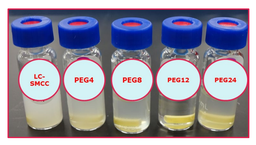
Understanding the Effect of Discreet PEG Linkers on ADC Structure and Binding

Agnieszka Lass-Napiorkowska, Jason Ramsay & Lisa McDermott
Process and Analytical Development | Life Science | Process Solutions | Actives & Formulation | MilliporeSigma
Piotr Kaczmarek, Leo Solorzano, Deepa Raghu, Yu Ting Hsu, Martin De Cecco & Omar Lamm
BioReliance Product Characterization Services | Life Science | Process Solutions Services | MilliporeSigma

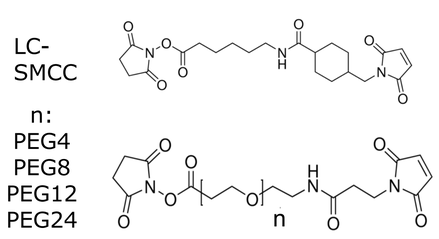
Introduction

For an antibody-drug conjugate, the choice of the linker can have a dramatic effect on both physical structure and biological activity. The increased hydrophilicity provided by a PEG linker can influence the potency, toxicity and pharmacokinetics of the ADC.
To investigate the chemical challenges that can be overcome by the right choice of the linker, we have explored the influence of a variety of PEG linkers on the structure of a model cysteine-bioconjugate. Specific sites of conjugation were identified by peptide mapping and changes in conformation were investigated by hydrogen/deuterium exchange mass spectrometry. The resulting impact on Fc receptor binding by surface plasmon resonance was also determined.
These results illustrate the importance of an appropriate linker and the need for thorough product characterization in order to understand the structure-function relationships of an ADC.

Schematic representation of ADC mimic (MSQC8): SigmaMAb (MSQC4) – LC-SMCC - dansylcadaverine



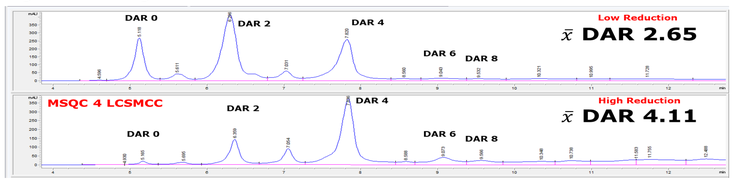
Visual representation of the DL solubility in the bioconjugation process during linker library preparation



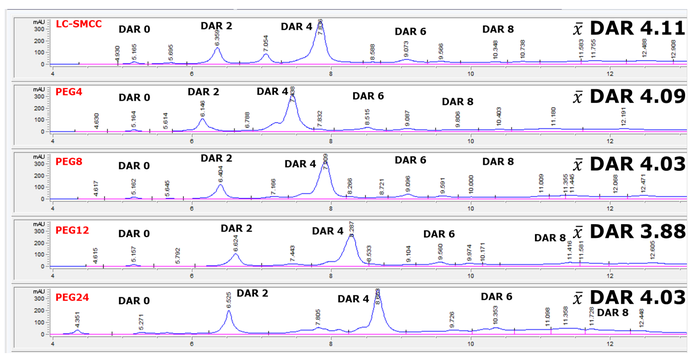
Structure of linkers used to prepare the ADC mimics

Process Development Analytical Results

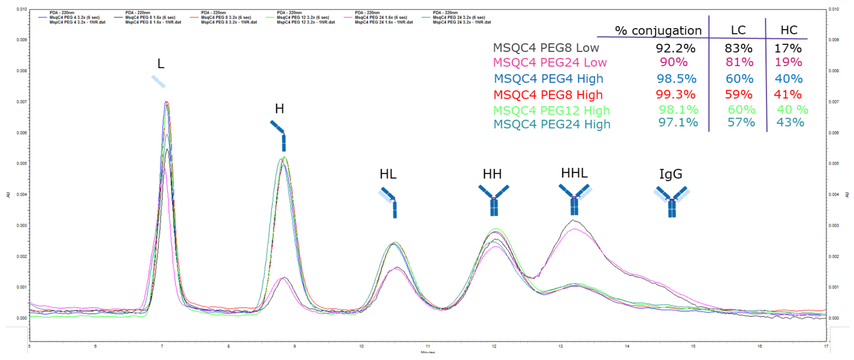
DAR calculations based on Hydrophobic Interaction Chromatography (HIC). Process development titrations with increasing reducing agent producing higher average DAR.



Optimized final process of PEG linker library and LC-SMCC model linker construct. PEG linker length correlates directly to the hydrophobic interactions seen chromatographically in retention time of discrete DAR species. Interestingly, the model linker LC-SMCC (Mw 447 g/mole; 16.2Å) is similar in hydrophobicity profile to the PEG8 linker (Mw 690 g/mole; 39.2Å), yet similar in mass to the PEG4 linker (Mw 513 g/mole, 24.6Å), though shorter.

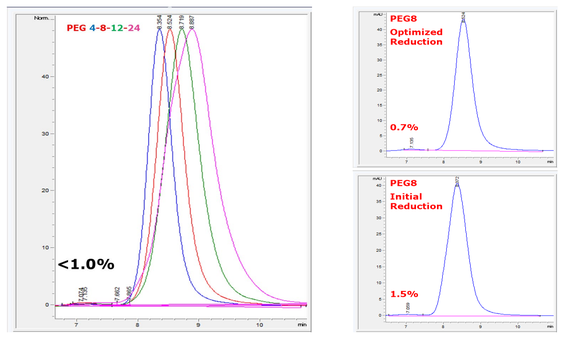


Capillary Gel Electrophoresis (CGE) can provide information about drug-linker distribution across heavy and light chain cysteine target sites.



Process Quality Results

PEG linker library size homogeneity by Size Exclusion Chromatography (SEC) demonstrates <1.0% total aggregation for all ADC constructs at concentrations of reducing agents optimized for conjugation of $\bar{x}$ DAR 4.

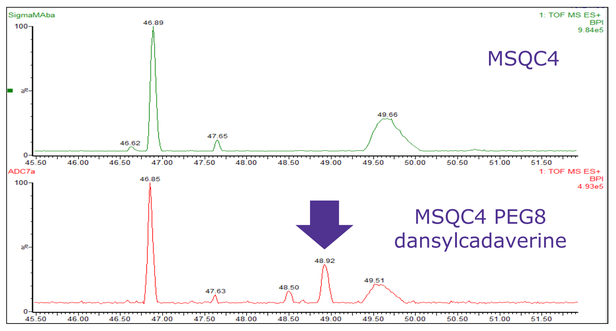


Structural Characterization: Sites of Conjugation

Peptide mapping by LC-MS confirmed that the conjugation occurred at the hinge region, at cysteines normally involved in inter-chain disulfide bonds. Site occupancy was greatest at Cys-224 on the heavy chain and Cys-217 on the light chain, indicating that the disulfide bond between the LC and HC was most susceptible to reduction. Occupancy at Cys-230/Cys-233 (HC) seemed to decrease with increasing linker length.

Linker	DAR	LC	HC		
		Cys-217	Cys-224	Cys-230 or Cys-233	Cys-230 & Cys-233
LC-SMCC	4.1	88%	100%	11%	15%
PEG4	4.1	59%	100%	9%	11%
PEG8	2.6	64%	100%	1%	-
PEG8	4.0	69%	100%	5%	3%
PEG12	3.9	71%	100%	5%	3%

Site occupancy of conjugate at cysteines in hinge region

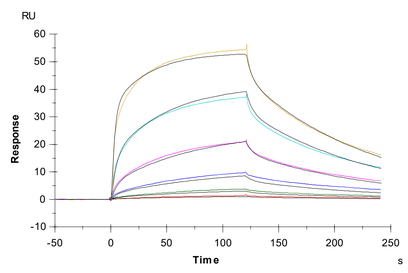


Zoomed chromatogram showing presence of a conjugated peptide in ADC mimic sample

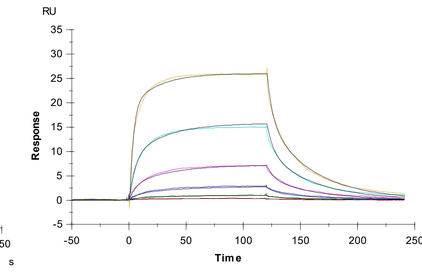
Fc Receptor Binding

The affinities of the ADC mimics for recombinant human FcγRIIIA (both V and F variants) and FcRn were determined by surface plasmon resonance (SPR) on a Biacore T200 instrument.

For FcγRIIIA, there is a general trend of decreased affinity (higher K_D, lower relative K_D) with increasing length of PEG linker. In addition, higher DAR tends to result in decreased affinity for FcγRIIIA. The differences are more pronounced for the V variant, which binds more strongly than the F variant.



Representative kinetic data for FcγRIIIA (V)-mAb interaction

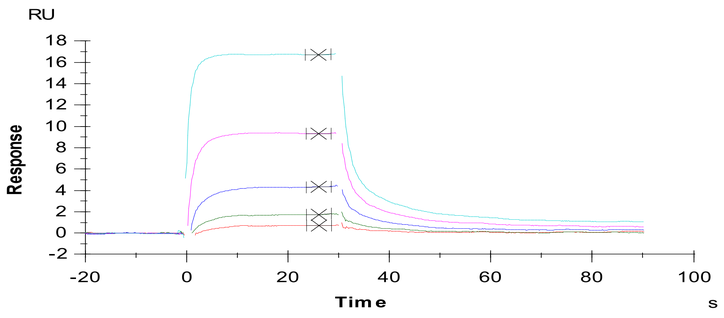


Representative kinetic data for FcγRIIIA (F)-mAb interaction

Linker	DAR	FcγRIIIA (V)		FcγRIIIA (F)		FcRn	
		K_D (nM)	Rel. K_D (%)	K_D (nM)	Rel. K_D (%)	K_D (nM)	Rel. K_D (%)
Reference	0	169	100	468	100	392	100
LC-SMCC	4.1	195	87	465	101	309	128
PEG4	4.1	211	80	500	94	299	132
PEG8	4.0	200	84	433	108	358	111
PEG12	3.9	230	74	572	82	361	110
PEG24	4.0	257	66	612	77	293	135
LC-SMCC	2.7	154	109	374	125	288	138
PEG8	2.6	192	88	474	99	333	119
PEG24	2.8	215	79	555	84	272	146

Relative K_D to the unmodified SigmaMAb is calculated using K_D = RS K_D/ Sample K_D.

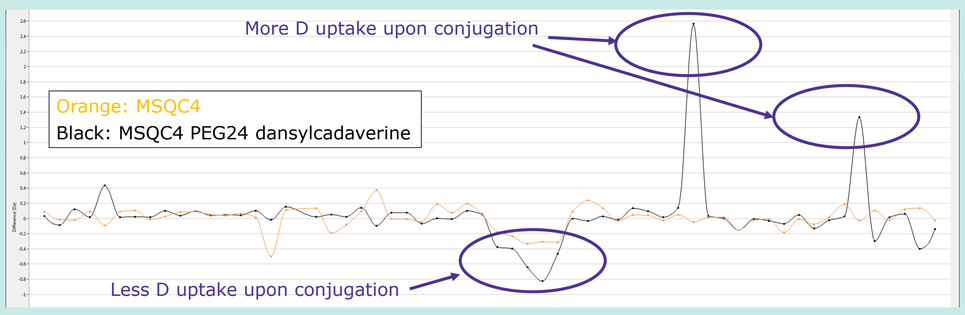
Interestingly, all of the ADC mimics exhibited increased affinity for FcRn. For FcRn, there was no clear association between relative K_D and the size of linker or DAR of the ADC mimic. Further work is required to assess the statistical significance of the above results.



Representative sensorgrams for FcRn-ADC mimic interaction

Higher Order Structure

The influence of linker size on higher order structure was investigated by hydrogen/deuterium exchange mass spectrometry.
Conjugation resulted in a decrease in D uptake in the HC(246-272) region (hinge region), possibly due to protection from the linker.
Conjugation also resulted in an increase in D uptake in HC(302-318) and HC(419-444), suggesting a change in conformation leading to greater solvent accessibility.
There were no significant differences between PEG4 and PEG24, indicating that the increasing linker size does not affect the overall structure of the mAb.



Deuterium uptake across the heavy chain backbone

Summary

Drawing on worldwide resources and expertise, MilliporeSigma can assist in the creation of a diverse set of ADCs (or other bioconjugates) for rapid screening of preferred constructs for a variety of research and development activities. From concept to conjugation to characterization, ADC Express™ and BioReliance® can provide high-quality material and supporting analysis to answer the specific questions that drive agile decision making. Making a library of constructs on a relatively small scale is affordable and can significantly accelerate selection of the best construct(s) at the beginning of the development process.
From these data the length of the PEG linkers doesn't seem to affect the DAR of the constructs based on LC/HIC, as well as distribution of the DL (HH vs HL) within the ADC species (CGE). Length of PEG linker does affect site of conjugation though (by increased steric hindrance it restricts the ability to conjugate linker/payload at neighboring site). Increased size also hinders interaction with Fcgamma, which impacts on effector function. Whilst conjugation may result in a conformational change of the mAb, this does not seem to be affected by linker size.

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