Managing Bioconjugate Heterogeneity Related to Drug-Linkers

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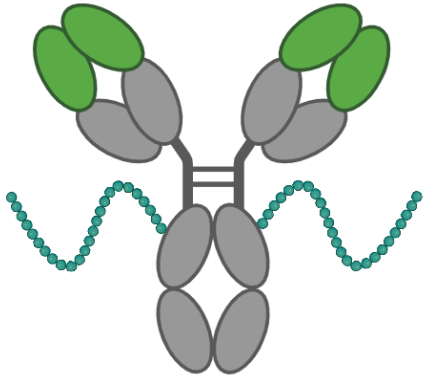
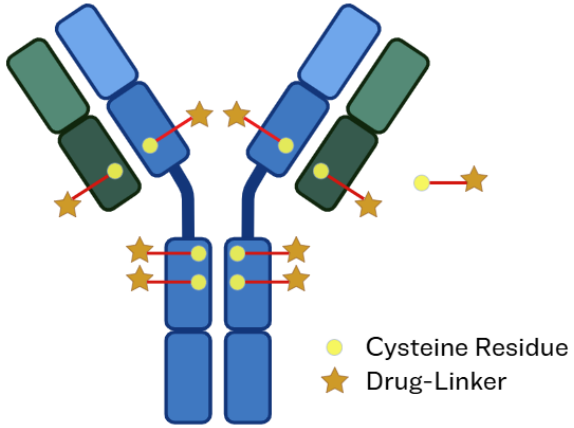
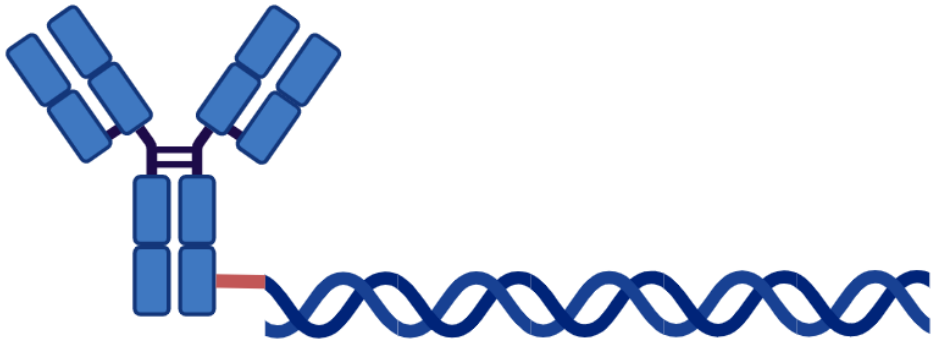
Introduction







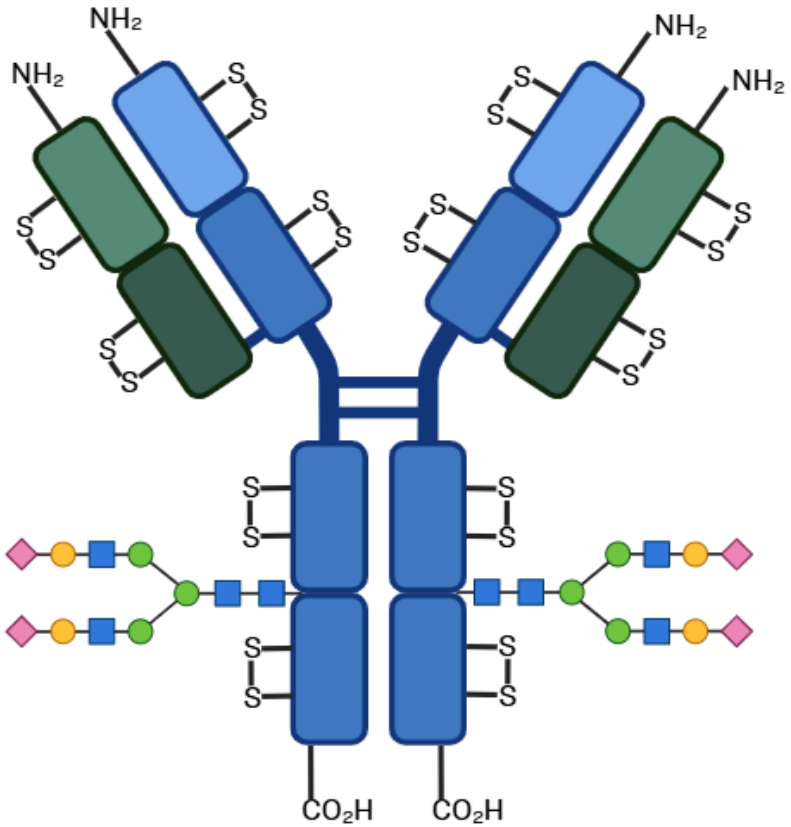
- Bioconjugates such as ADCs, AOCs, and antibody-peptide conjugates are becoming very common in the clinical space.
- These complex modalities present new challenges to development teams, while also offering opportunities to define new CMC and regulatory approaches.
- What practices from the existing large-molecule space could we apply to these hybrid molecules?
- Does it always make sense to apply small molecule guidance to the drug-linker, or is there room to innovate?

ICH Q6B: Related Substances vs Impurities

- Biotech/biological drug substances may include multiple molecular entities/variants due to biosynthetic processes and molecular characteristics.
- Variants from anticipated modifications (i.e., PTMs) are part of the desired product.
- When variants form during manufacturing and have properties comparable to the desired product, they are considered “product-related substances”, not impurities.
- Regulatory agencies expect individual and/or collective acceptance criteria for product-related substances.

Can we leverage the concept of a related substance for a biologic by generating evidence at the ADC DS but place the related substance control at the synthetic intermediate?

Some Common Forms of Biologic Heterogeneity



- Met oxidation
- Glycation
- High mannose
- Sialylation
- Disulfide scrambling
- Lys glycation
- Deamidation
- Pyroglutamation

There can be millions of individual species present…somehow, we manage it!

What about for the drug-linker?

Drug-Linker "Heterogeneity" – What's Happening?

Should drug-linkers be rigorously held to ICH Q3A limits even if actual dose of the toxin is very low?

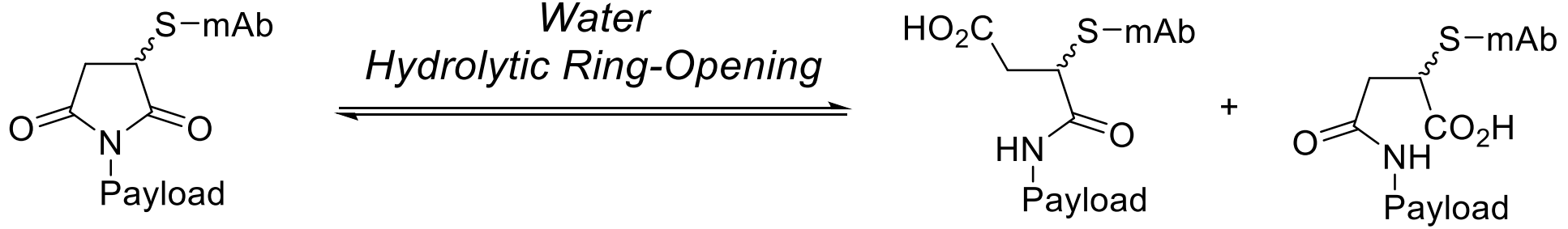
What is the mass of the impurity? 160 kDa or 400 Da?

Initial publication by Gong *et al*. identified/defined the "mass dilution" effect to support higher qualification limits, but it doesn't appear to have been widely accepted by HAs.[1]

Recent publication provided a more rigorous toxicological justification for 1.0% (w/w) qualification limits in drug-linkers for oncology.[2] HA alignment remains to be seen.

1: *AAPS PharmSciTech* **19**, 971–977 (2018)
2: Regulatory Toxicology and Pharmacology 164 (2026) 105974

Example 1 – Maleimide Conjugation Handle



Initial Thio-Succinimide Adduct

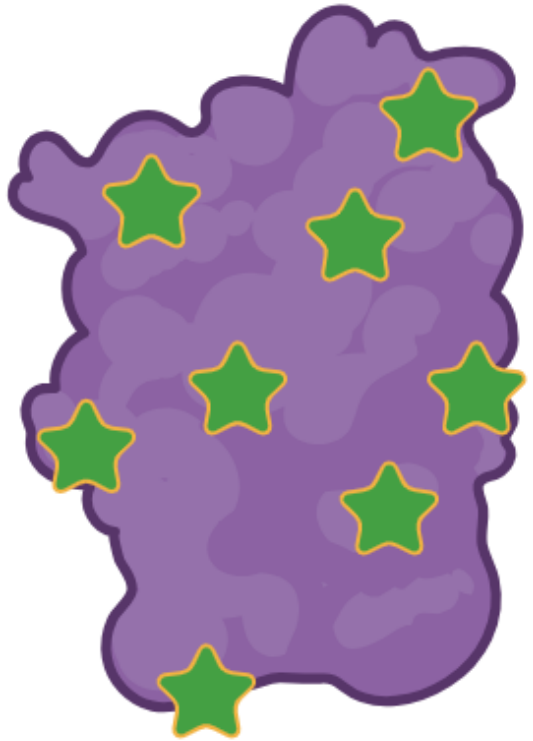
No control over stereochemistry.
Normally can't detect presence of stereocenter.

Equilibrium process at physiological pH.
No control over regiochemistry of opening.
Often can't detect the regiochemistry.
Charge and MS can reveal hydrolysis extent.

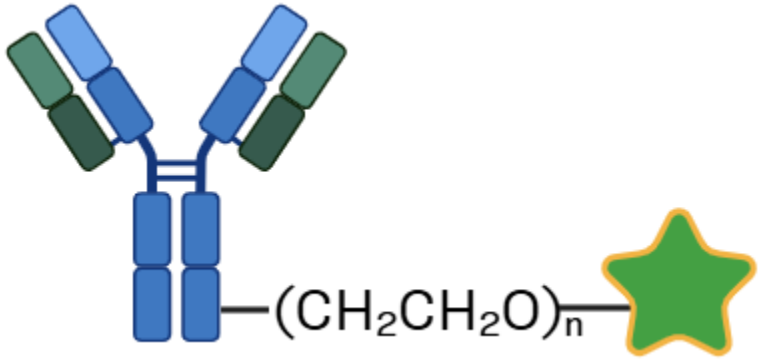
- DAR 8 ADC: Number of possible isomers = 6^8 (1,679,616 isomers!)
- Do these isomers have ANY impact on patient safety or product efficacy?
- This heterogeneity is an inherent property of the molecule and can’t be controlled practically.
- Not treated as impurities

Example 2 – Lysine Conjugation

- One of the original forms of bioconjugation was simply to expose a protein to an activated ester to form amide bonds (~40 solvent accessible lysine residues).
- Still used today and is used to manufacture 4 FDA-approved ADCs.
- Little/no control over site selectivity but largely reproducible.
- Reasonable control over *average* DAR using drug-linker stoichiometry control but result in highly heterogeneous mixture of DAR species.

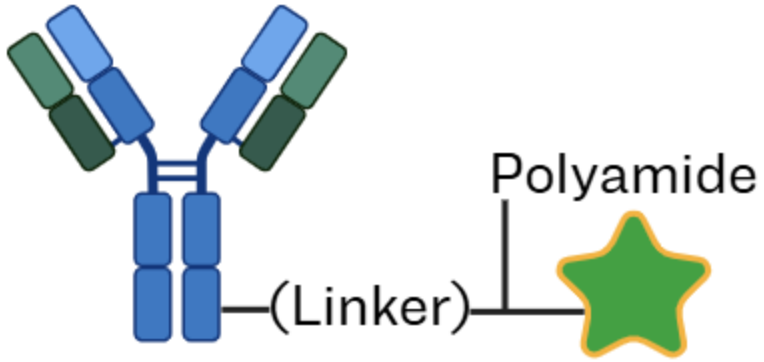


Example 3 – Linker Chain Length



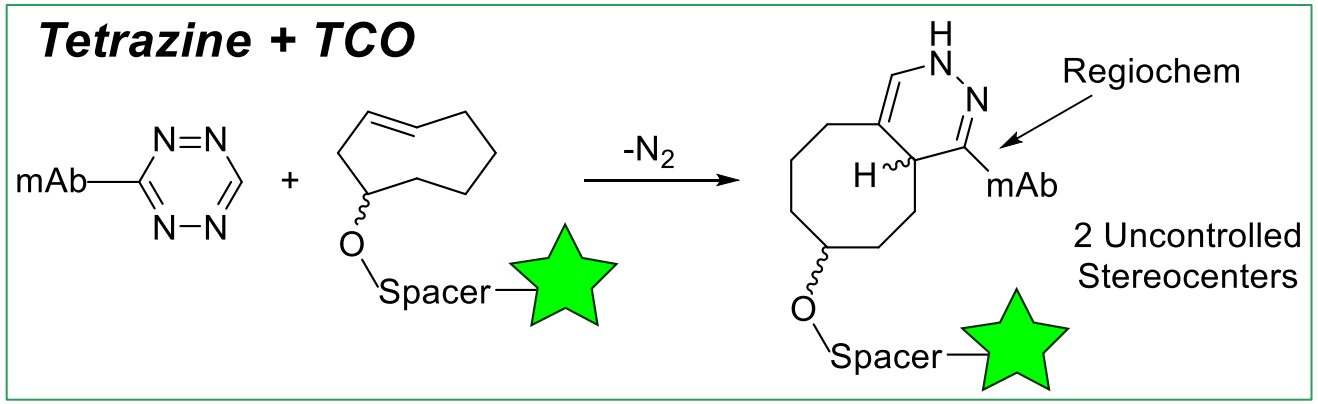
- For long PEG chain lengths, it can be very difficult or impractical to control polydispersity.
- Changing distance between mAb and payload.
- Many clinical conjugates have highly polydisperse PEG chain lengths – considered a property of the molecule.
- Changes to PEG sourcing that could impact polydispersity must be assessed.

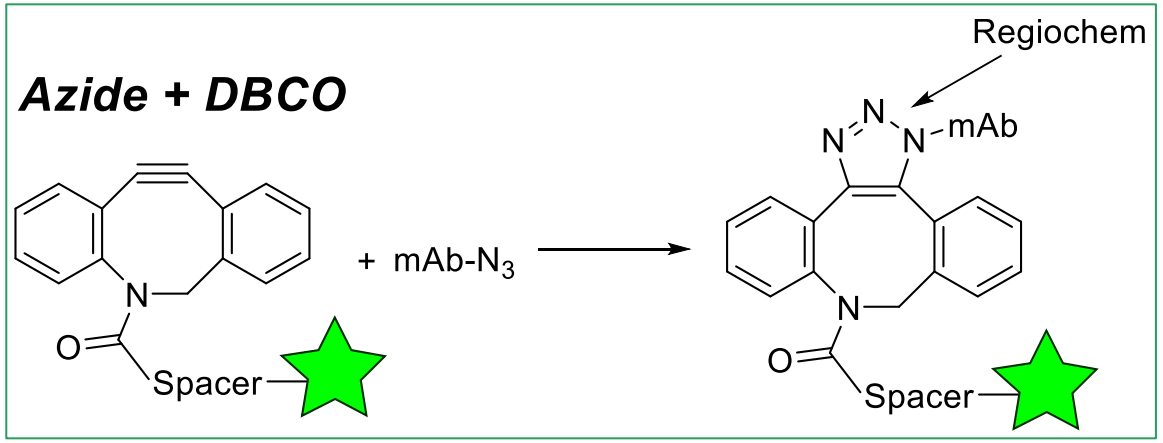
Example 4 – Linker Side Chain



- For polyamide hydrophobicity masking, precise control of chain length can be challenging.
- Distance between mAb and payload is constant, but extent of hydrophobic masking could differ.
- These are truly hybrid molecules since the polyamide may be manufactured using solid-phase peptide synthesis (SPPS).
- ICH Q3A not applicable?
  Follow peptides: 0.10% report, 0.50% ID, 1.0% qualify?
- Can this be applied to an RSM designation argument?
- Assessment of product performance and bracketing approach merited, but *should these homologues be treated as impurities?*

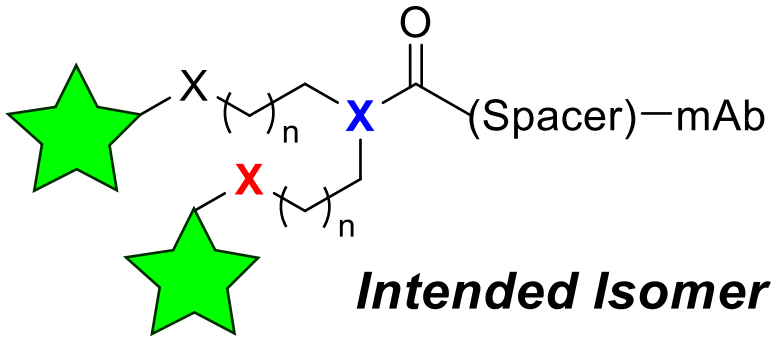
Example 5: Click Reactions



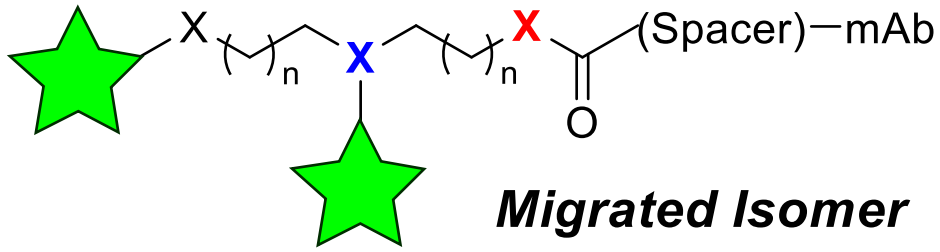


- Somewhat rare in clinical space, click reactions have gotten us “comfortable” with heterogeneity near the site of conjugation.
- Little/no ability to control stereocenters or regiochemistry – these inherently form mixtures.
- Trust that there is no issues with payload release or stability of conjugate.

Example 6 – Bifurcated Linker Acyl Migration



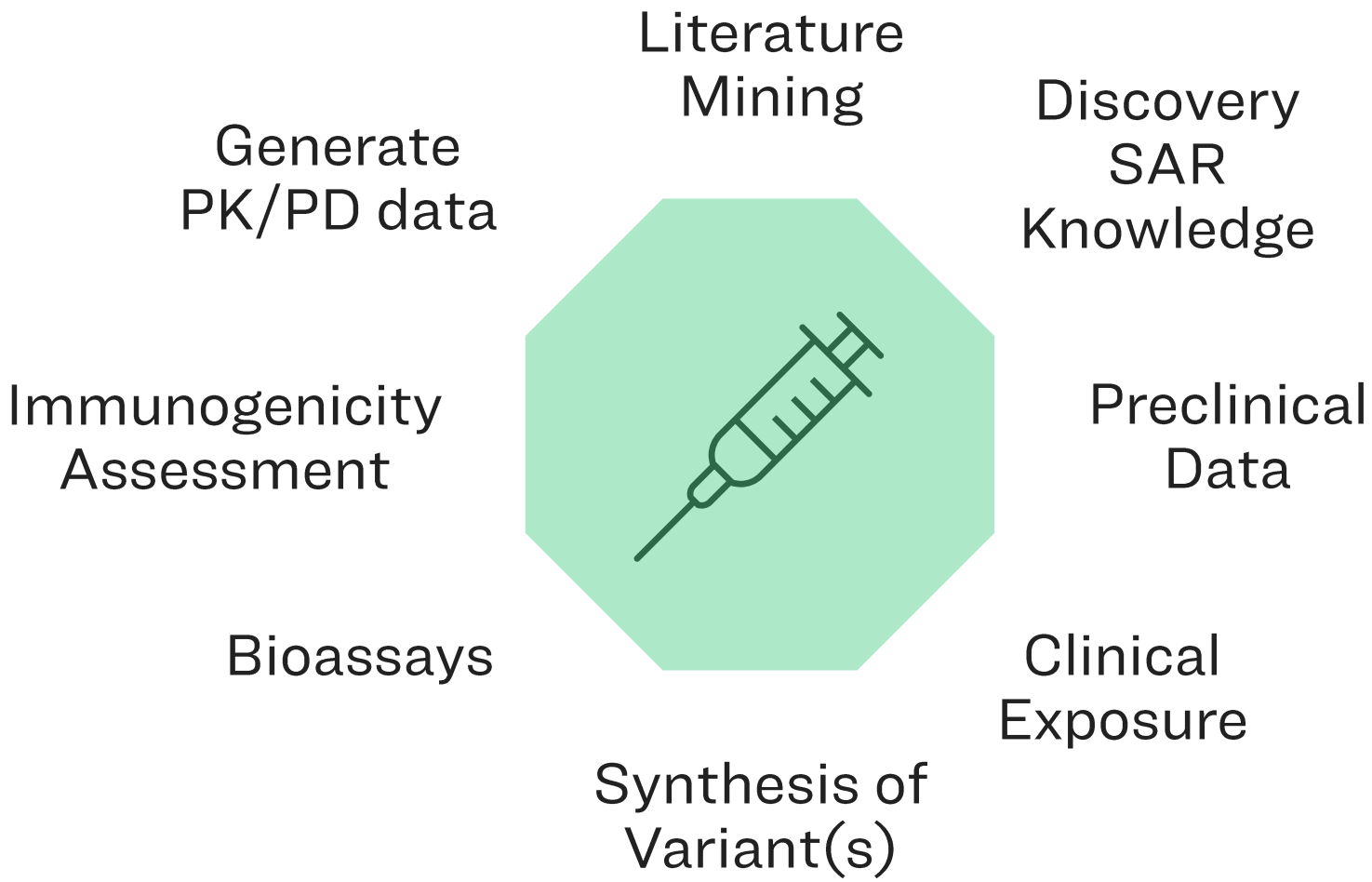
Intended Isomer



Migrated Isomer

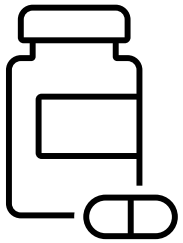
- Have seen examples analogous to this.
- Detection of isomerization can be challenging depending on the exact system.
- Assignment of the chemical structure can be VERY challenging – need good collaboration between synthetic chemists, analytical, and bioconjugation.
- Synthesis issues may be resolved by modifications to the process, but justification of these additional species must be made for the early phase materials until the corrections have been implemented.

Supportive Mechanisms



Suggested Path Forward and Wrap-Up

- In some cases, drug-linker related speciation at the DS is unavoidable.
  - This can often be distant from the payload or active drug site.
  - May be from inherent reactivity or from the synthetic process itself.
- If species doesn't present risk to patient and has similar efficacy, consider designation as a product related substance.
  - This is highly impactful for the drug-linker team.
  - Standards and rules will be needed – we should still STRIVE for a single, homogeneous DS while recognizing where limitations exist.
- This complexity is here now…we should choose to manage it with what makes scientific sense, not necessarily what we've done before.



Thank You!
Questions?