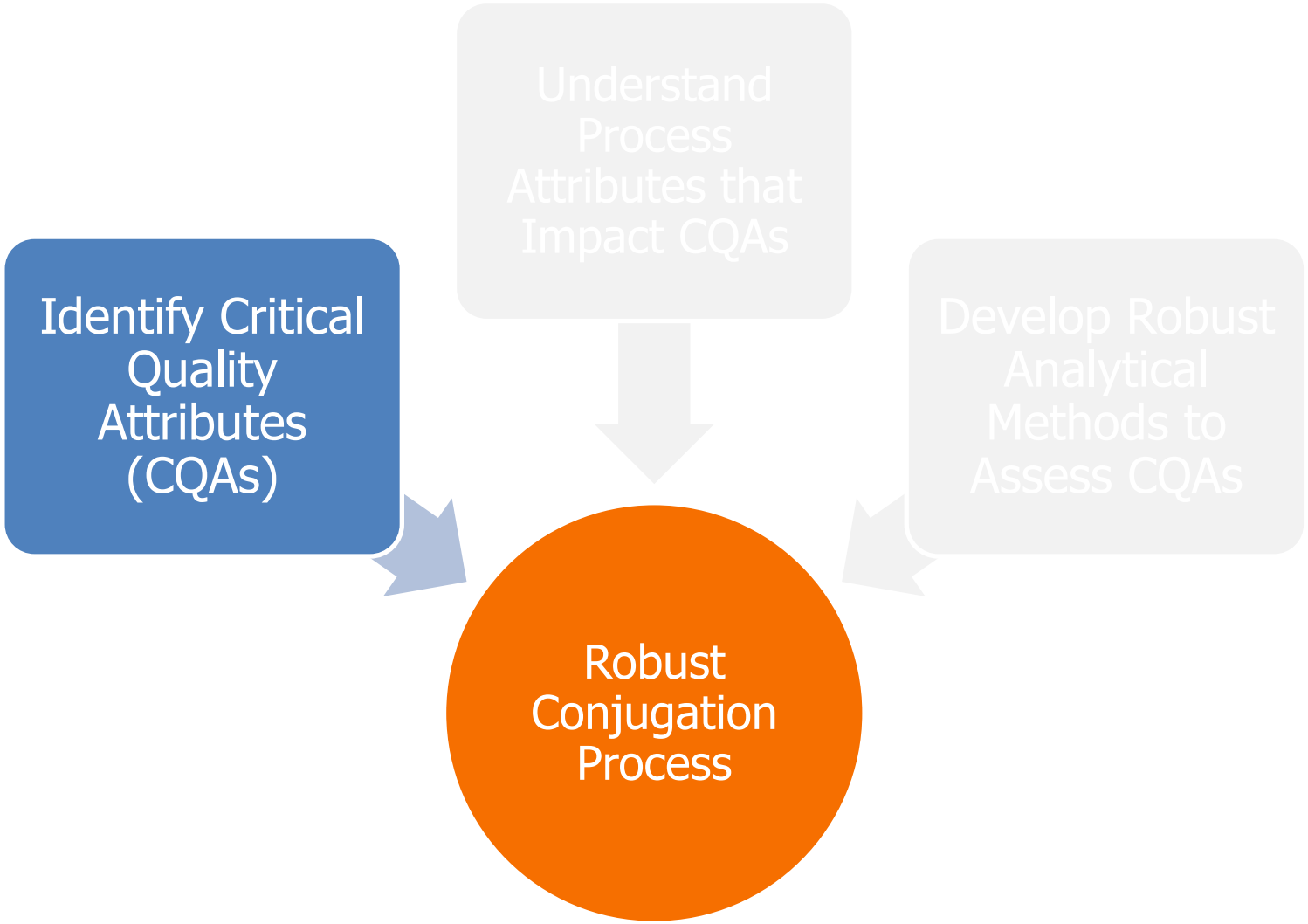
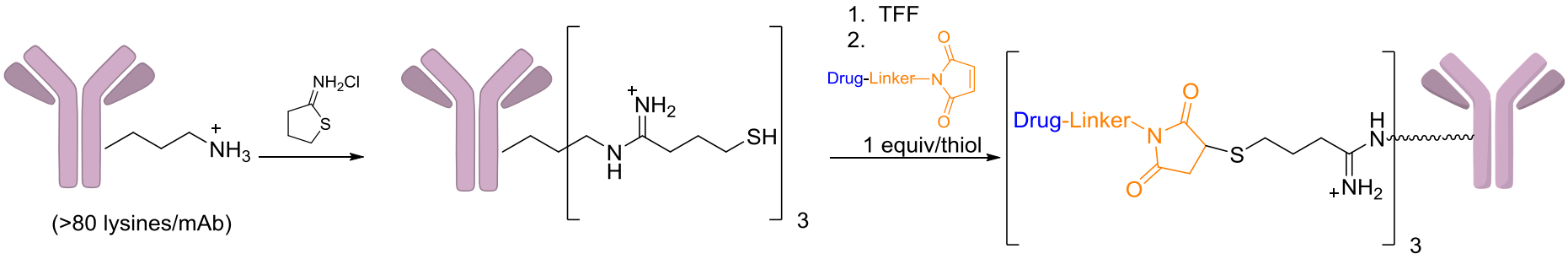
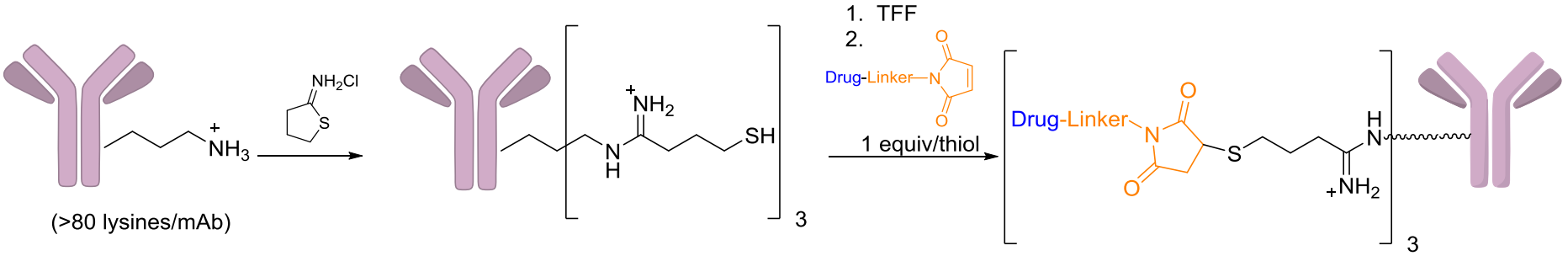


Case Study: 2-Iminothiolane-Based Lysine Conjugations



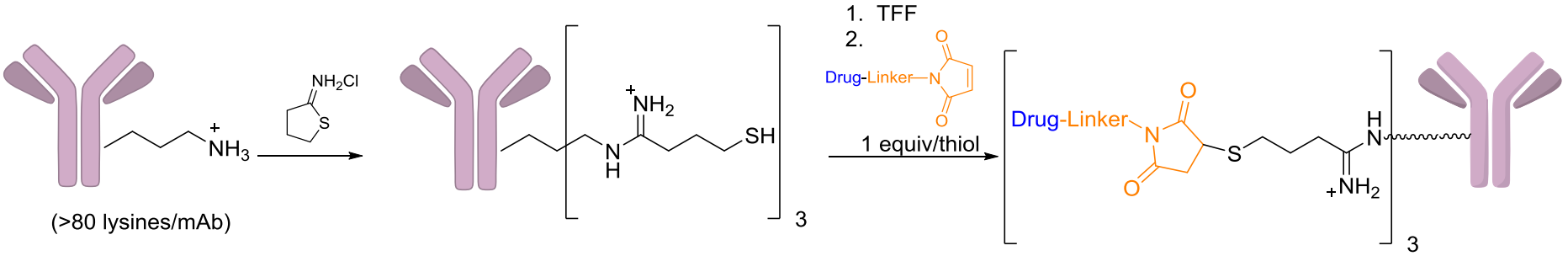
- Two step random lysine conjugation
- Intermediate thiolated antibody is purified by Tangential Flow Filtration (TFF) prior to conjugation with payload linker
- DAR is controlled by number of thiols on thiolated antibody intermediate

Defining Critical Quality Attributes



- Drug to antibody ratio (DAR)
- Aggregation
- Drug distribution
- Sites of conjugation
- Free (unconjugated) mAb
- Fragmentation
- Potency (cell killing and/or ELISA)
- Free drug
- Residual solvent

Defining Critical Quality Attributes



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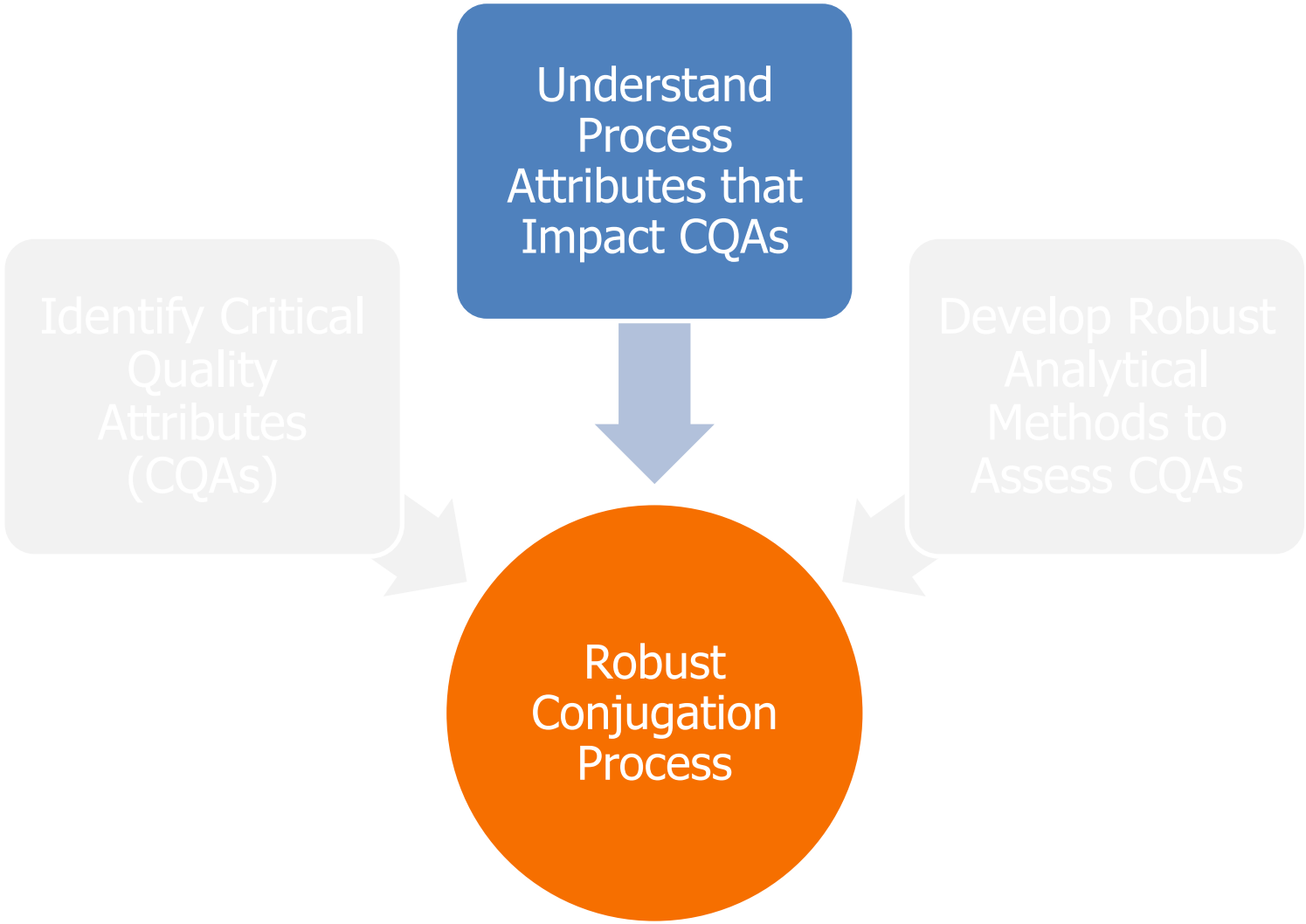
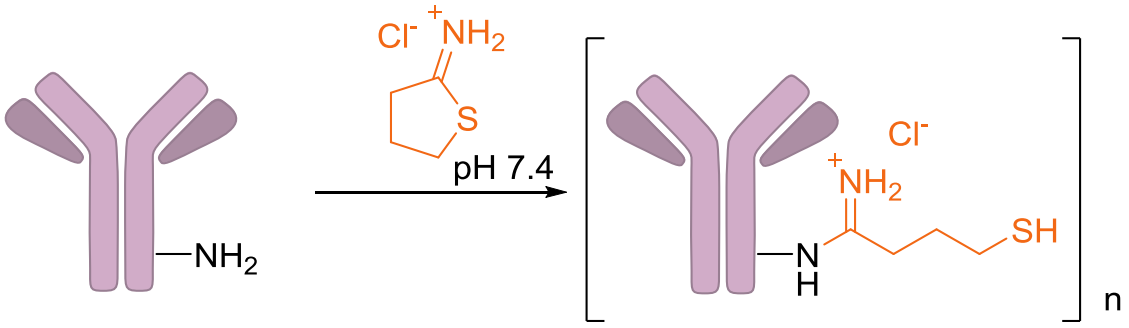
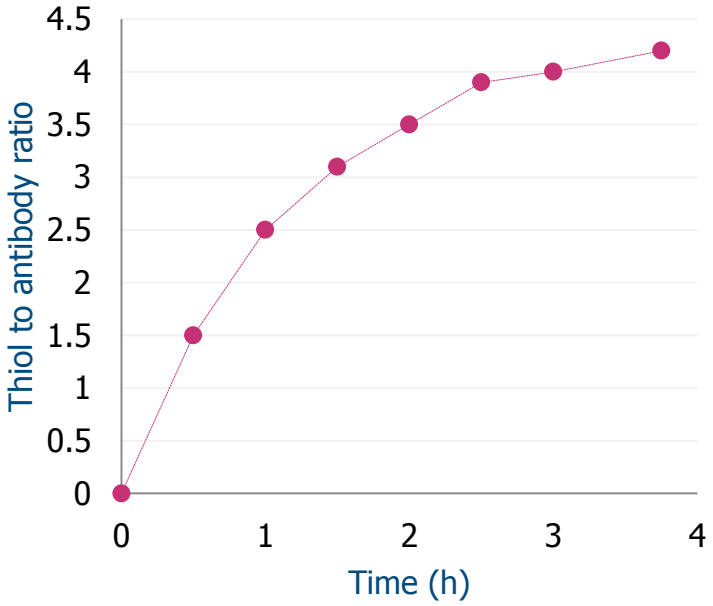


Thiolation of Antibody



Thiolation Reaction Profile



Traut et al. *Biochem.* **1973**, *12*, 3266

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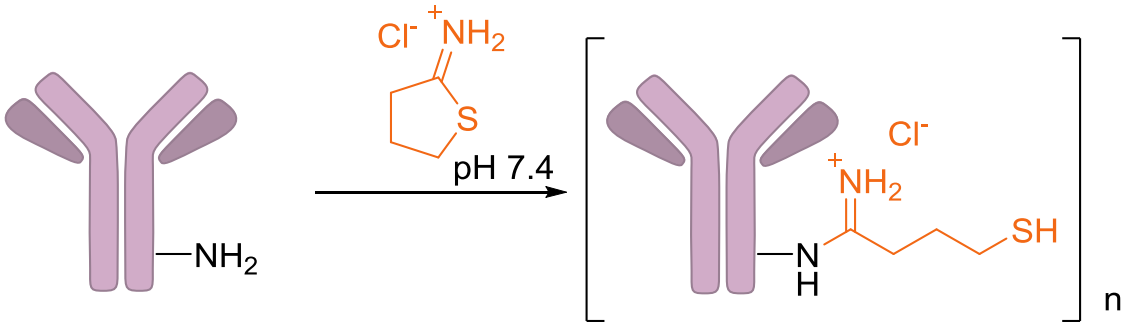

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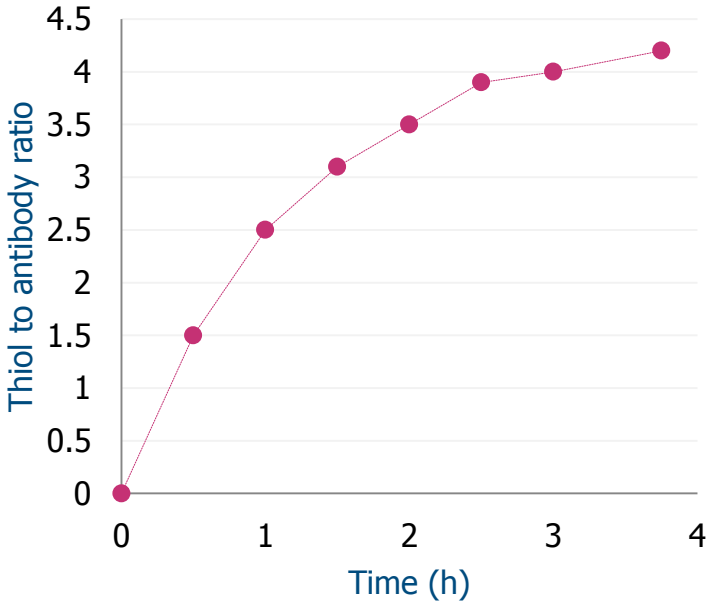
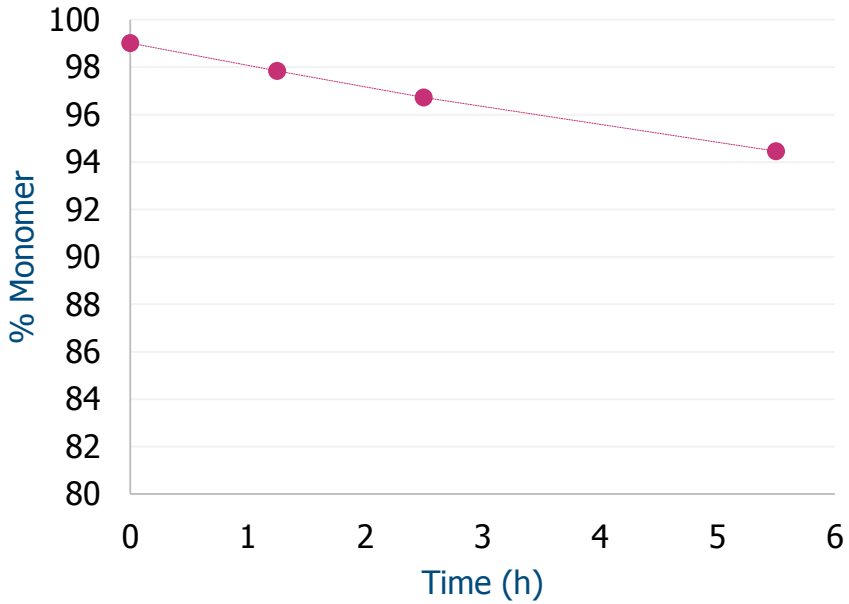


Aggregation Profile



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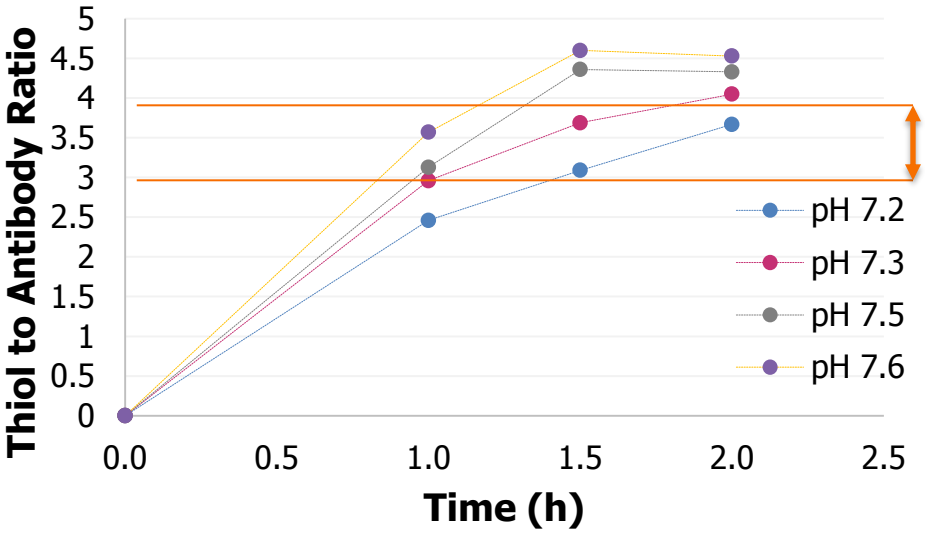
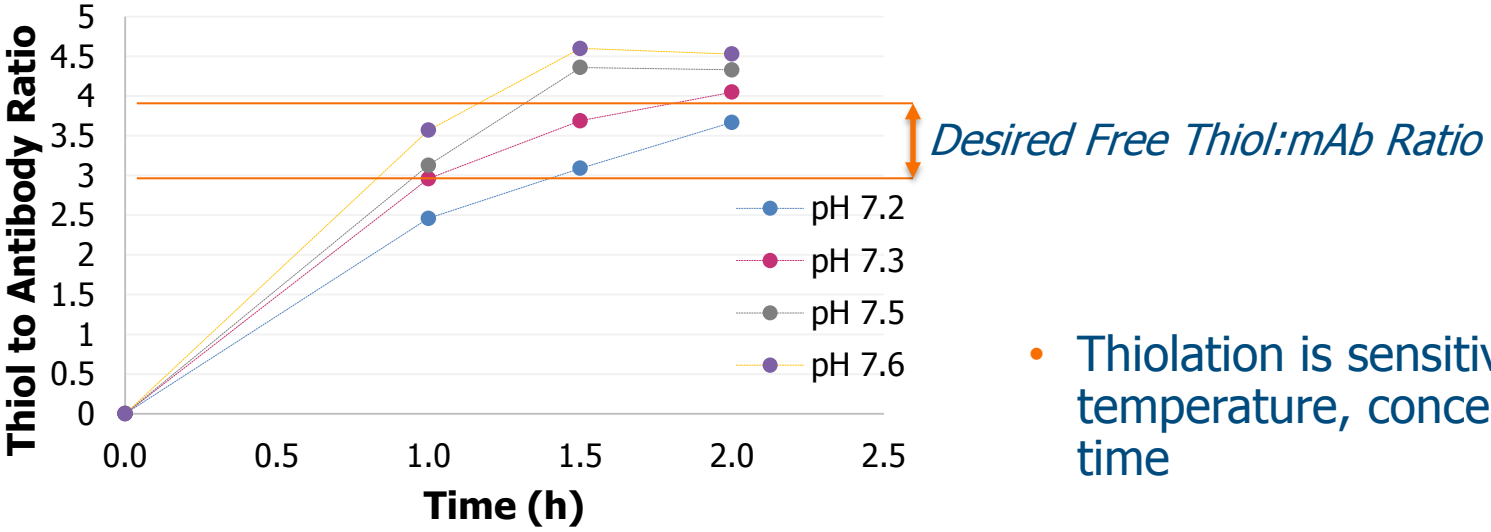
Key Thiolation Parameters



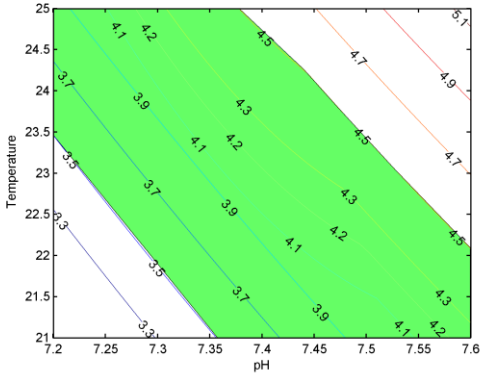
- Thiolation is sensitive to pH, temperature, concentration, and time

Key Thiolation Parameters

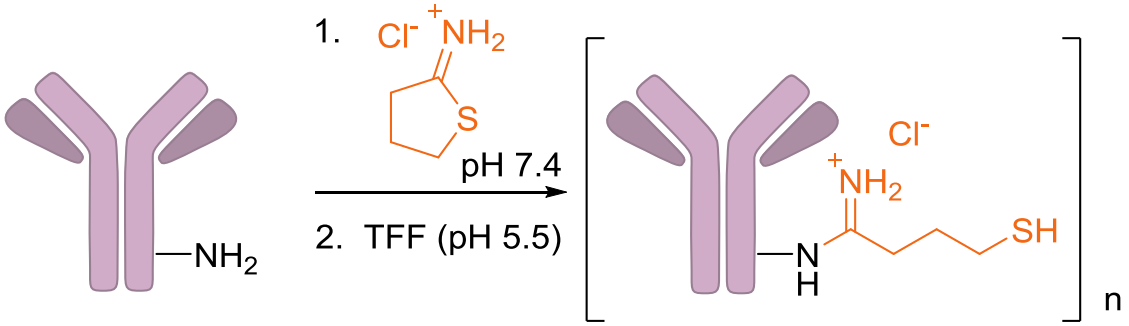


- Thiolation is sensitive to pH, temperature, concentration, and time

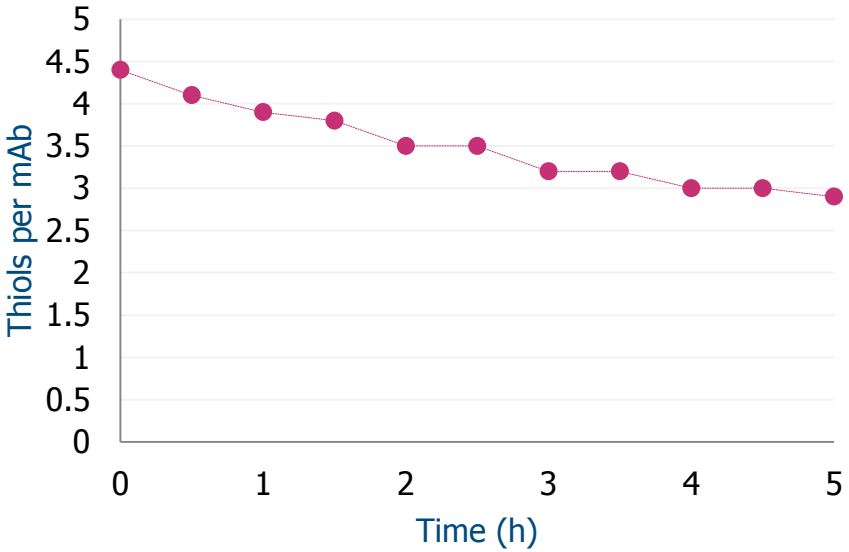
- Design of Experiment (DoE) was used to determine interplay between thiolation parameters (temperature and pH) and to help identify centerline reaction parameters



Stability of Quenched Thiolated Antibody



Thiolation Stability



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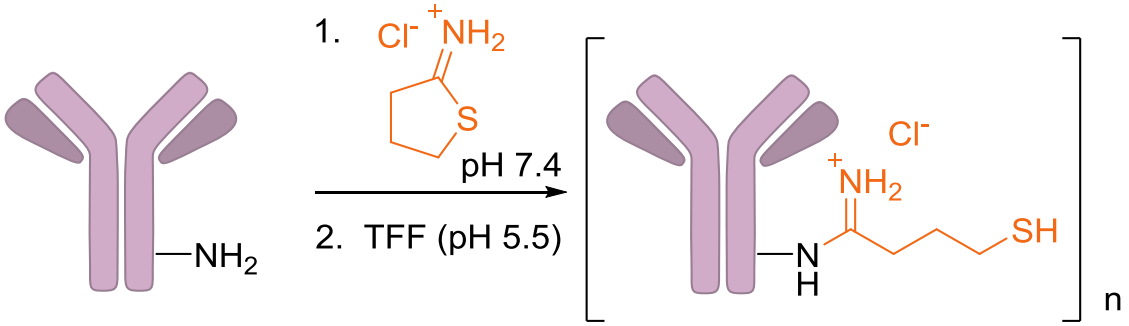

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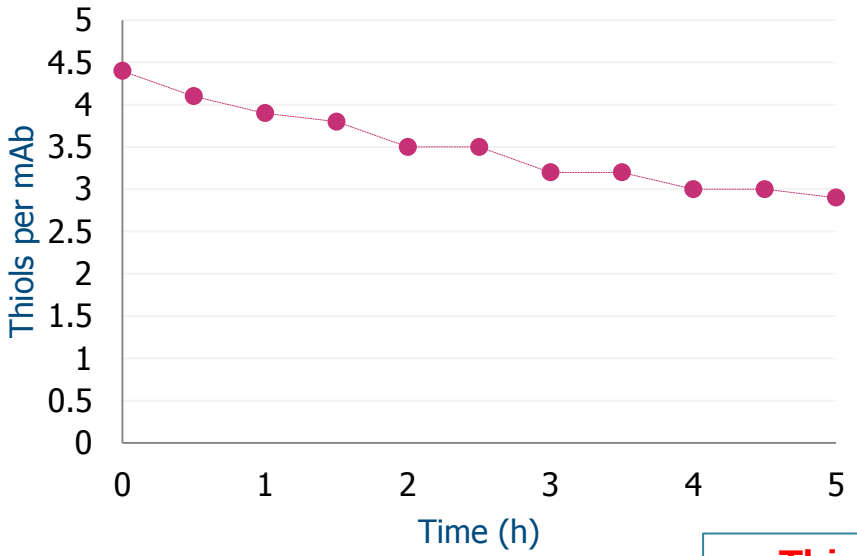
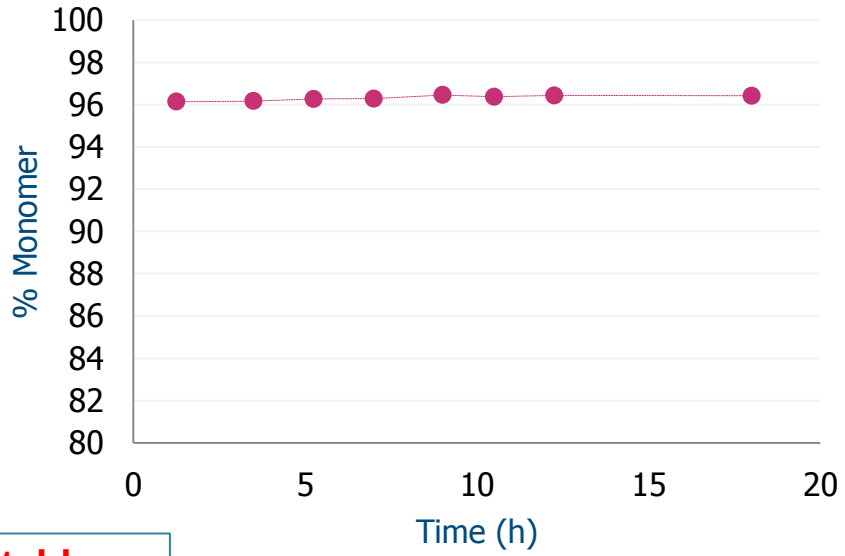
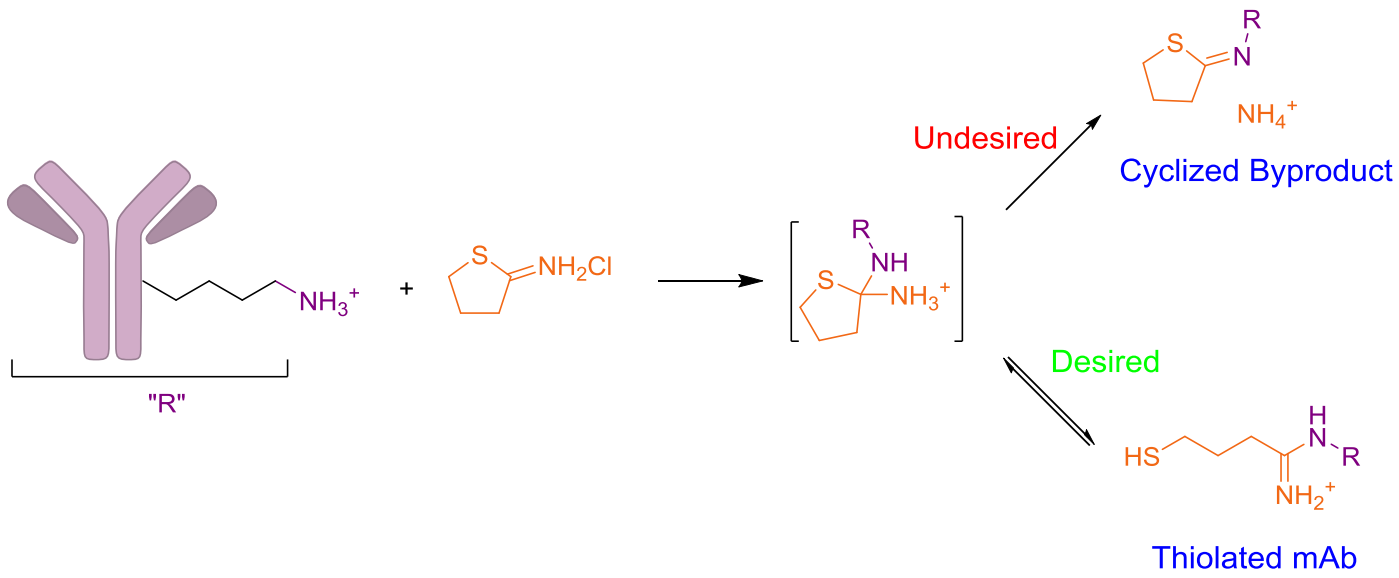


Aggregation Profile



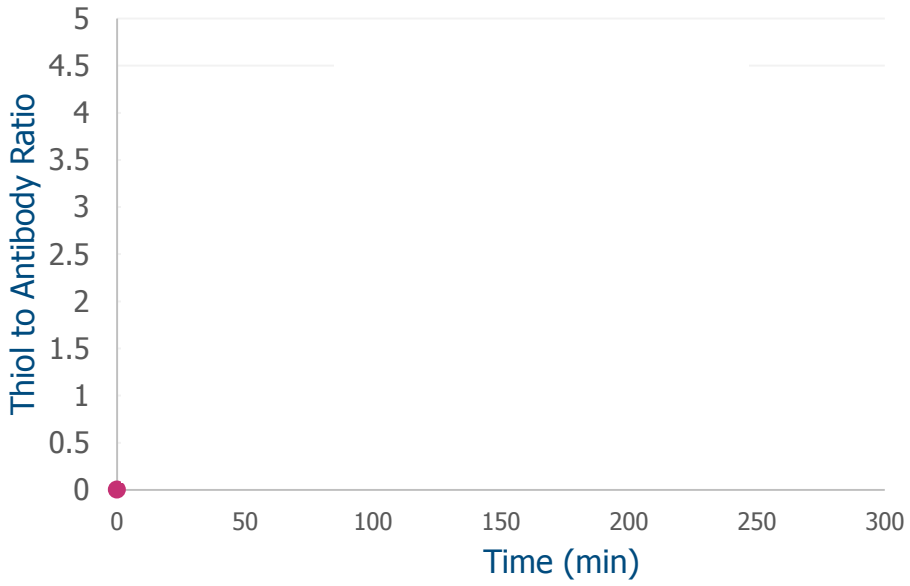
Thiols: unstable
Aggregation: stable

What Causes Thiol Decay?



- Desired thiolated antibody intermediate is produced through a complex equilibrium process that also affords undesired cyclized byproduct
- Thiolation and corresponding decay are impacted by temperature and pH

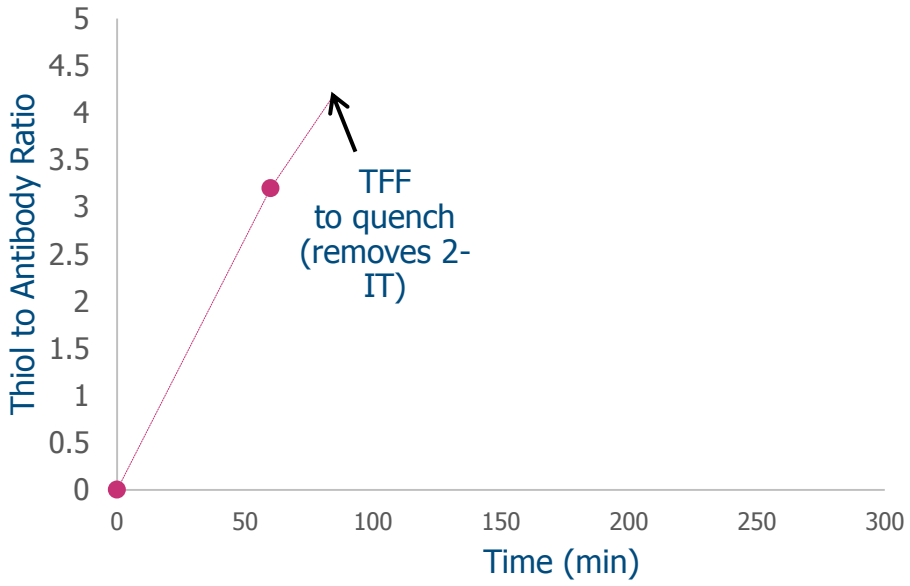
Thiolation Stability: Scale Up Impact



Key Scale Up Considerations to Ensure Achievement of DAR CQA:

- Thiolated mAb stability
- Time required for TFF (2-IT quench)

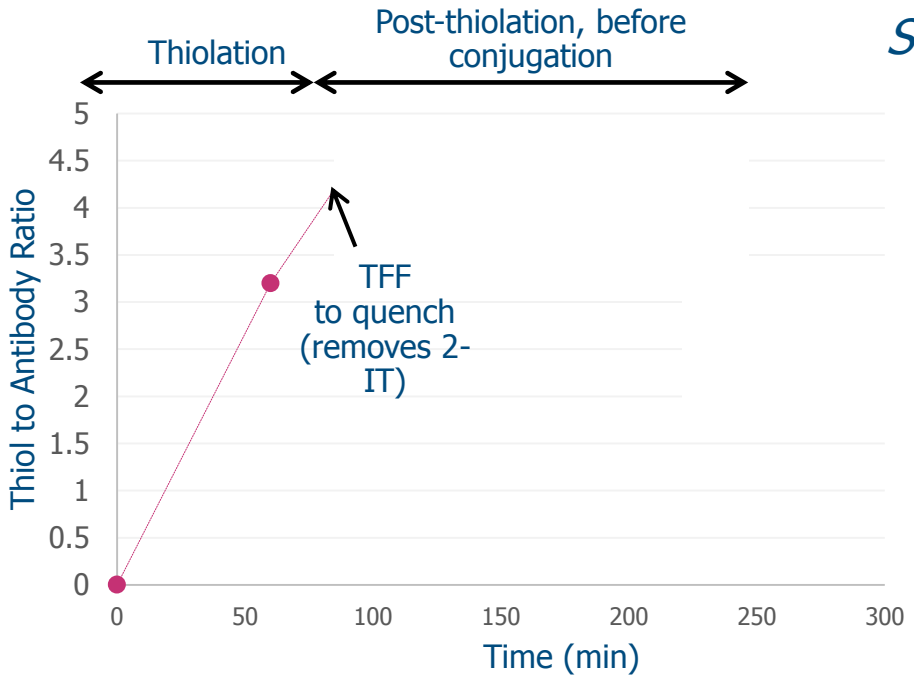
Thiolation Stability: Scale Up Impact



Issues:

- Thiolated mAb stability
- Time required for TFF (2-IT quench)

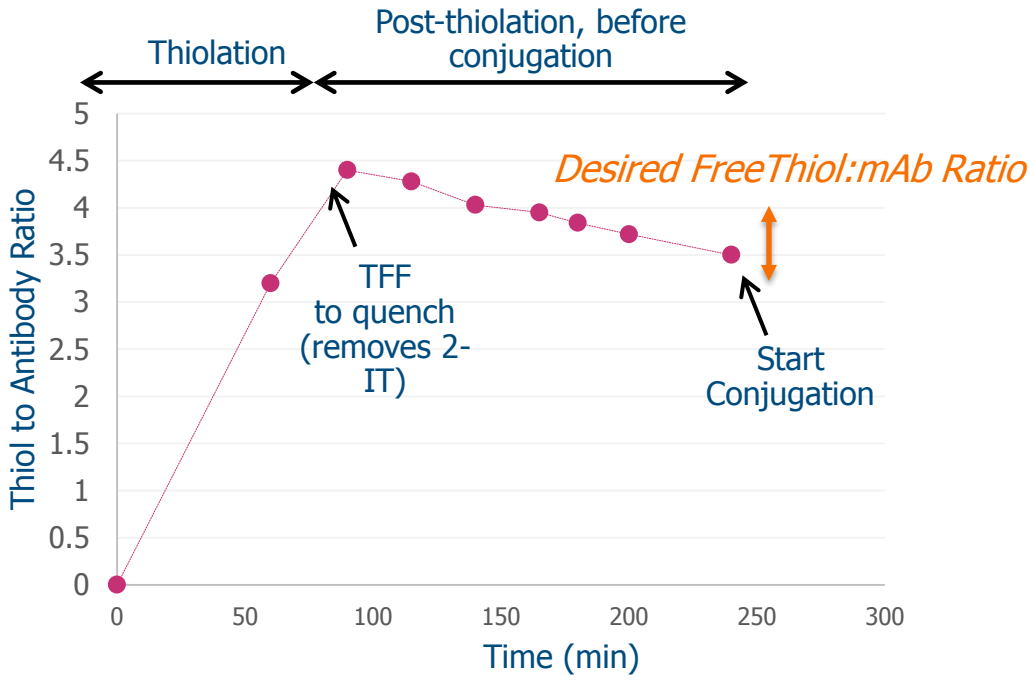
Thiolation Stability: Scale Up Impact



Solution: Take Advantage of Thiol Decay

- Overthiolate antibody
- Allow free thiols to decay to target level during TFF operation/hold time

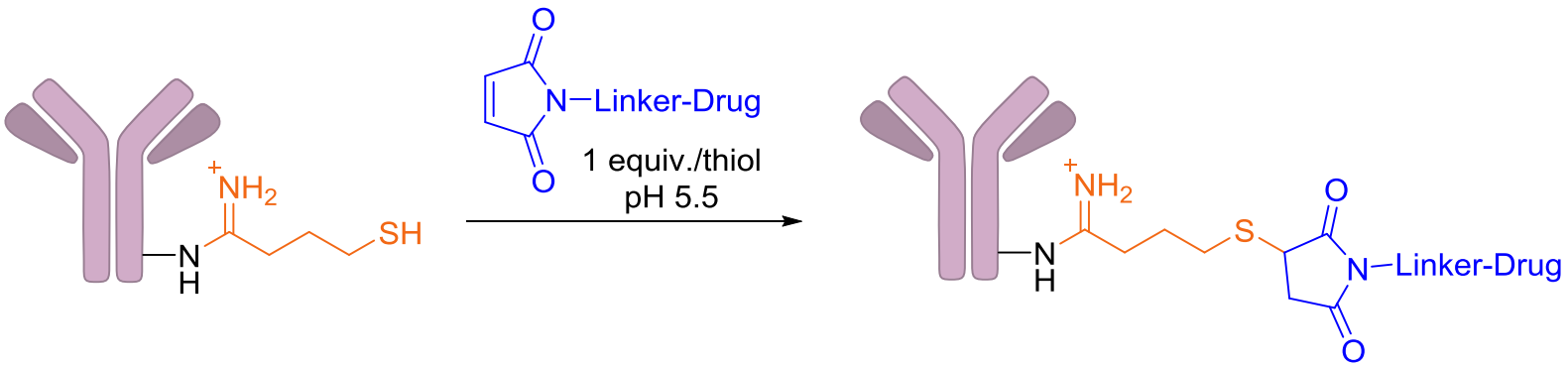
Thiolation Stability: Scale Up Impact



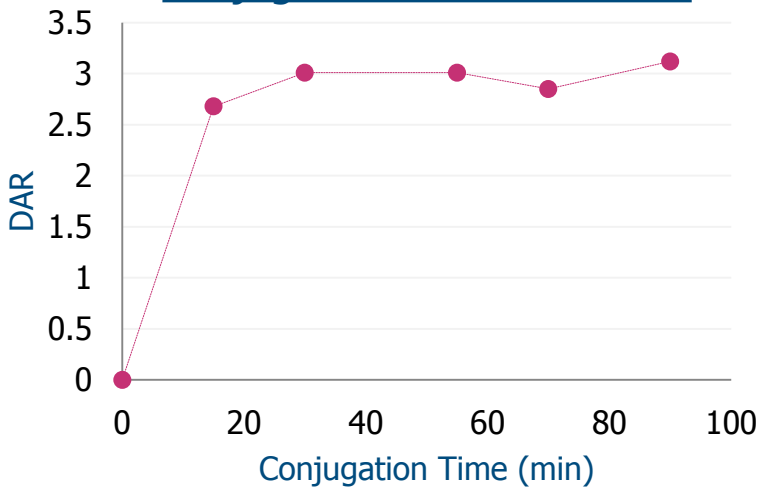
Successful Thiolation Scale Up Requires Understanding of:

- Thiolation parameters
- Rate of thiol decay
- Understanding of TFF parameters (TMP, crossflow rate, small molecule purge rate, pH change rate) and equipment

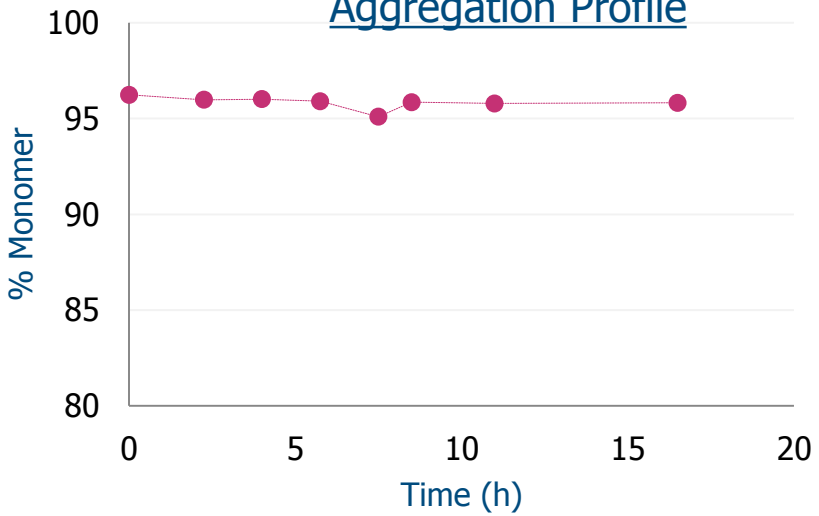
Conjugation Step



Conjugation Reaction Profile

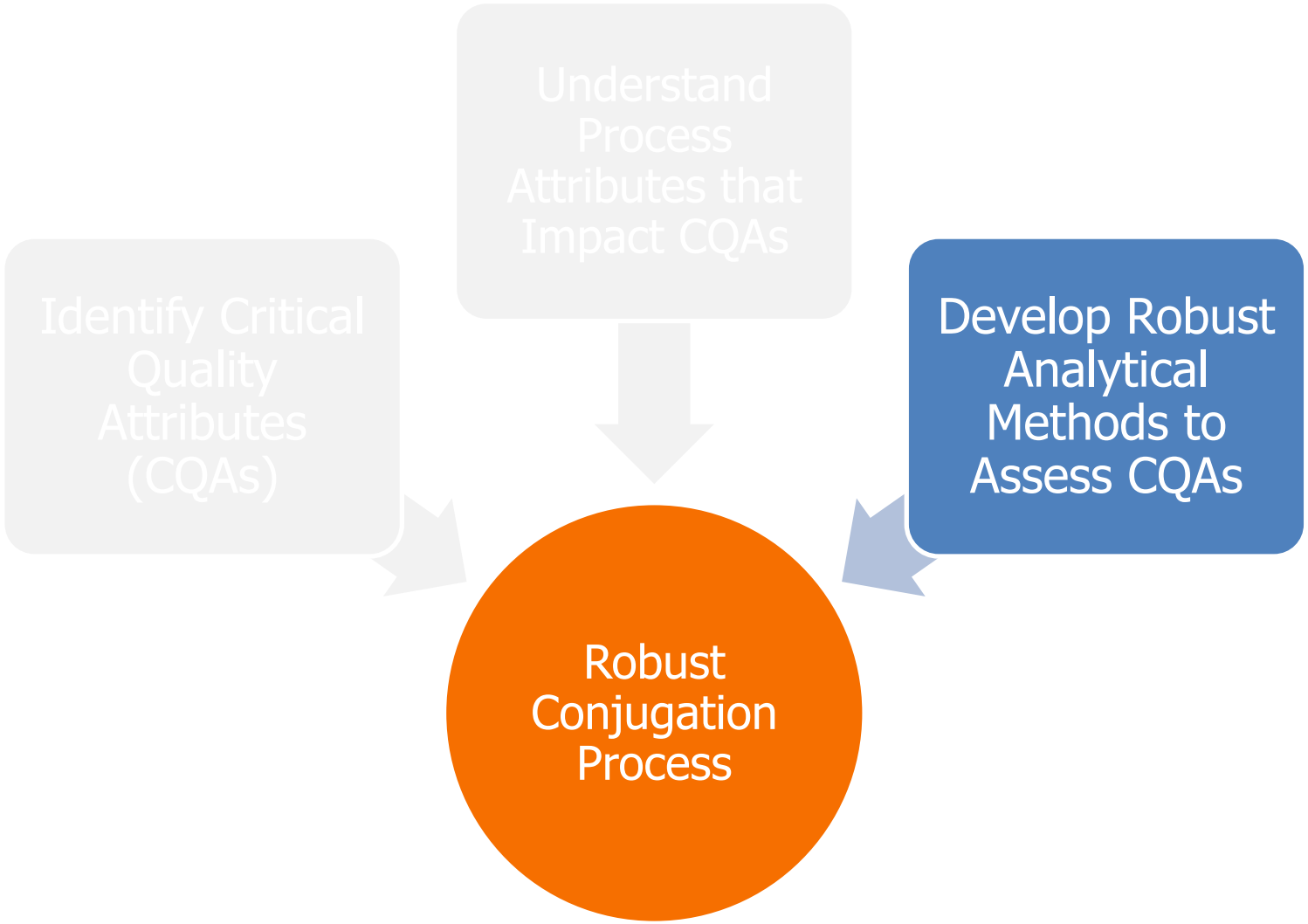


Aggregation Profile



- Maleimide in payload linker undergoes hydrolysis at pH>6
- Conjugation is rapid at pH 4.5-6.0
- Monomeric purity remains constant throughout thiolation step

Defining a Robust Conjugation Process



ADC Characterization

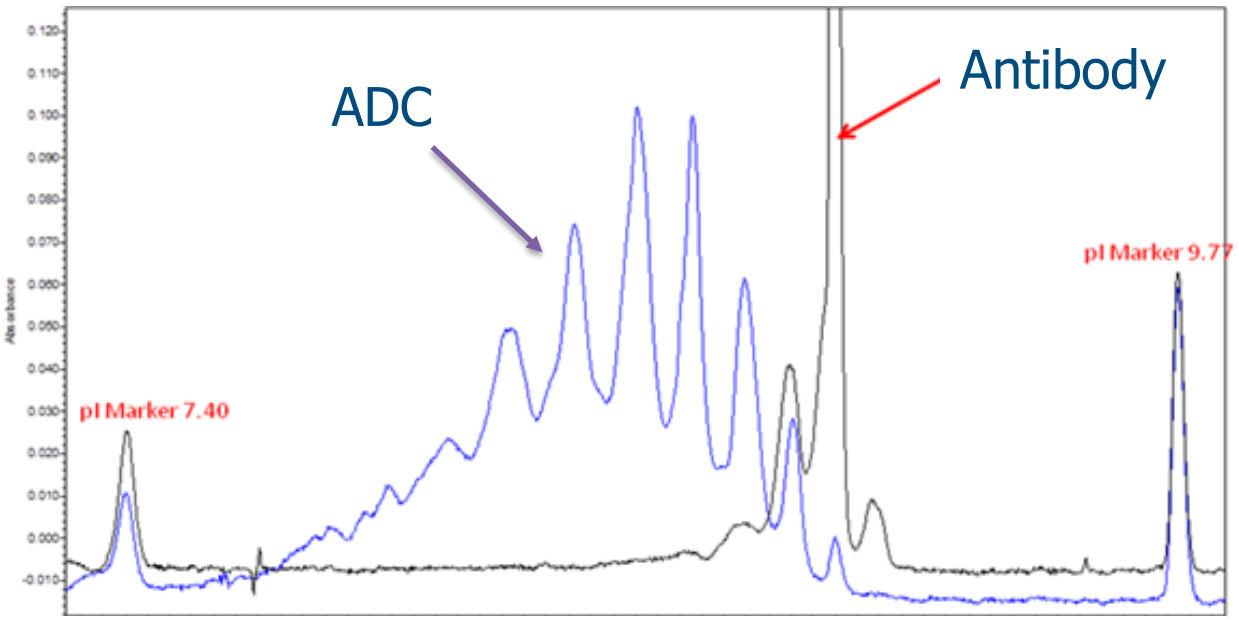
Characterization Method	Attribute
UV/Vis	DAR
SEC	Aggregation
Mass Spectrometry	Drug location and distribution
HIC	Unconjugated antibody
CE-SDS	Fragmentation
iCIEF	Charge variants
ELISA	Identity/Activity
Cell Based Assay	ADC activity
RP HPLC	Free drug
Glycosylation	Glycosylation pattern
Biophysical Characterization	Higher order structure/folding

A variety of analytical characterization techniques are needed to gain a comprehensive picture of the random ADC…

ADC Characterization

Characterization Method
UV/Vis
SEC
Mass Spectrometry
HIC
CE-SDS
iCIEF
ELISA
Cell Based Assay
RP HPLC
Glycosylation
Biophysical Characterization

For example, iCIEF can show the impact of conjugation on the antibody charge profile…

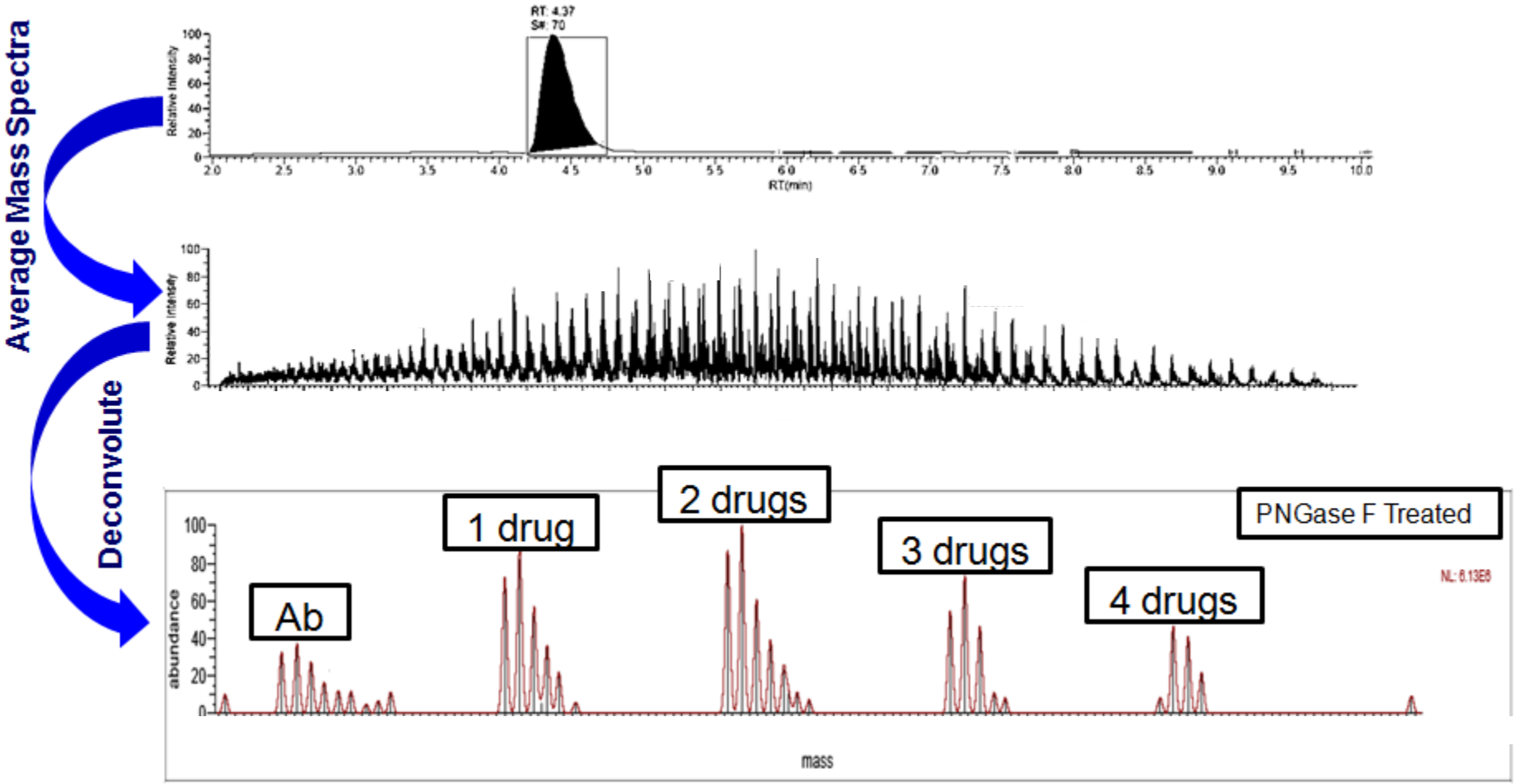


ADC Characterization

Characterization Method
UV/Vis
SEC
Mass Spectrometry
HIC
CE-SDS
iCIEF
ELISA
Cell Based Assay
RP HPLC
Glycosylation
Biophysical Characterization

…although, mass spectrometry techniques have provided the highest level of detail for these types of compounds

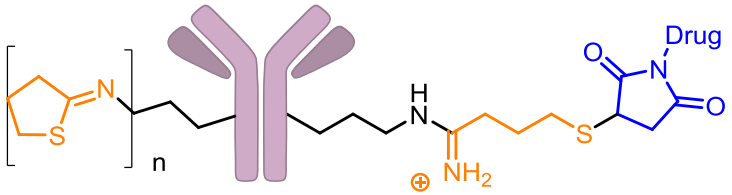
Intact Mass Analysis of an ADC

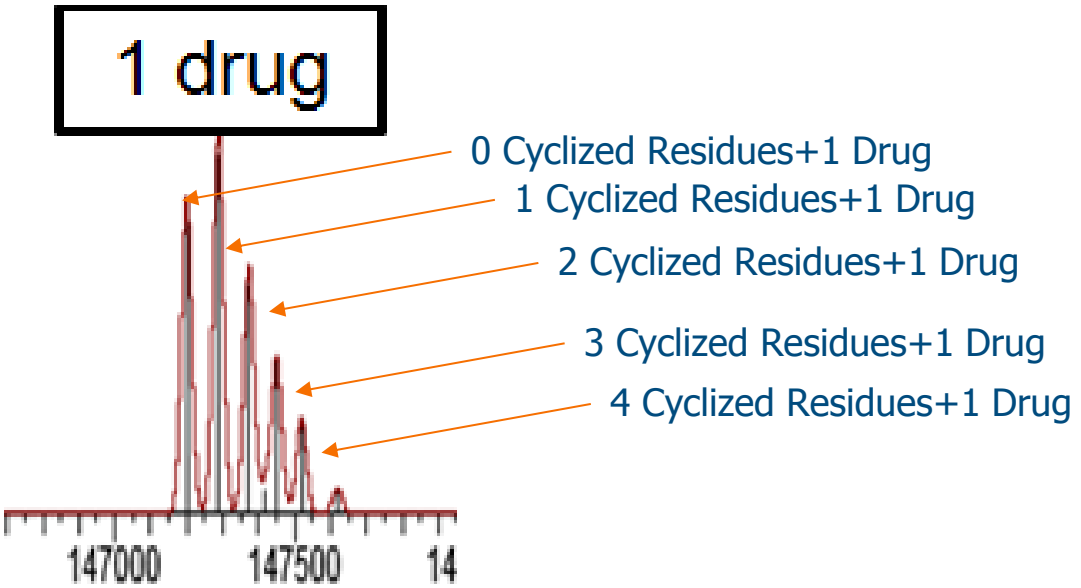


Higher level of detail vs traditional DAR measurement methods (UV spectroscopy)

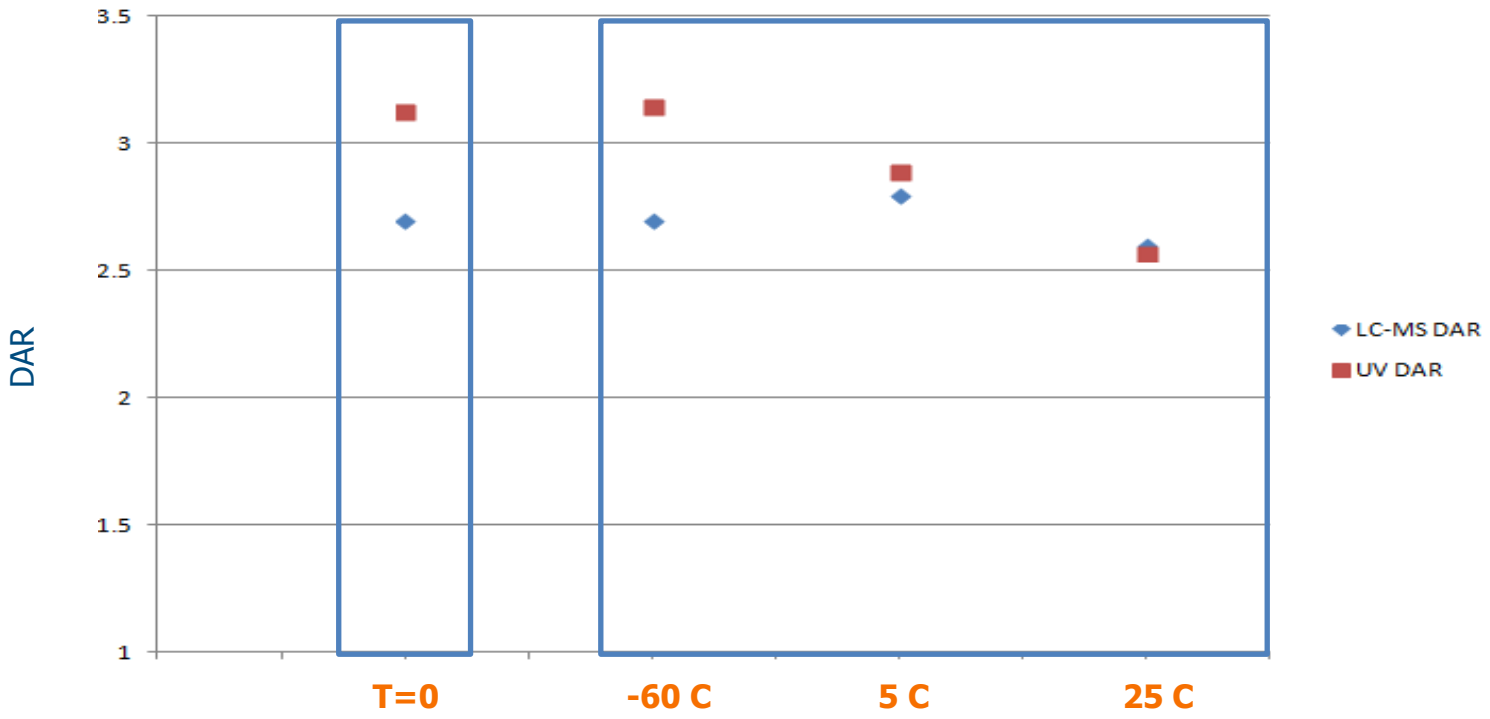
Determining Cyclized 2-IT Incorporation by Intact MS

- Each cyclized 2-IT residue adds 84 to the molecular weight of the ADC





Using Intact MS to Understand DAR

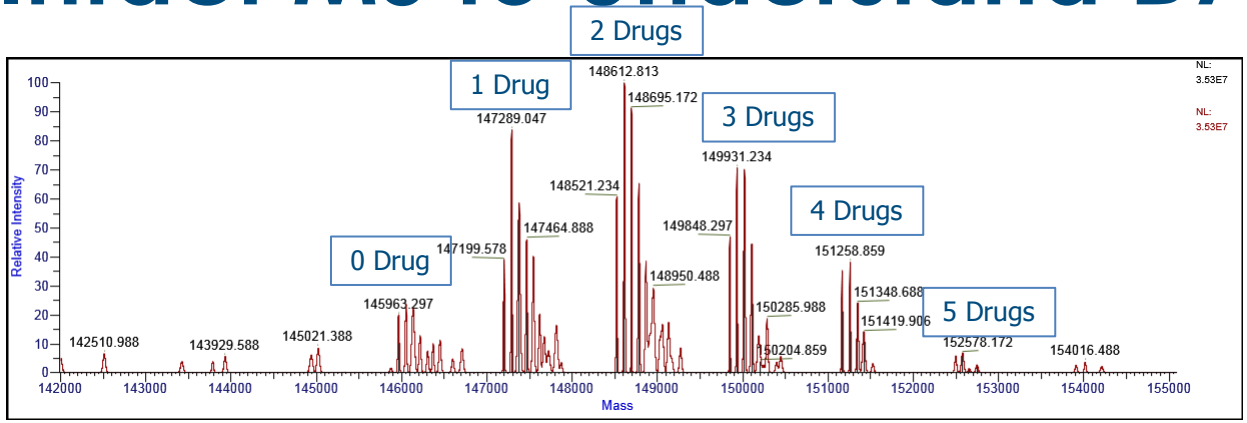


1 month stability data

- LC-MS DAR values are consistent across the series suggesting good stability of the drug product, while UV DAR decreases
- DAR values from the two techniques converge at the 1M, 25C condition.

Using Intact MS to Understand DAR

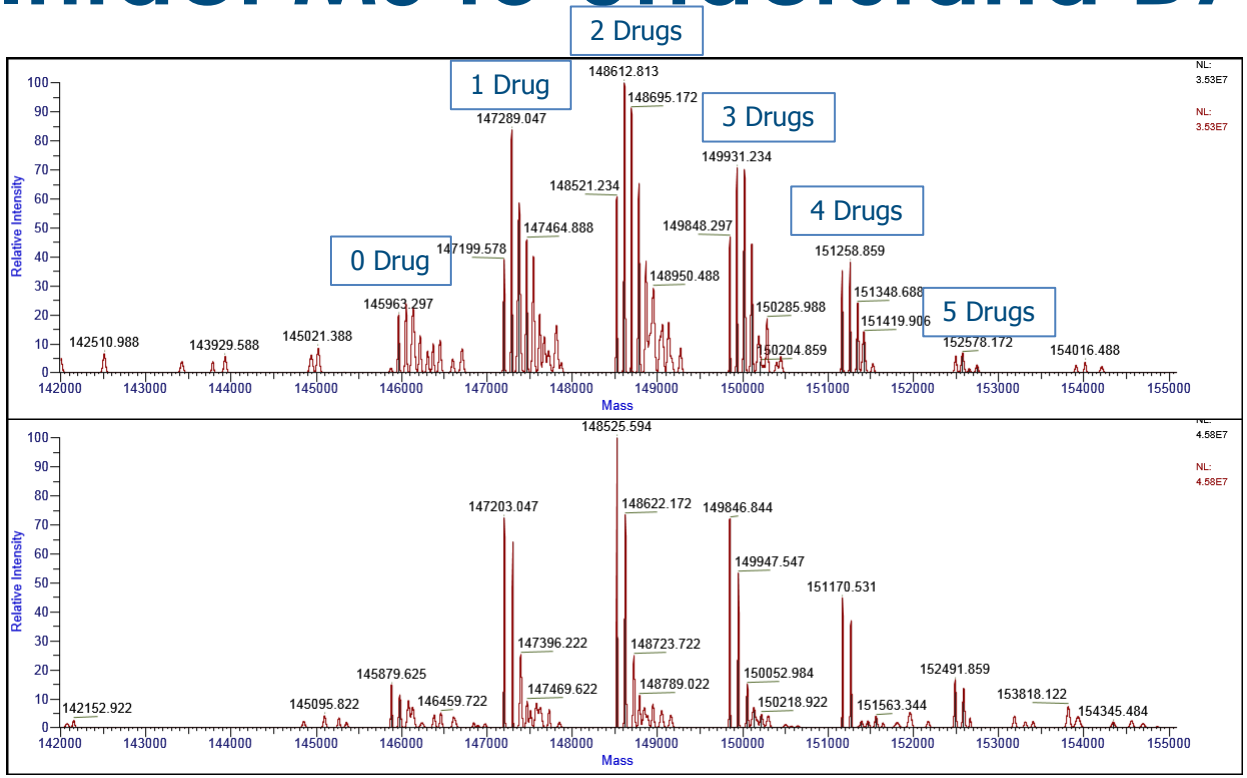
T=0



Using Intact MS to Understand DAR

T=0

7 Days/25°C

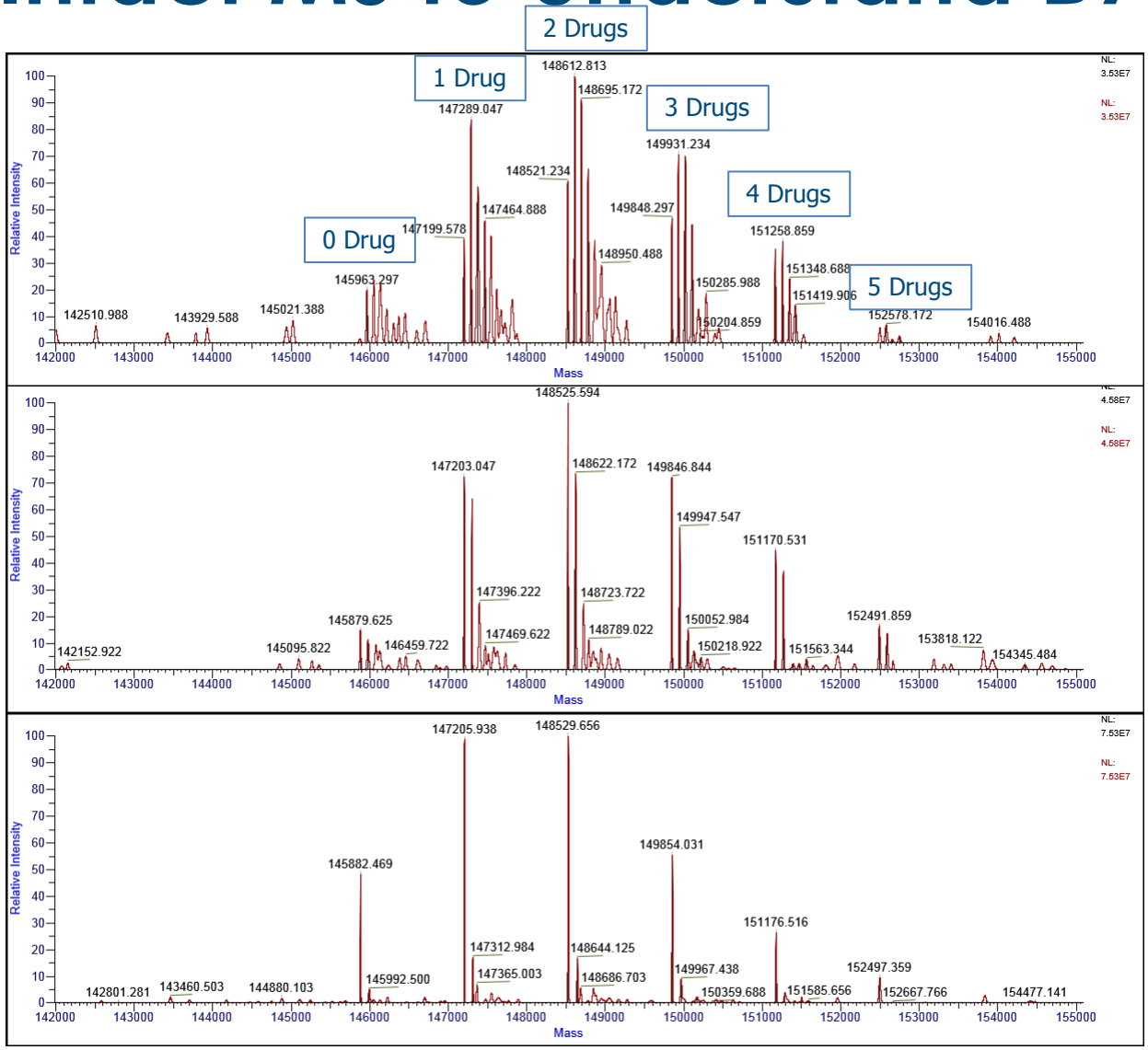


Using Intact MS to Understand DAR

T=0

7 Days/25°C

42 Days/40°C



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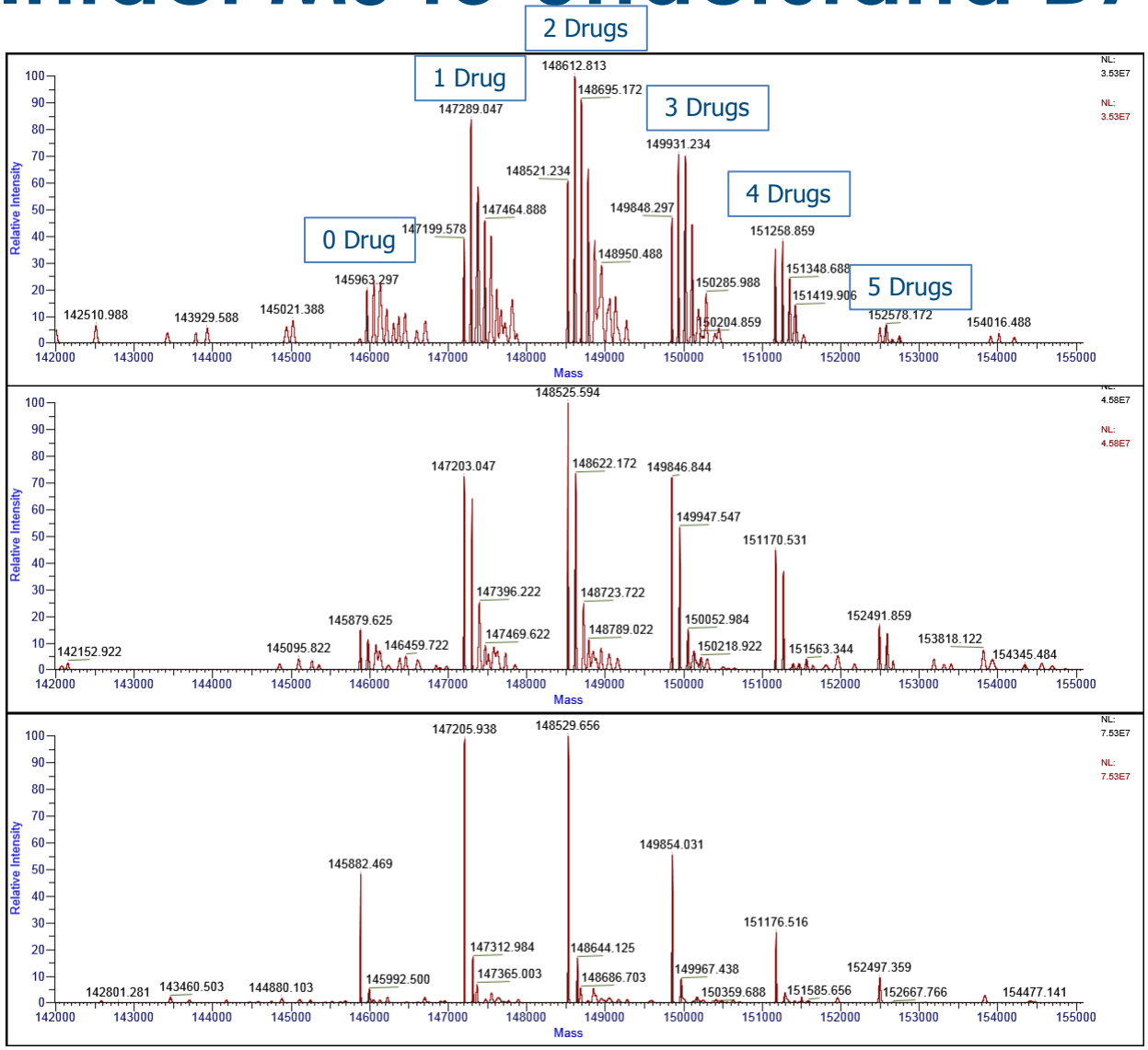
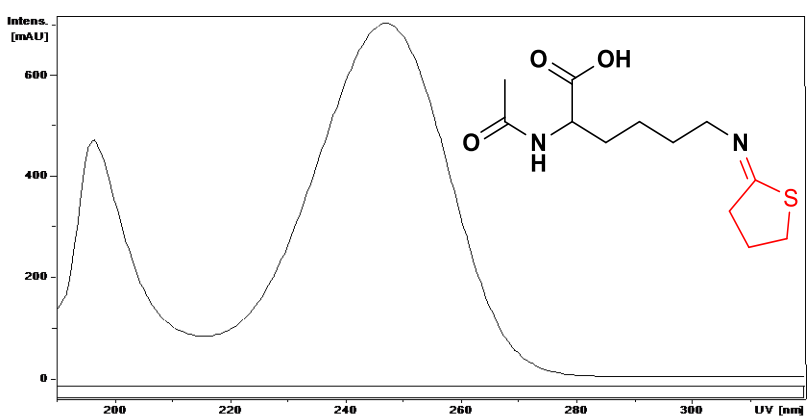
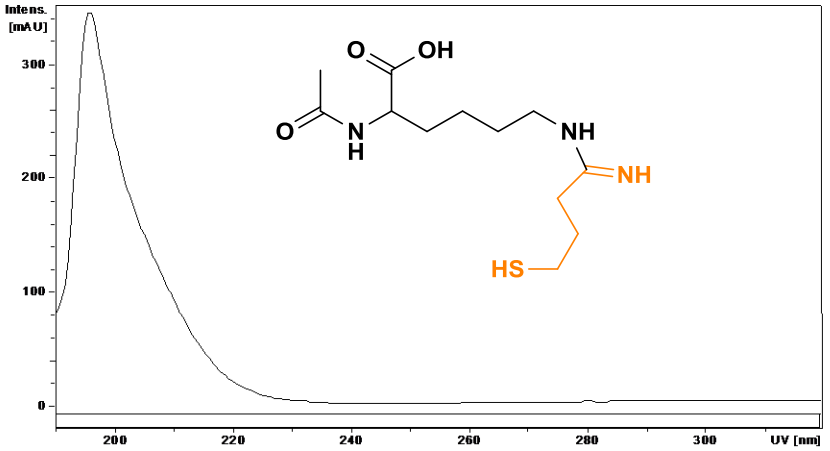


Decreasing Cyclized 2-IT Content

Explaining the DAR Discrepancy

- NAc lysine based model systems were used to show that cyclized 2-IT residues have a similar λmax as the payload (~245 vs 252 nm)





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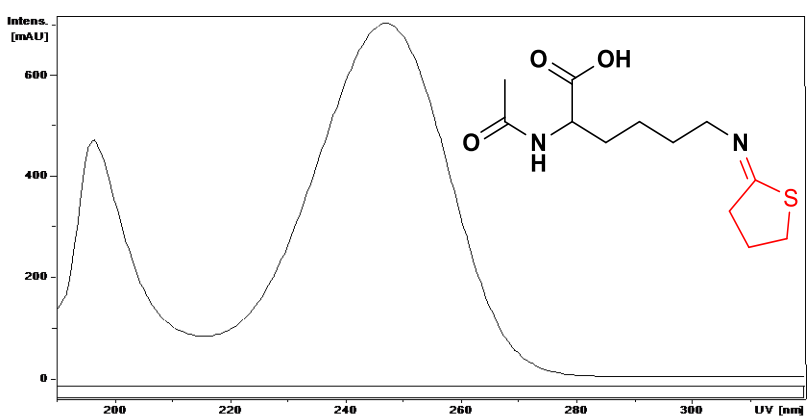
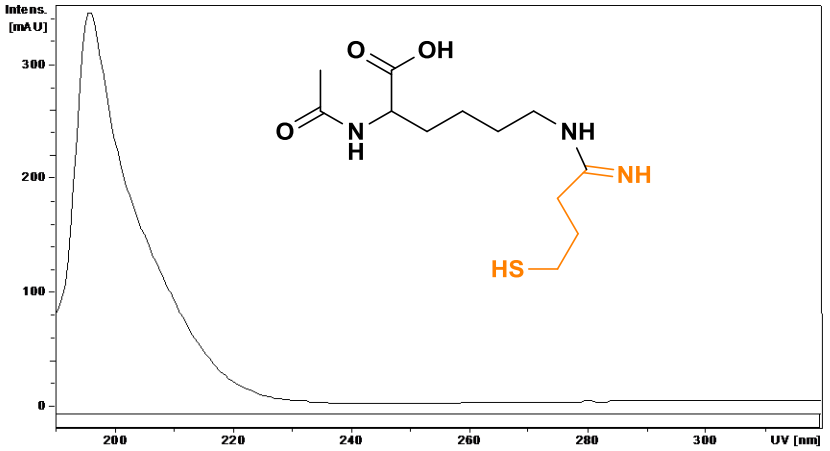
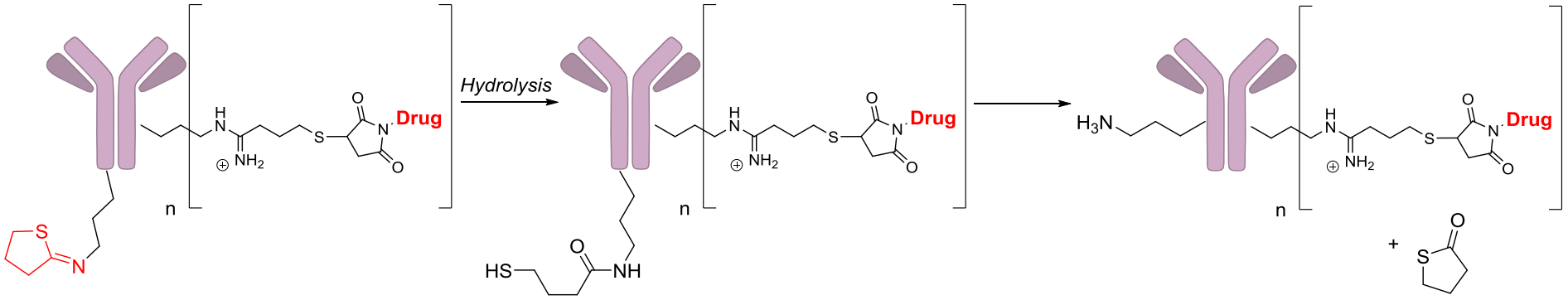




- These residues can open under stressed conditions resulting in apparent "DAR loss" by UV



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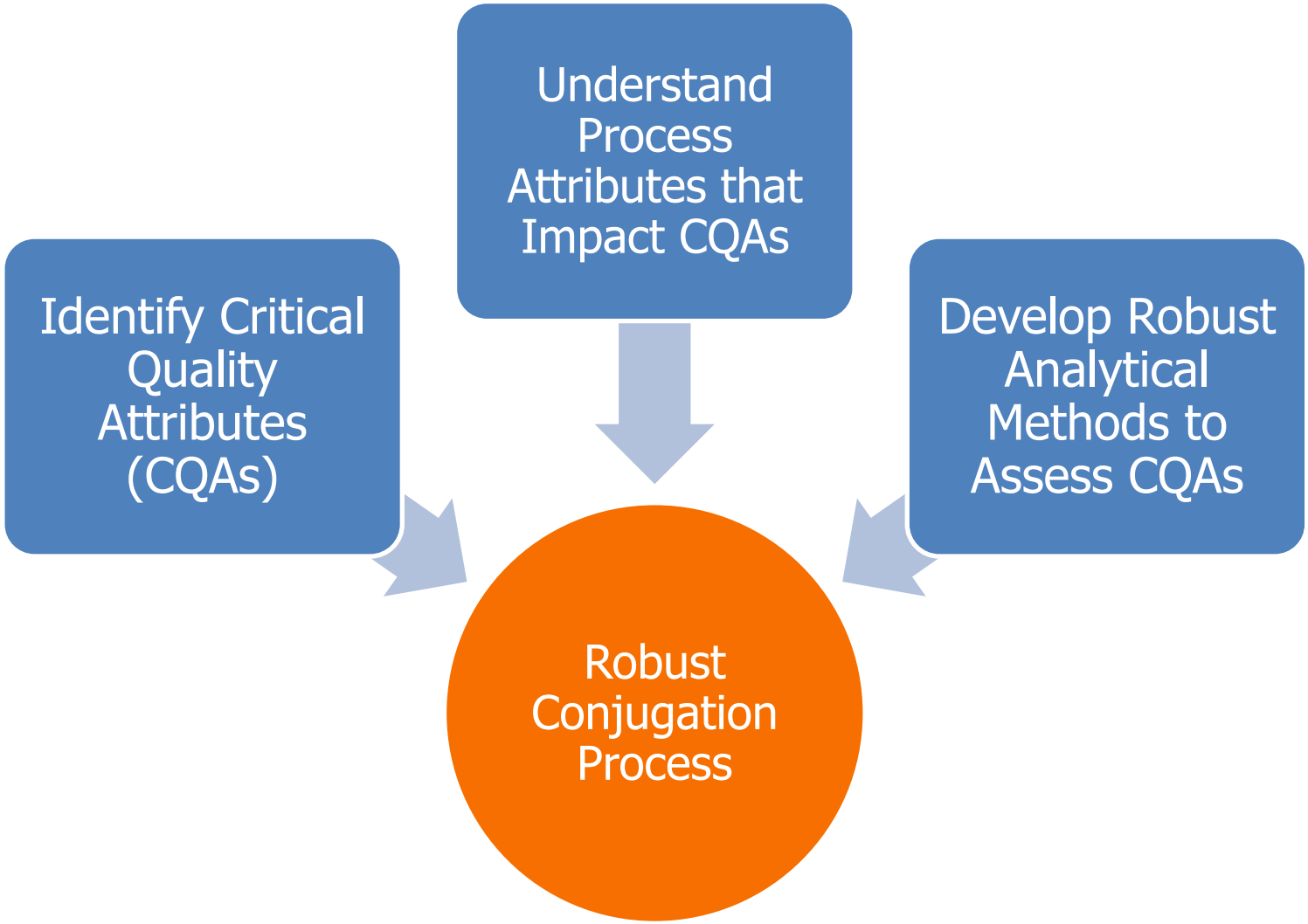

Acknowledgements

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