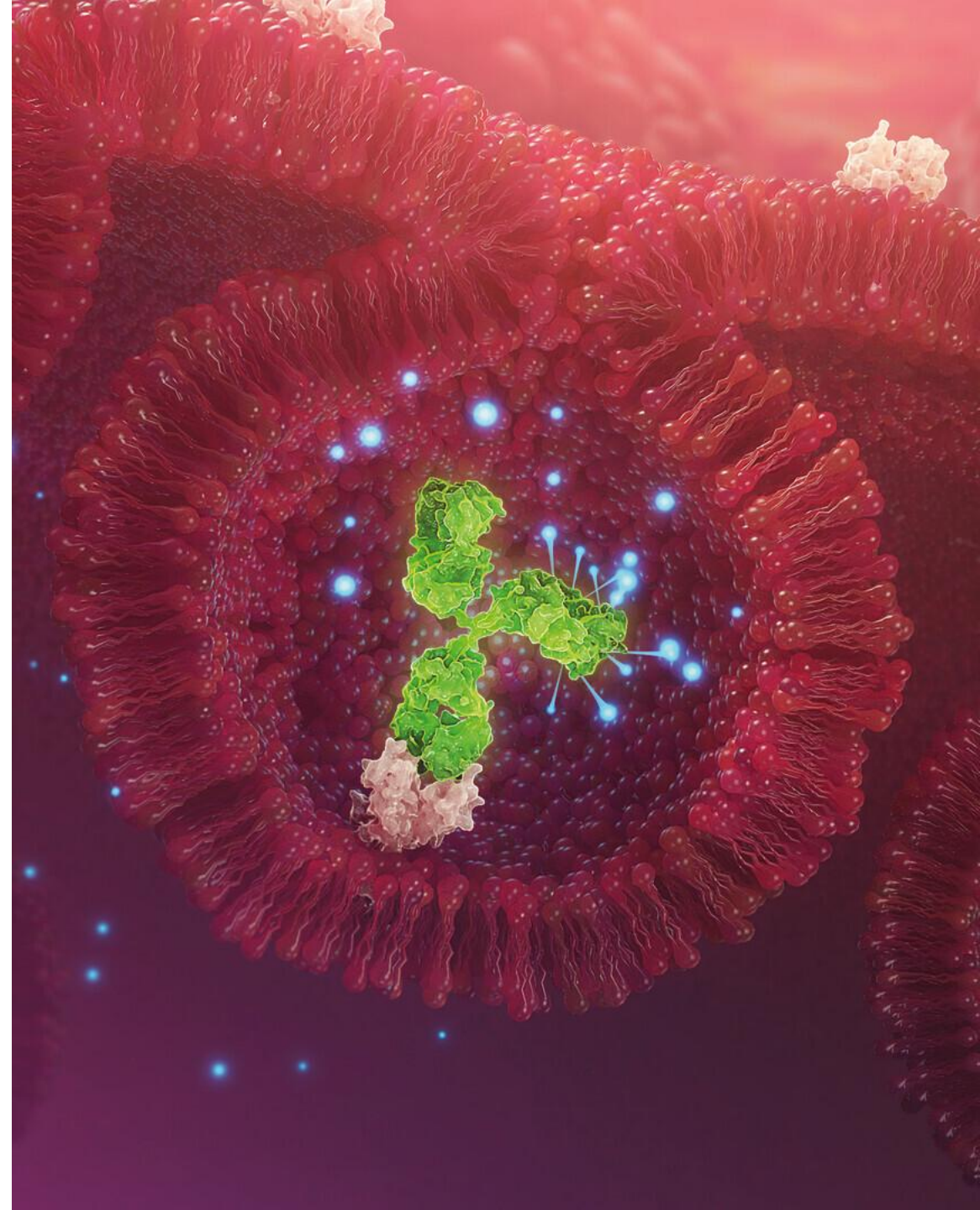




# Charge Variant Control Strategy for Late-Stage Antibody Drug Conjugates

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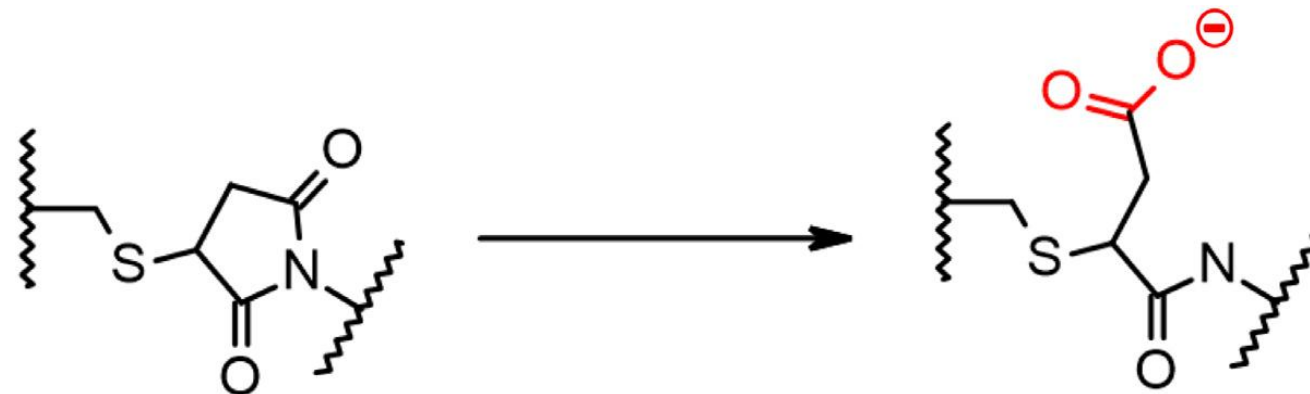


# Background

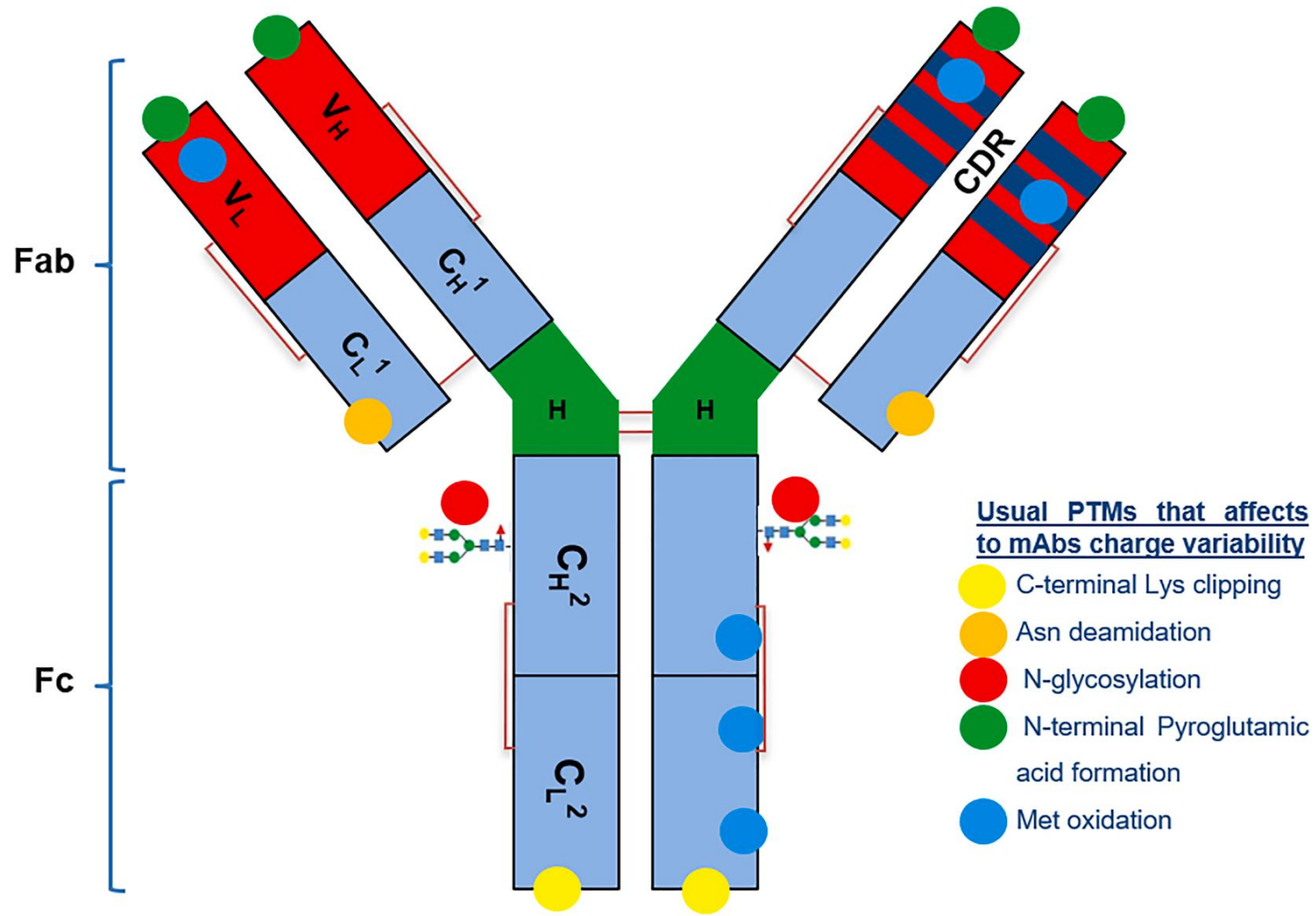


# ADC Conjugation

- Many antibody drug conjugate (ADC) drugs on the market and in clinical trials are conjugated at a cysteine residue and contain a succinimide-thioether functional group
- One of the possible degradation pathways of this succinimide-thioether functional group is irreversible ring hydrolysis to form a negatively-charged carboxylate functional group
- While the ring hydrolysis has been reported to be beneficial in vivo by increasing serum stability, it can have a significant impact on the charge profile



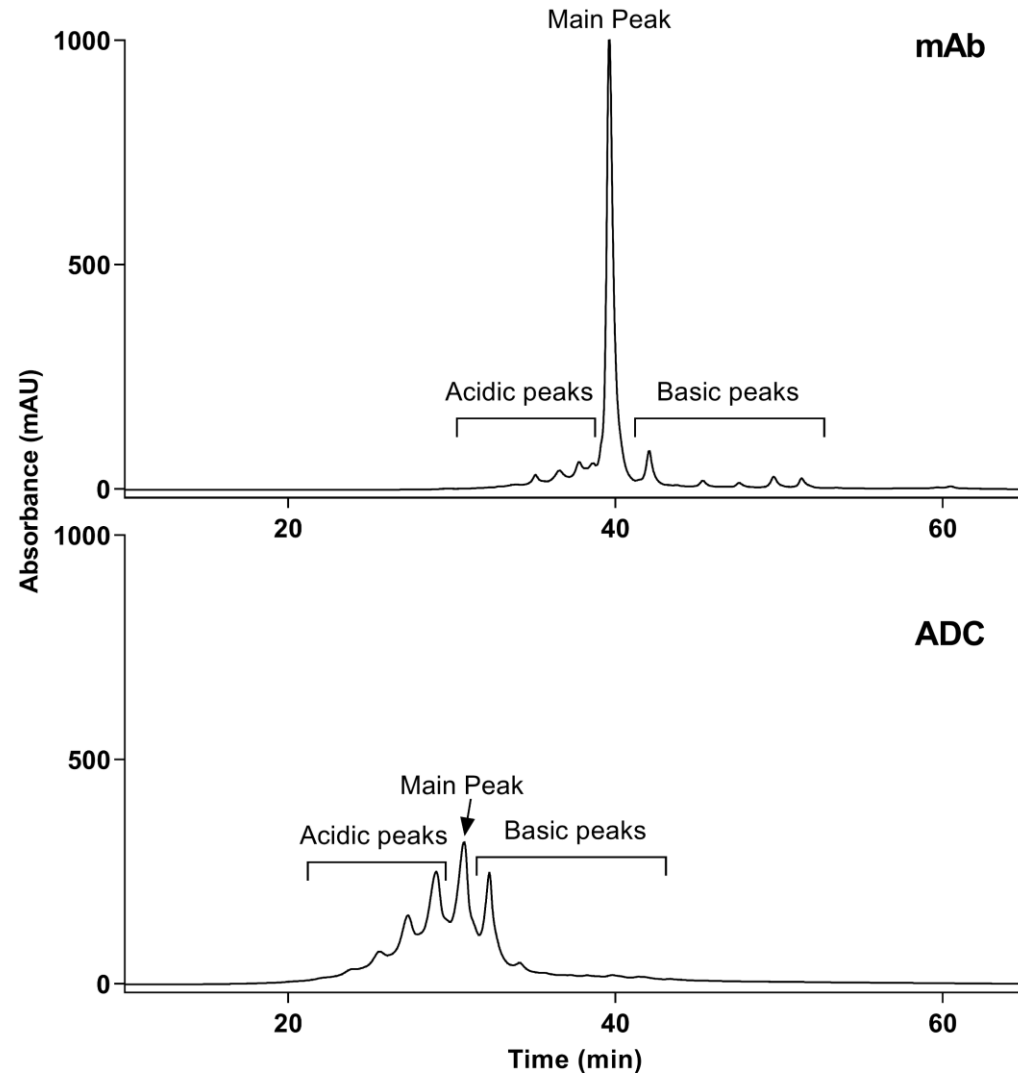
# What's In A mAb Charge Profile?



Martínez-Ortega, et. al., J Chromatogr B Analyt Technol Biomed Life Sci, 2022



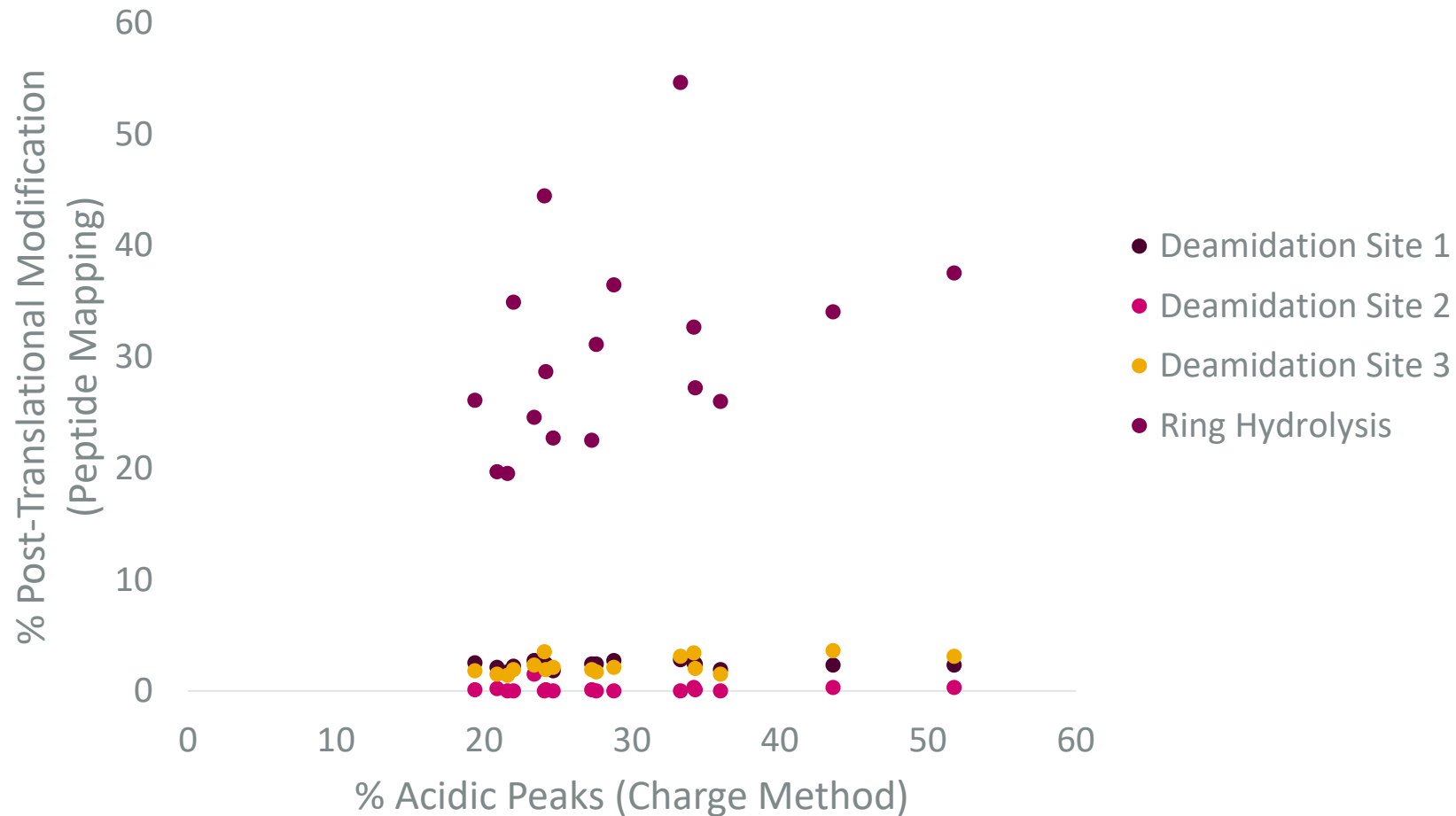
# What's In A (mAb vs. ADC) Charge Profile?



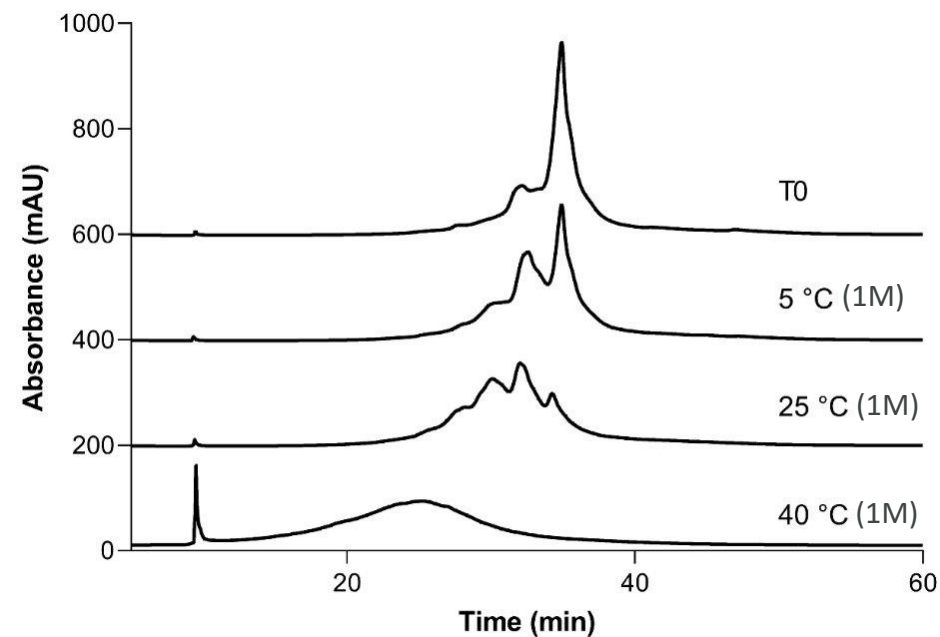
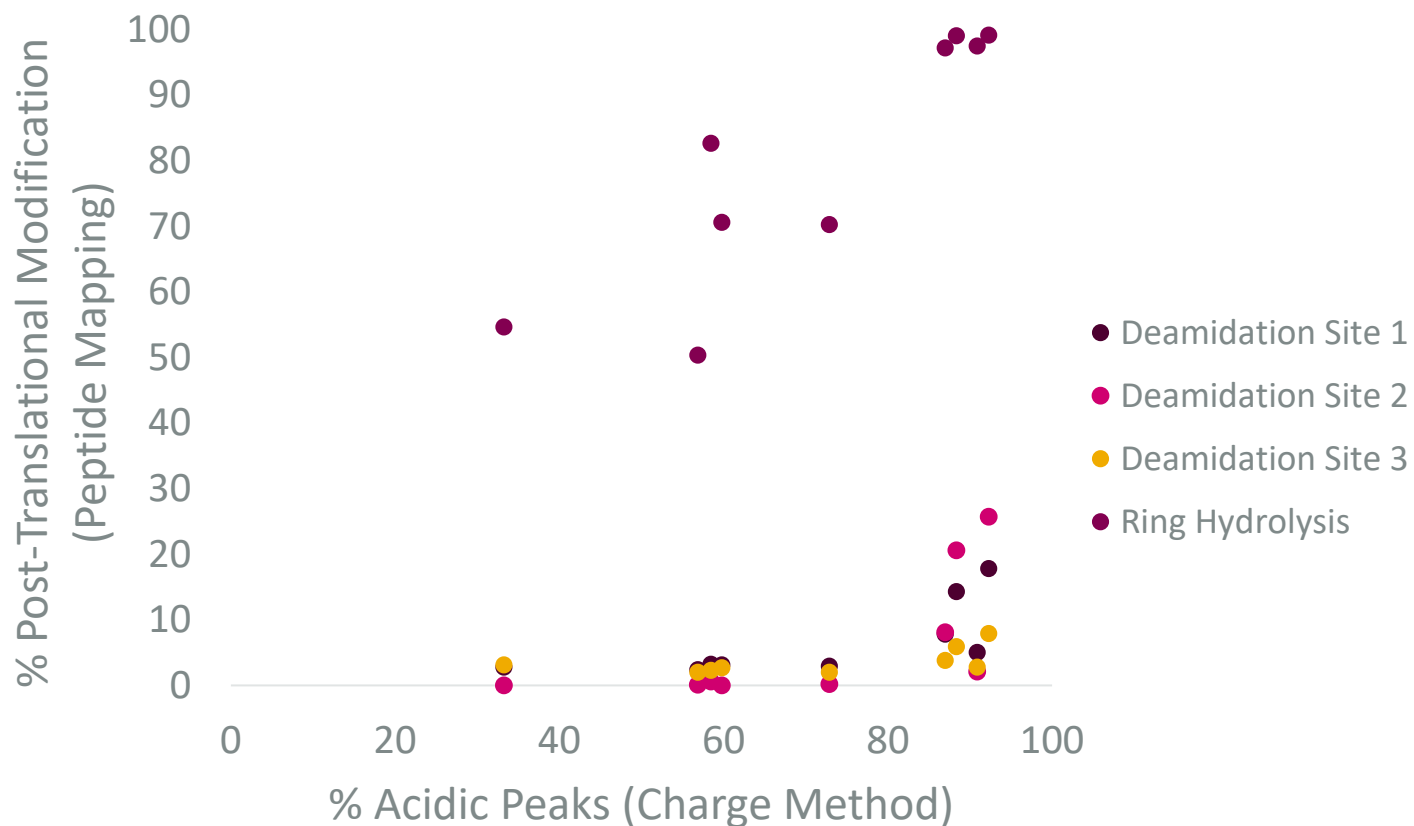
- Because the ring hydrolysis forms a negatively-charged carboxylate functional group, the ADC charge profile shifts to the acidic side, causing a broader charge distribution and increased acidic species when compared to the mAb
- The complex charge profile of the ADC makes protein modifications difficult to reliably measure and assess for any changes



# Changes in % acidic peaks seen at ADC release correlates with variability in succinimide ring hydrolysis rather than deamidation



# Increase in deamidation under thermal stress in the ADC are masked by the charge variants from the succinimide ring hydrolysis



Webb, et. al., J Pharm Sci, 2024

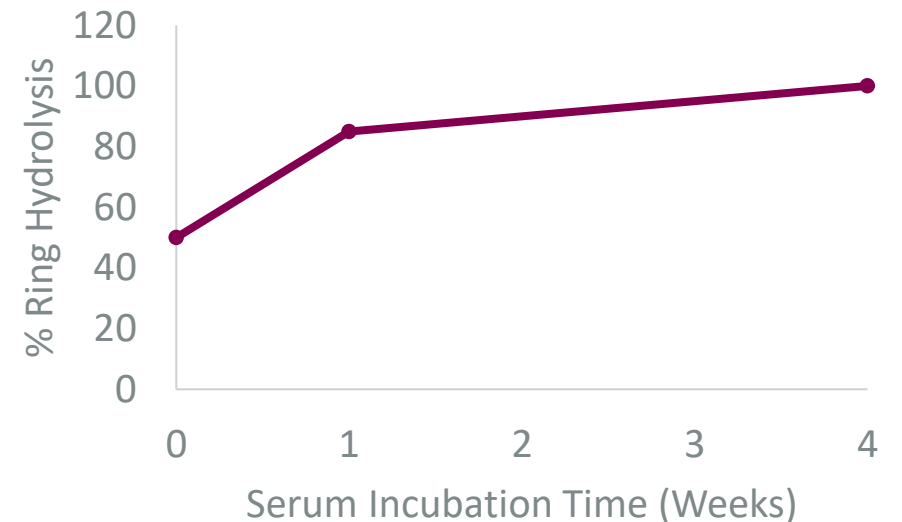
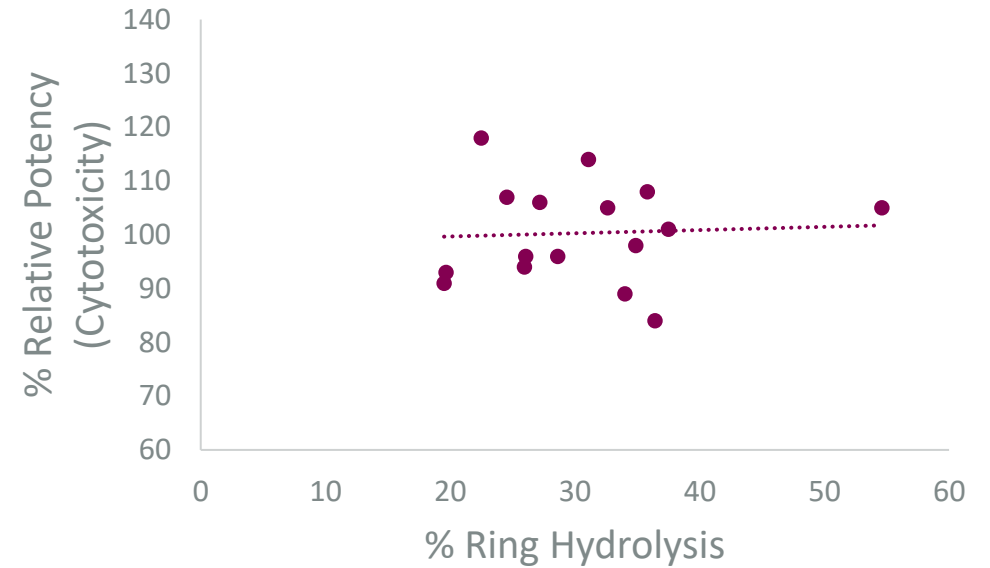


Understanding the relationship between charge variant profiles and the impact on safety and efficacy

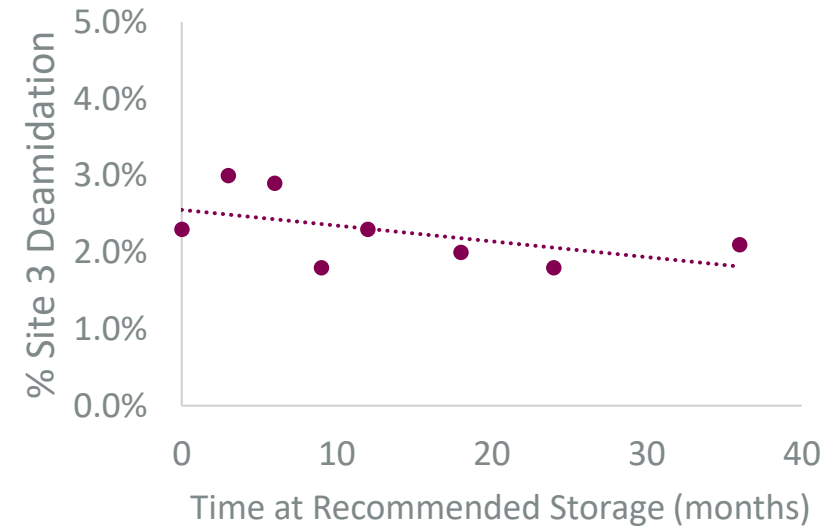
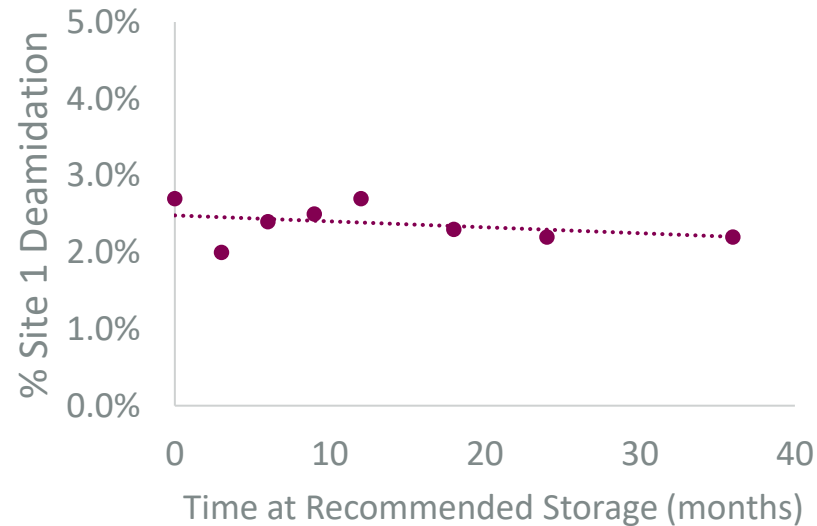
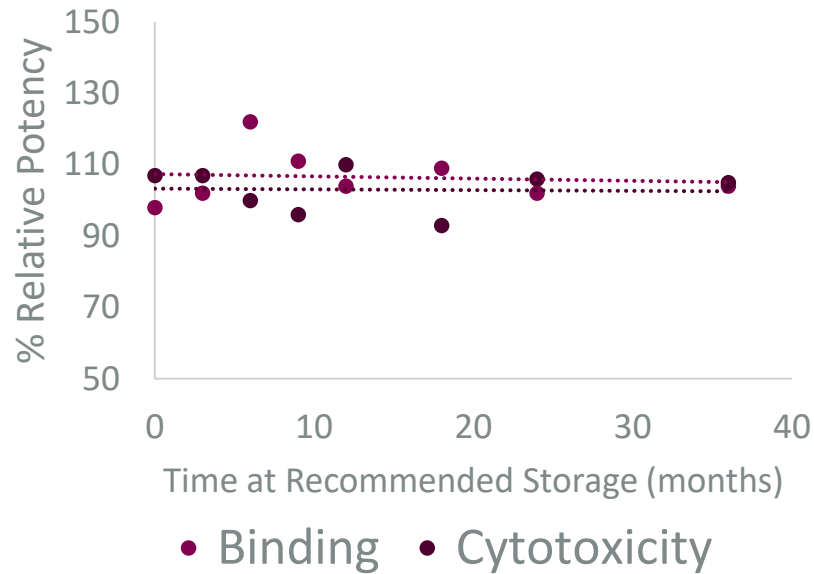


# Succinimide ring hydrolysis does not impact safety or efficacy of the ADC

- Ring hydrolysis has been demonstrated in the literature to:
  - Not impact cytotoxicity
  - Be beneficial in vivo as it prevents nonspecific deconjugation of the drug-linker from the ADC
- Succinimide ring hydrolysis has no correlated impact on potency for our ADC
- Ring hydrolysis happens quickly in vivo, so levels at release are inconsequential



# No changes in potency or protein-related charge variants are observed under recommended storage conditions for the ADC



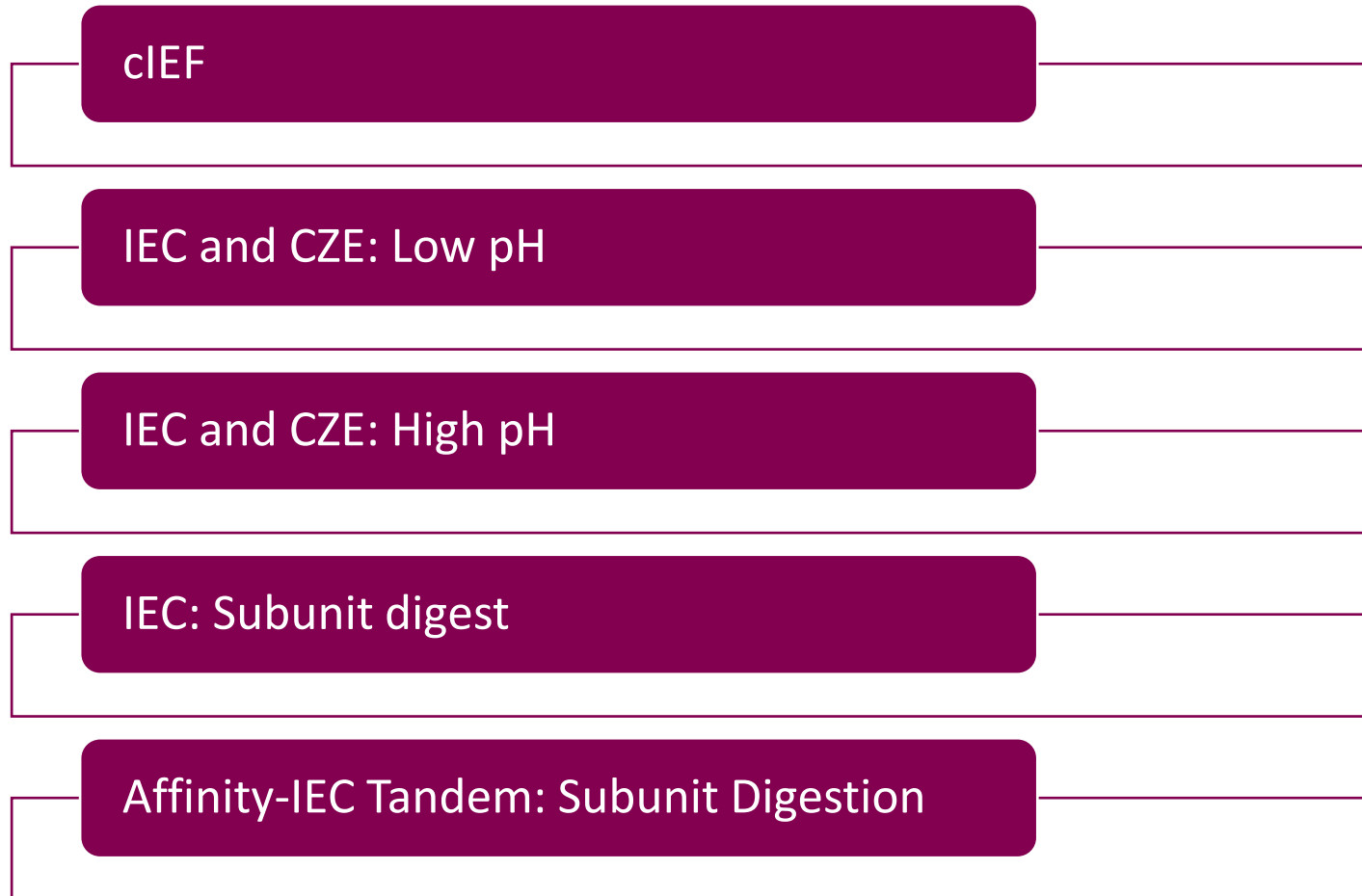
- Note: Results for Deamidation Site 2 not shown as results are all < LOQ



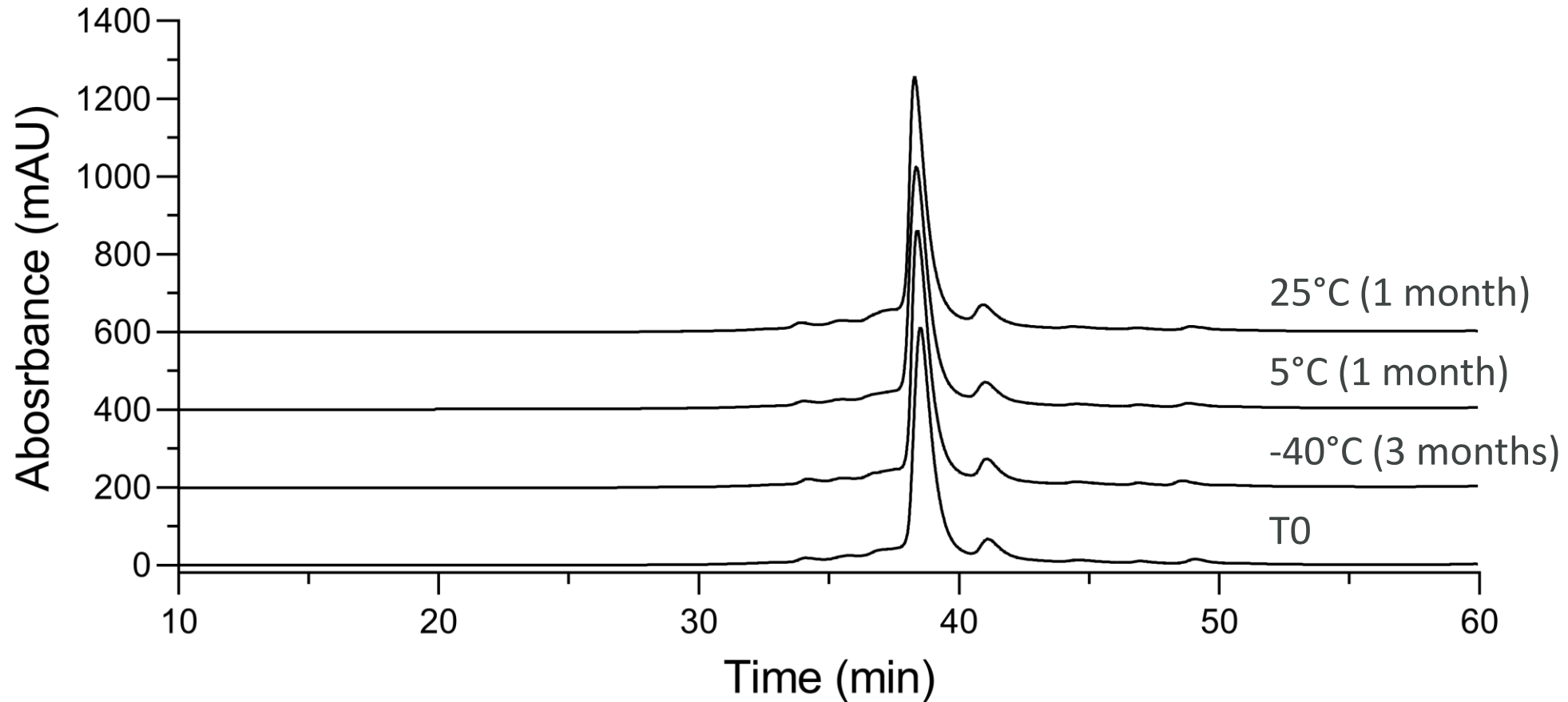
How can we control protein-related charge variants?



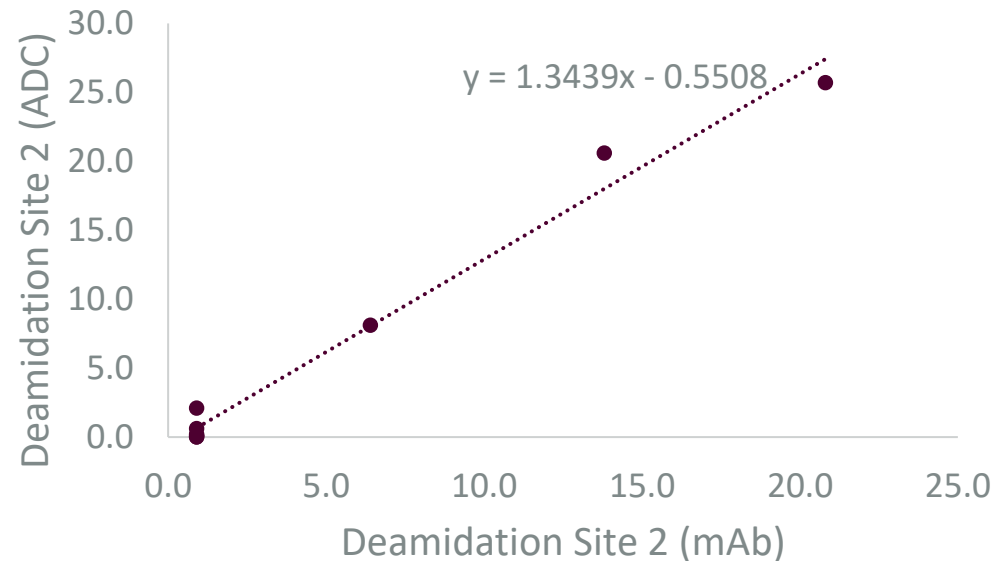
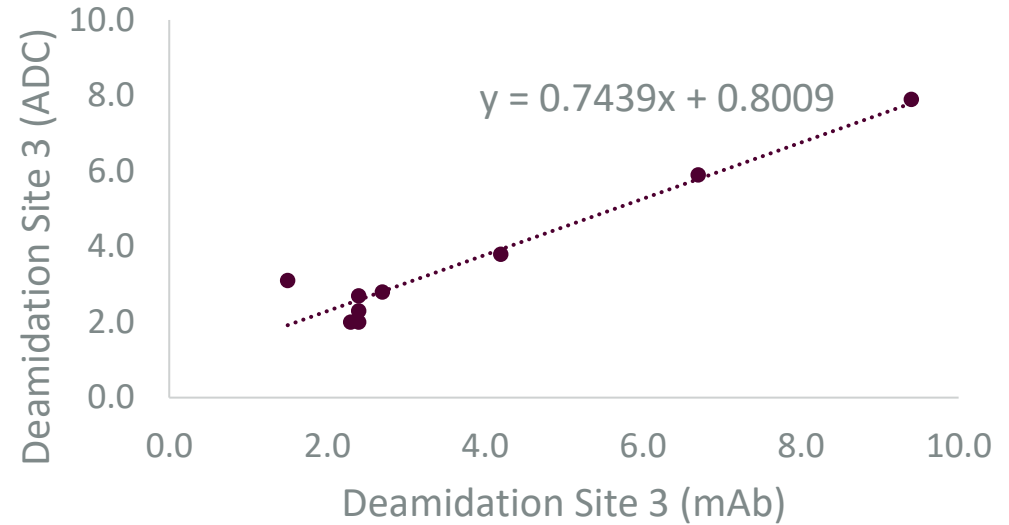
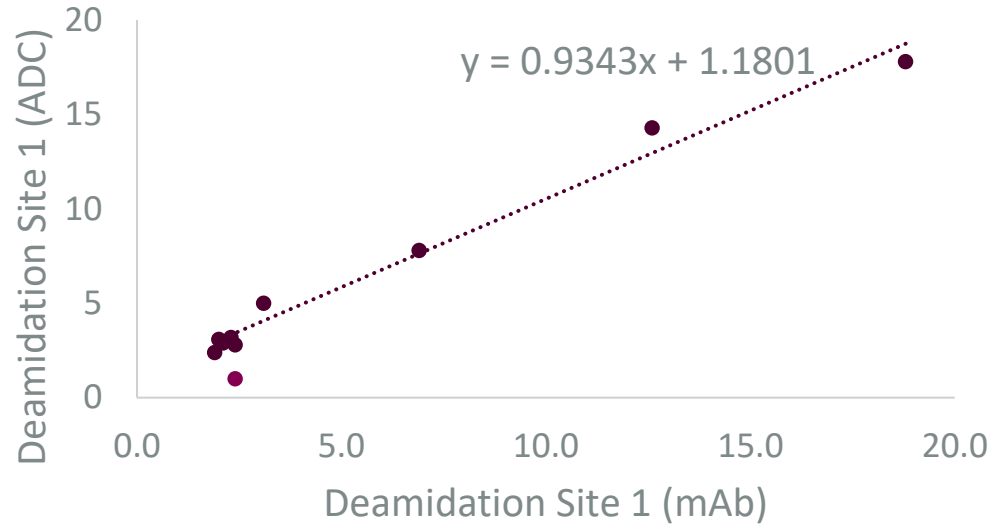
The implementation of a charge assay for release and stability monitoring of this ADC is not possible due to impact of the drug-linker on the charge profile.



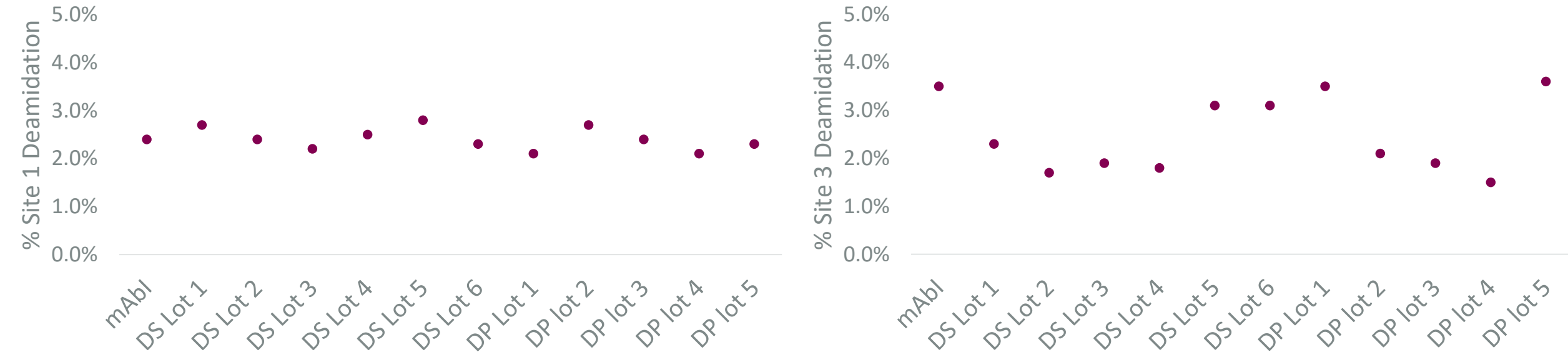
# The mAb charge profile is consistent at release and under recommended and accelerated storage conditions



Similar rates of deamidation are seen in the mAb and ADC under accelerated and stressed conditions.



# Neither the conjugation nor the fill finish processes impact protein-related charge variants



- Note: Results for Deamidation Site 2 not shown as results are all < LOQ



# Conclusions

- The development of an adequate charge profile method for quantifying protein-related charge variants is not possible for our ADC molecule due to masking from the succinimide ring hydrolysis
- Succinimide ring hydrolysis is not a critical quality attribute and thus does not need to be monitored
- Charge variant-related critical quality attributes are controlled at the mAb



Thank you!

