Toward Next-Generation ADC Platform tCAP for Site-Specific IgG Modification

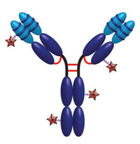
Hiroko Kawakami[1,2], Abdur Rafique[1], Shugo Tsuda[2], Taku Yoshiya[2,3] and Yuji Ito[1]

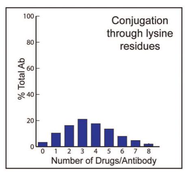
[1]Graduate School of Science and Engineering, Kagoshima University, Kagoshima, Japan.,
[2]Peptide Institute, Inc., Osaka, Japan., [3]Institute for Protein Research, The University of Osaka, Osaka, Japan

https://doi.org/10.17952/38EPS.2026.P561

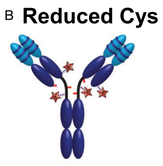
Traditional antibody conjugation

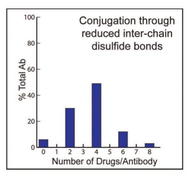
A **Amine**

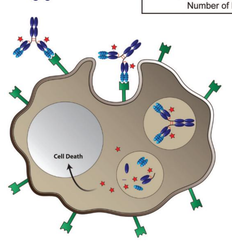




B **Reduced Cys**





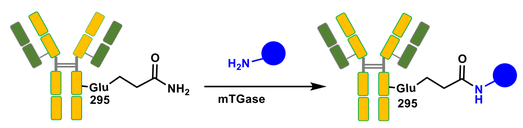


ADC

Product Name	Target Molecule	Average DAR	Modification Site	Indications / Remarks
Adcetris	CD30	4	Cys (Hinge region)	Hodgkin lymphoma, malignant lymphoma, etc.
Kadcyla	HER2	3.5	Lys residue (Random method)	HER2-positive breast cancer
Enhertu	HER2	8	Cys (Hinge region)	HER2-positive/low breast cancer, gastric cancer, etc.
Elahere	FRα	3.5	Lys residue (Random method)	Ovarian cancer
Datroway	TROP2	4	Cys (Hinge region)	Breast cancer, non-small cell lung cancer, etc.

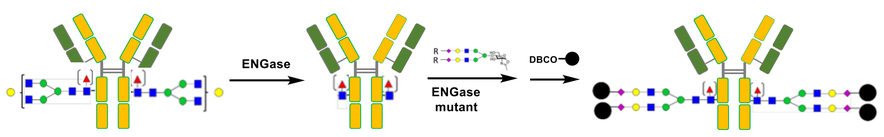
Site-specific IgG modification

Enzymatic modification



Glycans need to be trimmed by endoglycosidase (EndoS2)

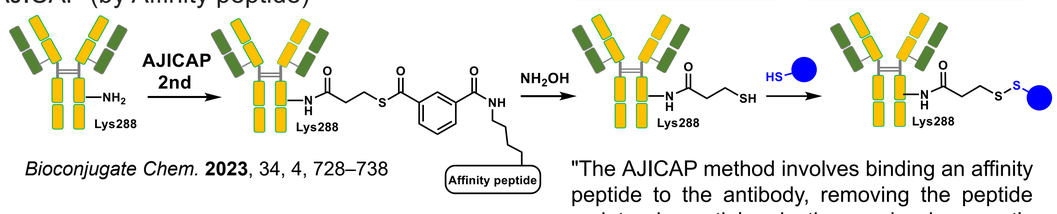
Bioconjugate Chem. **2024**, 35, 4, 465–471



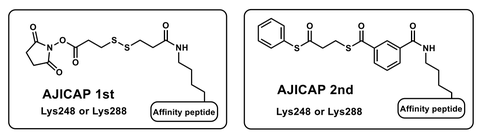
Bioconjugate Chem. **2019**, 30, 5, 1343–1355

Chemical modification

AJICAP (by Affinity peptide)



Bioconjugate Chem. **2023**, 34, 4, 728–738



"The AJICAP method involves binding an affinity peptide to the antibody, removing the peptide moiety via partial reduction, and subsequently introducing the payload in two steps."

Manabe *et al.* succeeded in producing homogeneous ADCs by trimming N-linked glycans with ENGase and attaching small molecules to the glycan moiety using an engineered variant of ENGase.

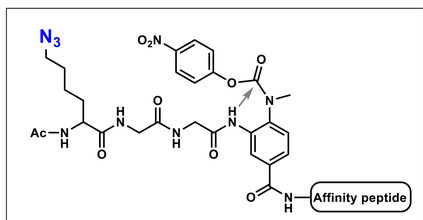
Mechanism of site-specific IgG modification " tCAP(N3)"

✓**Fmoc solid-phase peptide synthesis**

Affinity peptide

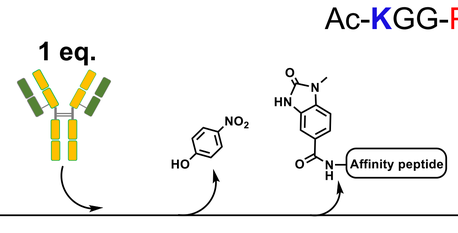
Ac-**K**GG-PMD(NO_2)-Gaba - NMQCQRRFYEALHDPNLNEEQRNAR**I**R**I**RSIRDDC-NH_2

The tCAP(N3) reagent, featuring a stable and self-activating precursor, enables site-specific conjugation of native IgGs.
This Fc-affinity peptide approach selectively transfers an Ac-Lys(N_3)-Gly-Gly payload to Lys248, producing divalently azidated antibody while preserving its biological function.

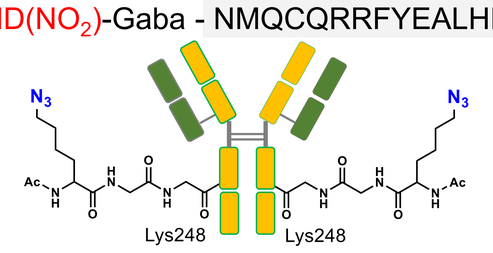


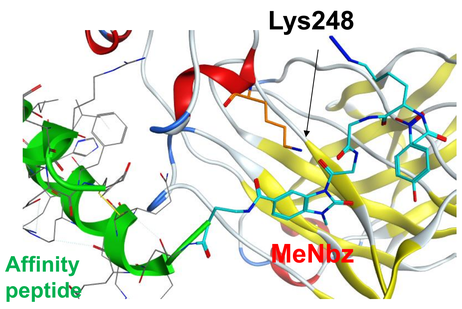
tCAP(N3) **5 eq.**

1 eq.



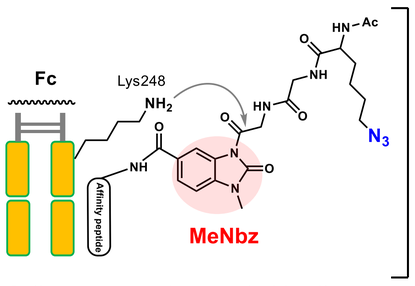
Site selective modification
rt., 2 h





Model of human IgG_1 complexed with the tCAP intermediate, highlighting the ε-amino group of Lys 248 in the Fc region.

Self-activation under neutral conditions

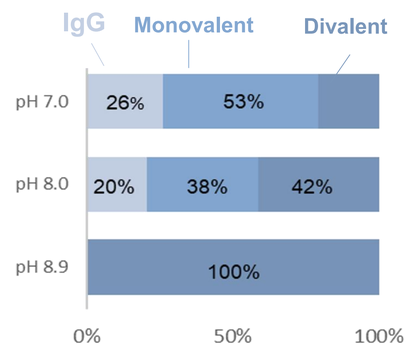


Active compound generated *in situ*

You can find modification protocol on our web site!!

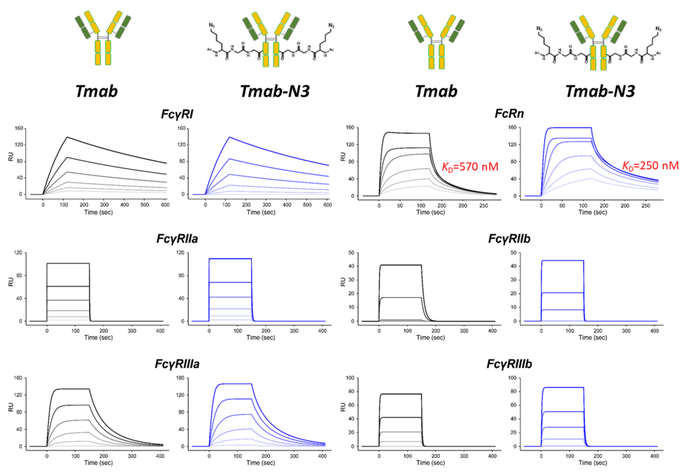
1) Pala-Pujadas,J. *et al.*, *Angew.Chem.Int.Ed.*, **2018**, 57,16120
2) Tsuda,S. *et al.*, *Chem.Commun.*, **2018**, 54,. 8861.

Effect of pH on IgG modification



The reaction was incomplete at pH 8.0 but proceeded to completion under more basic conditions (pH8.9), yielding divalently Tmab-N3.

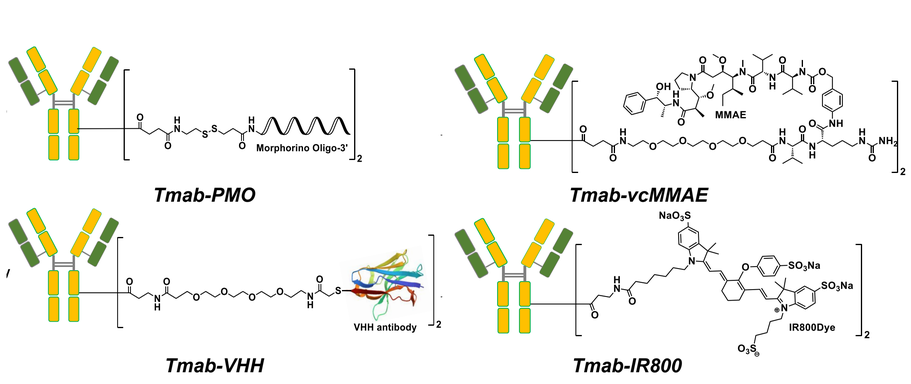
SPR analyses with Fc receptors



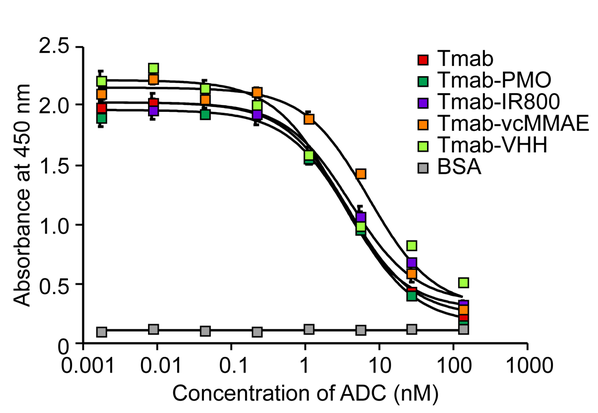
Surface plasmon resonance (SPR) analyses were performed to compare the binding affinities of Tmab with Tmab-N3 toward Fc receptors.

Several antibody conjugates

ADCs



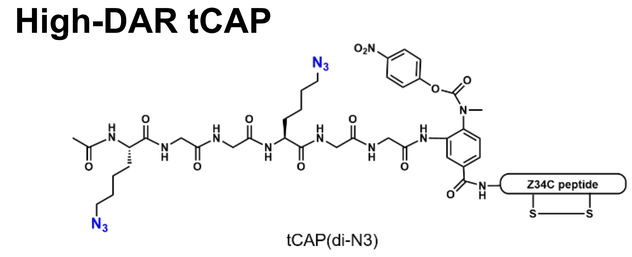
HER2 recognition of Tmab-based ADCs evaluated by competitive ELISA



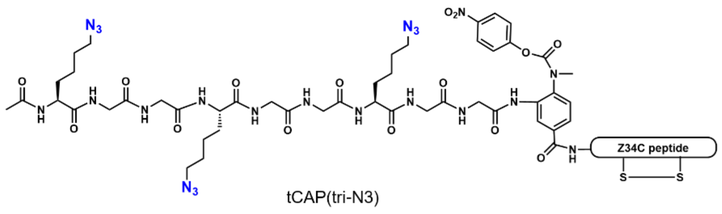
Several antibody conjugates were prepared from Tmab-N3 using strain-promoted alkyne azide–cycloaddition with a fivefold molar excess of DBCO-functionalized payloads.

Next-Generation ADC Platform tCAP

High-DAR tCAP

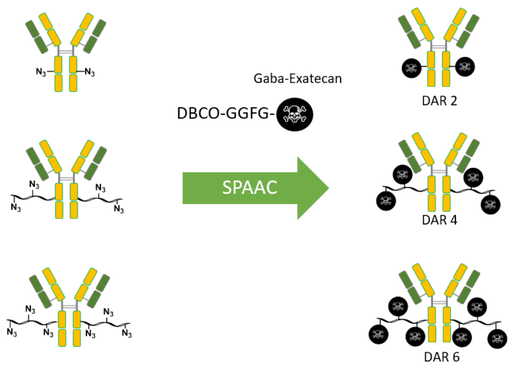


tCAP(di-N3)



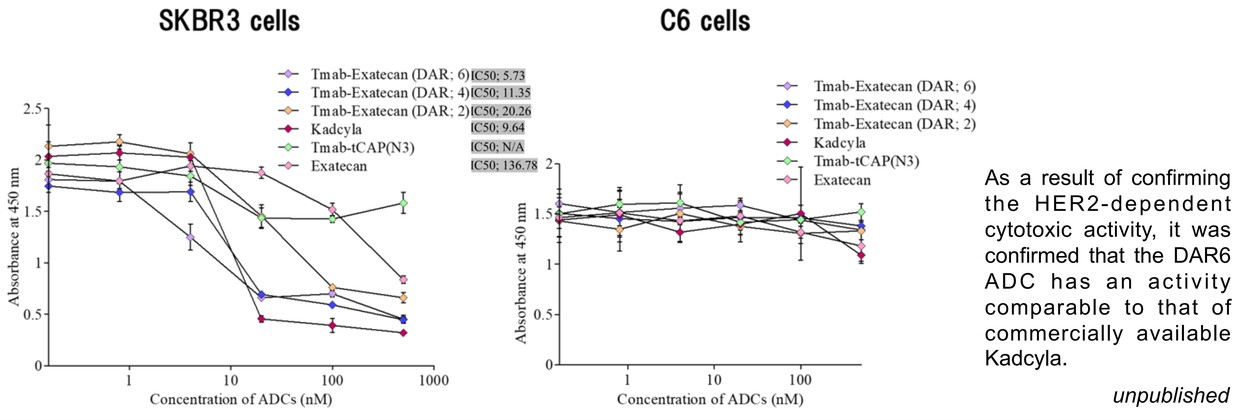
tCAP(tri-N3)

High-DAR ADC



ADCs prepared using tCAP(tri-N3) or tCAP(di-N3) easily achieved the preparation of DAR4 or DAR6 ADCs using multiple N3 groups as a foothold.

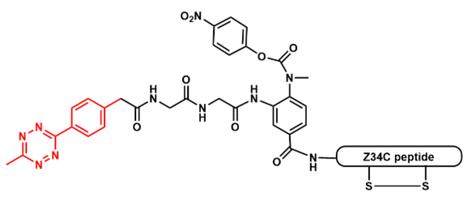
Cytotoxicities of the ADCs



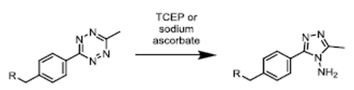
As a result of confirming the HER2-dependent cytotoxic activity, it was confirmed that the DAR6 ADC has an activity comparable to that of commercially available Kadcyla.

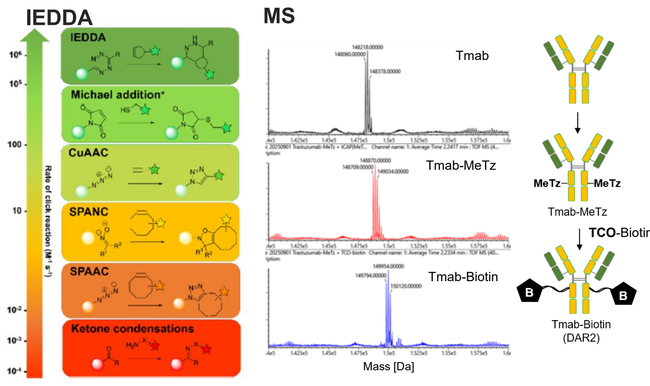
unpublished

tCAP(MeTz)



tCAP(MeTz)



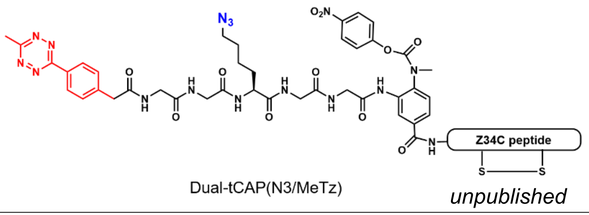


Bioconjugate Chem. **2025**, 36, 1054–1065

The conjugation reaction between MeTetrazine (MeTz) and trans-cyclooctenes (TCO) are faster than that between azide and DBCO; therefore, we also prepared tCAP(MeTz) for future challenging antibody modifications.

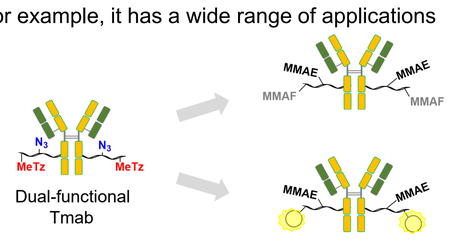
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Dual-functional tCAP



Dual-tCAP(N3/MeTz)

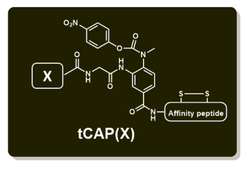
For example, it has a wide range of applications



unpublished

Summary

tCAP technology is expected to advance as a core platform for next-generation antibody drugs, enabling optimal DAR, linker, and payload design, along with multi-payload complex modalities.



Welcome collaboration!!
(kawakami@peptide.co.jp)

Patent filed (WO 2023/038082 A1).

[1] H. Kawakami, A. Rafique, S. Tsuda, A. Nagashima, Y. Nohara, N. Dozono, K. Sakamoto, S. Masuda, M. Kiyoshi, H. Shibata, A. Ishii-Watabe, T. Yoshiya, and Y. Ito, *Chem.Eur.J.*, **32**, e03144 (2026). DOI: 10.1002/chem.202503144
[2] K. Tsuji, Y. Miura, T. Kuwata, R. Matsuzaki, T. Kobayakawa, K. Matsumoto, Y. Ito, T. Yoshiya, S. Matsushita, and H. Tamamura, *ChemMedChem*, **21**, 500820 (2026). DOI: 10.1002/cmdc.202500820