Supporting Information for:

Characterization and Higher-Order Structure Assessment of an Interchain Cysteine-Based ADC: Impact of Drug Loading and Distribution on the Mechanism of Aggregation

Jianxin Guo[‡], Sandeep Kumar[‡], Mark Chipley[§], Olivier Marcq[#†], Devansh Gupta[+], Zhaowei Jin[‡], Dheeraj S. Tomar [‡], Cecily Swabowski[§], Jacquelynn Smith[§], Jason A. Starkey[§], Satish K. Singh[*‡]

[‡] Pharmaceutical R&D, [§] Analytical R&D, [#] Bioprocess R&D
Pfizer Inc., 700 Chesterfield Parkway West, Chesterfield, MO 63017, USA
[+] Department of Chemical and Biological Engineering, Princeton University, NJ 08544

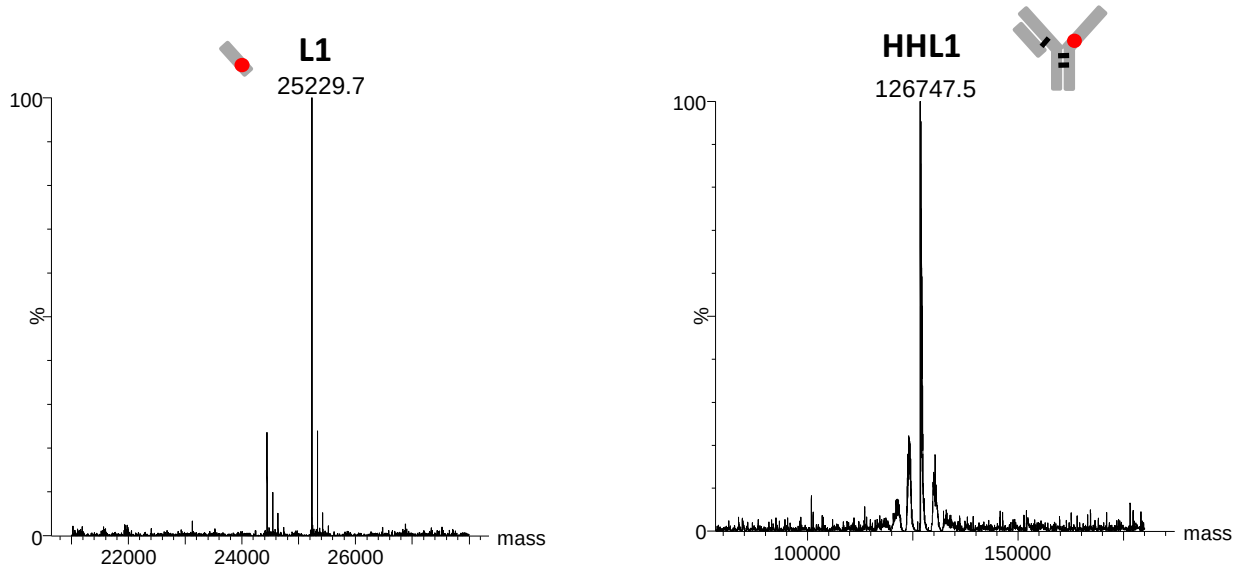
[†]Present Address: Agensys Inc., 1800 Stewart Street, Santa Monica, CA 90404

[*]To whom correspondence should be addressed:
Satish K Singh
Ph: (636) 247-9979
Fax: (860) 686-7768
satish.singh@pfizer.com

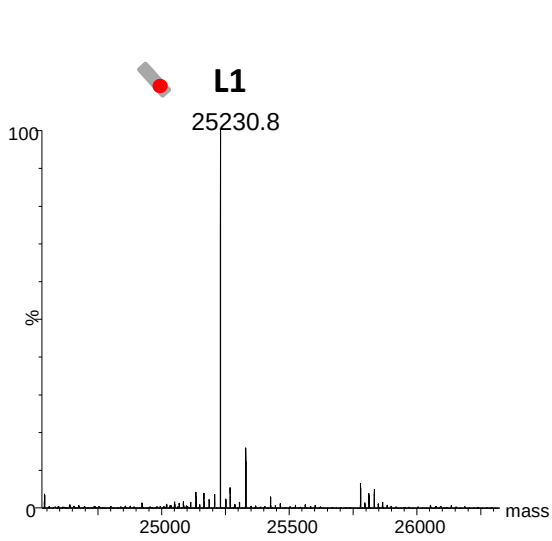
TABLE OF CONTENTS

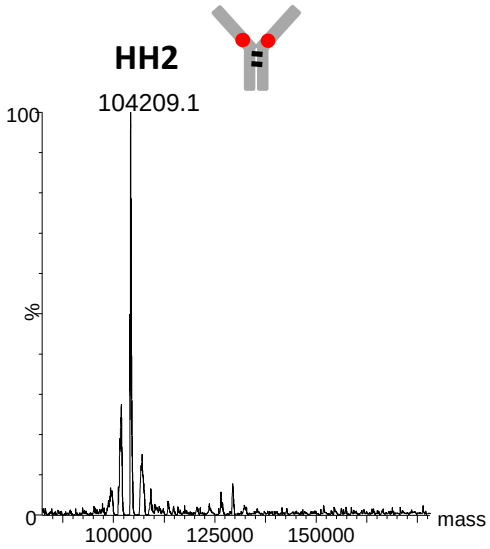
Figure S1. Characterization of conjugate forms and positional isomers of cysteine-based ADC. Mild thiol reduction and hinge-region Cys conjugation leads to a distribution of species observed in Figure 1 (main text). The HIC profile observed in Figure 2 (main text) illustrates the DAR distribution for this specific ADC. The corresponding HIC-resolved conjugate forms and positional isomers were elucidated through two-dimensional liquid chromatography/mass spectrometry. In the first dimension, samples were injected onto the HIC column and detected via UV following gradient elution. As each individual species elutes from the column, they are trapped to a phenyl-based stationary phase cartridge to desalt and buffer exchange into a suitable mobile phase for mass spectrometry. After sufficient desalting, samples are gradient eluted and analyzed by electrospray ionization time-of-flight mass spectrometry in the second dimension. Mass spectra are deconvoluted via maximum entropy for accurate mass determinations. The samples are eluted from the reversed-phase cartridge under acidic conditions with an aqueous/organic mobile phase, which dissociates all intermolecular interactions between the polypeptide chains. Under such denaturing, non-reducing conditions, the intact ADC, which primarily would be missing one or more inter-chain disulfide bonds due to hinge-region Cys conjugation, will dissociate into various polypeptide forms, including light chain (L), heavy (H) chain, HL, HH, HHL, etc., each of which contains a particular number of linker payloads, corresponding to the number of reduced inter-chain disulfide bonds. Again, assuming only hinge-region Cys conjugation, mass spectrometric analysis provides the number of linker-payloads per polypeptide chain, which then essentially allows the "sites" of conjugation to be determined when using the range of possible conjugate forms and positional isomers in Figure 1 (main text) as a guide. The mass spectra below indicate the predominant species observed in the HIC profile, based on Figure 1 (main text) structures and agreement of the observed and theoretical molecular mass values within a method-specified error tolerance (see Supplement Table 1). The primary species observed in Figure 2 (main text) are: (a) DAR 2a species, (b) DAR 4b species, (c) DAR 4a species, and (d) DAR 6a species.

a. DAR 2a Mass Spectra

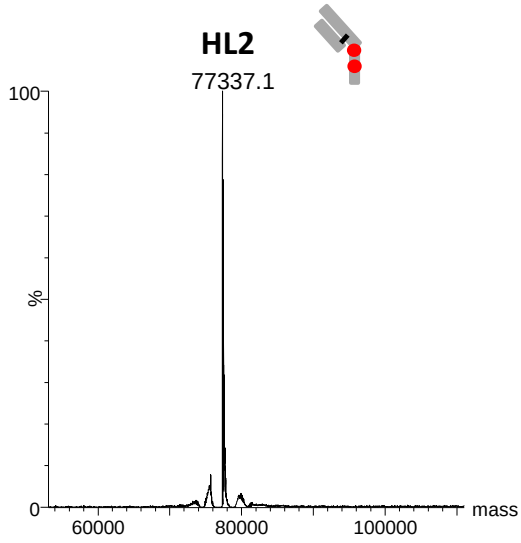


b. DAR 4a Mass Spectra

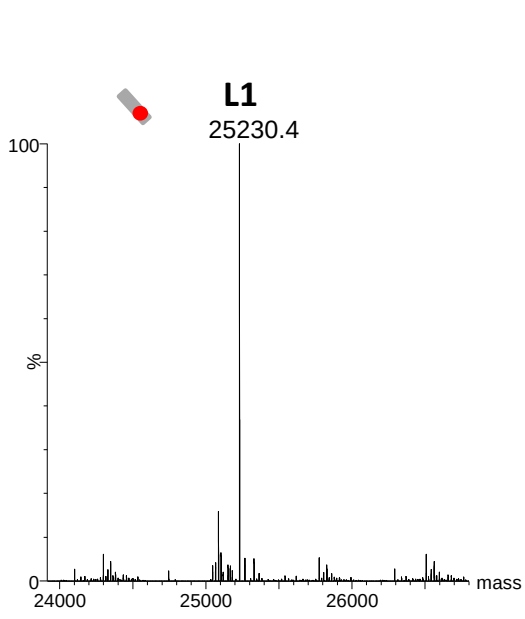




c. DAR 4b Mass Spectrum



d. DAR 6a Mass Spectra



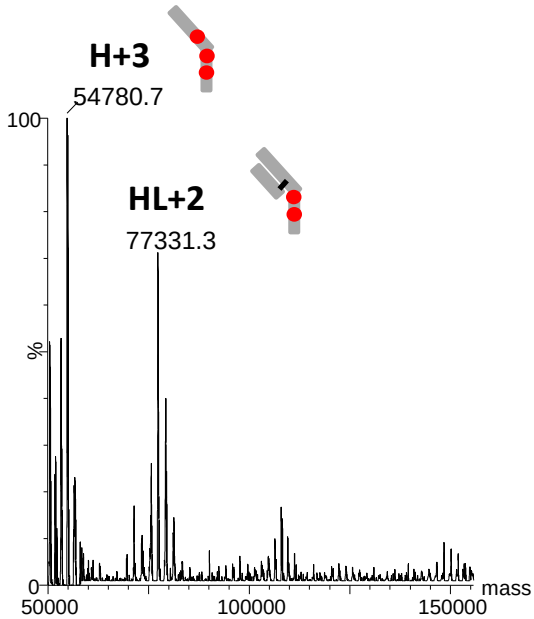


Figure S2. Solvent exposed polar (pink) and nonpolar (green) surface on parent mAb and ADCs at different DAR levels.