Characterization of ADCs in Serum and Formulation Buffer

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Biologics Discovery @ JNJ Innovative Medicine
Multispecifics and ADCs Characterization

Johnson&Johnson
Innovative Medicine

For CASSS 2024

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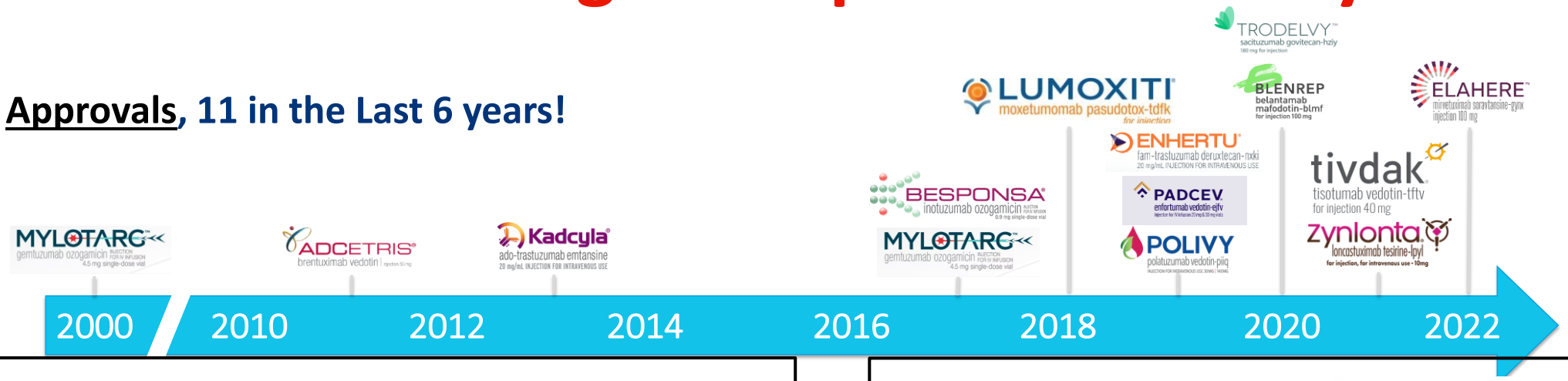
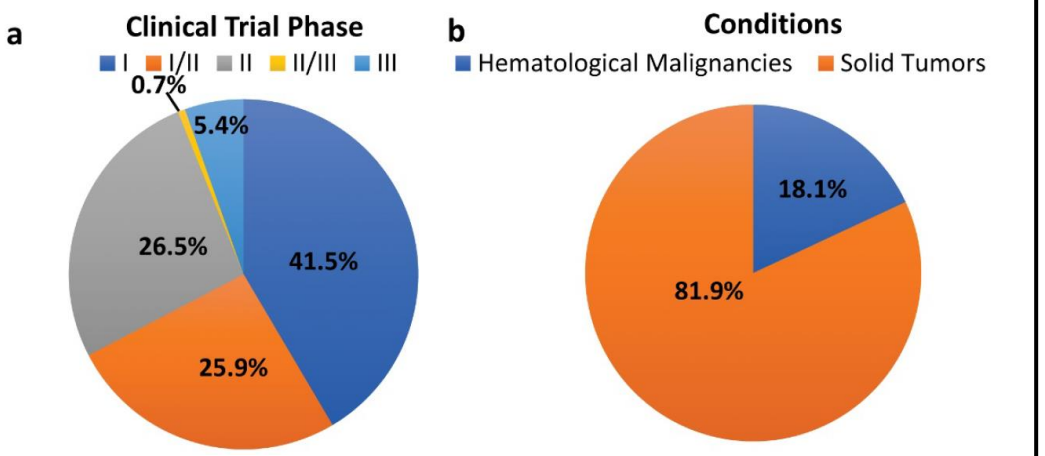
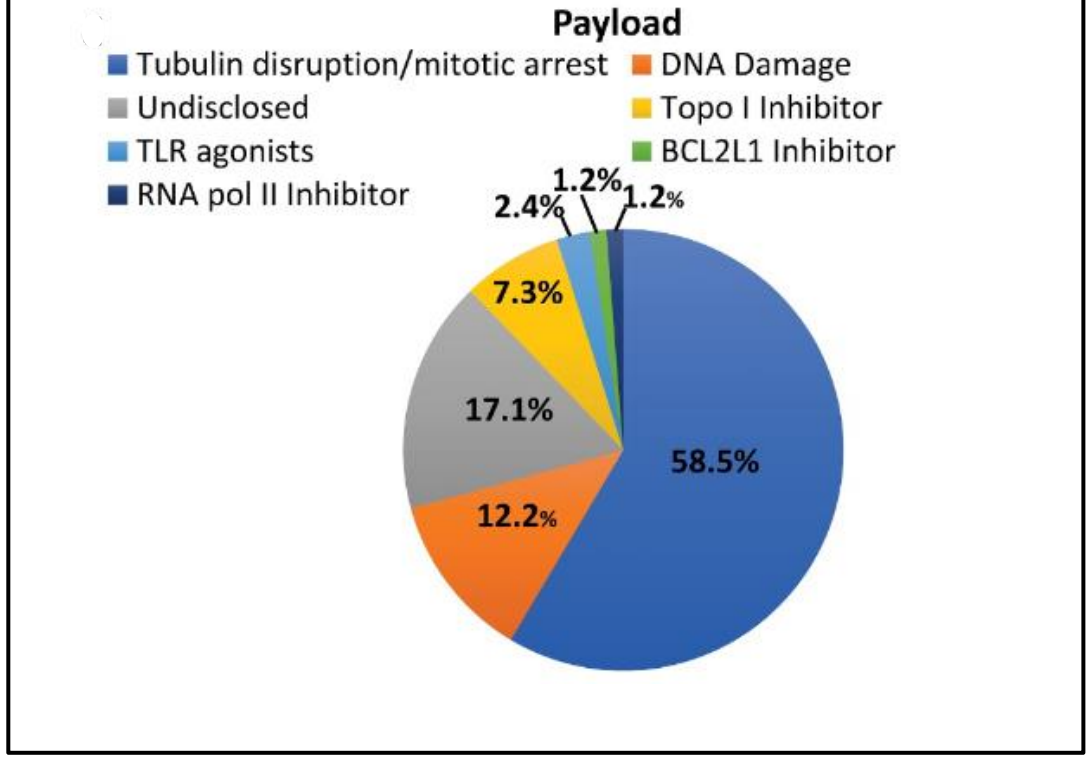
ADCs – Fast Growing Therapeutic Modality

14 Approvals, 11 in the Last 6 years!





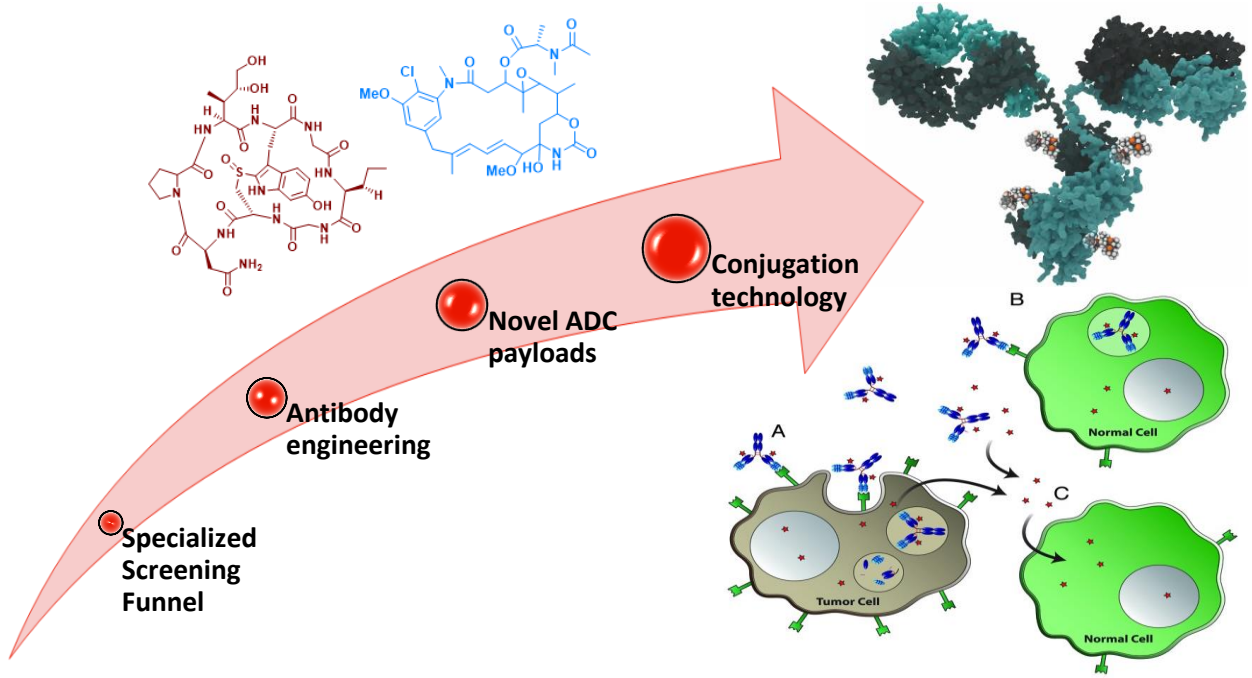
- > 300 clinical trials for ADC (~90% in Phase I/II)
- 133 ADC agents (~20 targets)
- ADCs have shown efficacy against solid tumors

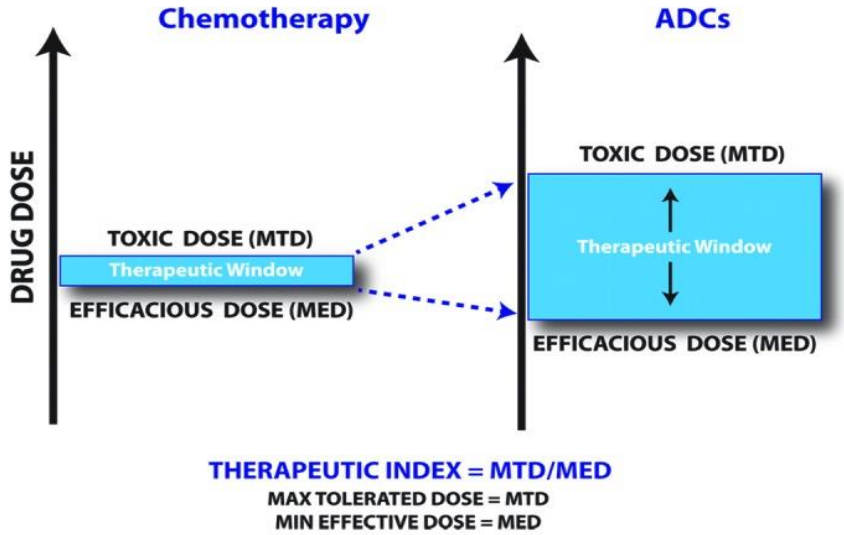


https://axispharm.com/antibody-drug-conjugatesadcs-list-approved-by-fda2000-2022/
Targeting cancer with antibody-drug conjugates: Promises and challenges, mAbs, 13:1, 1951427

ADCs – An Integrated Approach for Enhanced TI

- Major MoA of ADCs: target-dependent killing of tumor cells expressing higher than normal levels of target antigen.
- Target binding specificity of ADCs expands therapeutic windows by lowering the minimum effective dose (MED) and increasing the maximum tolerated dose (MTD).
- Free drugs can be released by ADC catabolism or by unstable labile linkers in the plasma, resulting in target-independent toxicity.
- Biophysics characterizations with fit-for-purpose analytics are critical for the detailed assessments of ADCs developability and payload-linker stability to advance novel protein-based therapeutics

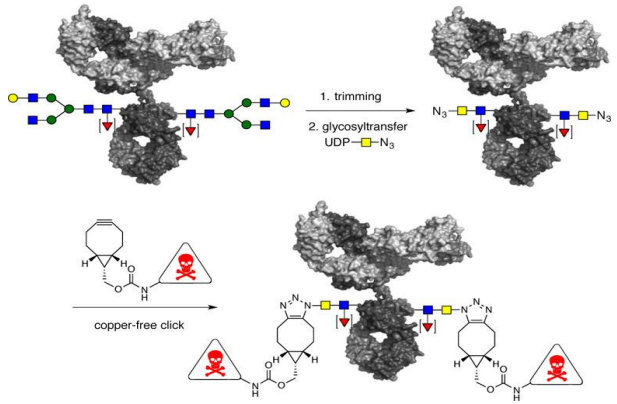




Panowksi et al. MAbs 2014, 6 (1), 34-45

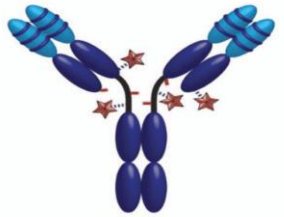
ADCs – Conjugation Chemistry

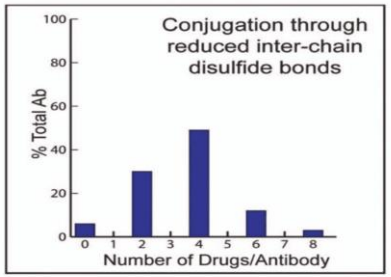
- Via glycan
  - Glycan-based antibody conjugation is achieved by introducing a chemically reactive moiety into the N-glycan, followed by conjugation to a payload carrying a matching chemically reactive group



Bioconjugate Chemistry, 26, 11, 2015, 2233-2242

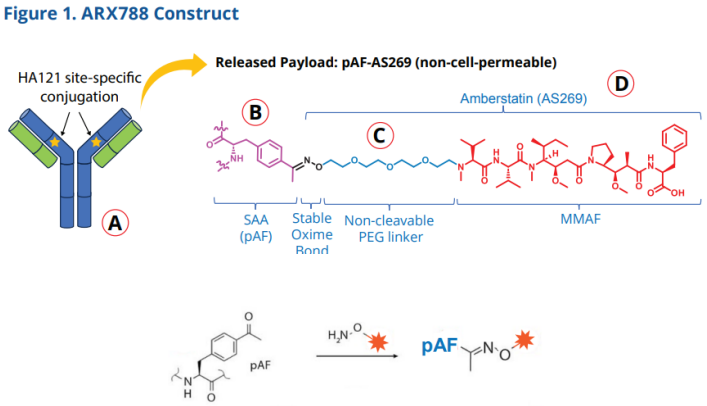
- Via Cys
  - The highly reactive thiol side chains provide an appealing route for conjugating toxic agents to antibodies.





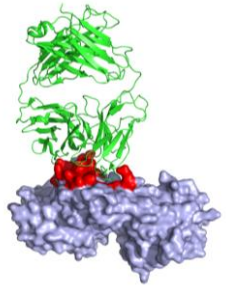
https://www.creative-biolabs.com/adc/cysteine-based-conjugation.htm

- Via unnatural AA
  - payload covalently conjugated to a synthetic amino acid (SAA), para-acetyl phenylalanine (pAF) in CH1 domain of the HC



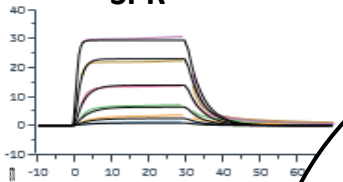
A Comprehensive Biophysical Toolbox for Detailed Characterization of ADC Target Interactions, Developability, and Metabolism

Target binding

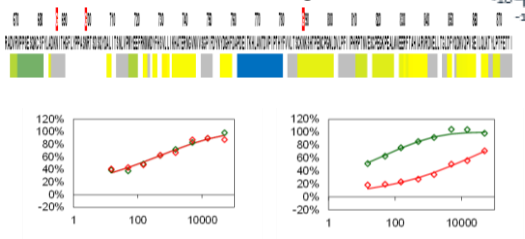


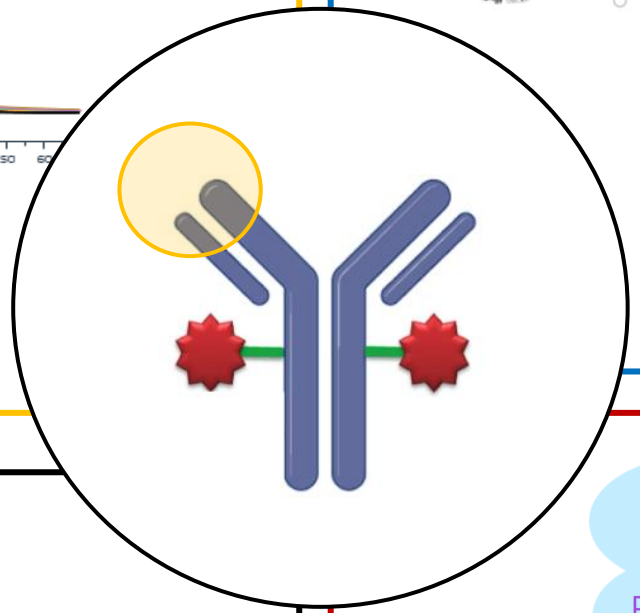
- Binding Epitope (HDX-MS)
- Target binding affinity, cross-species reactivity (SPR)
- Impacts of conjugation and stress on target binding (SPR)
- Non-specific binding/interaction (CIC, SPR)

SPR



HDX-MS

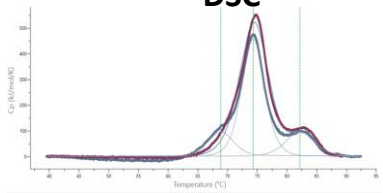




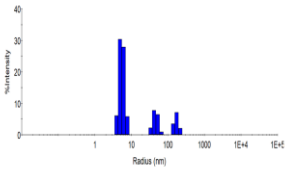
ADC biophysics properties and developability

- pI (cIEF), hydrophobicity (HIC), non-specific binding (CIC)
- Colloidal, physical, chemical, and thermal stability (SEC, AUC, nDSF, DSC, MS)
- Freeze/thaw stability (SEC)
- Serum stability (SEC, MS on DAR change)
- Concentratibility (SEC, DLS, AUC)
- PTM stress stability (SEC, SPR, MS, peptide mapping)

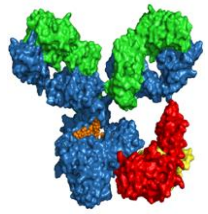
DSC



DLS

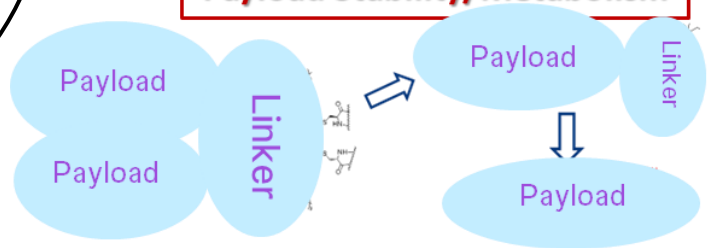


FcR binding



- FcRs, e.g, FcγR binding profile across species
- FcRn binding: conjugated vs. parental (SPR)
- FcγR binding: conjugated vs. parental (SPR)

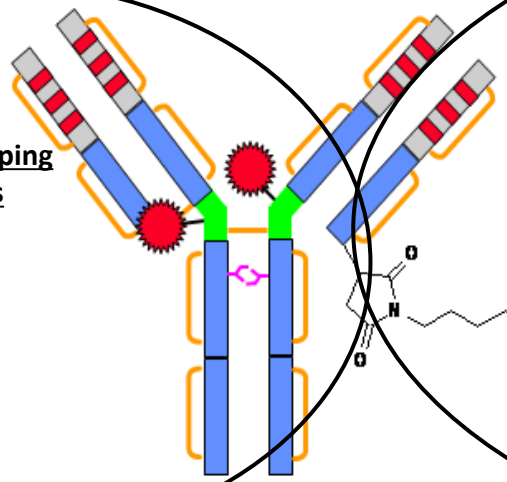
Payload Stability/Metabolism



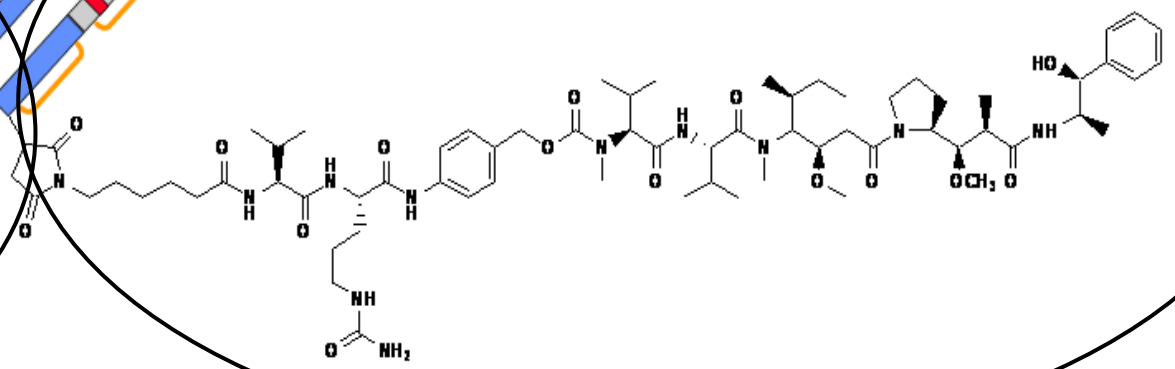
- Free conjugate moiety/payload in stock solution (MS, HIC)
- Conjugate stability under stress, pH, and temperature (MS)
- Conjugate stability at high concentration (MS)
- Conjugate stability in serum/plasma (MS)

Multi-tiered Mass Spec Analytical Approaches Allow for Molecular-level Characterization of ADC

Epitope/Paratope Mapping
Chemical Modifications
Peptide mapping
- Deamidation
- Isomerization
- Oxidation
- Conjugation site



Payload/Linker stability
- Deconjugated payload
- Payload metabolism
- Payload hydrolysis
- Ring opening of maleimide



DAR Characterization
HIC-MS, UV-MS, RP-MS
Clippings
SEC-MS, RP-MS

Higher-order Structure
Epitope/Paratope mapping (HDX-MS, MS-based protein footprinting)
Protein conformational dynamics (HDX-MS)

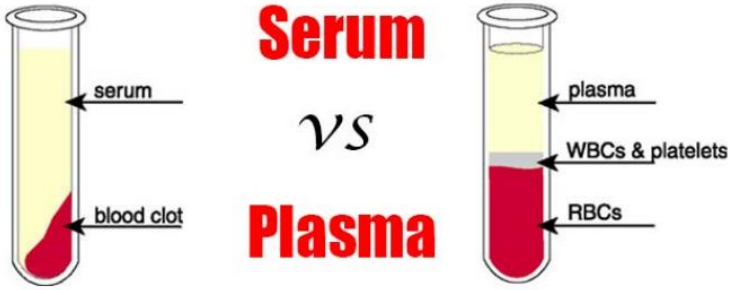
Brentuximab (chIgG1) -Cys-SH (Hinge)

Peptide linker -Cit-Val-

Auristatin E = (4 per IgG)

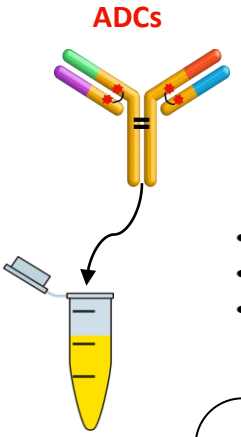
ADC Stability in Serum/Plasma

- ADC stability is extensively studied in formulation buffers
- ADCs could break down differently in serum/plasma from that in buffers
- In vitro serum stability can serve as a guide for in vivo study design and analysis
- Need a platform for evaluating molecule's serum stability by LC-MS early in Discovery
- Create a workflow that can be used to screen serum stability for ≥10 molecules to allow teams to select the molecules with the best stability



https://microbiologyinfo.com/difference-between-serum-and-plasma/

Serum Stability – Exemplary MS Workflow

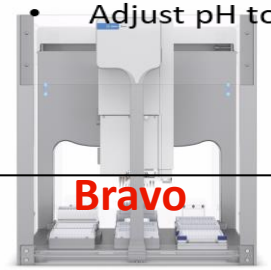


Human, Cyno, Mouse & PBS (0.1 mg/mL)
Incubated @37°C

Day 0
- Prepare samples
- Adjust pH to 7.0
- Collect 50 uL and Freeze @ -80C

Day 2
- Collect 50 uL and Freeze @ -80C
- Adjust pH to 7.0

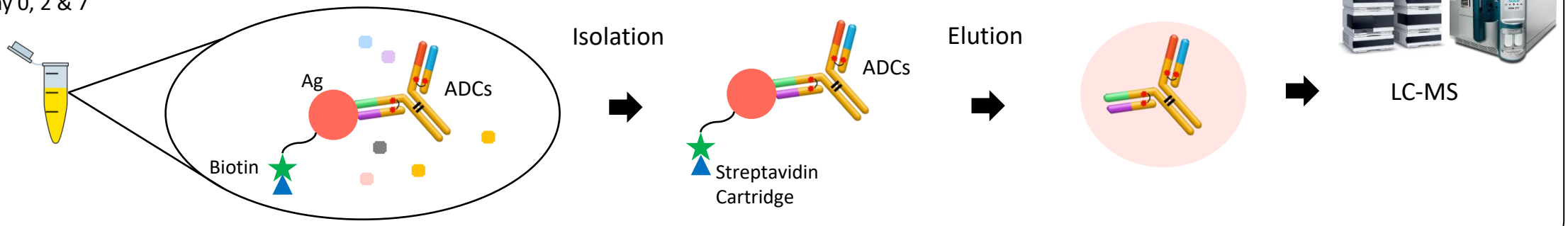
Day 4
- Adjust pH to 7.0



Day 7
- Collect 50 uL and Freeze @ -80C

Immunocapture of ADCs

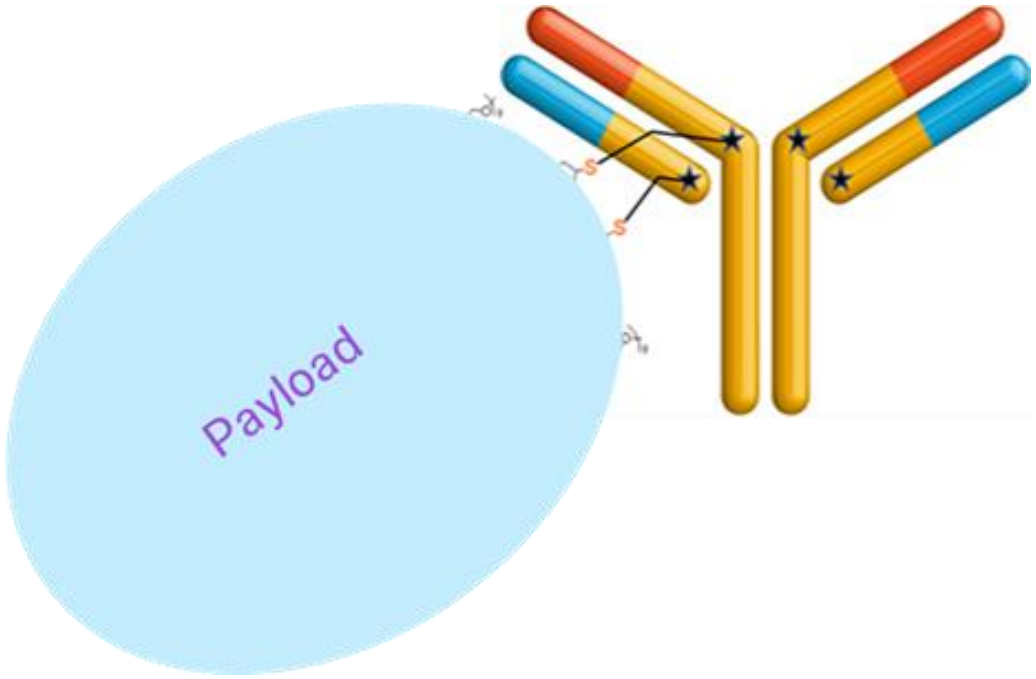
PBS & Serum/Plasma
Day 0, 2 & 7



Key points to consider:

- Maintain serum pH throughout the study
- EDTA helps to maintain pH but may prevent or promote payload loss

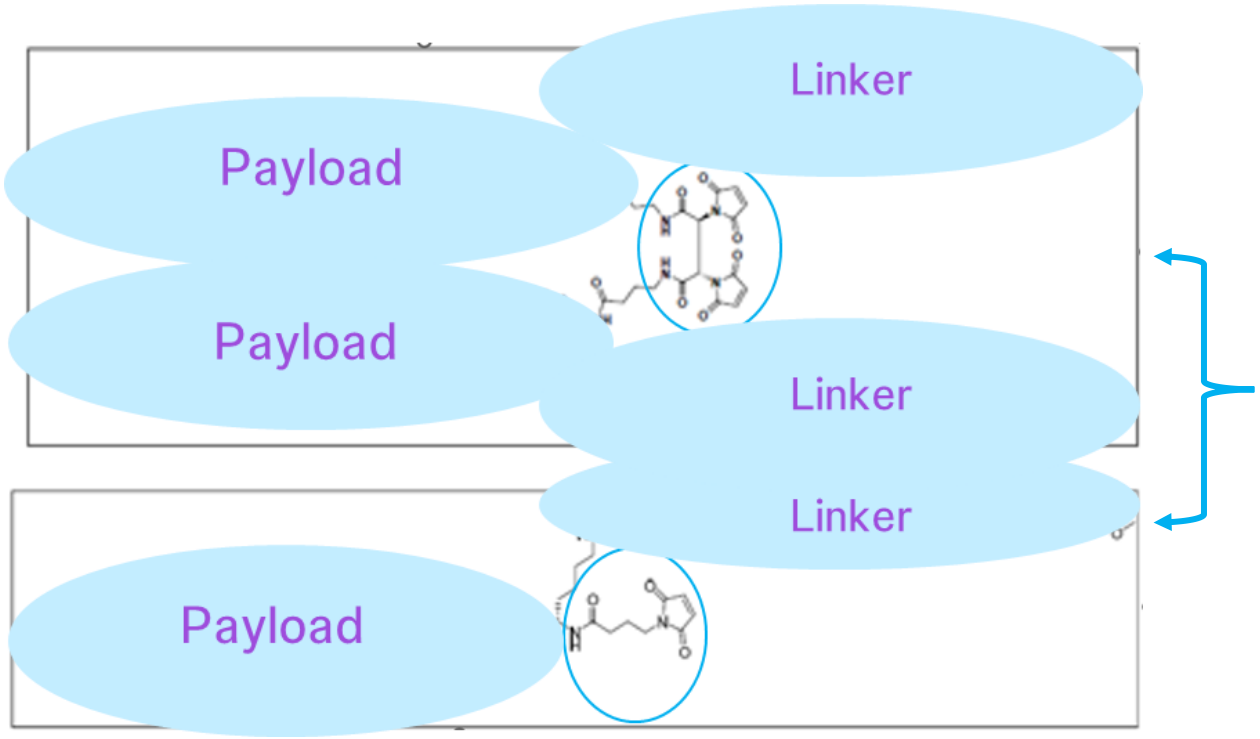
Case Study #1:
Bridged vs Un-bridged linker



Conjugation on interchain Cys

- Tublysin payload – bridged vs non-bridged
- IgG1 kappa
- Fc Silent

Tubulysin conjugates: Bridged vs Un-bridged linker



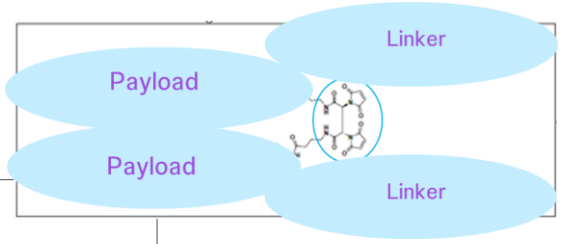
How different in serum stability?

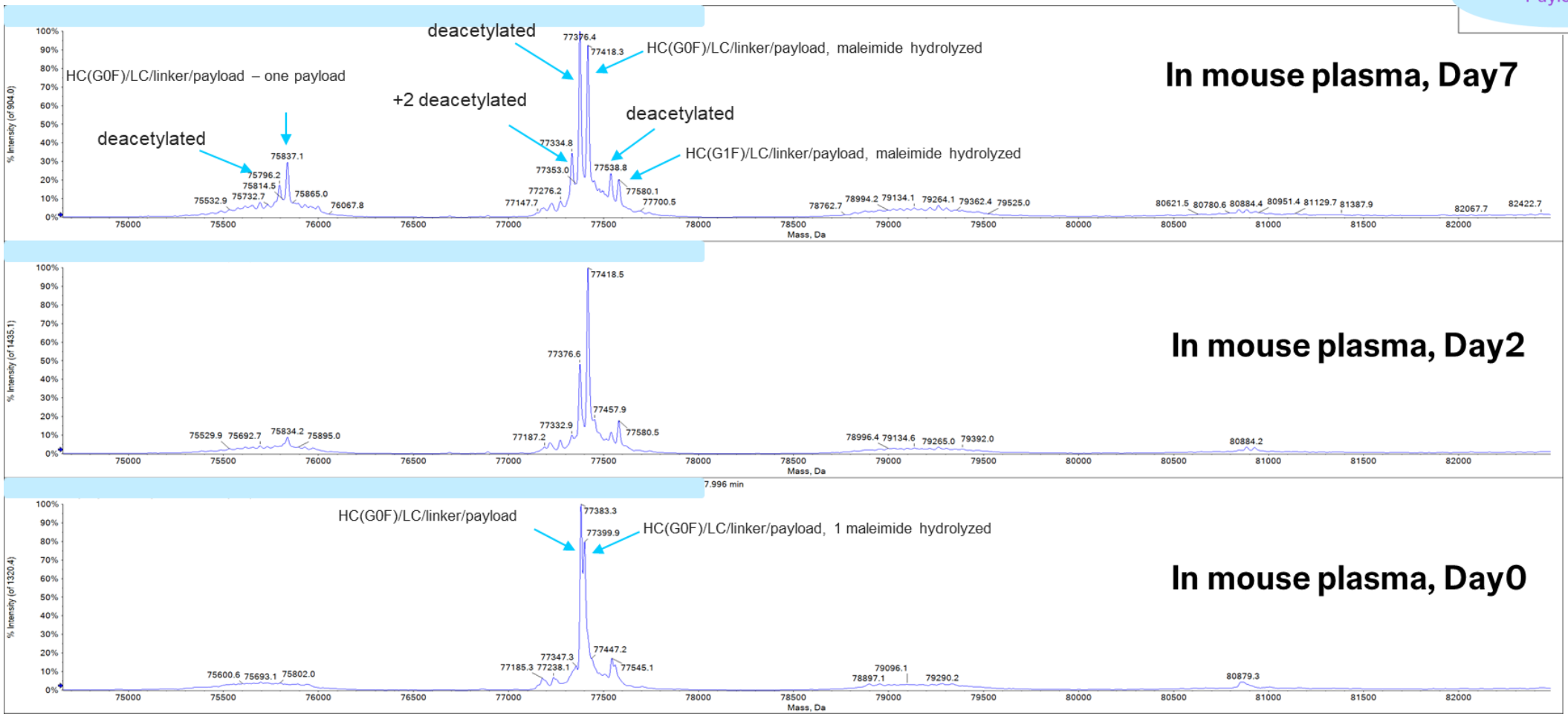
Potential degradation pathways:

- Linker-payload loss: transferred to serum albumin https://pubs.acs.org/doi/pdf/10.1021/acs.analchem.6b00976
- Deacetylation, conversion of –OAc to –OH (could result in >100 fold less active species; https://pubs.acs.org/doi/pdf/10.1021/acsmedchemlett.6b00195)
- Maleimide hydrolysis, not expected to have impact on payload activity

ADC Stability with Bridged Linker - In Mouse Plasma

Maleimide hydrolysis, deacetylation, and payload loss increase with the duration of incubation at 37°C

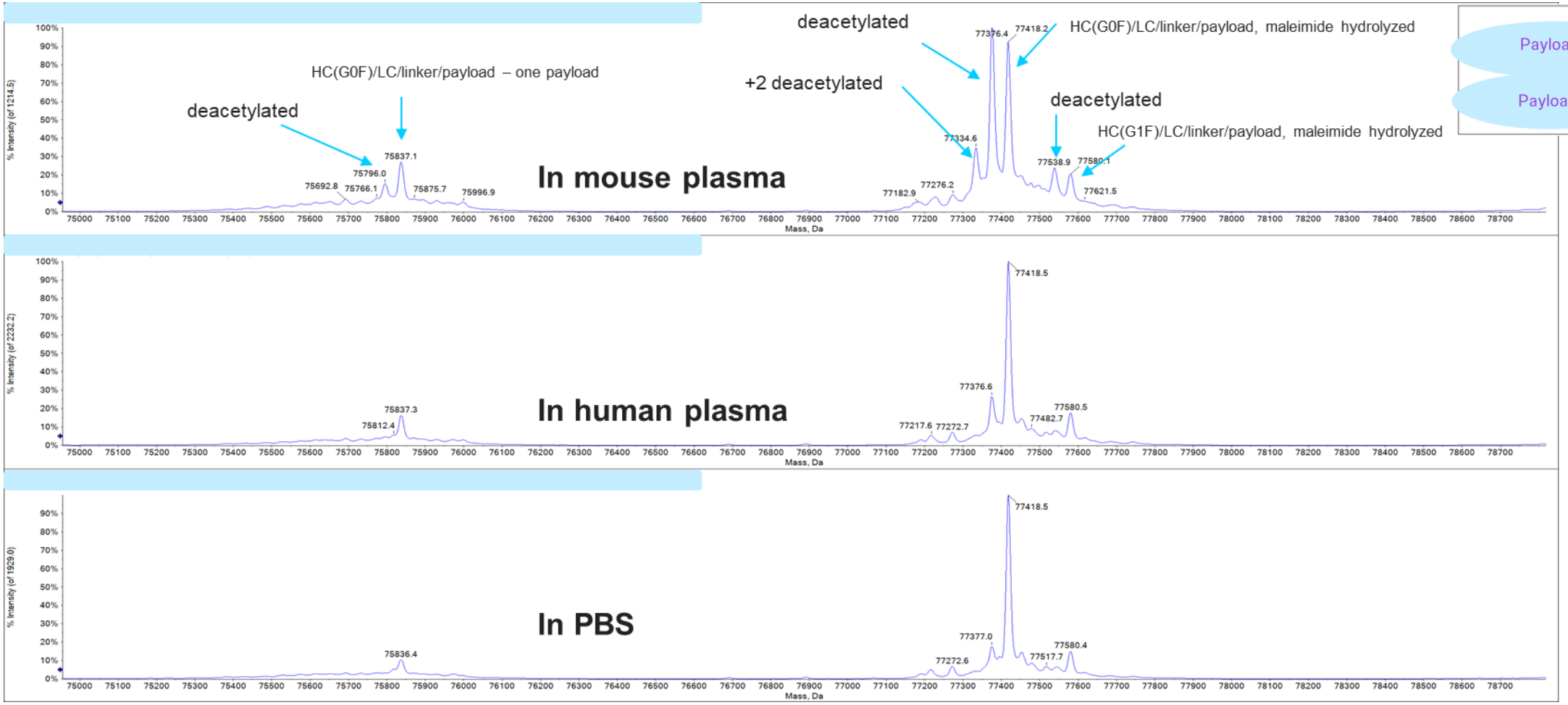


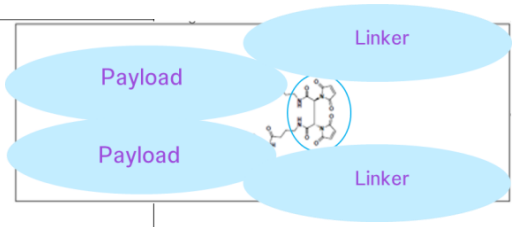


ADC Stability with Bridged Linker - Day 7 in Plasma vs PBS

Maleimide hydrolysis and deacetylation were observed in all matrices, most prominently in mouse plasma

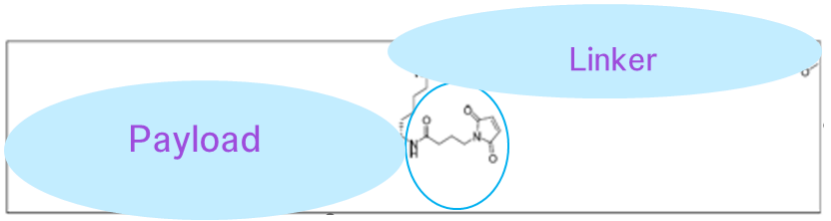
Minor payload loss was also observed

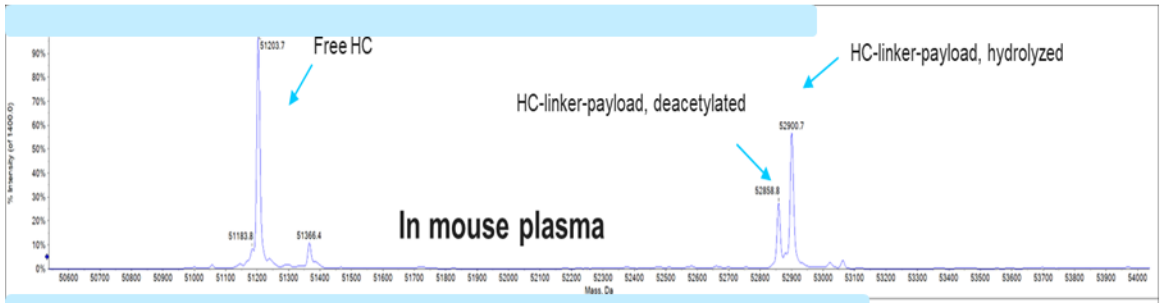


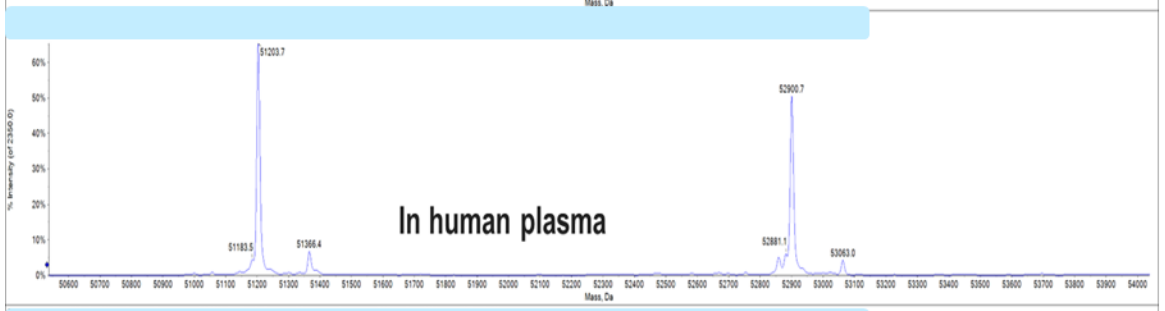


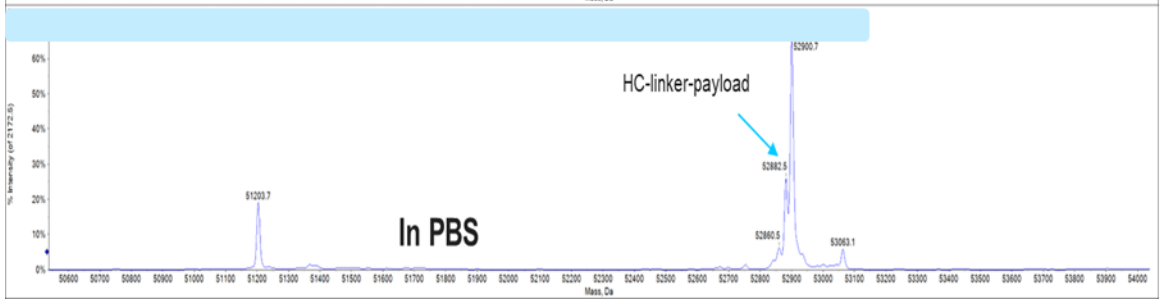
ADC Stability with Un-Bridged Linker - Day 7 in Plasma vs PBS

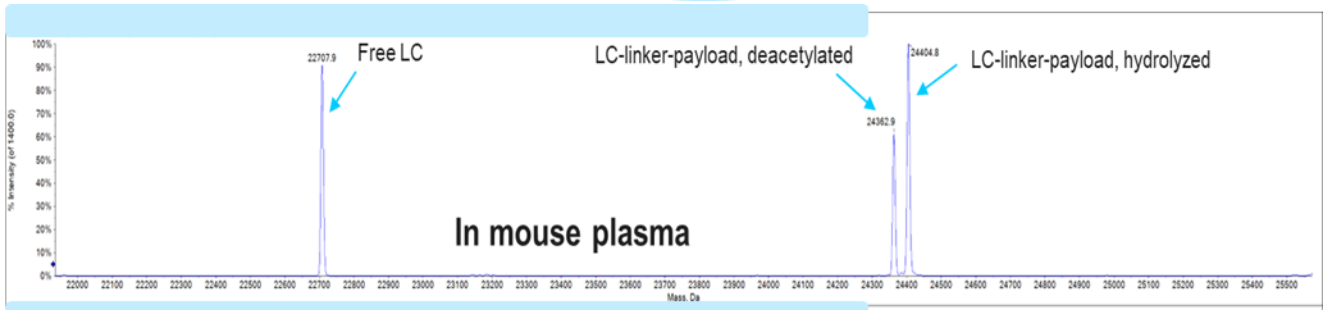
Maleimide hydrolysis and deacetylation were observed
Payload+Linker loss was much more significant than the one with bridged linker

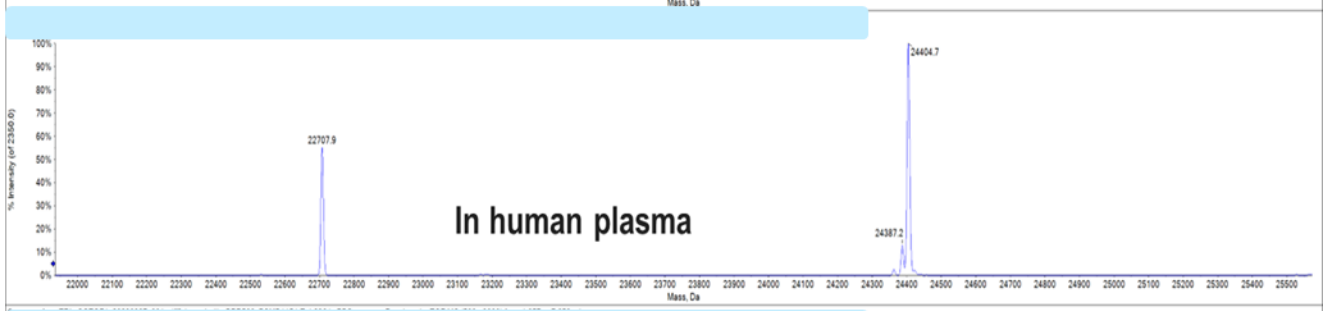


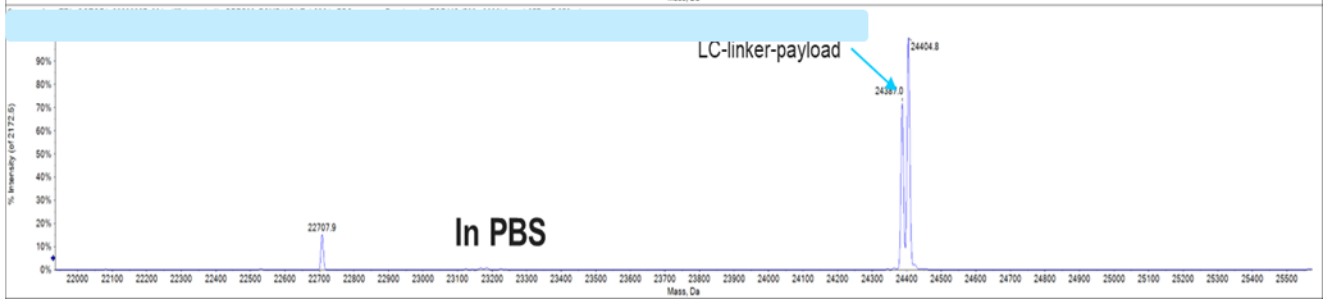








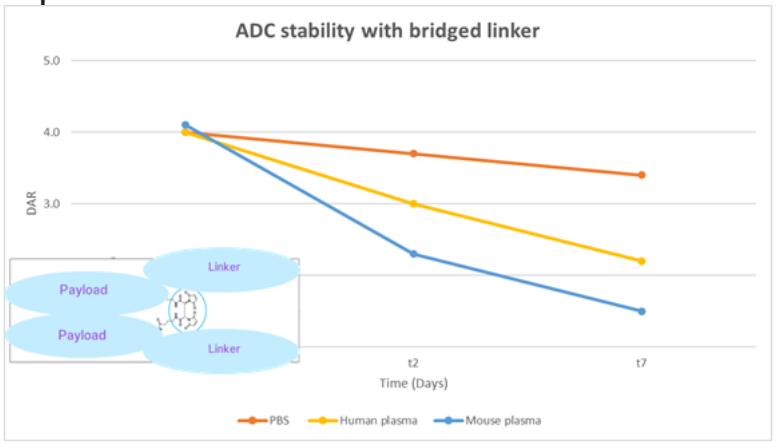


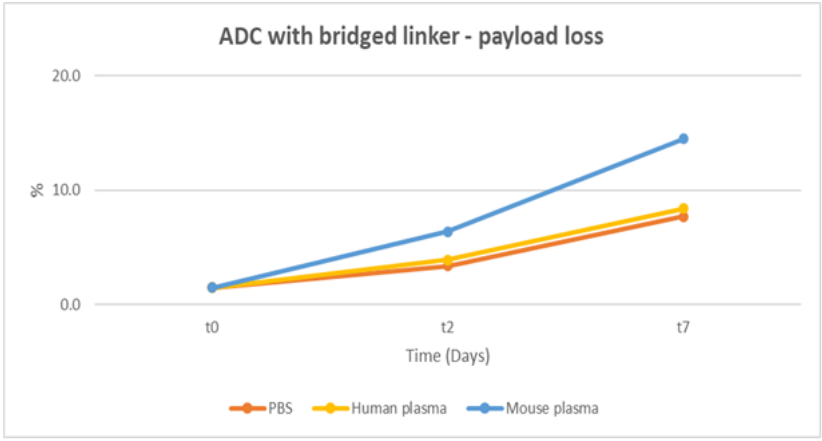


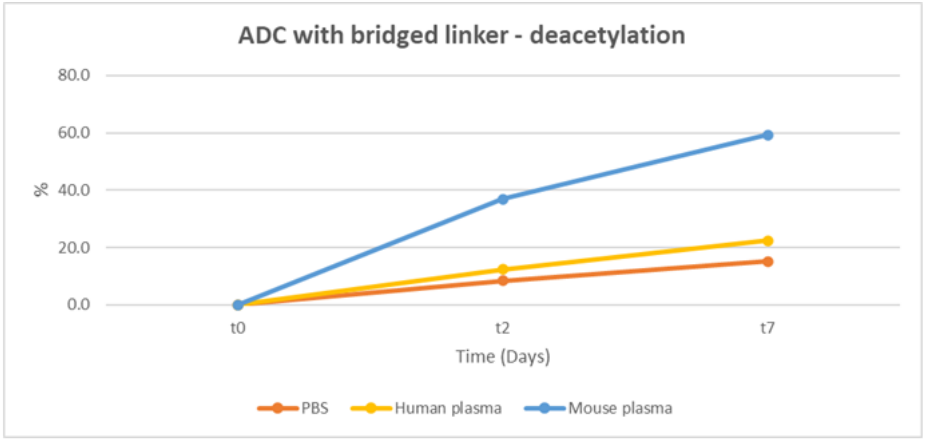
Summary: DAR Change in Plasma

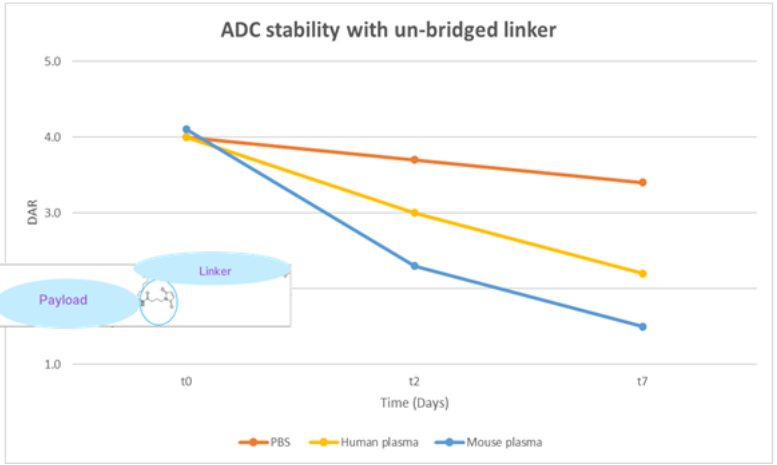
Both molecules showed similar trend of DAR change in respective matrix with most significant DAR change in mouse plasma

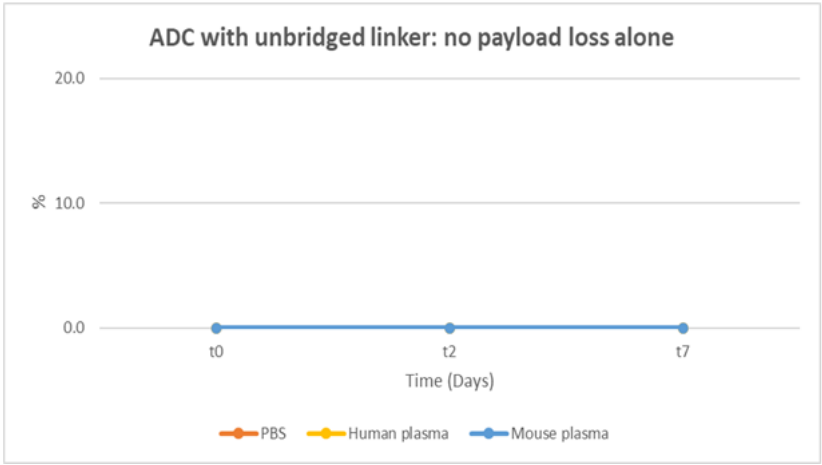
Hydrolyzed maleimide species still considered active and included for the DAR calculation. Deacetylated species were not counted as active species

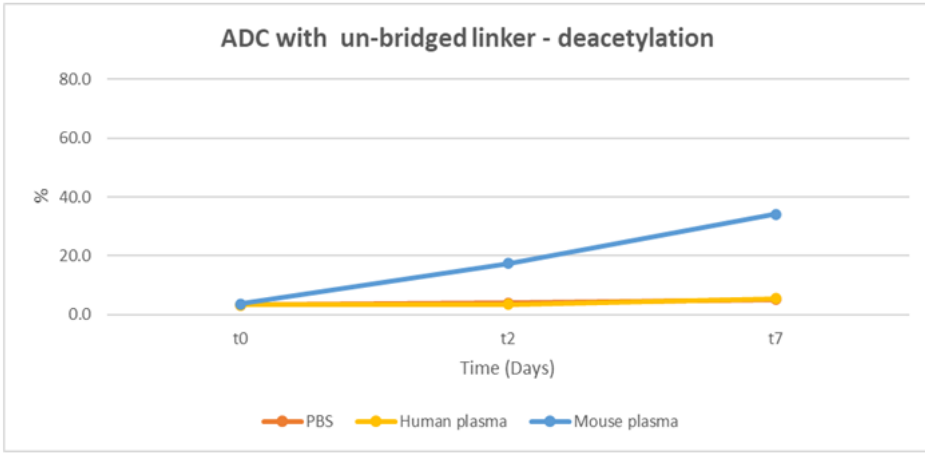






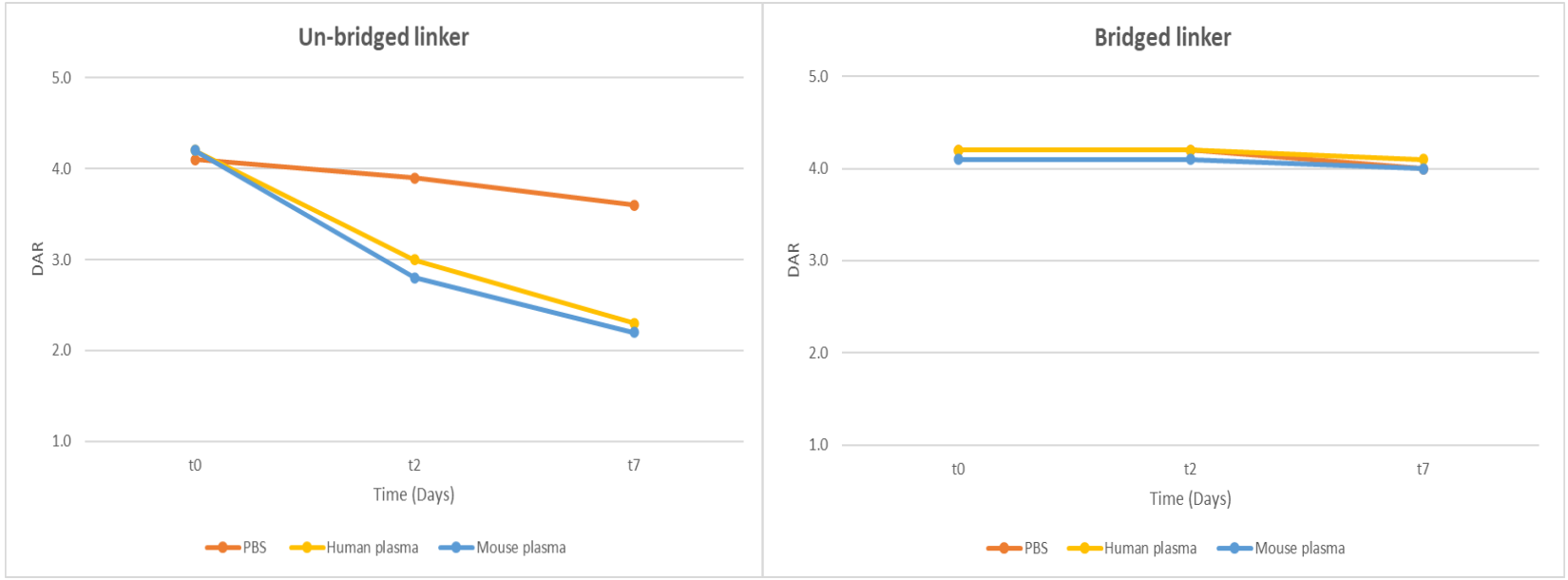




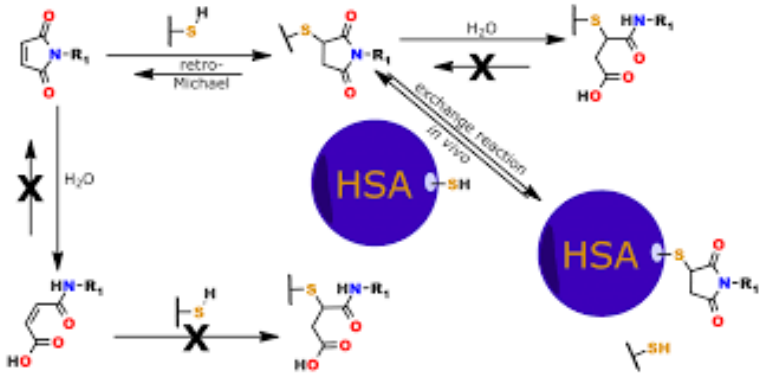


Bridged Linker Demonstrated Good Payload-Linker Stability

ADC with unbridged linker demonstrated poor payload-linker stability in serum/plasma – therefore de-selected for development

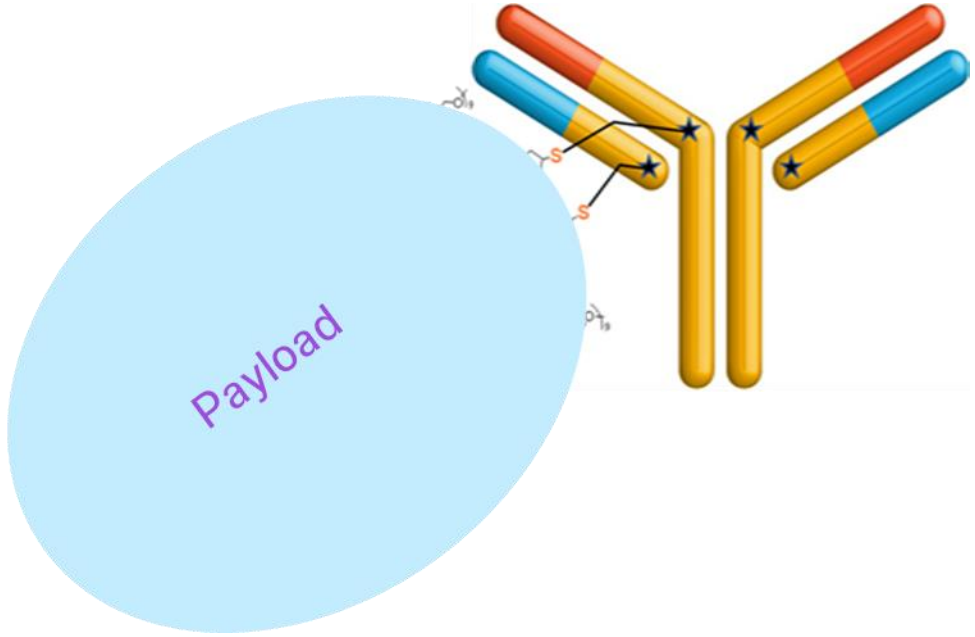


Where did the payload linker go? Lost to albumin in serum for the unbridged payload-linker
https://pubs.acs.org/doi/10.1021/acs.analchem.6b00976

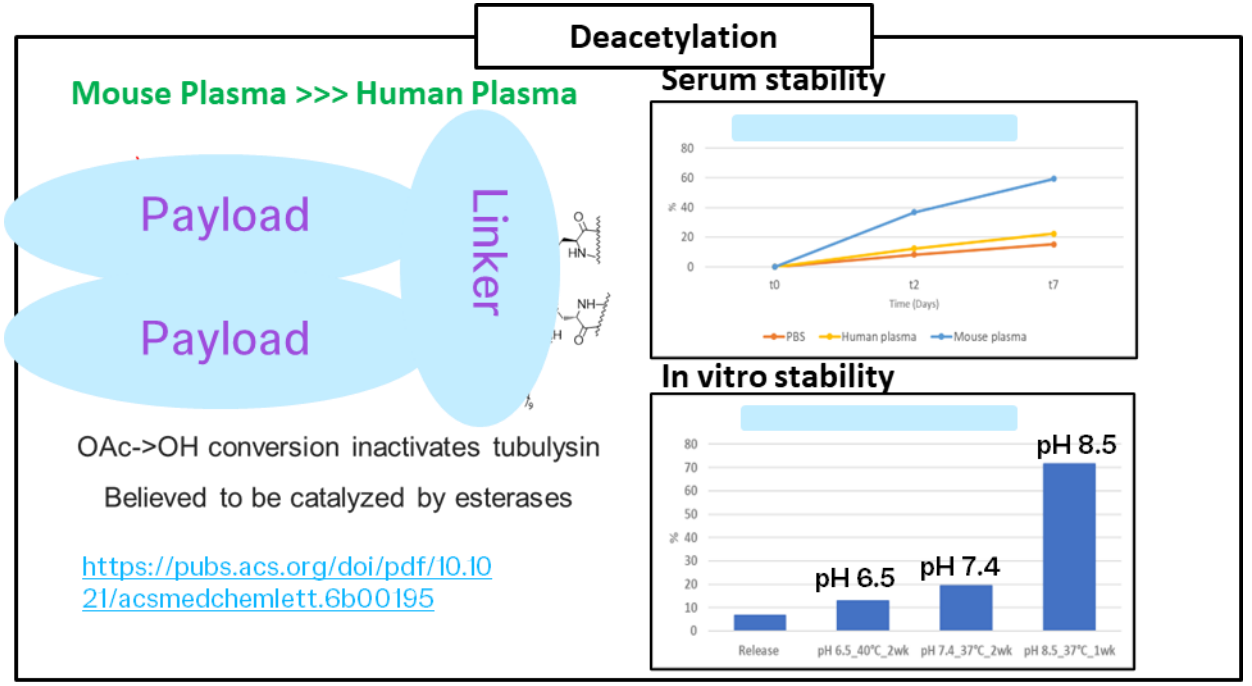


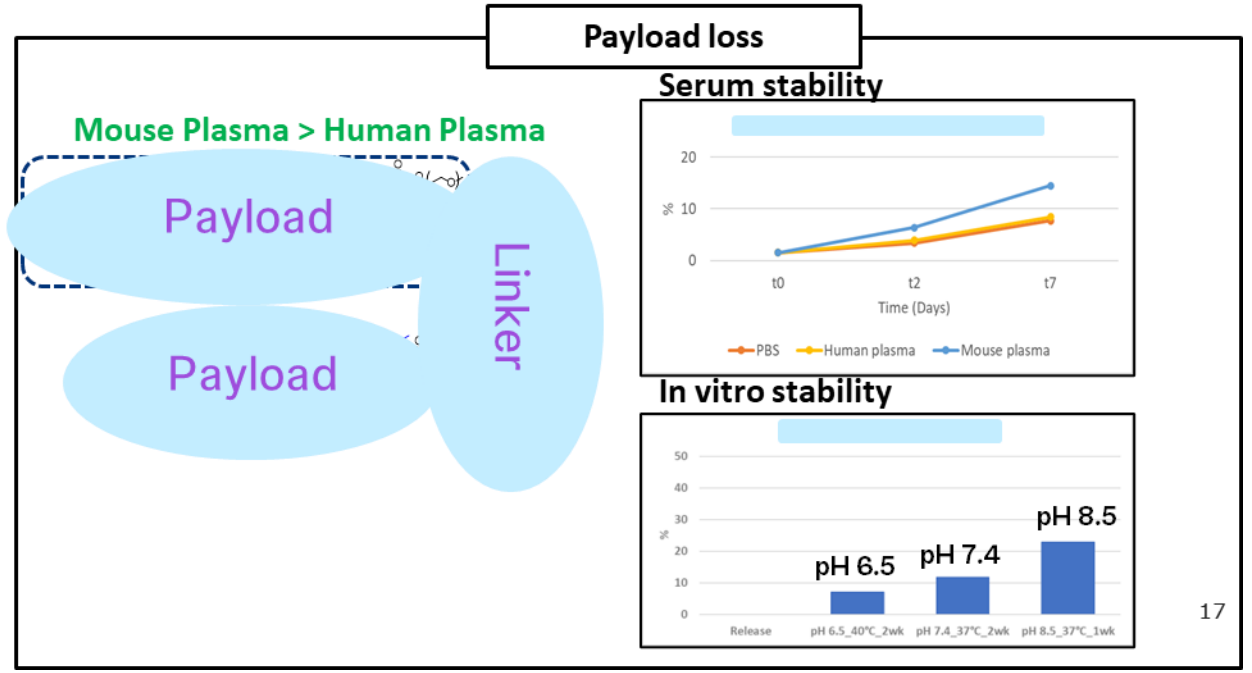
Note: Deacetylated species included in DAR calculation for this comparison

Summary:
Payload Stability and Metabolite

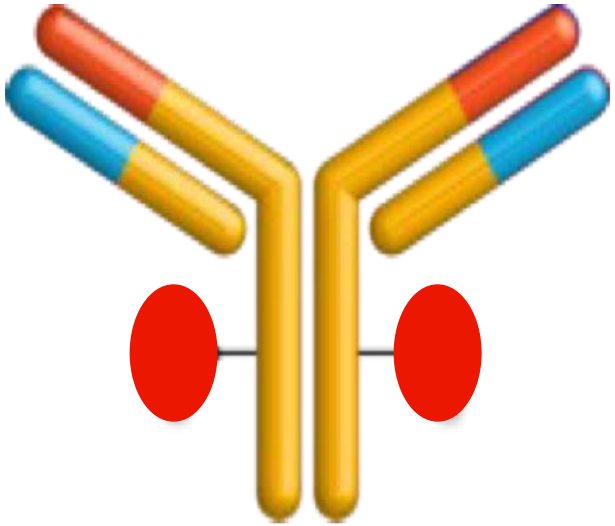


- ADC with unbridged linker demonstrated poor payload-linker stability in serum/plasma – therefore de-selected for development
- Both deacetylation and payload loss are accelerated via temperature and pH and should be considered as potential Critical Quality Attributes (CQAs)
- Deacetylation results in loss of payload activity





Case Study #2: Auristatin Payload

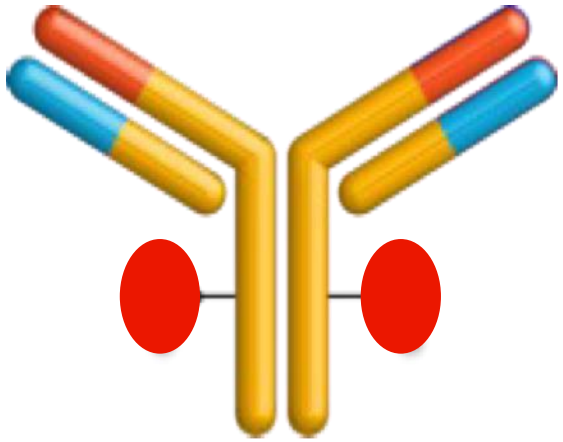


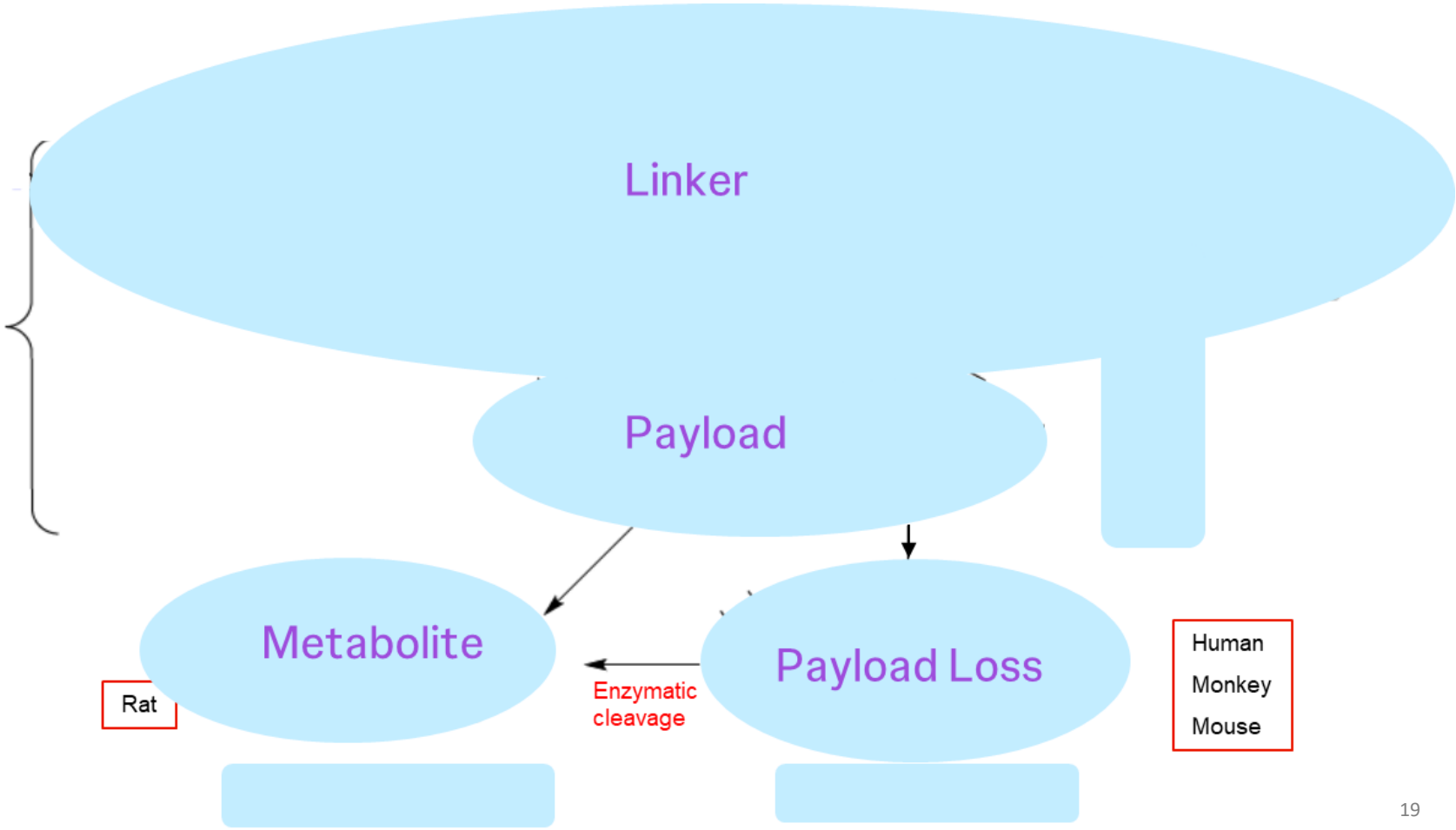
Conjugation via glycan

- Auristatin payload
- IgG1 kappa
- Fc Silent

Auristatin Payload

- Site specific Conjugation
- Multiple payloads on the same site
- How stable is the payload in serum/plasma?





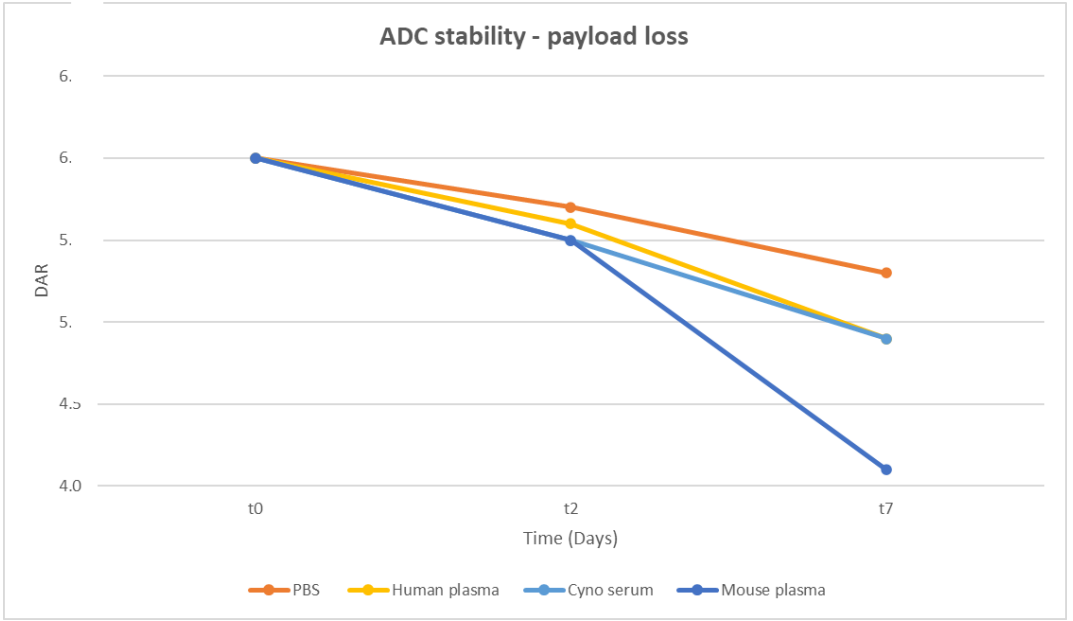
Auristatin Payload Stability – Day 7 in Plasma/Serum vs PBS

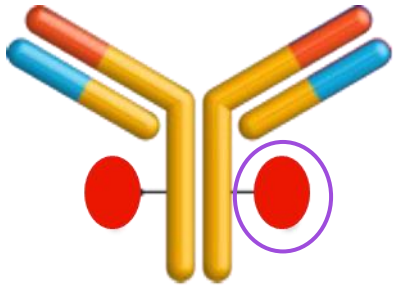
This ADC exhibits payload loss with linker left behind

Auristatin Payload Stability and Metabolite by MS

- This ADC exhibits payload loss in all matrices examined with the most significant loss in mouse plasma
- The molecule has similar stability in human plasma and cyno serum with the same amount of payload loss after 7 days at 37°C
- There was a nearly complete loss of DAR (96%) upon high pH stress, resulted in a 100-fold loss in cytotoxic activity upon high pH stress.
- The loss of DAR is solely attributed to the loss of payload. The entire linker structure remains intact and connected to the mAb

Serum stability





Free drug concentration:
0.064 uM (0.02%)

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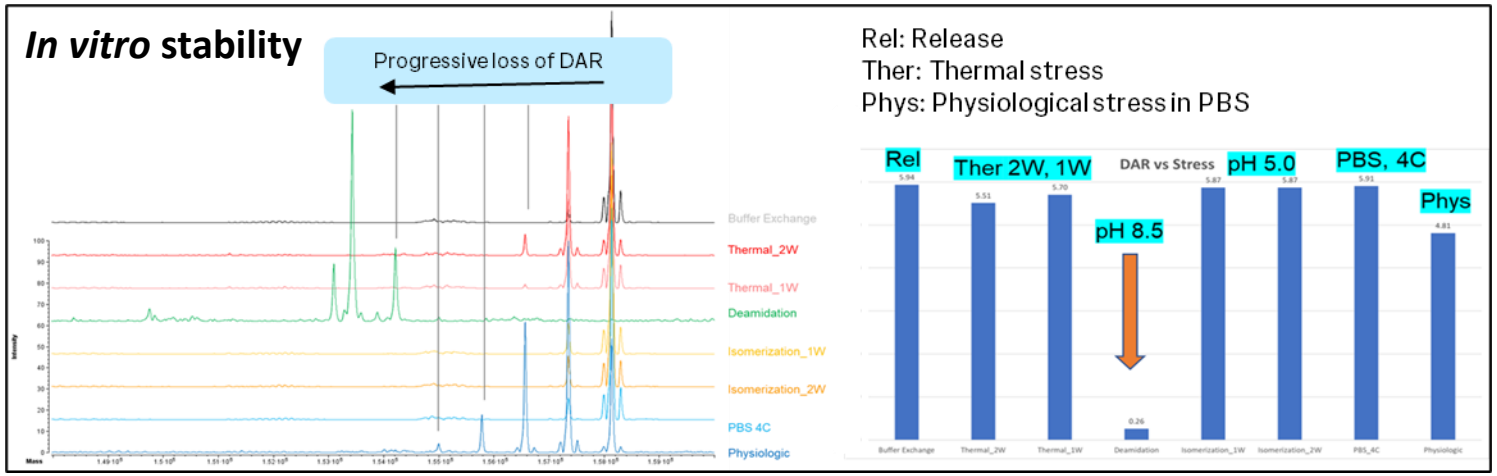
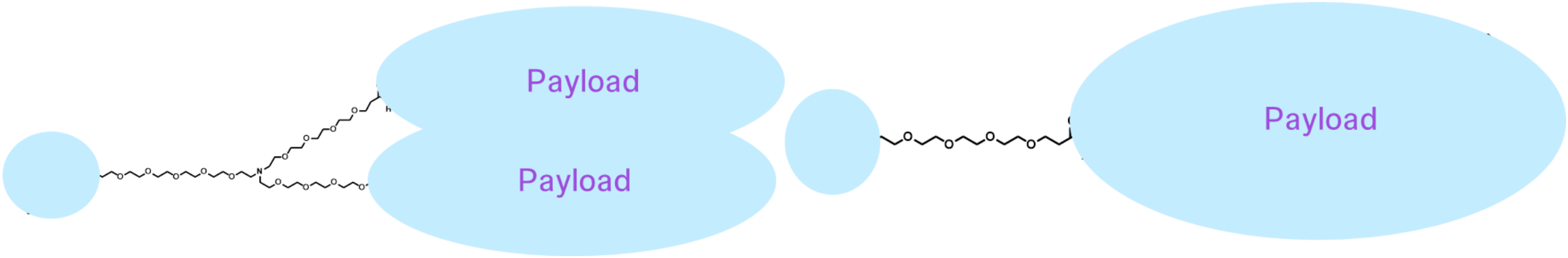
***In vitro* stability**

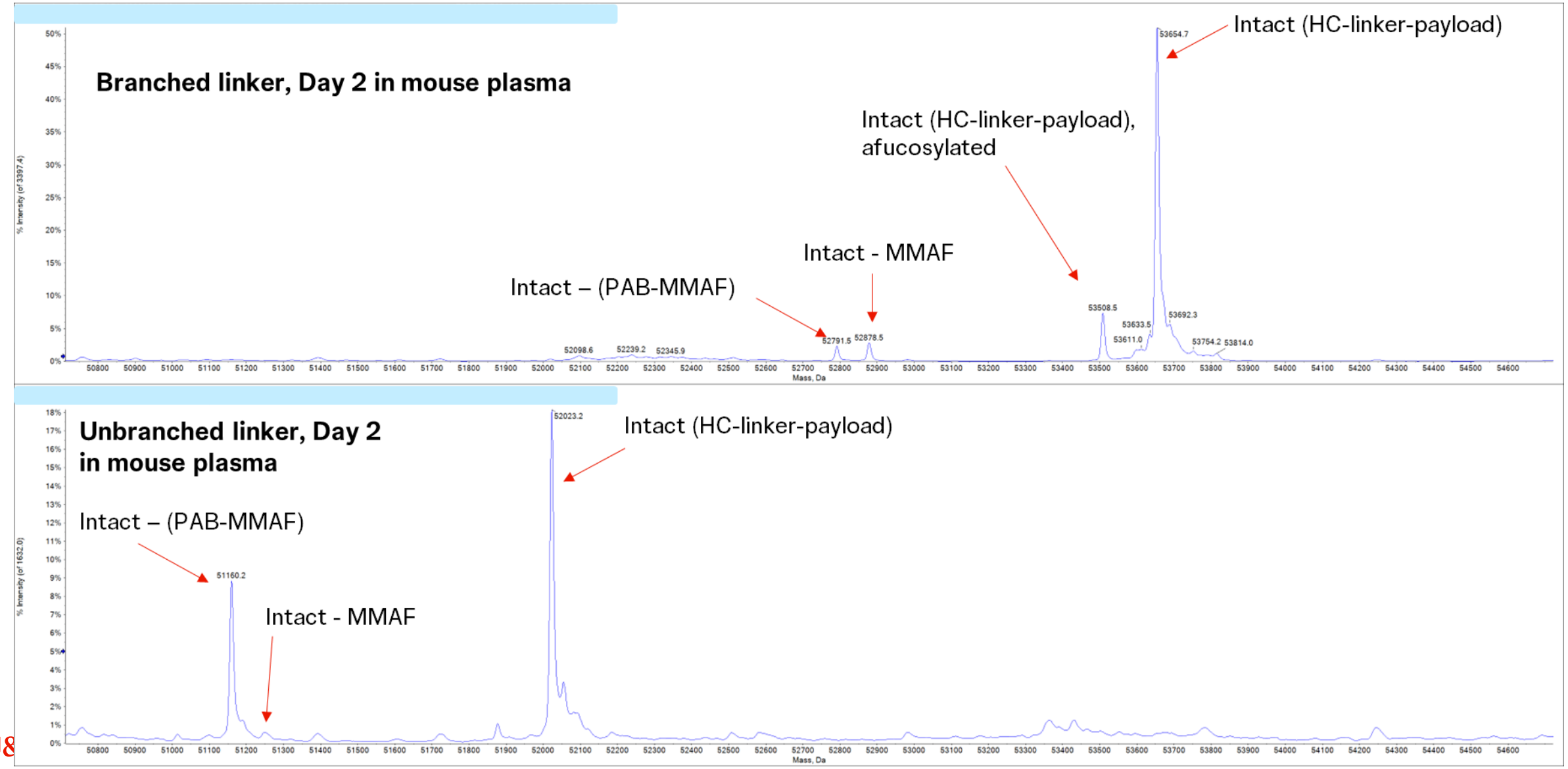


Case Study #3:
Branched vs Unbranched Linker



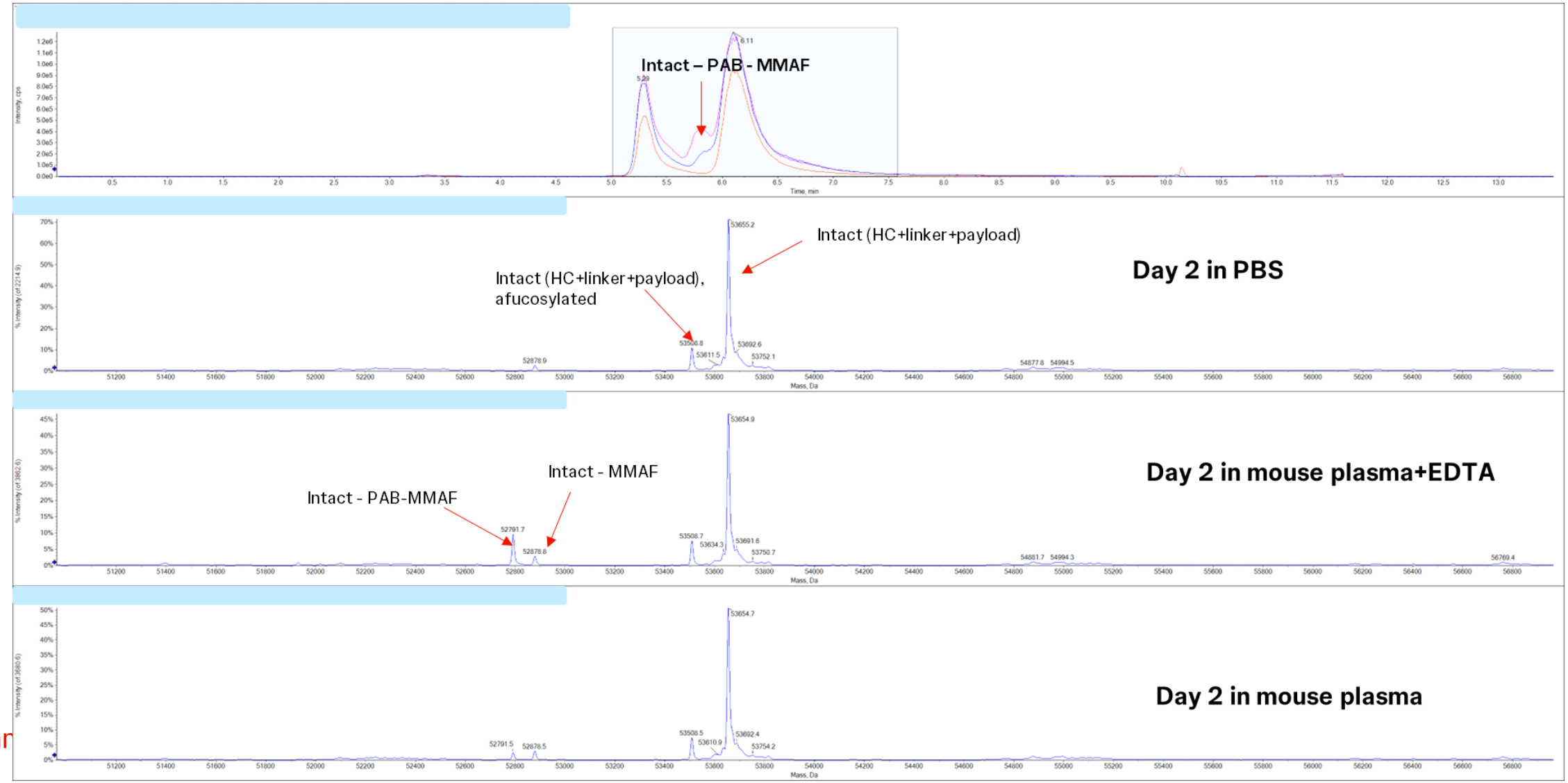
Linker: Branched vs Unbranched in Mouse Plasma

The molecule with branched linker is significantly more stable than the single linker in mouse plasma



Practical Consideration: EDTA Seems to Promote Payload Loss in This Case

EDTA is added to serum/plasma for maintaining pH during incubation but need to be aware of its effect



Summary

- Serum stability assessment provided decisive information for linker-payload selection in the bridged vs un-bridged linker case
- The ADCs via Cys conjugation with unbridged linker lose payload-linker altogether, presumably transferred to albumin in serum. The auristatin conjugates, on the other hand, lose payload but leave the linker behind
- Robust and sensitive mass spec-based assays for ADC stability assessment in early discovery allow lead payload-linker/ADCs selection and aided the design of PK studies and subsequent analytical strategy for the PK sample analysis.
- Comprehensive biophysics characterization to ensure success in Biologics/ADCs advancement.

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Therapeutic Discovery

Thank You for you Attention!