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A PHYSICOCHEMICAL APPROACH TO CHARACTERIZING ANTIBODY-DRUG CONJUGATES THROUGH STABILITY INTO TARGET VALIDATION

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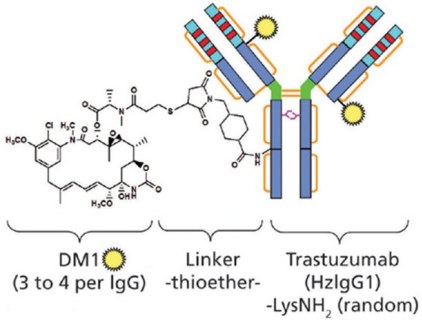
Background

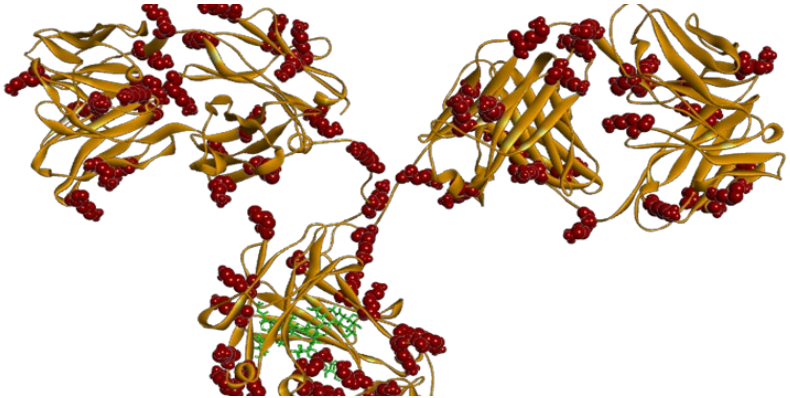


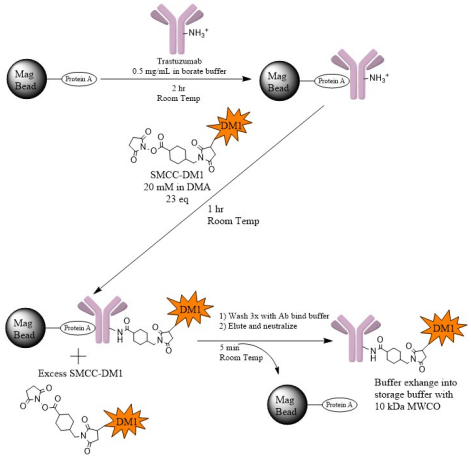
Cartoon of trastuzumab emtansine (T-DM1). Discovery Medicine, A. Beck et al., Discov. Med. 10 (53), 329–339 (2010).

- Trastuzumab (Herceptin®) is approved for use in human epidermal growth factor receptor HER2-positive cancers (ie breast, stomach). Maytansine, cytotoxic drug increases application -binds to tubulin to prevent microtubule formation.

- Antibody Drug Conjugates: A discriminatory therapeutic with high potency

Experimental

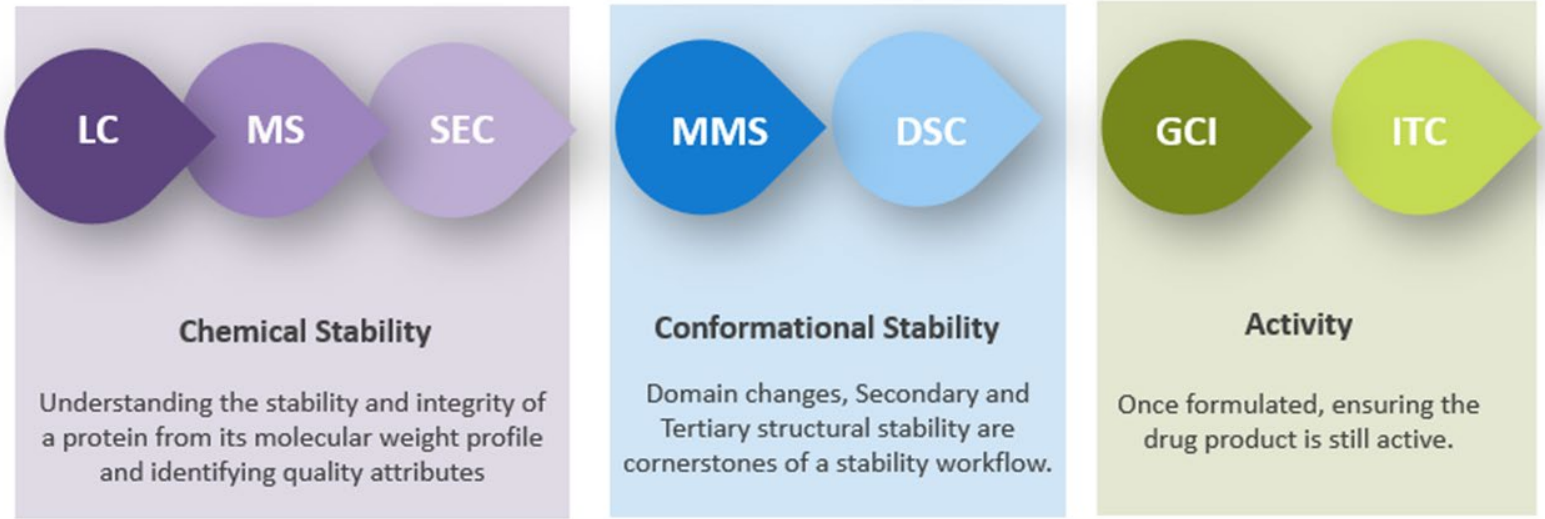




- On-bead or off-bead preparation.
- On-bead advantage: smaller batches, mL of 0.5 mg/mL
- On-bead question: Did the bead occlude favorable modification sites?
- Modification question: After adding a drug to the mAb does its conformation and binding profile change?

A Panel of Techniques to Answer the Questions

A panel of methods were used to establish comprehensive characterization of antibody-drug conjugates (ADCs).

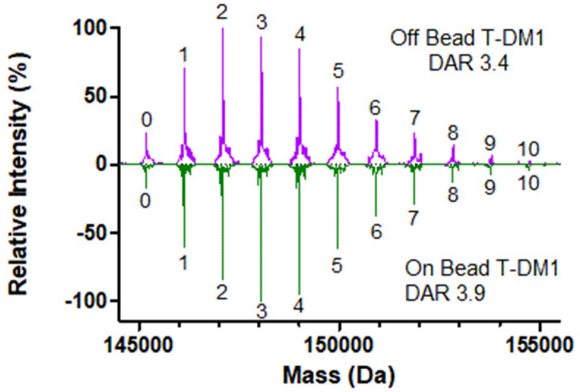


- Liquid Chromatography
- Mass Spectrometry
- Size Exclusion Chromatography
- Microfluidic Modulation Spectroscopy
- Differential Scanning Calorimetry
- Grating Coupled Interferometry
- Isothermal Titration Calorimetry

Biochemical Characterization

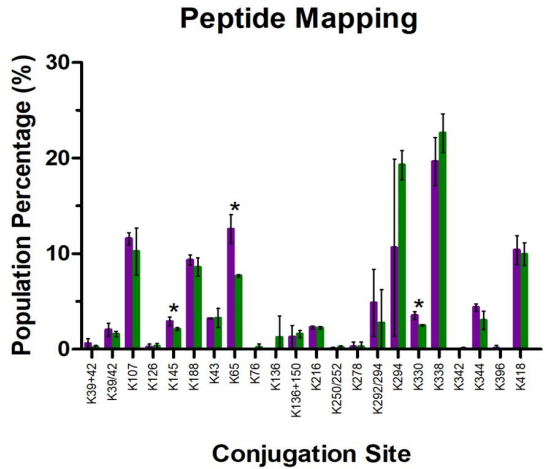
Intact LC MS: Drug-Antibody Ratio

- Waters Acquity UPLC with Xevo G2-QTof
- Sample Prep: deglycosylation & Desalting with a MassPREP column



Peptide Mapping: Modification ID

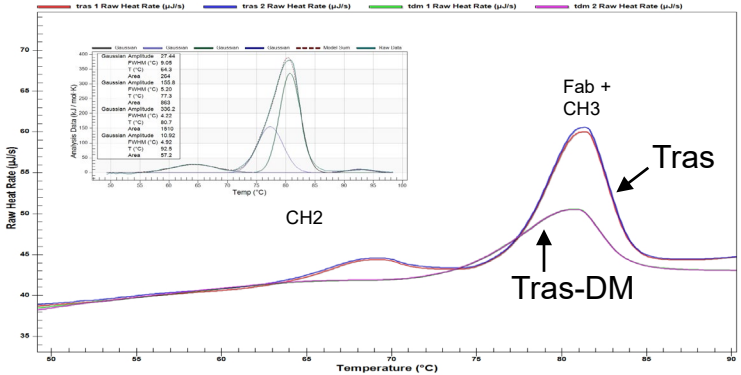
- Waters Acquity UPLC with Xevo G2-QTof
- Peptide Mapping After Trypsin Digest. Acquity UPLC with Xevo G2-S, QToF



Biophysical Conformational Stability and Characterization

Domain Stability & Heterogeneity: DSC

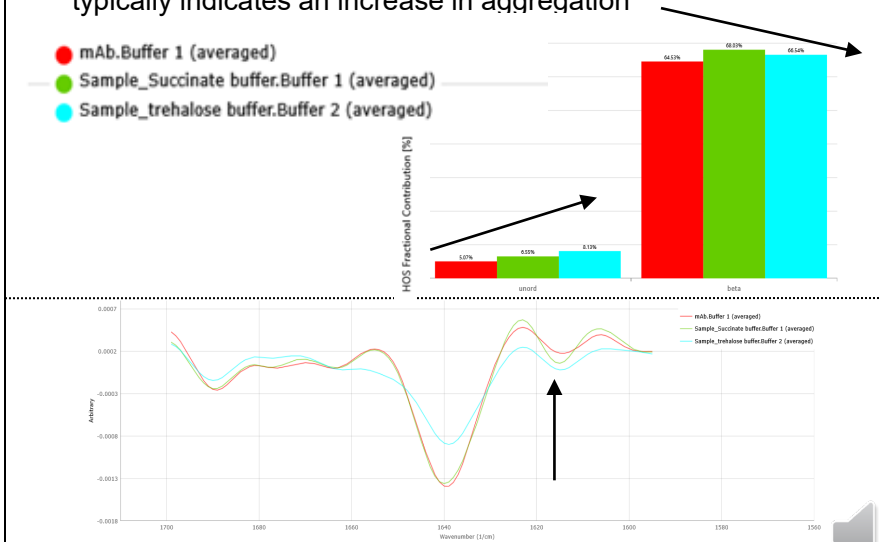
- TA Instruments NanoDSC with a Capillary Cell



	T_{max1} (°C)	FWHM1 (°C)	T_{max2} (°C)	ΔH_1 (kJ/mol)	ΔH_2 (kJ/mol)	ΔH_{total} (kJ/mol)
Tras(avg)	68.4	5.7	81.4	574	3185	3853
T DM (avg)	64.4	8.5	80.6	251	2389	2698

Secondary Structural Changes & Aggregation: MMS

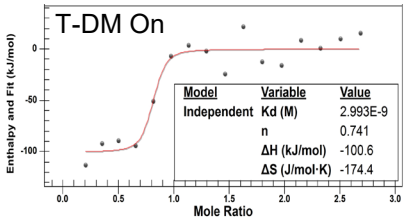
- RedShiftBio MMS
- loss of parallel beta sheet to anti-parallel beta sheet, which typically indicates an increase in aggregation

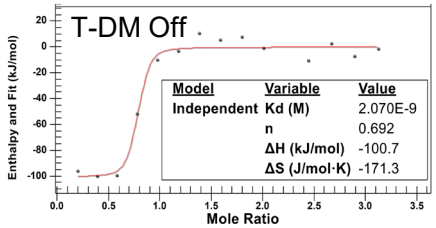


Binding Affinity and Stability

Affinity, Enthalpy, Entropy, Stoichiometry: ITC

- TA Instruments Affinity ITC LV
- Affinity, Enthalpy, Entropy, Stoichiometry

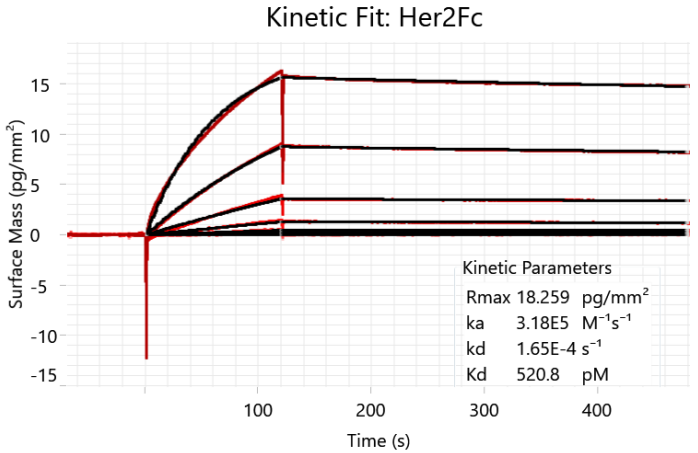




Avg Values (n=2)	K_d (nM)	n	ΔH (kJ/mol)
Tras	3 ± 1	0.8 ± 0.2	-101 ± 5
T-DM on	3.3 ± 0.3	0.64 ± 0.1	-99 ± 2
T-DM off	4 ± 2	0.66 ± 0.02	-98 ± 2

Affinity, k_{on}, k_{off}: GCI

- Creoptix WAVE GCI
- Amine-coupled
- k_{on} agreement, similar K_d for Her2Fc



Conclusion

- The complex was modified, but did it change?
  - Stability Changes
  - Binding remained Intact

- This type of combined biophysical and biochemical analysis amplifies and solidifies the confidence of the reported results while decreasing bias.

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