Site-specific Conjugation of Cell-penetrating Peptides: A Strategy to Ameliorate Antibody Therapy

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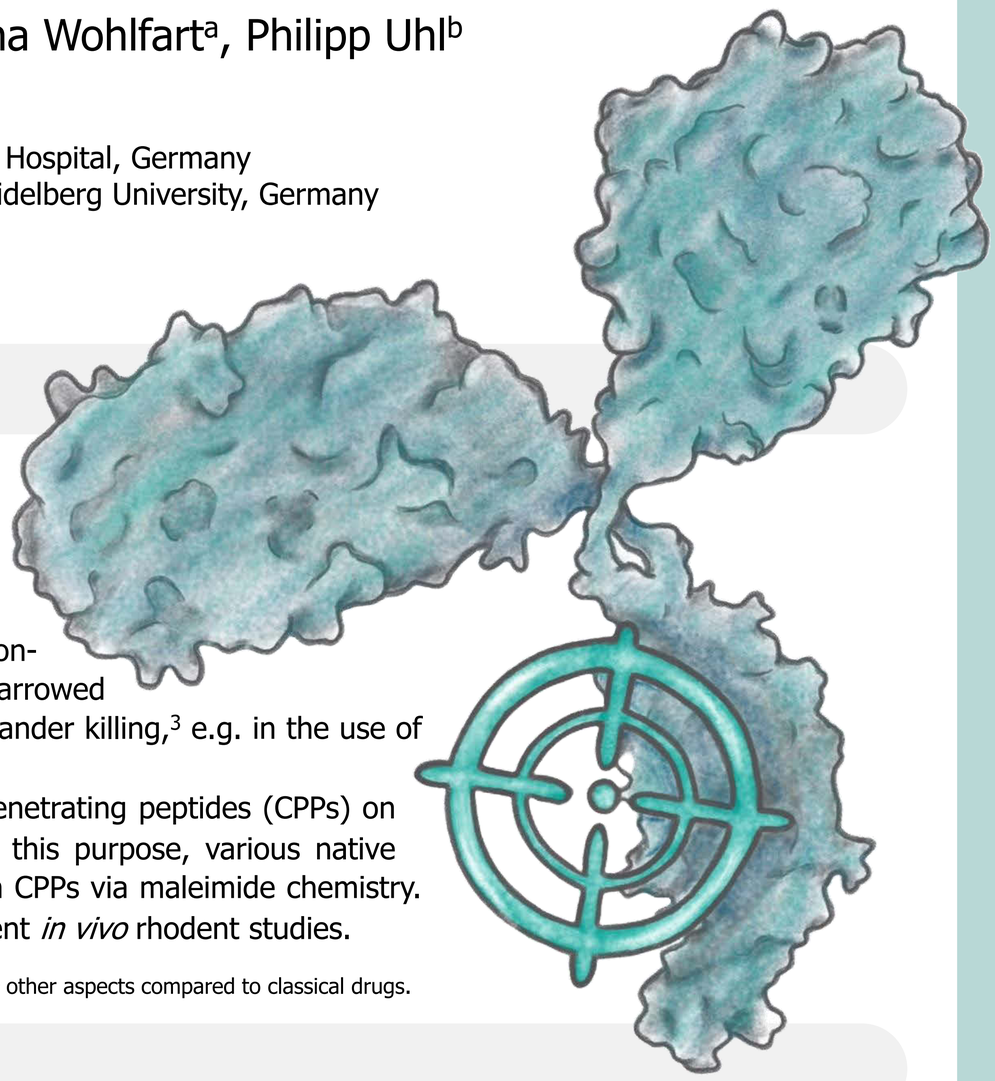
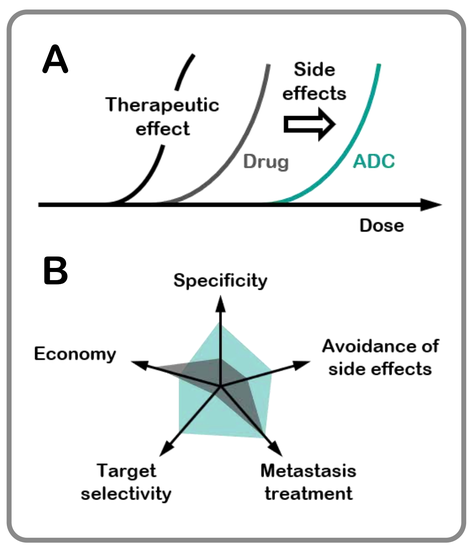


Motivation and Objective



Antibody drug conjugates (ADCs) have emerged as innovative therapeutics for the targeted therapy of cancer,[1] inflammation and infection.[2] To date, their synthesis mostly relies on random and heterogenous conjugation of the payload since site-specific labeling of monoclonal antibodies (mAbs) remains challenging and tedious.[3] As a consequence, broad drug-antibody ratio (DAR) distributions can lead to a narrowed therapeutic index[4,5] and cause undesired side-effects as a result of bystander killing,[3] e.g. in the use of cytostatic ADCs.

In this work, we aim to exploit the reported benefitial effects of cell-penetrating peptides (CPPs) on mAb internalization and biodistribution[6] under strict DAR control. For this purpose, various native off-the-shelf mAbs are conjugated site-specifically to a library of known CPPs via maleimide chemistry. The conjugates are characterized and labeled radioactively for subsequent *in vivo* rhodent studies.

Figure 1. ADCs are able to (A) broaden the therapeutic window and (B) enhance treatment in many other aspects compared to classical drugs.

Methods and Concept

To be able to obtain high yields of conjugates with defined DARs, the recently established AJICAP™ approach[7] was chosen. It relies on proximity-induced site-specific labeling of distinct lysine residues using affinity peptides equipped with a reactive organic moiety. For the analysis of the conjugates, Hydrophobic Interaction Chromatography (HIC) and Ellman's Assay are used.

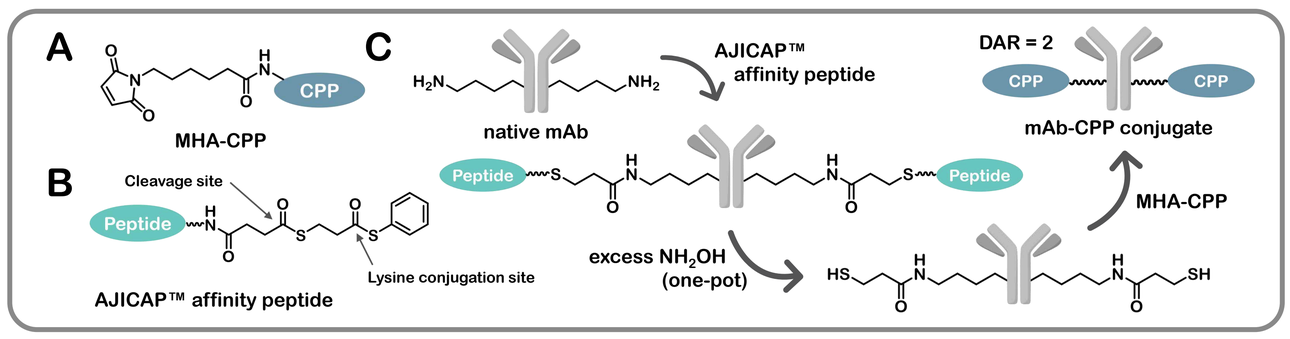


Figure 2. (A) Schematic general structure of CPPs coupled to maleimidohexanoic acid (MHA) for thiol conjugation. (B) Schematic structure of the AJICAP™ affinity peptide used in this work. (C) Modification of native mAbs is carried out in a three-step protocol.

Results

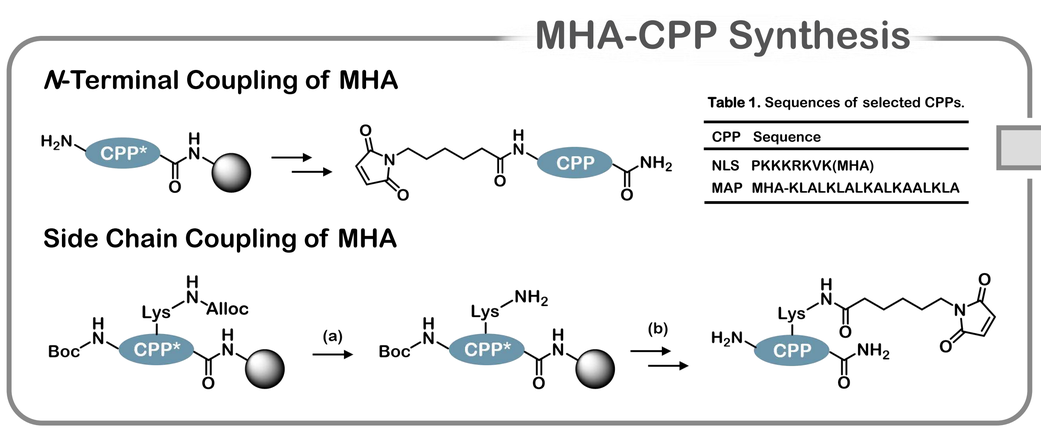


Figure 3. Alternative approaches for the solid phase synthesis of MHA-labeled CPPs. *N*-Terminal labeling of resin-bound peptide is achieved in two steps: HBTU-mediated coupling of MHA followed by simultaneous deprotection and resin cleavage using TFA and scavengers. Side chain coupling via lysine residues is carried out using orthogonal protection: (a) The *N*-allyloxycarbonyl (Alloc) protecting group is reductively cleaved from the Boc-protected resin-bound peptide using Pd(0) and BH_3NHMe_2; (b) MHA coupling is then performed prior to deprotection and resin cleavage using TFA and scavengers. *Indicates that the CPP side chains are protected with Fmoc/*t*Bu-compatible protecting groups.

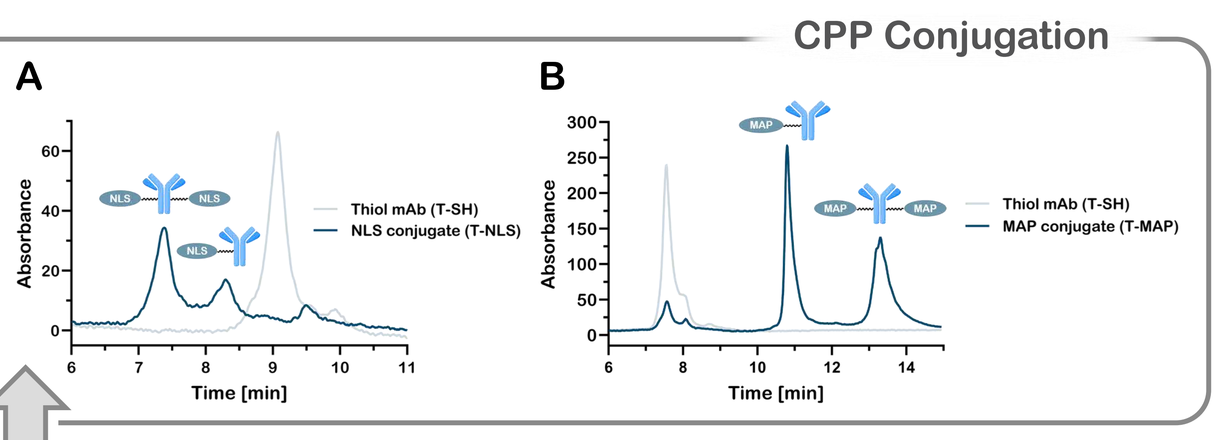


Figure 5. HIC chromatograms (TSKgel HIC-ADC column, 5 µm, 4.6 x 100 mm from Tosoh Bioscience) of Trastuzumab CPP conjugates (crude mixtures) show a decrease in retention times for (A) hydrophilic NLS and an increase for (B) hydrophobic MAP. Gradient: A: 1.1 M $(NH_4)_2SO_4$ in 100 mM phosphate buffer (pH 7.0) to B: 25% iPrOH in 100 mM phosphate buffer (pH 7.0); (A) 0% B for 2 min, then 0 to 30% B in 11 min; 1 mL/min; 5 µg sample. (B) 0% B for 2 min, then 0 to 50% B in 11 min and 50% B for 2 min; 1 mL/min; T-SH: 5 µg sample, T-MAP: 17.5 µg sample.

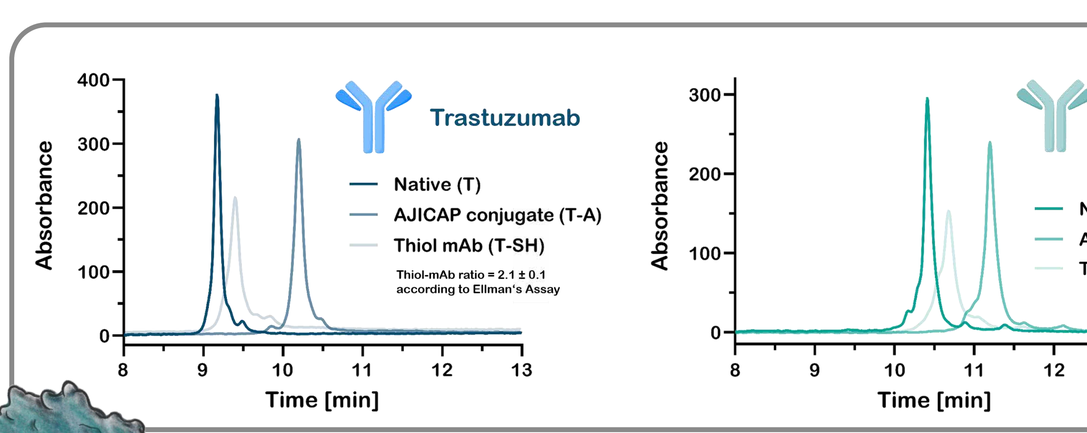
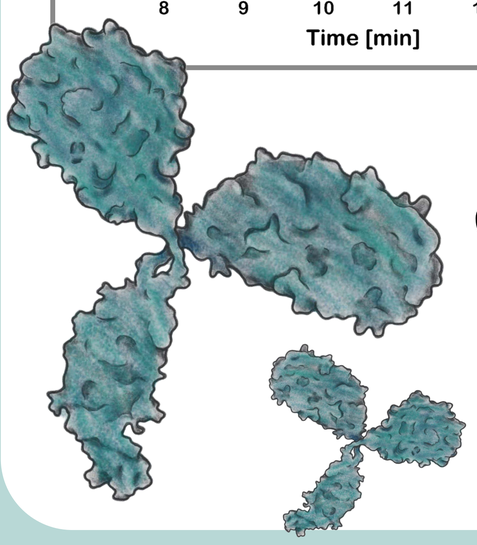


Figure 4. HIC chromatograms (TSKgel HIC-ADC column, 5 µm, 4.6 x 100 mm from Tosoh Bioscience) of different native off-the-shelf mAbs as well as their AJICAP™ derivatves. Gradient: A: 1.5 M $(NH_4)_2SO_4$ in 25 mM phosphate buffer (pH 6.0) to B: 25% iPrOH in 25 mM phosphate buffer (pH 6.0); 0% B for 2 min, then 0 to 50% B in 11 min; 1 mL/min; 5 µg sample.



Summary and Outlook

Site-specific modification of different CPPs using the AJICAP™ approach was accomplished for Trastuzumab, Rituximab and Matuzumab. Next, purification and *in vivo* testing of the CPP conjugates will be performed. We anticipate to obtain valuable data for the amelioration of ADC-based therapies.

References

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