Multi-Modal Biophysical Characterization of an Antibody-Drug Conjugate: Linking Conformational Properties to Function

Michael Piazza, PhD, Assoc. VP, Applications Development, Nicoya Lifesciences

Introduction

Antibody-drug conjugates (ADCs) combine the target specificity of monoclonal antibodies with the cytotoxic potency of small-molecule payloads. A key challenge encountered in the development of these therapeutics is that the conjugation chemistry can perturb antibody structure, stability, and target-binding function in ways that are difficult to characterize. Here we present an integrative biophysical characterization workflow that compares an unconjugated antibody to its corresponding ADC using three complementary methods: circular dichroism spectroscopy (Chirascan) to assess secondary and tertiary structural integrity, surface plasmon resonance (Alto) to quantify antigen-binding affinity and kinetics, and intrinsic DSF (SUPR-DSF) to evaluate stability. By layering conformational, interfacial, and thermal stability data, we identify which biophysical attributes shift upon conjugation and link these changes to functional consequences for target engagement. This multi-technique approach demonstrates how combining orthogonal biophysical methods provides a complete picture of ADC critical quality attributes, supporting more informed decision-making in ADC development.

Integrated Solution for Kinetic, Stability & Structural Analysis

Higher order structure (HOS), thermal stability, and binding kinetics were systematically evaluated using a joint instrumentation suite comprising: Digital Surface Plasmon Resonance (Digital SPR™), differential scanning fluorimetry (SUPR-DSF™), and circular dichroism (CD) spectroscopy (Chirascan™ V100).



ADCs: Sac & Sac-TMT

Sacituzumab (Sac; also known as hRS7) is a humanized IgG1 monoclonal antibody that binds trophoblast cell-surface antigen 2 (TROP2), a transmembrane glycoprotein overexpressed in many epithelial cancers and associated with tumor progression. (citation) The same antibody sequence forms the targeting scaffold of sacituzumab tirumotecan (Sac-TMT; also known as SKB264 or MK-2870), in which approximately seven to eight molecules of the belotecan-derived (camptothecin core) topoisomerase I inhibitor KL610023 are attached through a 2-methylsulfonyl pyrimidine-containing linker to cysteines generated by reduction of interchain disulfide bonds. With its high drug loading and cysteine-based conjugation, the Sac-TMT must walk a fine line between accommodating substantial chemical modification and retaining the characteristic immunoglobulin architecture of the parent antibody.

Samples

- Sacituzumab (MedChemExpress, Cat # HY-P99045)
- Sacituzumab tirumotecan (MedChemExpress, Cat # HY-164789)
- TROP2 (MedChemExpress, Cat # HY-P70457)

CD: HOS Comparability

To assess the effect of payload conjugation on antibody structure, Sac and Sac-TMT were characterized by far-UV & near-UV/visible CD spectroscopy. Four independent replicates of each were measured on a Chirascan V100.

Far-UV CD showed the characteristic β-sheet-rich antibody profile for both Sac and Sac-TMT. After normalization by absolute area, weighted spectral difference (WSD) analysis detected a reproducible difference in spectral shape: all four Sac-TMT measurements exceeded the reference ±2 SD quality range (Z = 4.70–9.08).

Taken together with secondary structure deconvolution calculations (SSD; not shown), these results support retention of the global antibody fold alongside a subtle conjugation-associated difference.

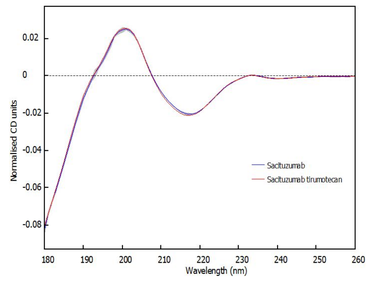


Figure 1: Mean far-UV CD spectra of Sac and Sac-TMT after baseline correction and absolute-area normalization. Gray shading indicates the Sac reference ±2 SD.

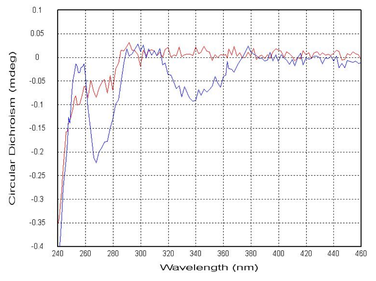


Figure 2: Mean, baseline-corrected near-UV/visible CD spectra of Sac (red) and Sac-TMT (blue).

Table 1: Z-scores from the far-UV CD HOS comparison following absolute-area normalization. Reference quality range: Z-score −2 to +2 SD. All Sac-TMT replicates exceeded the upper limit.

	Rep 1	Rep 2	Rep 3	Rep 4	Mean ± SD
Sac	0.02	1.30	-1.13	-0.19	0.00 ± 1.00
Sac-TMT	4.70	9.08	7.75	6.03	**6.98 ± 1.92**

DSF: Thermal Stability

The effect of payload conjugation on thermal stability was assessed by intrinsic differential scanning fluorimetry (DSF) on SUPR-DSF. Temperature-dependent fluorescence spectra of Sac and Sac-TMT were used to compare thermal denaturation profiles and assess the effects of conjugation and sample stress. Sac-TMT retained most of its thermal stability after conjugation and oxidative stress. Non-reversibility was investigated by modulated scanning fluorimetry (MSF), which uses heating & cooling cycles to measure the non-reversibility onset temperature (T_{nr}), indicating when thermal stress begins to cause irreversible structural changes. MSF data revealed that the lower thermal unfolding temperatures observed for Sac-TMT do not translate into an increased propensity for irreversible aggregation.

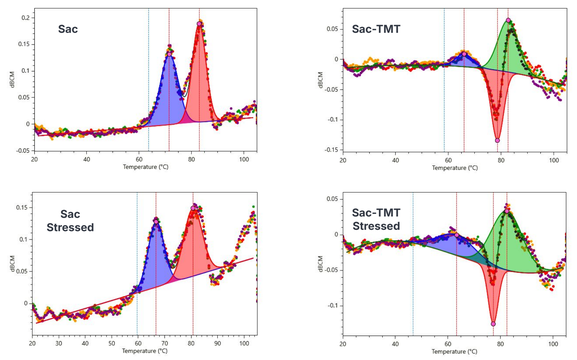


Figure 3: dBCM thermal denaturation profiles of Sac and Sac-TMT under biological conditions and oxidative stress

	T_{onset} (°C)	T_{m1} (°C)	T_{m2} (°C)	T_{m3} (°C)
Sac	63.62 ± 0.22	71.50 ± 0.05	82.89 ± 0.06	-
Sac Stressed	59.63 ± 0.32	66.74 ± 0.06	80.68 ± 0.22	-
Sac-TMT	58.46 ± 1.70	65.99 ± 0.18	78.57 ± 0.04	82.70 ± 0.12
Sac-TMT Stressed	46.74 ± 0.51	63.33 ± 0.64	77.22 ± 0.13	82.42 ± 0.29

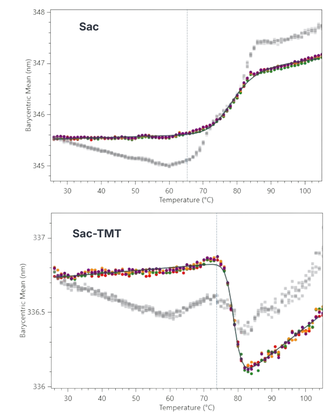
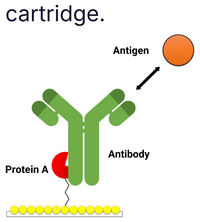


Figure 4: MSF-derived BCM plots of Sac and Sac-TMT under biological conditions

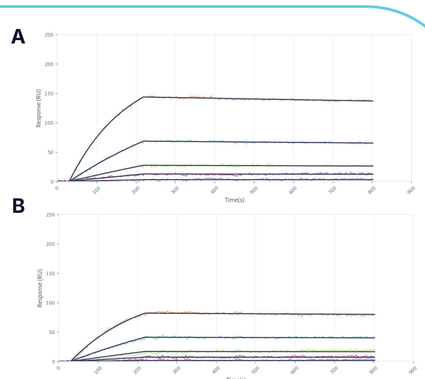
	T_{nr} (°C)
Sac	65.34 ± 0.20
Sac-TMT	73.75 ± 0.12

SPR: Kinetics Characterization

Digital SPR was used to compare the binding kinetics of the parent antibody Sac and a drug-conjugated version Sac-TMT against TROP2 using an Fc capture assay. Digital SPR offers powerful time, labor and reagent savings for running kinetics on up to 72 unique interactions per cartridge. For each analyte, Digital SPR measures five (5) unique concentrations by automatically performing 3X serial dilutions on cartridge.



The measured KD values of 1.51 nM and 1.42 nM, respectively, indicate that conjugation of the cytotoxic payload did not compromise the antibody's affinity for TROP2.



	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
Sac	4.84×10^4	7.25×10^{-5}	1.51
Sac-TMT	5.12×10^4	7.34×10^{-5}	1.42

Figure 5: Representative kinetic fits for (a) Sac and (b) Sac-TMT binding to TROP2 on Digital SPR

Conclusion

Digital SPR, CD, and SUPR-DSF collectively confirm that Sac-TMT retains the core binding affinity (K_D = 1.42 nM vs. 1.51 nM) and global β-sheet fold of parent Sac. Minor spectroscopic shifts in CD and localized thermal destabilization in DSF reflect the expected effects of payload attachment without compromising overall stability or aggregation resistance. The instrumentation suite from Nicoya is able to successfully validate Sac-TMT as a structurally intact, high-affinity ADC candidate.

Learn how Digital SPR, SUPR-DSF and Chirascan V100 can take your discoveries to the next level. Visit our website or contact us:

info@nicoyalife.com