Structural Characterization of a Monoclonal Antibody (mAb) - Maytansinoid Immunoconjugate

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Supporting Information 1

Preparation of DM1-SMCC. DM1, SMCC, diisopropylethylamine, and dry chloroform were charged to a 100 mL round bottom flask equipped with a magnetic stir bar. The mixture was stirred at room temperature overnight. The mixture was then concentrated to a residue at 40 °C and reduced pressure to yield a solid residue. The residue was purified by preparative HPLC (Luna Silica, 21.2 x 150 mm, 5 μm column; 20 mL/min isocratic elution; 20% acetonitrile/ methyl tert-butyl ether).

Preparation of ADC. Dimethylacetamide (DMAC) and mAb were combined in a glass vial equipped with a magnetic stirrer. The mixture was stirred at room temperature. DM1-SMCC was added as a solution in DMAC, and the mixture was stirred for 70 minutes. Glycine was charged, and the mixture was stirred at room temperature for an additional 4 h.

Supporting Information 2

DAR Determination. UV/Vis, cIEF, and LC-TOF-MS were used to determine the average DAR of the ADC in this study. The basis for quantitative analysis in the UV/Vis spectrophotometric assay is the Beer–Lambert law. The Beer–Lambert law can also apply to a multicomponent system if these components have different absorption spectra and there are no interactions among these components. The total absorbance of the solution at a given wavelength, λ, is the sum of the individual absorbance values for each species. DM1 and the mAb have distinct absorption maxima, and the presence of DM1 does not affect the absorption of the mAb and vice versa (Fig. 1). Therefore, ADC can be considered a two-component mixture, DM1 and the mAb.

$$A_{252} = \left(C^{DM1} \times \varepsilon_{252}^{DM1} + C^{mAb} \times \varepsilon_{252}^{mAb}\right) l$$

$$A_{280} = \left(C^{DM1} \times \varepsilon_{280}^{DM1} + C^{mAb} \times \varepsilon_{280}^{mAb}\right) l$$

$$C^{DM1} = \left(\frac{A_{280} \times \varepsilon_{252}^{mAb} - A_{252} \times \varepsilon_{280}^{mAb}}{\varepsilon_{280}^{DM1} \times \varepsilon_{252}^{mAb} - \varepsilon_{252}^{DM1} \times \varepsilon_{280}^{mAb}}\right)/l$$

$$C^{mAb} = \left(\frac{A_{280} \times \varepsilon_{252}^{DM1} - A_{252} \times \varepsilon_{280}^{DM1}}{\varepsilon_{280}^{mAb} \times \varepsilon_{252}^{DM1} - \varepsilon_{252}^{mAb} \times \varepsilon_{280}^{DM1}}\right)/l$$

$$DAR = \frac{C^{DM1}}{C^{mAb}}$$

where A is the absorbance, ε is the molar extinction coefficient, l is the path length, and C is the molar concentration.

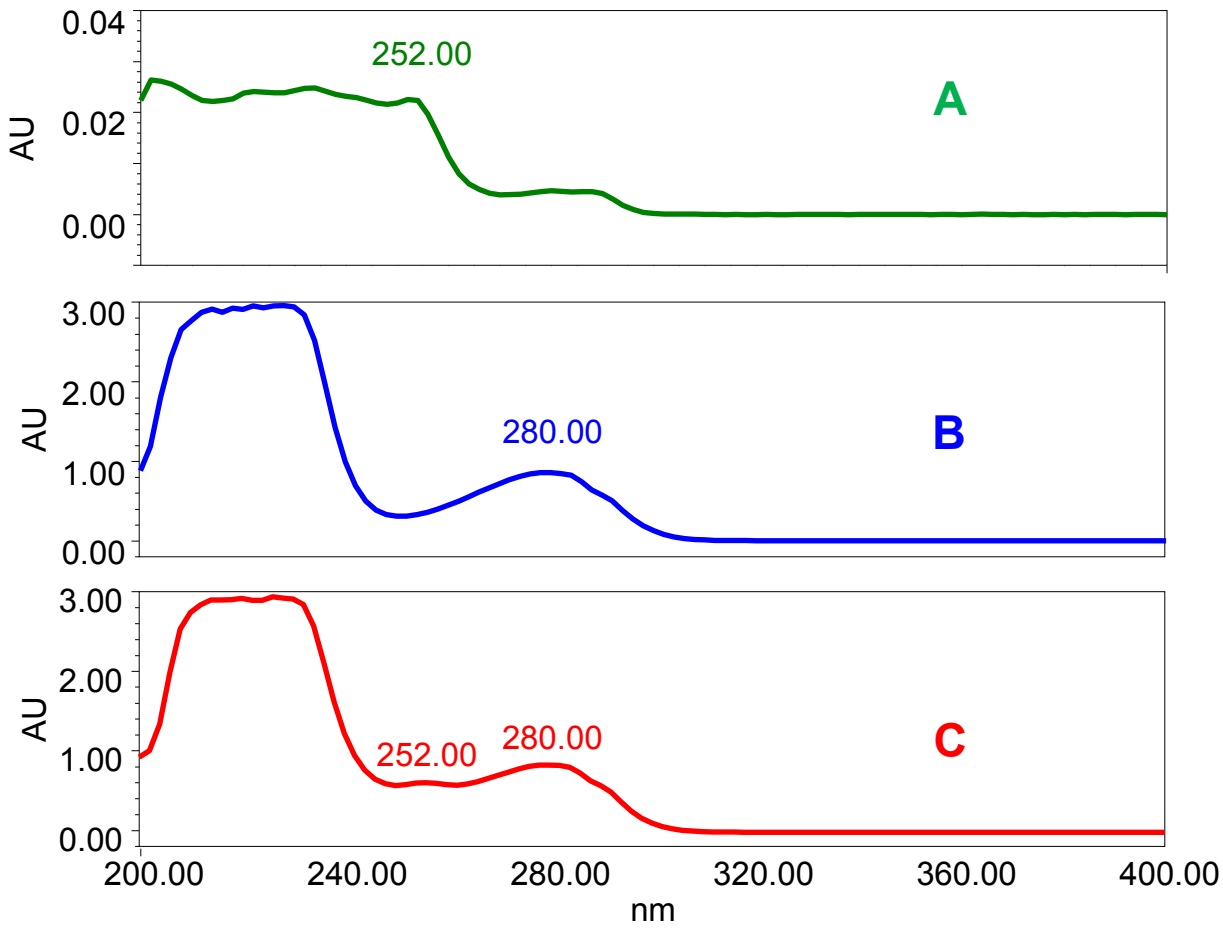


Fig. 1. UV/Vis Absorbance Spectra of DM1 (A), mAb (B), and ADC (C).

LC-TOF-MS was used to determine the drug load distribution (%) and the DAR value of the ADC. To determine the drug load distribution (%) for each mAb with drug load *i*, the mass peaks in the deconvoluted spectra (Figure 2B in manuscript) were integrated to obtain the individual peak areas, then the peak area of each mAb with drug load *i* was divided by the sum of all the peak areas (Eq.1 in manuscript).

To calculate the average DAR, first, the % drug load distribution of each mAb with drug load *i* was multiplied by its corresponding drug load *i*. The resulting value represents the weighted

contribution of each mAb with drug load *i* species to the ADC profile. Second, all these values were summed to calculate the weighted average DAR of the ADC (Eq.2 in manuscript).

cIEF was performed on a Convergent Bioscience iCE280 Analyzer with detection at 280 nm. ADC can be considered a two-component mixture, DM1 and mAb. Therefore, the extinction coefficient of each mAb with drug load *i* is:

$$\varepsilon_{280}^{mAb \cdot iDM1} = \varepsilon_{280}^{mAb} + i \times \varepsilon_{280}^{DM1}$$

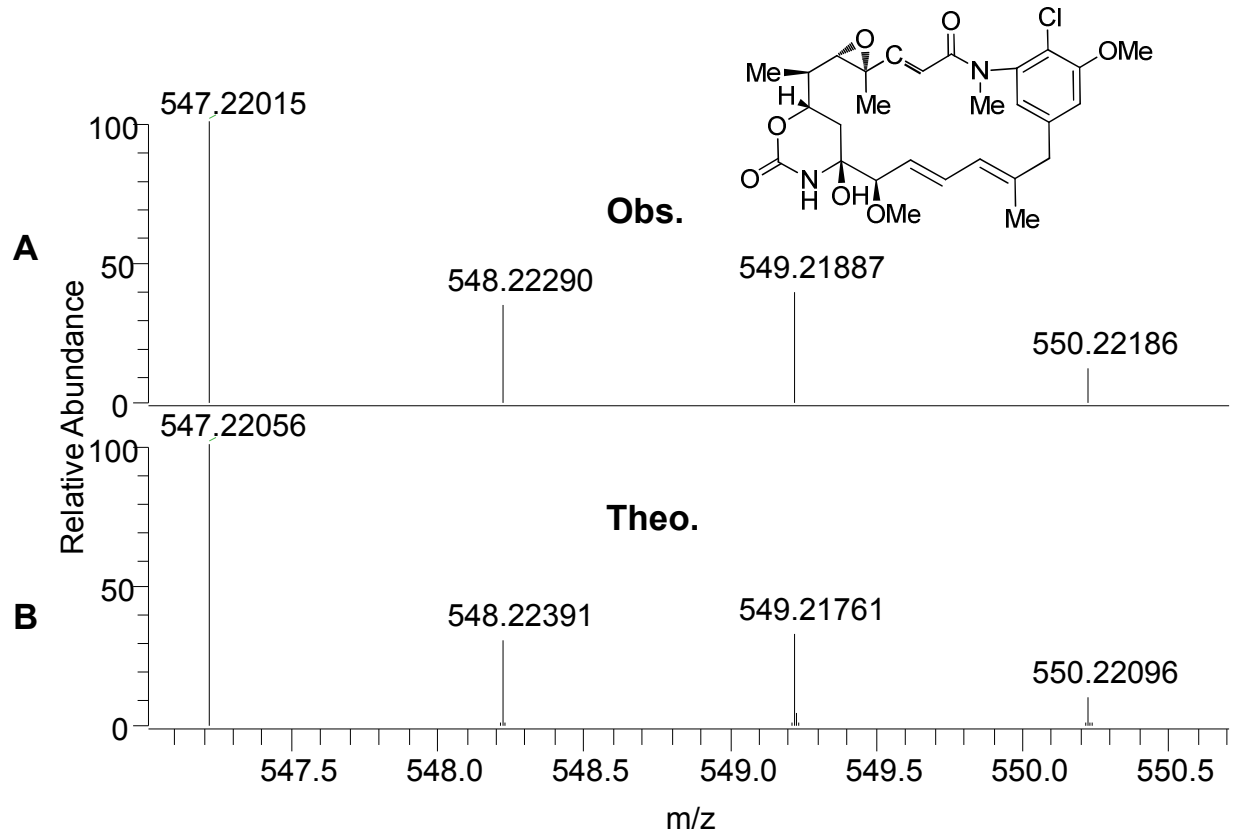
Where ε_{280}^{mAb} and ε_{280}^{DM1} are the molar extinction coefficients of mAb and DM1, respectively. The peak area of each mAb with drug load *i* was integrated from the cIEF UV profile of ADC (Figure 2A in manuscript) and was divided by the corresponding molar extinction coefficient ($\varepsilon_{280}^{mAb \cdot iDM1}$). The normalized peak area was then used to calculate the drug load distribution and DAR as described above.

Supporting Information 3

LC/ESI-MS/MS Analysis. A Thermo Scientific LTQ Orbitrap Velos high resolution mass spectrometer (San Jose, CA) with collision induced dissociation (CID) fragmentation was used to characterize the tryptic digests. The ESI source voltage was set at 4.5 kV, and the capillary temperature was set at 250°C. The mass spectrometer was set up to acquire one high-resolution full scan at 60,000 resolution (at m/z 400), followed by five concurrent data-dependent MS/MS scans of the top five most abundant ions (normalized collision energy 35 – 60%). Alternatively, a Thermo Scientific Q-Exactive Orbitrap high resolution mass spectrometer (San Jose, CA) with higher-energy collisional dissociation (HCD) (normalized collision energy 25%) was used to characterize the DM-attached peptides. The ESI source voltage was set at 4.0 kV, and the capillary temperature was set at 250°C. The mass spectrometer was set up to acquire one high-resolution full scan at 140,000 resolution with scan range of 300-2000 m/z, followed by ten data-dependent MS/MS scans of the top ten most abundant ions.

Supporting Information 4

Comparison of MS spectrum of DM1 fragment ion generated from HCD fragmentation (A) and the theoretical MS spectrum of the DM1 fragment (B)



Supporting Information 5

Base peak chromatograms of tryptic digest of the naked antibody (A) and the XIC of the DM1 fragment ions from the HCD MS/MS spectra of the LC/MS-MS profile.