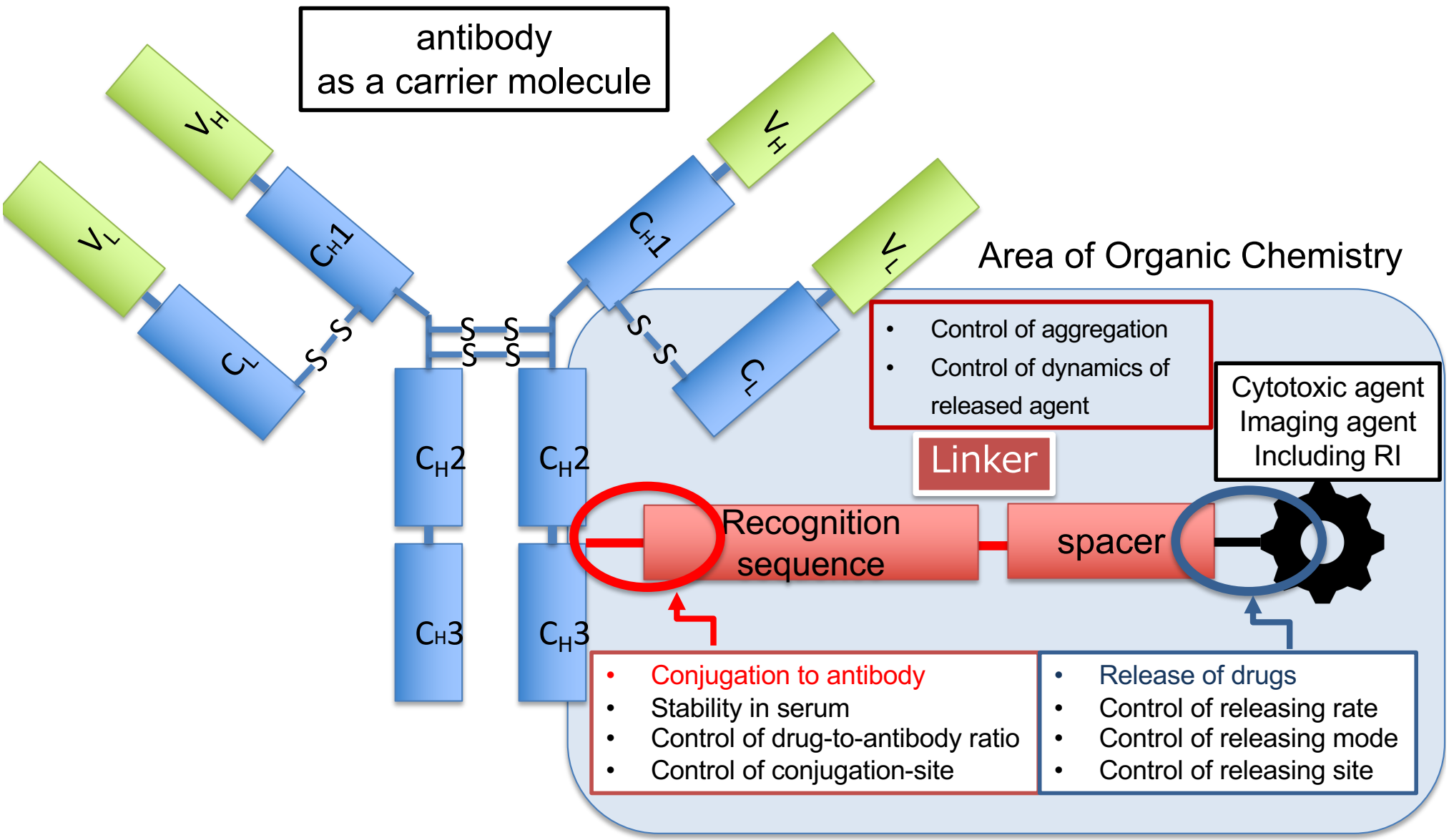
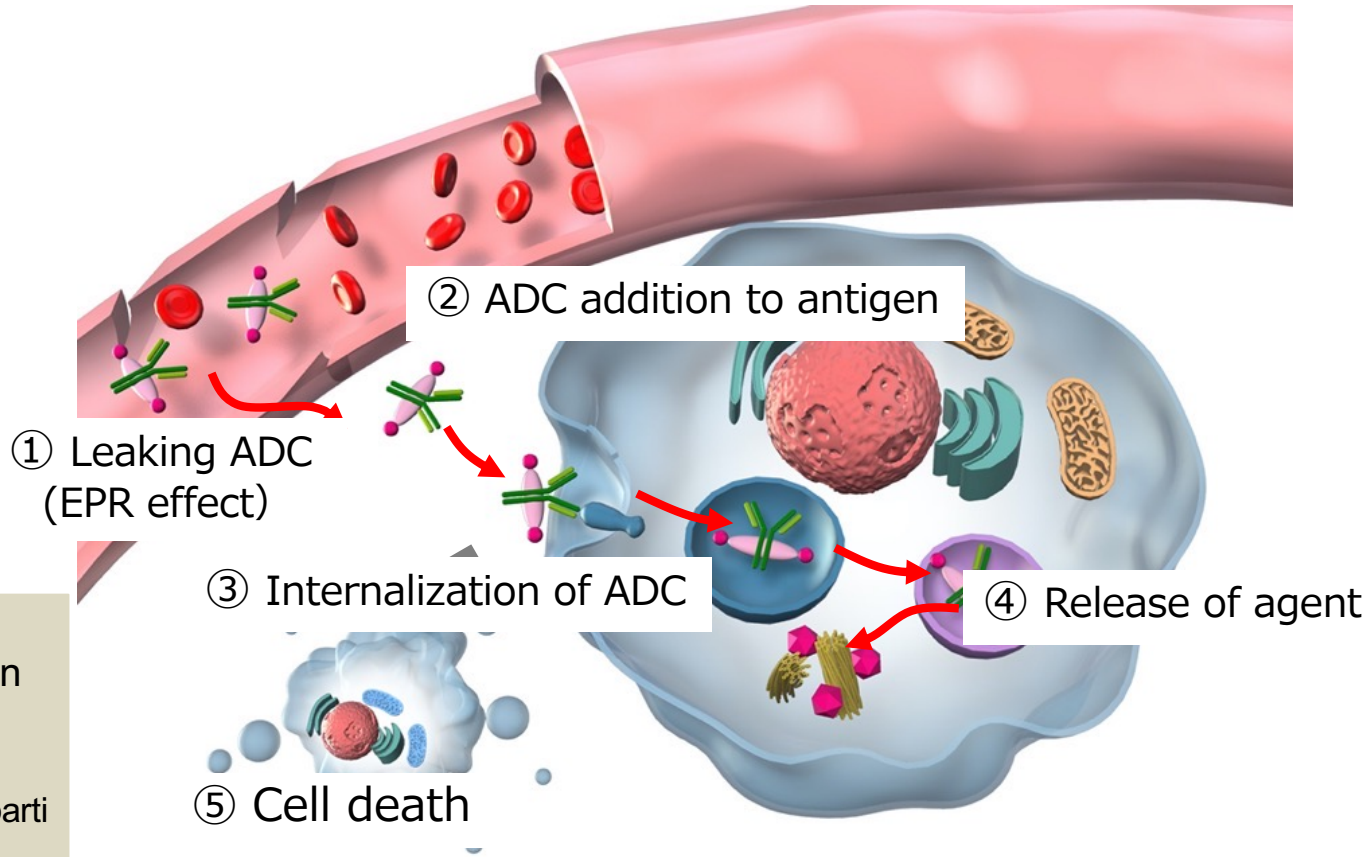
Antibody-drug conjugate (ADC)

Structure of ADC
(**A**ntibody-**D**rug **C**onjugate: ADC)



Next-generation antibody from organic chemistry

Mode of Action of ADCs

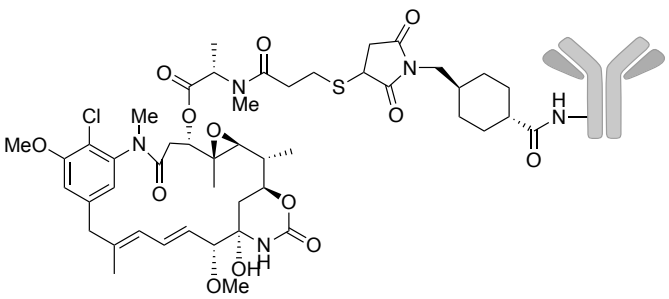


EPR Effect
(Enhanced permeation and retention)

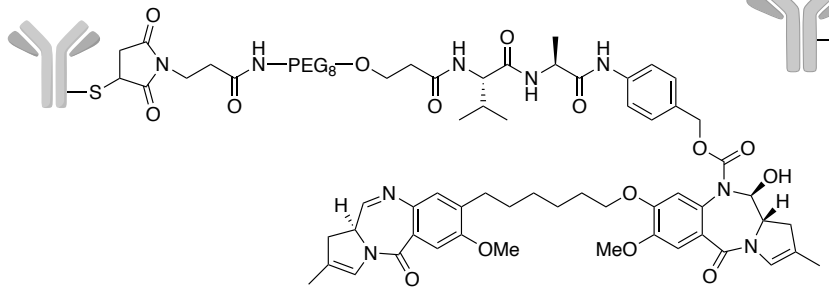
Molecules of certain sizes (typically liposomes, nanoparticles, and macromolecular drugs) tend to accumulate in tumor tissue much more than they do in normal tissues.
Proposed by Dr. Matsumura and Maeda in 1986.

	Antibody	Low-molecular agent
Selectivity	**Extremely high**	Low in general
Target	Antigen on cell surface	**Proteins inside cells as well as on surface**

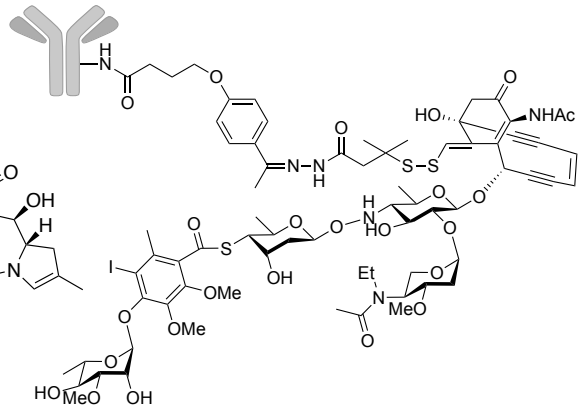
Administrated ADCs (as of October 2021)



Trastuzumab emtansine

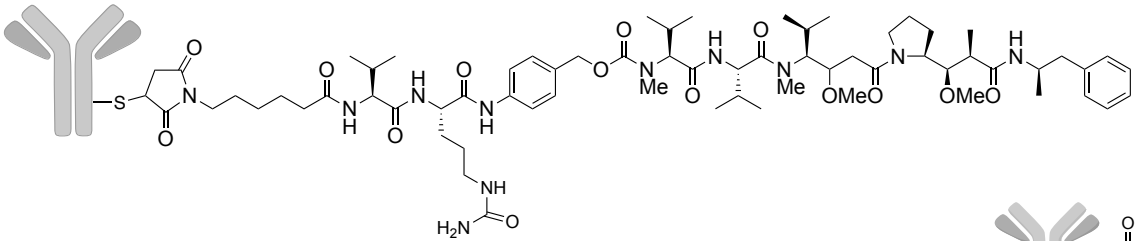


Loncastuximab tesirine



Gemtuzumab ozogamicin

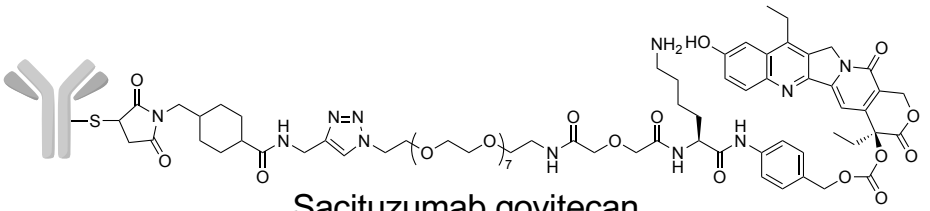
Inotuzumab ozogamicin



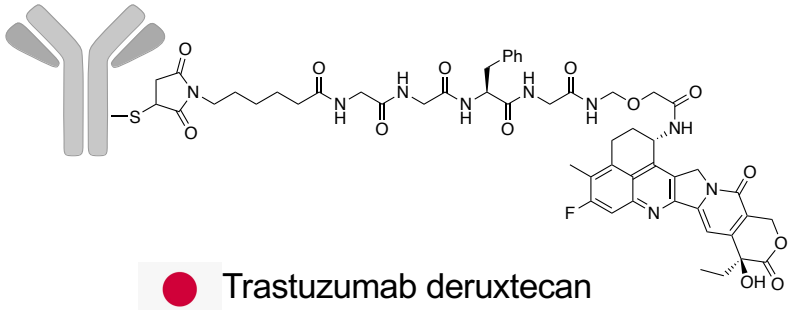
Brentuximab vedotin

Polatuzumab vedotin

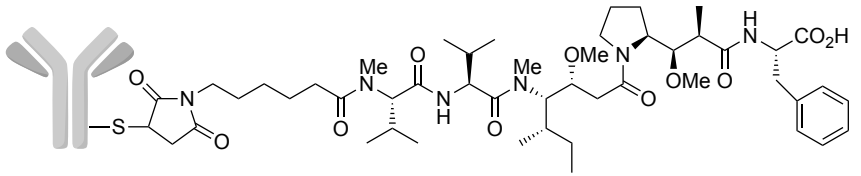
● Enfortumab vedotin



Sacituzumab govitecan



● Trastuzumab deruxtecan



Belantamab mafodotin

Only three (two) ADCs (red highlighted) were administrated in 2017.
The number of administrated ADCs is increasing.
The number of ADCs in pipeline is more than 80.

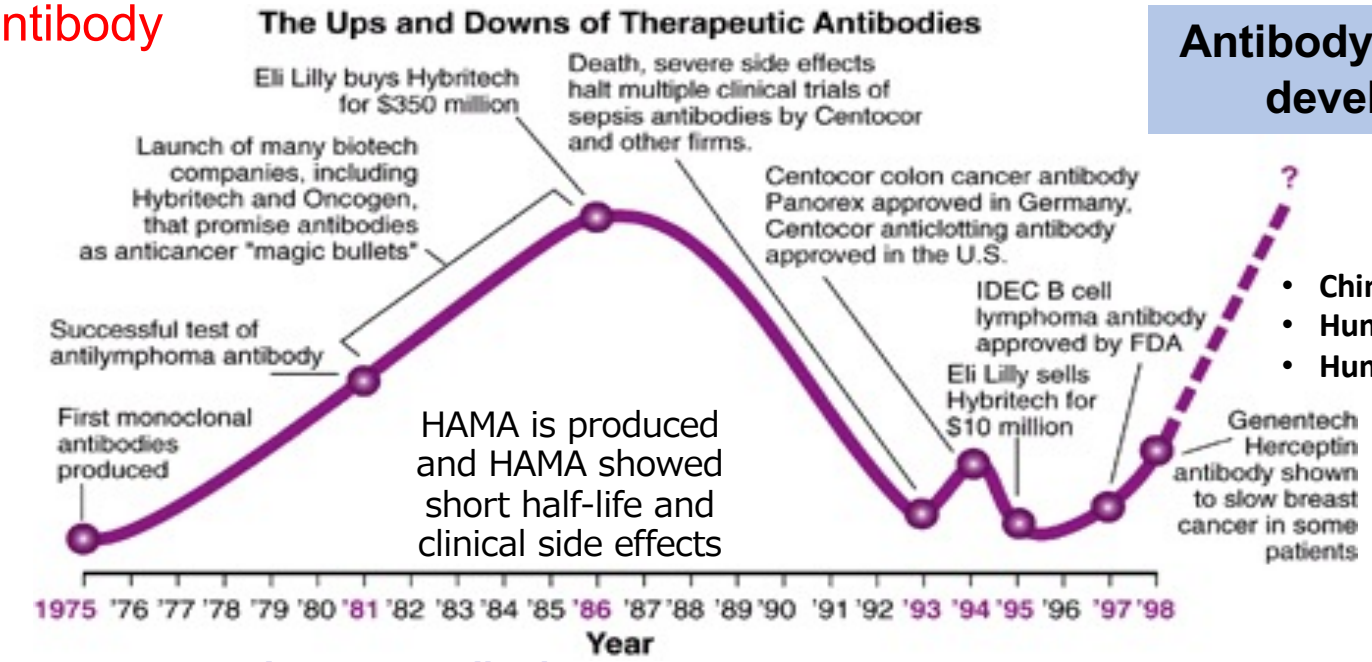
Antibody discoverer : Paul Ehrlich

"If the antibody is conjugated by toxin and reach the target cell, it will be able to kill the target cell."

Why have antibody-drug conjugates remained unexplored to date?

History of ADC Development

Problems in antibody



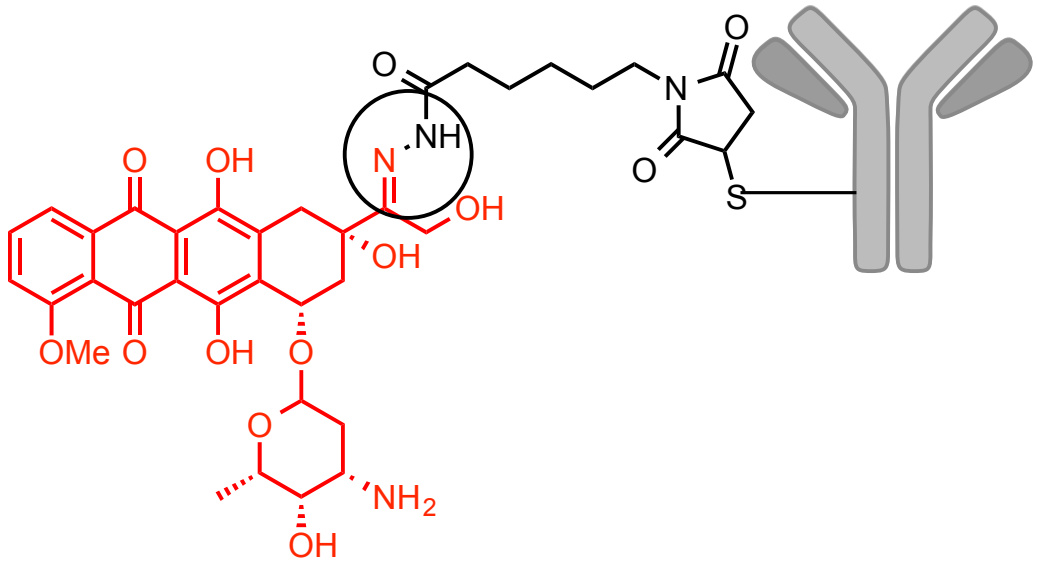
Antibody-technology development

- **Chimeric antibodies**
- **Humanized antibodies**
- **Human antibodies**

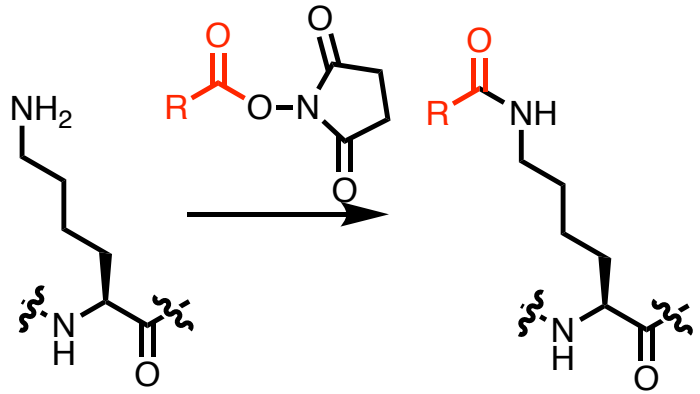
HAMA : Human Anti Mouse Antibody

Problems in linker

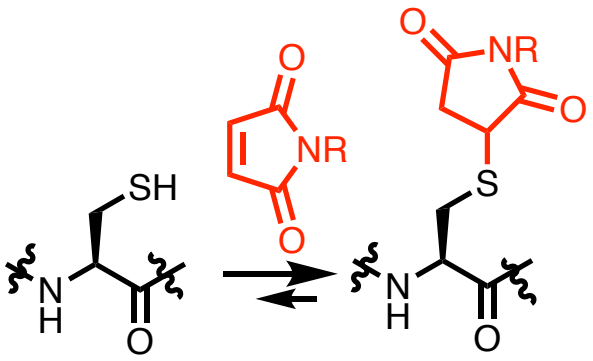
Hydrolyzed in undesired area
Unable selective/specific release



Limitation of classical conjugation



Amide formation at Lys

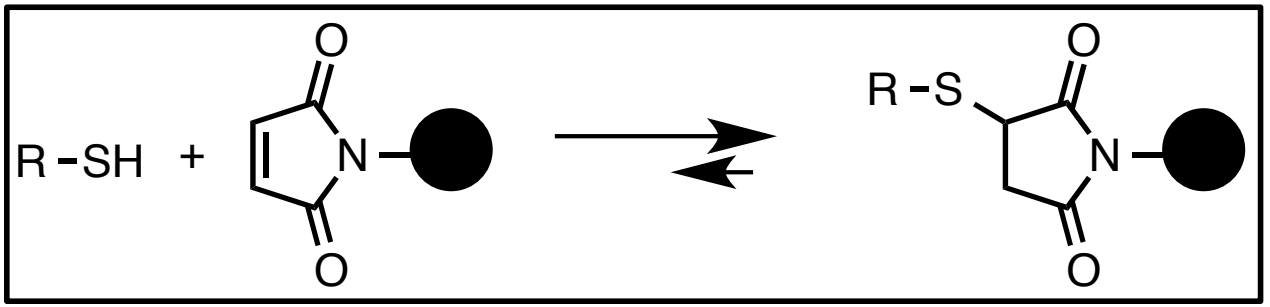


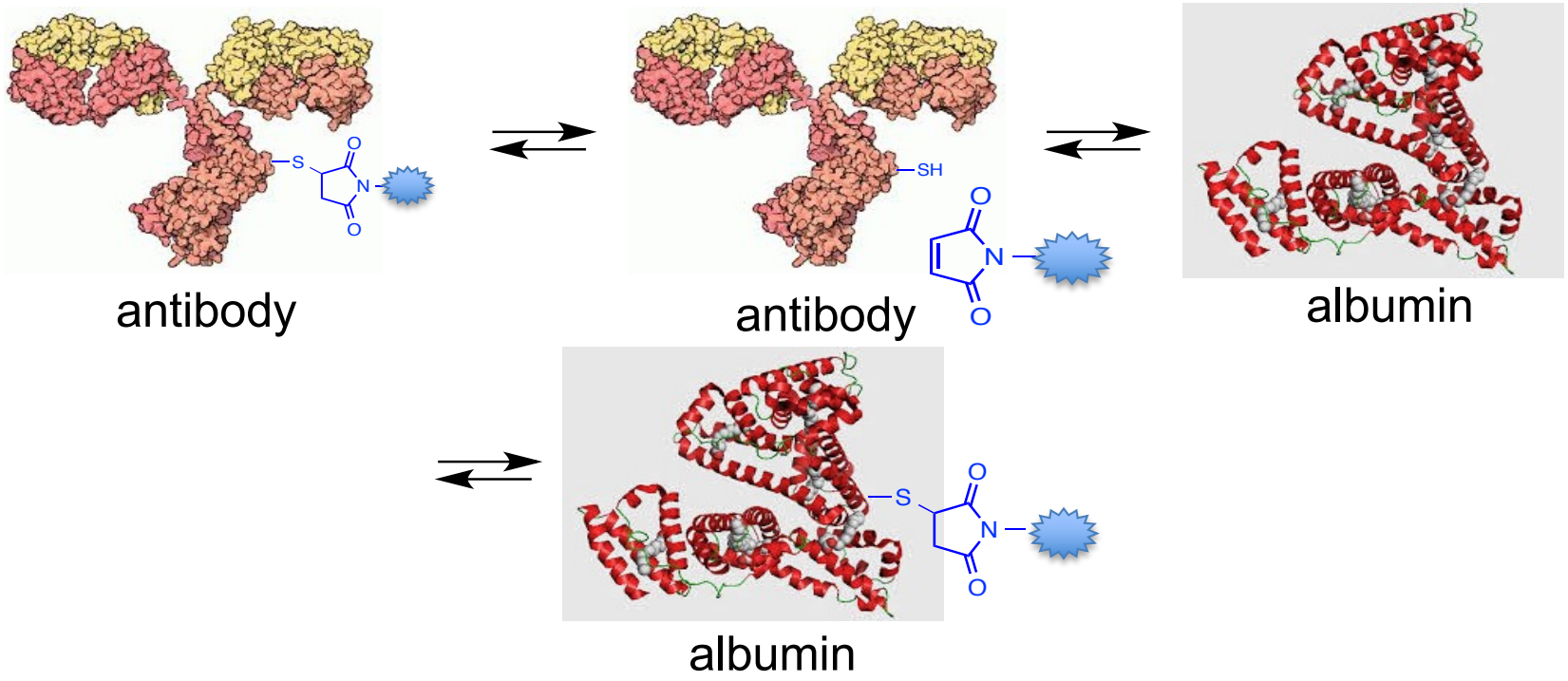
Michael addition at Cys

	Features	Disadvantages
Lys linkage	• Amino group is highly reactive. • 90 Lys in one antibody • Lys is hydrophilic. • Lys is located in surface.	• Control of conjugation-site and drug to antibody ratios are difficult. Numerous (>10^6) ADCs are generated. • Payload is conjugated to antibody binding site. • Conjugation at amino group may caus aggregation and alters pharmacokinetics/pharmacodynamics.
Cys linkage	• Number of Cys is less than Lys.	• Conjugation at amino group may caus aggregation and alters pharmacokinetics/pharmacodynamics. • Bond between Maleimide and SH group is not stable. Michael reaction is reversible.

Homogeneous ADC cannot be prepared.

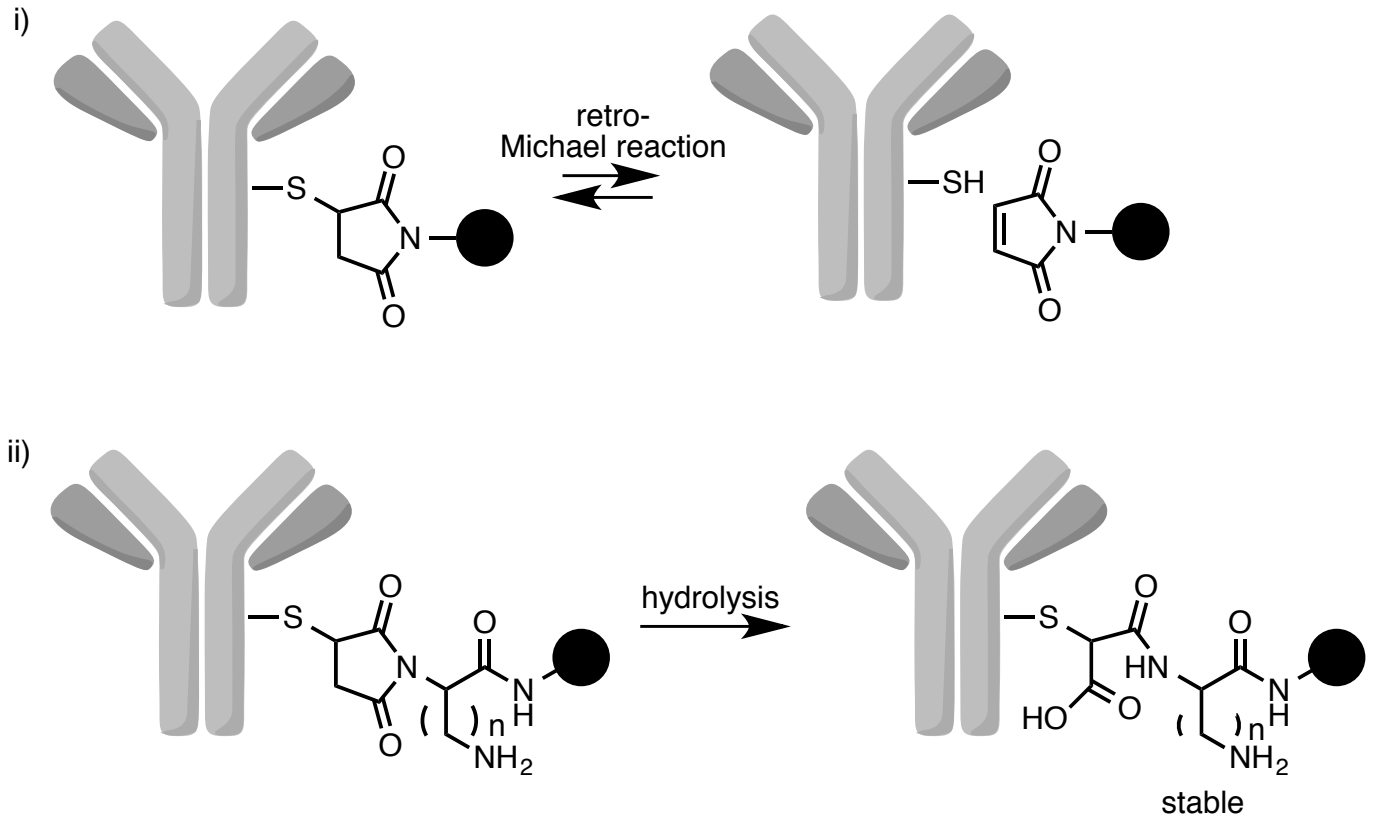
Stability between SH and maleimide bond is limited.





S. C. Alley *et al. Bioconj. Chem.* **19**, 759 (2008)

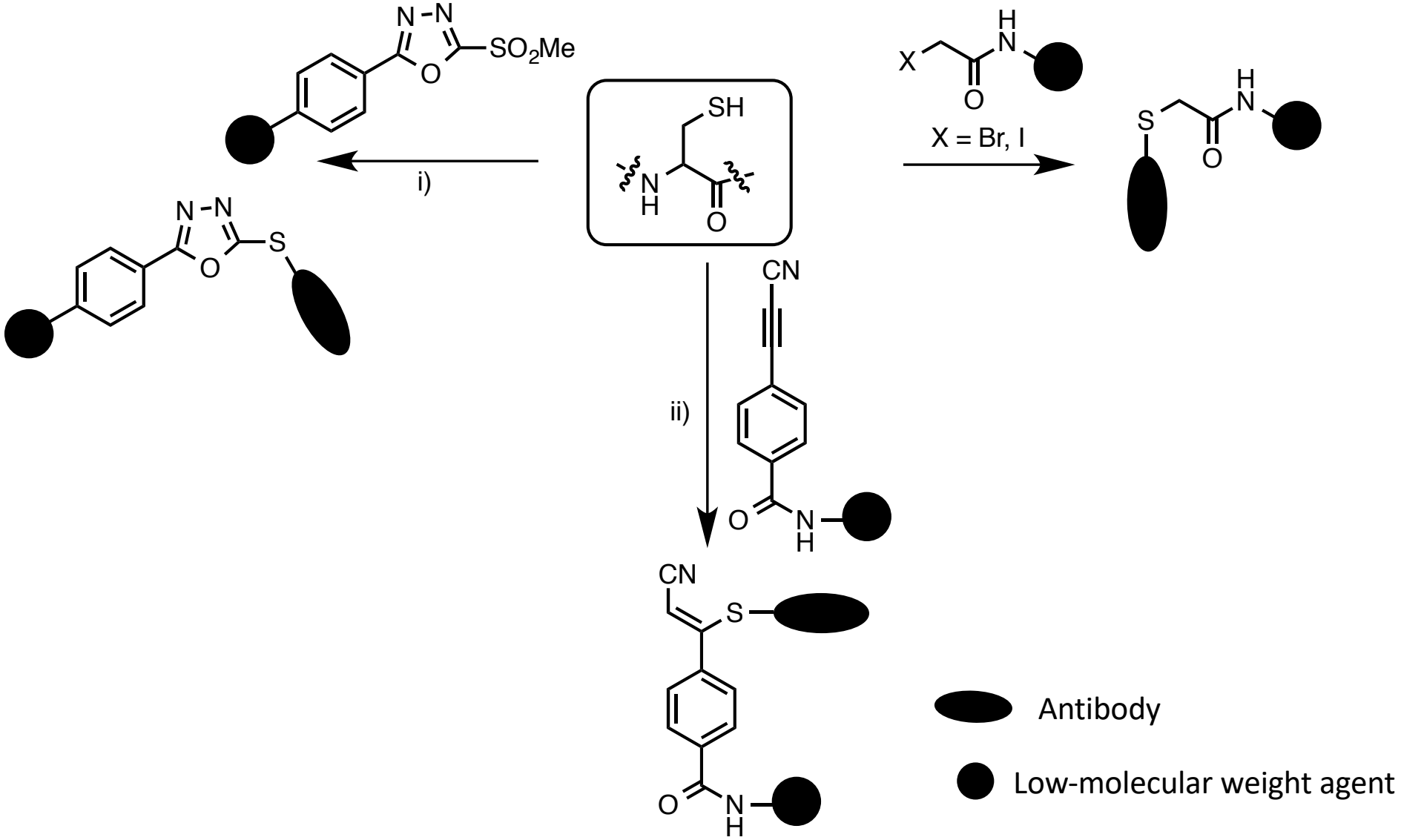
Stability enhancement by hydrolysis of maleimide group



i) Instability between SH group and malemide group
ii) Stabilization of hydrolysis of maleimide group; addition of hydrolysis enhancement group

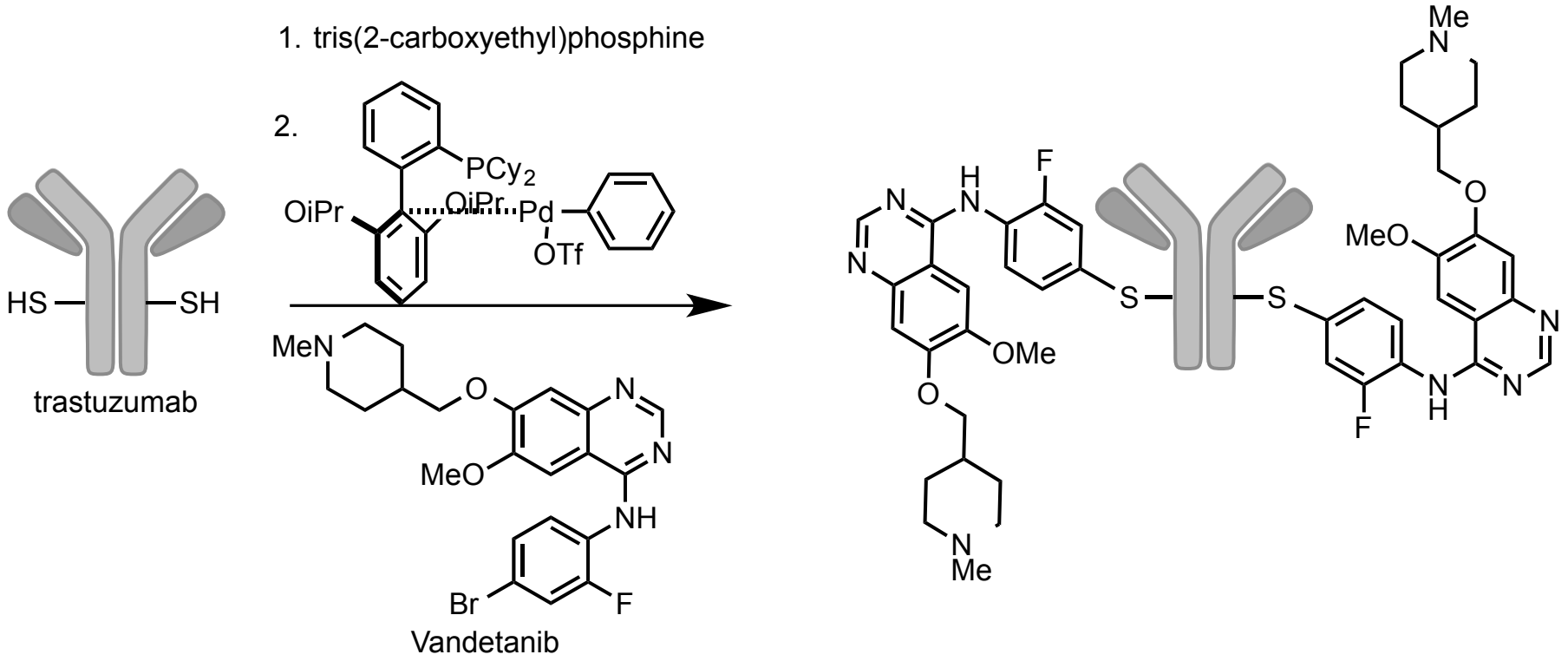
R. P. Lyon *et al. Nat. Biotech*. **32**, 1059 (2014)

Other conjugation via SH group



i) J. T. Patterson *et al. Bioconj. Chem*. **25**, 1402 (2014)
ii) S. Kolodych *et al. Bioconj. Chem.* **26***,* 197 (2015)

Conjugation at SH group by transition metal catalyzed reaction

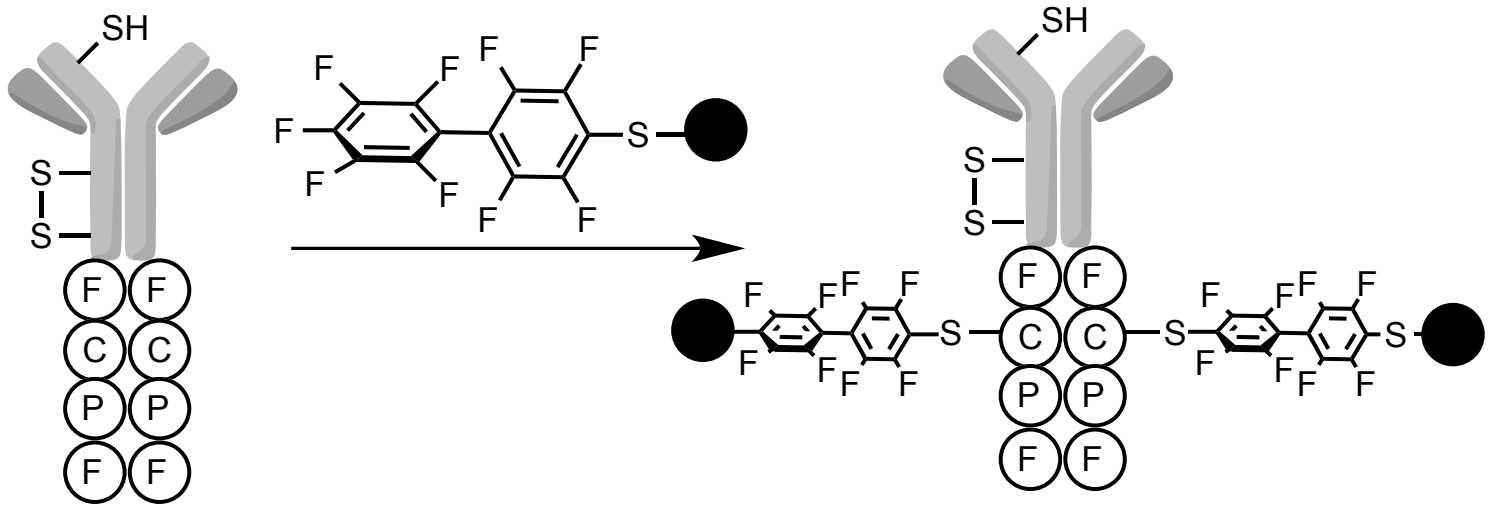


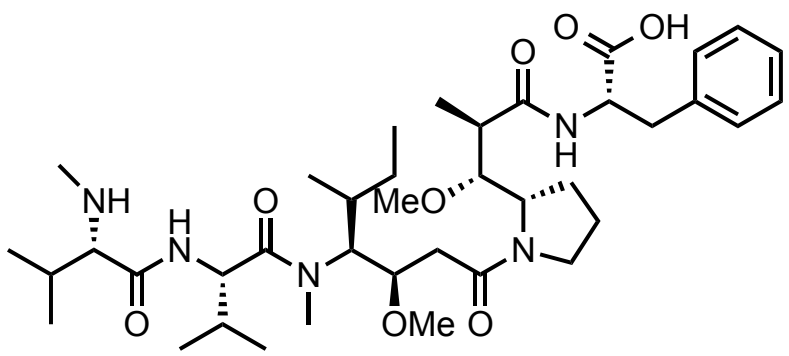
- The reaction was quick, completed within 5 min.
- Catalyst is stable at room temperature.
- SH group has higher reactivity than amino group and hydroxy group in this reaction.

E. V. Vinogradova *et al. Nature*, **526***,* 687 (2015)

S_NAr reaction by fluorine-substituted biphenyl group

Sequence specific, not amino acid specific
More selective conjugation is possible.





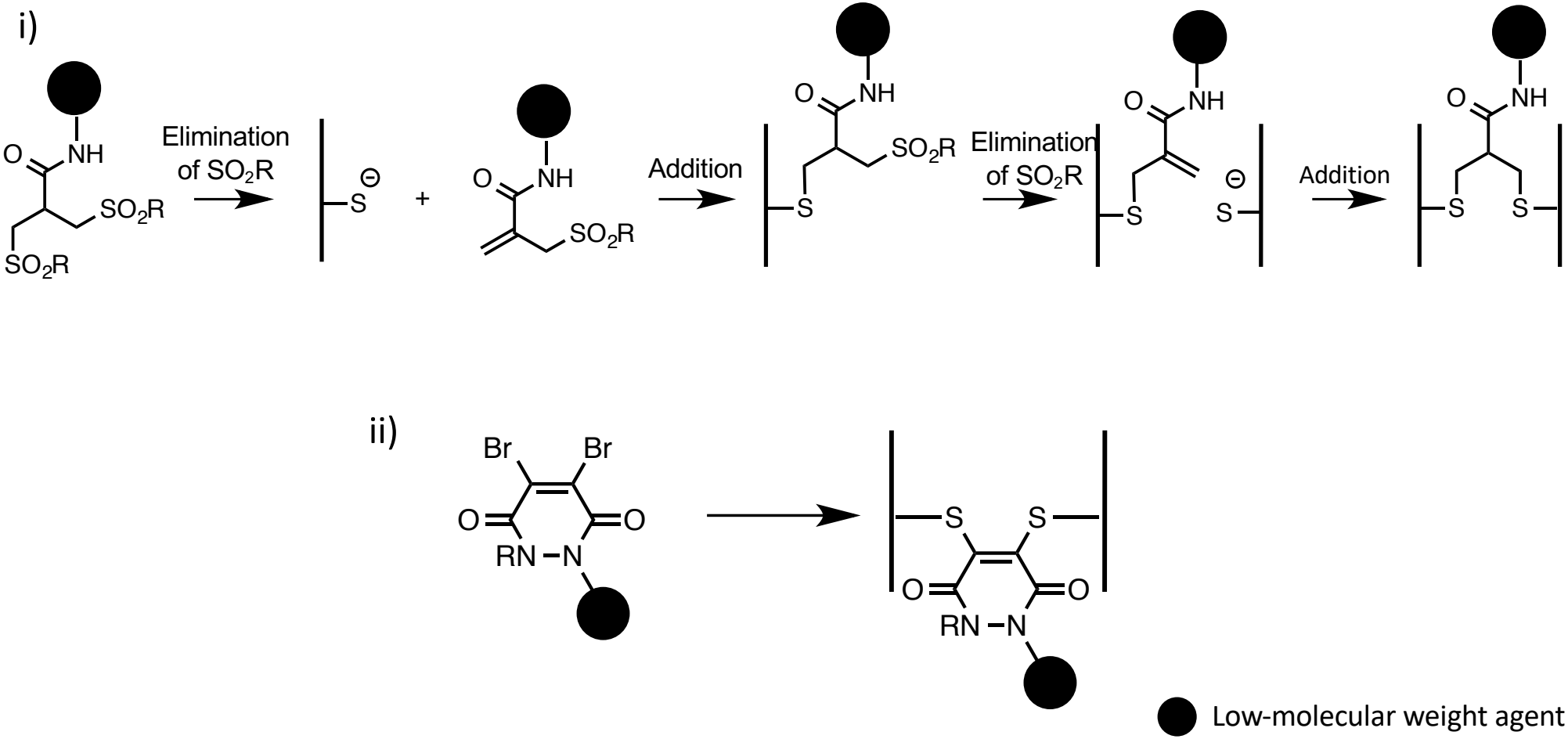
MMAF

C. Zhang *et al. Nat. Chem*. **8**, 120 (2016)

Homogeneous ADC

Disulfide-type conjugation

Disulfide structure in antibody is maintained.

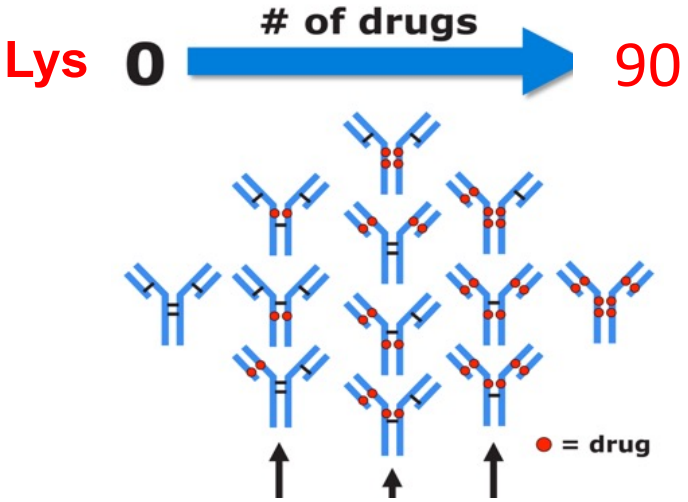


G. Badescu *et al*. *Bioconj. Chem*. **25**, 1124 (2014)

L. Castañeda *et al. Chem. Commun*. **49**, 8187 (2013)

Homogeneous ADC

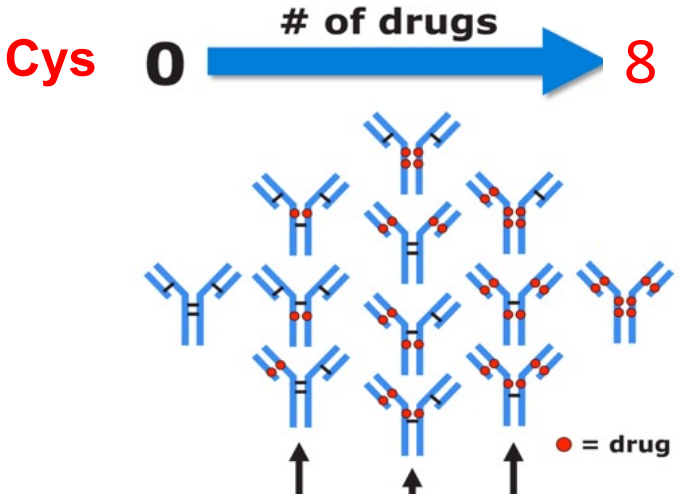
Heterogeneous ADCs by classical conjugation



Difference of conjugation sites

Drug antibody ratio	Number of ADCs
0	1
1	90
2	4005
3	117,480
4	2,555,190
5	43,949,268
6	622,614,630
7	7,471,375,560
8	77,515,521,435

10^{10}



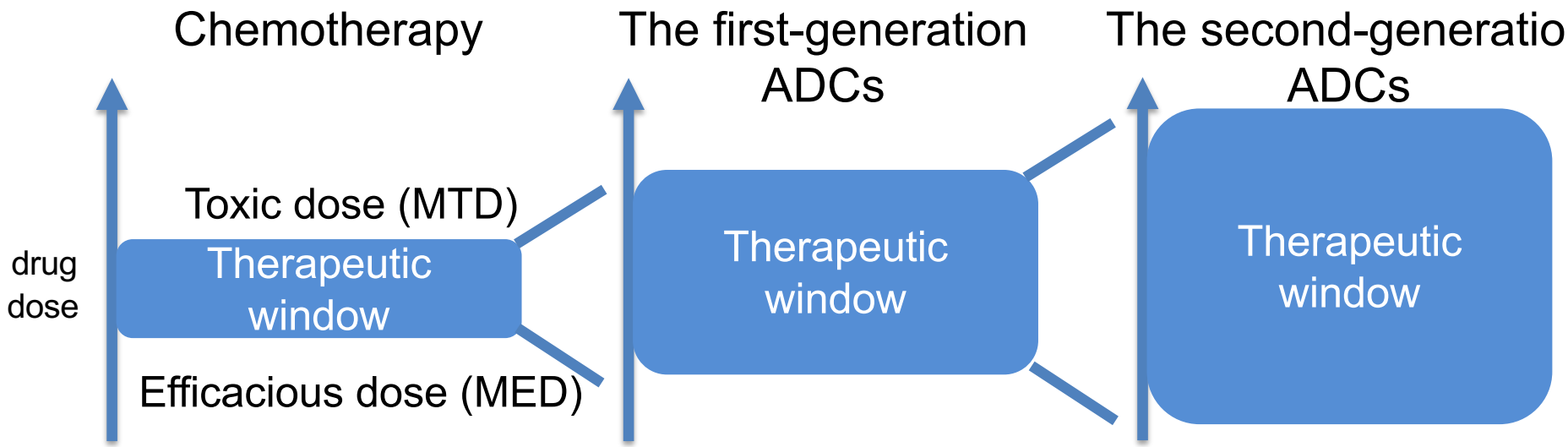
Difference of conjugation sites

Drug antibody ratio	Number of ADCs
0	1
1	8
2	28
3	56
4	70
5	56
6	28
7	8
8	1

256

Homogeneous ADCs is desired.

1. Therapeutic window is increased.



- Selective delivery to cancer site
- Potent cytotoxic drug

- Site-selective conjugation
- Release mechanism

Therapeutic window = MTD/MED

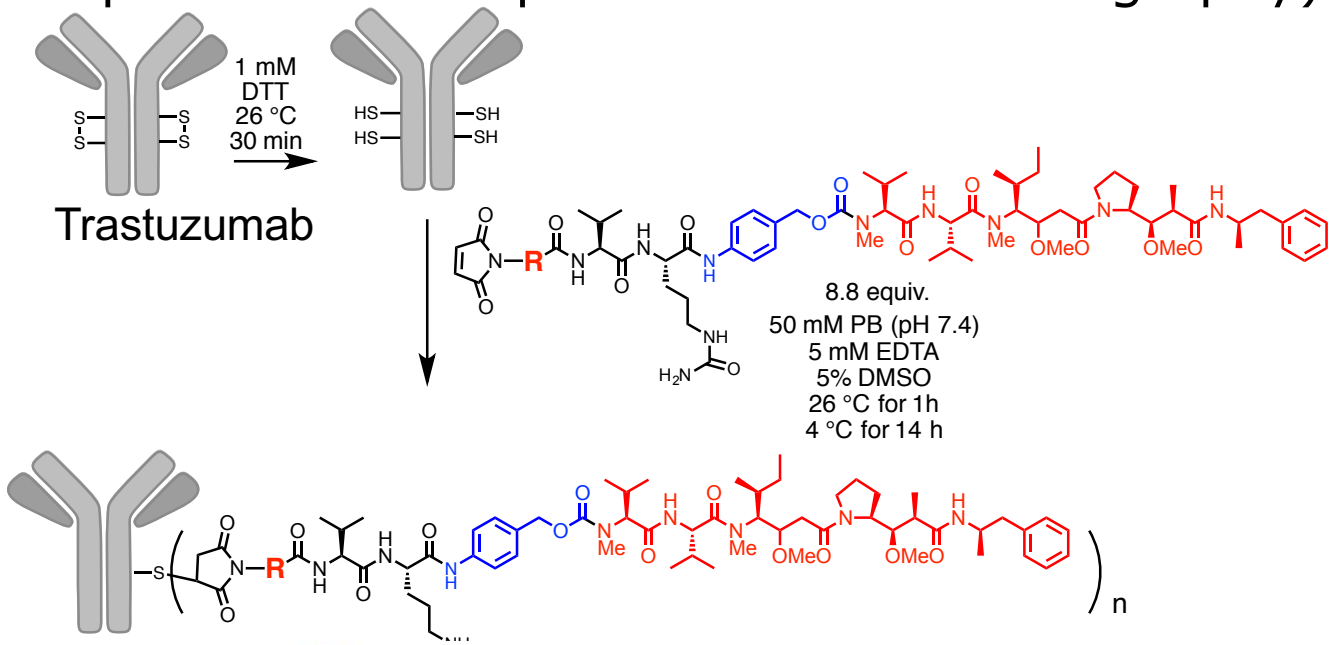
2. Reproducibility of synthesis and efficacy.

3. Responded to regulation.

Homogeneous ADC

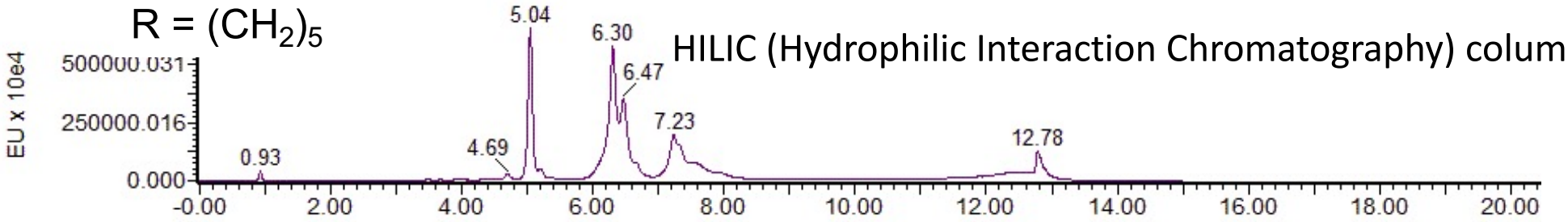
Our data

Heterogeneity of classical ADC preparation between SH and maleimide UPLC (Ultra-performance liquid column chromatography) analyses

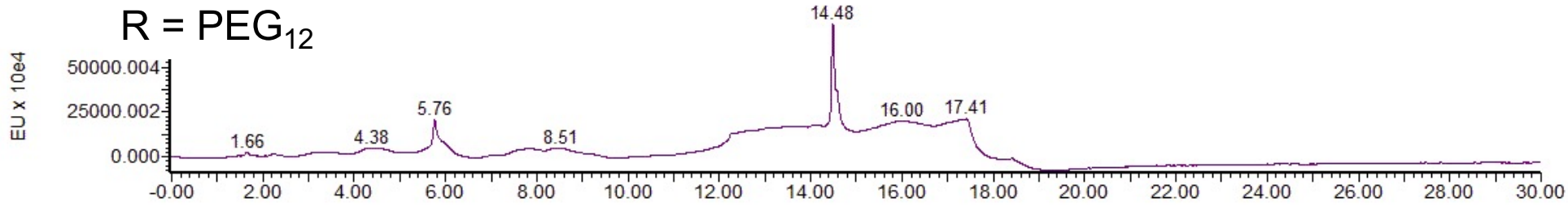


R = $(CH_2)_5$

HILIC (Hydrophilic Interaction Chromatography) column

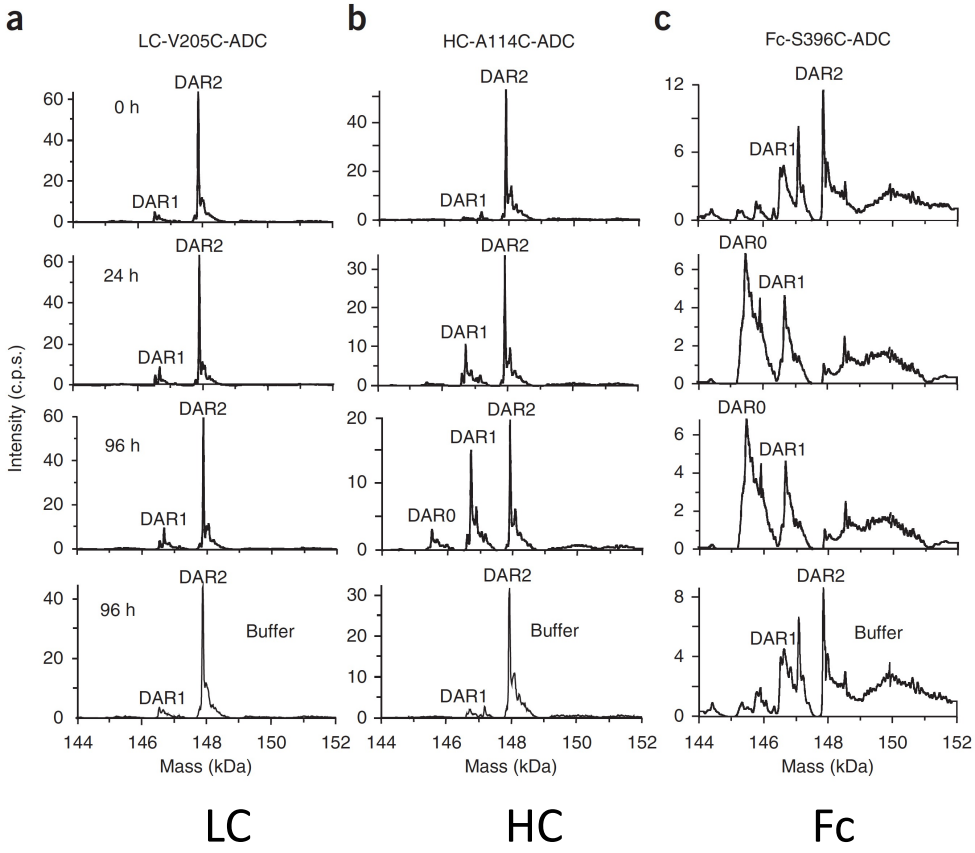


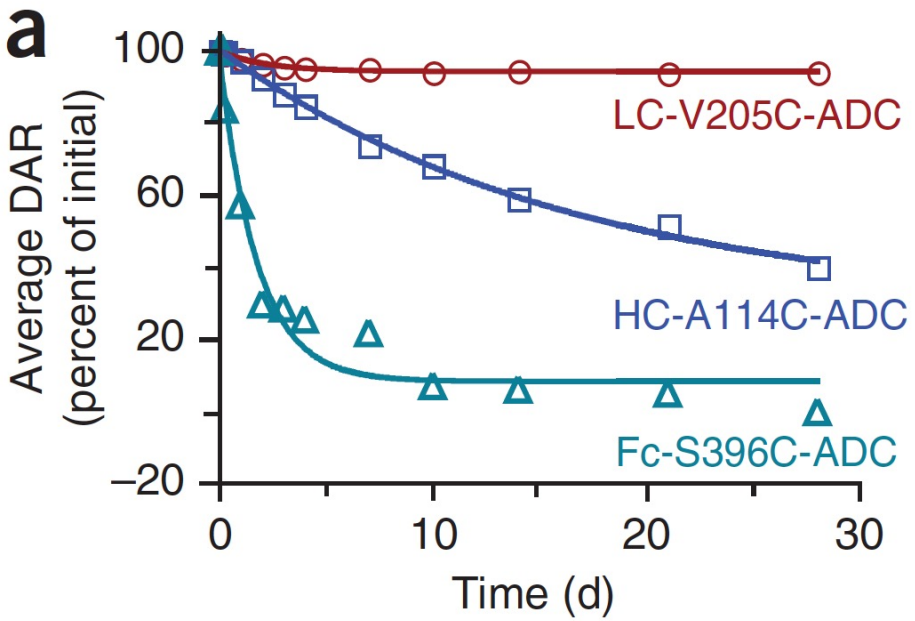
R = PEG_{12}



Glycoprotein BEH Amide 300 A, 1.7µm ex 280 nm, em320 nm

ADC stability is different by conjugation-site

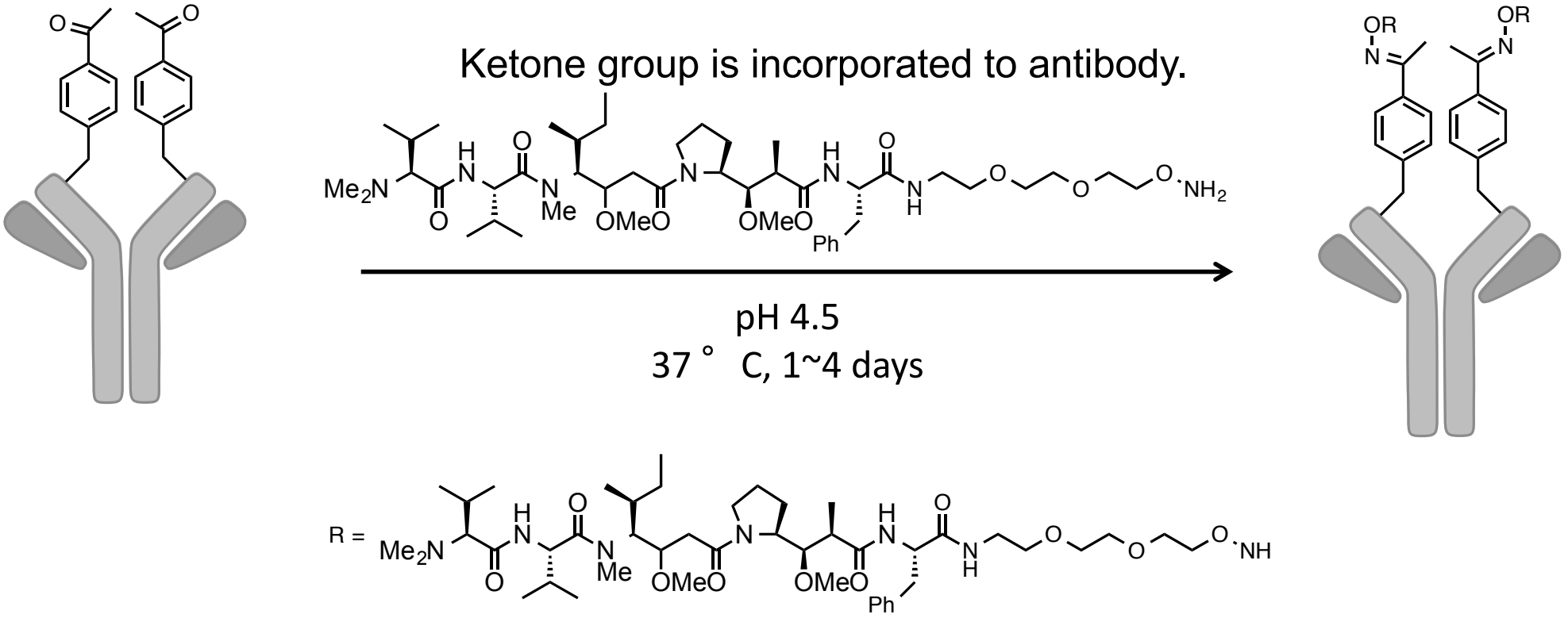




B.-Q. Shen *et al*. *Nat. Biotech*. **30**, 184 (2012)

Homogeneous ADC preparation: 1

Non-natural amino acid incorporation by *in vitro* protein synthesis



J. Y. Axup *et al*. *Proc. Natl. Acad. Sci. USA*. **109,** 16101 (2012)

Azide version

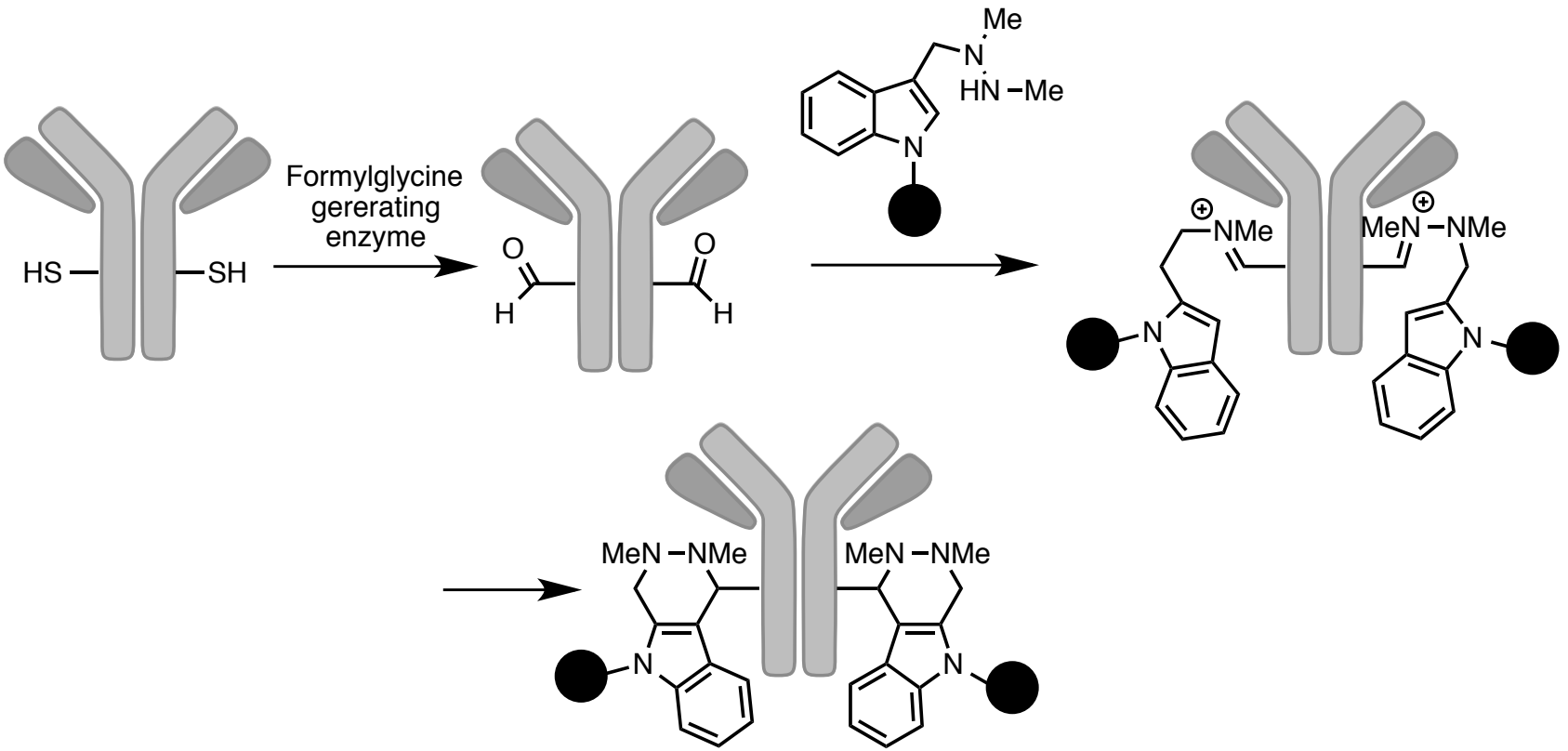
E. S. Zimmerman *et al*. *Bioconj. Chem*. **25***,* 351 (2014)

Homogeneous ADC preparation: 2

Formyl group generation by formylglycine generating enzyme and Hydrazino-Pictet-Spengler reaction

Formylglycine generating enzyme:
Cys in Leu-Cys-Thr-Pro-Ser-Arg sequence is changed to formyl group

Formyl group is generated.



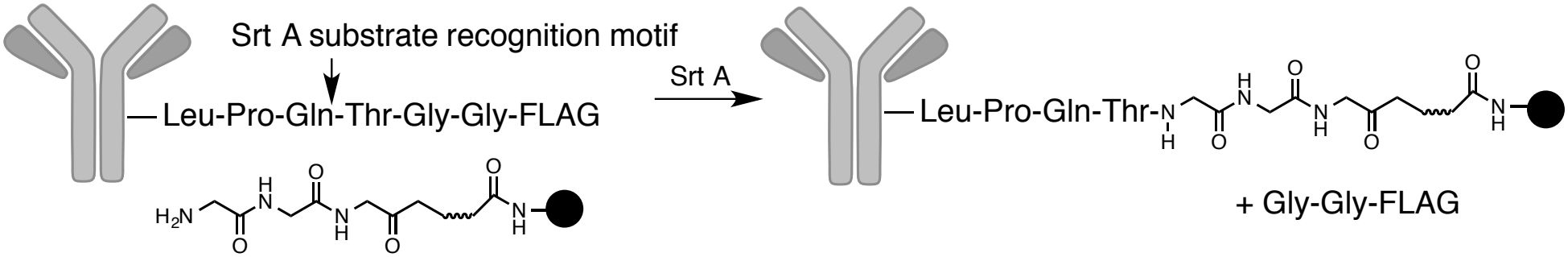
M. J. Appel, C. R. Bertozzi, *ACS Chem. Biol*. **10**, 72 (2015)

Homogeneous ADC preparation: 3

Site-selective conjugation by Sortase

Enzymes can recognize peptide sequence.
Site-specific conjugation is possible.

Sortase: The bond between Thr and Gly in Leu-Pro-any-Thr-Gly sequence.



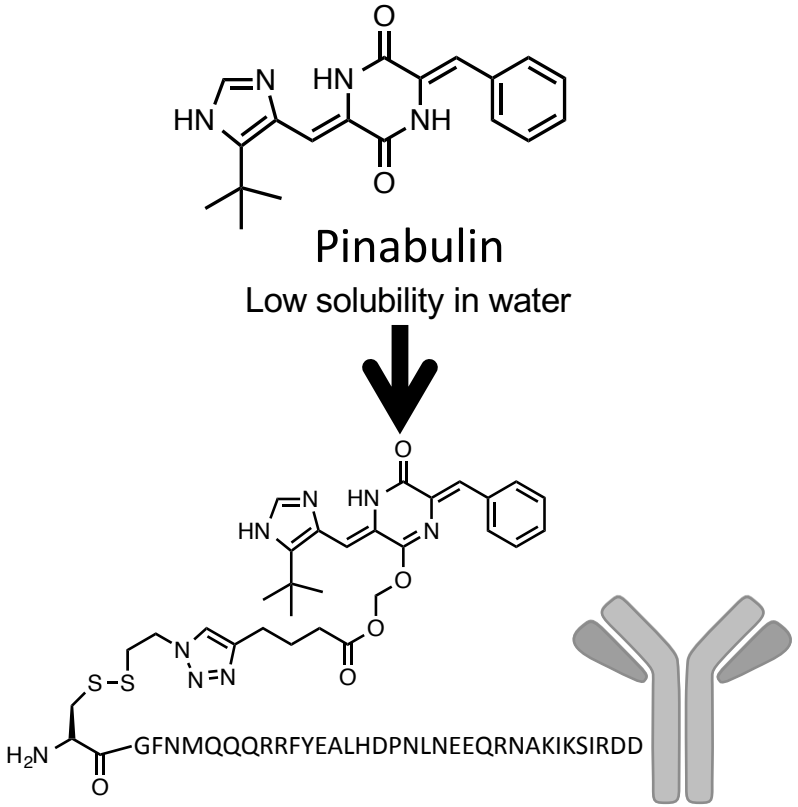
Reaction is reversible because product has also Leu-Pro-any-Thr-Gly sequence.

S. Jeger *et al*. *Angew. Chem. Int. Ed*. **49**, 9995 (2010)

N. M. Paterson *et al*. *Angew. Chem. Int. Ed*. **53**, 6115 (2014)

Homogeneous ADC preparation: 4
Fc region binding peptide

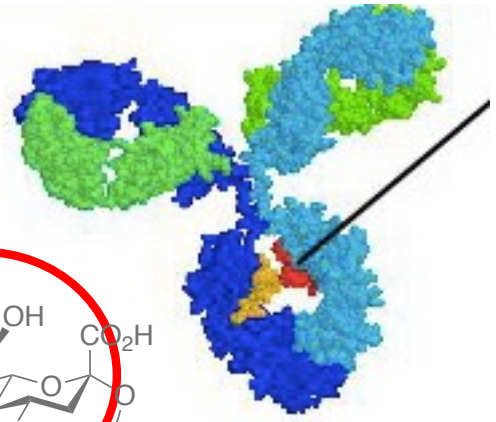
Z33 = FNMQQQRRFYEALHDPNLNEEQRNAKIKSIRDD



Binding efficacy is not interfered because peptide Z33 binds only to Fc region.

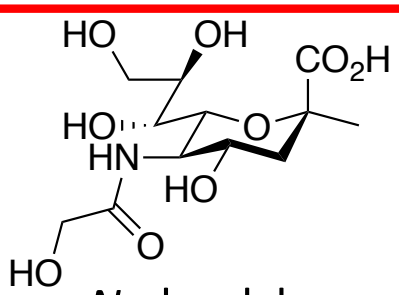
K. Mugumura *et al*. *Bioconj. Chem.* **27**, 1606 (2016)

Homogeneous ADC preparation: 5
N-glycan structure in antibody

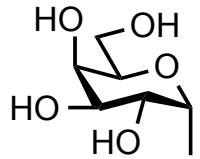


N-linked glycan at Asn297

Immunogenicity
from
Non-human type glycans

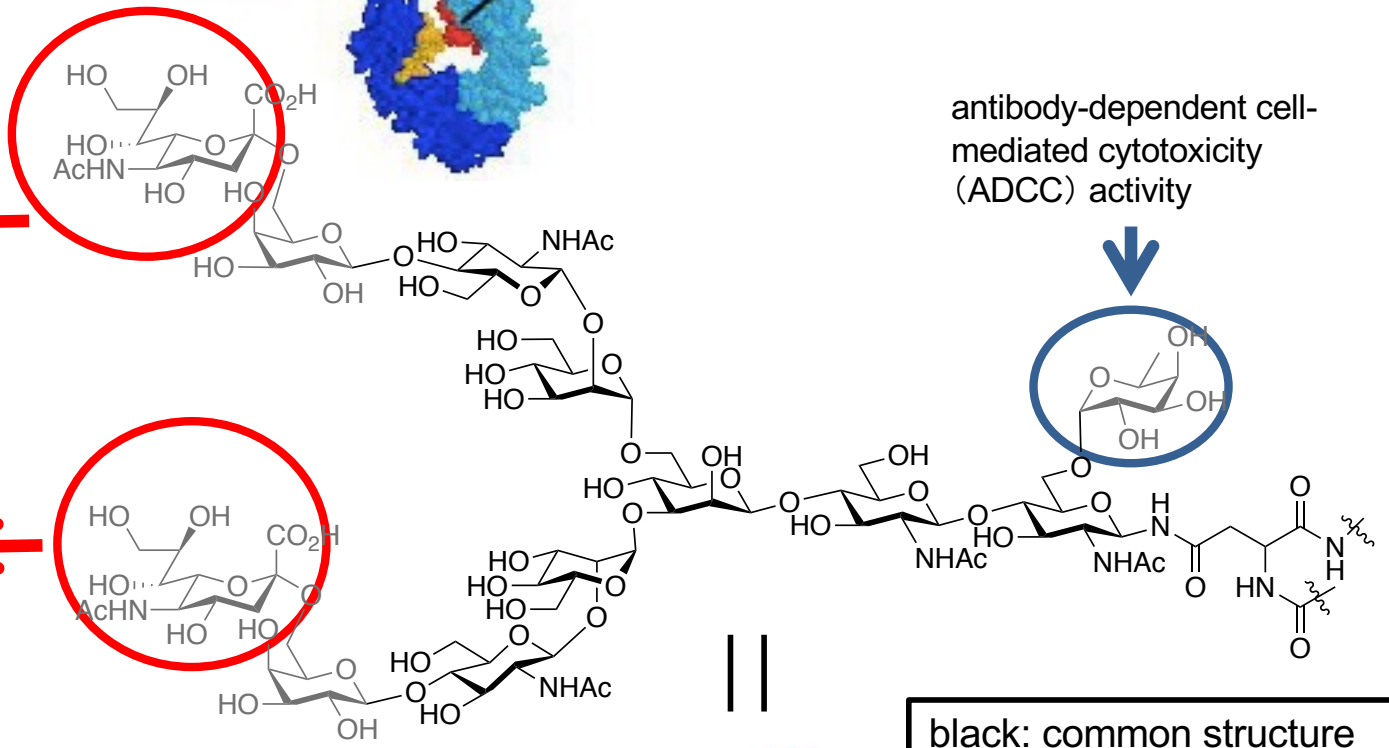


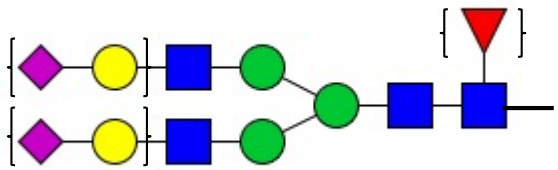
N-glycolyl
neuraminic acid



Galactose

antibody-dependent cell-mediated cytotoxicity
(ADCC) activity

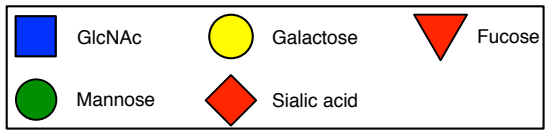




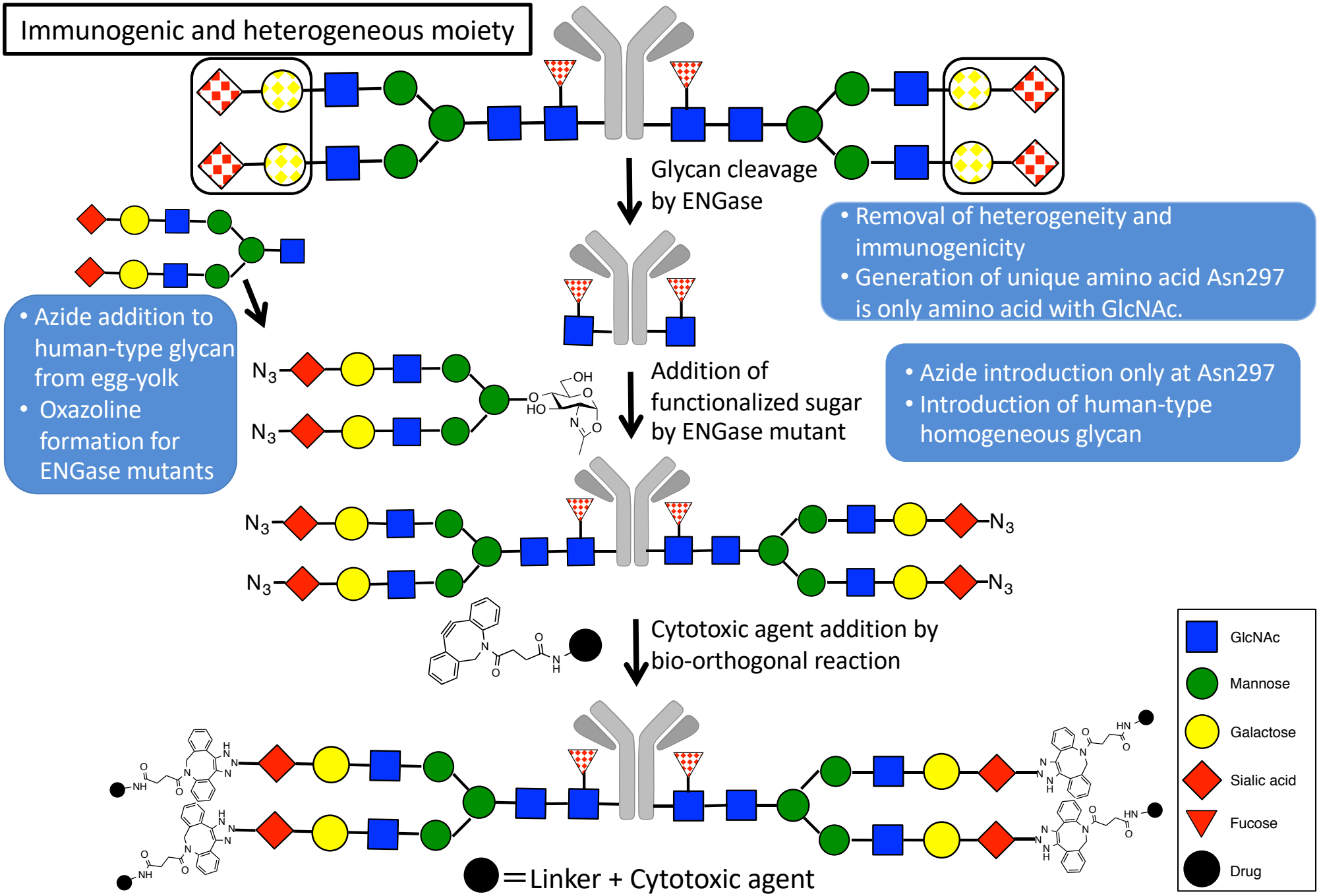
Asn

black: common structure
grey: heterogeneous structure

N-glycan is heterogeneous and affects to antibody functions.

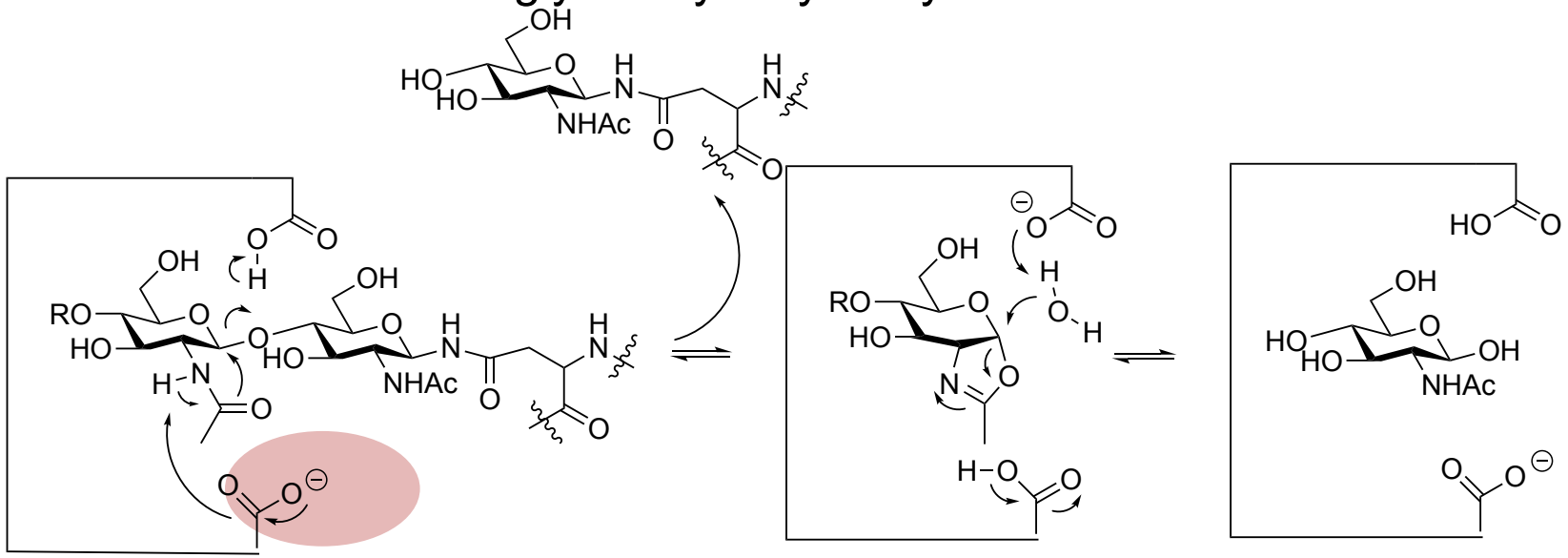


Homogenous *N*-glycan conjugate ADC strategy

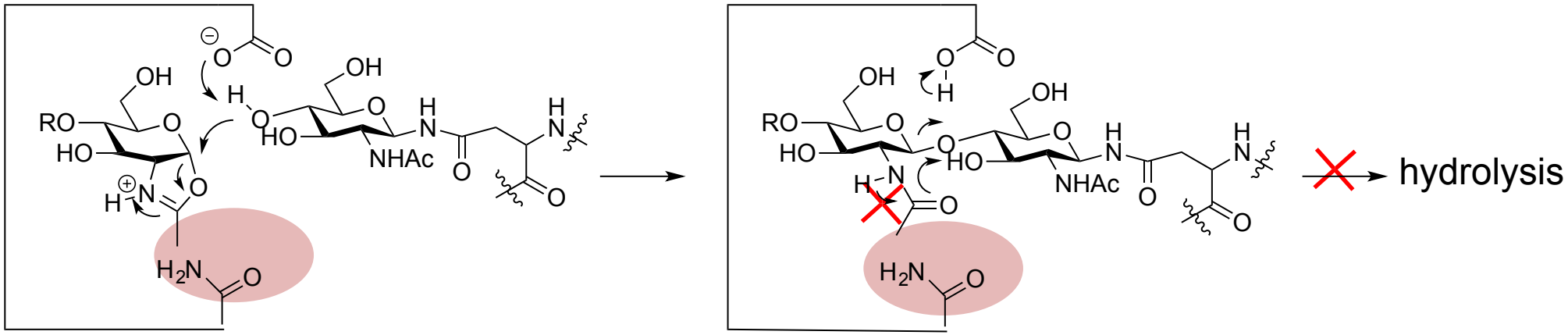


Reaction mechanism in N-glycan hydrolysis by ENGase

Oxazoline is intermediate in *N*-glycan hydrolysis by ENGase.



Mutated ENGase can transfer glycan, yet surppress hydrolytic activity.
Glycan oxazoline can be used as a glycan donor.

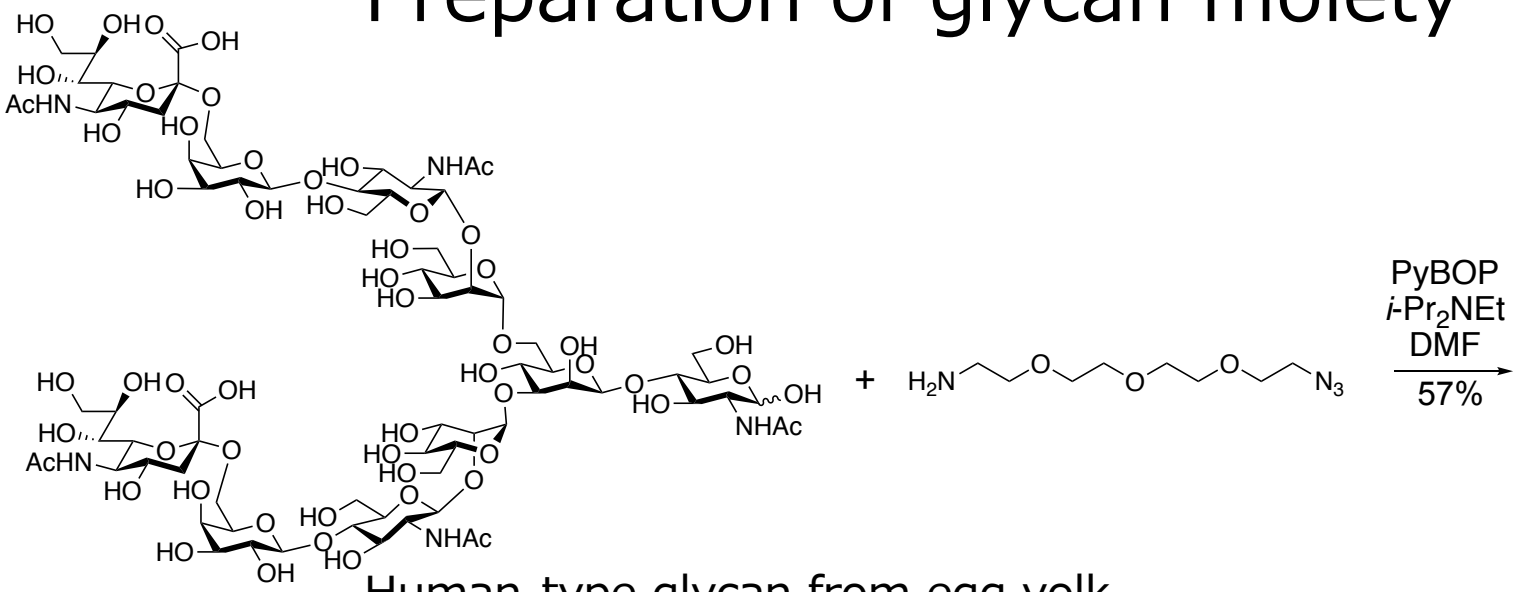


M. Fijita *et al. Biochim. Biophys. Acta*. **2001**, *1528*, 9: M. Umekawa *et al*. *J. Biol. Chem*. **2011,** *285*, 511: W. Huang *et al. J. Am. Chem. Soc*. **2012**, *134*, 12308.
S. Manabe *et al. R. Soc. Open Sci*. **2018**, *5*, 171521.

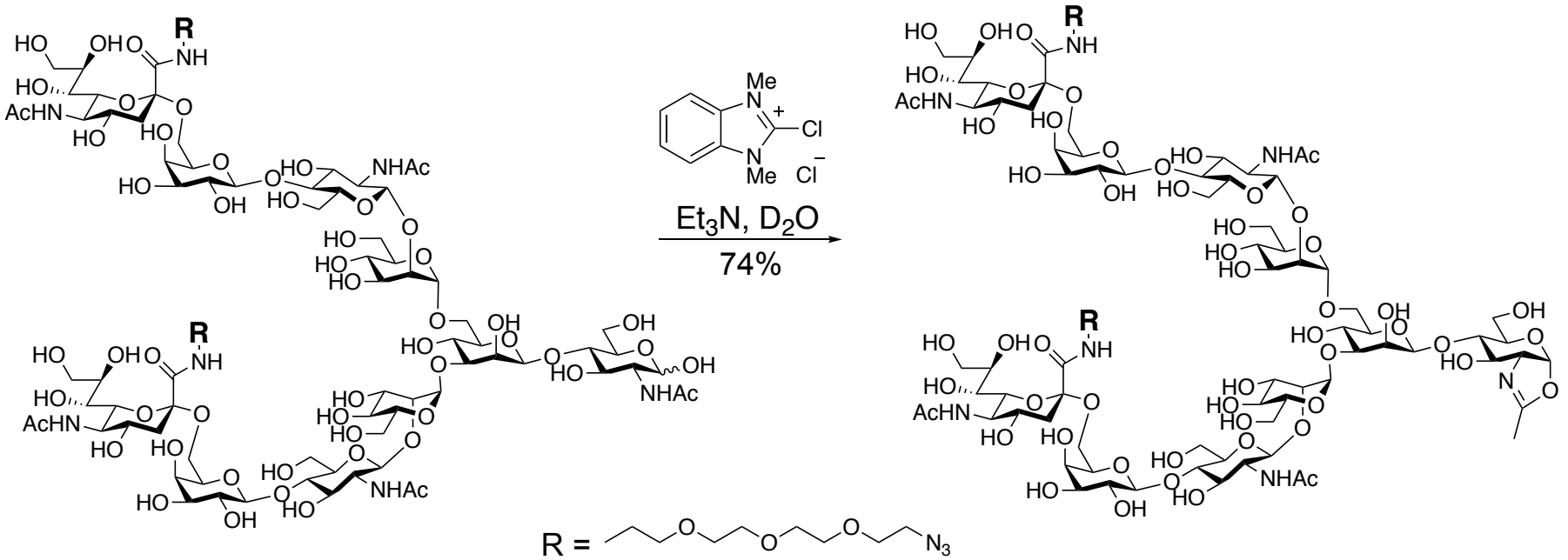
Homogeneous ADC

Our data

Preparation of glycan moiety



Human-type glycan from egg yolk



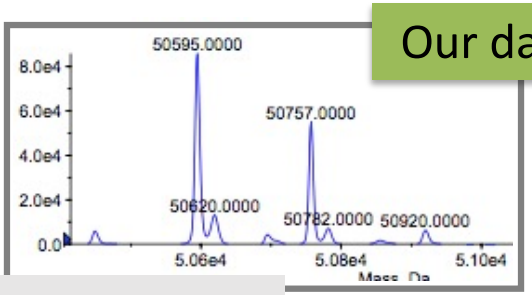
M. Noguchi *et al. Helv. Chim. Acta*. **95**, 1928（2012)

S. Manabe *et al. HeteroCycles*, **97**, 1203 (2018)

Homogeneous ADC

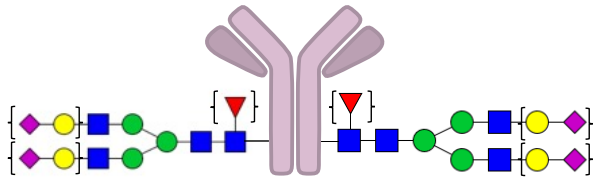
Homogeneity in *N*-glycan ADC

Our data



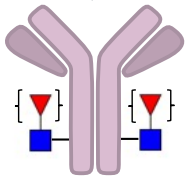
HILIC analyses

ESI-MS analyses

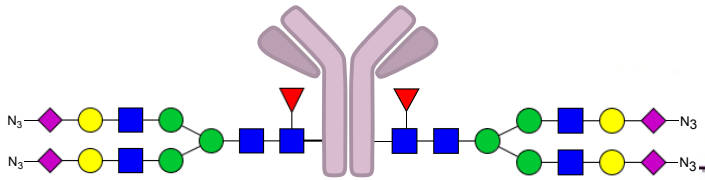


Trastuzumab

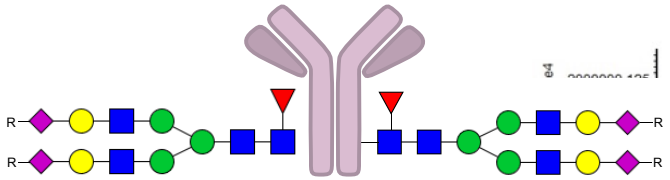
糖鎖除去 endoS

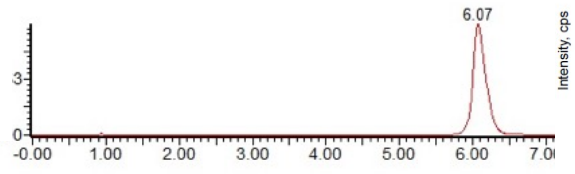


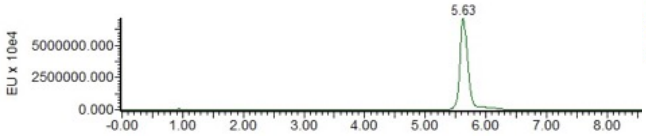
糖鎖付加 endoS D233Q



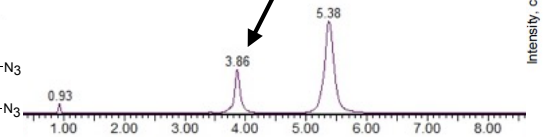
payload 付加

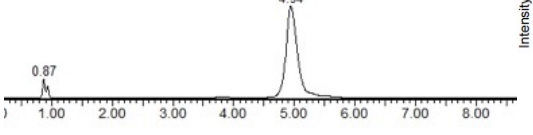


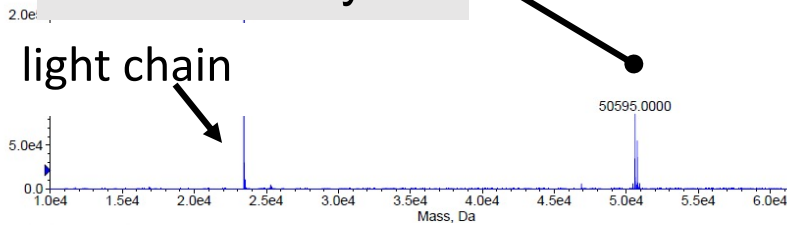


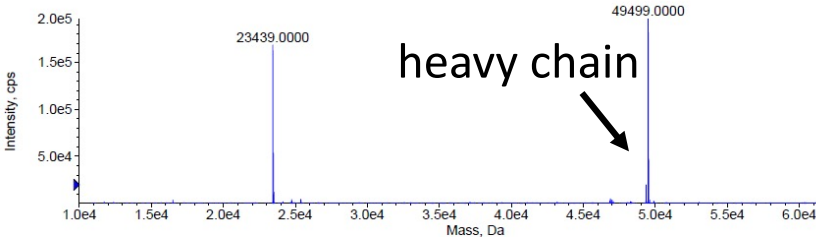


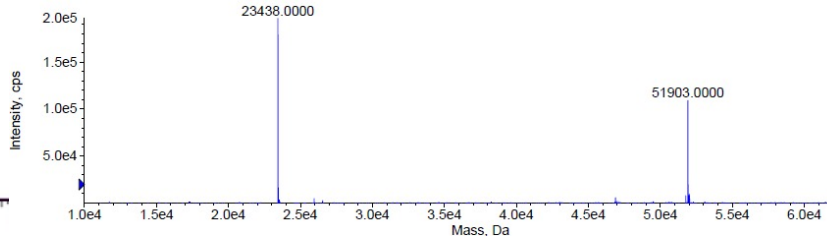
endoS D233Q

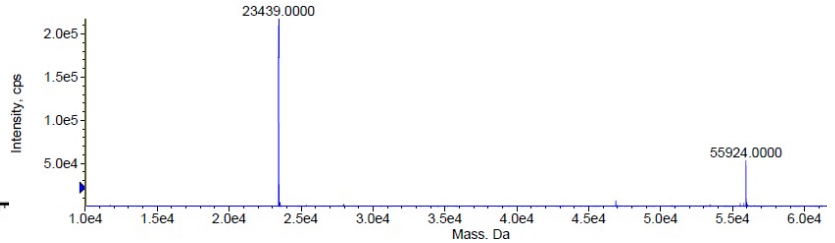












Glycoprotein BEH Amide 300 A
1.7μm ex 280nm, em 320nm

After DTT treatment

S. Manabe *et al. Bioconj. Chem.* **30**, 1343 (2019)

Homogeneous ADC

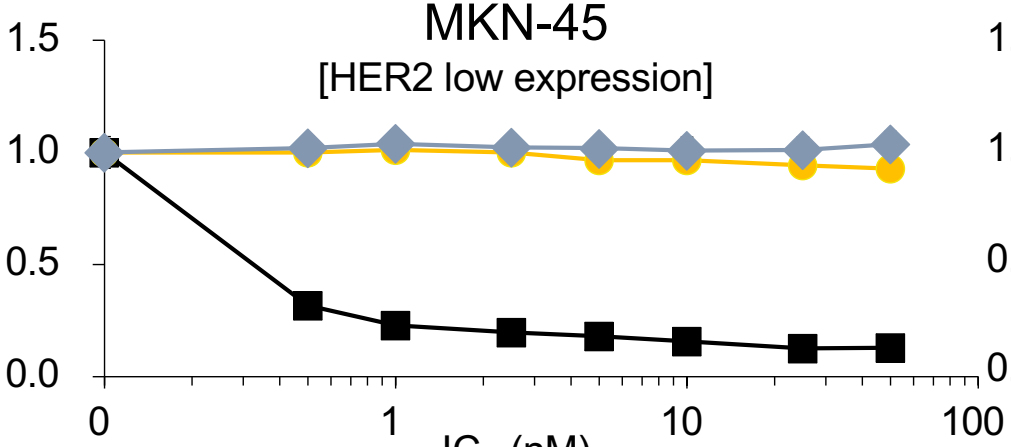
in vitro cytotoxicity

Our data

Gastric cancer cell line

MKN-45

[HER2 low expression]

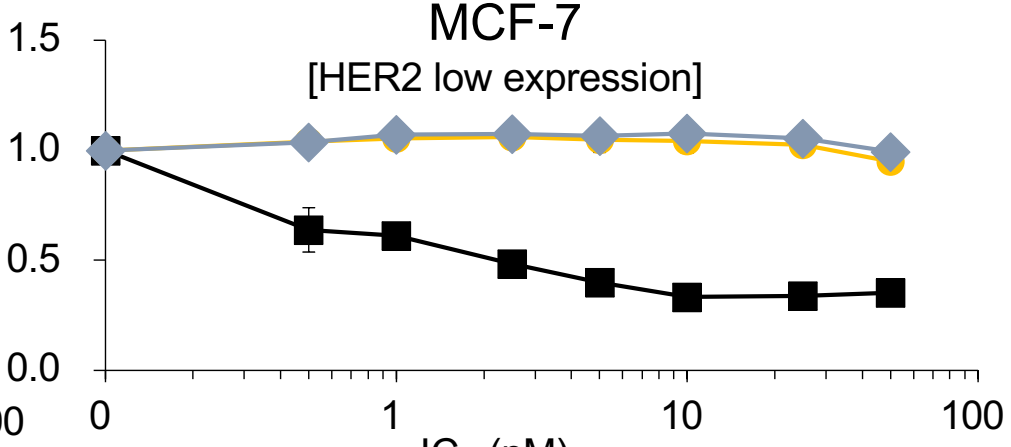


IC_{50}(nM)
MMAE: 0.3
Trastuzumab:-
Trastuzumab-ADC: -

Breast cancer cell line

MCF-7

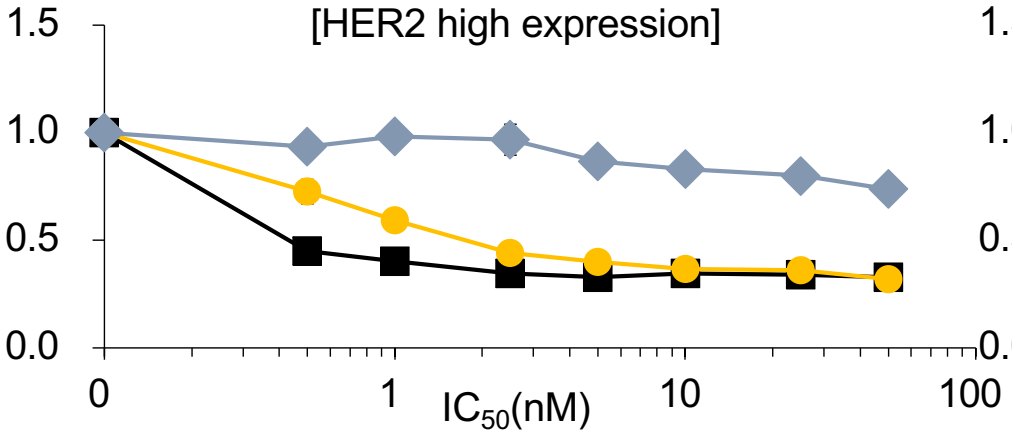
[HER2 low expression]



IC_{50}(nM)
MMAE: 2.2
Trastuzumab:-
Trastuzumab-ADC: -

N-87

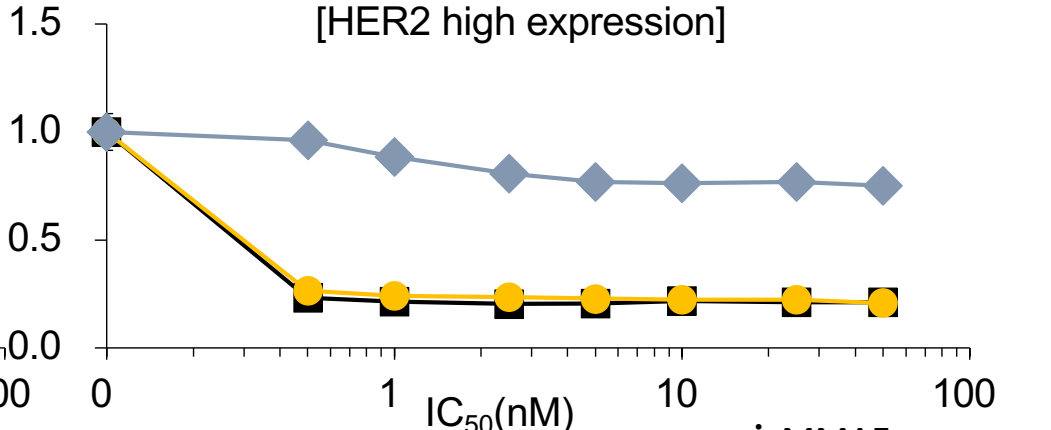
[HER2 high expression]



IC_{50}(nM)
MMAE: 0.4
Trastuzumab:-
Trastuzumab-ADC: 1.8

SK-BR-3

[HER2 high expression]



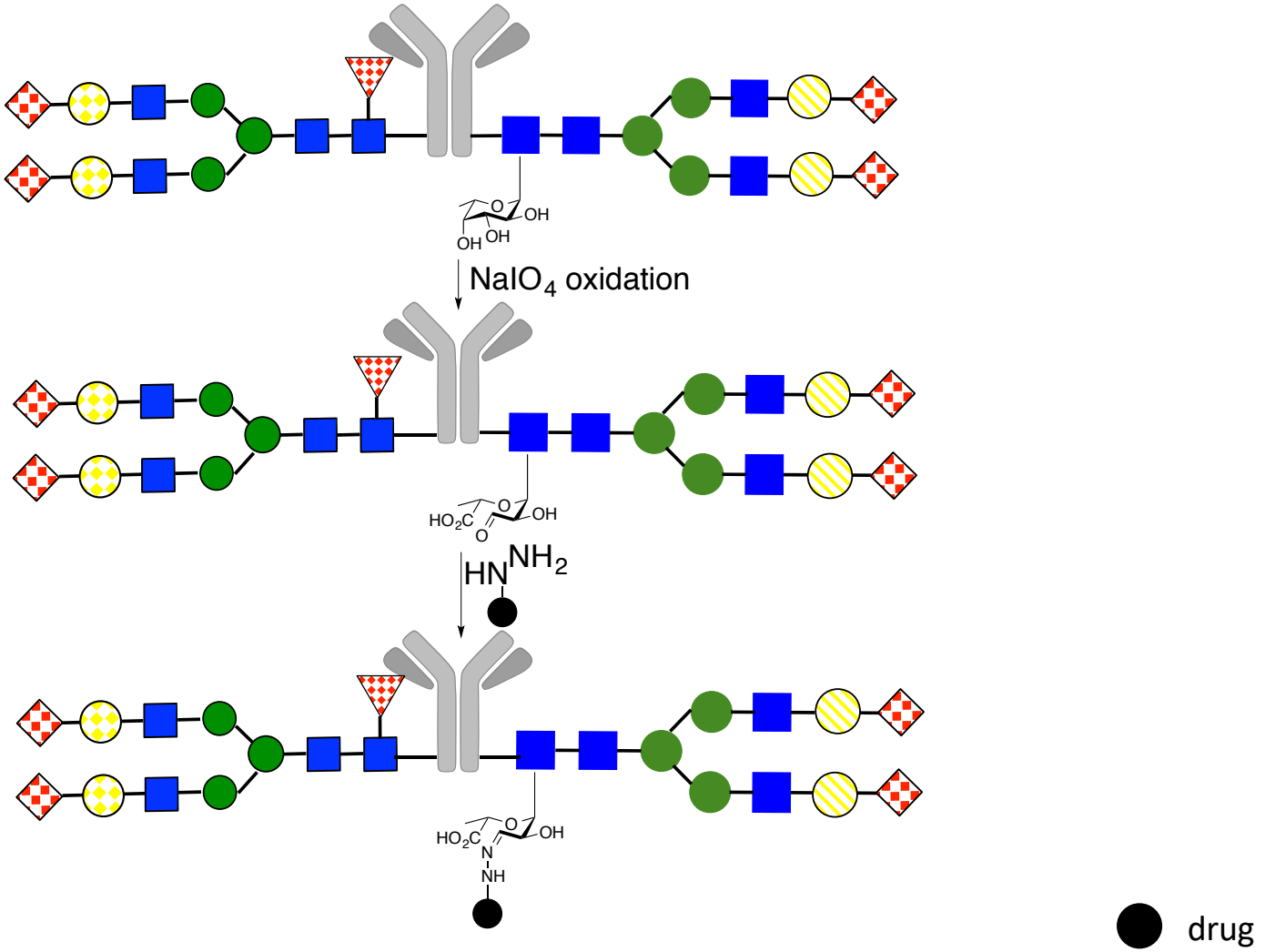
IC_{50}(nM)
MMAE: 0.3
Trastuzumab:-
Trastuzumab-ADC: 0.3

Homogeneous ADC

Other examples of glycan-conjugated ADC

cis-diol oxidative cleavage and conjugation to formyl group

Formyl group is generated.

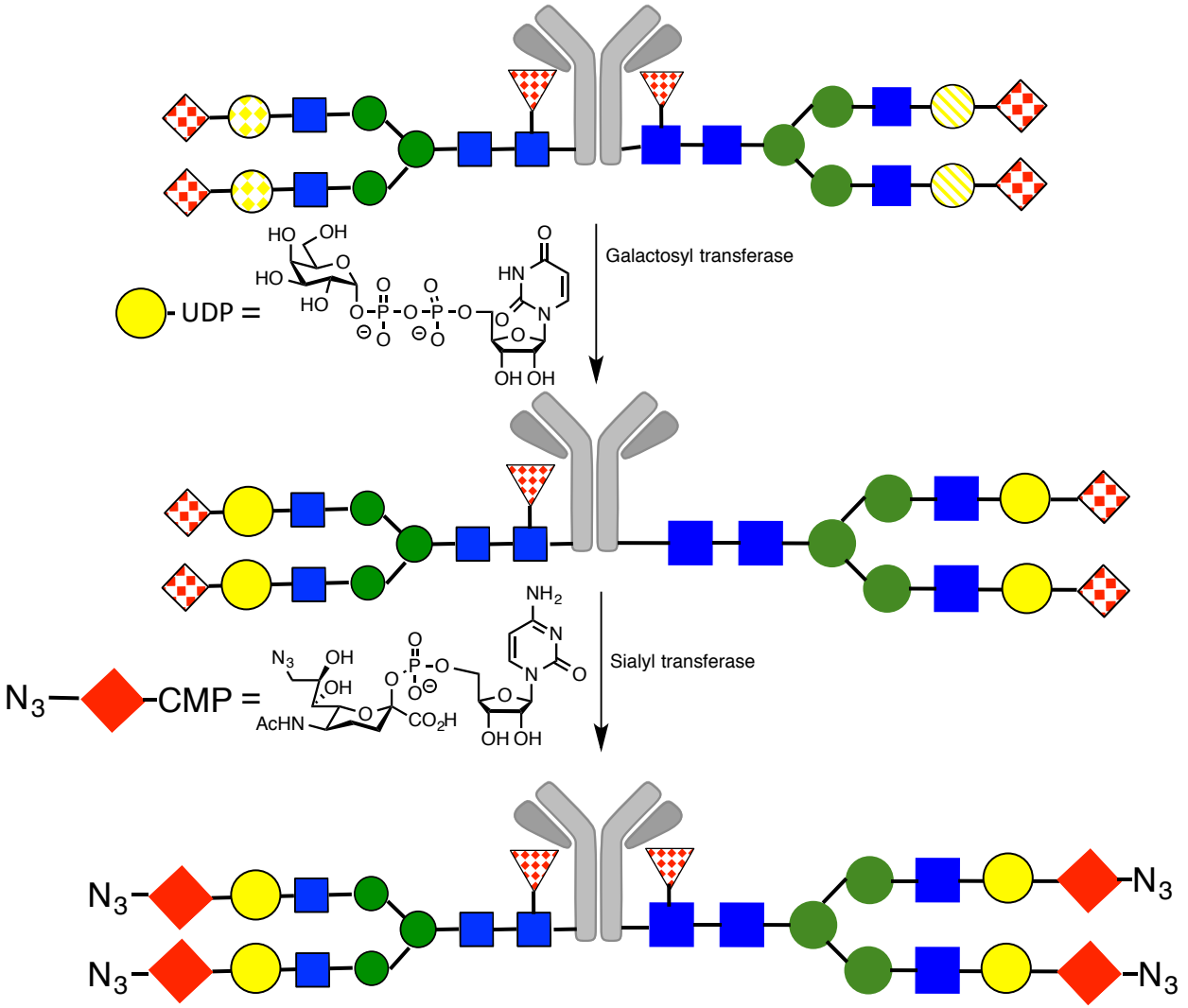


Concern to Met and Cys under oxidative conditions.

K. Züberbuhler *et. al. Chem. Commun.* **48,** 7100 (2012)

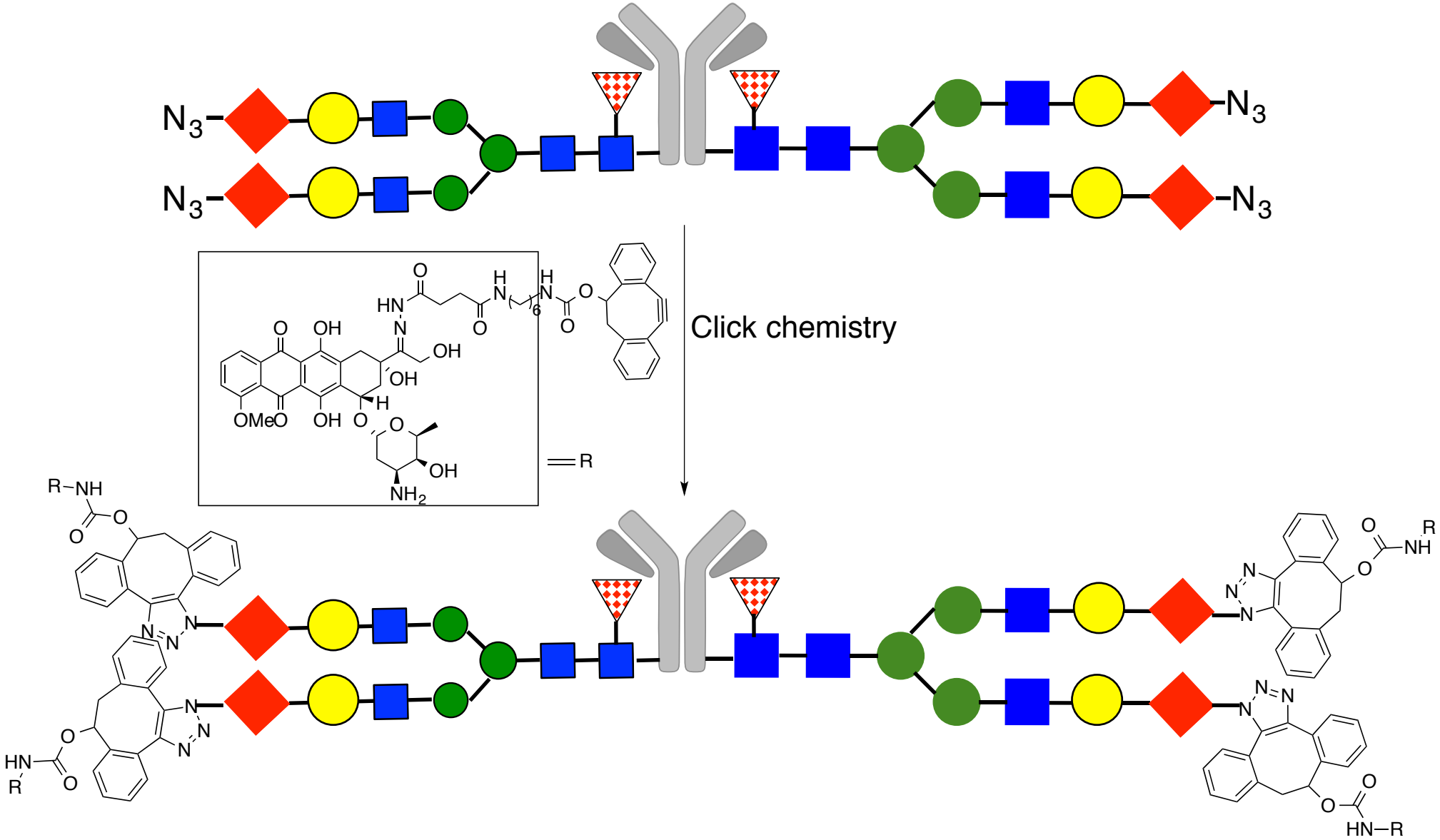
Other examples of glycan-conjugated ADC

Sialic acid transferase and azide-incorporation CMP-sialic acid



Other examples of glycan-conjugated ADC

Sialic acid transferase and azide-incorporation CMP-sialic acid

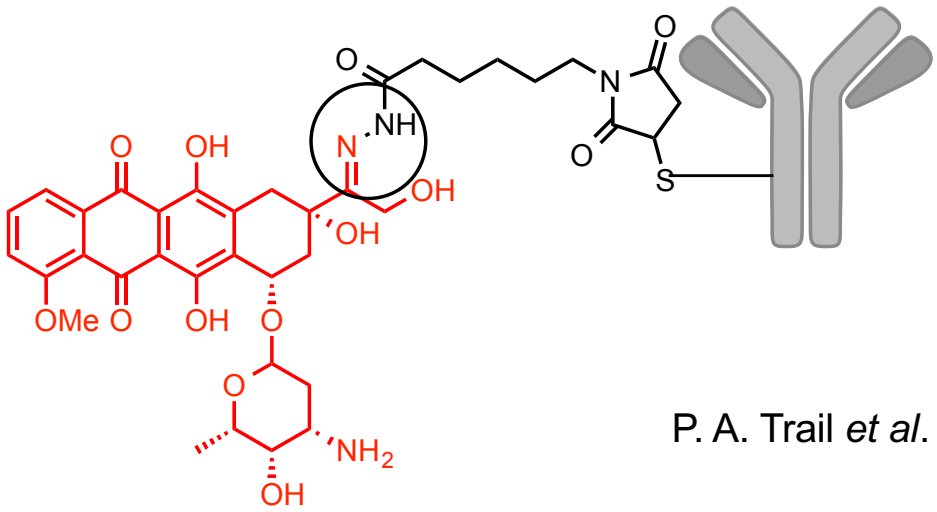


X. Li *et al. Angew. Chem. Int. Ed.* **53,** 7179 (2014)

Release mechanism

Linker at initial stage ADC

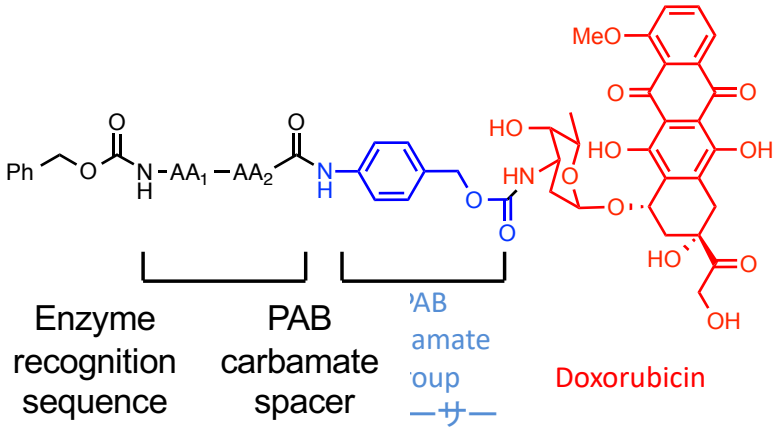
Unstable in serum by hydrolysis.



P. A. Trail *et al*. *Science*, **261**, 212 (1994)

Gradually release and specific/selective release is important in ADC.
Enzymatic cleavage is desired.

Release rate control
Cathepsin cleavable linker



	AA1	AA2	PAB carbamate	$t_{1/2}$
1	Phe	Lys	-	Not released
2	Phe	Lys	+	8 min
3	Val	Cit	+	630 min

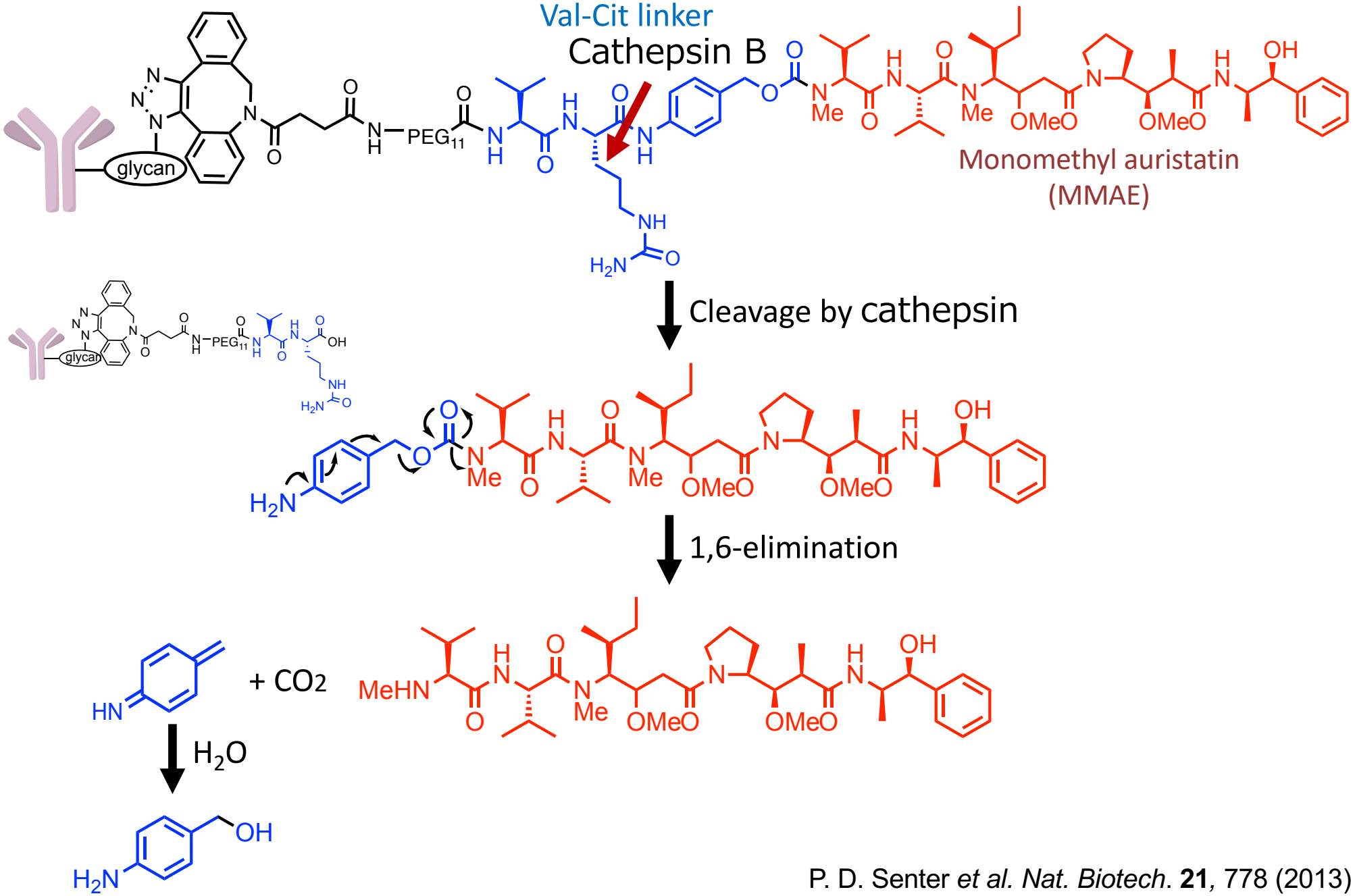
G. M. Dubowchik *et al. Bioconj. Chem*. **13,** 855 (2002)

- The most common linker.
- Drug is released in CRISPR-Cas9 knockout of cathepsin B cell.
- The cleavage rate of cathepsin cleavable linker by cathepsin S is faster than that of cathepsin B.
- The drug is also released outside the cell (caused by cathepsin, which is originally present in the lysosome, leaking out of the cell due to lesions?).

N. G. Caculitan *et al.*, *Cancer Res*. **77**, 7027 (2017)
M Dorywalska *et al*., *Mol. Cancer Ther.* **15**, 958 (2016)

Release mechanism

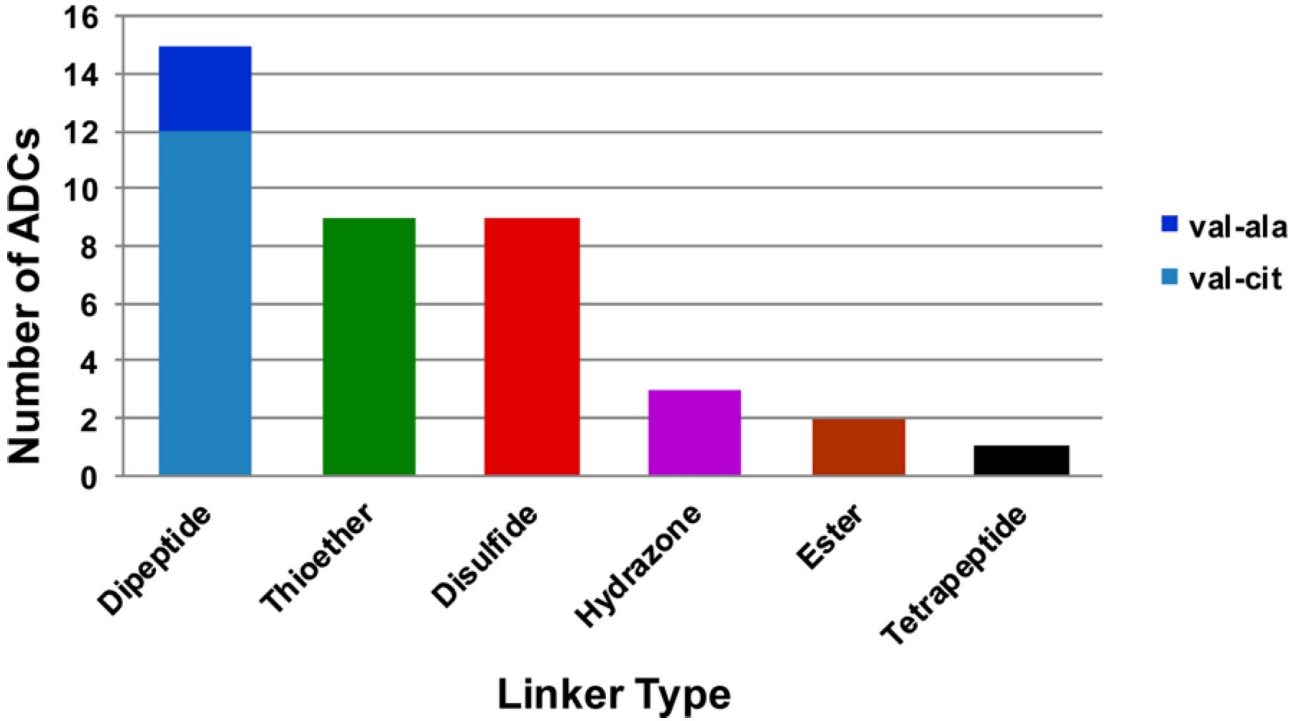
Linker design



P. D. Senter *et al. Nat. Biotech.* **21**, 778 (2013)

Linkers in present ADCs

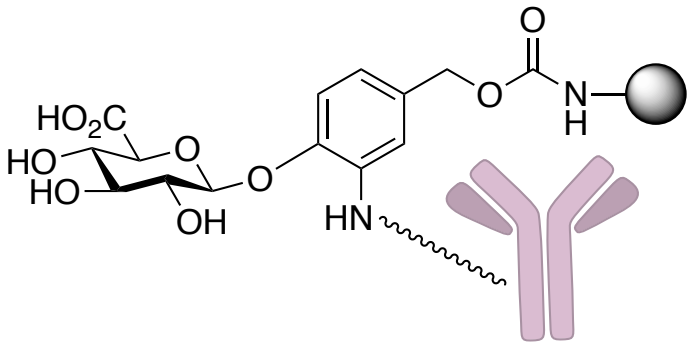
Linker variation is limited.



V. J. Chari, *ACS Med. Chem. Lett*. **7**, 974 (2016)

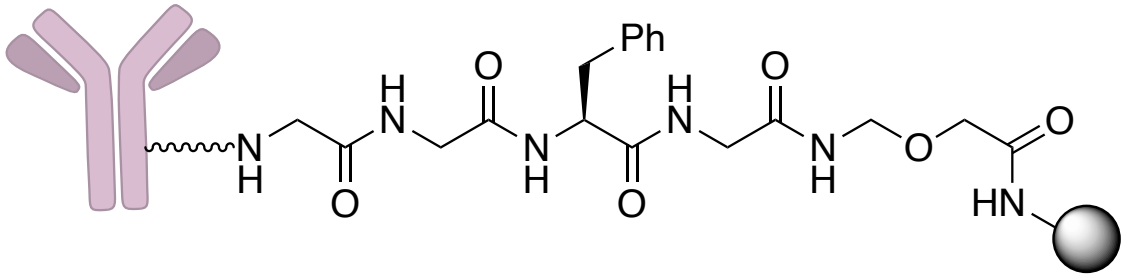
Various hydrolysis mode

Glucuronidase cleavage



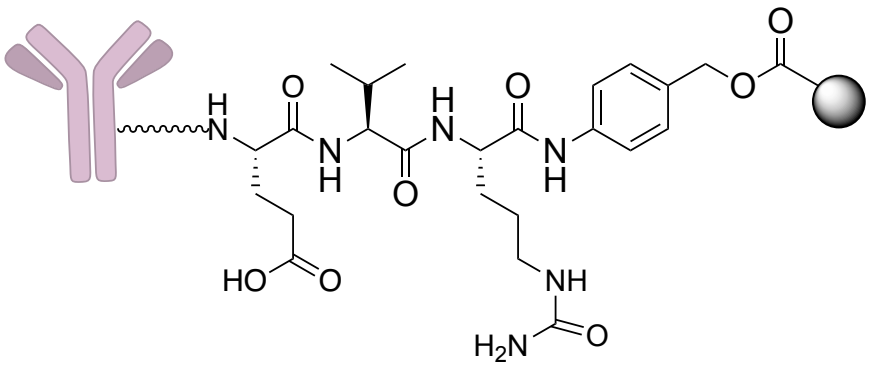
S. C. Jeffrey *et al*. *ACS Med. Chem. Lett*. **1**, 277 (2010)

Protease cleavage



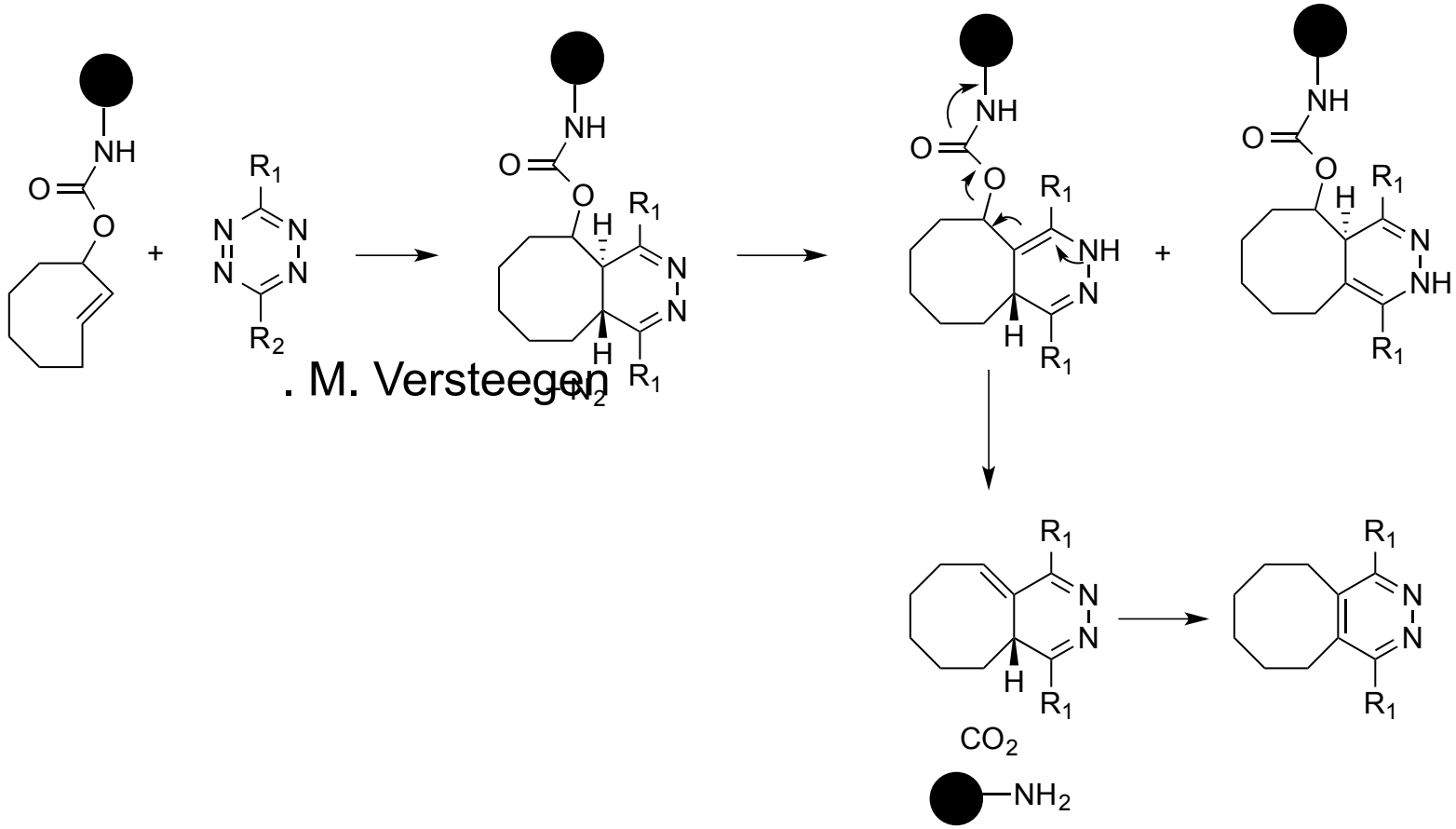
Y. Ogitani *et al. Clin. Cancer Res*. **22**, 5097 (2016)

Catepsin cleavage
More stable than Val-Cit to esterase in mice



Y. Anami *et al. Nature Commun*. **9**, 1 (2018)

Release by strain-promoted inverse electron-demand Diels-Alder Cycloaddition "Click to release"



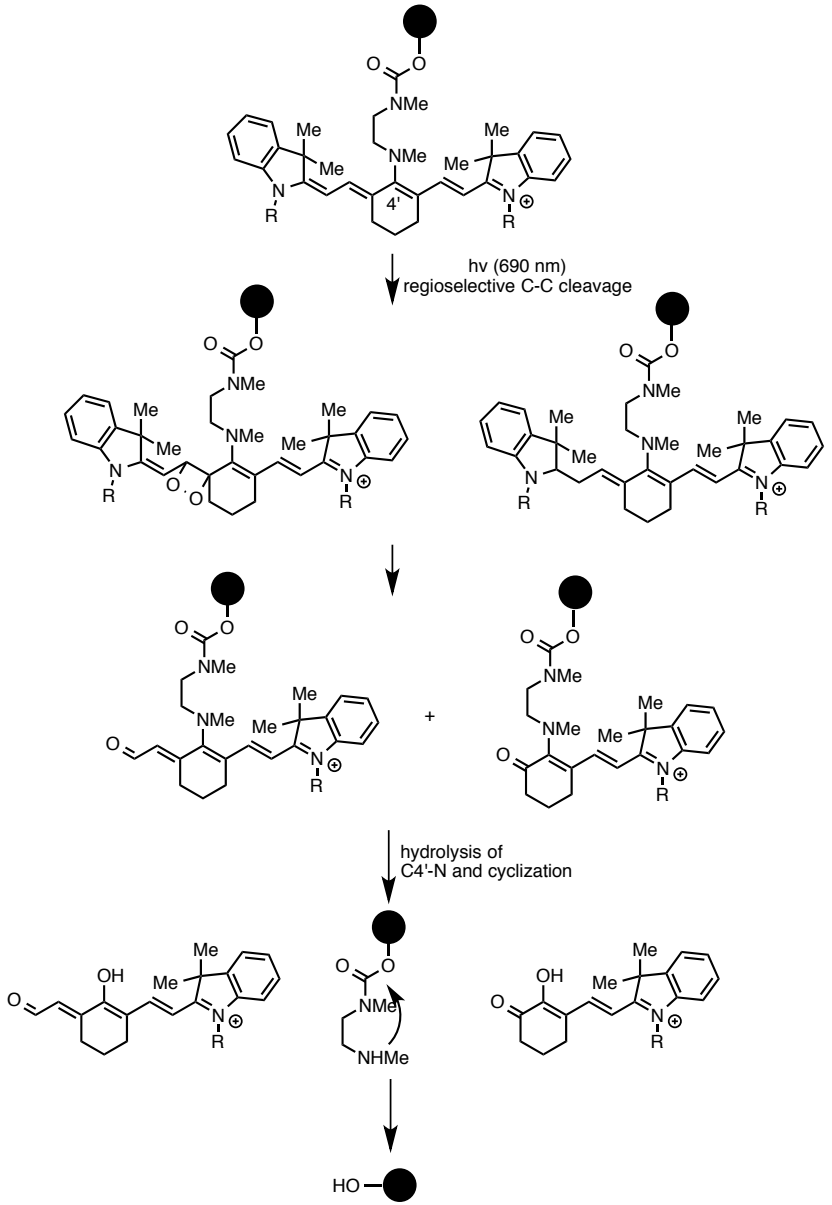
. M. Versteegen

R. M. Versteegen *et al*. *Angew. Chem. Int. Ed*. **52***,* 14112 (2013)

R. Rossin *et al*., *Bioconj. Chem.* **27***,* 1697 (2016)

J. Li, P. R. Chen, *Nat. Chem*. **12***,* 129 (2016)

Release mechanism by near infrared fluorescence

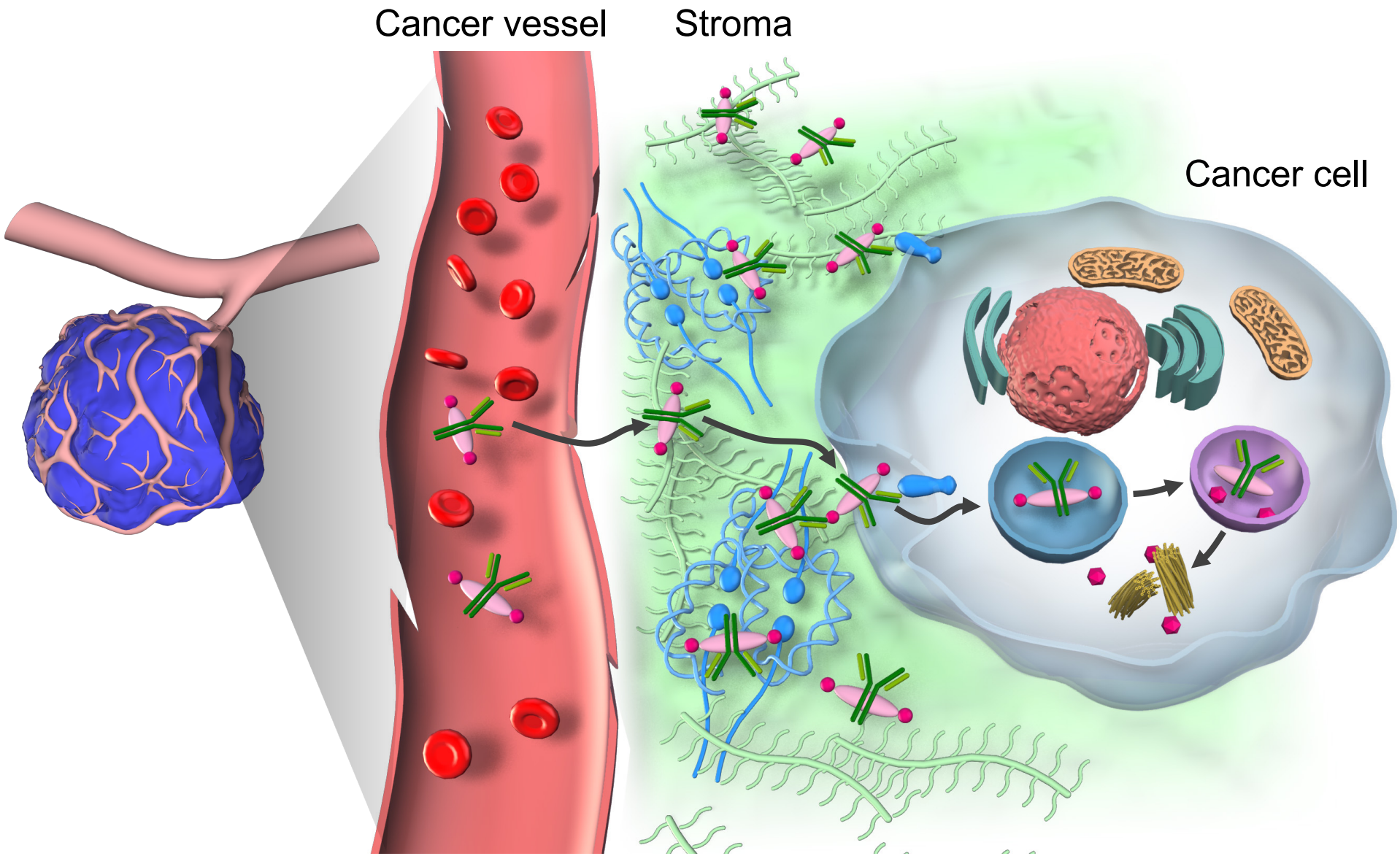


R. R. Nani *et al. Angew. Chem. Int. Ed*. **54**, 13635 (2015)

Release mechanism

Limitation of conventional antibody medicine

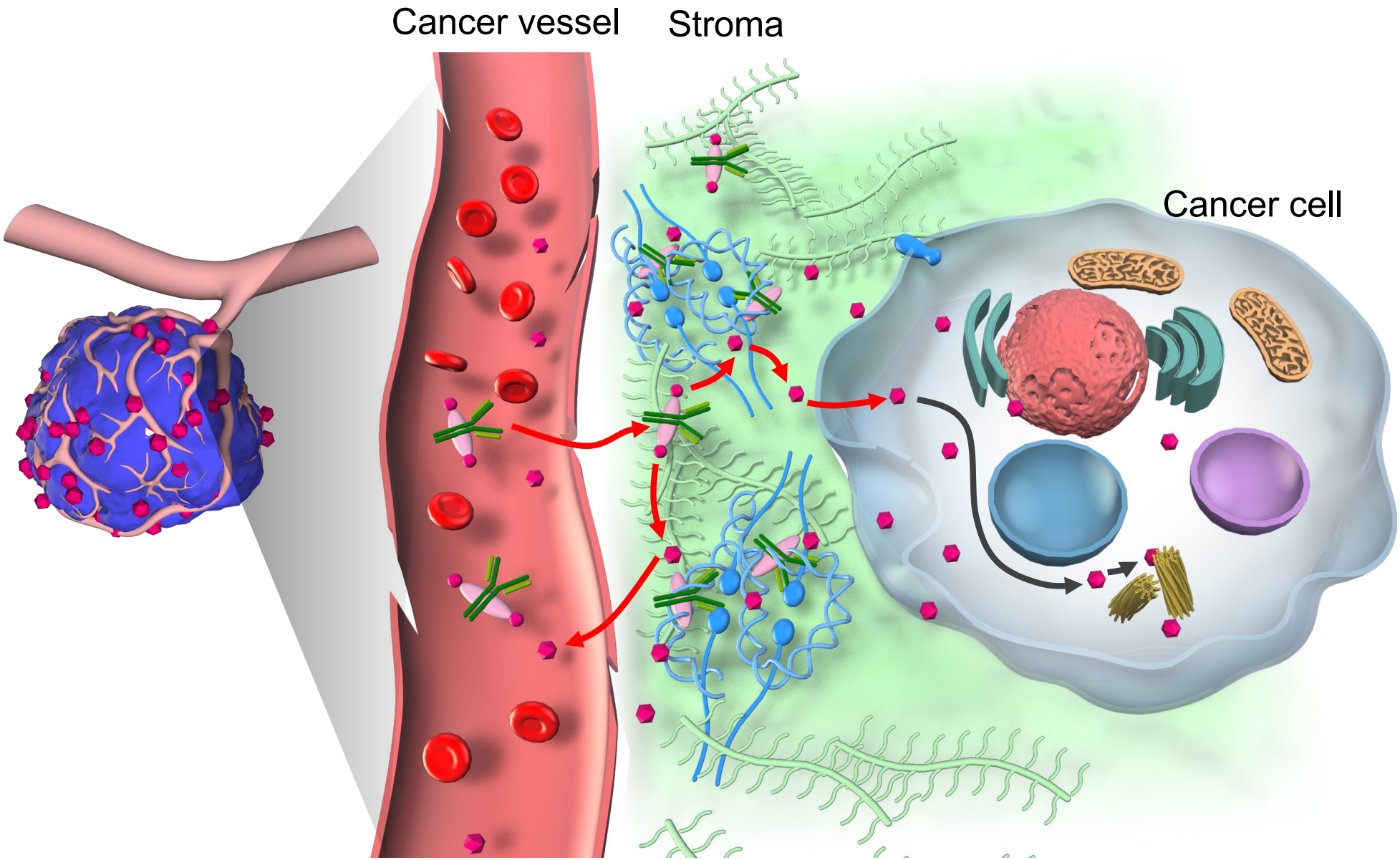
Stroma inhibits antibody to cancer cell from cancer vessel



Release mechanism

Cancer stromal targeting (CAST) strategy

Collaboration with Dr. Matsumura's group @NCC

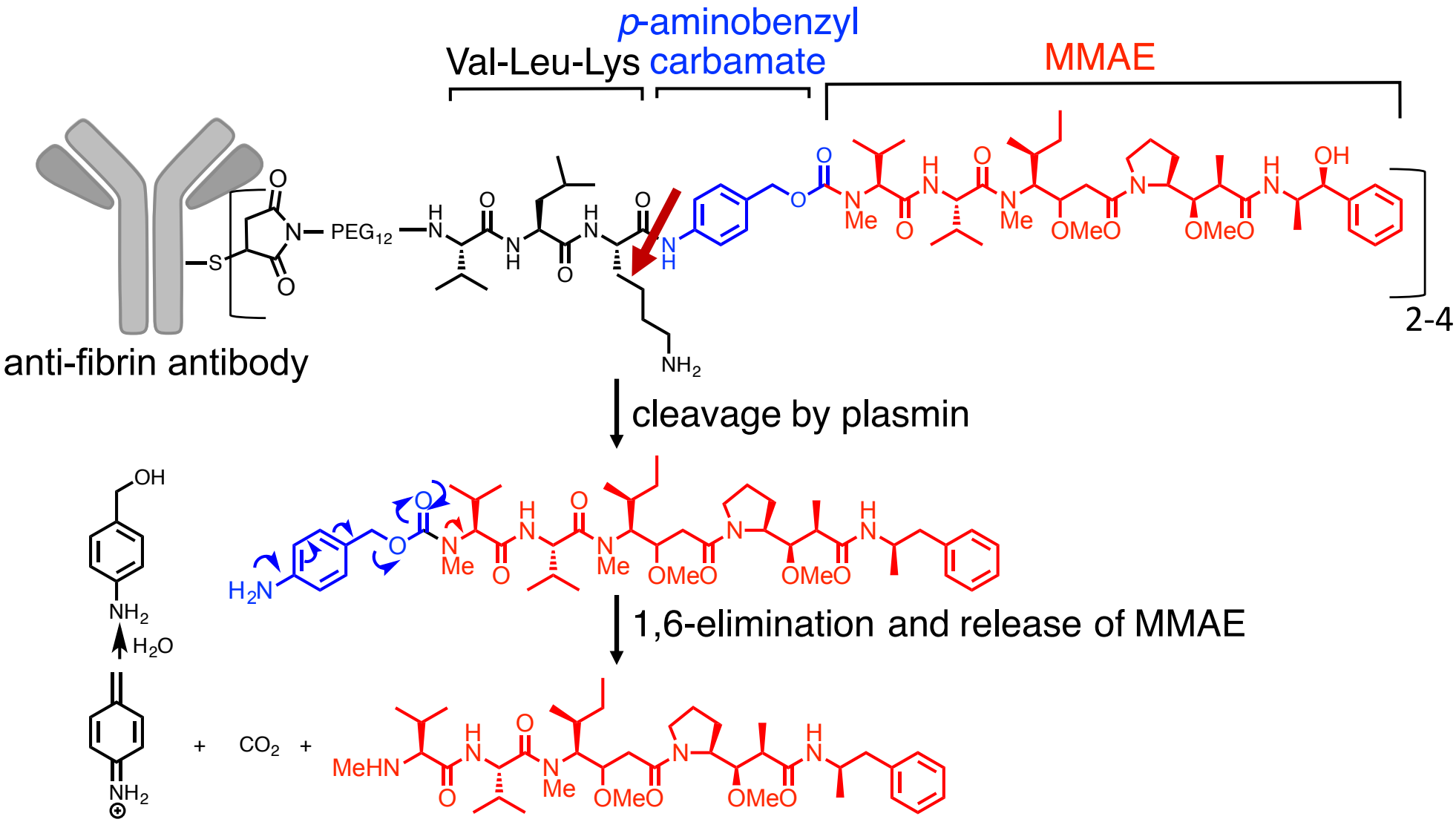


H. Fuchigami *et al. Sci. Rep.* **8**, 14211 (2018)

Release mechanism

Our data

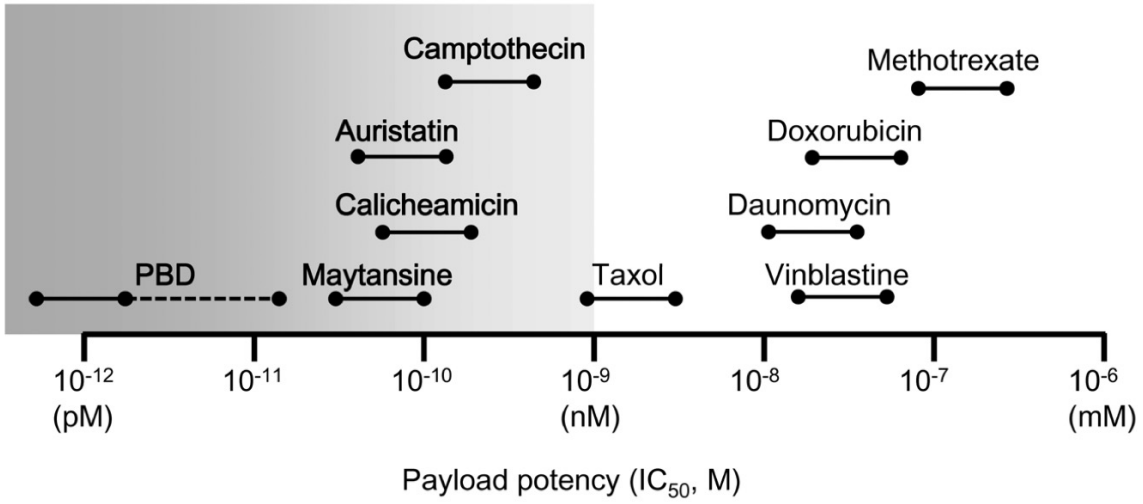
Design of plasmin-cleavable linker



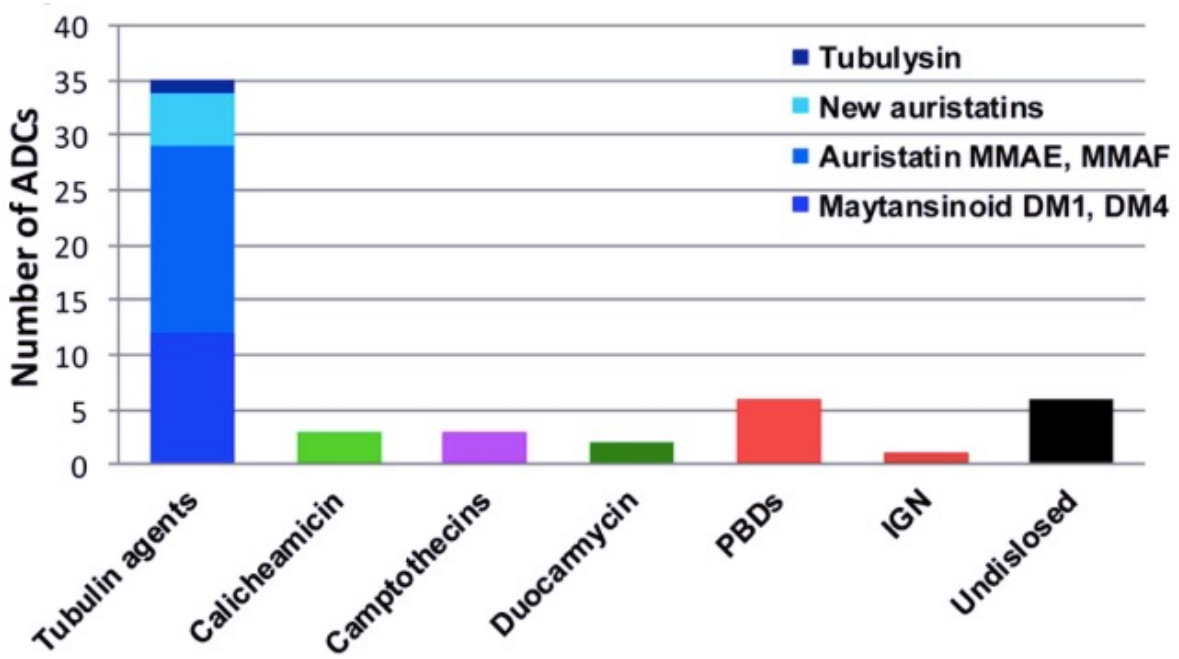
Plasmin is specific enzyme for cleavage. Plasmin is generated from plasminogen. Outside fibrin, anti-plasmin inhibitor deactivates plasmin.

Compound

Low-molecular compounds for ADCs



T. Nakada *et al. Chem. Pharm. Bull*. **67**, 173 (2019)



V. J. Chari, *ACS Med. Chem. Lett*. **7**, 974 (2016)

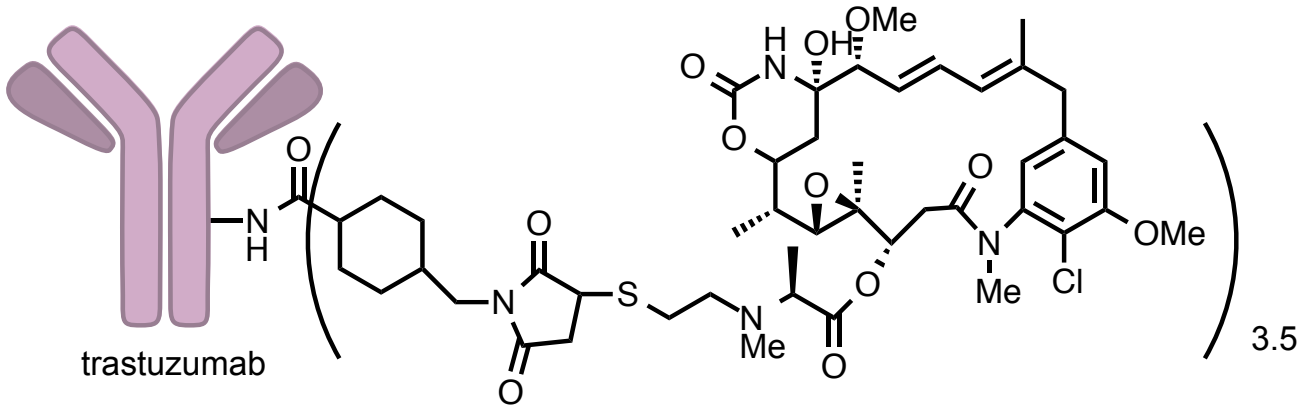
Compound

Release mechanism

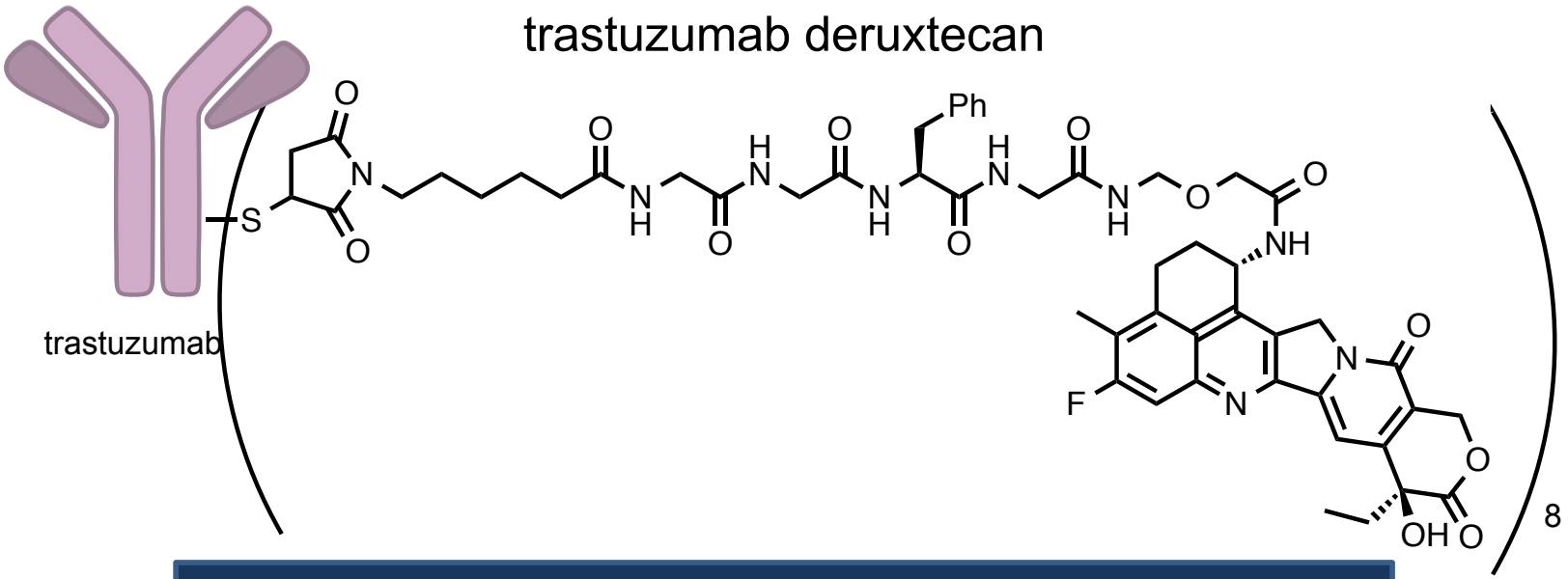
Importance of payload

Antibody is the same.

trastuzumab emtansine



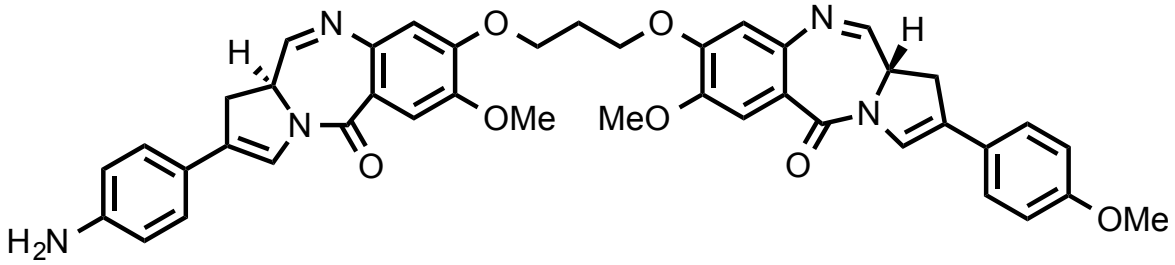
trastuzumab deruxtecan



More effective ADC can be generated by changing payload.

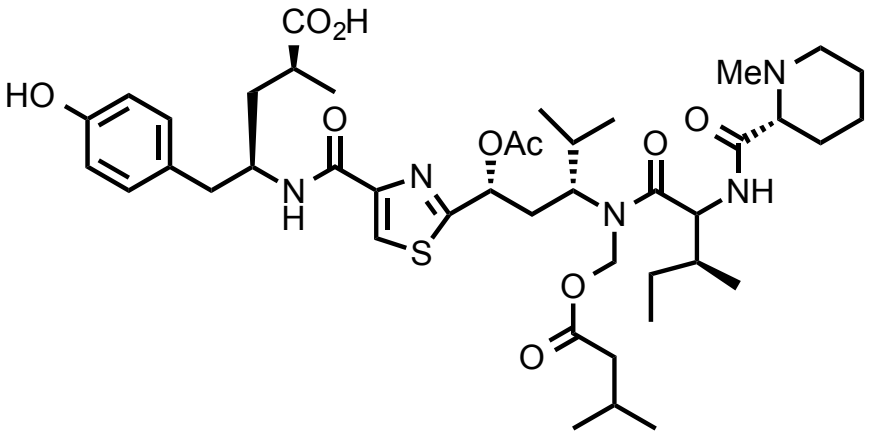
Compound

Potent payload in ADC:1

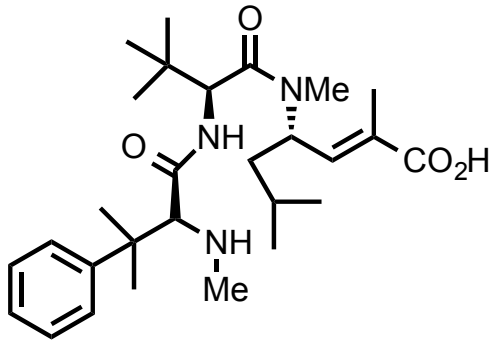


Pyrrolobenzodiazepine Dimer derivative

Free drugs	Karpas 299	L540cy	L428	786-0	Caki-1
SGD-1882 (PBD)	0.003	0.002	0.01	0.01	0.0004
MMAE	0.3	0.6	0.7	4	0.8



Tubulysin A

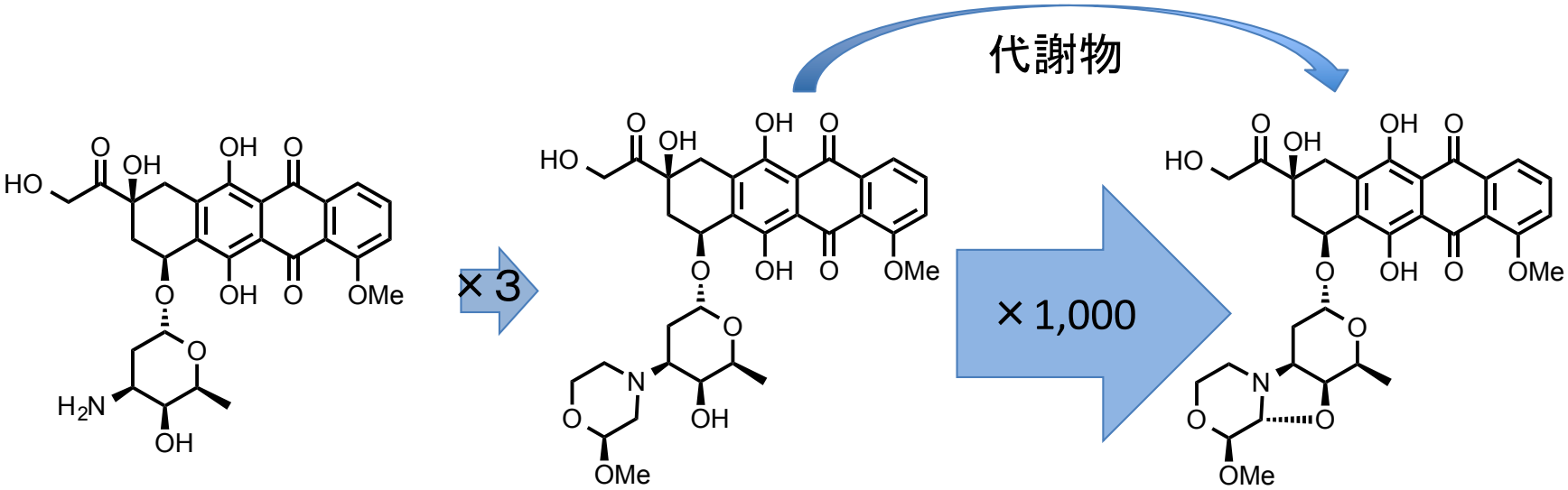


Taltobulin

IC_{50} = 2 nmol

Compound

Potent payload in ADC:1



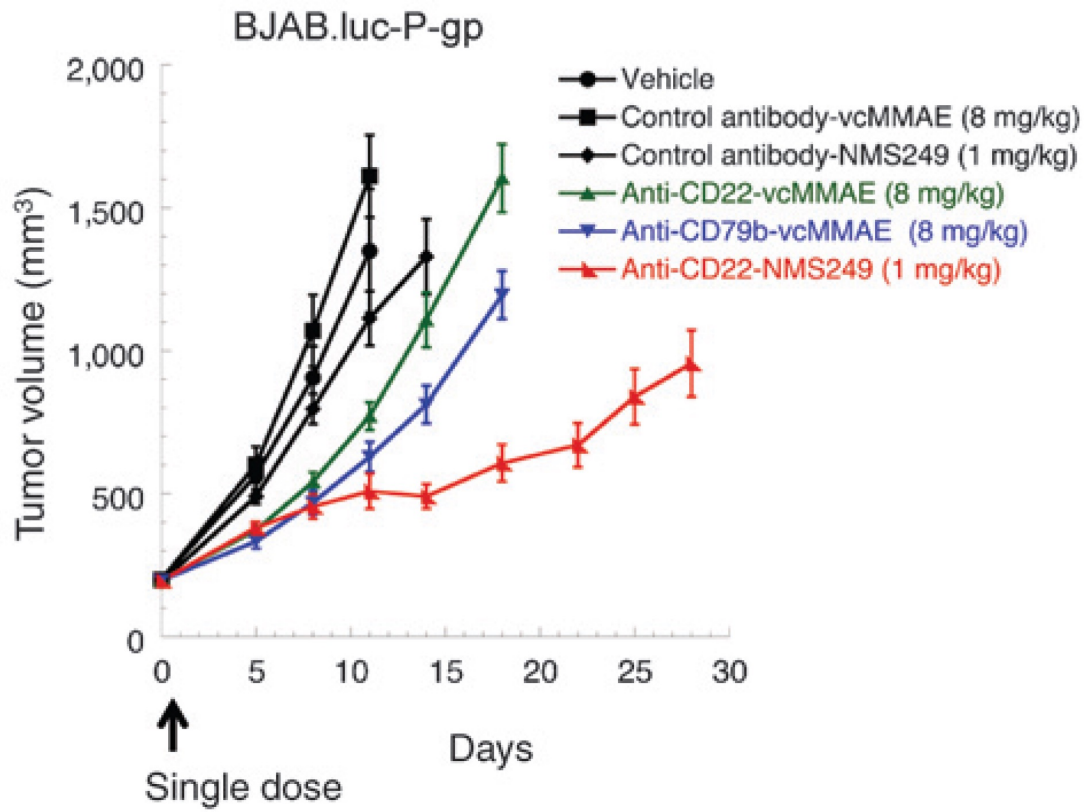
IC_{50} (nM)

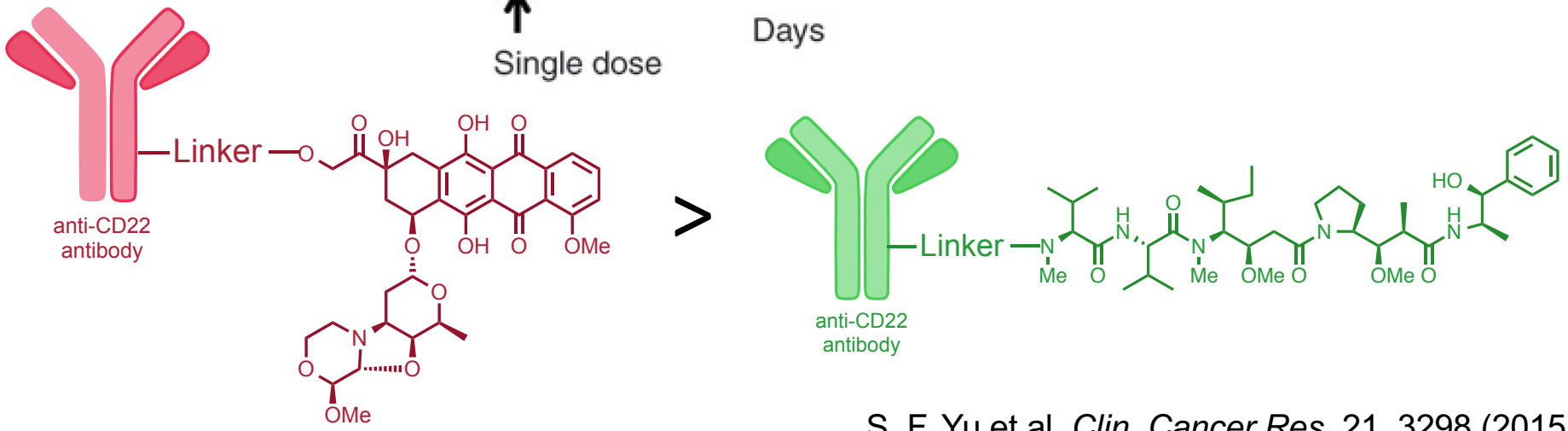
cell line	Doxorubicin	Nemorubicin	PNU-159682
HT-29 (colon)	1,279	578	0.577
A2780 (ovarian)	1,717	468	0.390
DU145 (prostate)	443	193	0.128
EM-2 (myeloblast)	521	191	0.081
Jurkat (T lymphocyte)	181	68	0.086
CEM (lymphoblast)	391	131	0.075

Until about three years ago, it was thought that it was sufficient to use strong drugs in vitro, but at present, there are many cases of patients dropping out due to strong side effects.
In some cases, the effects *in vitro* and in vivo are reversed.

Compound

Potent payload enhances ADC efficacy.





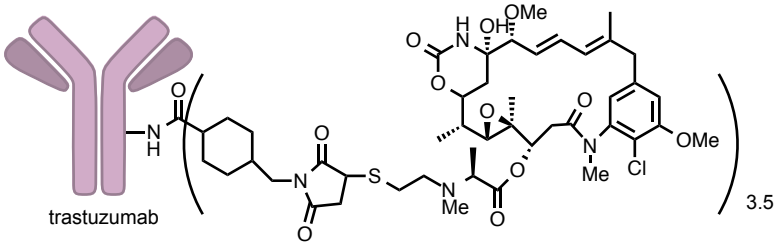
S. F. Yu et al. *Clin. Cancer Res*. 21, 3298 (2015)

Compound

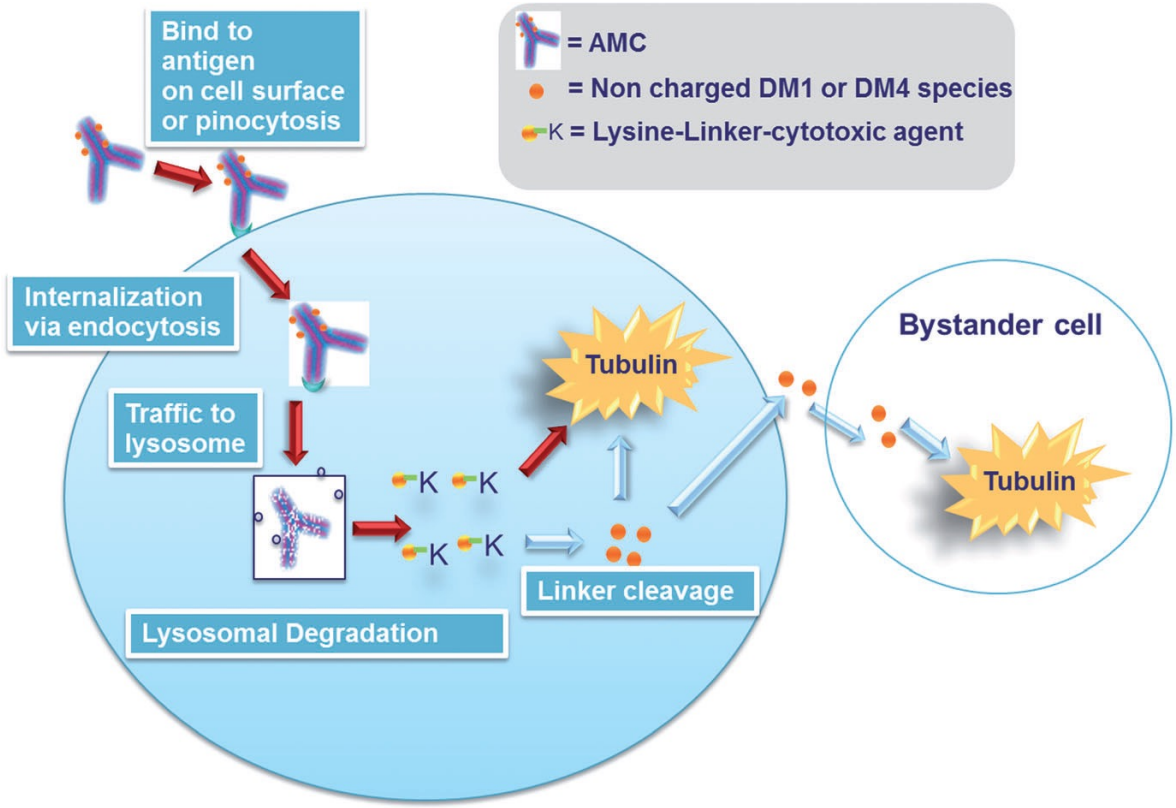
Release mechanism

Control of released drug
Without Bystander effect

trastuzumab emtansine



By adding an amino group to released drug, penetration through hydrophobic cell membranes is prevented.
The aim is to decrease adverse effect.



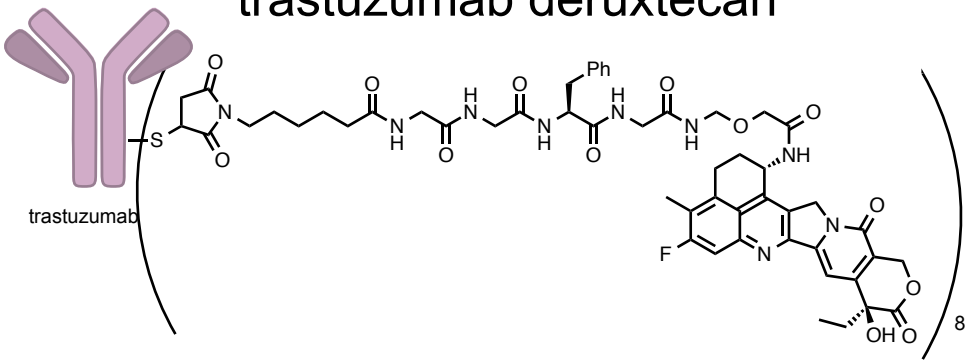
From R. V. J. Chari *et al. Angew. Chem. Int. Ed*. **53**, 3796 (2014)

Compound

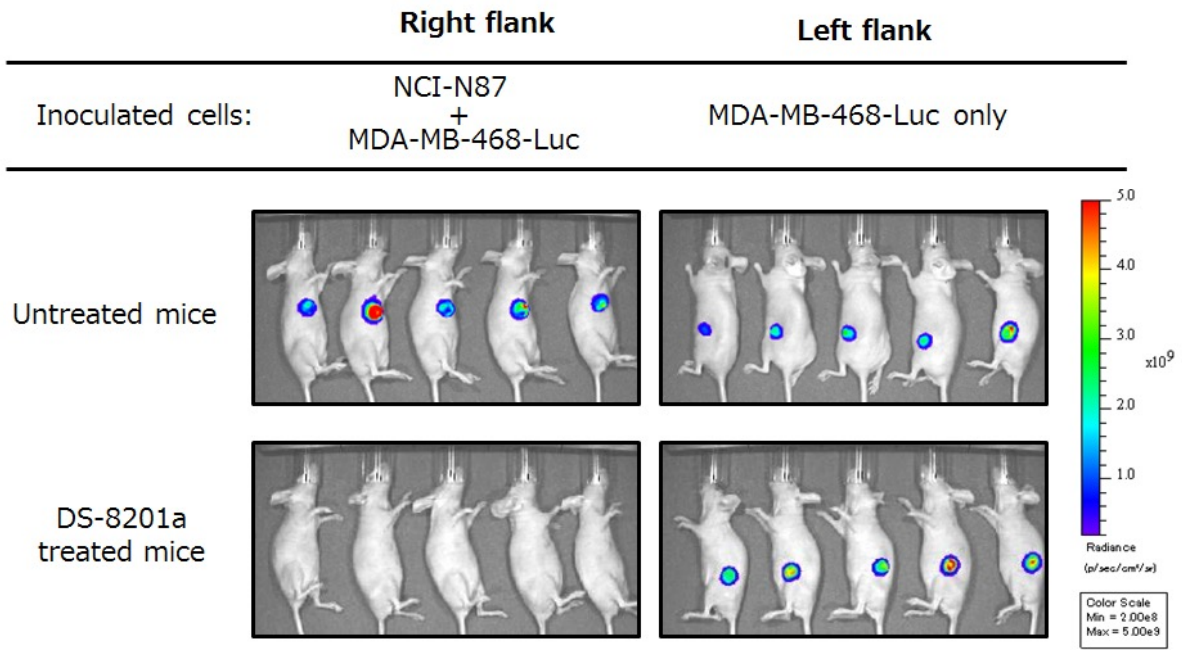
Release mechanism

Control of released drug With Bystander effect

trastuzumab deruxtecan



The drug released into the HER2-positive cells acts on the neighboring HER2-negative cells.
This strategy is effective to heterogeneous cancer microenvironment by bystander effect.



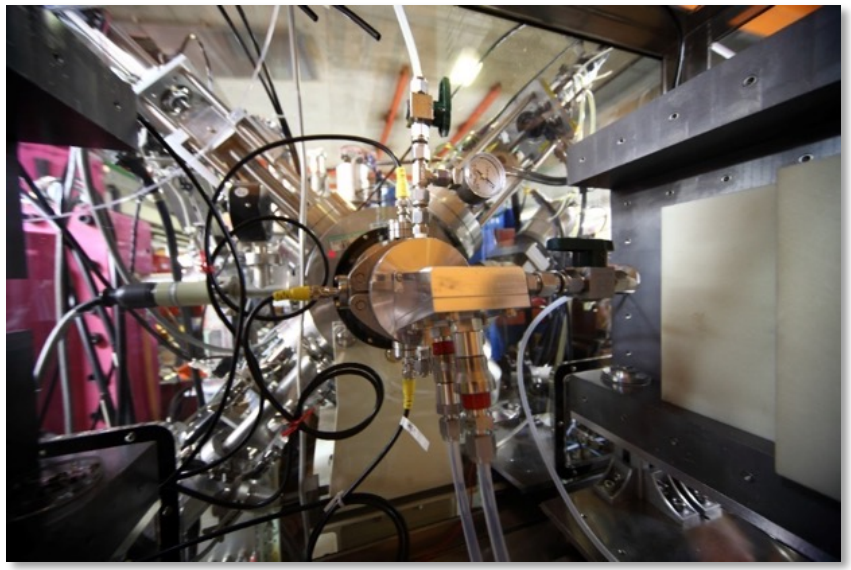
HER2 negative cells MDA-MB-468-Luc
HER2 positive cells NCI-N87

From T. Agatsuma, *Yakugaku Zasshi* **137**, 545 (2017)

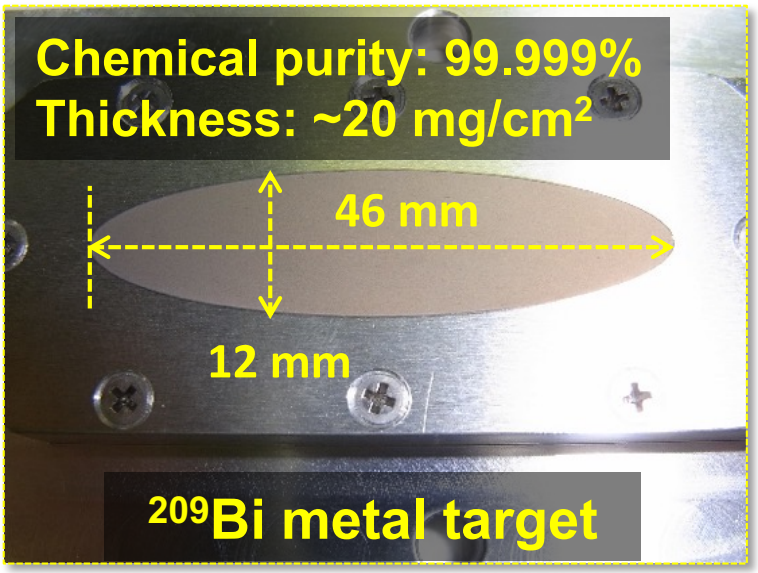
Compound

^{211}At production in RIKEN

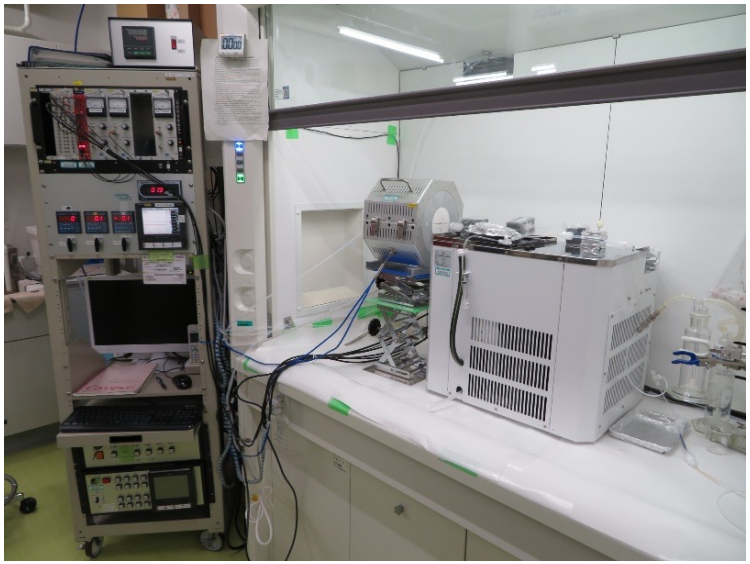
AVF cyclotron



^{211}At production apparatus



^{209}Bi target

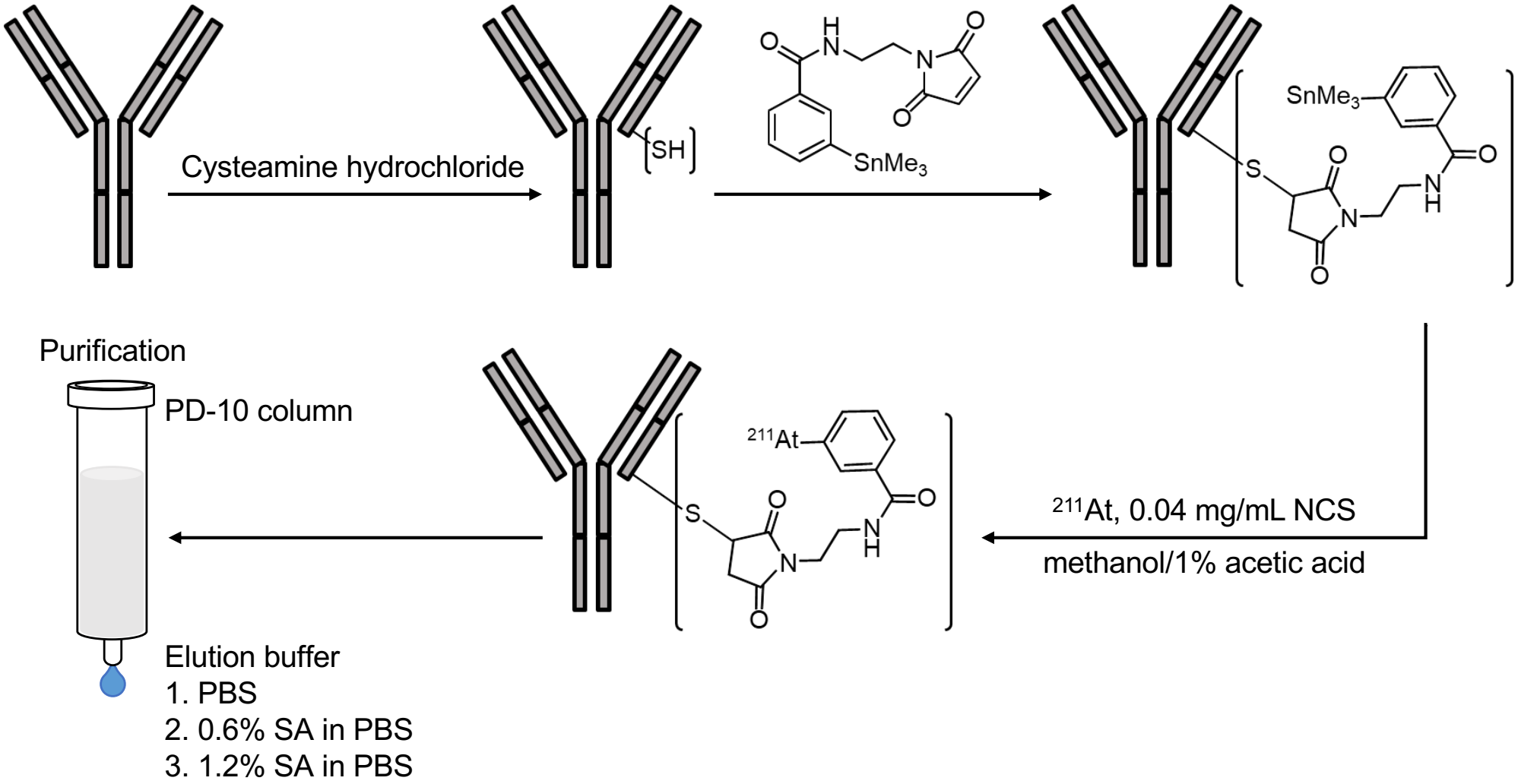


^{211}At purification apparatus

Compound

Our data

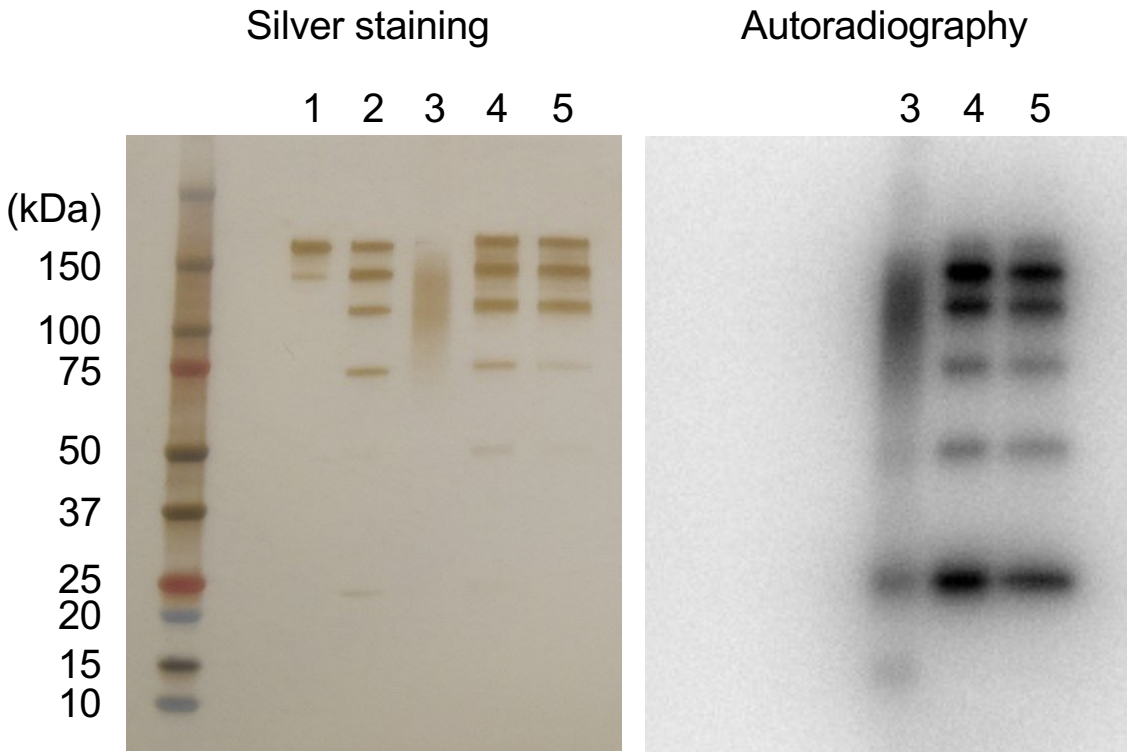
^{211}At conjugation methodology



Compound

Our data

Sodium ascorbate protects ^{211}At-labeled IgG



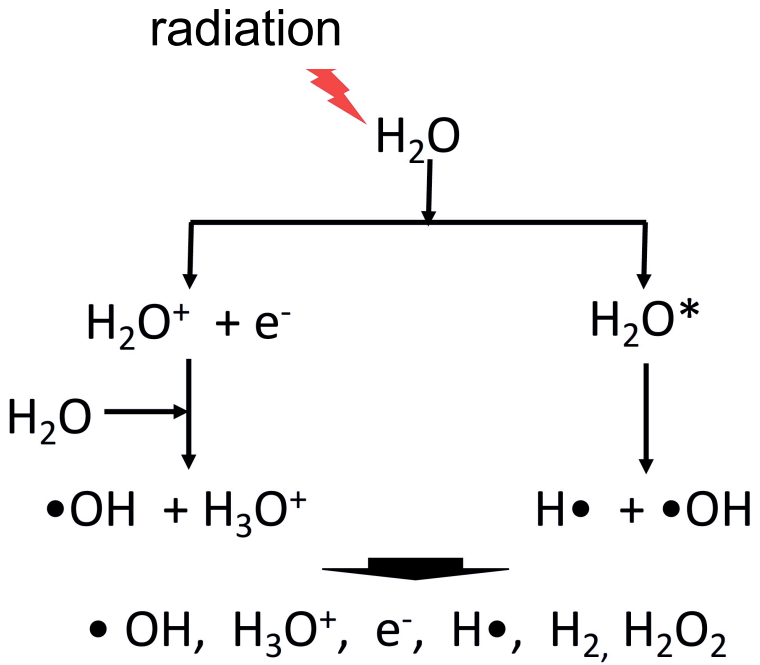
1: anti-TF mAb
2: Sn-anti-TF mAb
3: ^{211}At-anti-TF mAb (SA −)
4: ^{211}At-anti-TF mAb (0.6% SA)
5: ^{211}At-anti-TF mAb (1.2% SA)

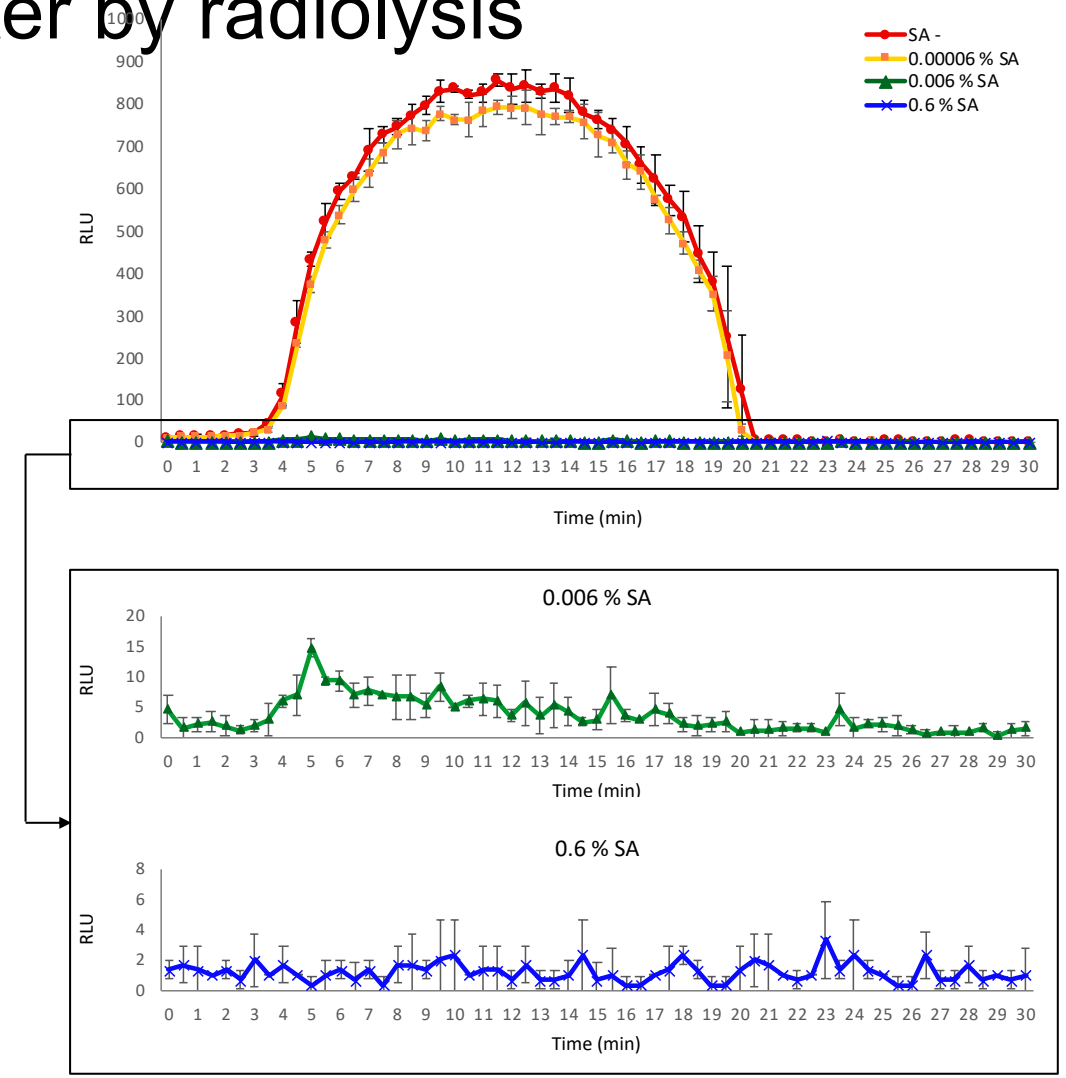
S. Manabe *et al. ACS Omega,* **6**, 14887 (2021)

Compound

Our data

Generation and quenching of reactive oxygen species from water by radiolysis



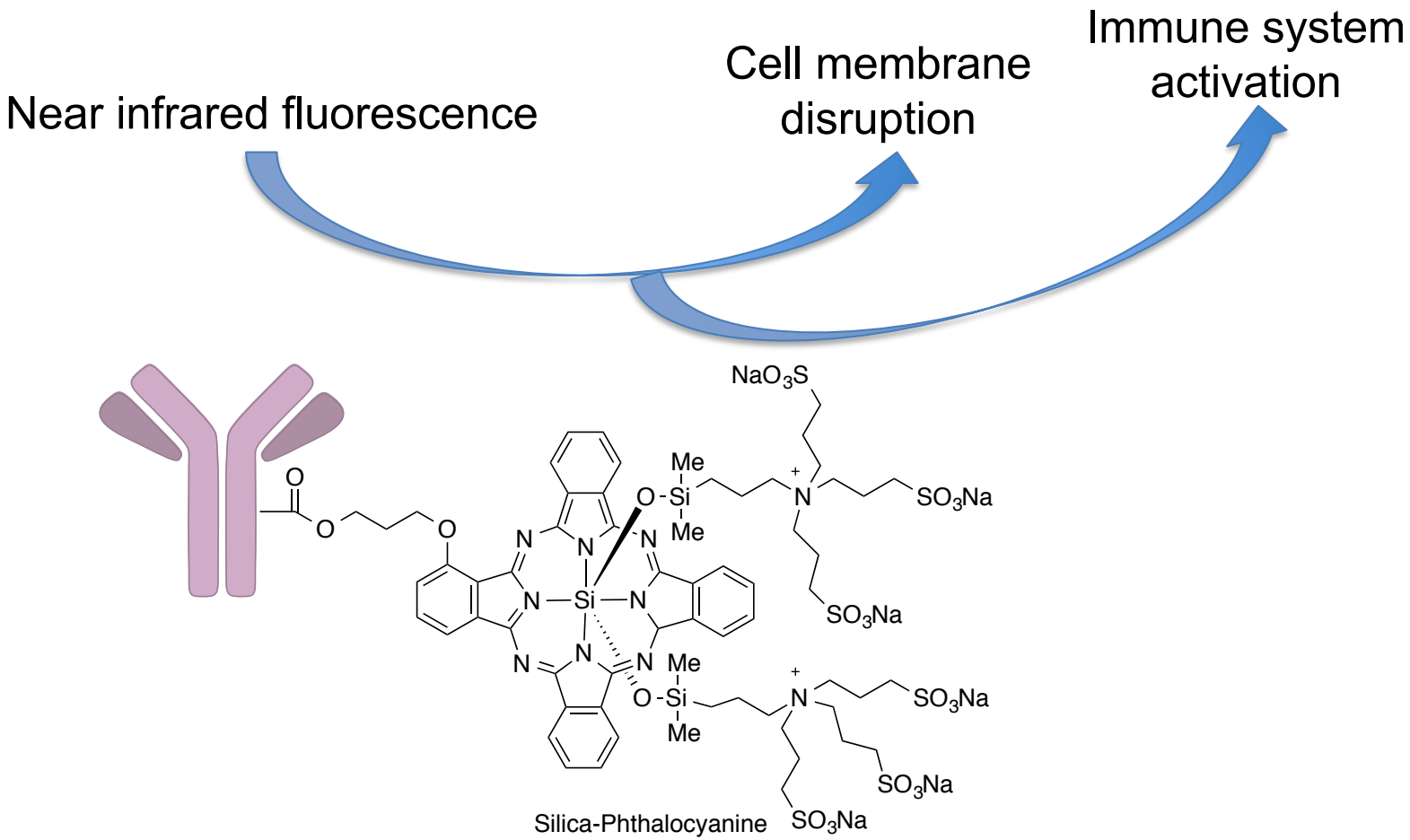


- The radioactive decomposition of water produces reactive oxygen species.
- Reactive oxygen species can be detected by the luminol assay.
- The reactive oxygen species are quenched by the addition of sodium ascorbate.

Compound

Photoimmunotherapy

Cetuximab sarotalocan sodium



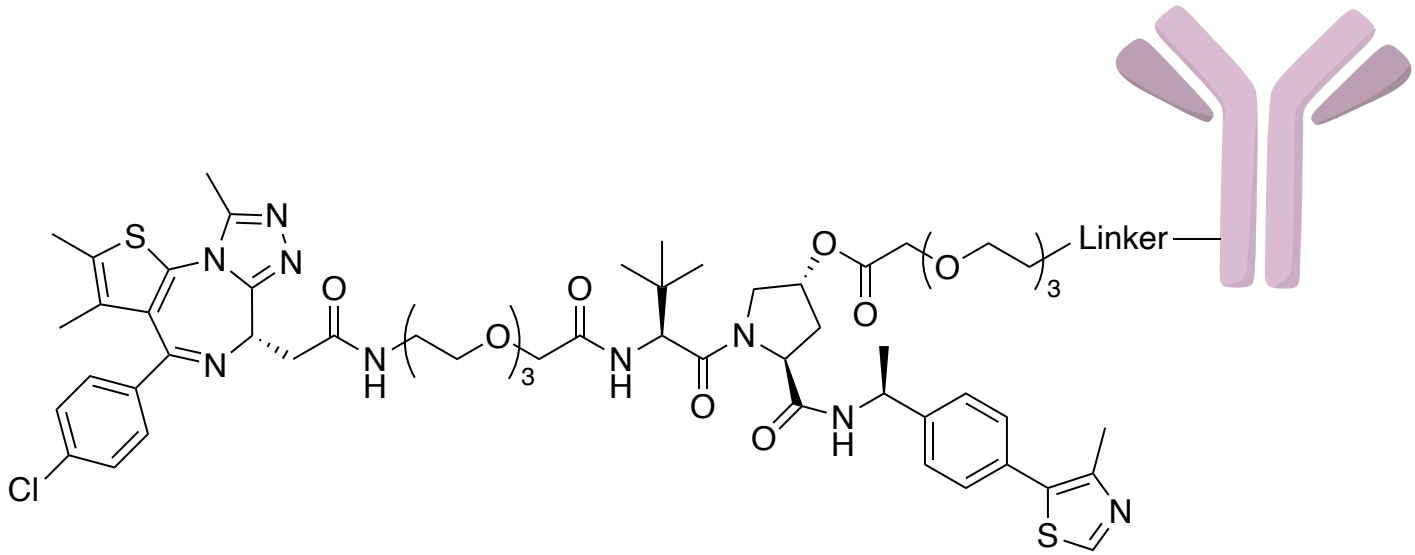
Insoluble compounds can be used for ADC.

It is not only the destruction of cells by reactive oxygen species caused by light.
It activates the immune system. → It also shrinks tumors in distant sites.

M. Itsunaga *et al. Nat. Med*. **17**, 1685 (2011)

Compound

PROTAC（Proteolysis targeting chimera）on ADC

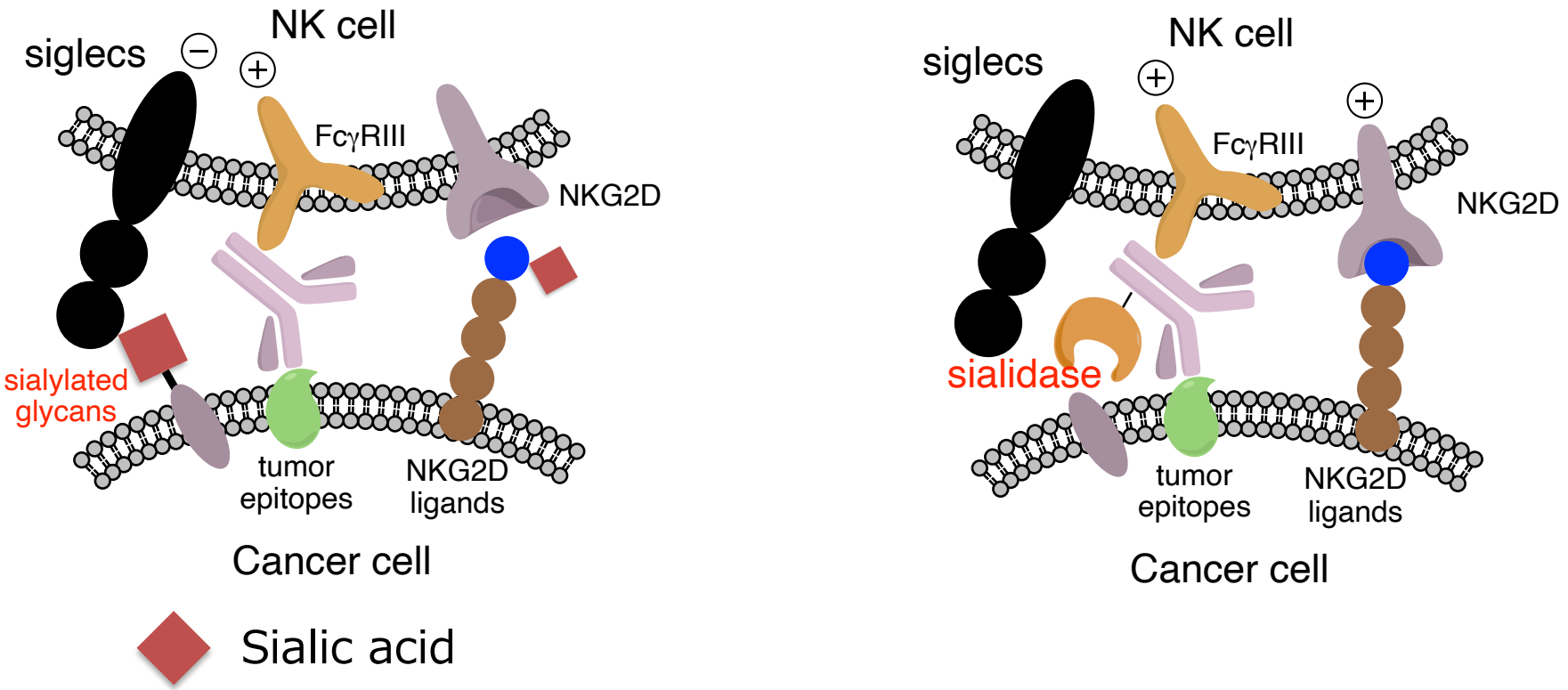


M. Maneiro *et al. ACS Chem. Biol.* **15**, 1306 (2020)

Certain undesirable proteins can be removed by PRPTAC compound.
PRPTAC can also target proteins other than conventional enzymes and receptors.

Compound

Enzyme can be immobilized to antibody.



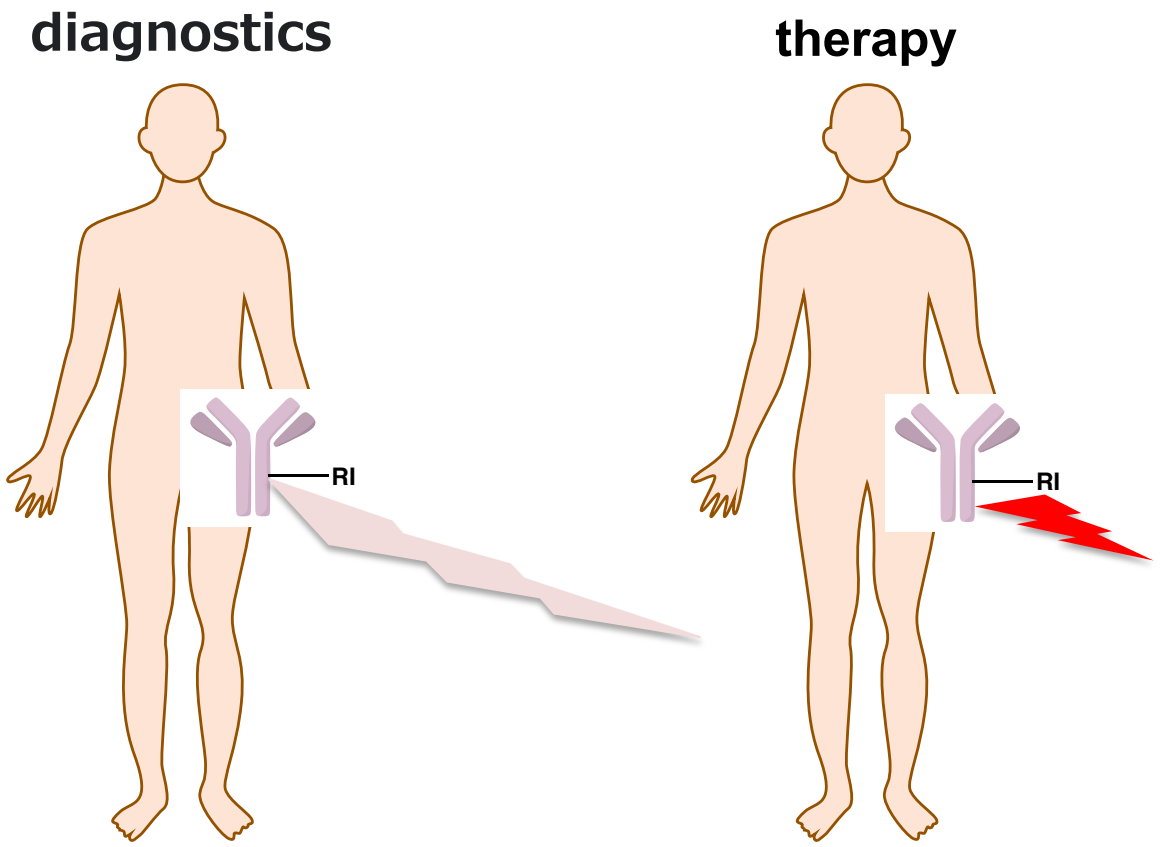
Sialic acid on the cell surface is associated with cancer.
Addition of sialidase to antibodies removes sialic acid on the cell surface, inhibits the inactivation of NK cells by siglec, and promotes the activation of NK cells by NKG2D.

C. R. Bertozzi *et al. PNAS,* **113**, 10304 (2016)

Compound

Theranostics

Therapy and diagnostics at once!



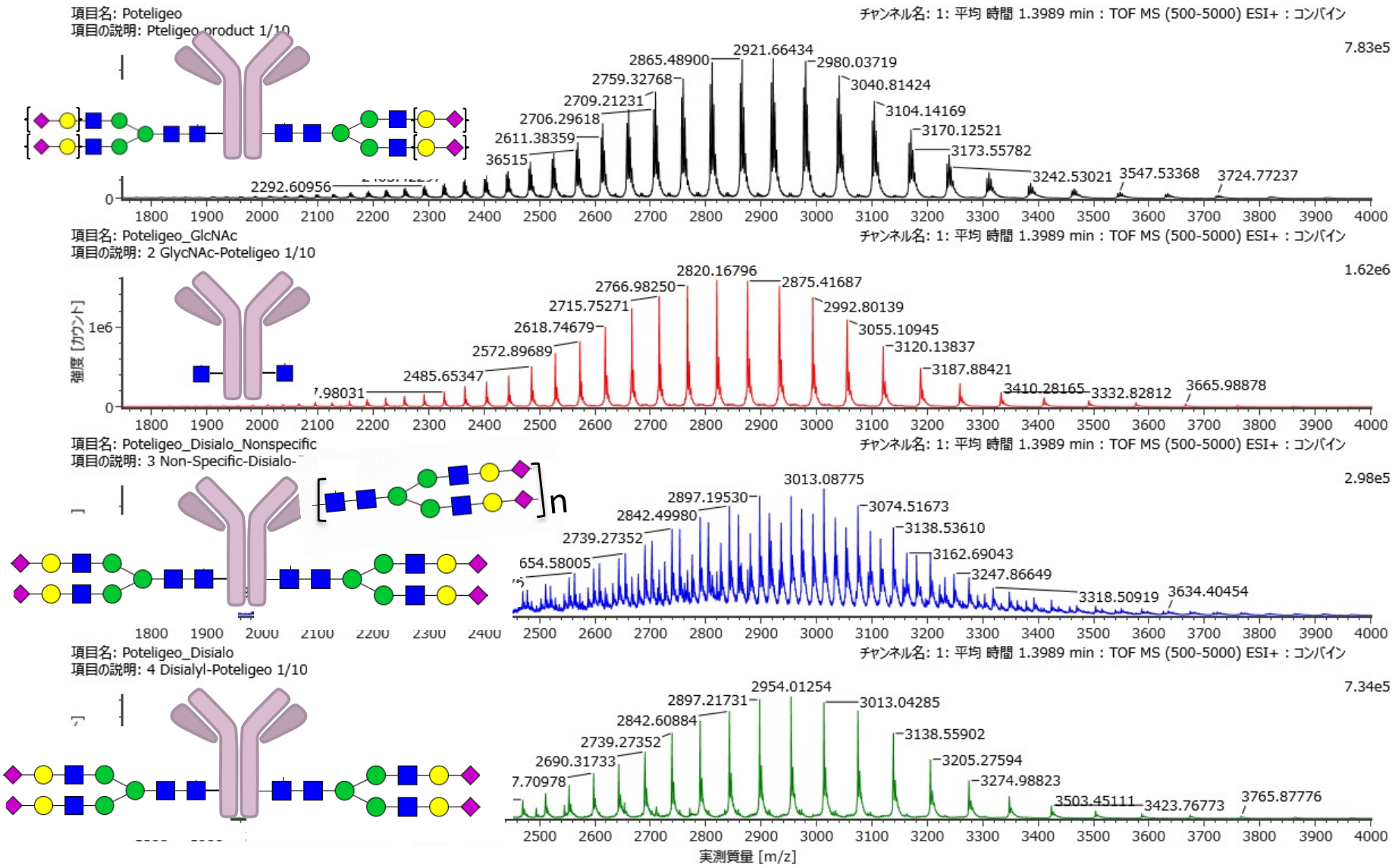
Longer wavelength,
but low energy RI
γ ray

Shorter wavelength,
but high energy RI
α particle

Characterization

Our data

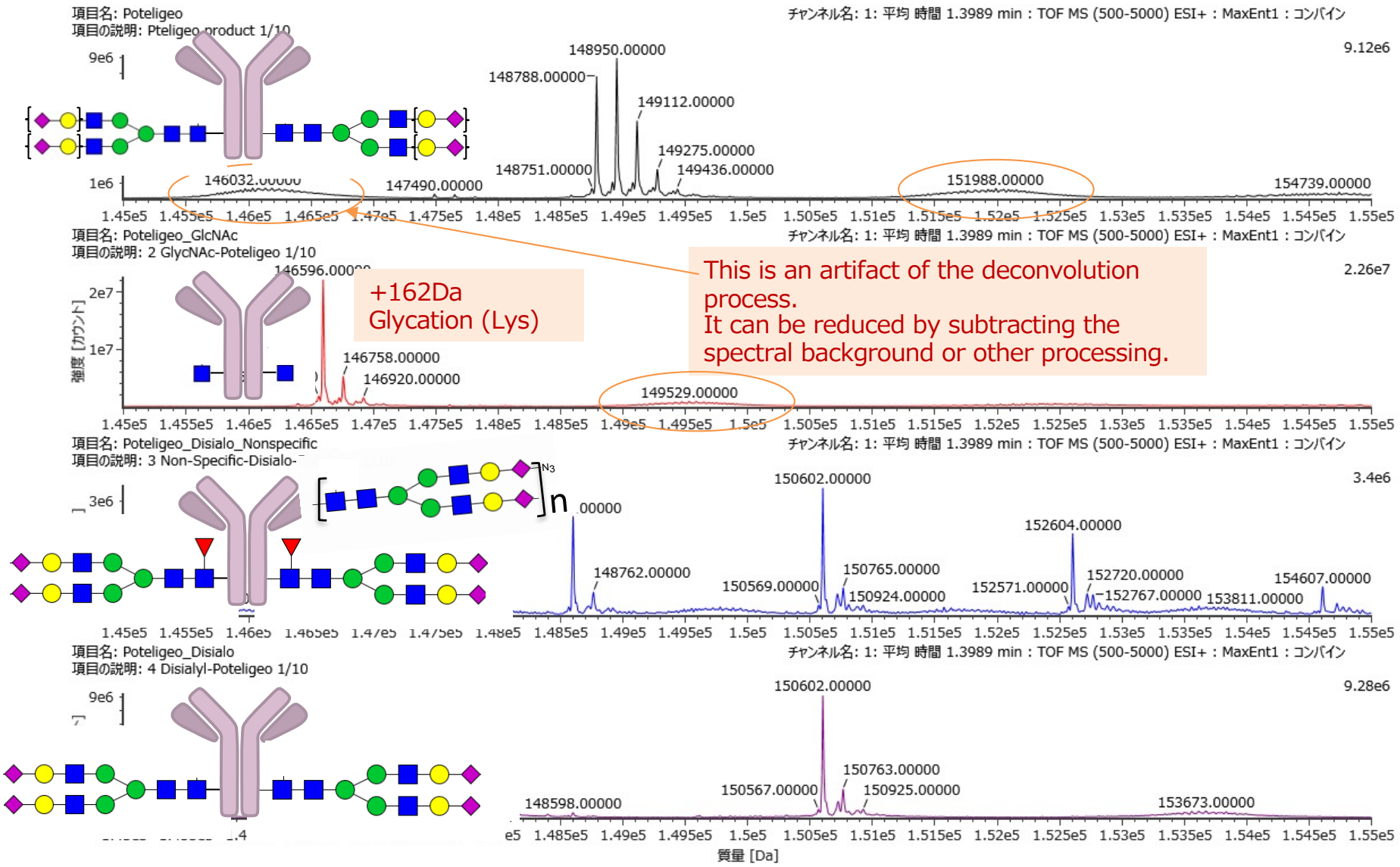
Characterization of ADC
ESI MS Spectra（raw data）



S. Manabe *et al. Bioconj. Chem.* **30**, 1343 (2019)

Our data

Characterization of ADC
ESI MS Spectra（after decomvolution）



S. Manabe *et al. Bioconj. Chem.* **30**, 1343 (2019)

Purification and analysis modes by antibody, protein, and ADC

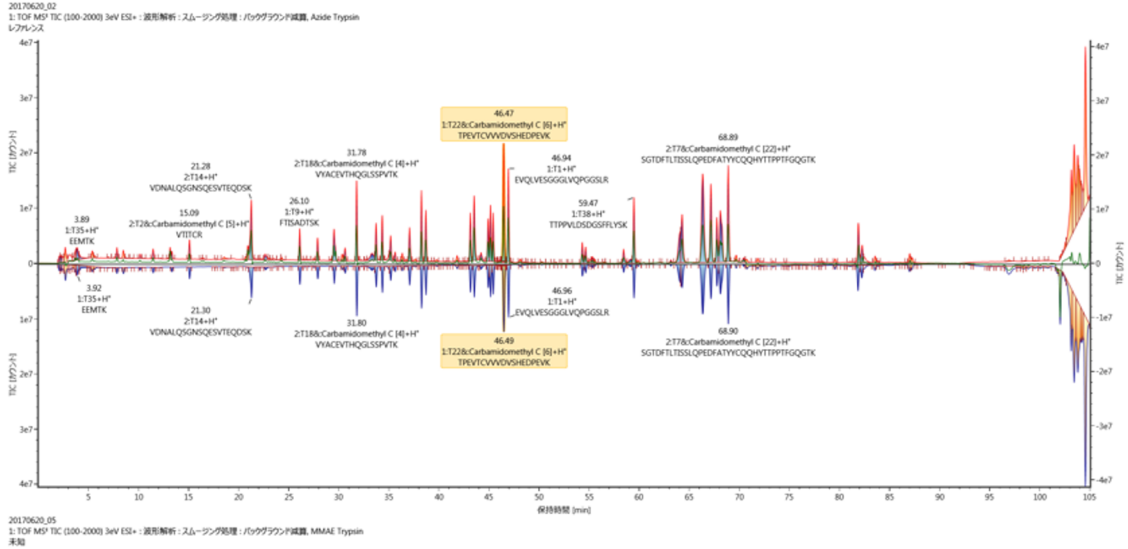
Reverse phase	Pretreatment of enzymatically fragmented antibodies for MS and peptide mapping C4 type is recommended than C18
HIC	Hydrophobic Interaction Chromatography A separation mode based on the principle that the hydrophobic portion of an analyte, such as a protein, is adsorbed and retained by the hydrophobic groups on the packing material surface in a high salt concentration eluent. It is used for the separation of ADCs conjugated with hydrophobic payload.
HILIC	Hydrophilic interaction column chromatography It is suitable for highly polar compounds such as sugar chains.
Ion-exchange	Cationic exchange type and anionic exchange type Glu/Asp/Tyr　Lys/His
Gel filtration	Molecular size
Affinity	For instance, Protein A column chromatography is used for antibody purification (protein A binds to Fc region.).

Characterization

Proof of ADC homogeneity

Our data

Total Ion Chromatogram



Coverage map

S. Manabe *et al. Bioconj. Chem.* **30**, 1343 (2019)

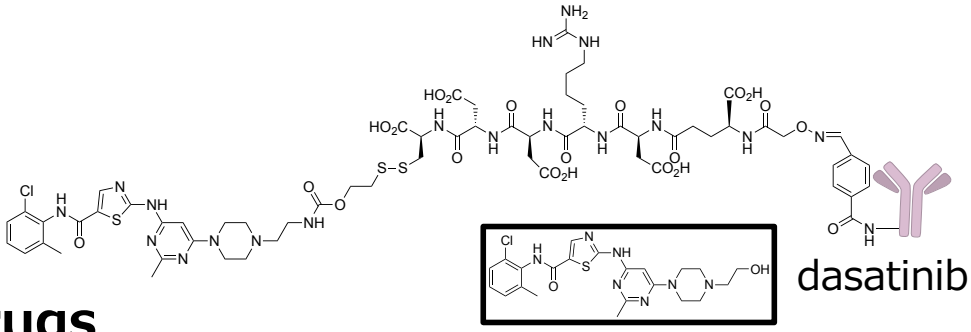
Thanks to Nihon Waters!

ADCs for various diseases

Immunosuppression

- anti-TNFα antibody+ novel steroid
- anti-CXCR4 antibody+dasatinib（Tyr kinase inhibitor)

R. E. Wang *et al. J. Am. Chem. Soc*. **137**, 3229 (2015)

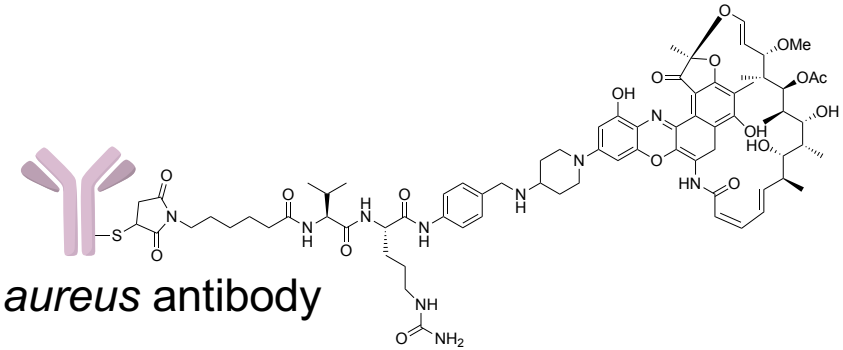


anti-CXCR4 antibody

dasatinib

Antibacterial drugs

methicillin-resistant *staphylococcus aureus* S. M. Lehar *et al. Nature*, **527**, 323 (2015)

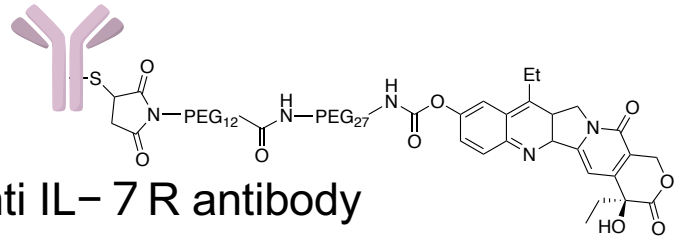


Rifalogue
(antibiotic)

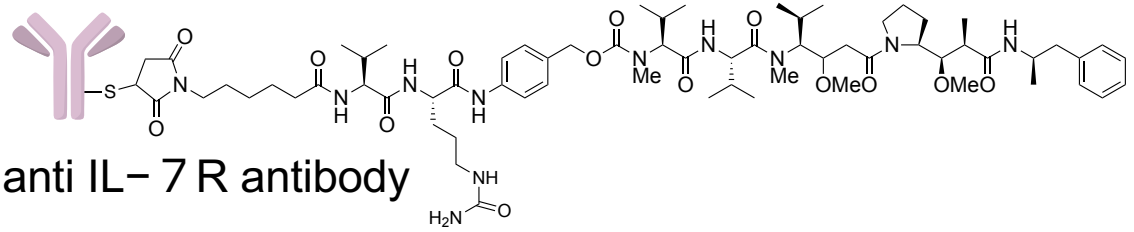
anti-*S. aureus* antibody

Immunosuppression Type II Diabetes

M. Yasunaga et *al. Sci. Rep.* **7**, 10735 (2017)



anti IL−7R antibody



anti IL−7R antibody

ADC and Cancer Immunity

Immunogenic cell death by released payload

A group of molecules called DAMPS (damage-associated molecular patterns), such as HMGB1 (High-Mobility Group Box 1), calreticulin, and ATP, released from cancer cells injured by MMAE or exatecan, can cause secondary cancer cell injury by activating T cells via dendritic cells and macrophages.

Synergistic effects with immune checkpoint inhibitors

T. Iwata *et al. Mol. Cancer Ther.* **17**, 1494 (2018)
T. Iwata *et al. PLoS One* **14**, e0222280 (2019)

PEG in ADC

- Increase the number of drugs per antibody by increasing the PEG chain length.

  Increasing the number of drugs per antibody results in faster metabolism of the ADC in the liver due to the hydrophobicity of the drug. Therefore, a number of 4 or less drugs per antibody is generally considered appropriate. However, it is possible to increase the number of drugs per antibody by increasing the PEG chain length.

  R. P. Lyon *et al*. *Nature Biotech*. **33***,* 733 (2015)

- Branched PEG can increase the number of drugs per antibody.

  M. Yasunaga *et al*. *Cancer Sci*. **102***,* 1396 (2011)

- Hydrophilic PEG prevents ADC from aggregation.

  T. Nakata *et al. Bioorg. Med. Chem. Lett*. **15,** 1542 (2016)

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- S. Manabe, “Cancer Drug Delivery Systems based on the Tumor Microenvironment”, Yasuhiro Matsumura and David Tarin eds. Springer Japan, 93–123 (2020)
- S. J. Walsh *et al. Chem. Soc. Rev.* **50**, 1305 (2021)
- J. Z. Drago *et al*. *Nat. Rev. Clinical Oncol.* **18***,* 327-344 (2021)
- Y. Jin *et al*. *Pharmacol. Ther.,* **229**, 107917 (2022)