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tCAP conjugate: an affinity peptide-based antibody conjugation system to generate highly functional antibody

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High functional drug with DDS by tissue-specific targeting antibody AccumBody®

Targeting/delivering antibody

AccumBody® biologics
Tissue-specifically accumulating antibody with cytokine, antibody fragment, nucleic acid medicine

ADC (antibody drug conjugate)
anticancer agent・toxin・radio isotope

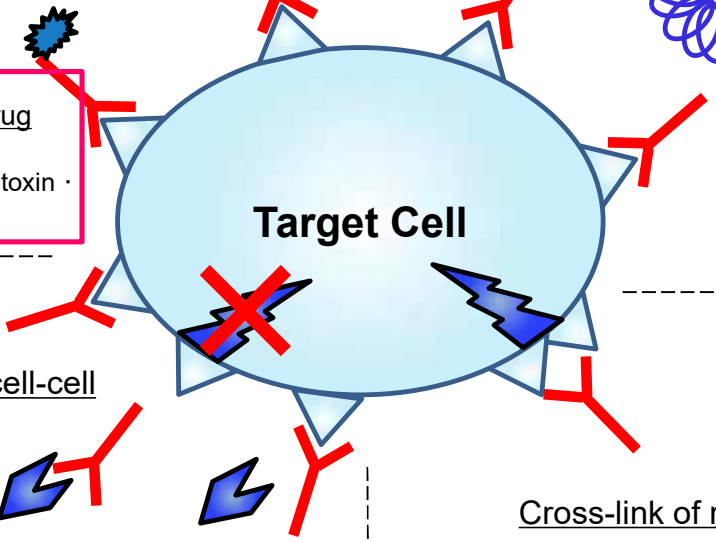
Killing (cytotoxic) antibody

NK cells

complement

ADCC
Antibody- dependent cell-mediated cytotoxicity

CDC
Complement-dependent cytotoxicity



Inhibition of cell-cell adhesion

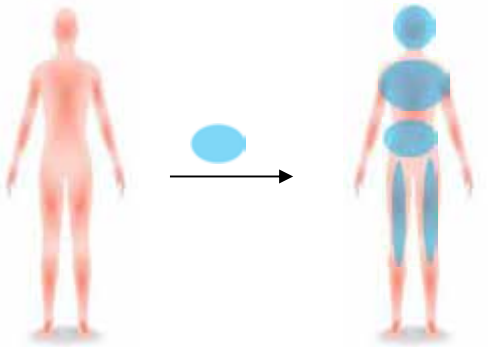
Cross-link of receptor

Inhibition of ligand-receptor binding

Blocking antibody

Signaling antibody

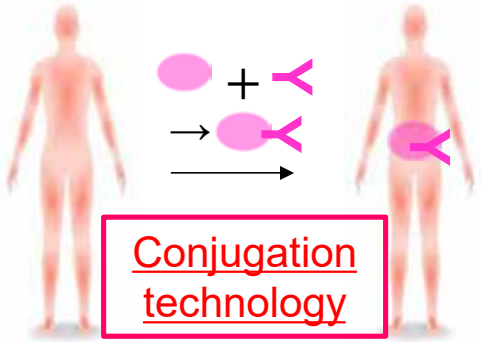
Normal drug



Distributed through whole body

-Low therapeutic efficacy
-Side effects

Drug with DDS by tissue-specific targeting antibody, AccumBody®

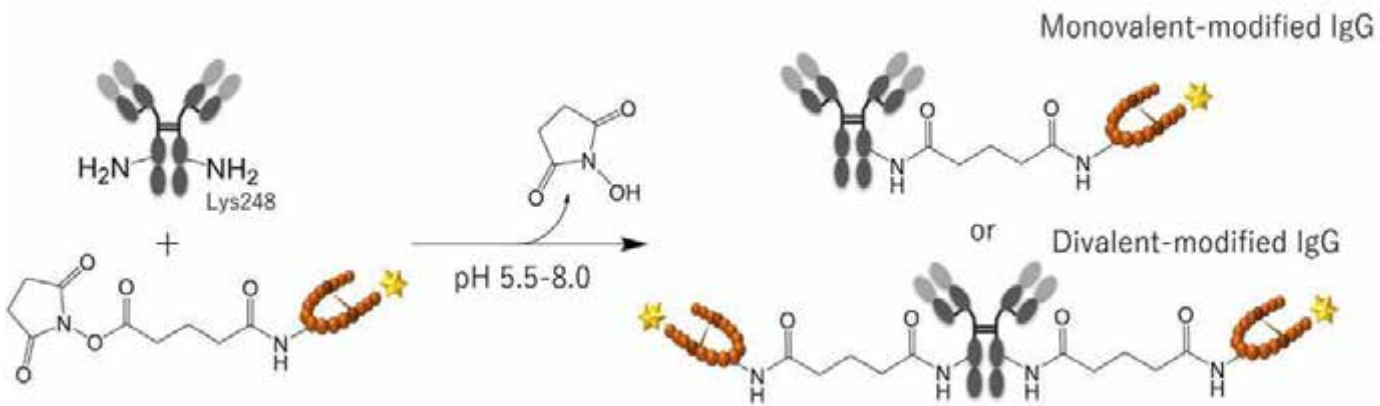


Conjugation technology

Distributed only in target organs

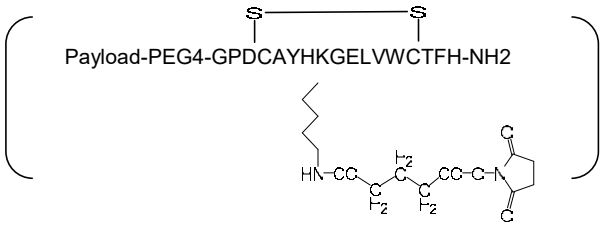
-High therapeutic efficacy
-Low side effects

CCAP （Chemical conjugation by affinity peptide）

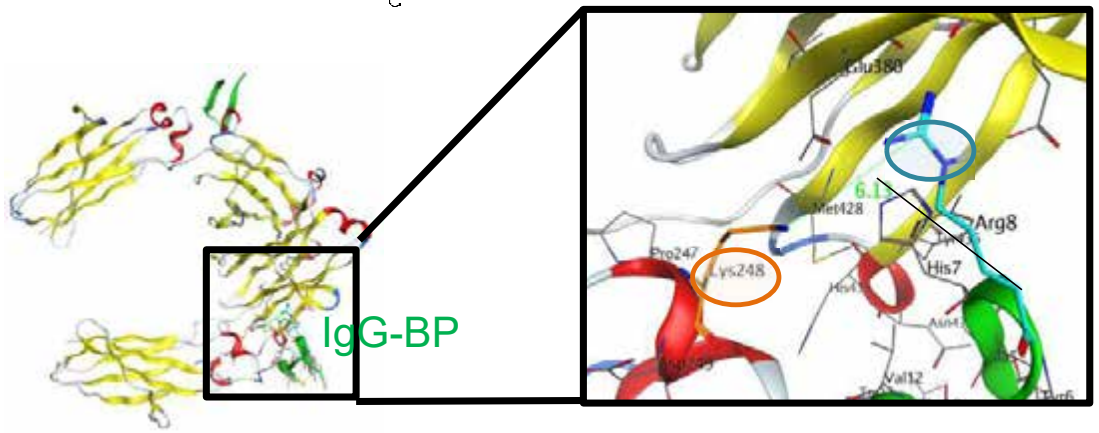


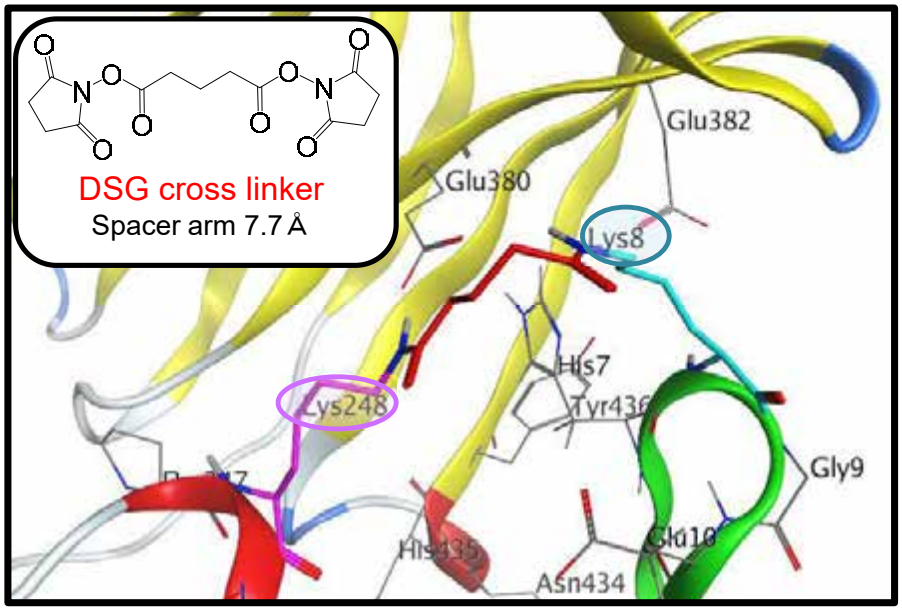
Kishimoto, S. et al. Bioconjug. Chem. 30, 698–702 (2019).

Patent-filed as WO2016186206.

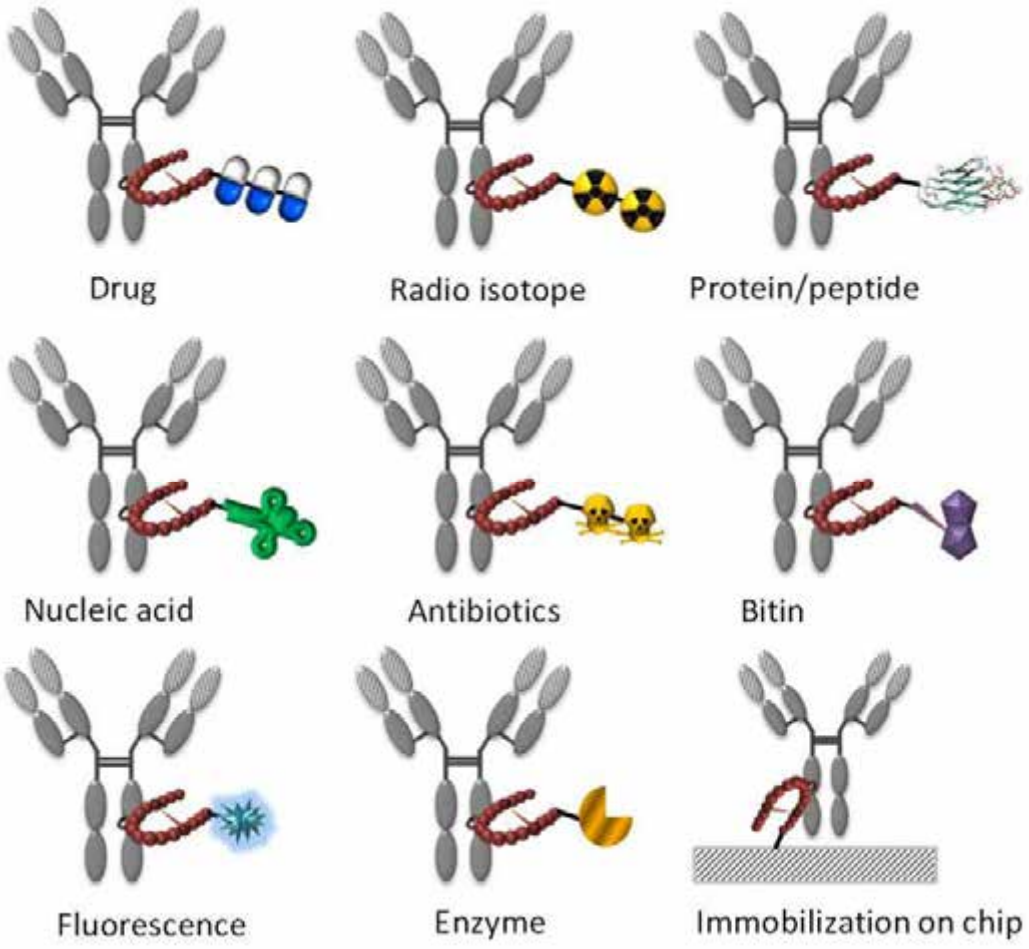


Hunan IgG-Fc and IgG-BP complex



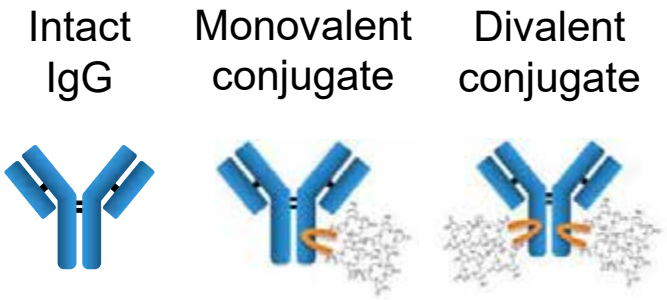


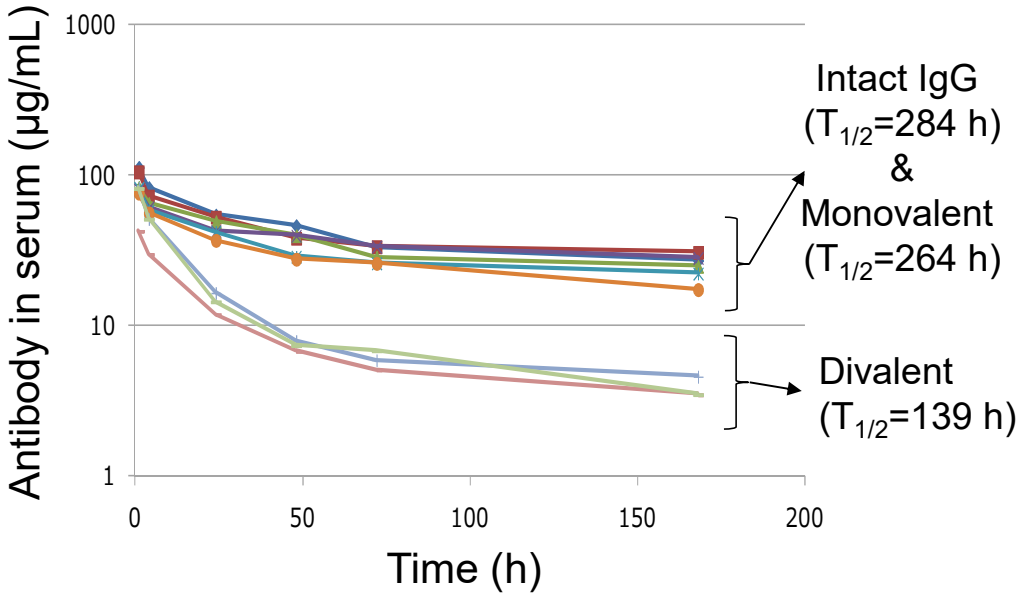
Various ADC prepared by CCAP



Serum half-life and Fc receptor binding ability of CCAP conjugates

[Dose] 5 mg/kg, I.V.
[Animal] Slc: ICR male, 5 weeks old
[Sampling] 1, 4, 24 h, 2, 3 and 7 d

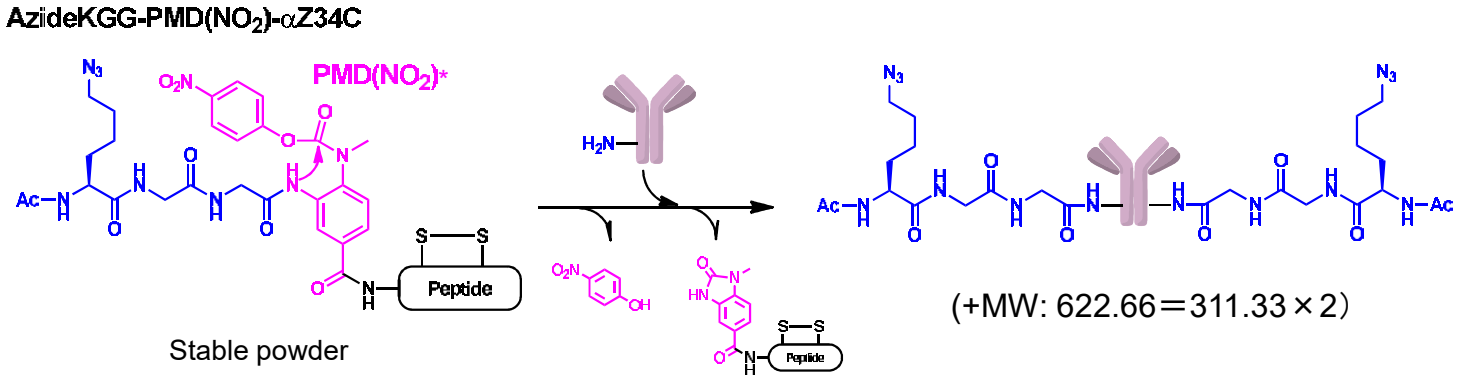




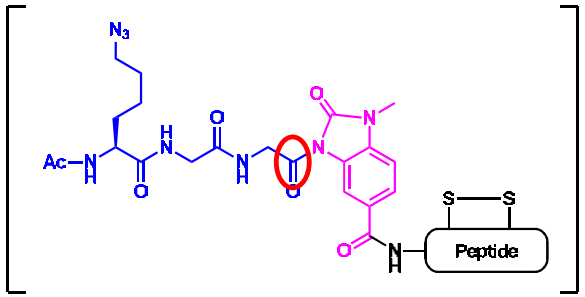
	Trastuzumab (Intact IgG)	Monovalent	Divalent
FcRn	ka=1.2 × 10^{6} (/Ms) kd=4.0 × 10^{-2} (/s) K_D=36 nM	ka=5.6 × 10^{5} (/Ms) kd=3.3 × 10^{-2} (/s) K_D=59 nM	No binding
FcγR Ⅰ	ka=2.3 × 10^{5} (/Ms) kd=5.7 × 10^{-3} (/s) K_D=24 nM	ka=3.1 × 10^{5} (/Ms) kd=5.4 × 10^{-3} (/s) K_D=17 nM	ka=2.9 × 10^{5} (/Ms) kd=4.8 × 10^{-3} (/s) K_D=16 nM
FcγR Ⅲ A	ka=9.4 × 10^{4} (/Ms) kd=1.5 × 10^{-2} (/s) K_D=160 nM	ka=8.2 × 10^{4} (/Ms) kd=8.4 × 10^{-3} (/s) K_D=100 nM	ka=1.1 × 10^{5} (/Ms) kd=5.0 × 10^{-3} (/s) K_D=45 nM

Next-generation CCAP technology: tCAP

	Z34C :	CH3CO-FNMQCQRRFYEALHDPNLNEEQRNAKIKSIRDDC-NH2
CCAP→	αZ34C :	DSG-FNMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH2
tCAP→	αZ34C :	Cargo-MeNbz-Gaba-NMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH2



Self-activation under neutral conditions via intramolecular cyclization



Active compound generated *in situ*

Traceless site selective modification

* Phenoxycarbonylated *N*-methyl-diaminobenzene (PMD):
1) Pala-Pujadas, J. et al., *Angew.Chem.Int.Ed.*, **57**,16120 (2018);
2) Tsuda, S. et al., *Chem.Commun.*, **54**, 8861 (2018).

Next-generation CCAP technology: tCAP

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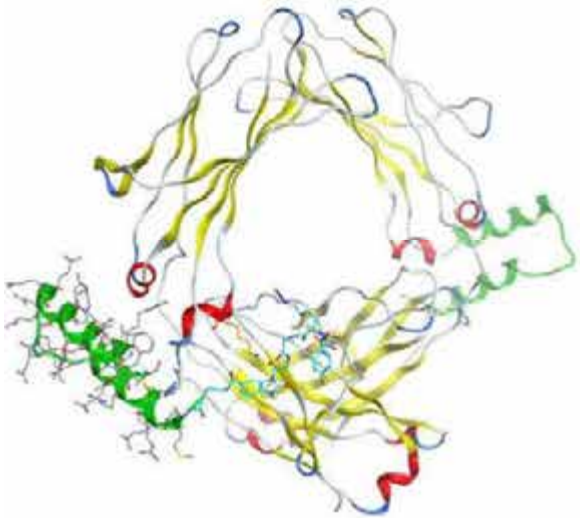
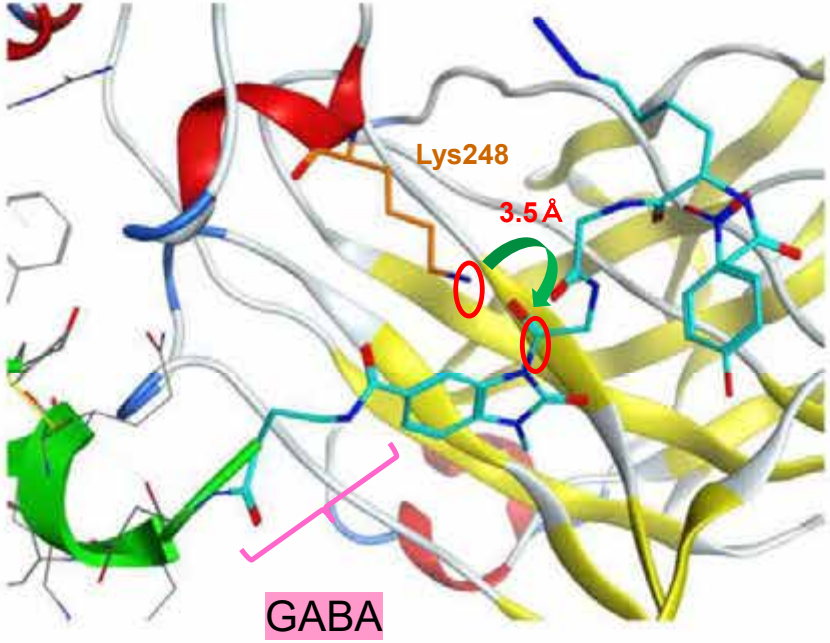
tCAP→	αZ34C :	Cargo-MeNbz-Gaba-NMQCQRRFYEALHDPNLNEEQRNARIRSIRDDC-NH2



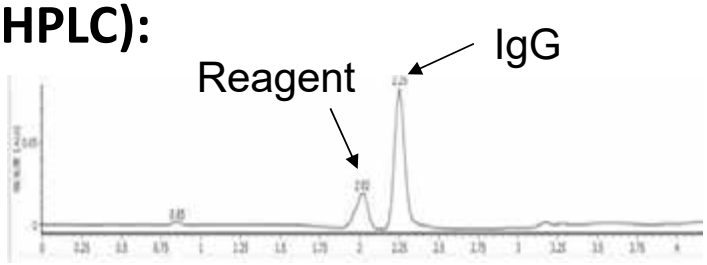


pH dependent reaction of tCAP reagent with human IgG1

Analytical method:

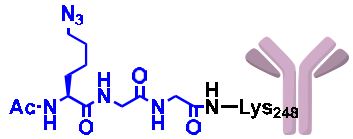
Antibody (15.6 μM) + 5 folds excess reagent
→At different pH and R.T. for 2 h →LC-MS

LC (RP-HPLC):

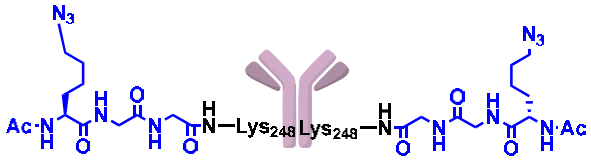


Intact IgG

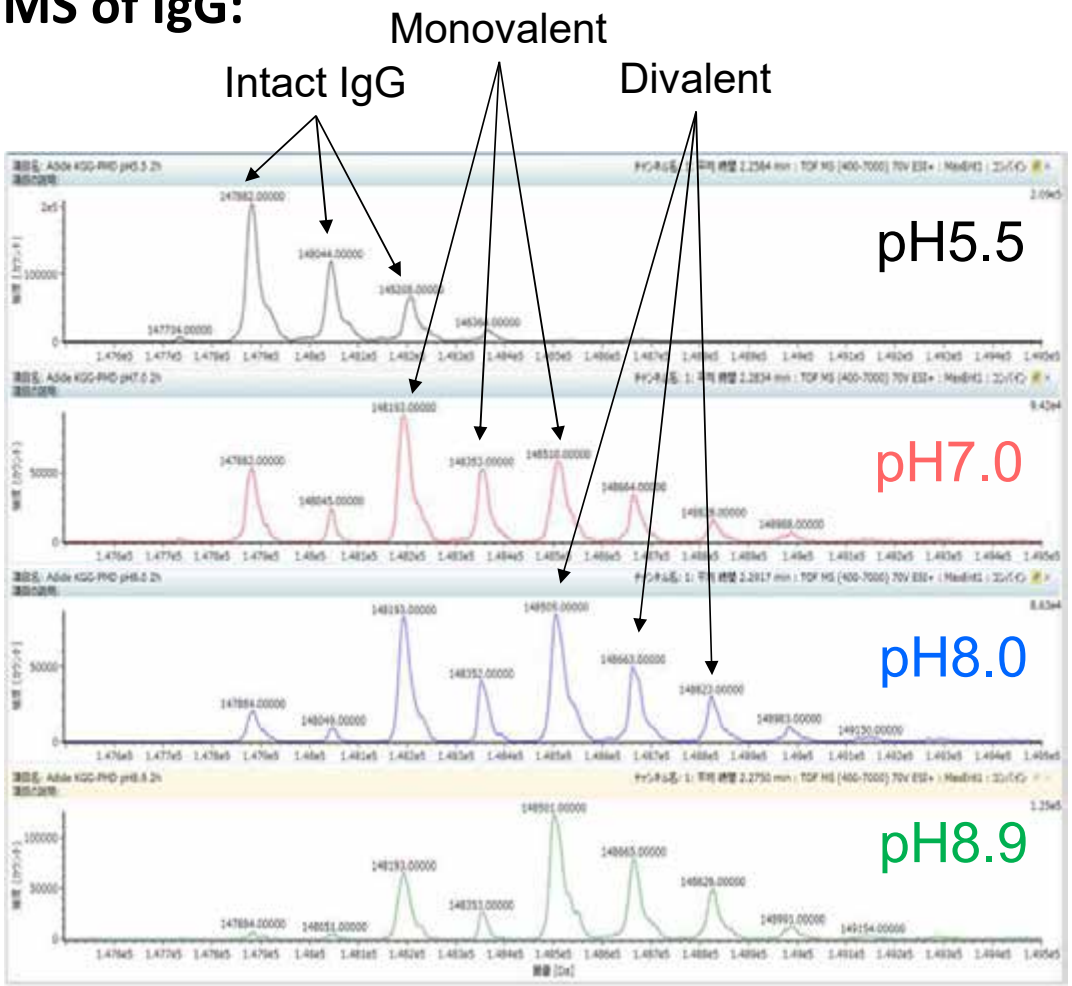
Monovalent



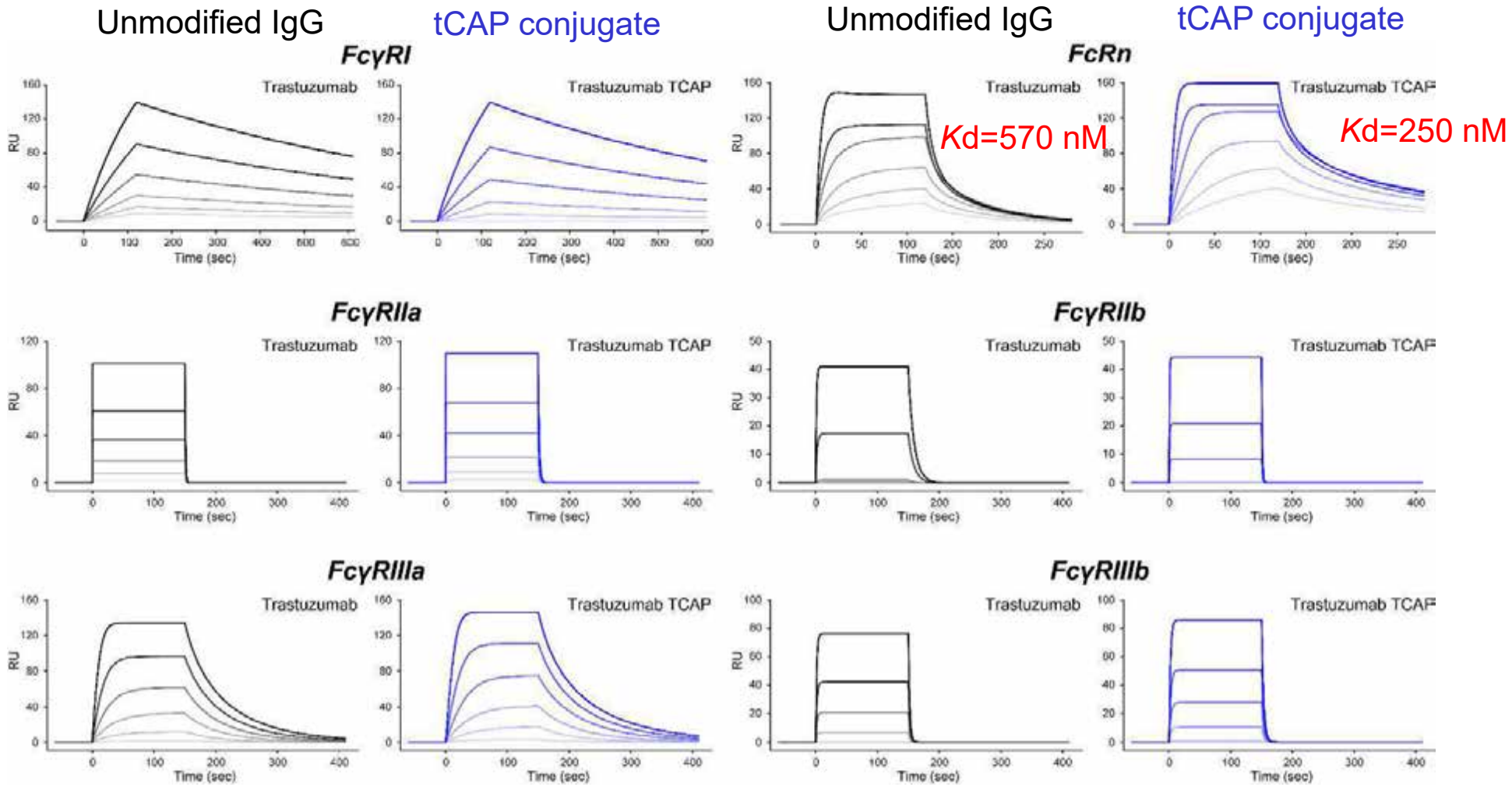
Divalent



MS of IgG:

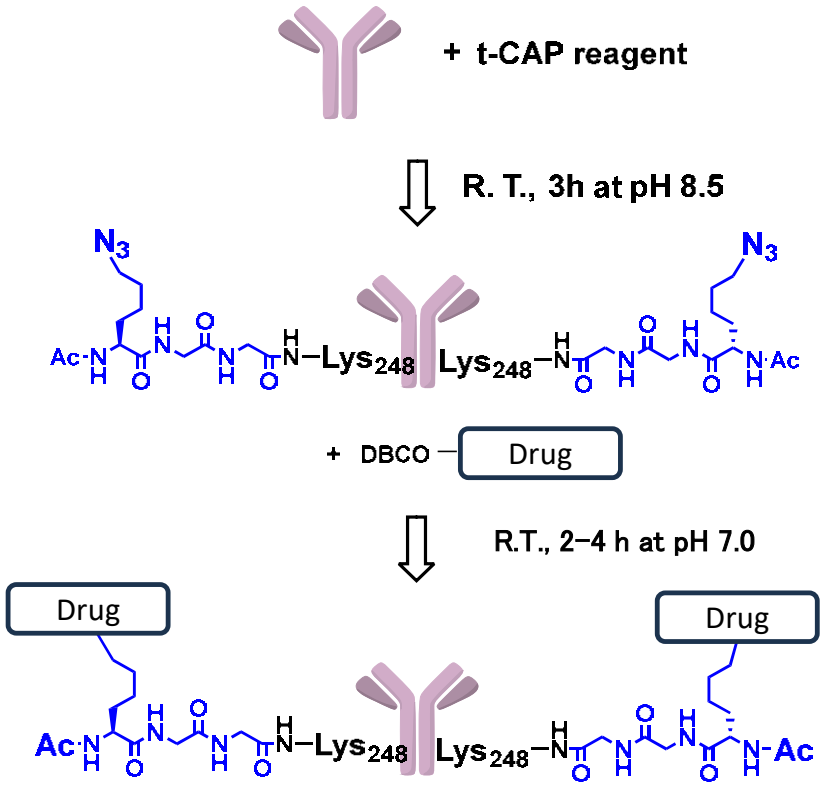


Kinetic binding of tCAP-modified IgG (Trastuzumab) for FcγR and FcRn on BIAcore

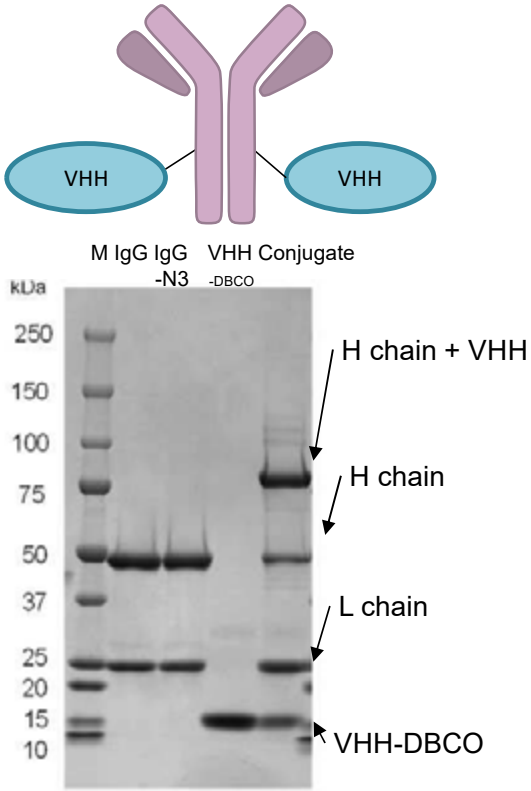


Preparation of Bispecific antibody and AOC by t-CAP

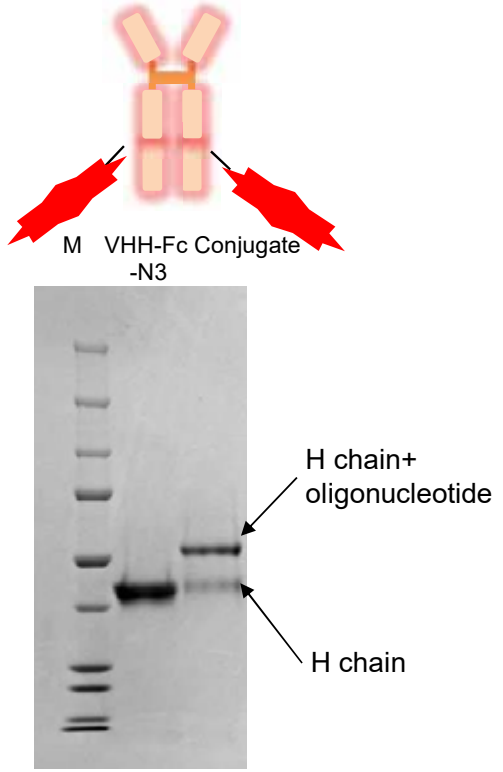
(A) Preparation of conjugate by t-CAP



(B) Bispecific antibody (IgG + VHH)

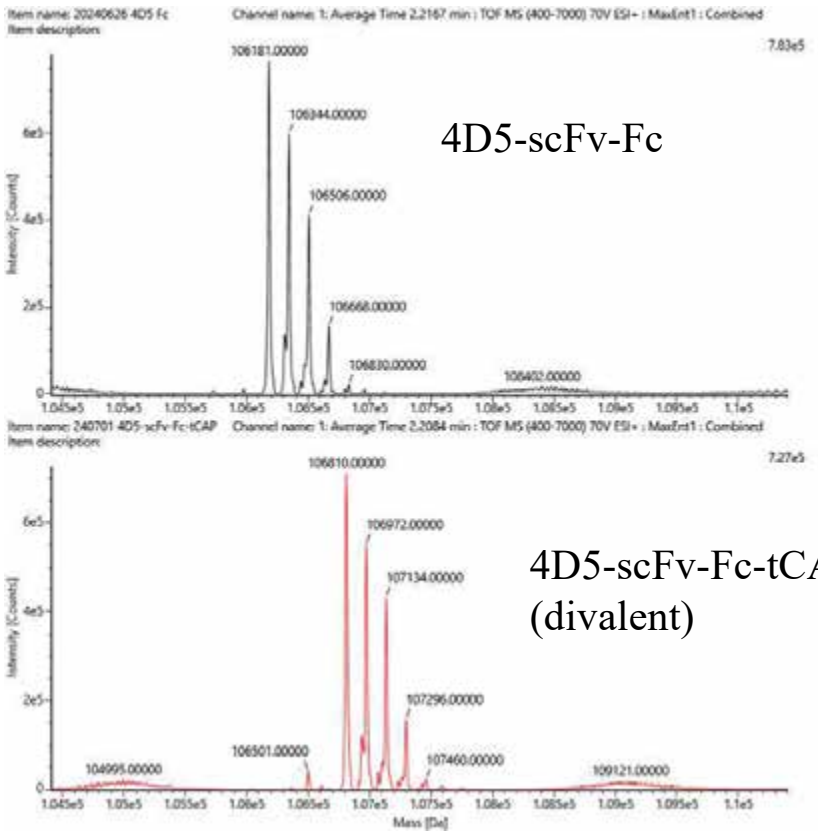


(C) Antibody oligonucleotide conjugate (AOC) (VHH-Fc + oligonucleotide)

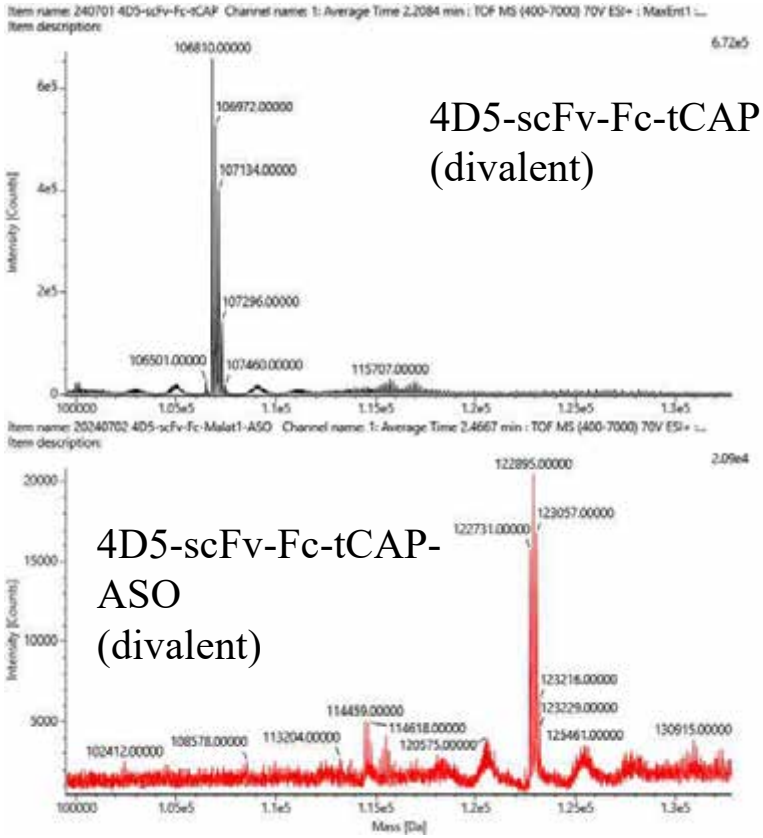


LC-MS analysis of ASO antibody conjugates by tCAP

Attachment of an azide group to 4D5 scFv-Fc by tCAP

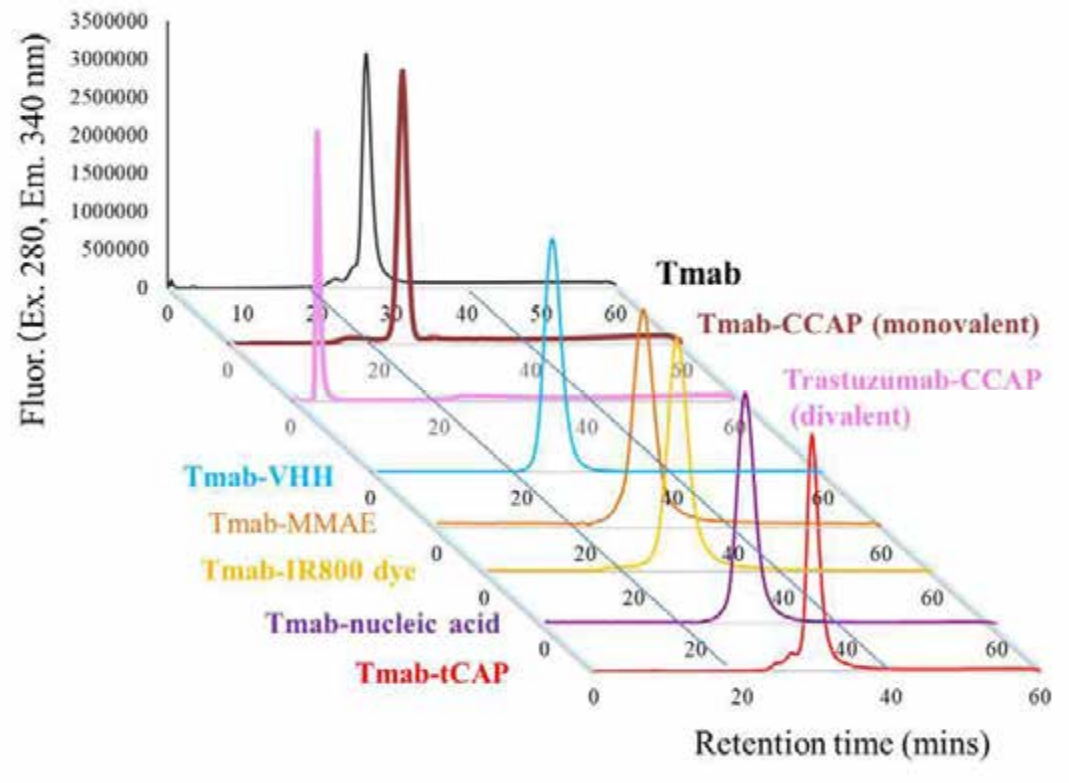


Introduction of oligonucleotide into scFv-Fc antibody by click reaction



FcRn binding of tCAP conjugates with payloads

FnRn binding evaluation by FnRn- immobilized column*



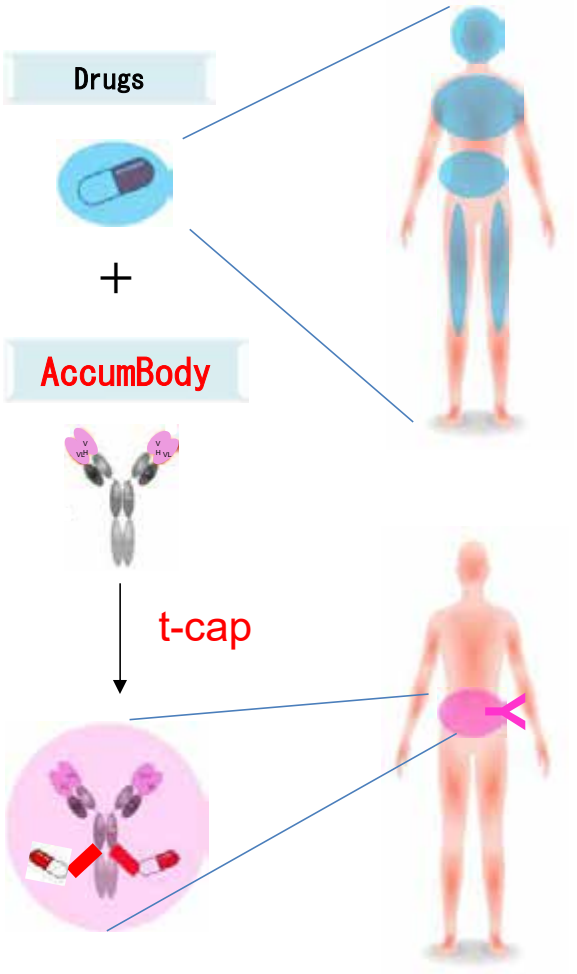
*Bindings with FnRn was evaluated using an FnRn-immobilized column (β version) from Tosoh Corp.

Development of Tissue-Specific Migrating Antibody, AccumBody and Its Application to Next-Generation antibody drug

Drug

Tissue-Specific Migrating Antibody 『AccumBody』

Next-Generation antibody drug



Distributed in whole body

Novel drug candidates for unmet medical needs

C.N. : multiple sclerosis
Muscle : muscular dystrophy
Bowel : IBD

Distributed in target tissue only

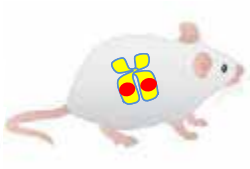
- High therapeutic efficacy with small side effects

Collaborators
Professor Y. Kazuki (Tottori Univ.)
Professor K. Tomizuka (Tokyo Phar. Univ.)

Isolation of AcuumBody® by antibody phage library

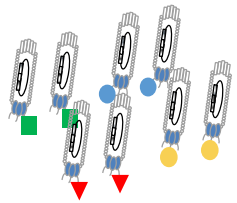
Immunization with tissue, or protein antigens

Fully human antibody-producing TC animals



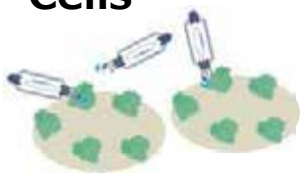
Alpaca

Construction of antibody phage library



In vitro and/or in vivo panning

Cells

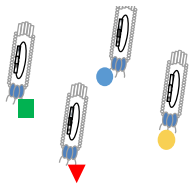


Mice

Panning

Recovery

Proliferation of phages



The enriched phages

Identification of antibodies by NGS analysis

NGS analysis of H chain

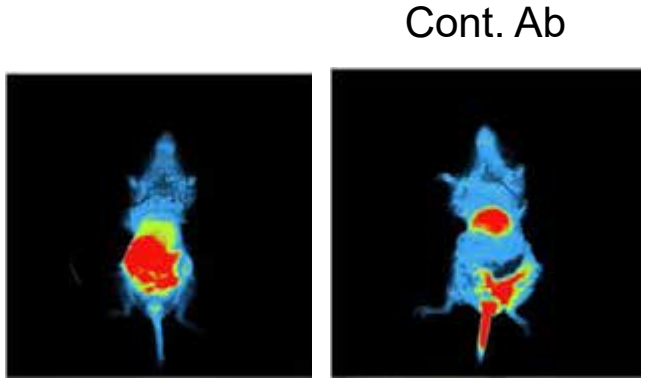
(Illumina Miseq)

Phylogenetic tree analysis on SOPRA program

Identification of specific antibody sequences

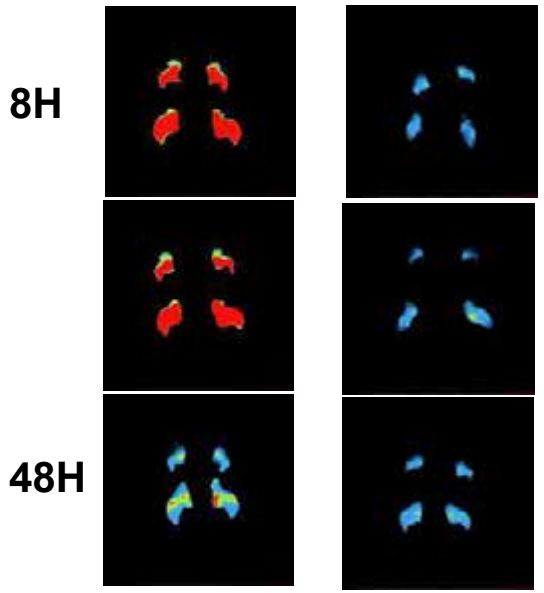
Tissue specific targeting AccumBody®

AcuumBody®-Bowel
(Anti GPA33 antibody)



After tail vein administration to Balb/c mice at 5 mg/kg, the mice were perfused and opened at 24 hours for fluorescence imaging.

AccumBody®-Muscle



Balb/c mice were perfused at each time after i. v. injection at 5 mg/kg and the muscles of the upper and lower limbs were subjected to fluorescence imaging.

Summary

1. tCAP, a site-specific modification method using antibody Fc-affinity peptides was developed. This method is a useful modification method for the preparation of ADCs, because it does not affect the antigen binding and Fc function including Fc receptor bindings.

2. AccumBodies that target antigens specifically expressed in the brain, bowel and muscle were developed. AccumBodies can deliver the drug to diseased tissues and are useful as DDS tools for highly effective therapeutics.

3. For AccumBody-bowel and -muscle, the technology to promote cellular uptake are necessary to develop. A common issue for the future is the development of cleavable linkers for ADCs.