Antibody-drug conjugates

Literature Talk

Oct 8, 2024

Esther Kang

MacMillan Group

Annual Conference in 2022

ASCO® AMERICAN SOCIETY OF CLINICAL ONCOLOGY

Annual Conference in 2022

The plenary session at the ASCO 2022 annual meeting saw that rarest of things at a scientific conference: a standing ovation.
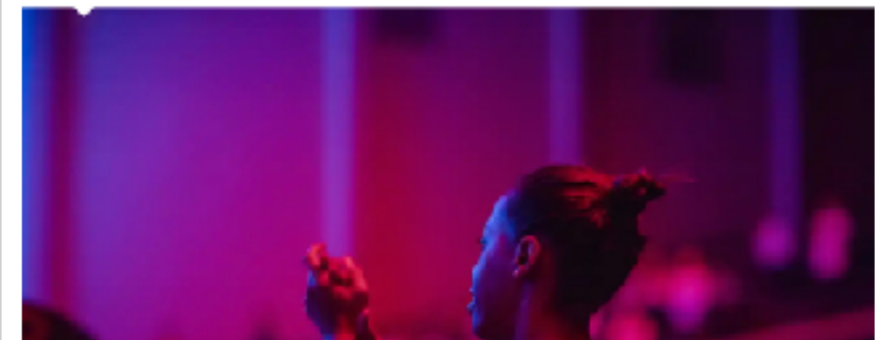
The New York Times

Breast Cancer Drug Trial Results in 'Unheard-Of' Survival

For some patients with metastatic tumors not significantly affected by other forms of chemotherapy, the treatment halted their cancer's growth.

A Standing Ovation! Results From DESTINY-Breast04 trial in Breast Cancer

ASCO® AMERICAN SOCIETY OF CLINICAL ONCOLOGY
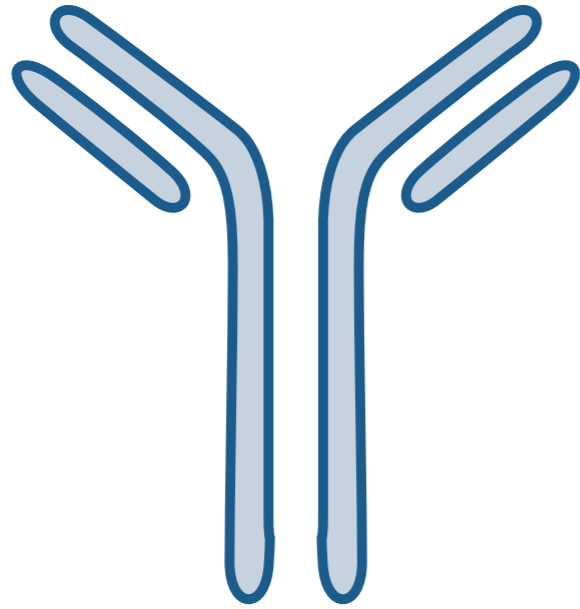
Annual Conference in 2022

Breast cancer trial plenary session

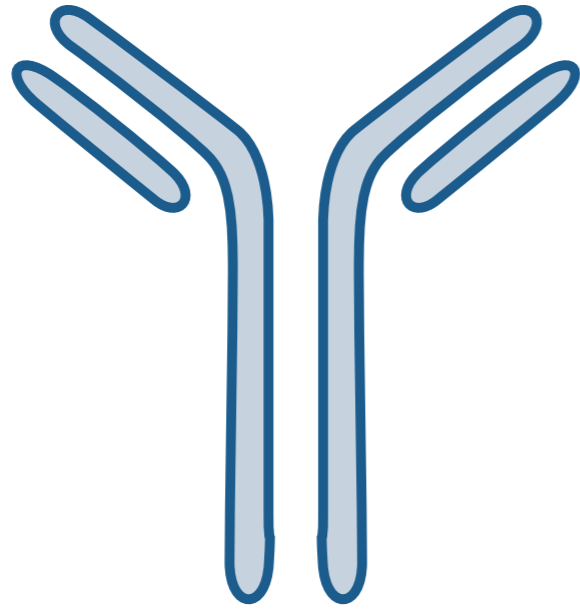*Antibody-drug conjugate (ADC)*

What is antibody-drug conjugate?

What is antibody-drug conjugate?

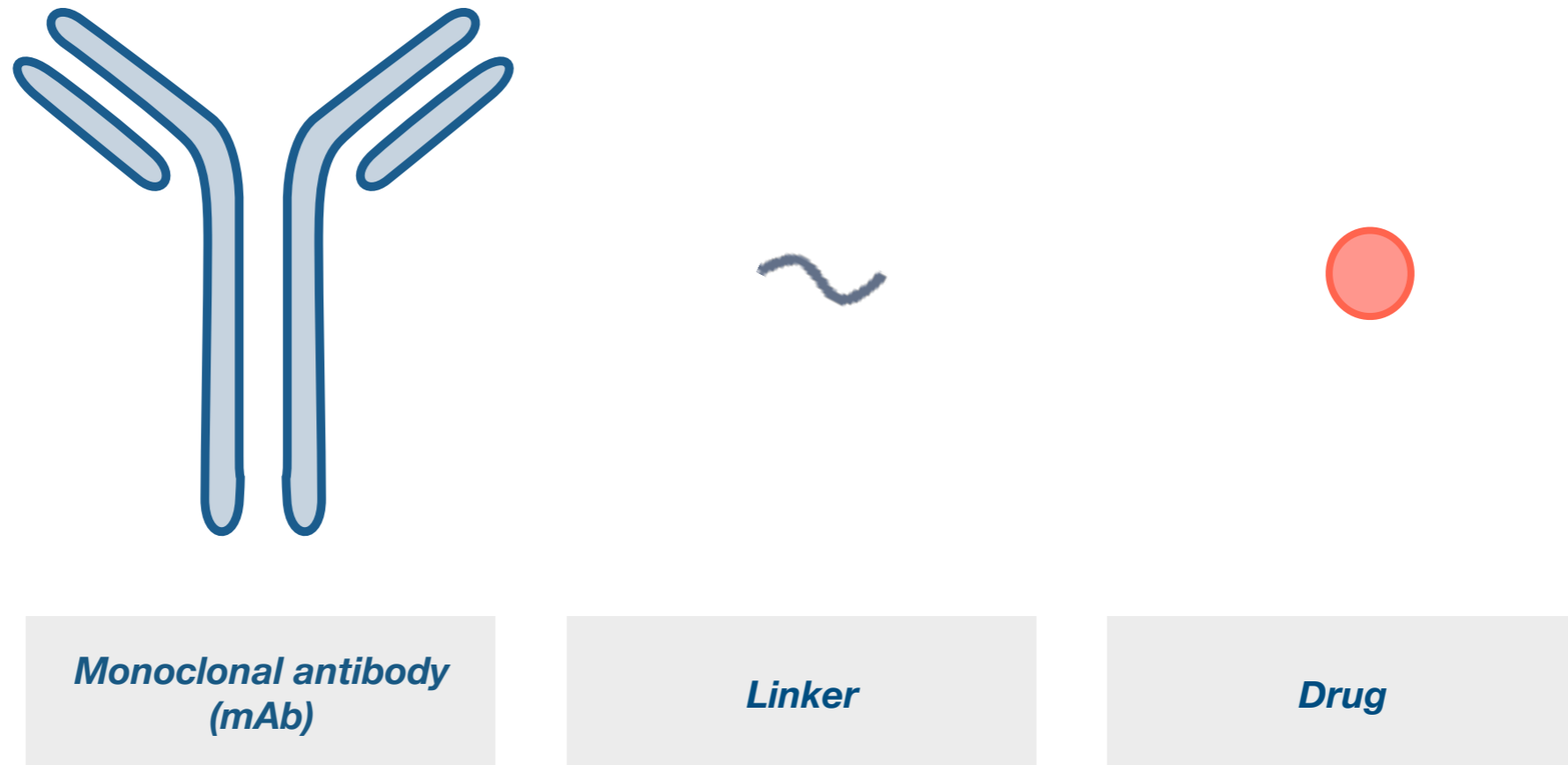**Monoclonal antibody (mAb)**

What is antibody-drug conjugate?
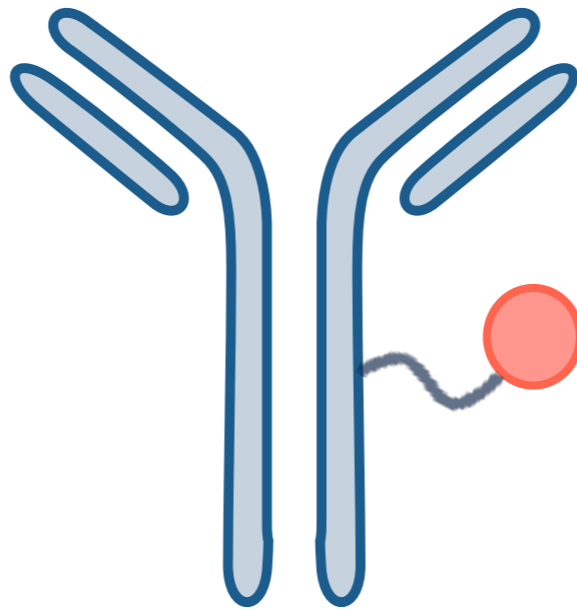
Monoclonal antibody (mAb)

Drug

What is antibody-drug conjugate?

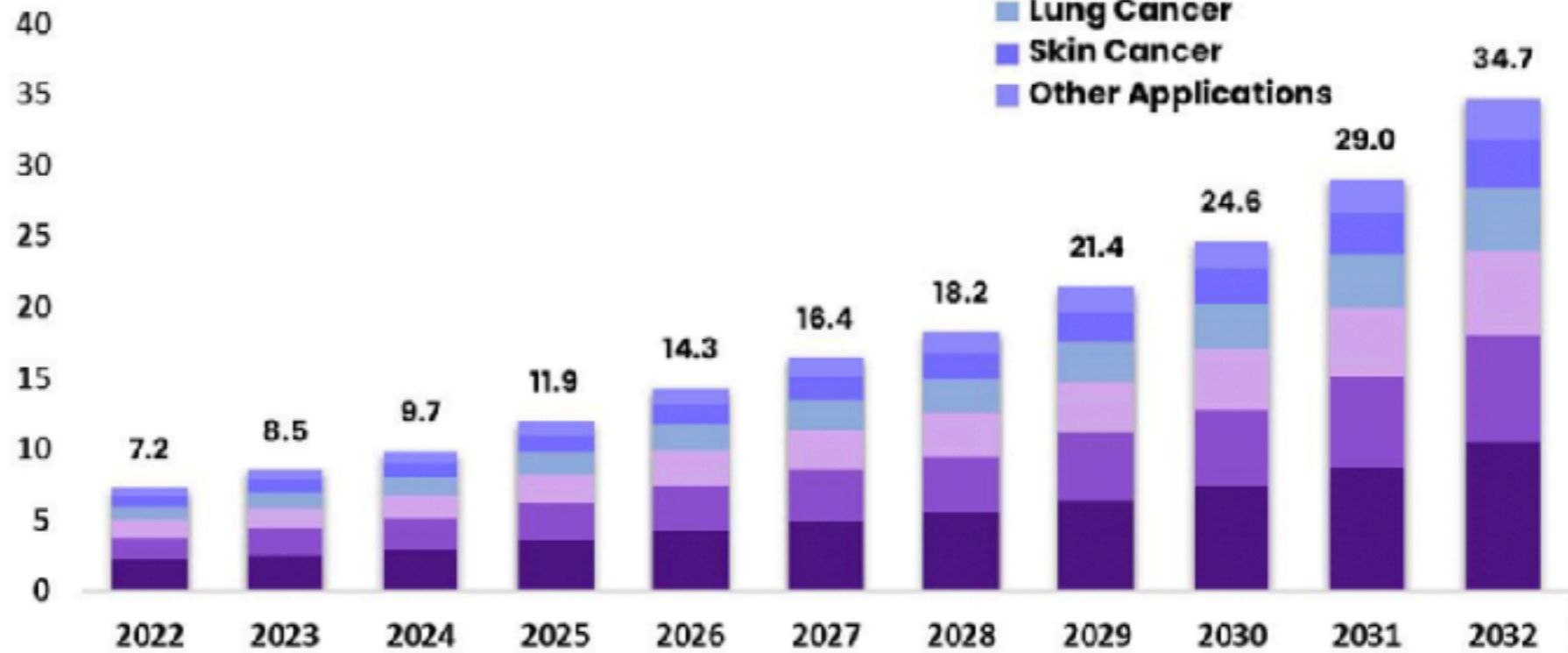| Monoclonal antibody (mAb) | Linker | Drug |

What is antibody-drug conjugate?

3 main components

- Antibody

- Linker

- Drug

Global Antibody Drug Conjugates Market

Size, by Application, 2022-2032 (USD Billion)

Legend:
- Breast Cancer
- Blood Cancer
- Ovary Cancer
- Lung Cancer
- Skin Cancer
- Other Applications

Bar values: 2022: 7.2, 2023: 8.5, 2024: 9.7, 2025: 11.9, 2026: 14.3, 2027: 16.4, 2028: 18.2, 2029: 21.4, 2030: 24.6, 2031: 29.0, 2032: 34.7

Enhertu™

2023 net revenues

$2.57 bil

(Breast cancer)

Kadcyla™

2023 net revenues

$2.22 bil

(Breast cancer)

Market.us
Annual reports from Daiichi Sankyo and Genentech

Outline

- **Introduction**

    - **Structural features of ADC**
    - **Mechanism of Action**
    - **FDA-approved ADCs**

- **'A' in ADC**

    - **Antibody alone as a drug**
    - **Characteristics of antibody drugs**
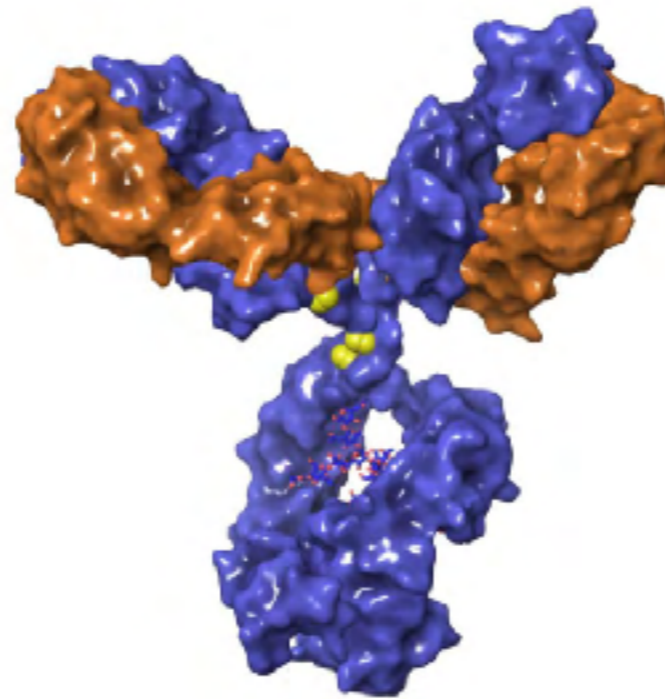   - **'A' versus 'ADC'**

- **'C' in ADC**

    - **Bioconjugation**
    - **Cleavable or non-cleavable**

- **'D' in ADC**

    - **Types of 'D'**
    - **Bystander effect**
    - **Other types of 'D'**

Antibody-drug conjugate
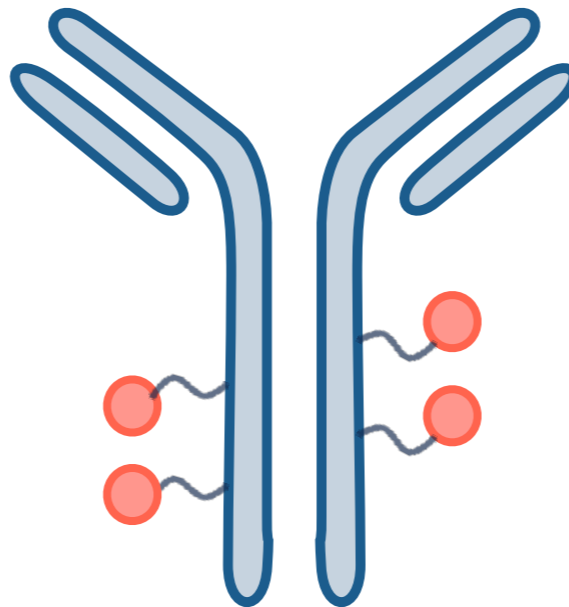## Structural feature

Antibody   :   Linker-Drug

150,000 Da   :   1,000 Da

Antibody-drug conjugate

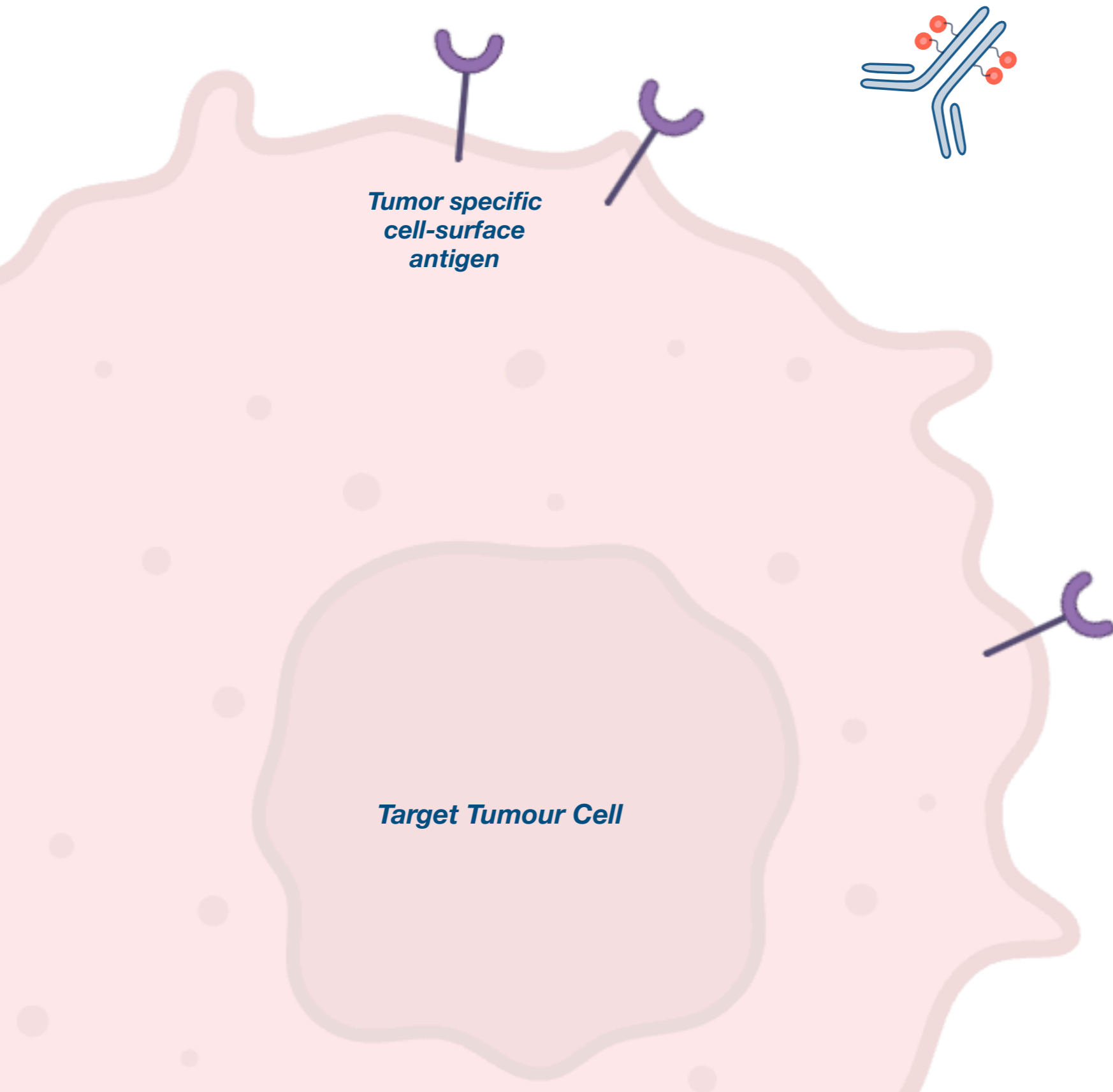## Structural feature: Drug-to-Antibody Ratio (DAR)

Drug-to-Antibody Ratio (DAR)

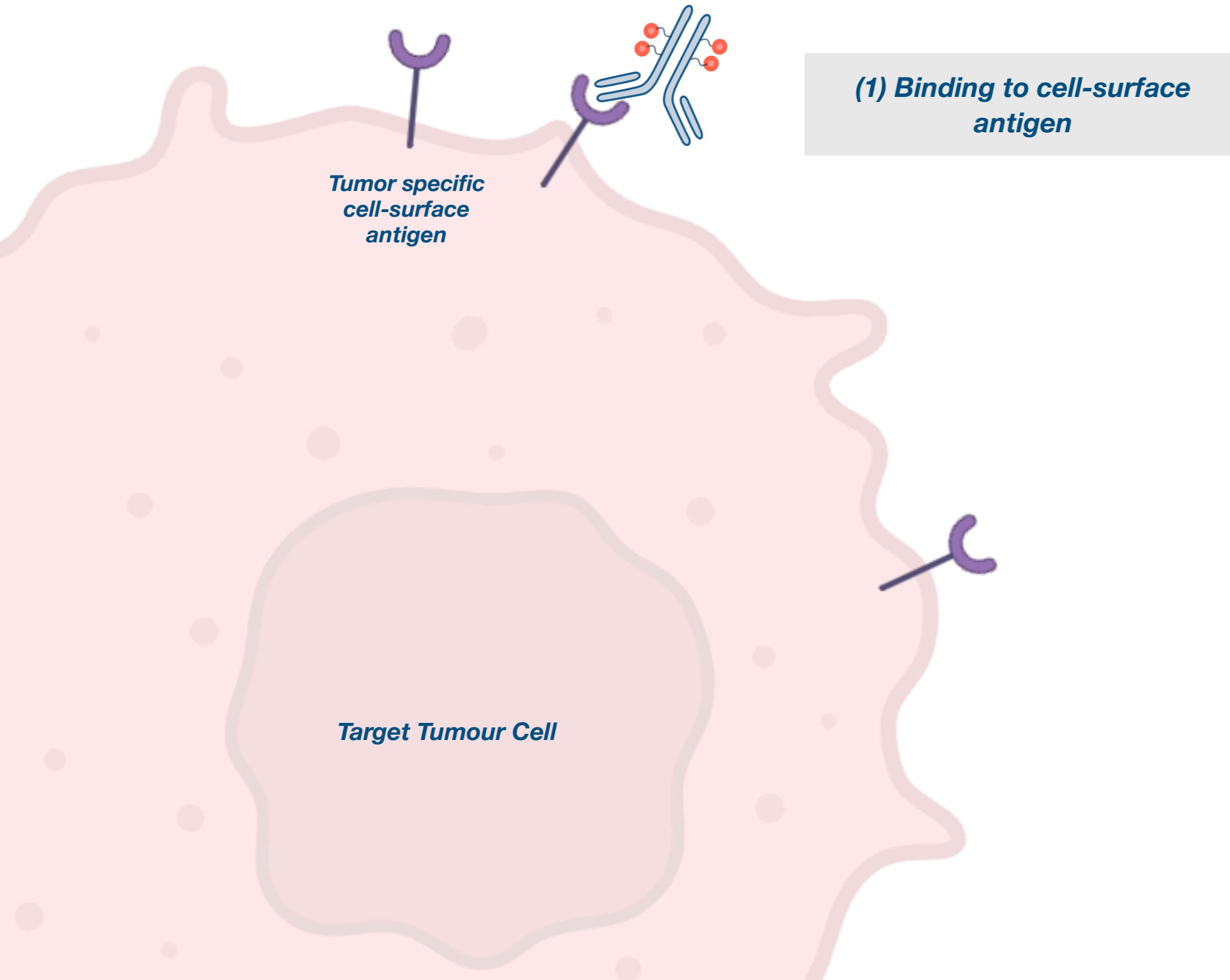*Average 3.5 - 4*
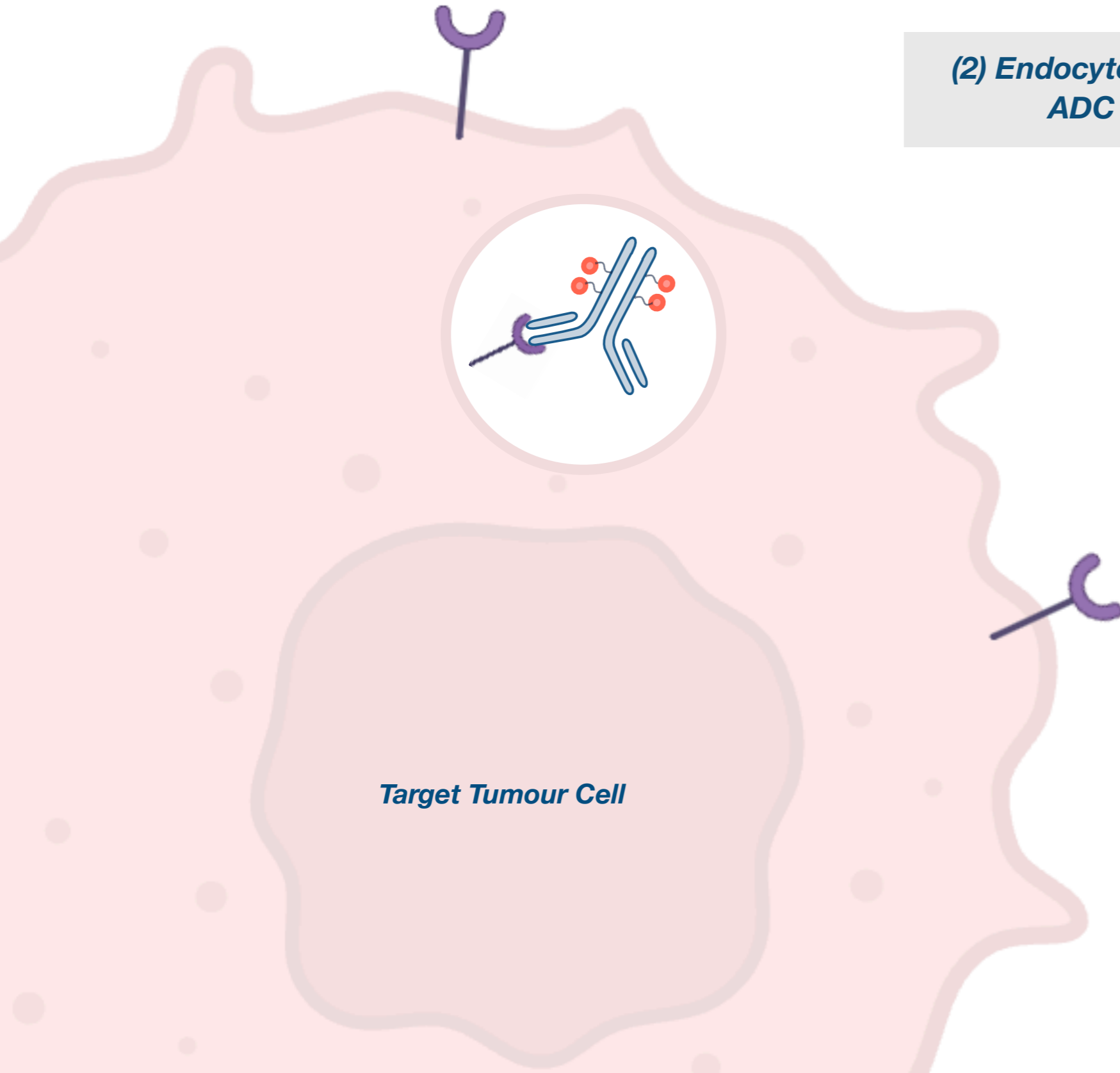
Antibody-drug conjugate

Mechanism of Action

Tumor specific cell-surface antigen

Target Tumour Cell

Antibody-drug conjugate

Mechanism of Action

(1) Binding to cell-surface antigen

Tumor specific cell-surface antigen

Target Tumour Cell

Antibody-drug conjugate
Mechanism of Action

(2) Endocytosis of antigen-ADC complex

Target Tumour Cell

Antibody-drug conjugate

Mechanism of Action
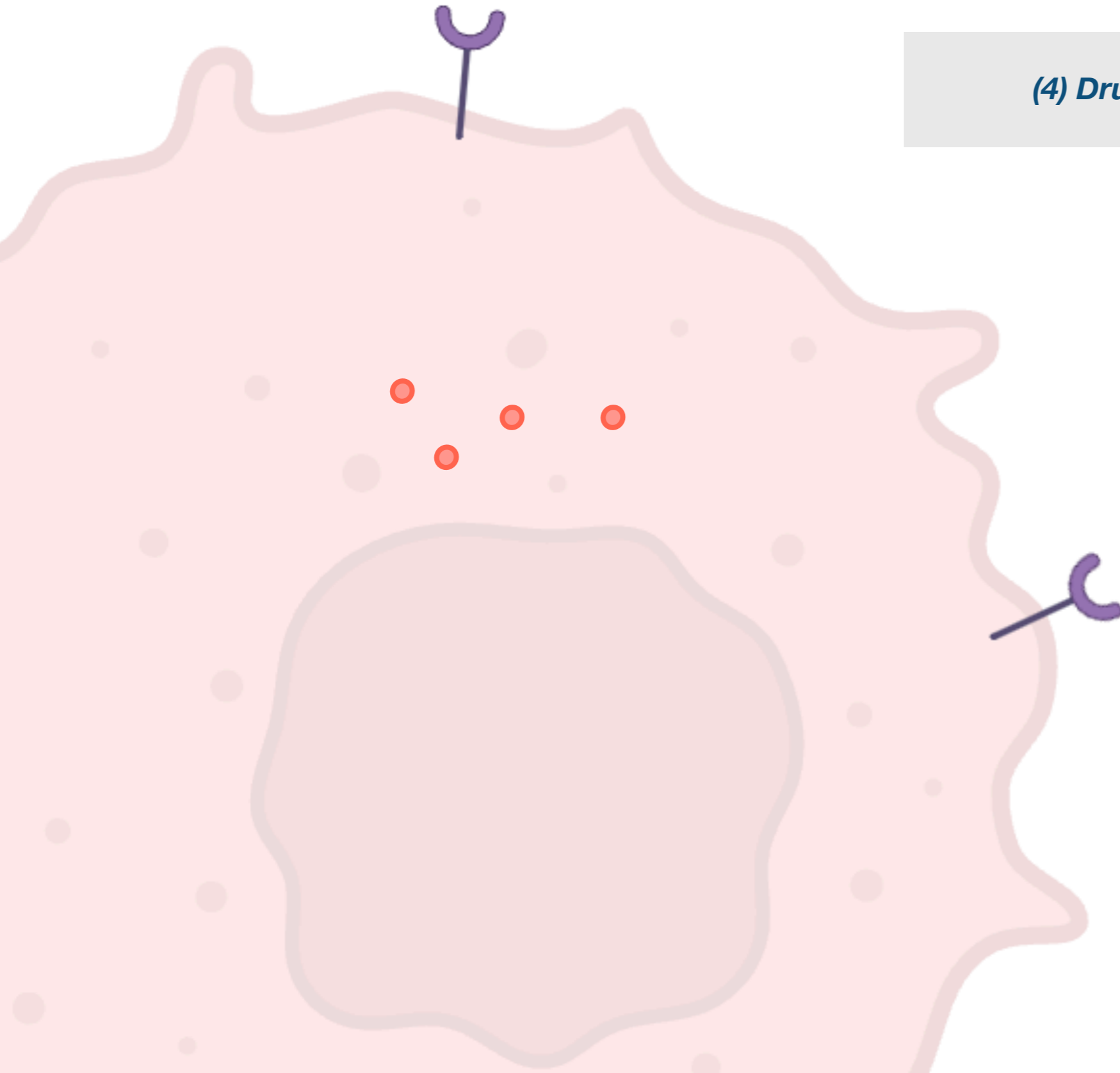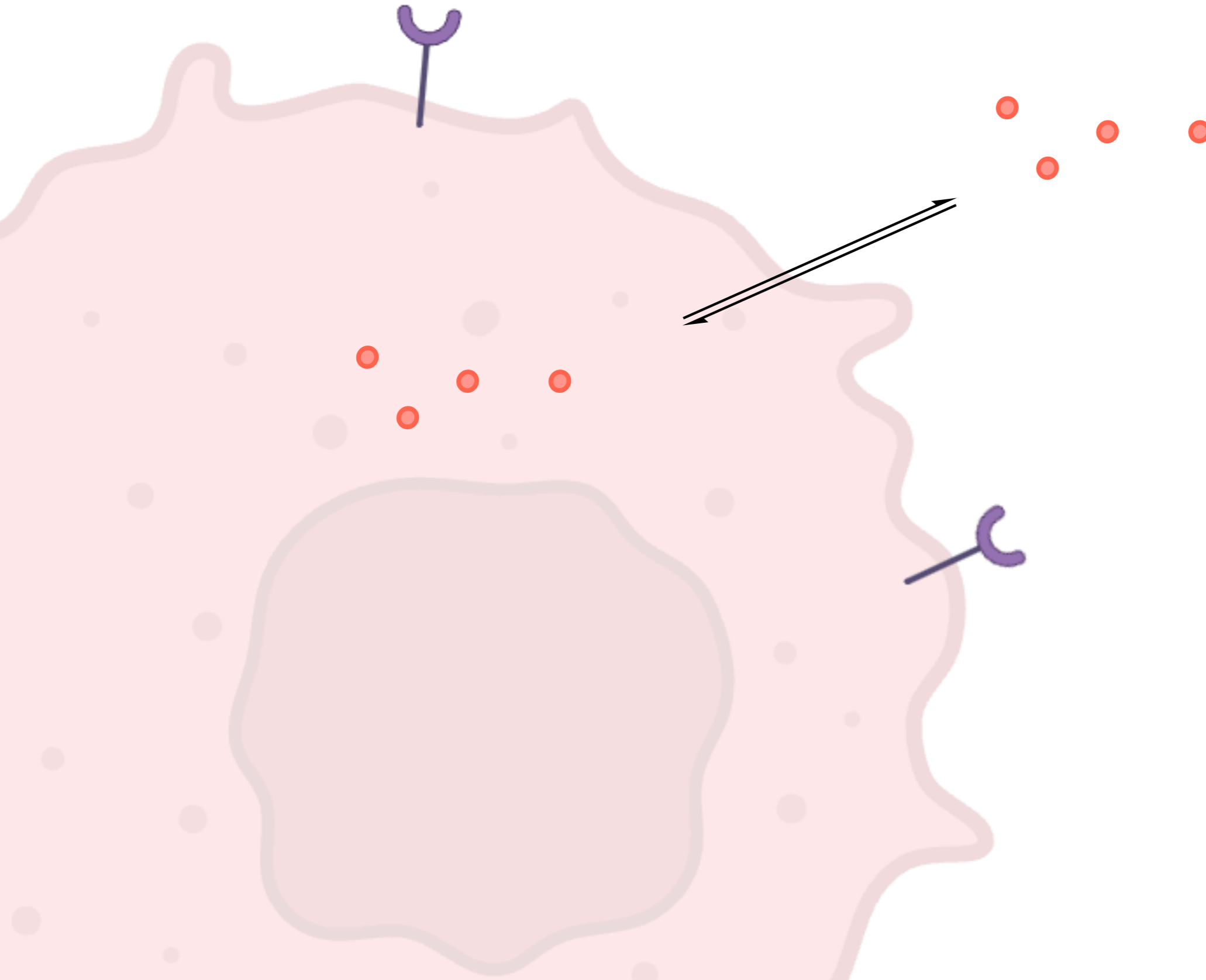
(3) Lysosomal Degradation or Linker Cleavage
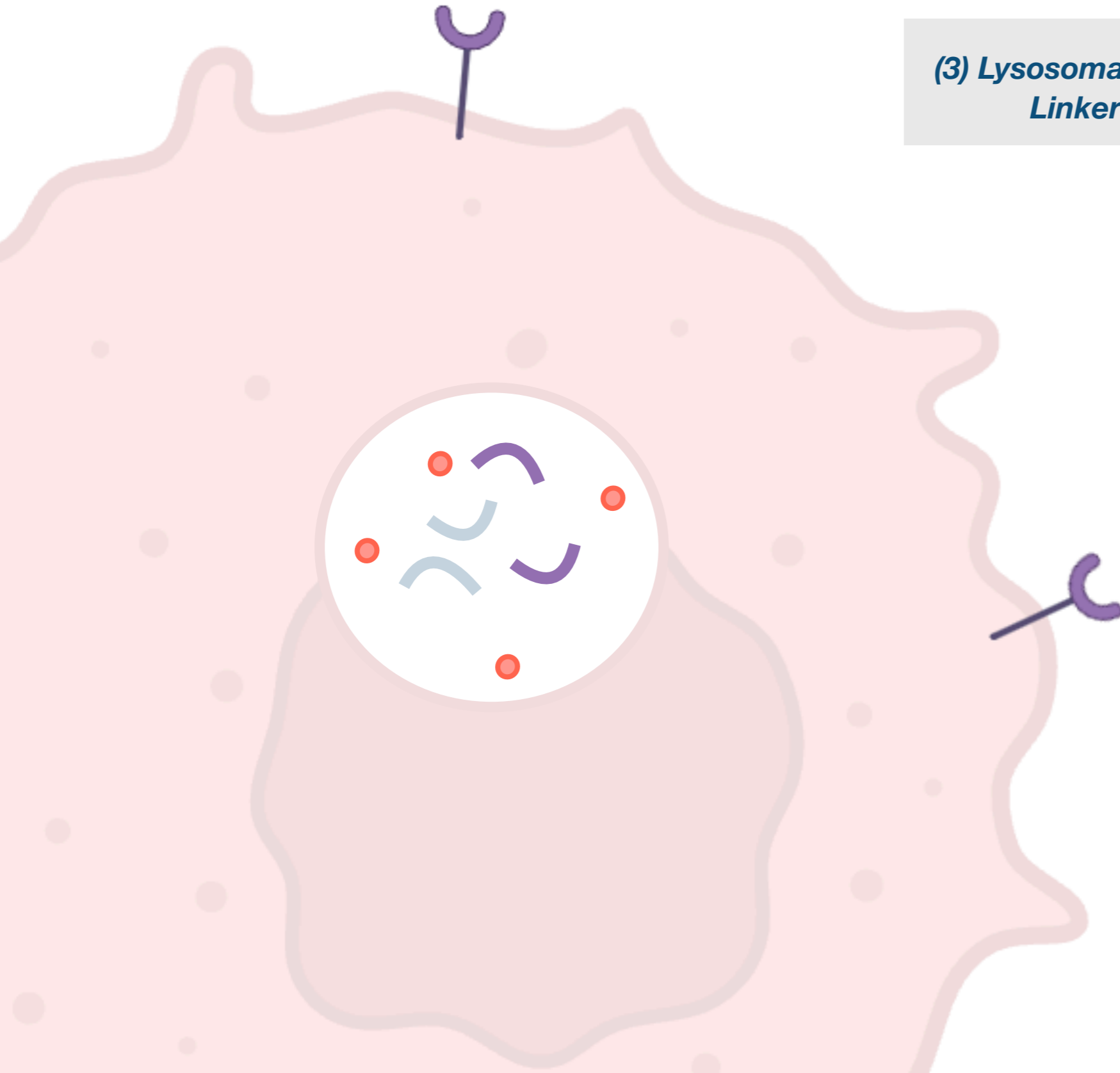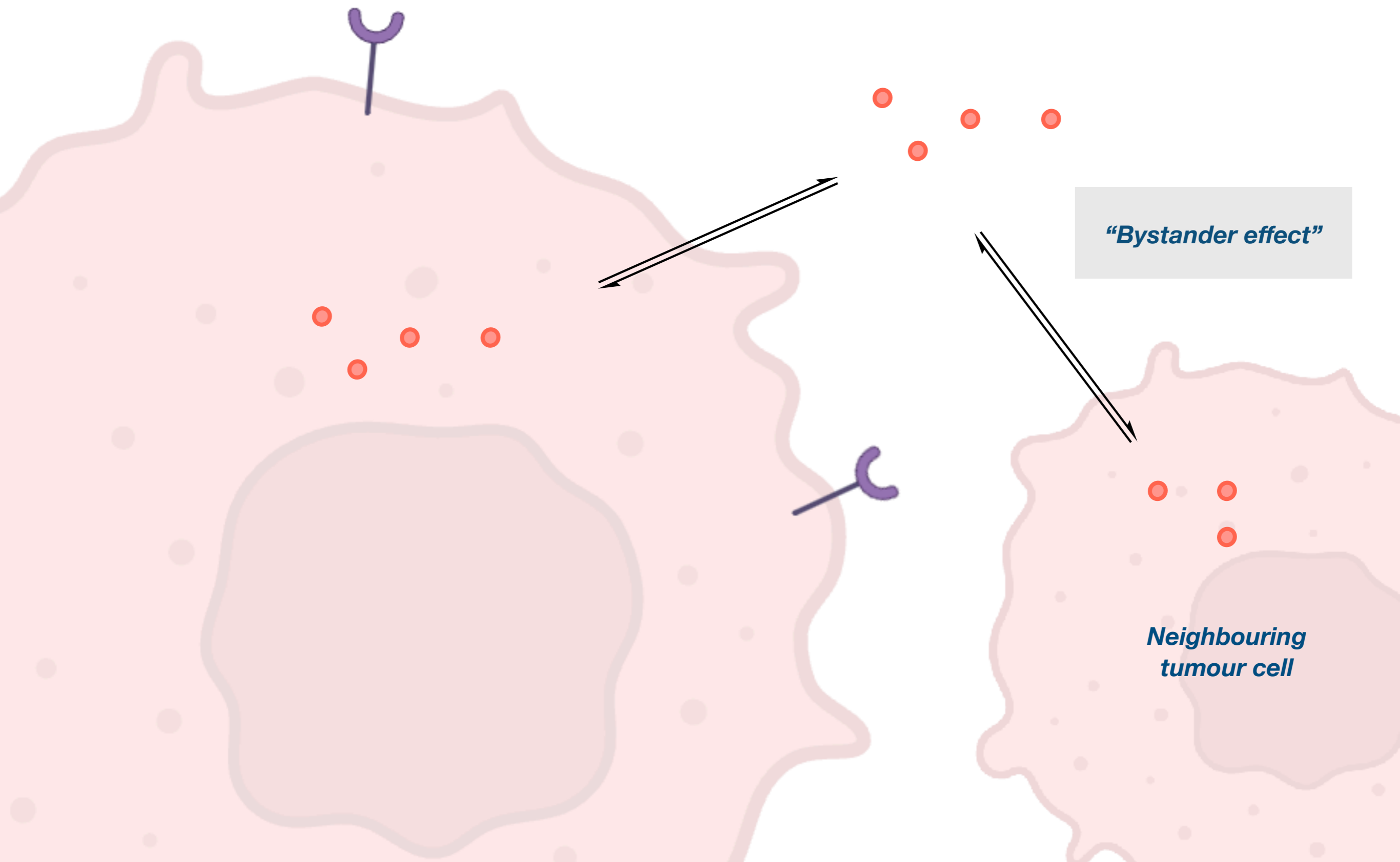
Antibody-drug conjugate

Mechanism of Action

(4) Drug release

Antibody-drug conjugate
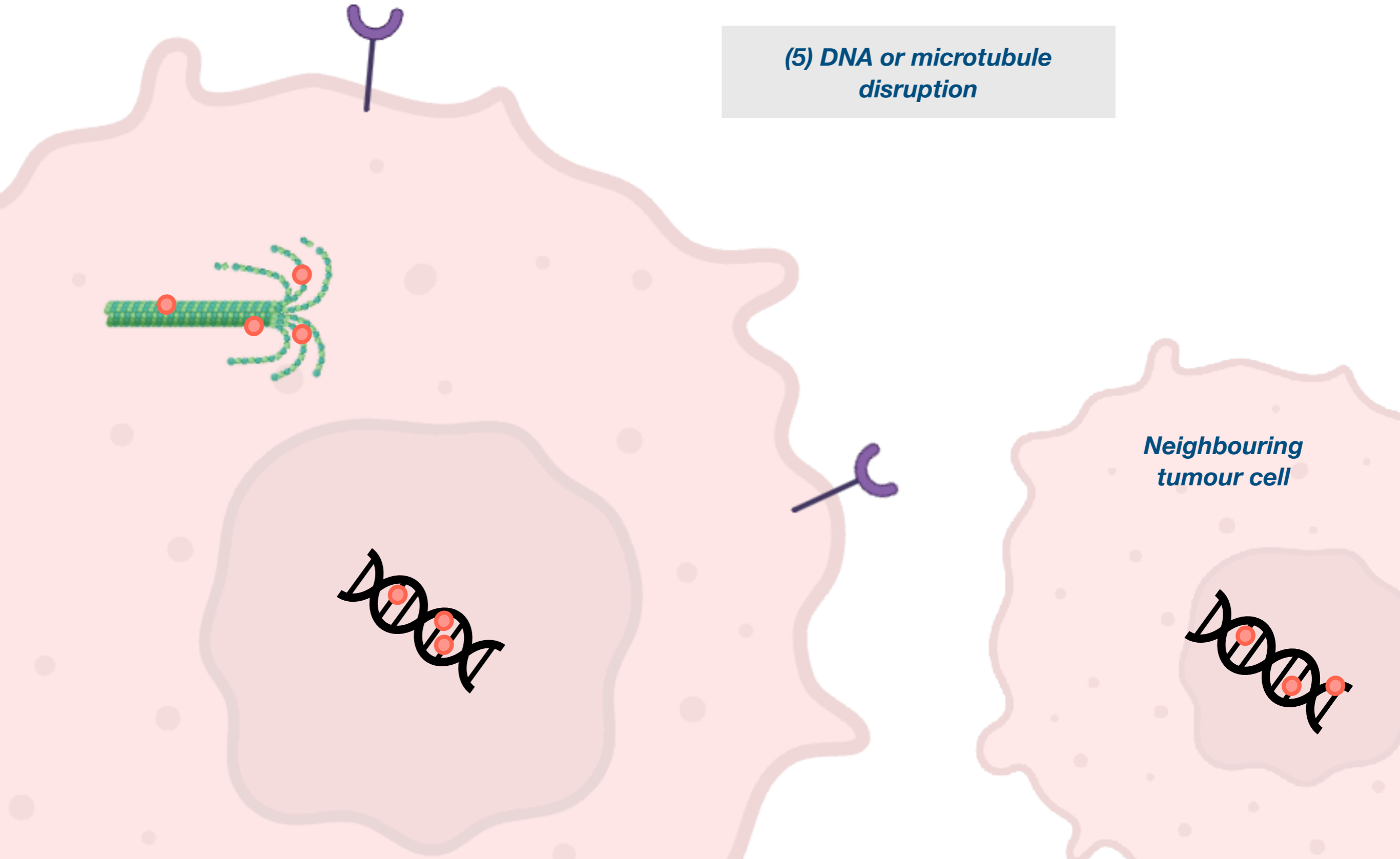## Mechanism of Action

Antibody-drug conjugate

Mechanism of Action

"Bystander effect"

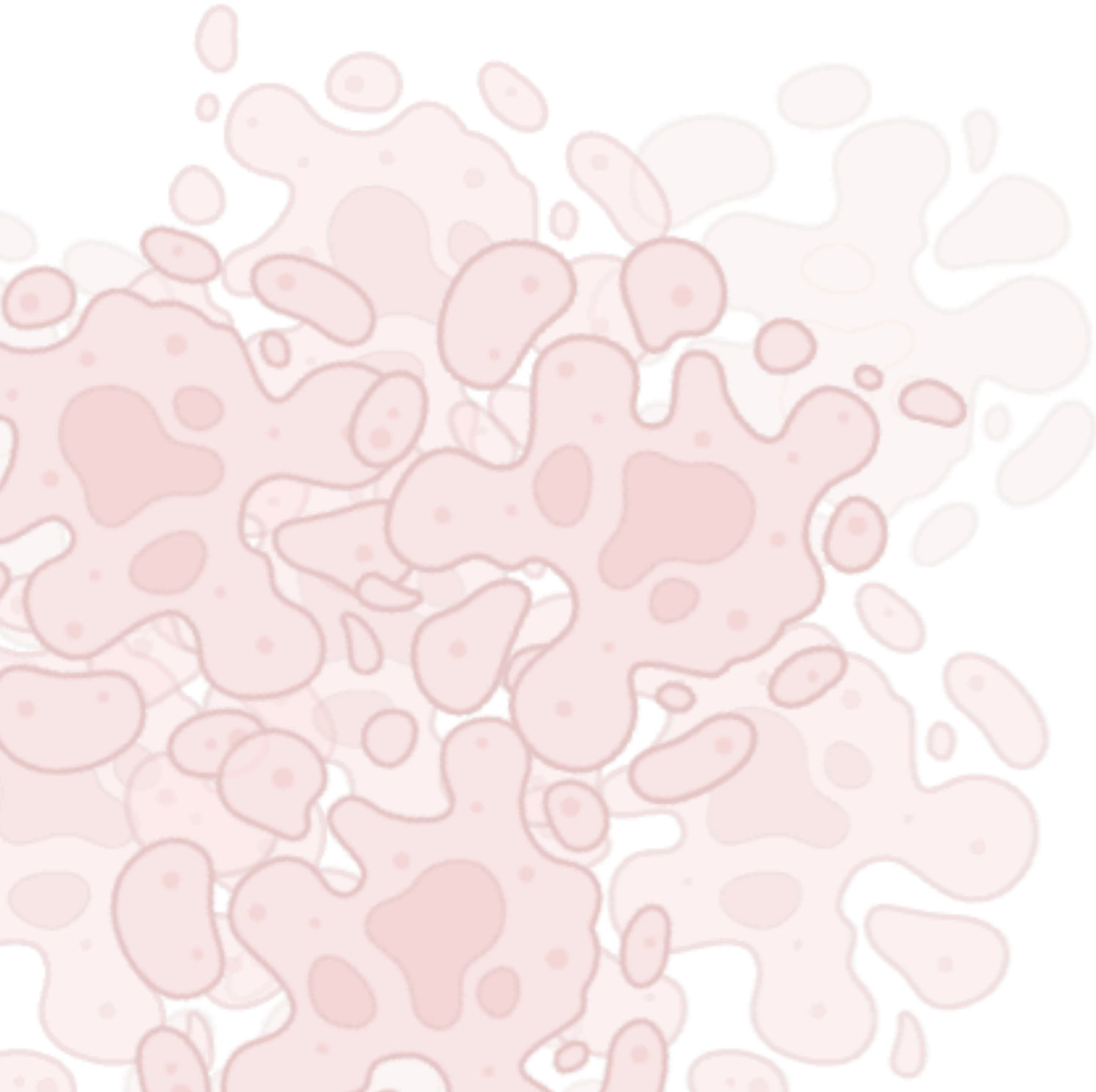Neighbouring tumour cell

Antibody-drug conjugate
Mechanism of Action

(5) DNA or microtubule disruption

Neighbouring tumour cell

Antibody-drug conjugate

Mechanism of Action

(6) Cell death

FDA-approved ADCs

12 in the market

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™ (First approval in 2000)

Besponsa™

Adcetris™
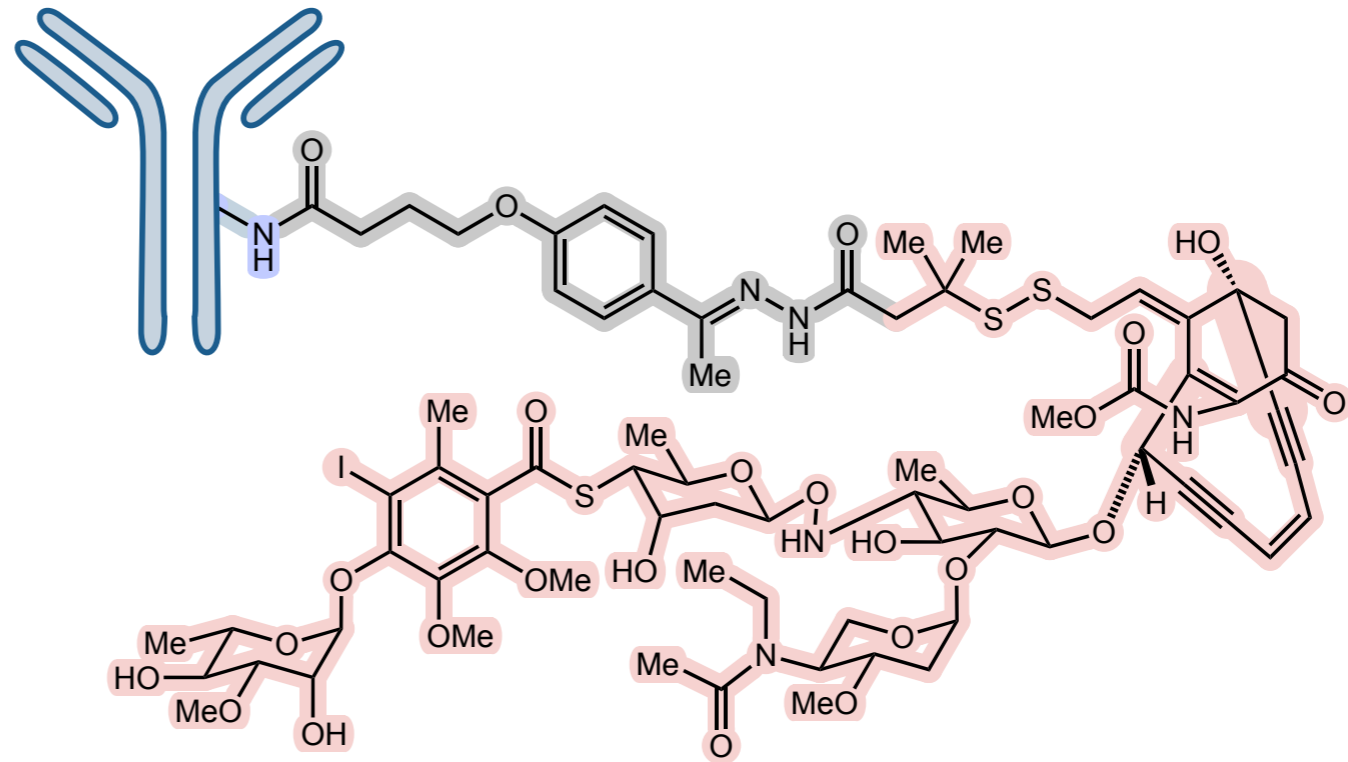
Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

	Antibody	*Indication*	*Developer*
Mylotarg™ *(Gemtuzumab ozogamicin)*	*anti-CD33 mAb*	*acute myeloid leukemia*	*Pfizer*
Besponsa™ *(inotuzumab ozogamicin)*	*anti-CD22 mAb*	*acute lymphoblastic leukemia*	*Pfizer*

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™
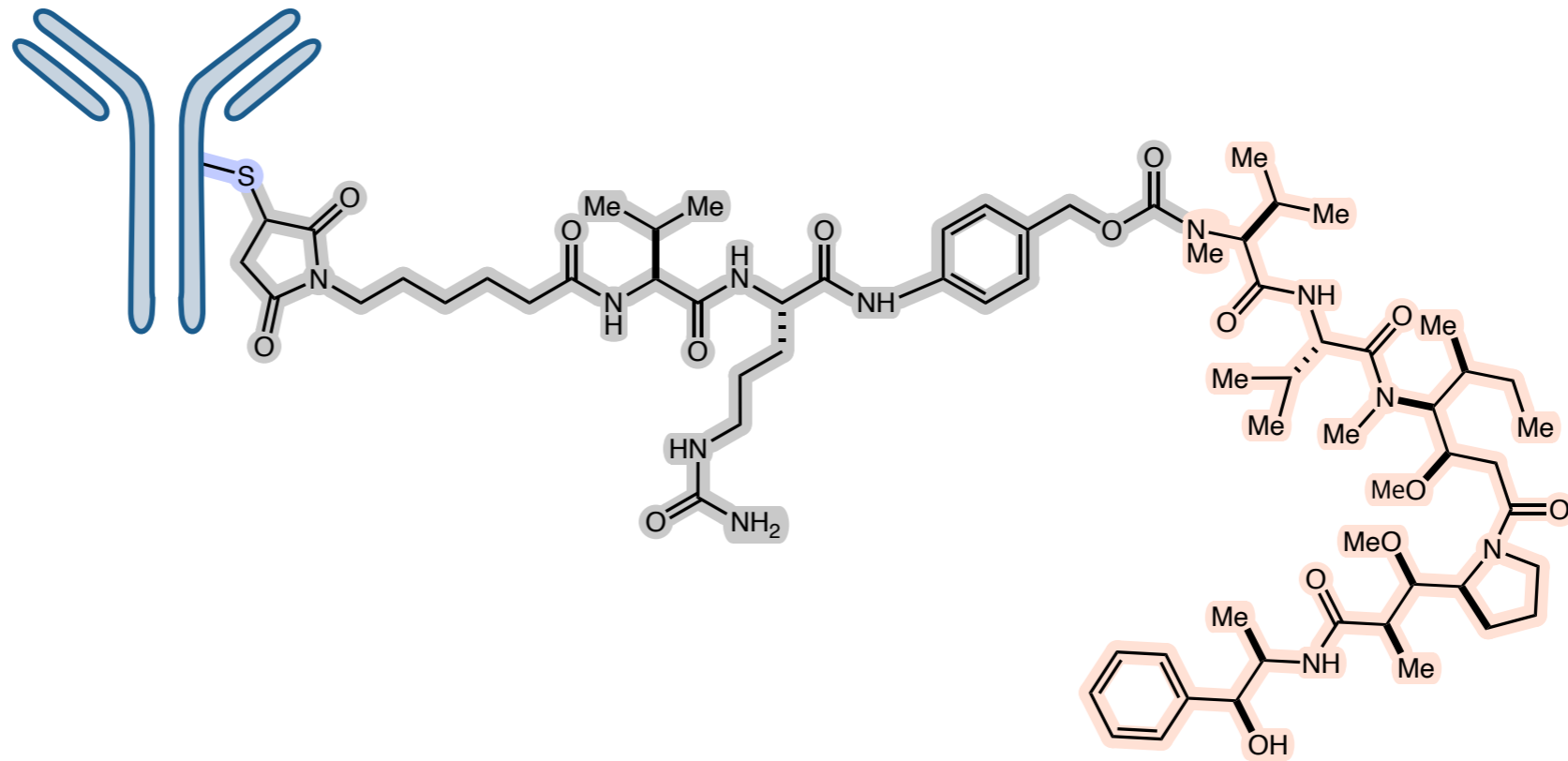
	Antibody	**Indication**	**Developer**
Adcetris™ *(Brentuximab vedotin)*	*anti-CD30 mAb*	*Hodgkin's Lymphoma*	Pfizer
Polivy™ *(Polatuzumab vedotin)*	*anti-CD79b mAb*	*Diffuse large B-cell lymphoma*	Genentech
Padcev™ *(Enfortumab vedotin)*	*anti-Nectin4 mAb*	*Urothelial Cancer*	astellas
Tivdak™ *(Tisotumab vedotin)*	*anti-TF mAb*	*Cervical Cancer*	Pfizer

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™

Besponsa™

Adcetris™
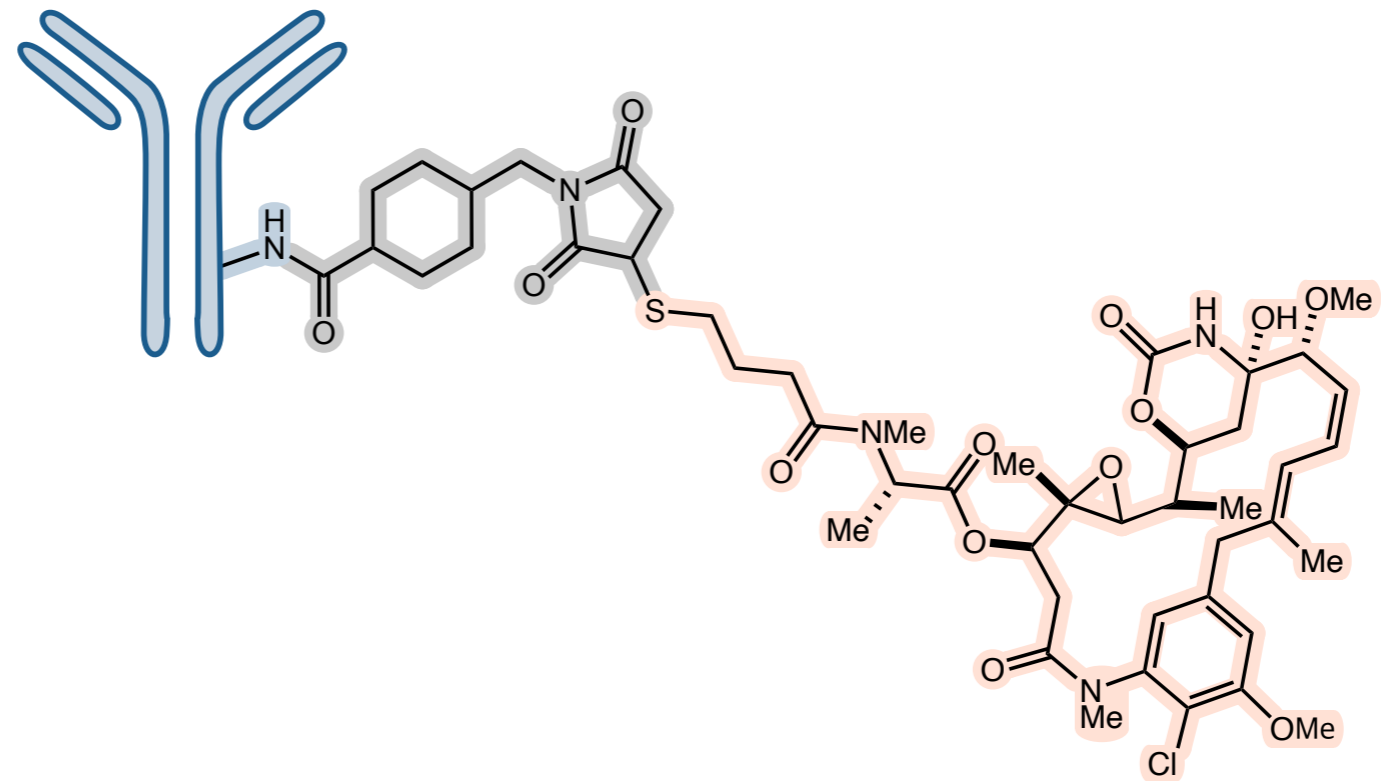
Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

	Antibody	Indication	Developer
Kadcyla™ (Trastuzumab emtansine)	Trastuzumab	Breast cancer	Genentech

FDA-approved ADCs

Antibody - *Linker* - *Drug*

Mylotarg™

Besponsa™

Adcetris™

Polivy™
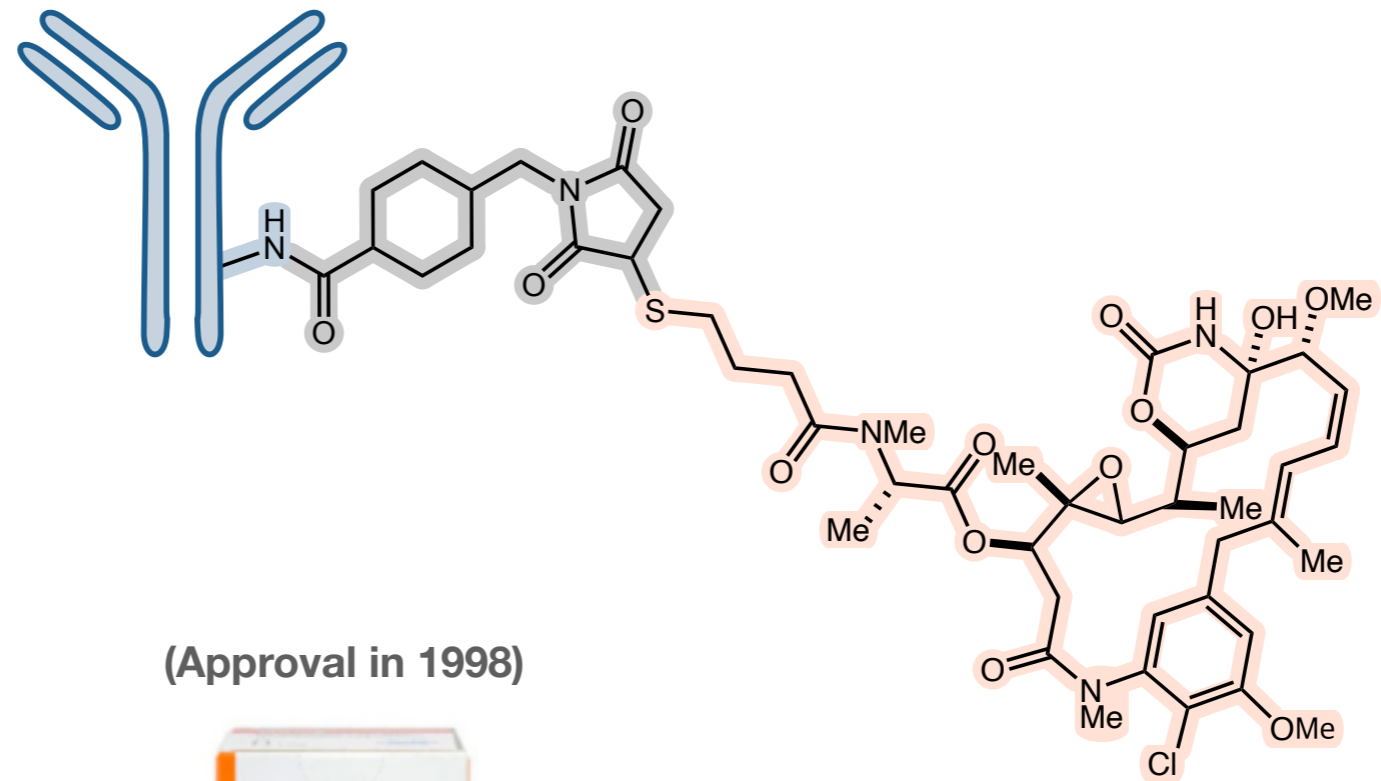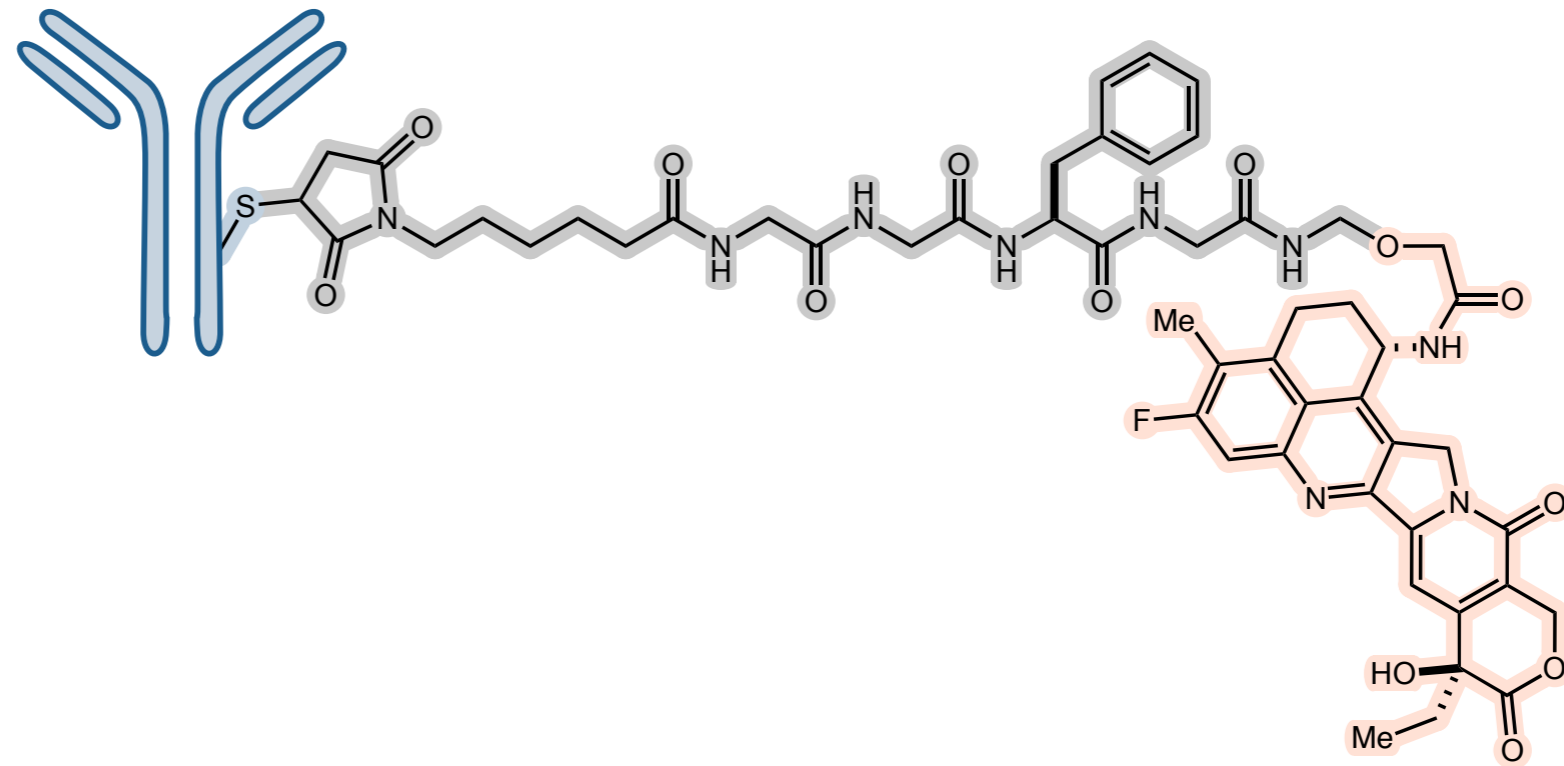
Padcev™

Tivdak™

Kadcyla™ **(Approval in 2013)**

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

(Approval in 1998)

Herceptin® 150 mg
powder for concentrate for
solution for infusion
Trastuzumab

150 mg

For intravenous use after
reconstitution and dilution

	Antibody	**Indication**	**Developer**
Kadcyla™ *(Trastuzumab emtansine)*	*Trastuzumab*	*Breast cancer*	**Genentech**

FDA-approved ADCs

Antibody - *Linker* - *Drug*

Mylotarg™
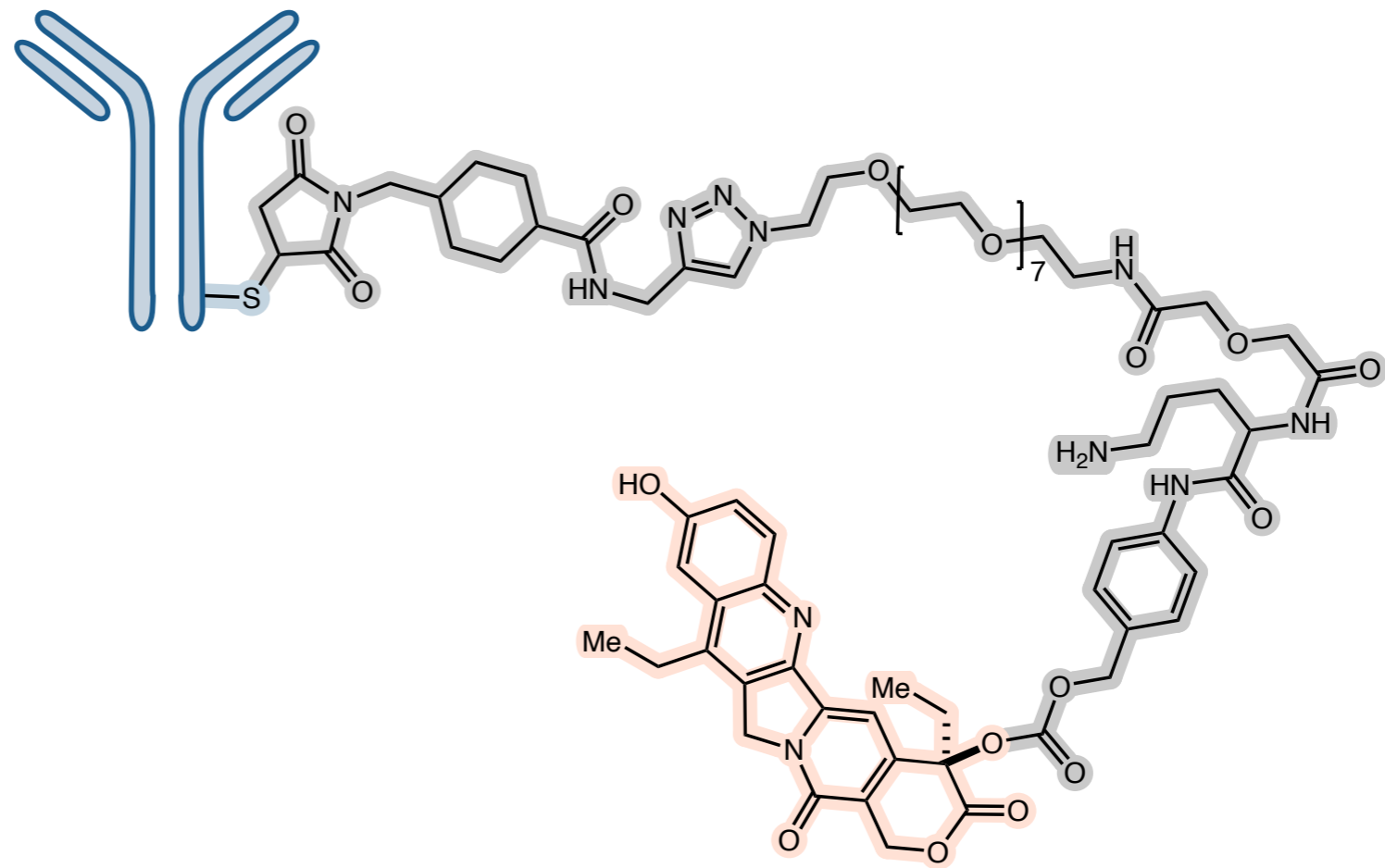
Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

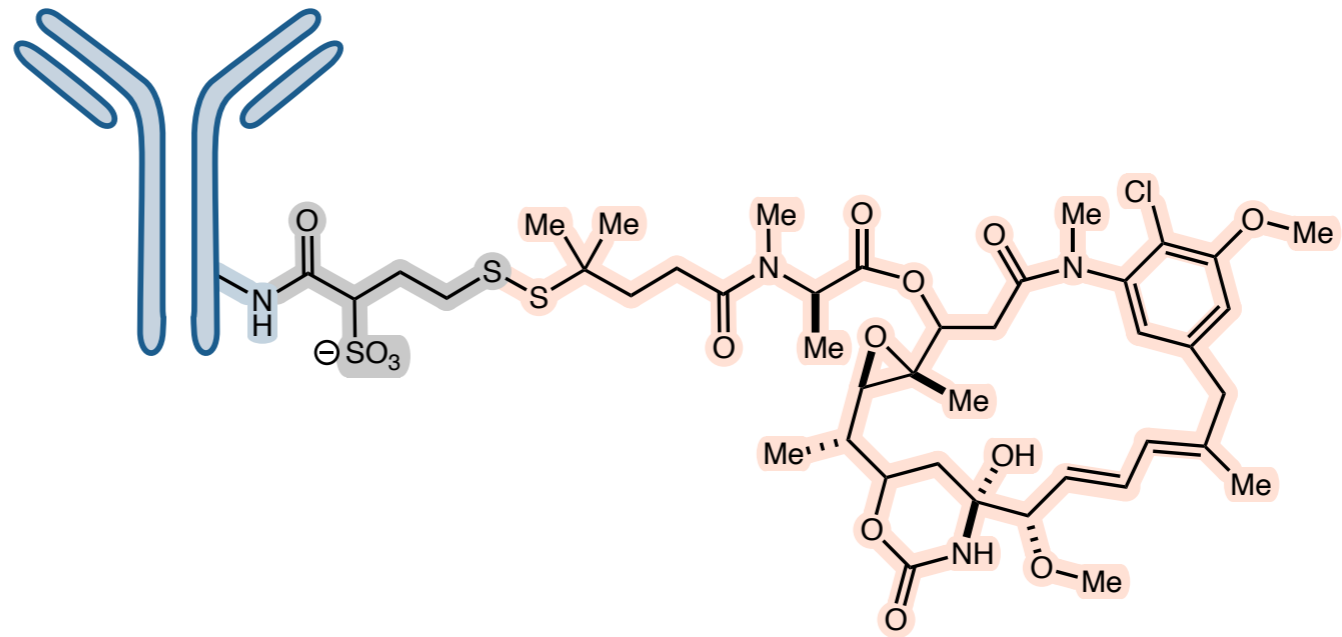Trodelvy™

Zynlota™

Blenrep™

Elahere™

	Antibody	Indication	Developer
Enhertu™ (Trastuzumab deruxtecan)	Trastuzumab	Breast cancer Solid cancer	Daiichi-Sankyo

FDA-approved ADCs

Antibody - *Linker* - *Drug*

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

	Antibody	*Indication*	*Developer*
Trodevly™ (Sacituzumab govitecan)	anti-Trop2 mAb	Triple-negative breast cancer	GILEAD

FDA-approved ADCs

Antibody - *Linker* - *Drug*

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

	Antibody	Indication	Developer
Elahere™ (Mirvetuximab soravtansine)	anti-Folate Receptor alpha mAb	Ovarian cancer	immun•gen.

Elahere™ (Approval in 2022)
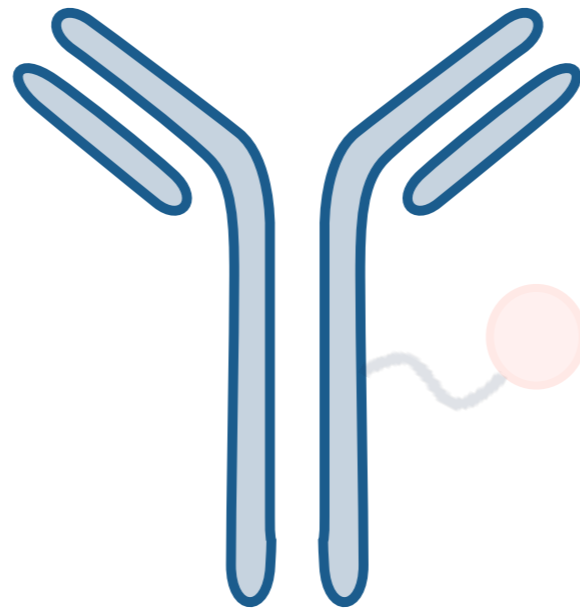
'A' in ADC

Antibody

- **Antibody alone as a drug**

- **Characteristics of antibody drugs**

- **'A' versus 'ADC'**

'A' in ADC
Antibody
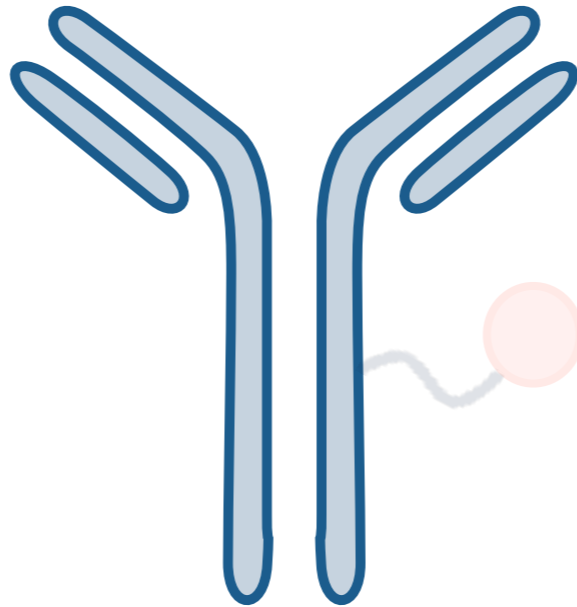
– **Antibody alone as a drug**

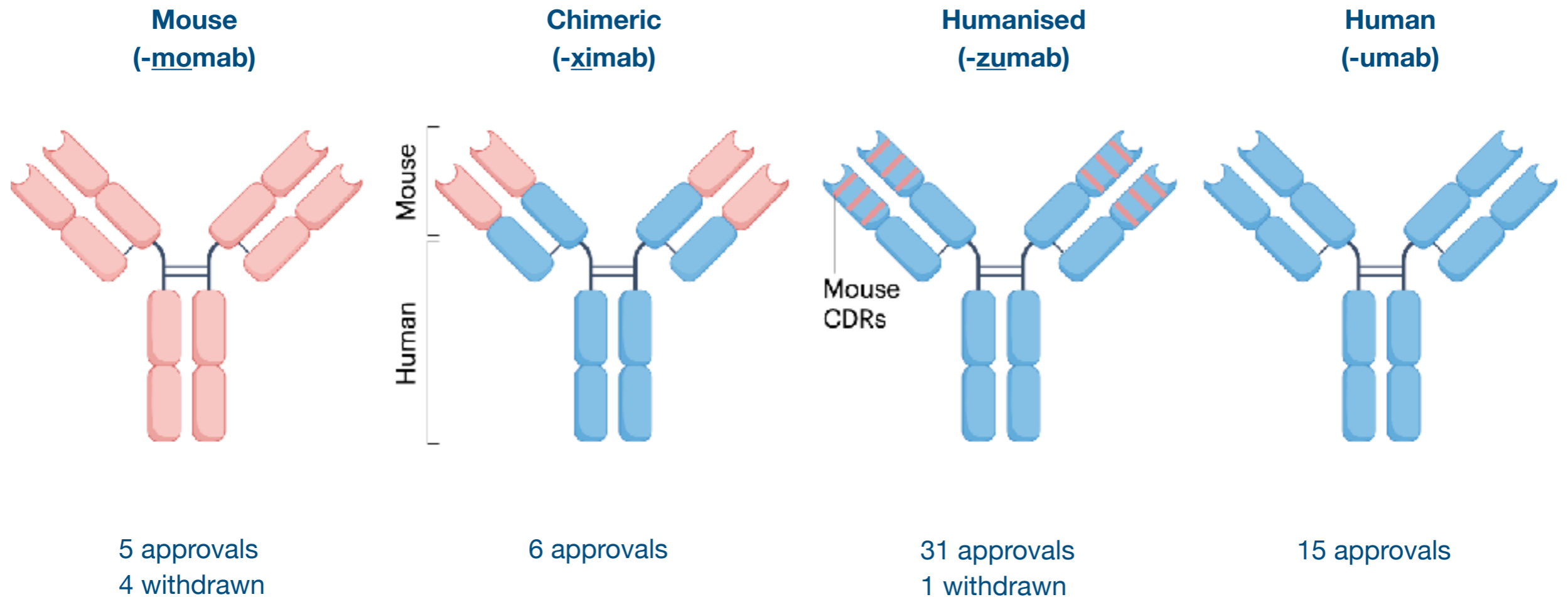– *Characteristics of antibody drugs*

– *'A' versus 'ADC'*

'A' in ADC

Structural features of antibody

Fab (Fragment antigen-binding)
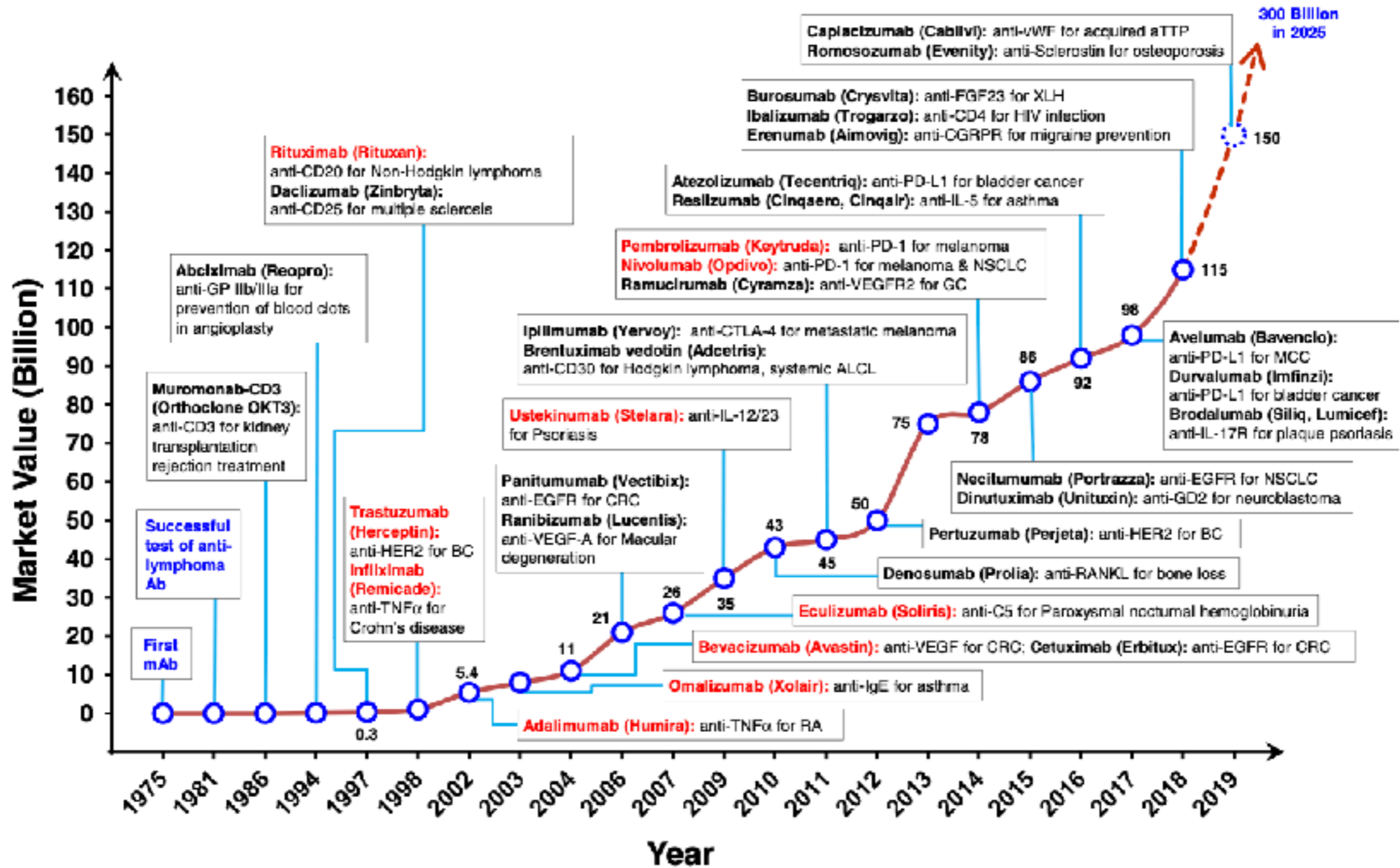
Fc (Fragment crystallisable)

CDRs (complementarity-determining region)

Variable region

Constant region

Heavy chain

Light chain

V_H, V_L, C_H1, C_L, C_H2, C_H3

―― : Disulfide bond

K. M.; Gabelli, S. B.; Ho, M.; Van Elsas, A.; Zhou, S. *Nat. Rev. Cancer* **2024**, *24* (6), 399–426
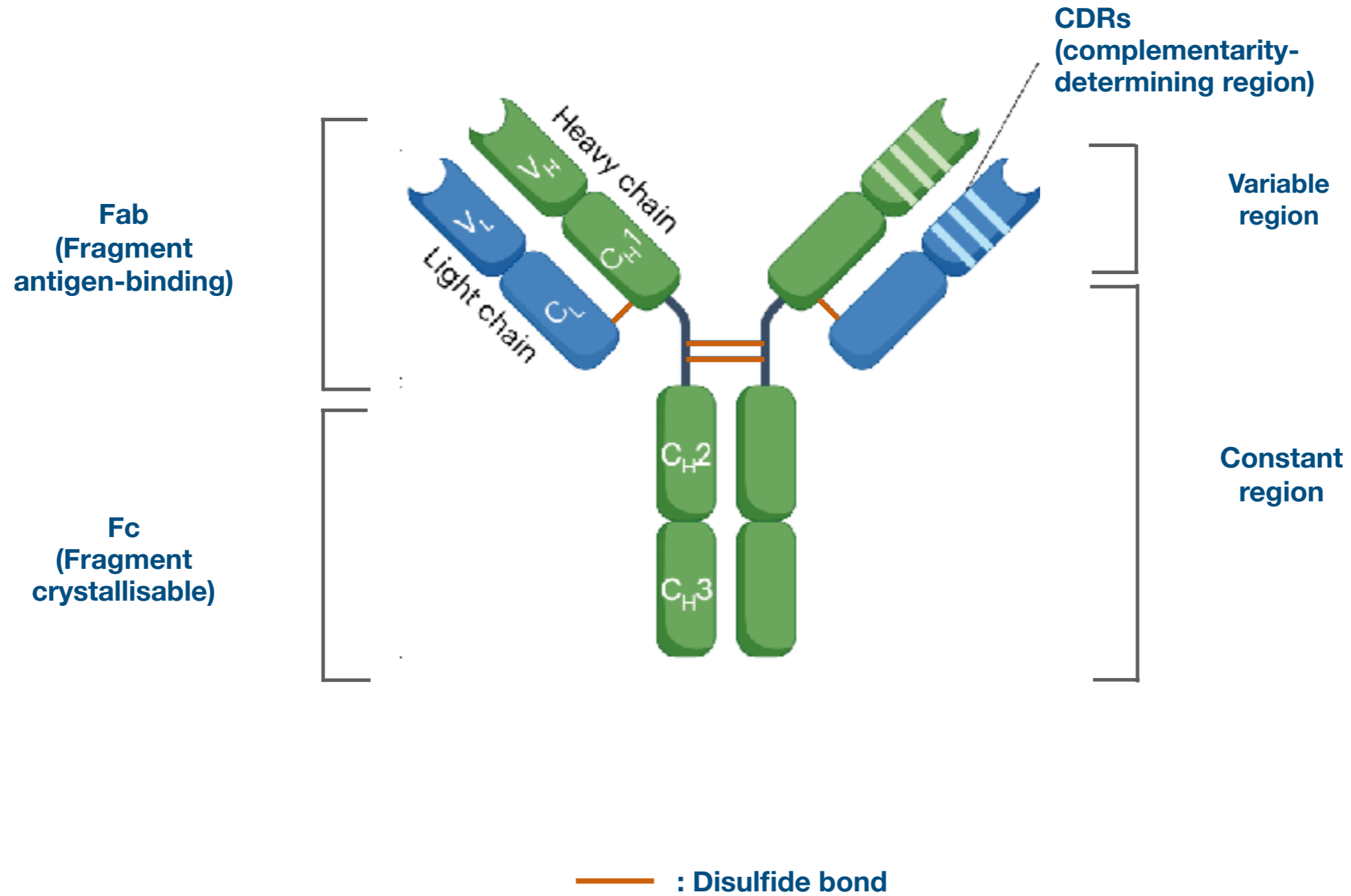
Antibody alone as a drug
How antibody could become a therapeutic modality

Mouse
(-momab)

Chimeric
(-ximab)

Humanised
(-zumab)

Human
(-umab)

Mouse

Human

Mouse
CDRs

5 approvals
4 withdrawn

6 approvals

31 approvals
1 withdrawn

15 approvals

- *Hybridoma system (developed in 1970s) allowed for production and selection of highly specific mouse mAb*

- *Antibody engineering enabled to graft a **human antibody constant region** to a **mouse antibody variable region***

- *Transgenic mouse models and phage display systems in 1990s enabled the generation of **fully human antibodies***

- ***For ADCs, 10 out of 12 ADCs use humanized antibodies***

K. M.; Gabelli, S. B.; Ho, M.; Van Elsas, A.; Zhou, S. *Nat. Rev. Cancer* **2024**, *24* (6), 399–426

Antibody alone as a drug

Successful development of therapeutic antibodies and their applications

As of 2024 2Q, more than 130 mAb therapies are approved

Lu, R.-M.; Hwang, Y.-C.; Liu, I.-J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. *J. Biomed. Sci.* **2020**, *27* (1), 1

Antibody alone as a drug

Successful development of therapeutic antibodies and their applications

Top 10 drugs by revenue forecasts

Rank	Product	Company	Pharmacological class	2024 worldwide sales forecast (US$)
1	Keytruda	Merck & Co.	Anti-PD1 mAb	27.19 billion
2	Ozempic	Novo Nordisk	GLP1 receptor agonist	16.13 billion
3	Dupixent	Sanofi/Regeneron	Anti-IL-4/IL-13 mAb	13.45 billion
4	Eliquis	Bristol Myers Squibb/Pfizer	Factor Xa inhibitor	13.31 billion
5	Biktarvy	Gilead Sciences/Yuhan	HIV INSTI/NRTI/NtRTI	12.57 billion
6	Darzalex	Johnson & Johnson	Ant-CD38 mAb	11.98 billion
7	Opdivo	Bristol Myers Squibb/Ono	Anti-PD1 mAb	11.33 billion
8	Comirnaty	Pfizer/BioNTech	SARS-CoV-2 vaccine	10.79 billion
9	Gardasil	Merck & Co./CSL	HPV vaccine	10.03 billion
10	Skyrizi	AbbVie	Anti-IL-23 mAb	9.93 billion

5 out of 10 are mAb therapies

Verdin, P. *Nat. Rev. Drug Discov.* **2024**, *23*

'A' in ADC
Antibody
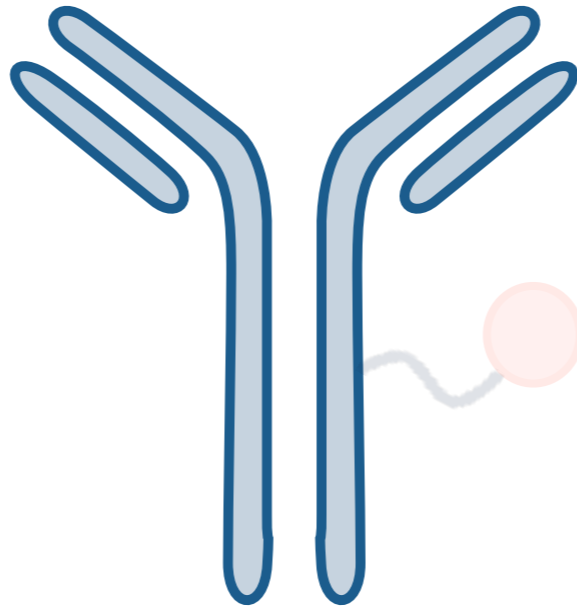
- *Antibody alone as a drug*

- **Characteristics of antibody drugs**

- *'A' versus 'ADC'*

Characteristics of antibody drugs

Target specificity

$K_d = 10^{-9} - 10^{-12}$ M

Cancer cell
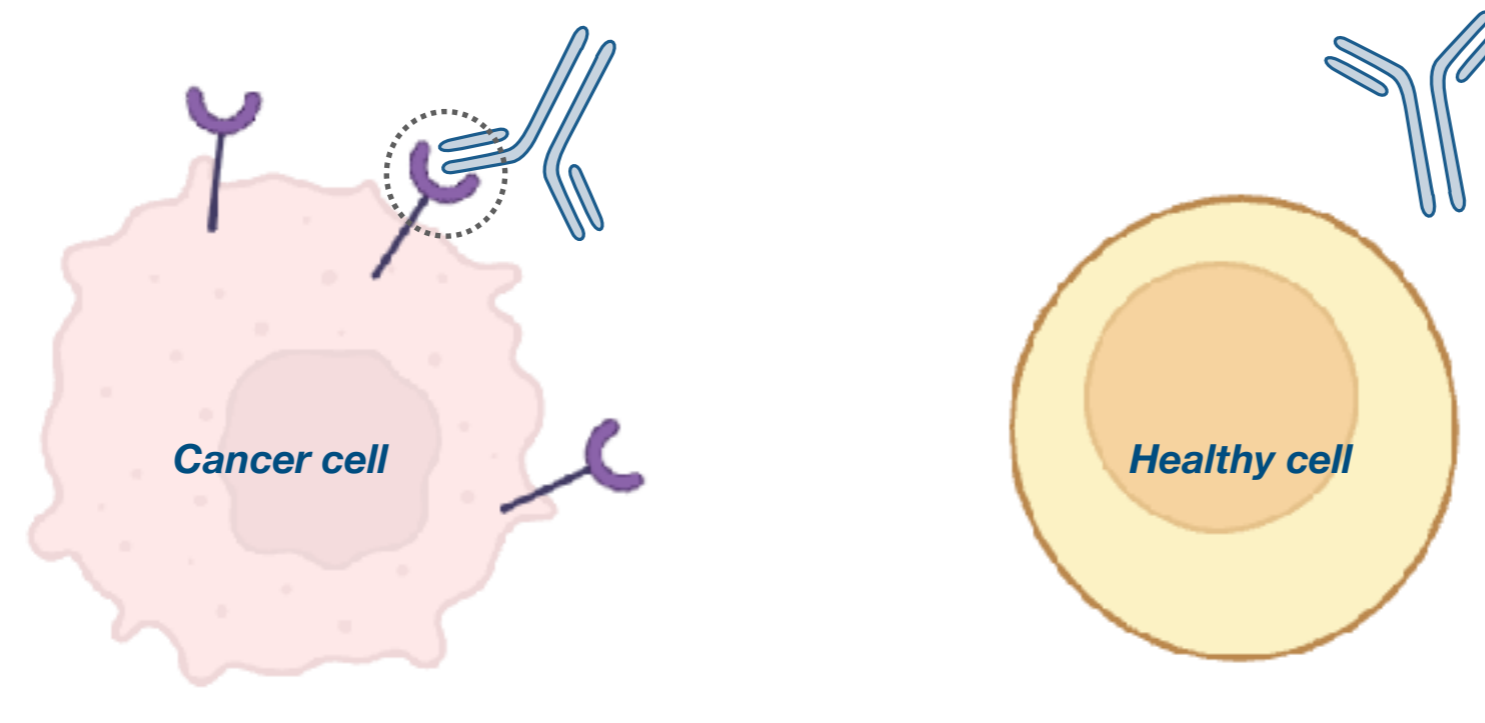
Healthy cell

Targets only pathological cells while sparing healthy cells

Miller, N. L.; Clark, T.; Raman, R.; Sasisekharan, R. *Front. Mol. Biosci.* **2023**, *10*

Characteristics of antibody drugs

Less adverse events and longer half-life
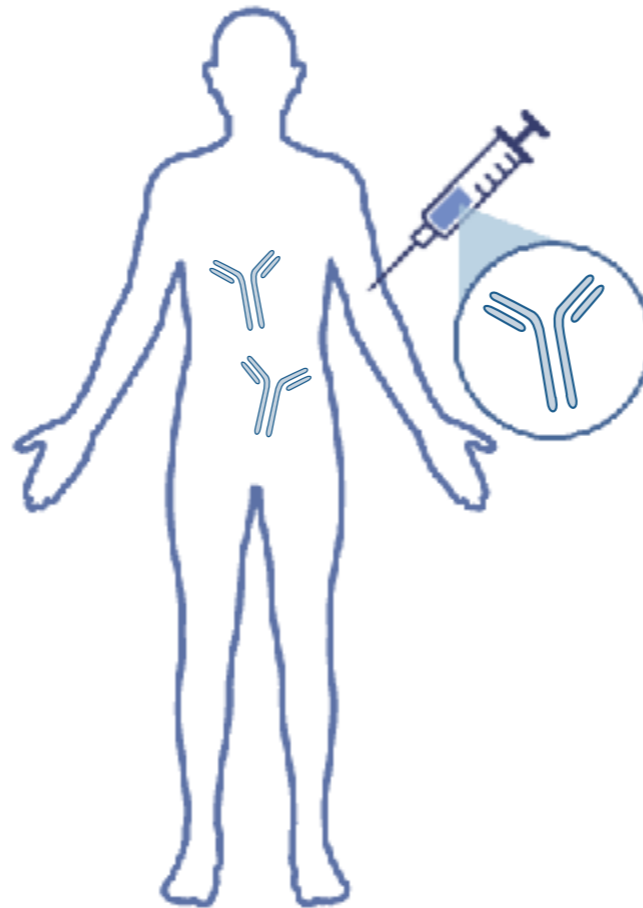
c.f. Half-life of small-molecule drugs (e.g. Taxol)

13-52 hours
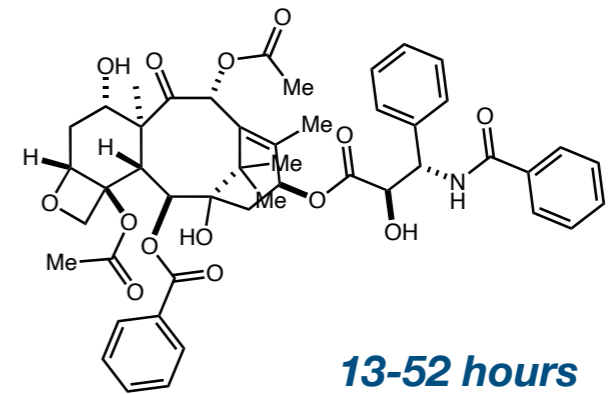
Significantly reduced immunogenicity

Less likely to be cleared by our body's immune system

Longer half-life: 2-4 weeks

Less dosing frequency, fewer side-effects

Characteristics of antibody drugs

Less adverse events and longer half-life

FcRn (neonatal Fc receptor)-mediated recycling
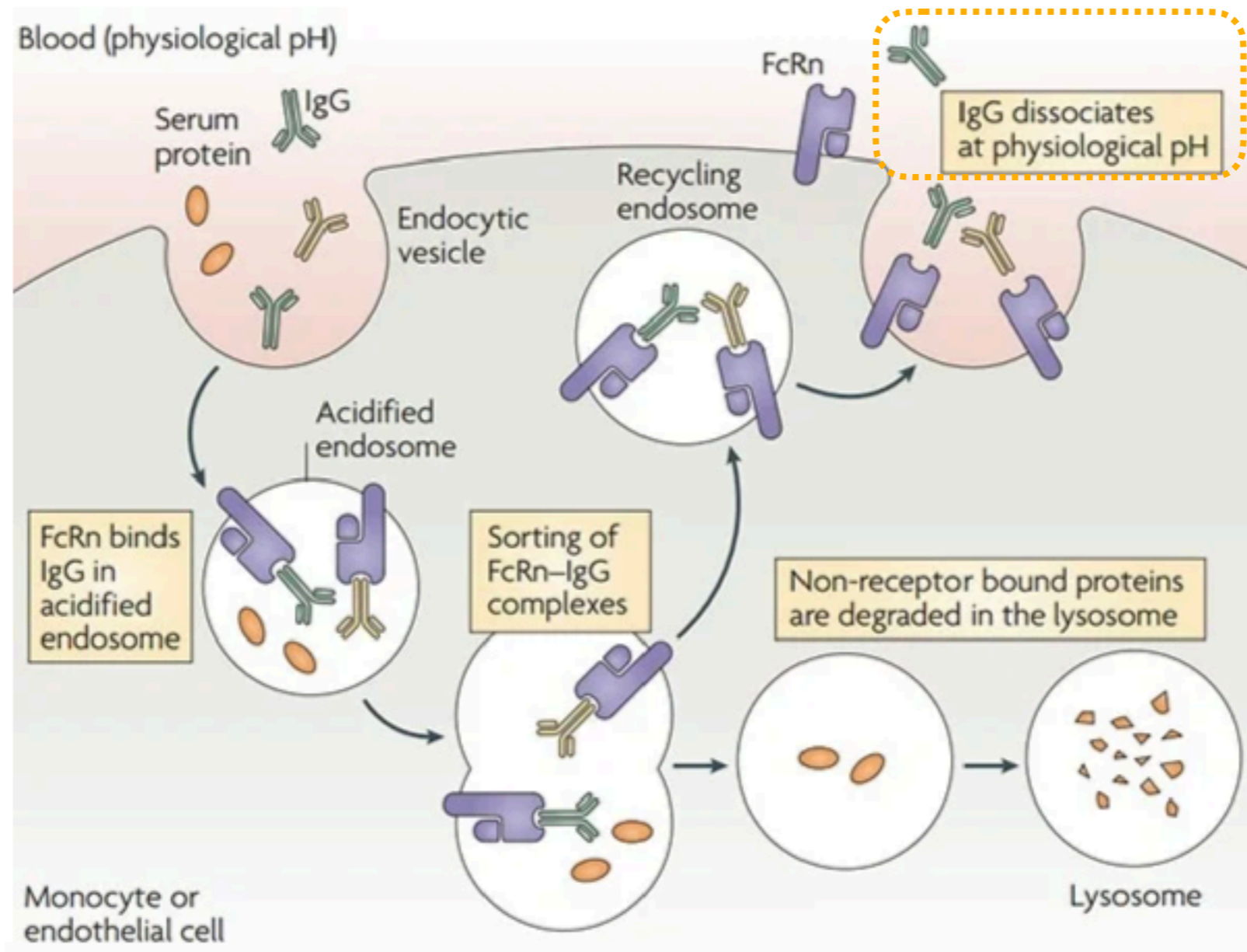
FcRn-mediated recycling can extend the half-life antibody (2-4 weeks)

Characteristics of antibody drugs
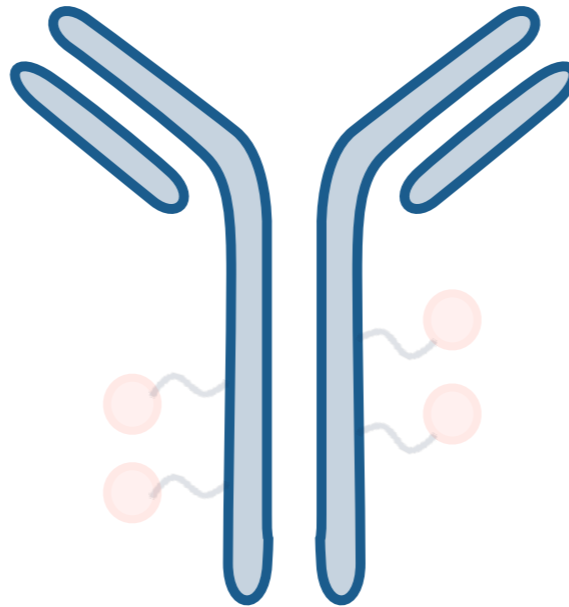
Target specificity

Less adverse events and longer half-life

Characteristics of antibody drugs
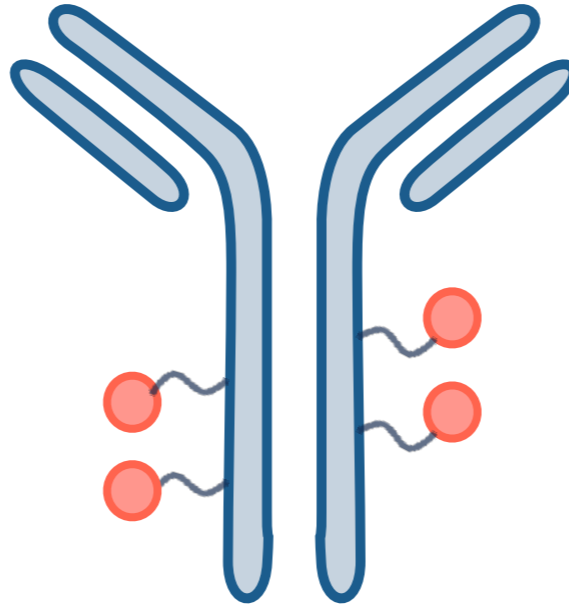
Target specificity

Less adverse events and longer half-life

Enables the delivery of highly cytotoxic drugs to target sites
Extends the half-life of small-molecule drugs attached to mAb

Characteristics of antibody drugs
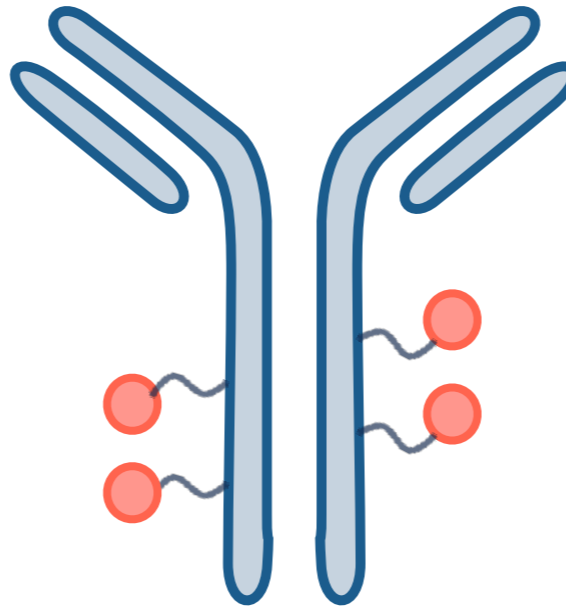
Target specificity

Less adverse events and longer half-life

Enables the delivery of highly cytotoxic drugs to target sites
Extends the half-life of small-molecule drugs attached to mAb

	MMAE alone	Antibody-MMAE
Half-life of MMAE (**'D'** *of 4 FDA-approved ADCs*)	2.5 h	3 days

Chang, H.P.; Cheung, Y.K.; Shah, D.K. *J. Clin. Med.* **2021**, *10*, 1332

Characteristics of antibody drugs

What 'A' is suitable for 'ADC'
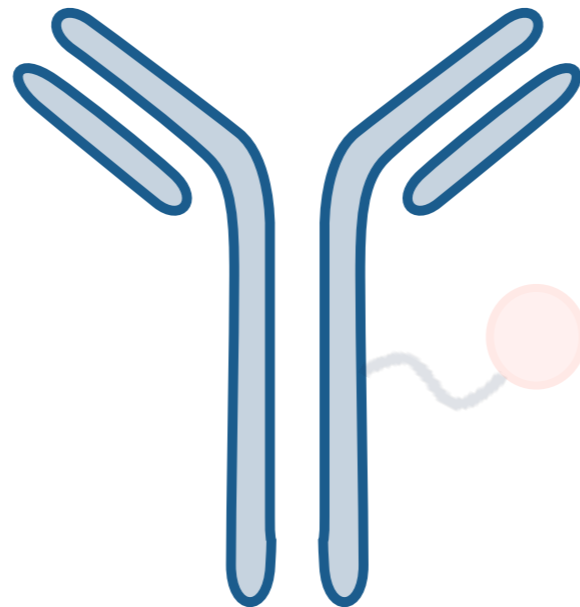
✓ *Well-elucidated MOA:* **endocytosis (internalisation)** *is necessary*

✓ *Its target should be* **cell surface antigens** *that are abundantly expressed on cancer cells, and absent on healthy cells*

'A' in ADC
Antibody
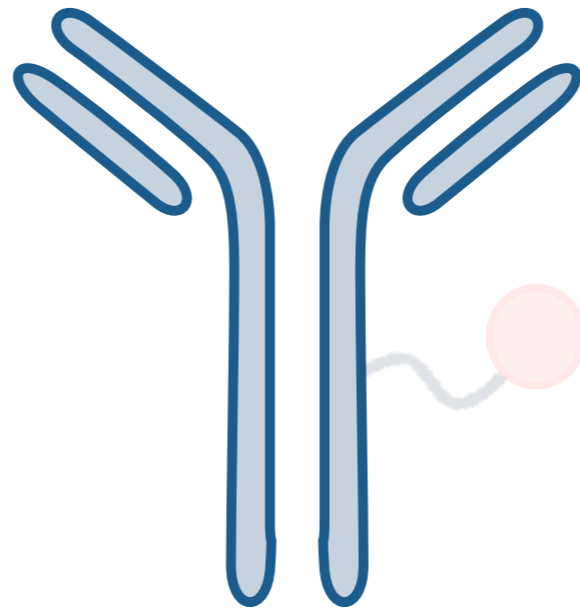
- Antibody alone as a drug

- Characteristics of antibody drugs

- **'A' versus 'ADC'**

'A' alone versus 'ADC'
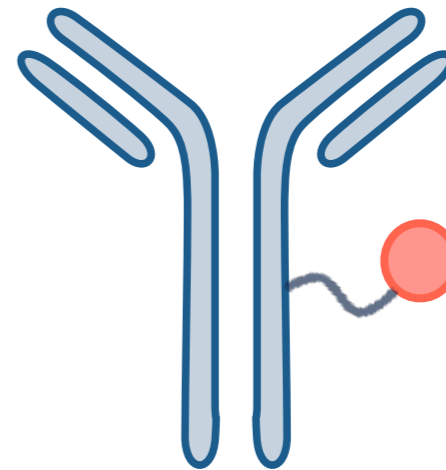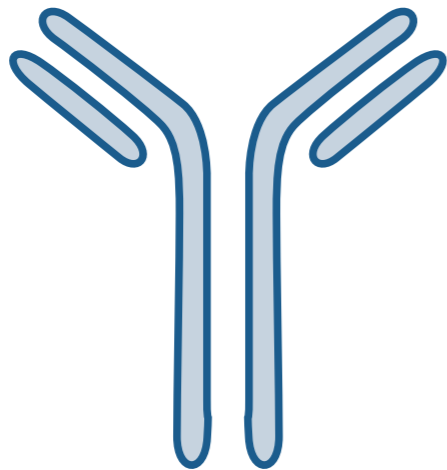
'A' alone versus 'ADC'

HerceptinTM versus KadcylaTM

HerceptinTM
(Trastuzumab, T)

KadcylaTM
(T-DM1)
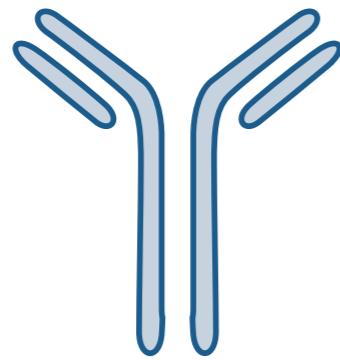
Genentech

Genentech
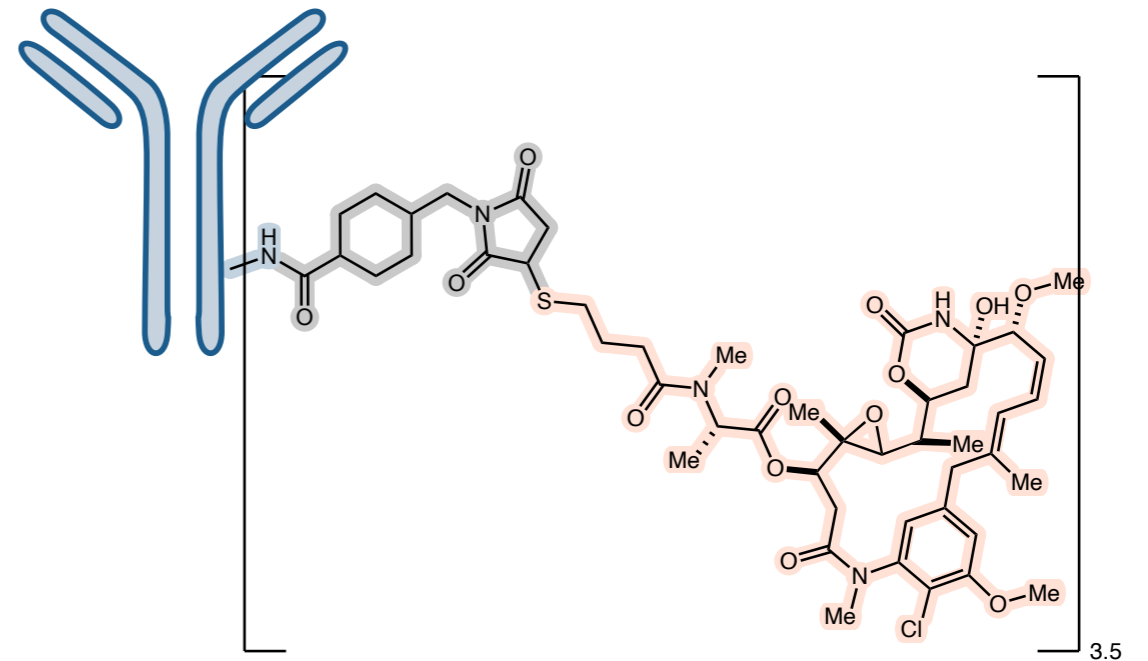
Same 'A' (trastuzumab)

Herceptin™ versus Kadcyla™
KATHERINE trial

Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer

G. von Minckwitz, C.-S. Huang, M.S. Mano, S. Loibl, E.P. Mamounas, M. Untch, N. Wolmark, P. Rastogi, A. Schneeweiss, A. Redondo, H.H. Fischer, W. Jacot, A.K. Conlin, C. Arce-Salinas, I.L. Wapnir, C. Jackisch, M.P. DiGiovanna, P.A. Fasching, J.P. Crown, P. Wülfing, Z. Shao, E. Rota Caremoli, H. Wu, L.H. Lam, D. Tesarowski, M. Smitt, H. Douthwaite, S.M. Singel, and C.E. Geyer, Jr., for the KATHERINE Investigators*

ABSTRACT

BACKGROUND

Patients who have residual invasive breast cancer after receiving neoadjuvant chemotherapy plus human epidermal growth factor receptor 2 (HER2)–targeted therapy have a worse prognosis than those who have no residual cancer. Trastuzumab emtansine (T-DM1), an antibody–drug conjugate of trastuzumab and the cytotoxic agent emtansine (DM1), a maytansine derivative and microtubule inhibitor, provides benefit in patients with metastatic breast cancer that was previously treated with chemotherapy plus HER2-targeted therapy.

METHODS

We conducted a phase 3, open-label trial involving patients with HER2-positive early breast cancer who were found to have residual invasive disease in the breast or axilla at surgery after receiving neoadjuvant therapy containing a taxane (with or without anthracycline) and trastuzumab. Patients were randomly assigned to receive adjuvant T-DM1 or trastuzumab for 14 cycles. The primary end point was invasive disease–free

Trastuzumab

HER2

Breast cancer cell
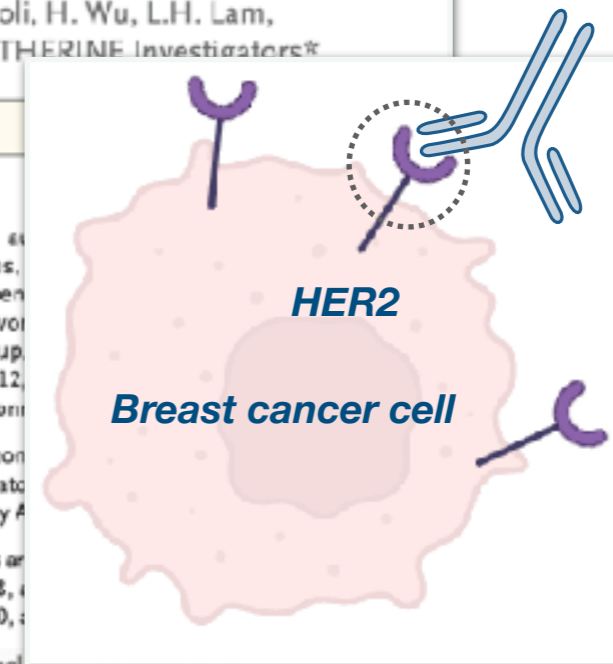
Herceptin™ versus Kadcyla™

KATHERINE trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

**Kadcyla™**
(T-DM1)

743 patients

Herceptin™ versus Kadcyla™

KATHERINE trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

Kadcyla™
(T-DM1)

743 patients

	No. of Patients	No. of Events (%)	3-Yr Invasive Disease–free Survival, %
T-DM1	743	91 (12.2)	88.3
Trastuzumab	743	165 (22.2)	77.0

Unstratified hazard ratio for disease recurrence or death, 0.50 (95% CI, 0.39–0.64) P<0.001

Invasive Disease–free Survival (%) — *Months since Randomization*
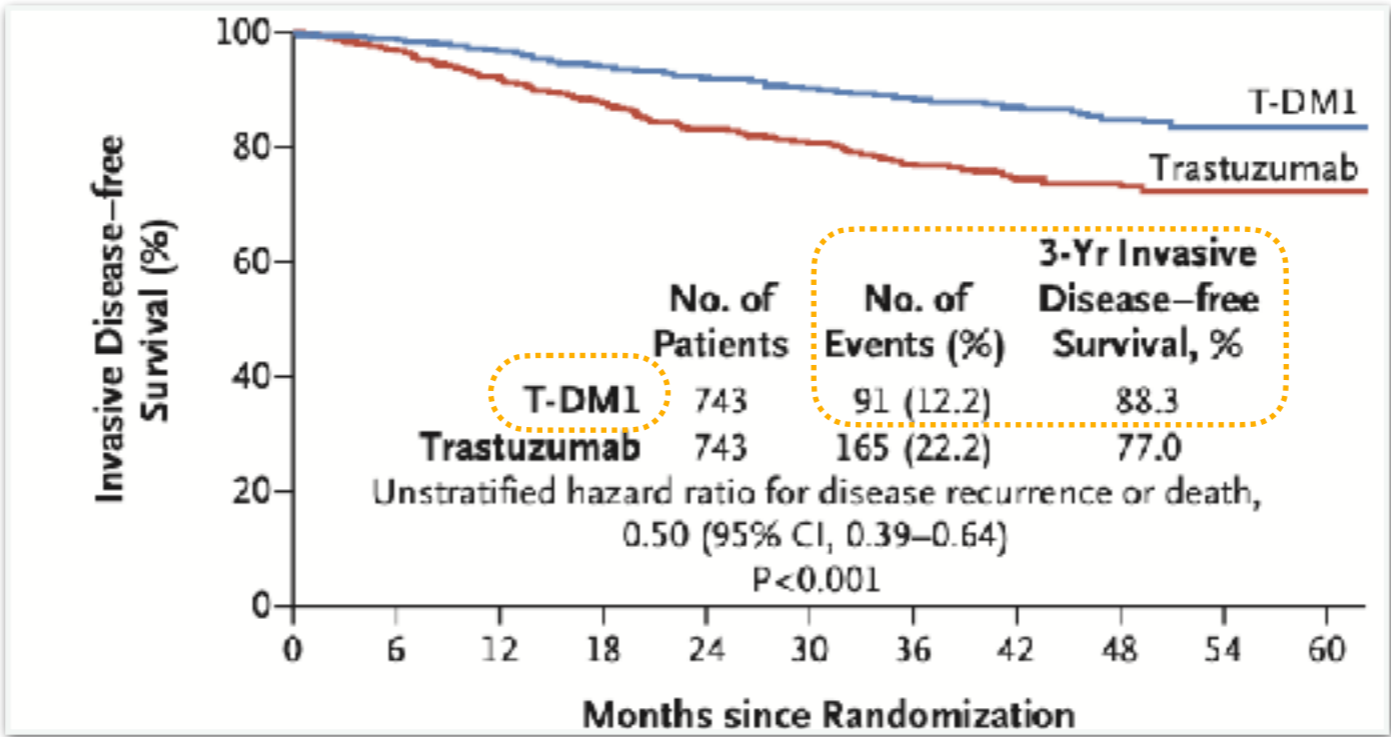
Risk of recurrence of invasive breast cancer or death was 50% lower with T-DM1 than with trastuzumab alone

Herceptin™ versus Kadcyla™

KATHERINE trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

Kadcyla™
(T-DM1)

743 patients

(8 years later…)

Herceptin™ versus Kadcyla™

KATHERINE trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

Kadcyla™
(T-DM1)

743 patients

(8 years later…)

461 (62.0%) patients were alive

521 (70.1%) patients were alive

Antibody-drug conjugate
Linker
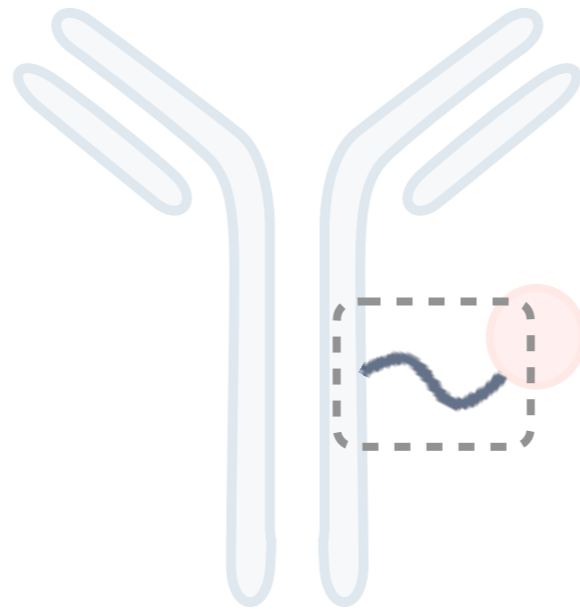
- **Bioconjugation**

- **Non-cleavable or cleavable**

Antibody-drug conjugate
Linker
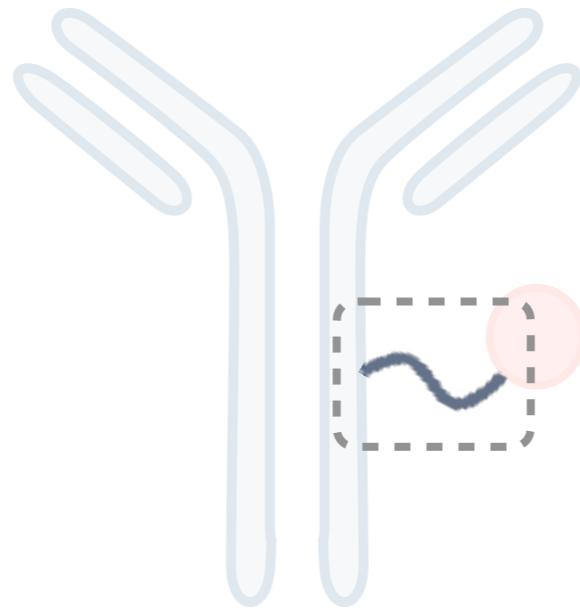
– Bioconjugation

– Non-cleavable or cleavable

Linker

Bioconjugation
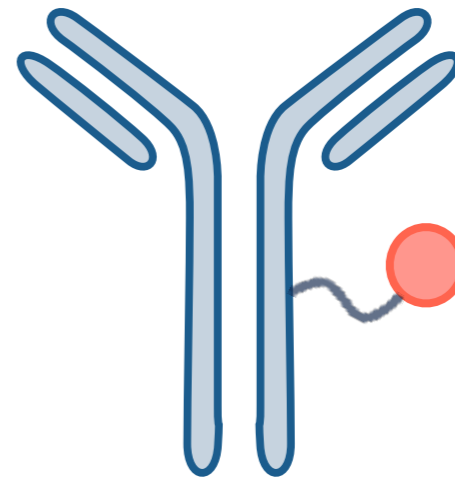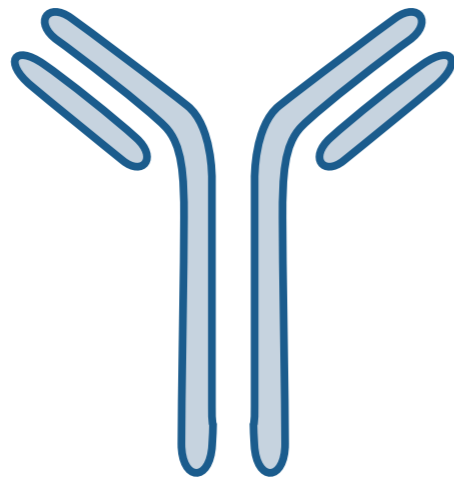
Linker

Bioconjugation
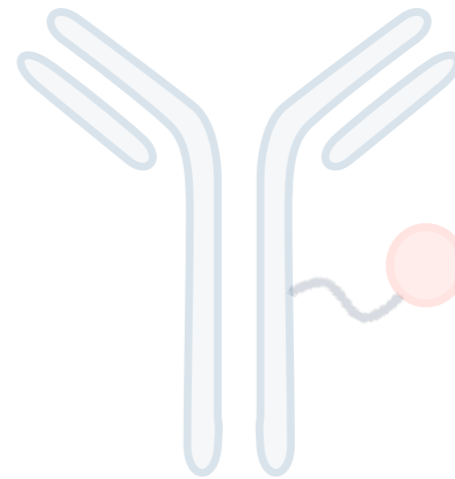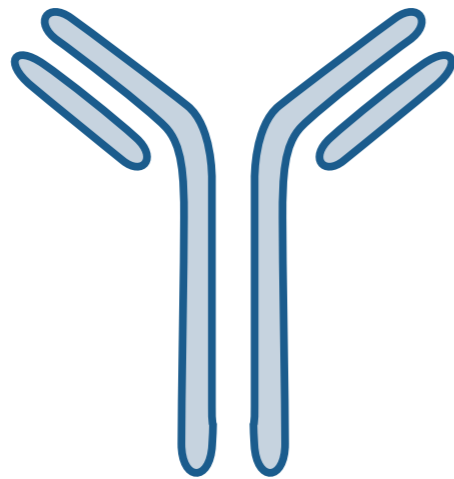
Biomolecules are sensitive

Linker
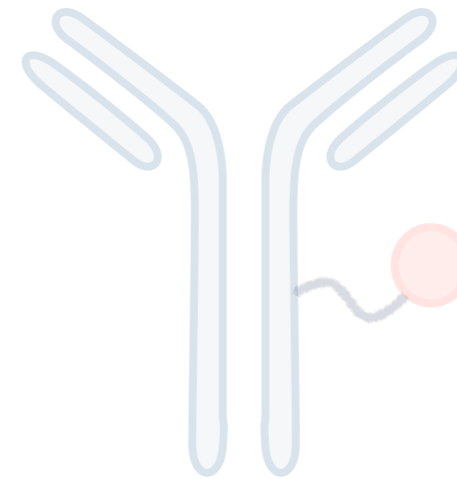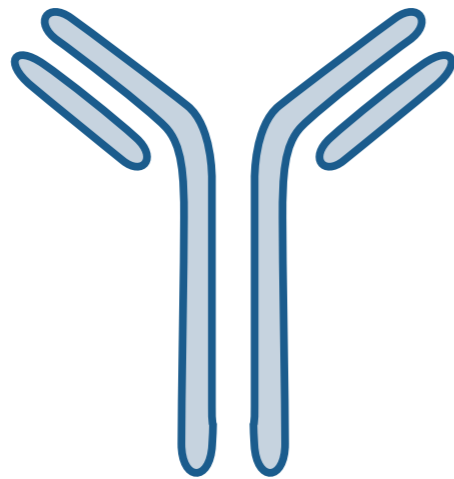
Bioconjugation

Biomolecules are sensitive

Linker
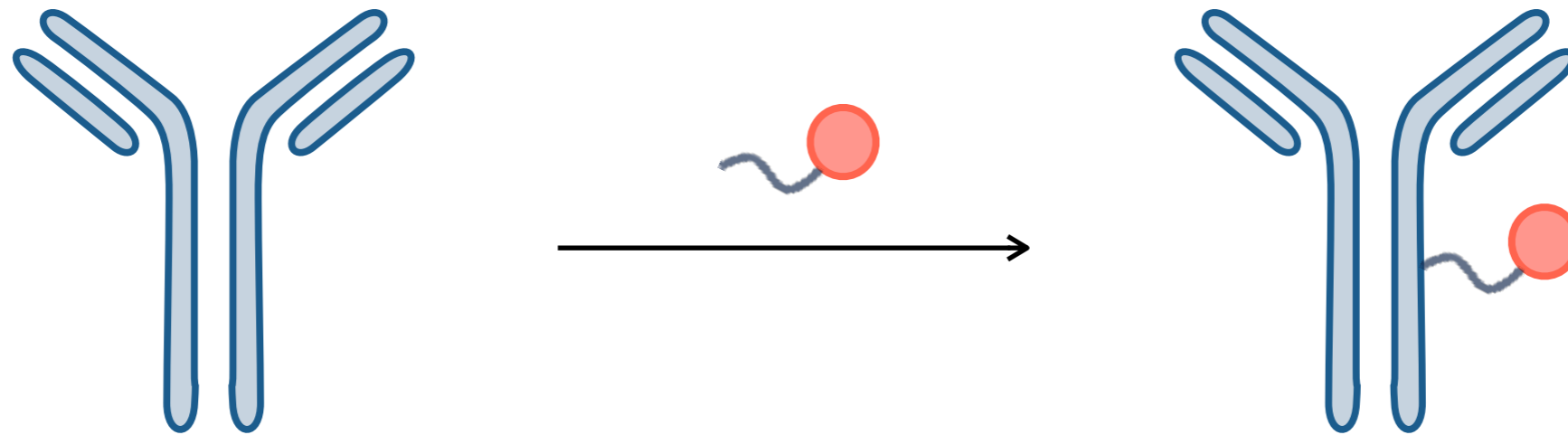
Bioconjugation

100 L ADC reactor (for conjugation)

"Shaken, not stirred"

Linker

Bioconjugation
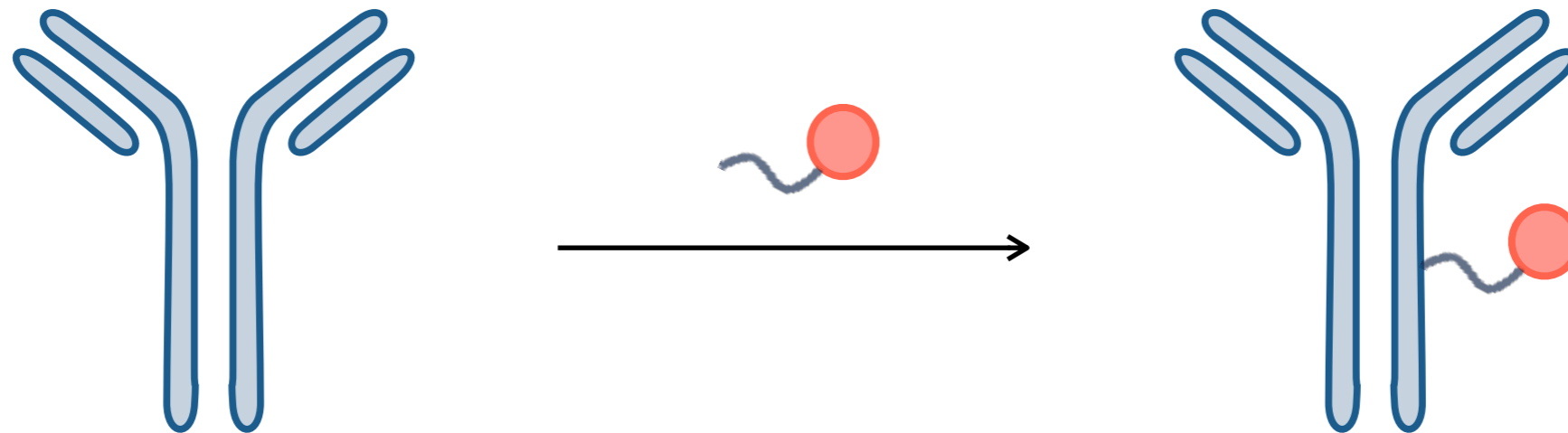
Bioconjugation Criteria

✓ *To avoid antibody aggregation*

✓ *To minimise antibody denaturation*

✓ *To minimise the degradation of linker-drug compounds*

✓ *To ensure product stability*
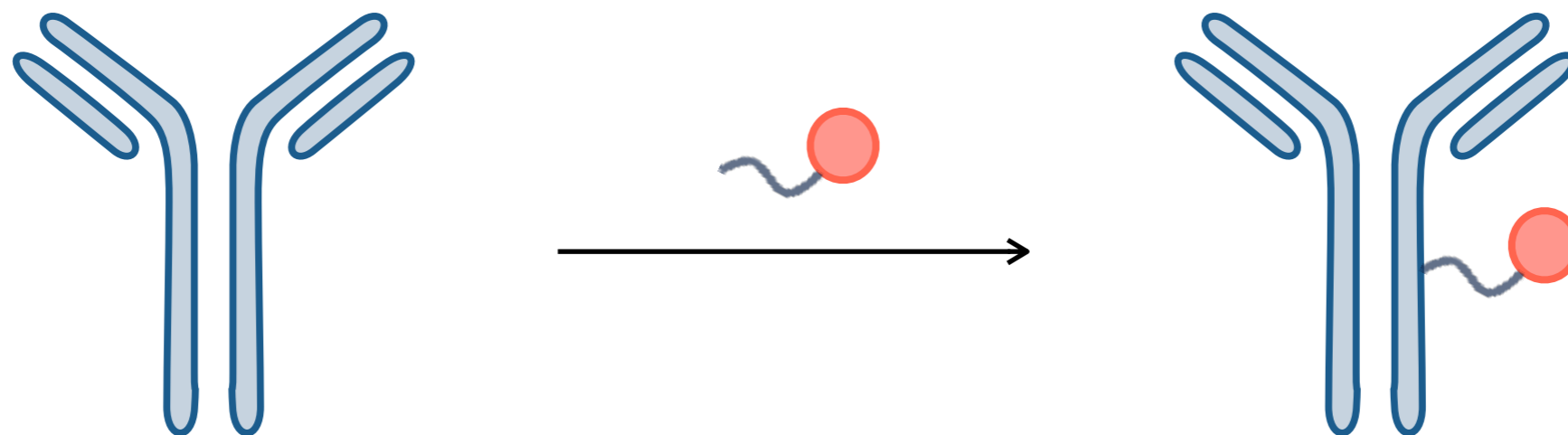
Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker

Bioconjugation

Bioconjugation Criteria

✓ *Aqueous*

✓ *pH neutral*

✓ *Below 37 °C*

✓ *Low concentration*

✓ *Atom-economic*

✓ *Rapid*

Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker
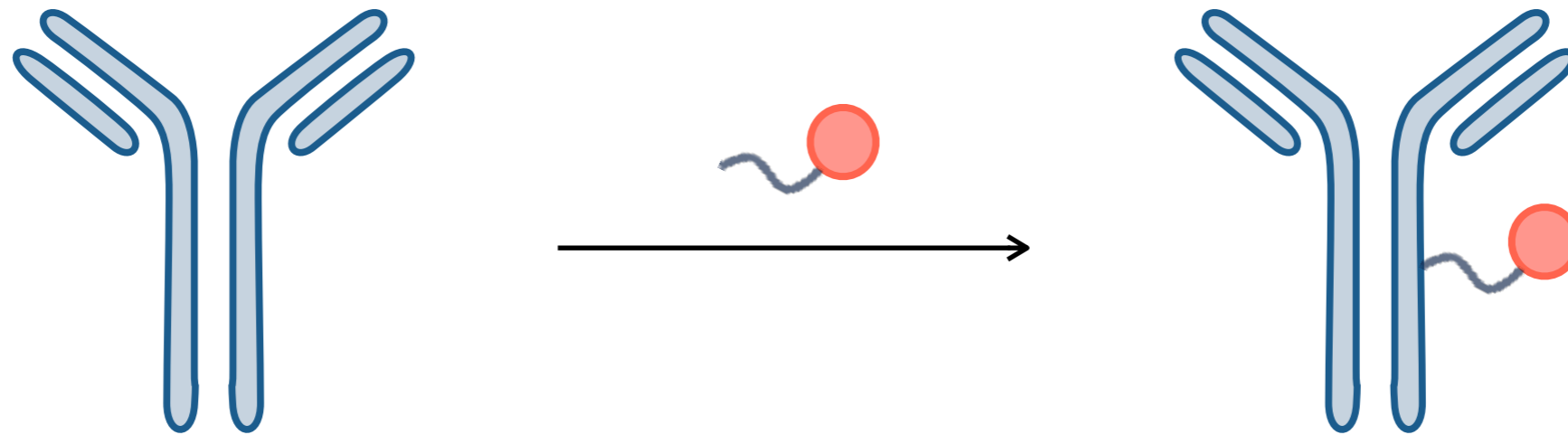
Bioconjugation

Bioconjugation Criteria

✔ **Aqueous**

✔ **pH neutral**

✔ **Below 37 °C**

✔ **Low concentration**

✔ **Atom-economic**

✔ **Rapid**

Mild, physiological condition that does not disturb the quaternary structure of antibody
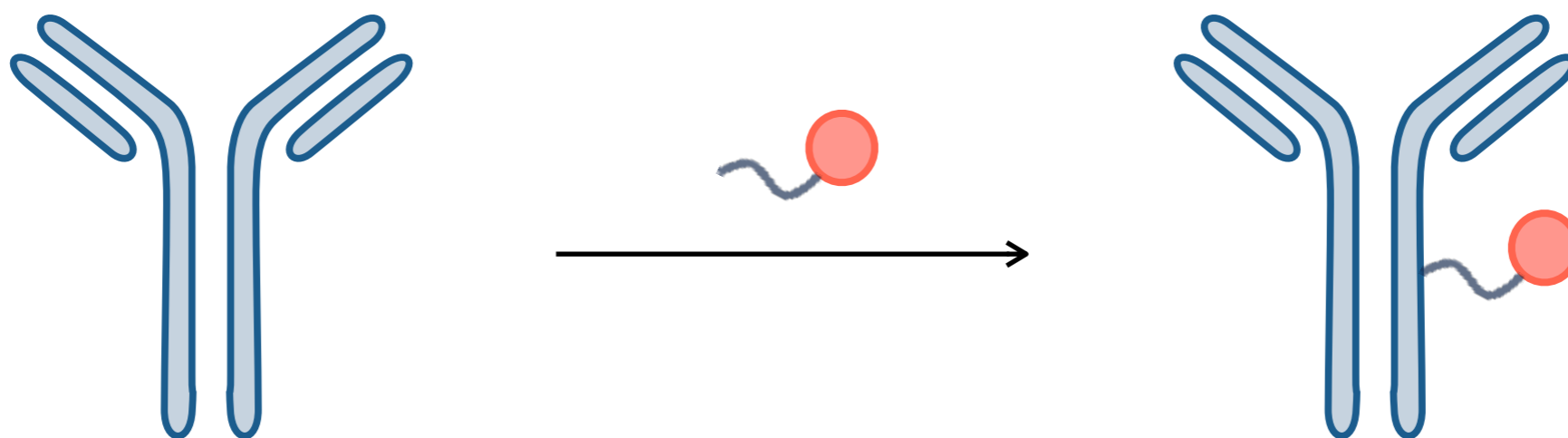
Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker

Bioconjugation

Bioconjugation Criteria

✔ *Aqueous*

✔ *pH neutral*

✔ *Below 37 °C*

✔ *Low concentration*

✔ *Atom-economic*
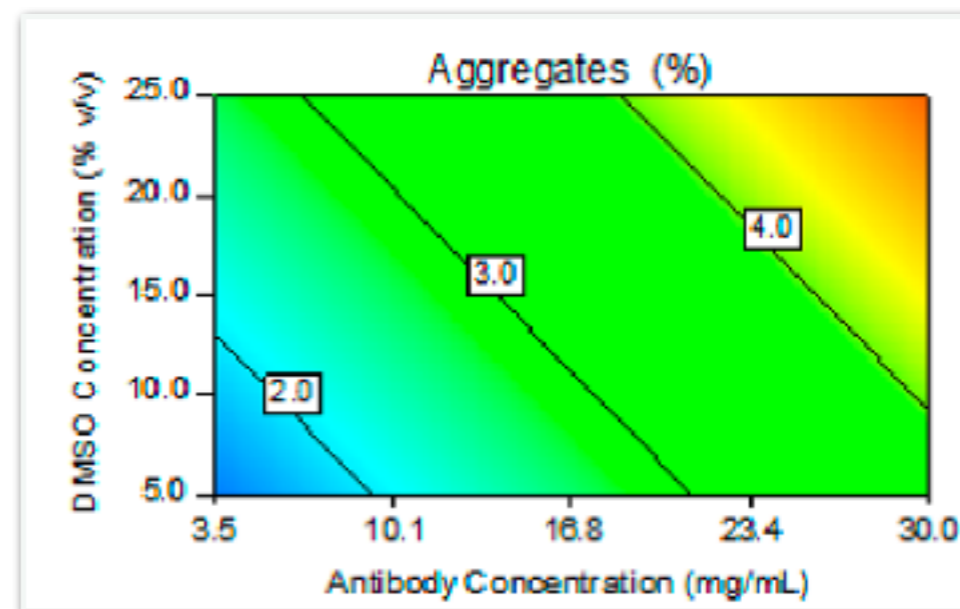
✔ *Rapid*

Aggregation(%) rises with organic solvent (% v/v) and Ab concentration (mg/mL)
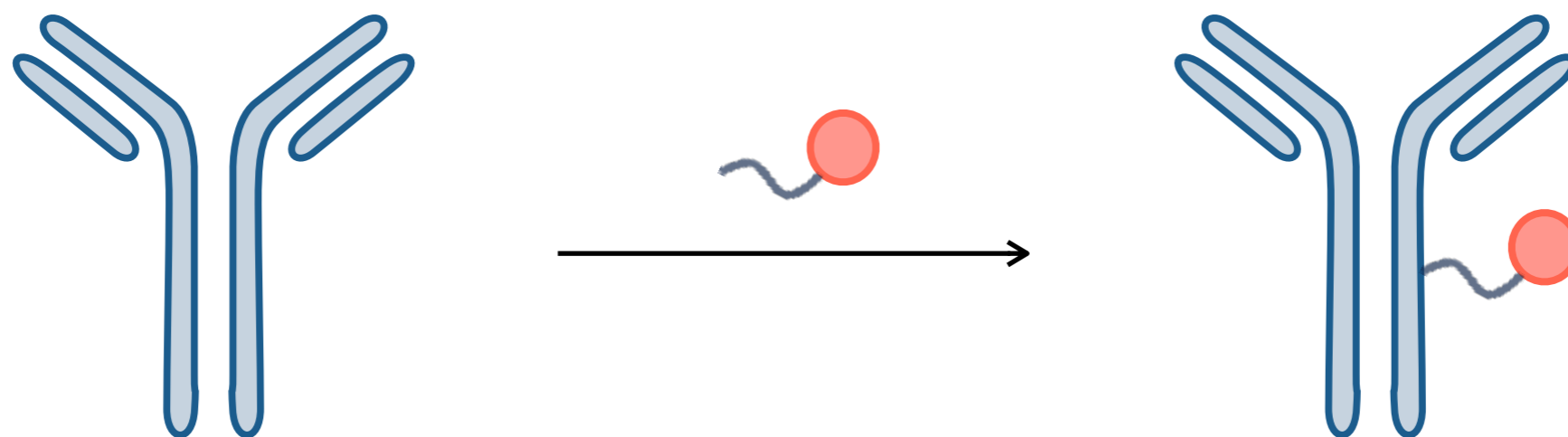
Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

Linker

Bioconjugation

Bioconjugation Criteria

✔ **Aqueous**

✔ **pH neutral**

✔ **Below 37 °C**

✔ **Low concentration**

✔ **Atom-economic**

✔ **Rapid**

Common production condition

1-5 mg/mL of mAb
(5-30 μM)
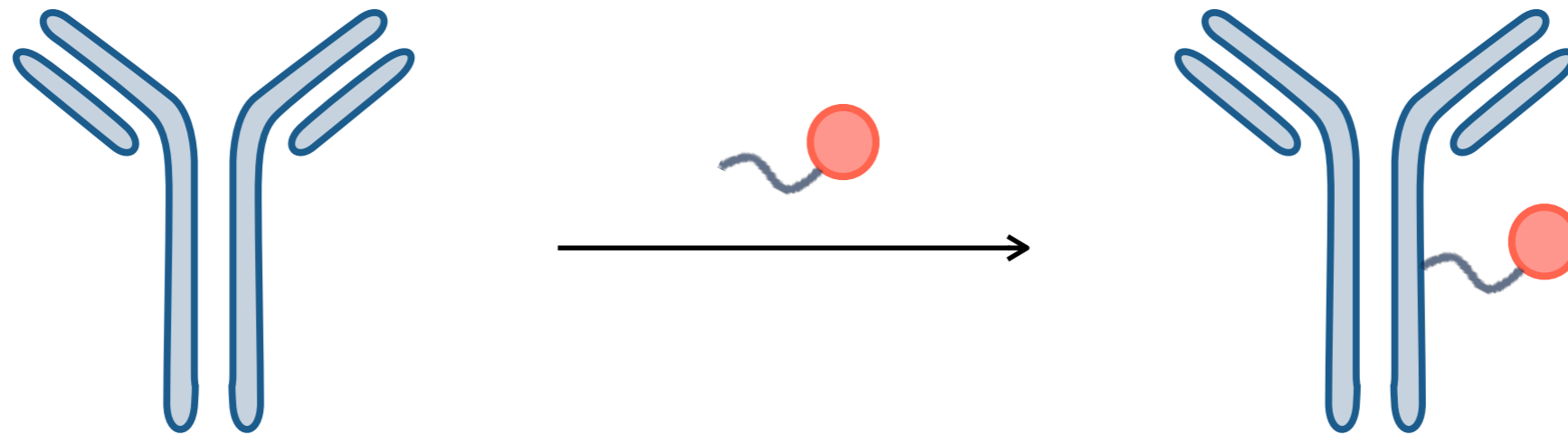
Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

Linker

Bioconjugation

Bioconjugation Criteria

- ✔ **Aqueous**
- ✔ **pH neutral**
- ✔ **Below 37 °C**
- ✔ **Low concentration**
- ✔ **Atom-economic**
- ✔ **Rapid**

Molar equivalence

**: **

~ 1 : 50
(DAR ~ 4)
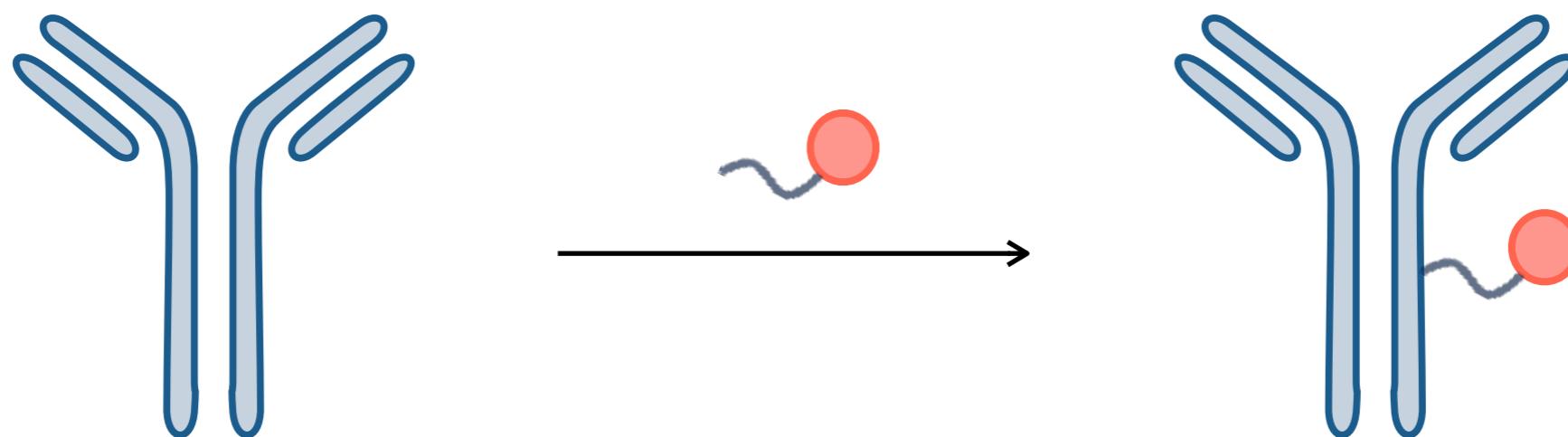
Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

Linker
Bioconjugation

Bioconjugation Criteria

✔ **Aqueous**

✔ **pH neutral**

✔ **Below 37 °C**

✔ **Low concentration**

✔ **Atom-economic**

✔ *Rapid*

Rate constant = [mAb][Linker-Drug]

Second-order rate constant (k_2) > 10 $M^{-1}s^{-1}$

With this kinetics,
when [Antibody] = 10-50 μM,
97% conversion within 1 h,
with 10 equiv. of linker-drug to
achieve a DAR of 1

'C' in ADC

Lysine-NHS ester conjugation

$$k_2 \sim 10^3 \ M^{-1}s^{-1}$$

Cline, G. W.; Hanna, S. B. *J. Org. Chem.* **1988**, *53* (15), 3583–3586

'C' in ADC

Lysine-NHS ester conjugation

$k_2 \sim 10^3 \ M^{-1} s^{-1}$

Kadcyla™
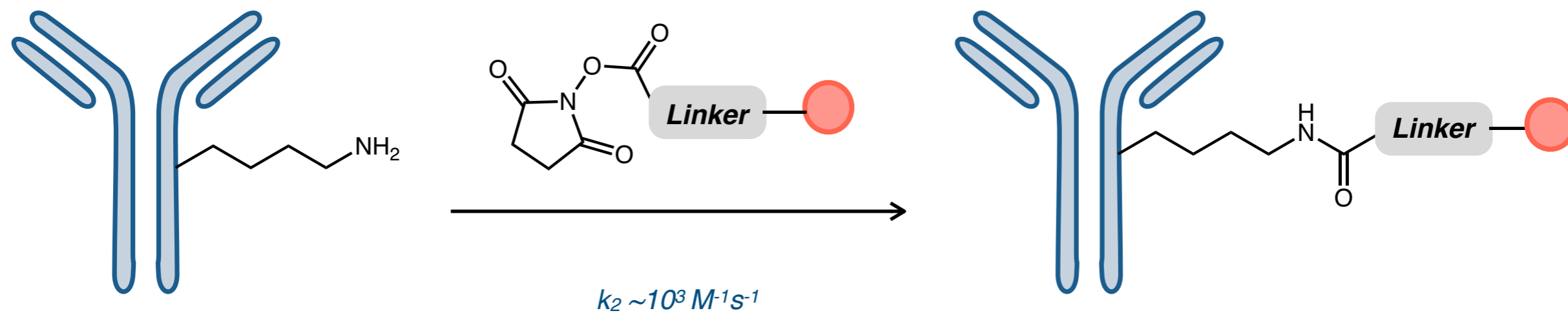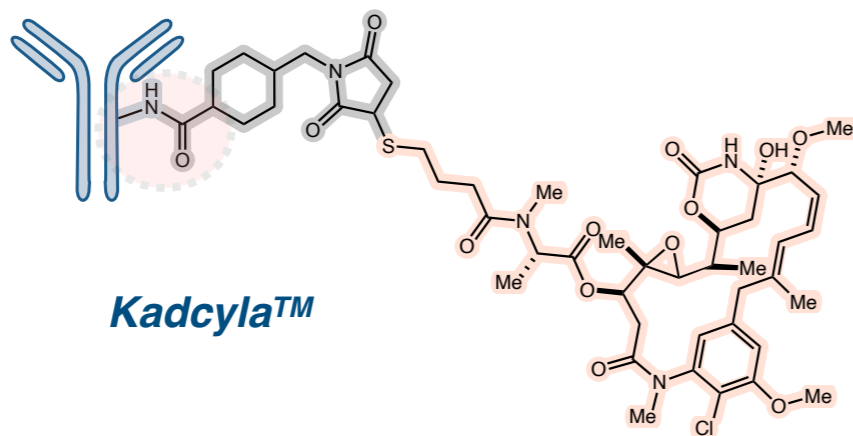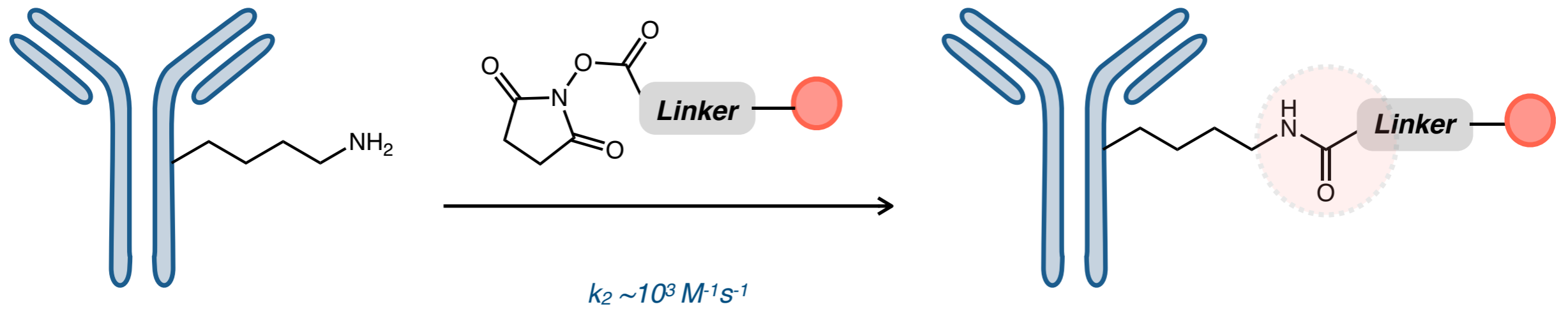
Mylotarg™, Besponsa™
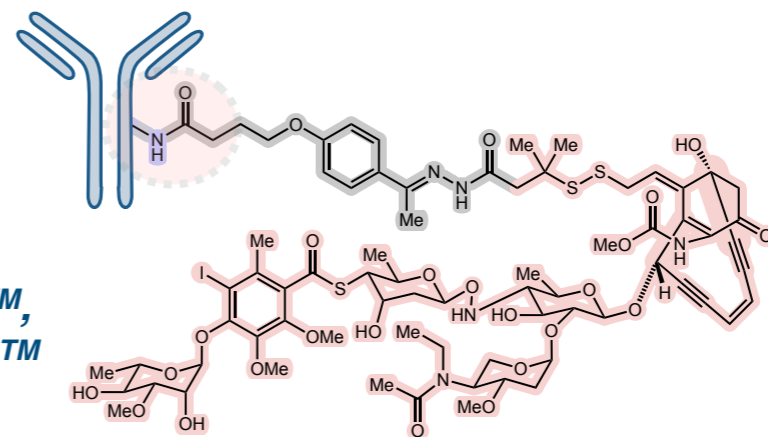
Lysine-NHS ester conjugation
Regioselectivity and chemoselectivity
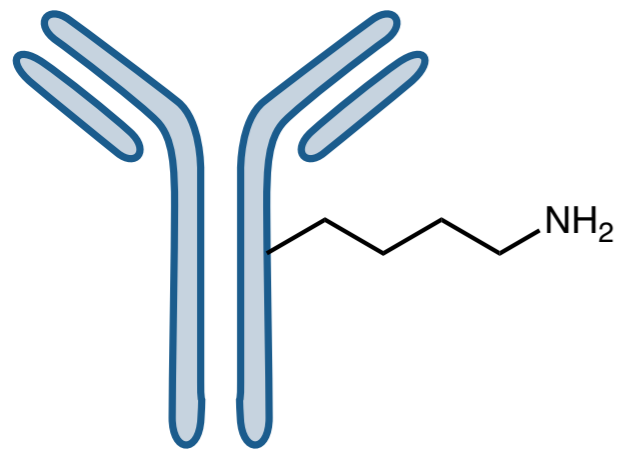
~ 80 accessible lysine sites

$k_2 \sim 10^3\ M^{-1}s^{-1}$

Linker

Linker
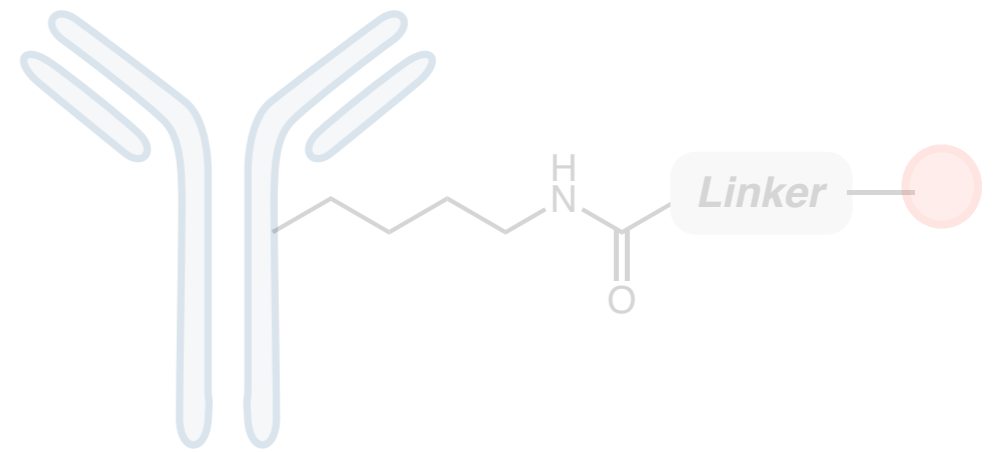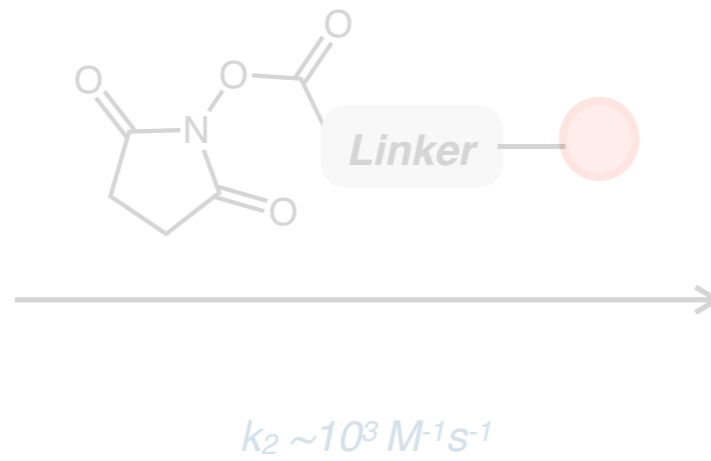
Lysine-NHS ester conjugation
Regioselectivity and chemoselectivity
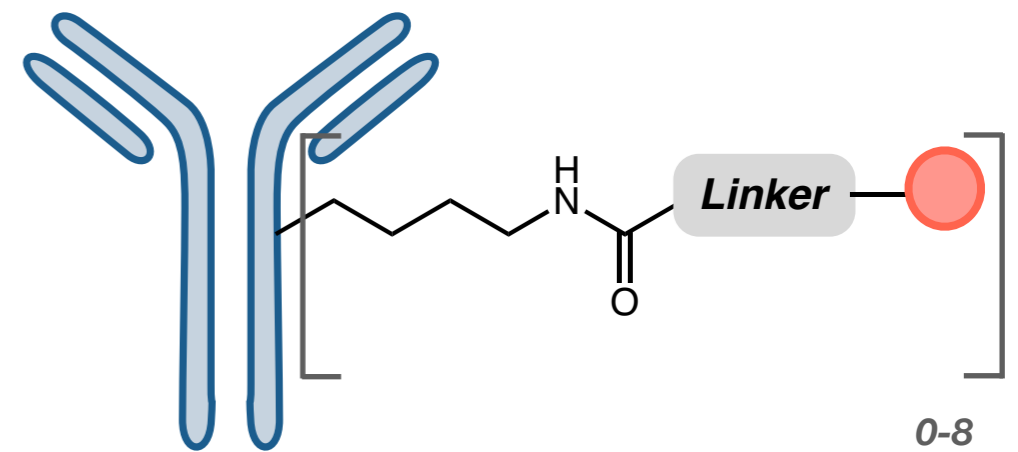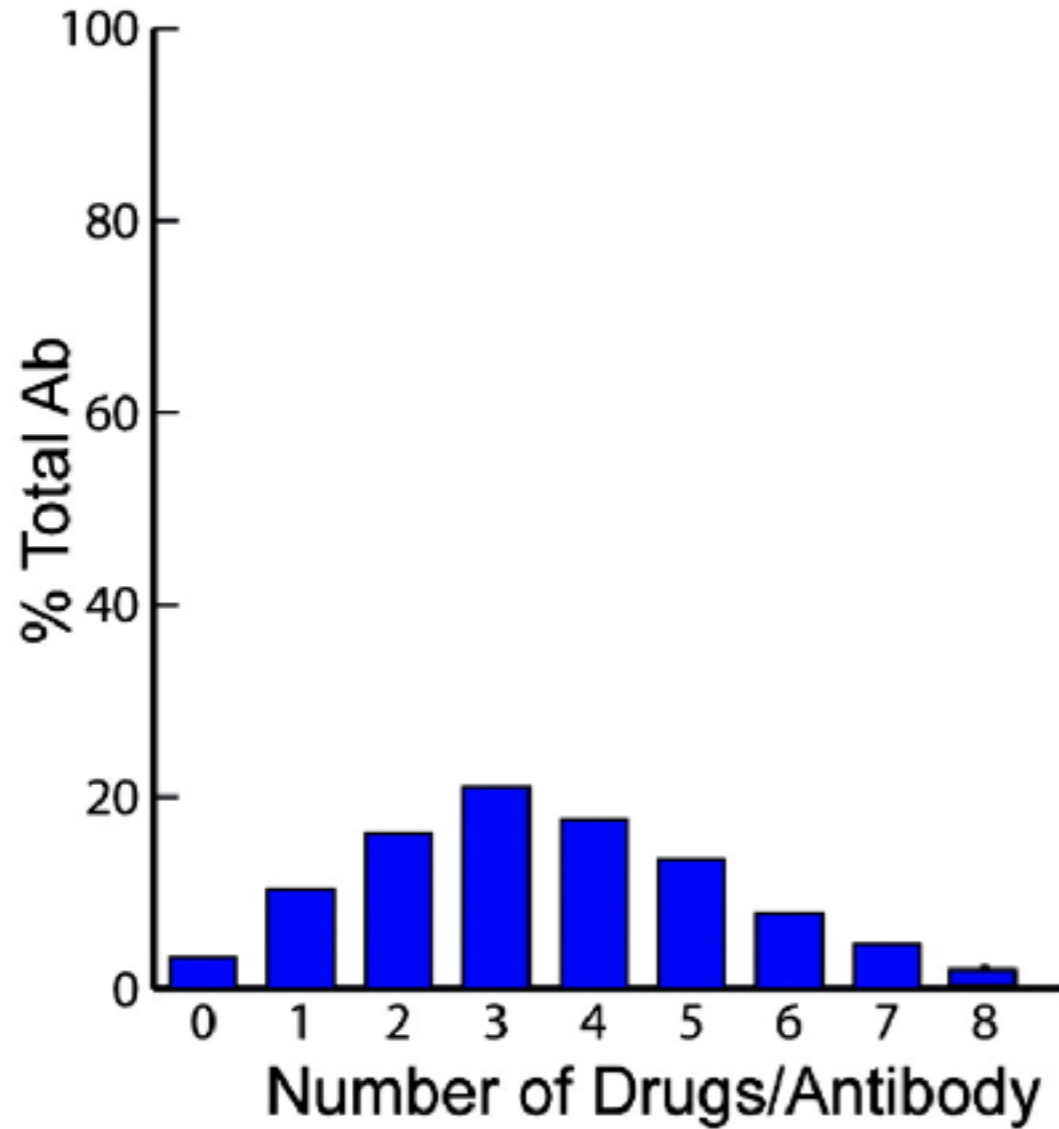
Heterogeneous mixtures with different DARs

Lysine-NHS ester conjugation

Regioselectivity and chemoselectivity
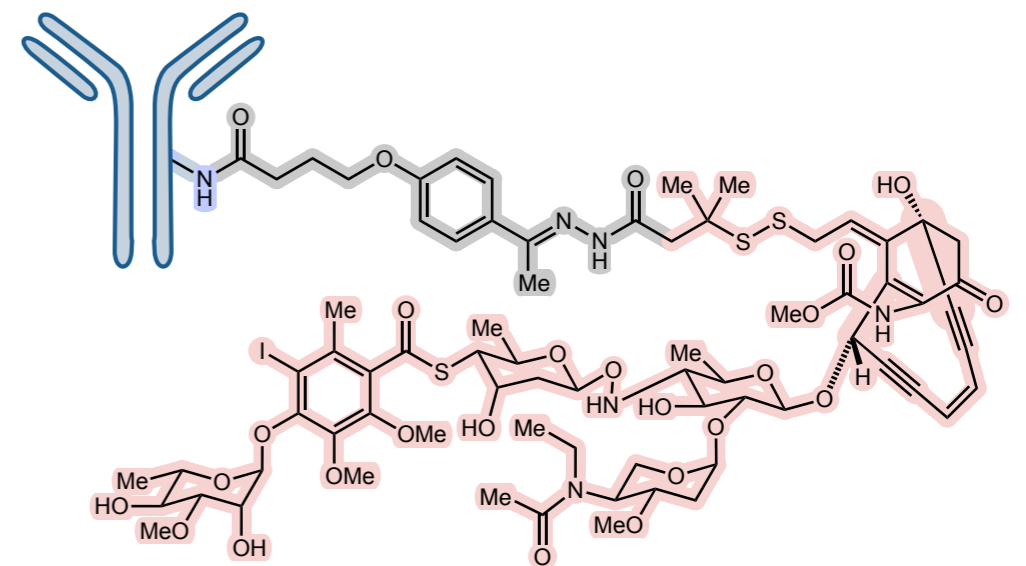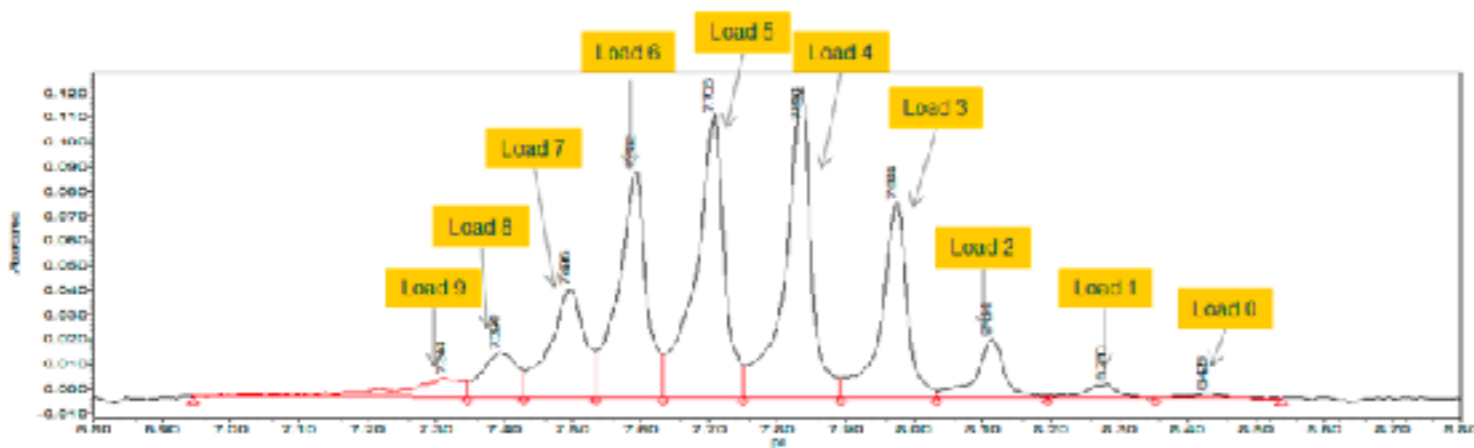
Mylotarg™   **Average DAR = 1.5**

Besponsa™   **Average DAR = 6**

ADCs with the same DAR are likely to be regioisomers

Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

'C' in ADC

Cysteine-maleimide conjugation
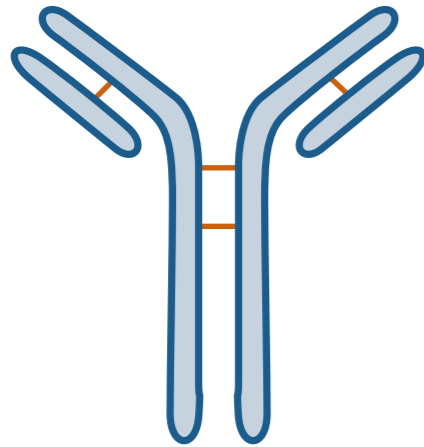
**4 Disulfide bonds
(IgG1)**

'C' in ADC

Cysteine-maleimide conjugation
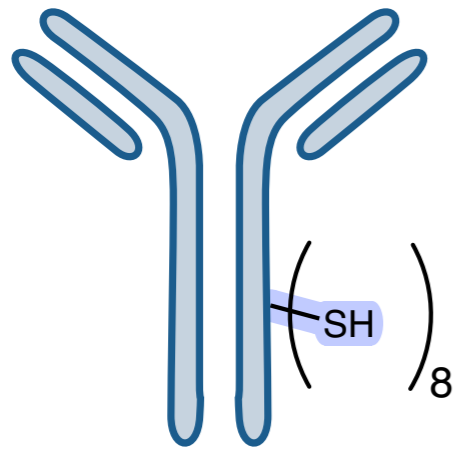
Reduced (with DTT/TCEP)

'C' in ADC

Cysteine-maleimide conjugation

Reduced (with DTT/TCEP)

$k_2 \sim 10^2\ M^{-1}s^{-1}$

Linker

Linker

F.-J. Chen, J. Gao, *Chem. Eur. J.* **2022**, *28*, e202201843

'C' in ADC

Cysteine-maleimide conjugation

$k_2 \sim 10^2\ M^{-1}s^{-1}$

Reduced (with DTT/TCEP)

c.f. Iodoacetamide

F.-J. Chen, J. Gao, *Chem. Eur. J.* **2022**, *28*, e202201843
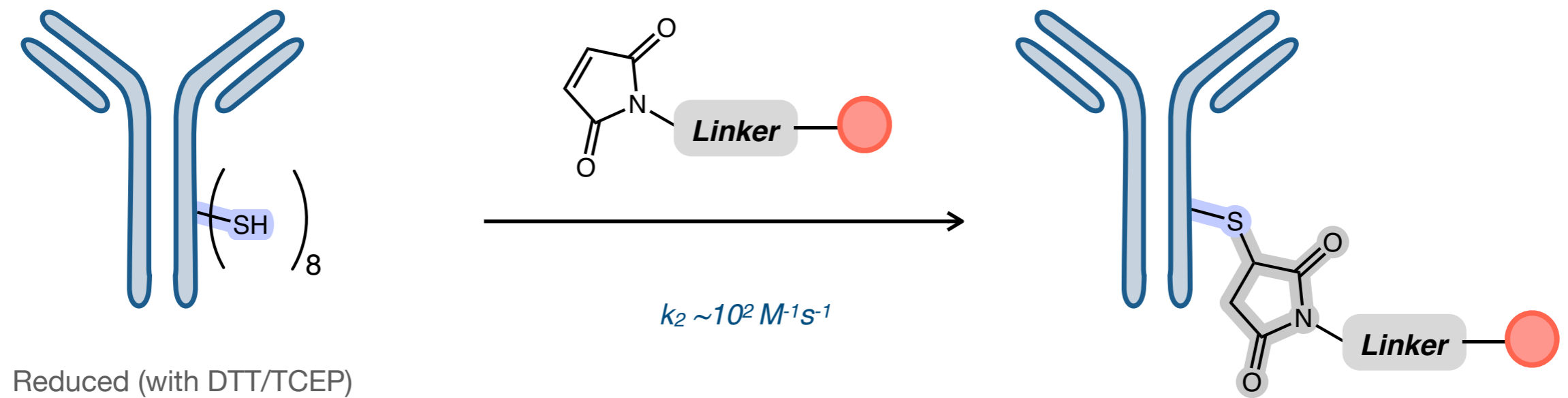
'C' in ADC

Cysteine-maleimide conjugation

Reduced (with DTT/TCEP)

$k_2 \sim 10^2\ M^{-1}s^{-1}$
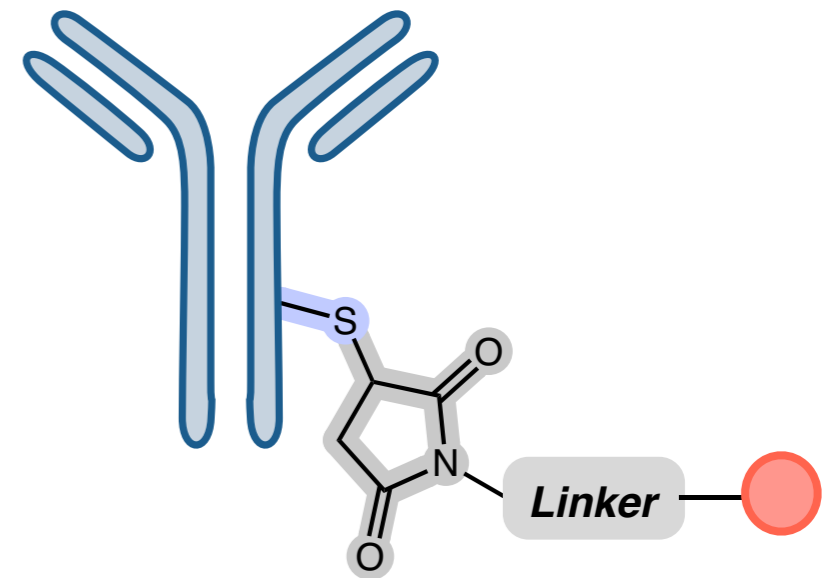
c.f. Iodoacetamide
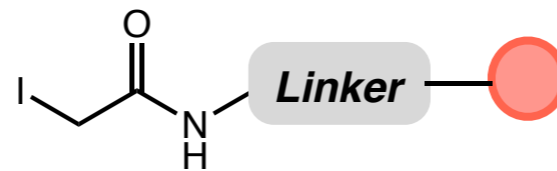
$k_2 \sim 0.6\ M^{-1}s^{-1}$

More than 160-fold slower

9 out of 12 FDA-approved ADCs use cysteine-maleimide conjugation

'C' in ADC

Cysteine-maleimide conjugation
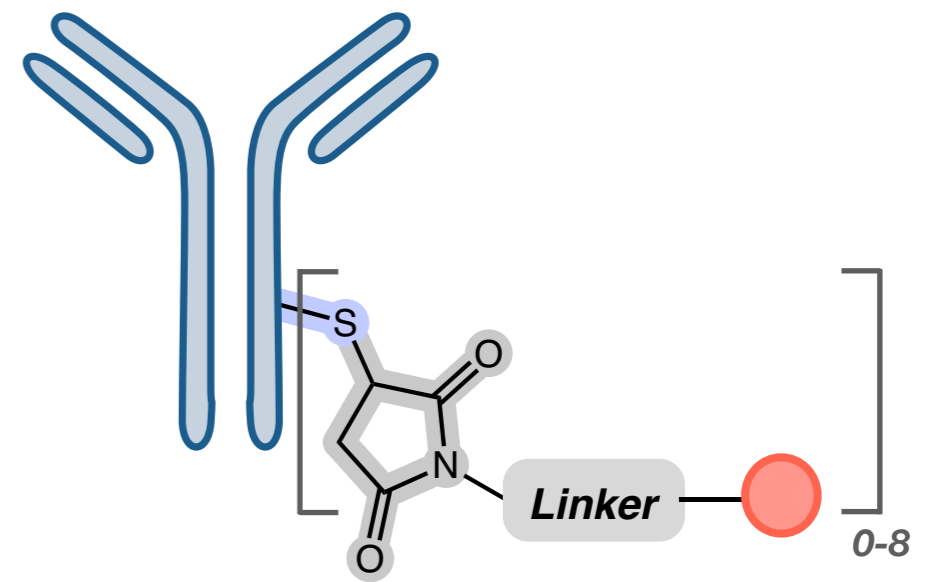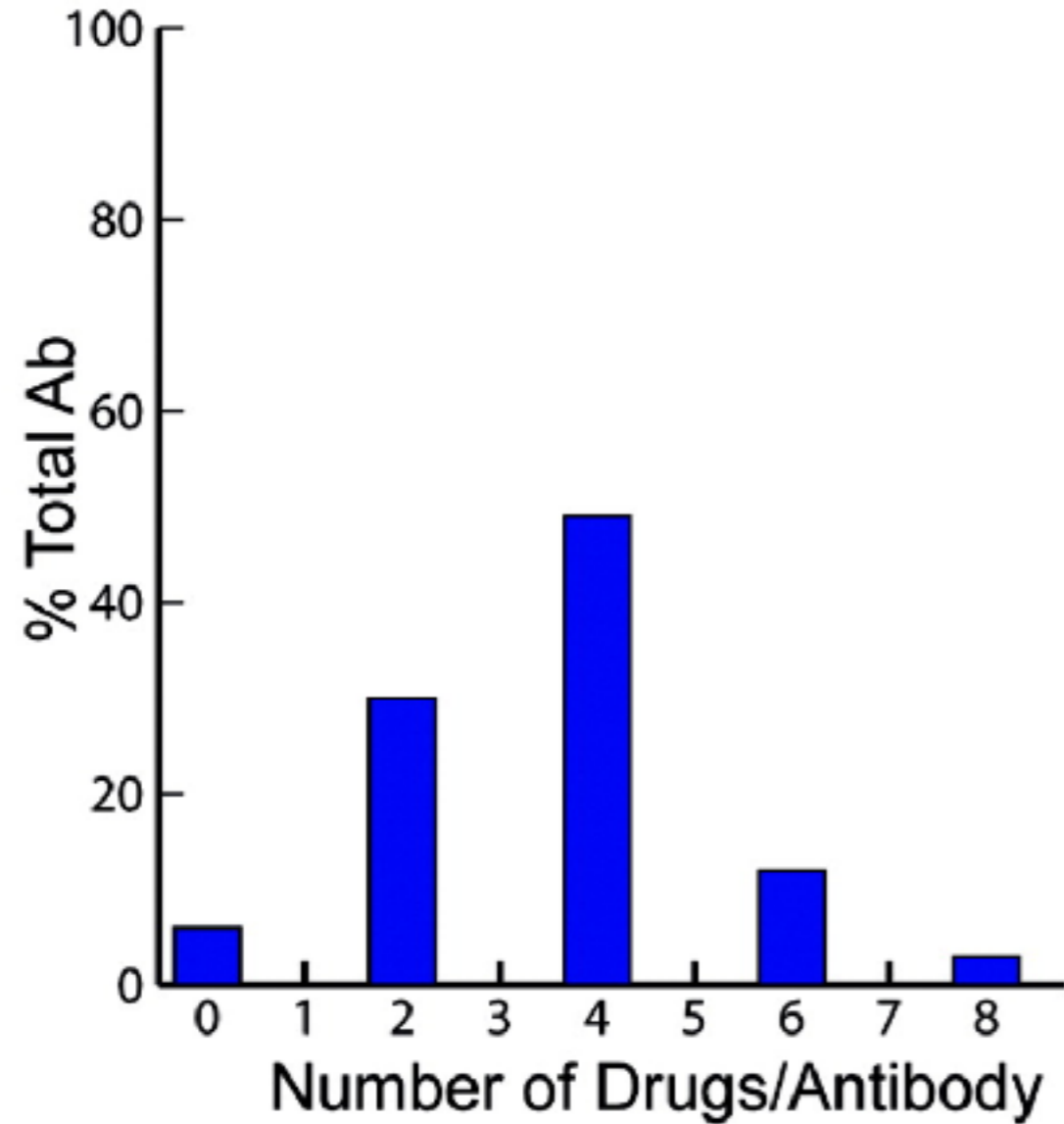
Less heterogeneity compared to the lysine conjugation method

Cysteine-maleimide conjugation
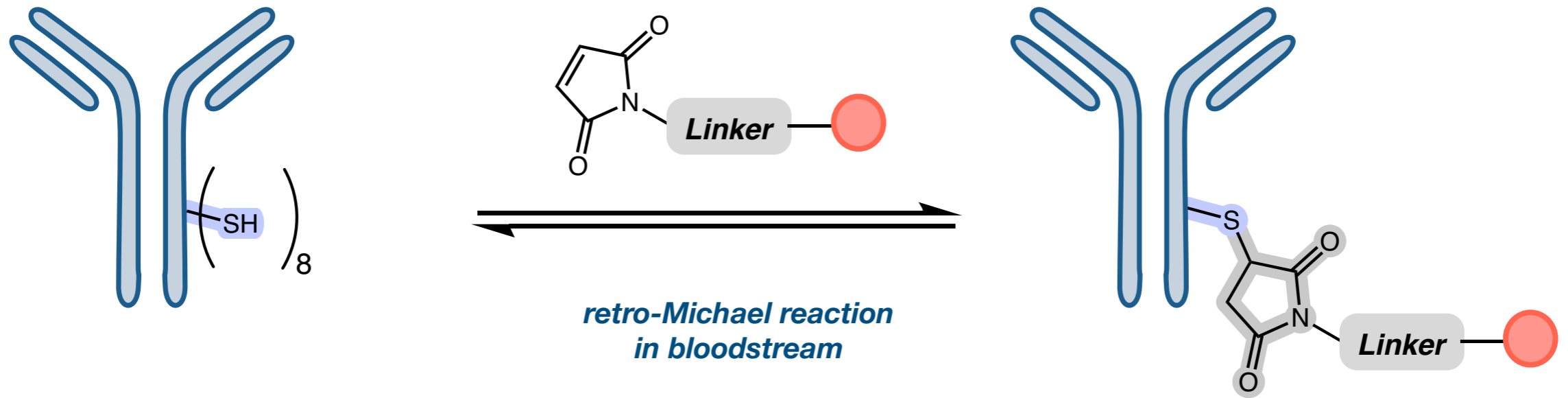
Deconjugation via retro-Michael reaction

Linker

retro-Michael reaction
in bloodstream

Cysteine-maleimide conjugation
Deconjugation via retro-Michael reaction

Off-target toxicity

Linker

SH)₈

retro-Michael reaction in bloodstream

Δ = 1342 Da
≈ mc-VC-payload

S

Linker

100% drug loss after 144 hours in human plasma

Anal. Chem. **2016**, *88* (9), 4979–4986

Cysteine-maleimide conjugation
Deconjugation via retro-Michael reaction

Off-target toxicity

retro-Michael reaction in bloodstream

Linker

Linker
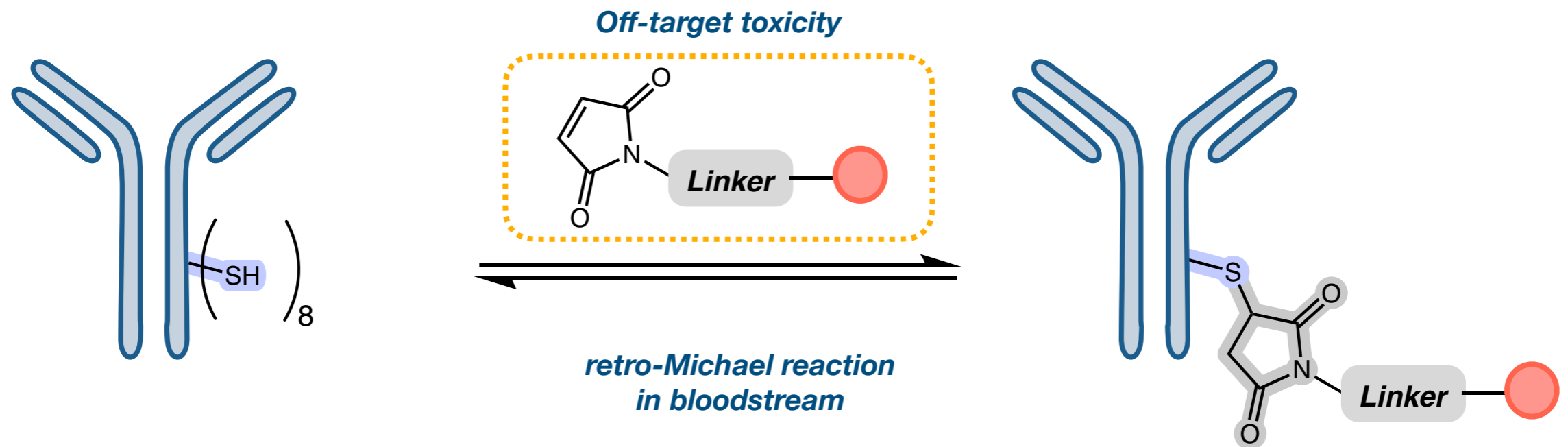
Key G3/4 toxicities of ADCs in the clinic are likely off-target and related to payload

Masters, J. C.; Nickens, D. J.; Xuan, D.; Shazer, R. L.; Amantea, M. *Invest. New Drugs* **2018**, *36* (1), 121–135

Cysteine-maleimide conjugation

Formation of diastereoisomers
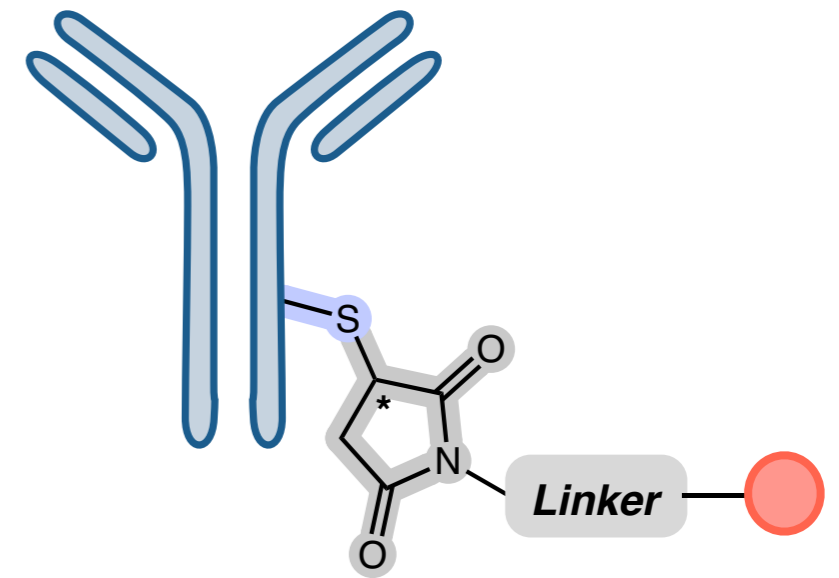
Cysteine-maleimide conjugation

Formation of diastereoisomers

Hydrophobic interaction chromatogram (HIC)

(analytical/purification technique in ADC)

Multiple peaks in chromatogram (Different hydrophobicity profiles)
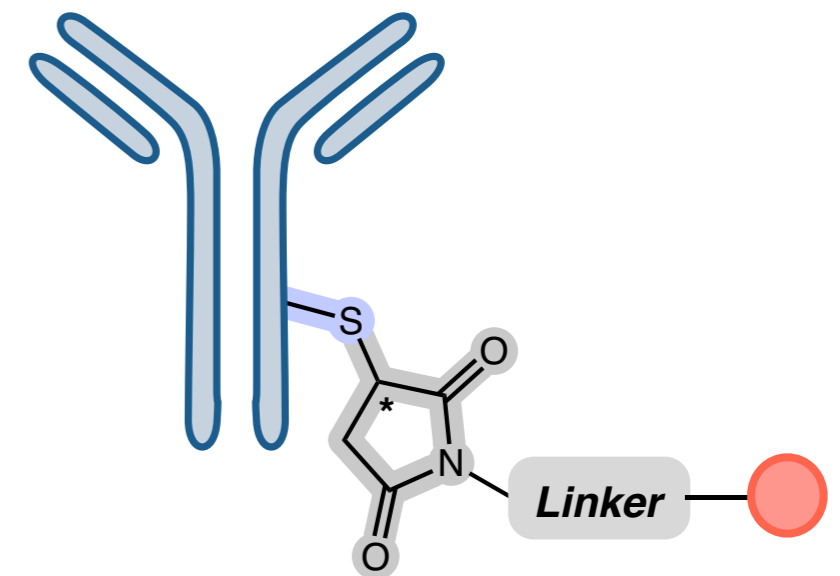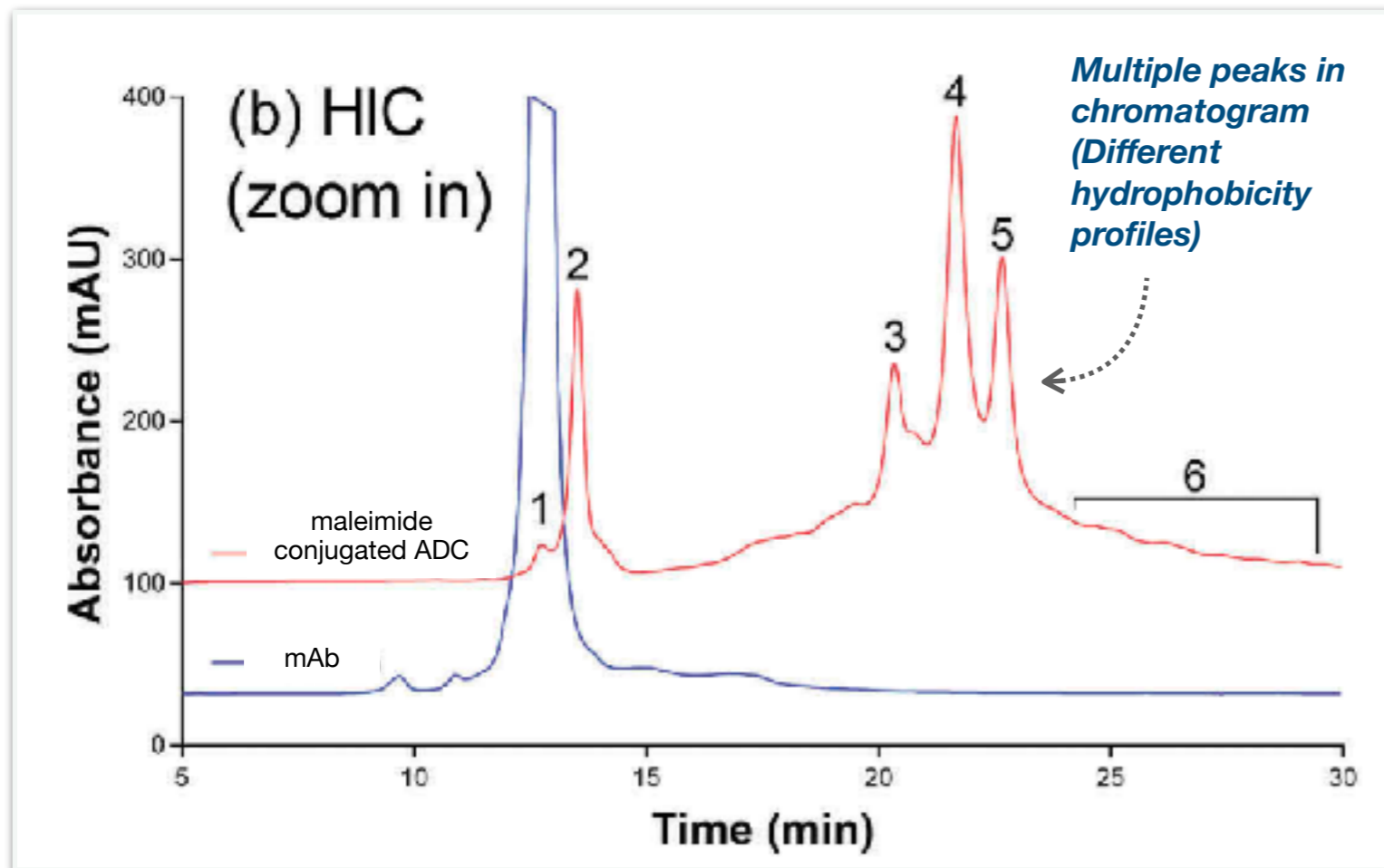
Stereoisomeric conjugation sites result in **differences in the three-dimensional orientation** *of the linker-drug payload on the antibody*

Complicates analysis/separation of ADCs

Parthemore, C.; Korman, S.; Thompson, C.; Wendeler, M.; Liu, D. *mAbs* **2019**, *11* (6), 1064–1076.

Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC
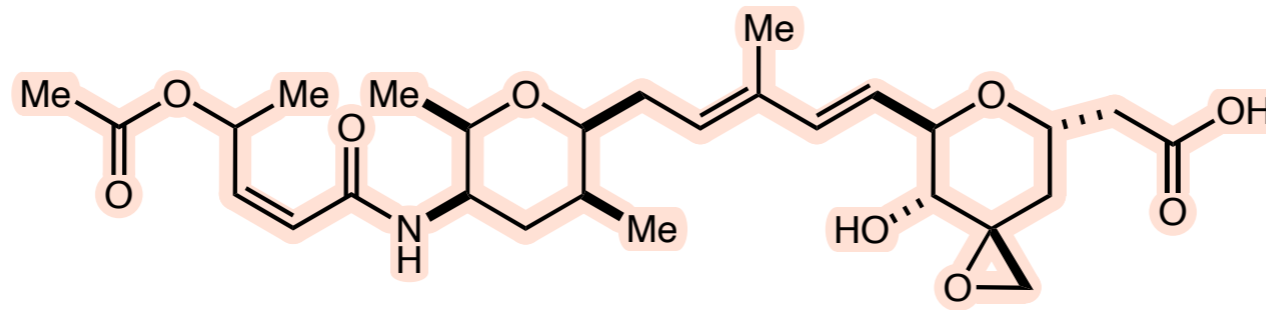
Thailanstatin A

$IC_{50} = 650\ nM$

Puthenveetil, S.; Sapra, P.; Gerber, H. P.; Ma, D.; Graziani, E. I. *Bioconjug. Chem.* **2016**, *27* (8), 1880–1888
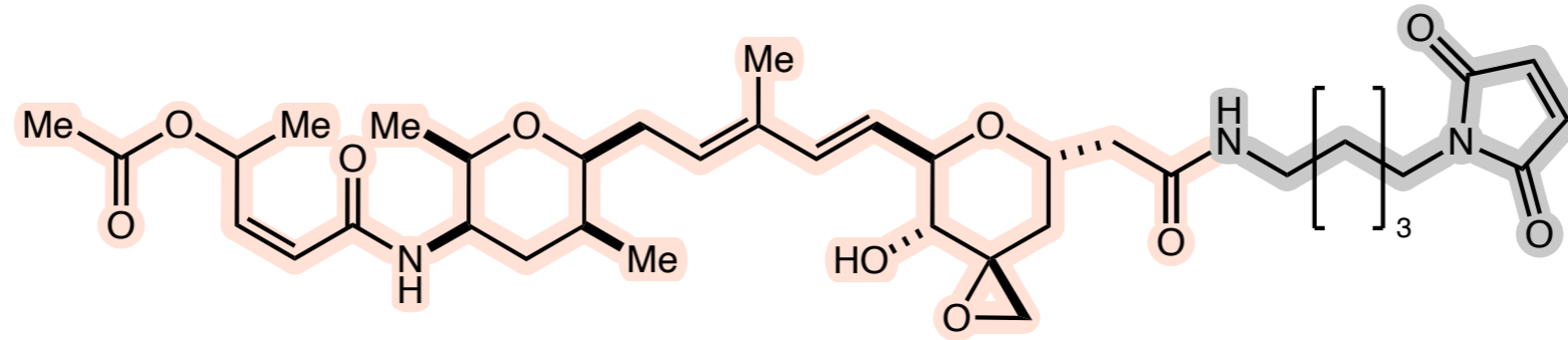
Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC

Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC

Electron-poor dienophile
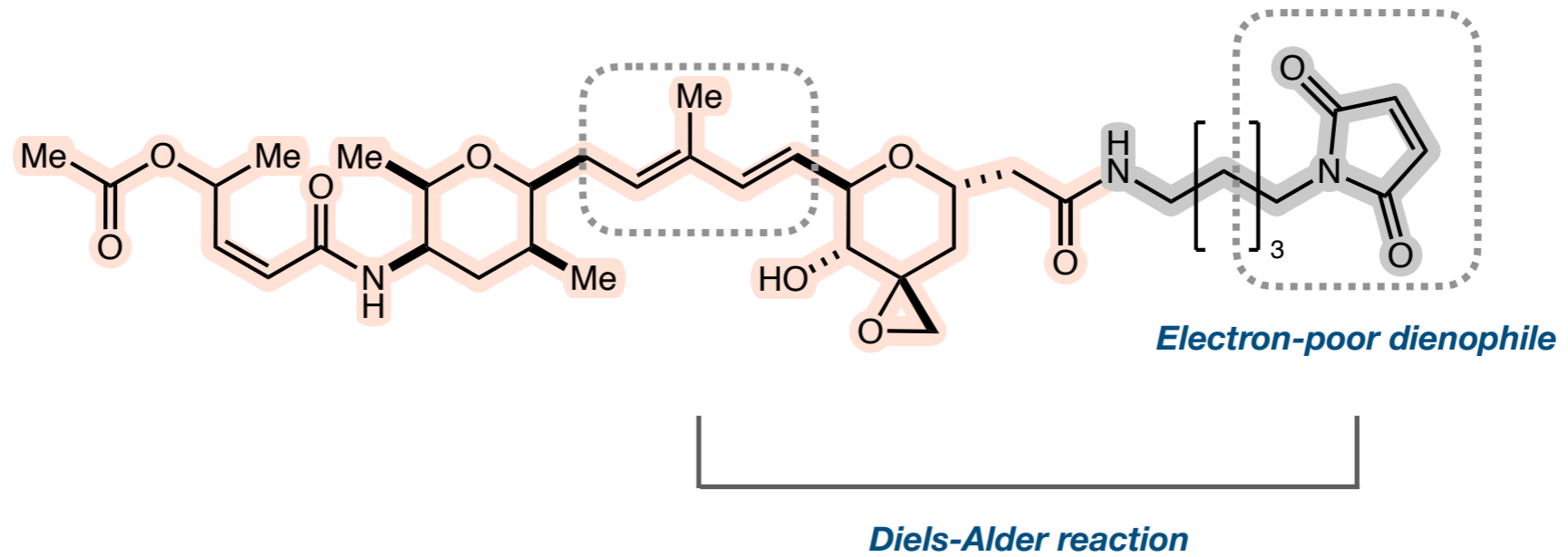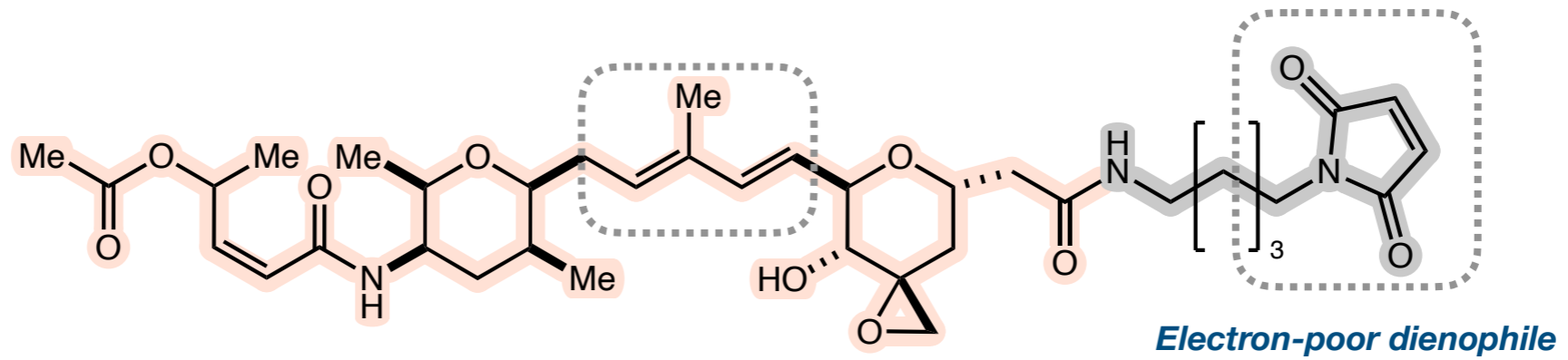
Diels-Alder reaction

Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC

Electron-poor dienophile

Diels-Alder reaction

Desired product (ADC)

Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC

Electron-poor dienophile

Diels-Alder reaction

Desired product (ADC)
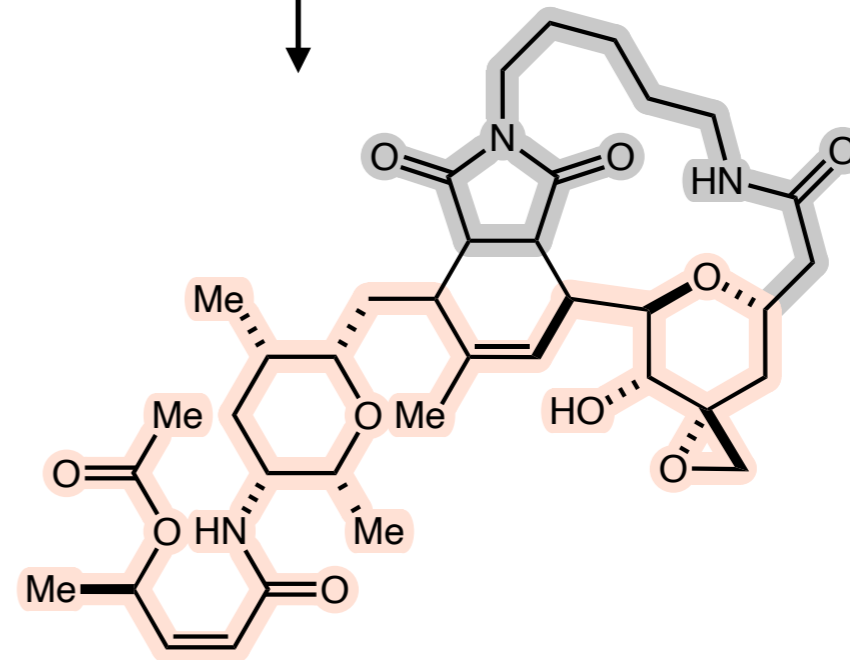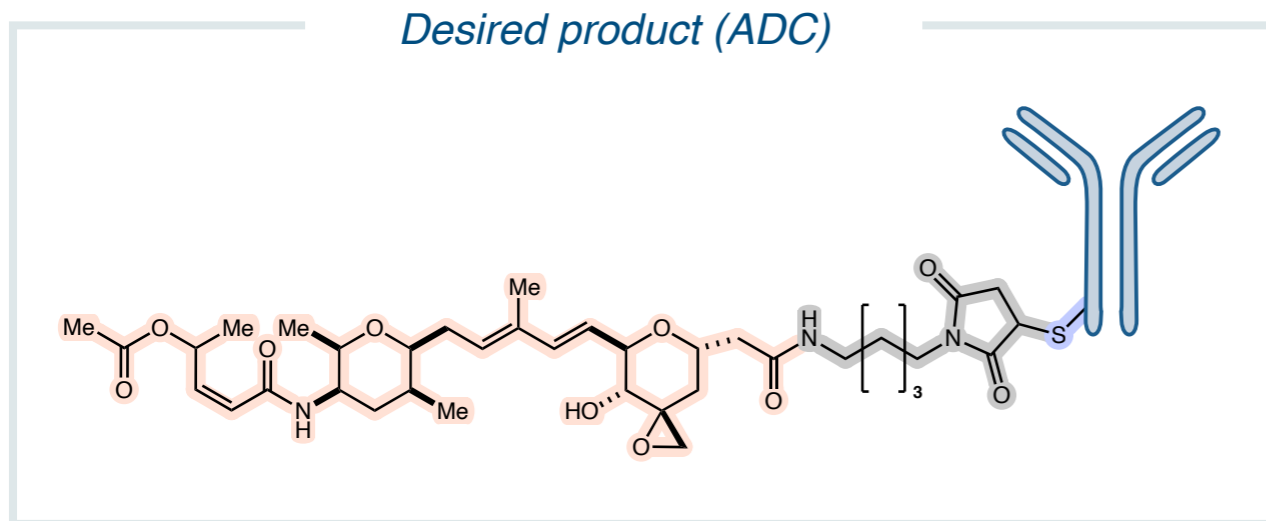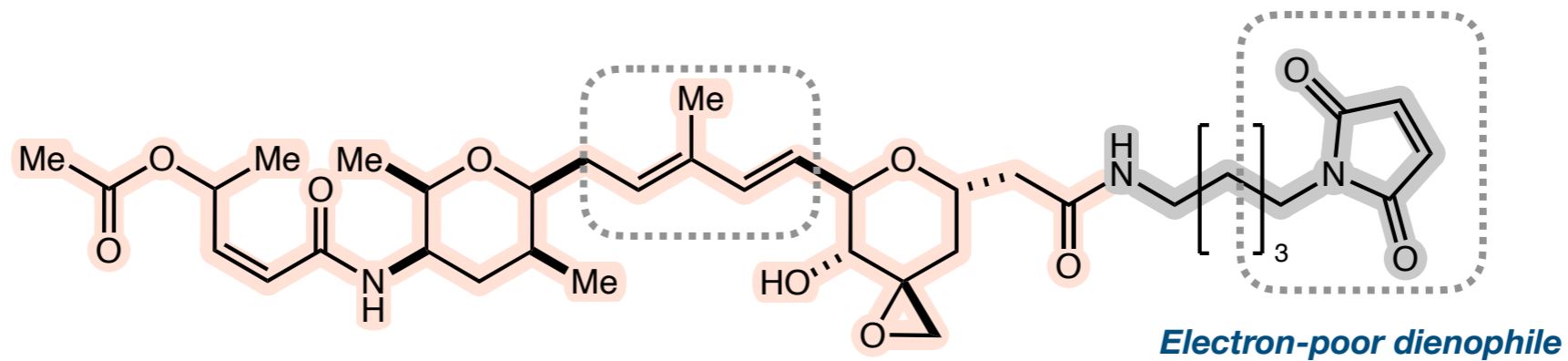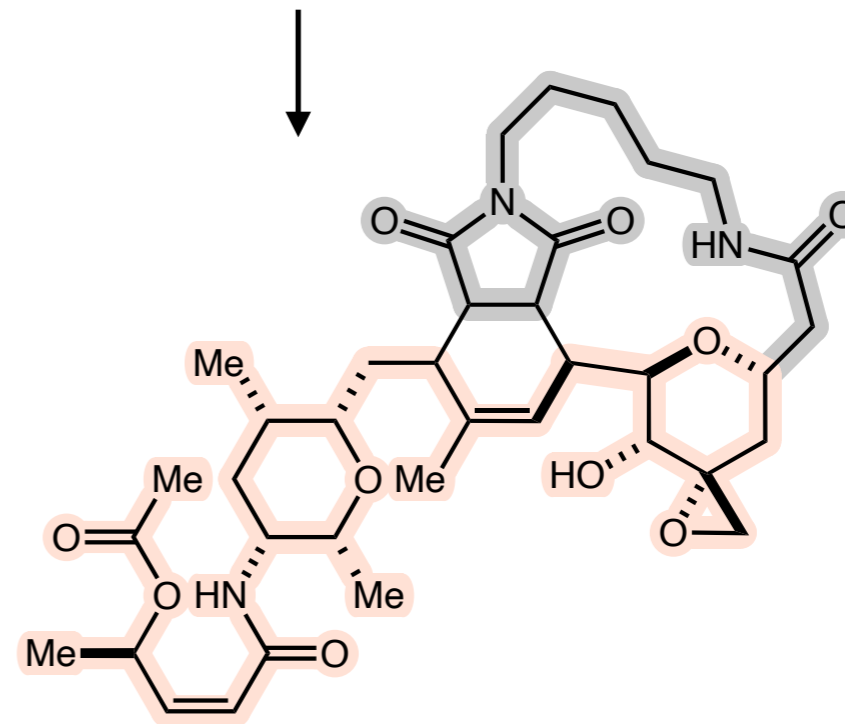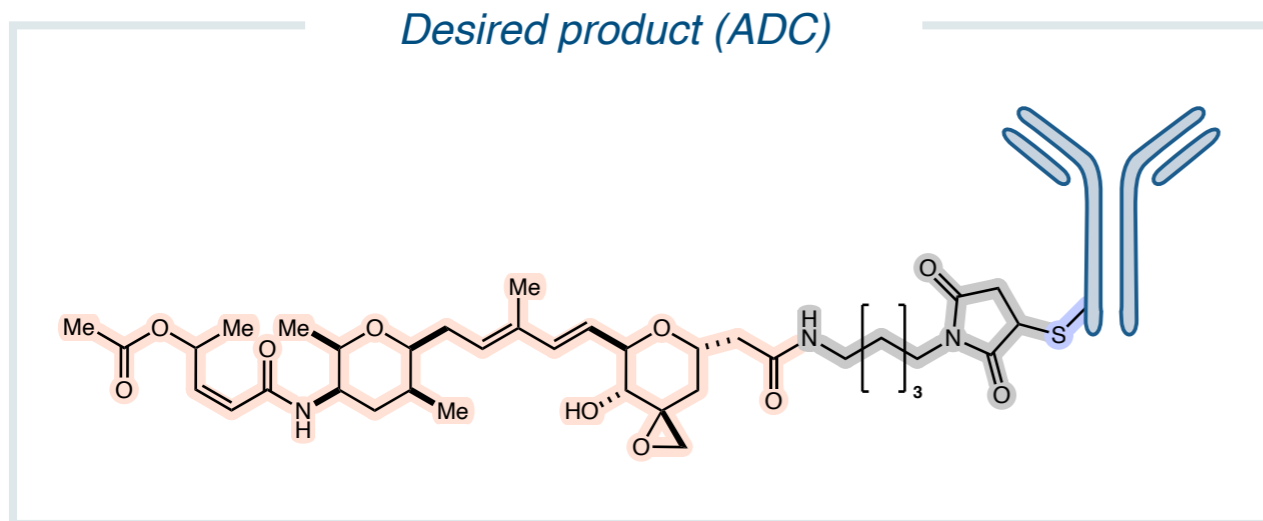
Maleimide bioconjugation linker is not compatible with diene-containing 'D'

'C' in ADC

Conjugation through antibody engineering
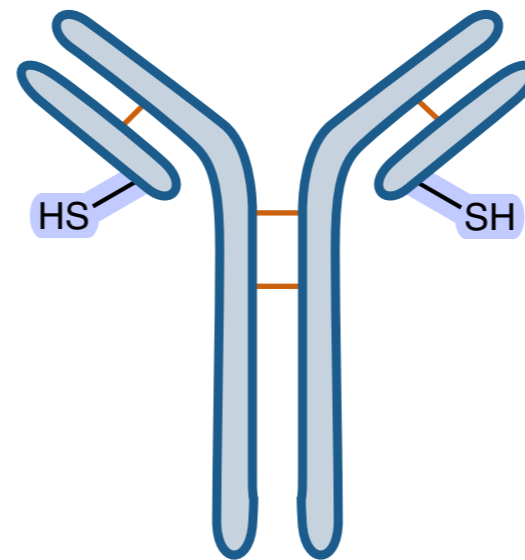
THIOMAB™ antibody

2 engineered cysteines

Conjugation through antibody engineering
THIOMAB Technology
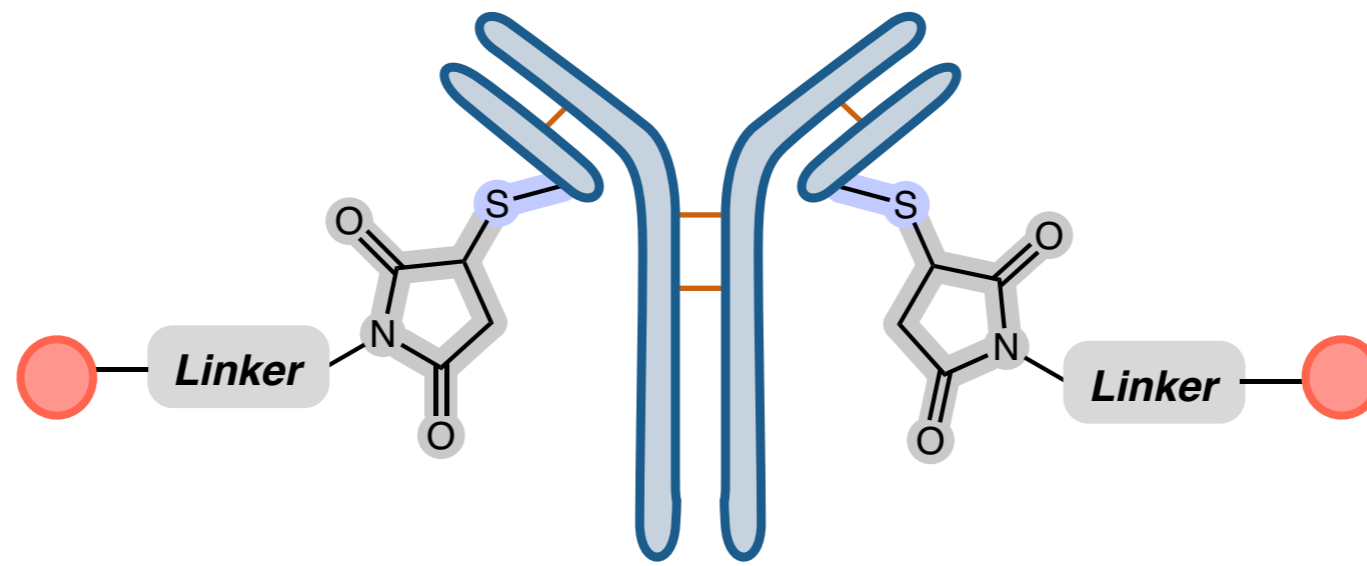
THIOMAB™ antibody

Conjugation through antibody engineering

THIOMAB Technology

Highly homogenous DAR

92.1%
DAR = 2

THIOMAB™ antibody

Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.
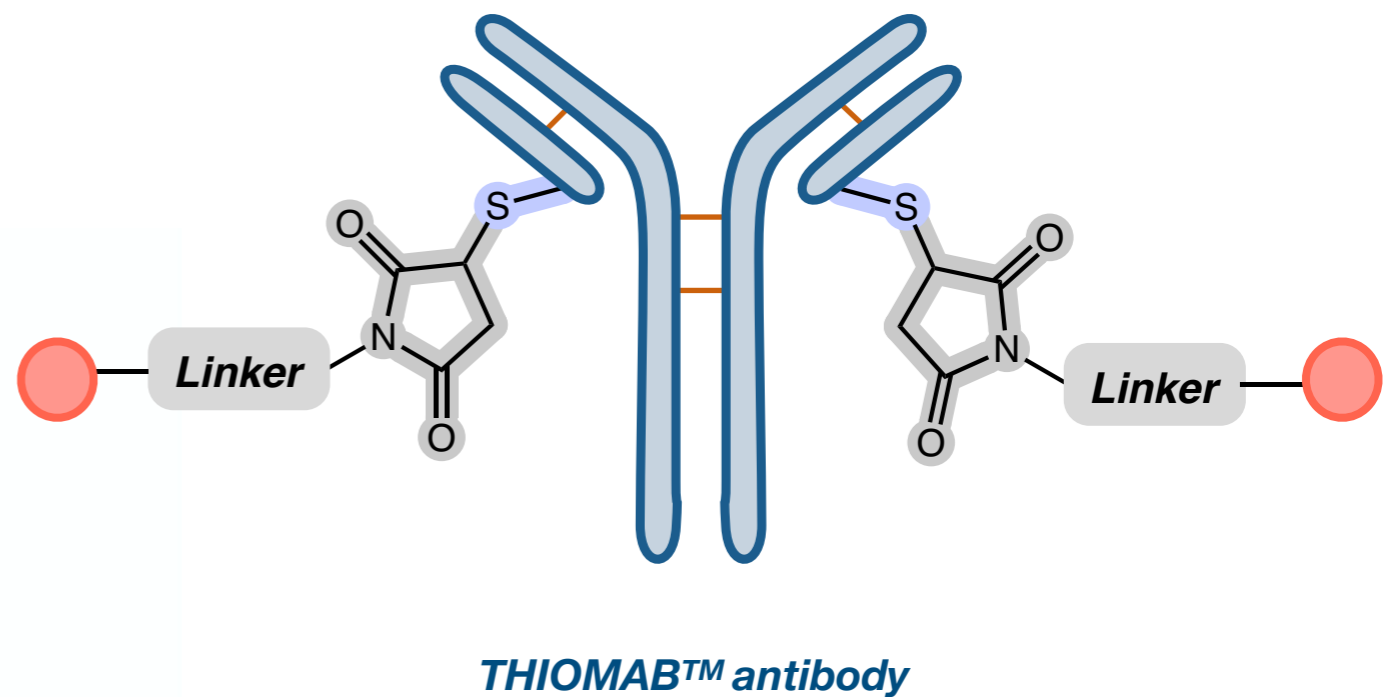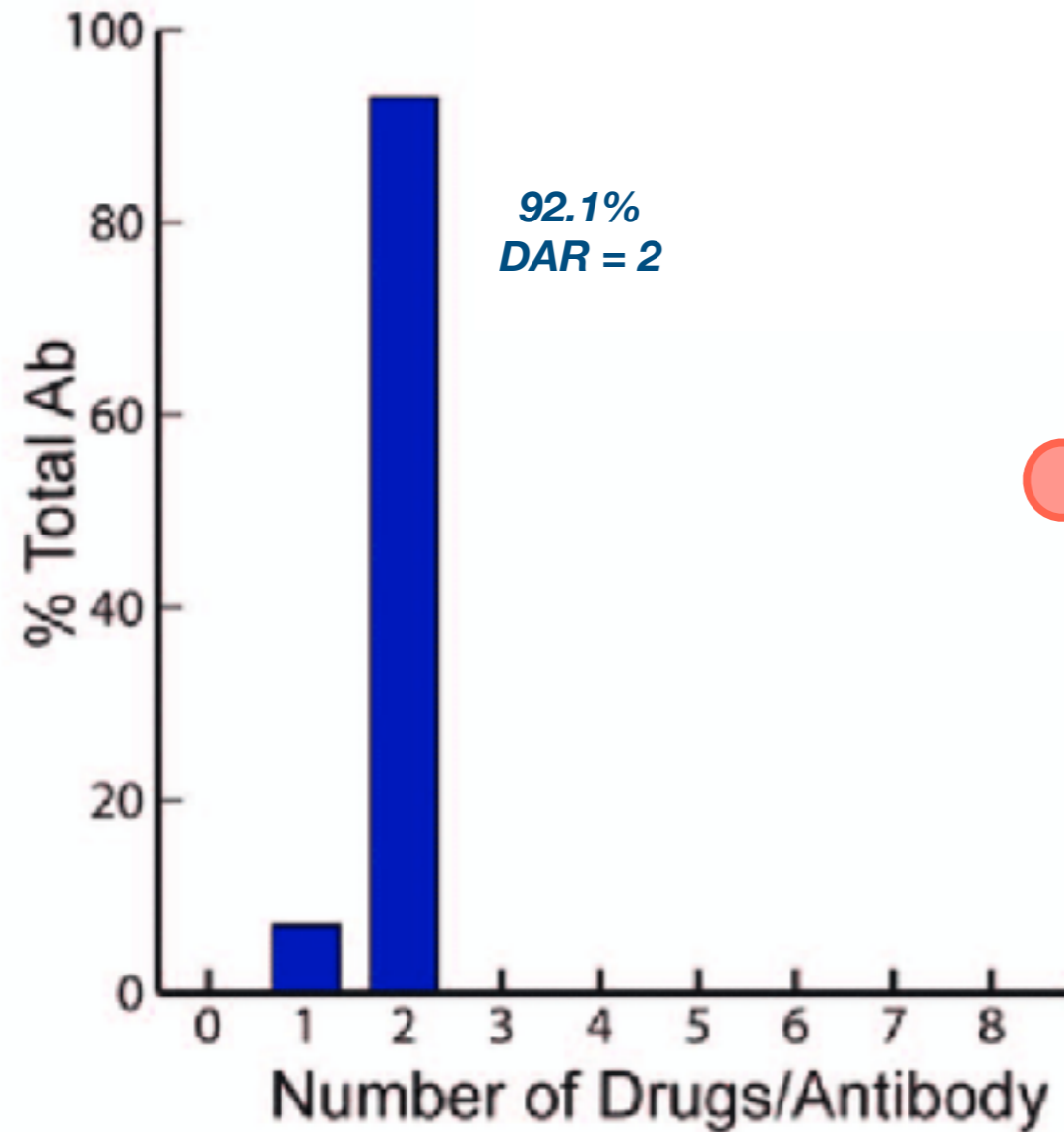
Conjugation through antibody engineering

THIOMAB Technology

Limitations

- **Antibody scrambling**
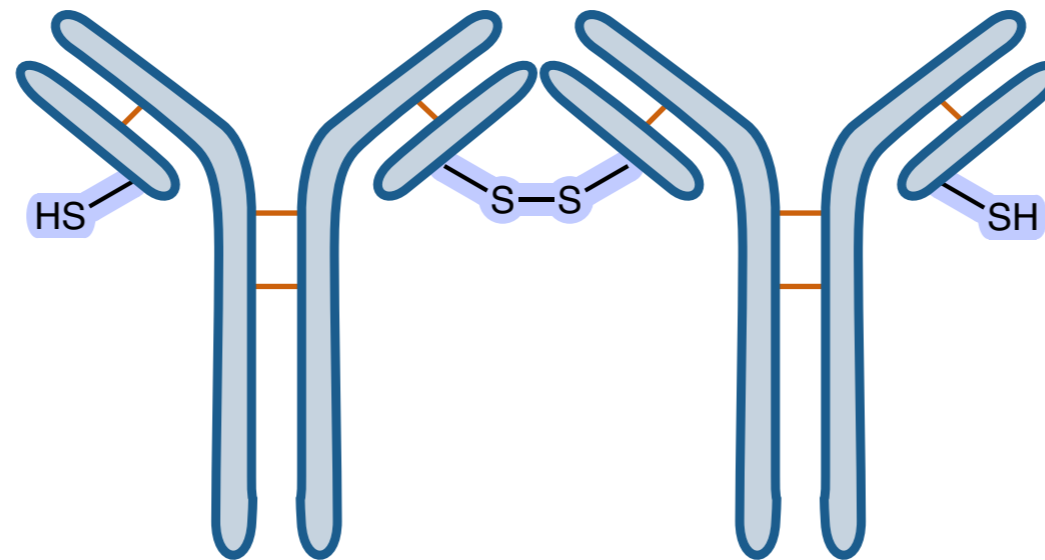
Wrong disulfide bond formation between the two Fabs in the antibody

- **DAR limited to 2 and does not solve the inherent issues of maleimide chemistry**

- **Engineered antibody: increased risk of immunogenicity**

Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.

Antibody-drug conjugate
Linker
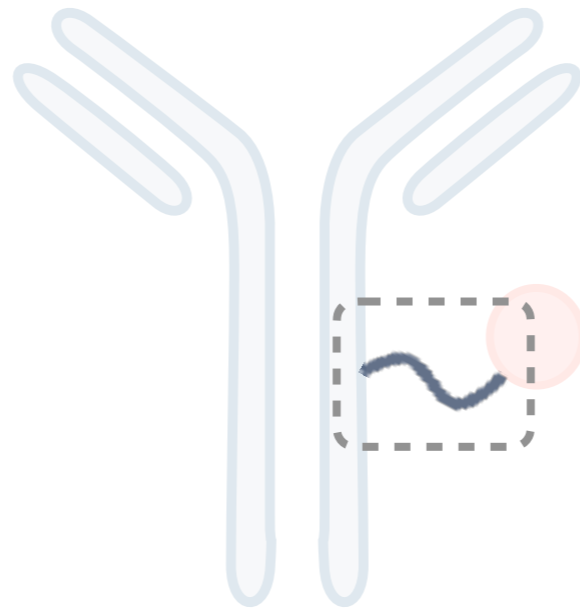
- *Bioconjugation*

- **Non-cleavable or cleavable**

'C' in ADC

Cleavable or non-cleavable

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

"Non-cleavable"

Tivdak™

"Cleavable"

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

'C' in ADC

Non-cleavable

Kadcyla™
(Trastuzumab emtansine)
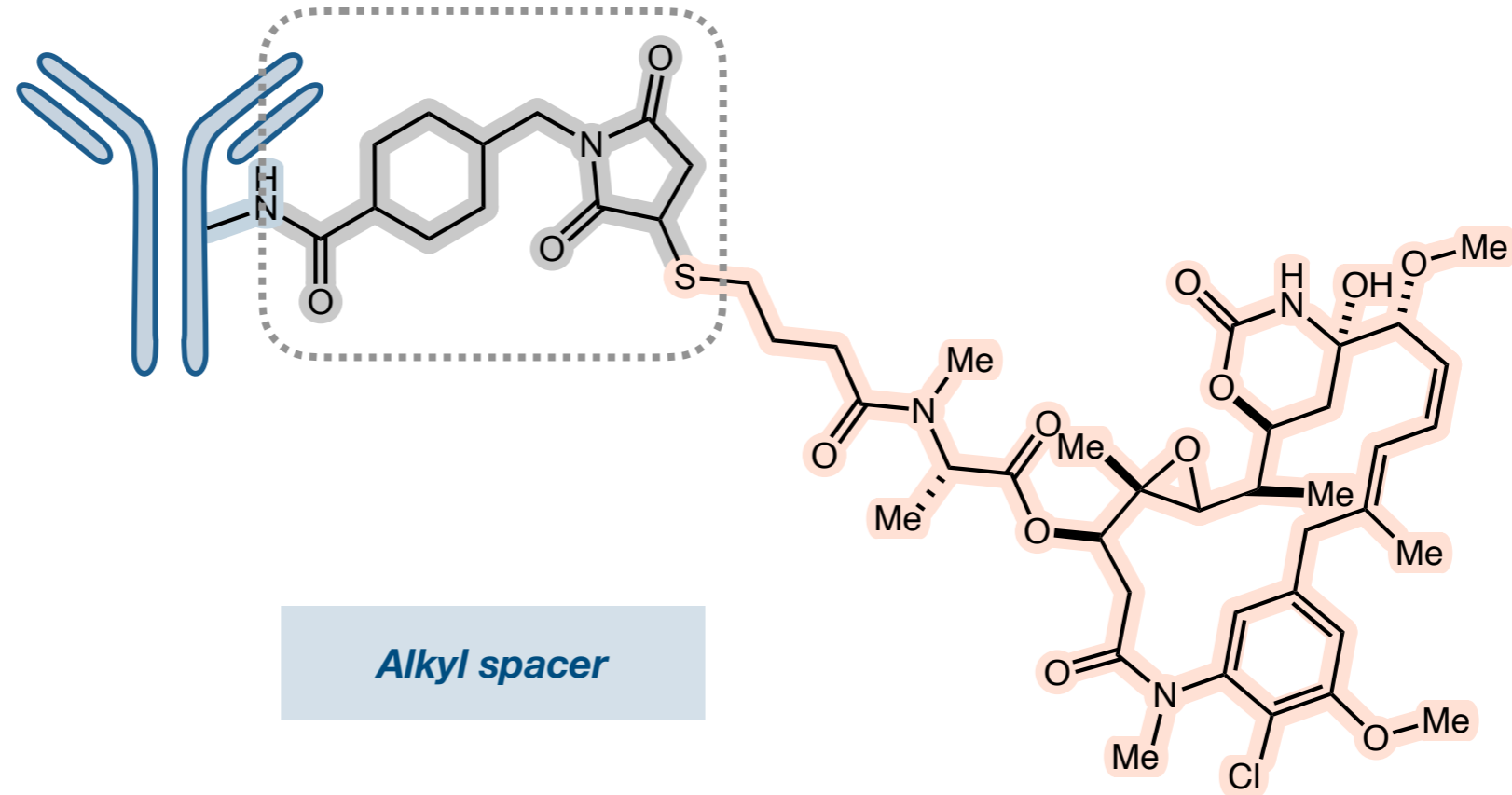
Alkyl spacer

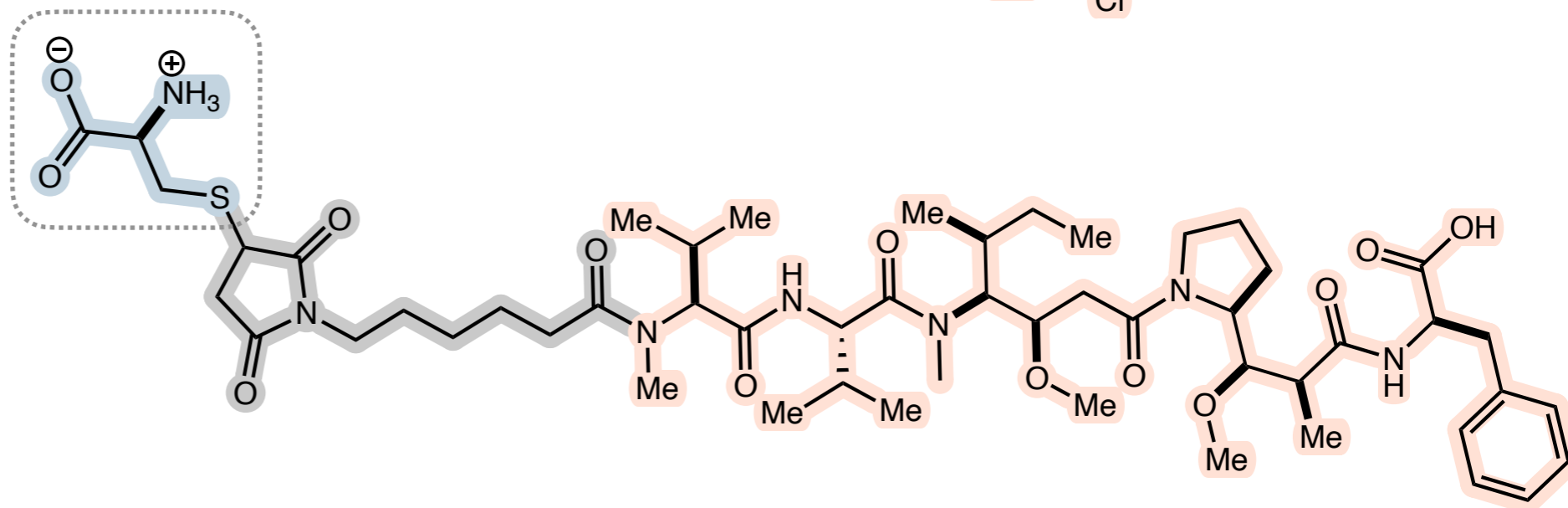**Blenrep™**
(Belantamab mafodotin)
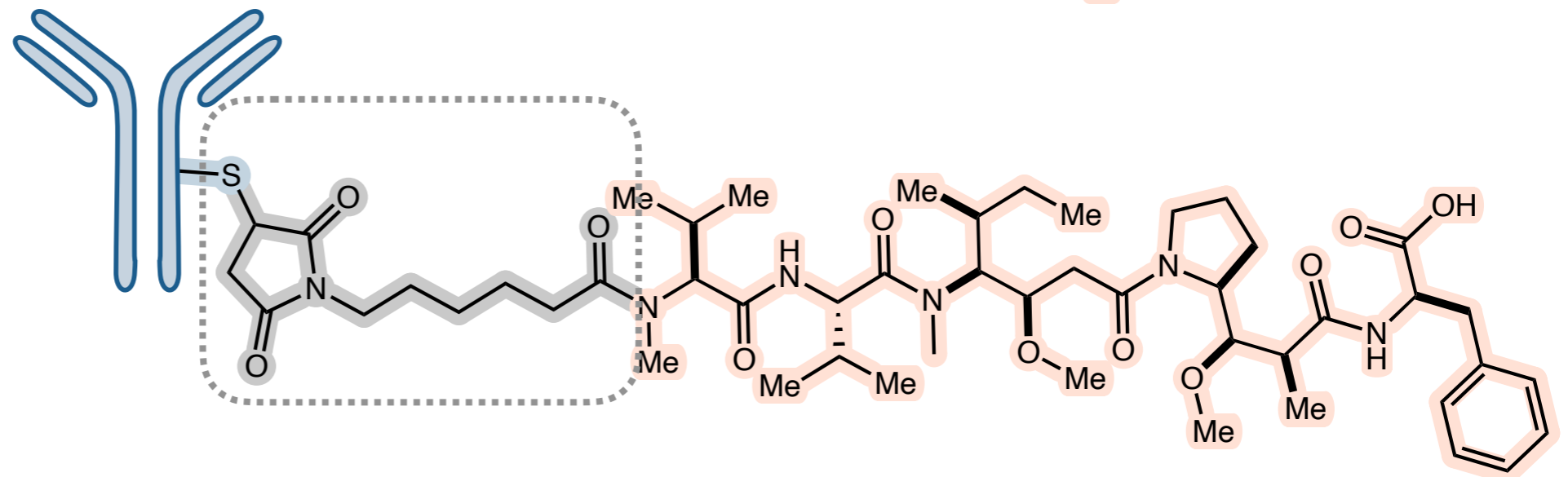
Non-cleavable linker

Lysosomal degradation with amino acid appendage

Kadcyla™
(Trastuzumab emtansine)
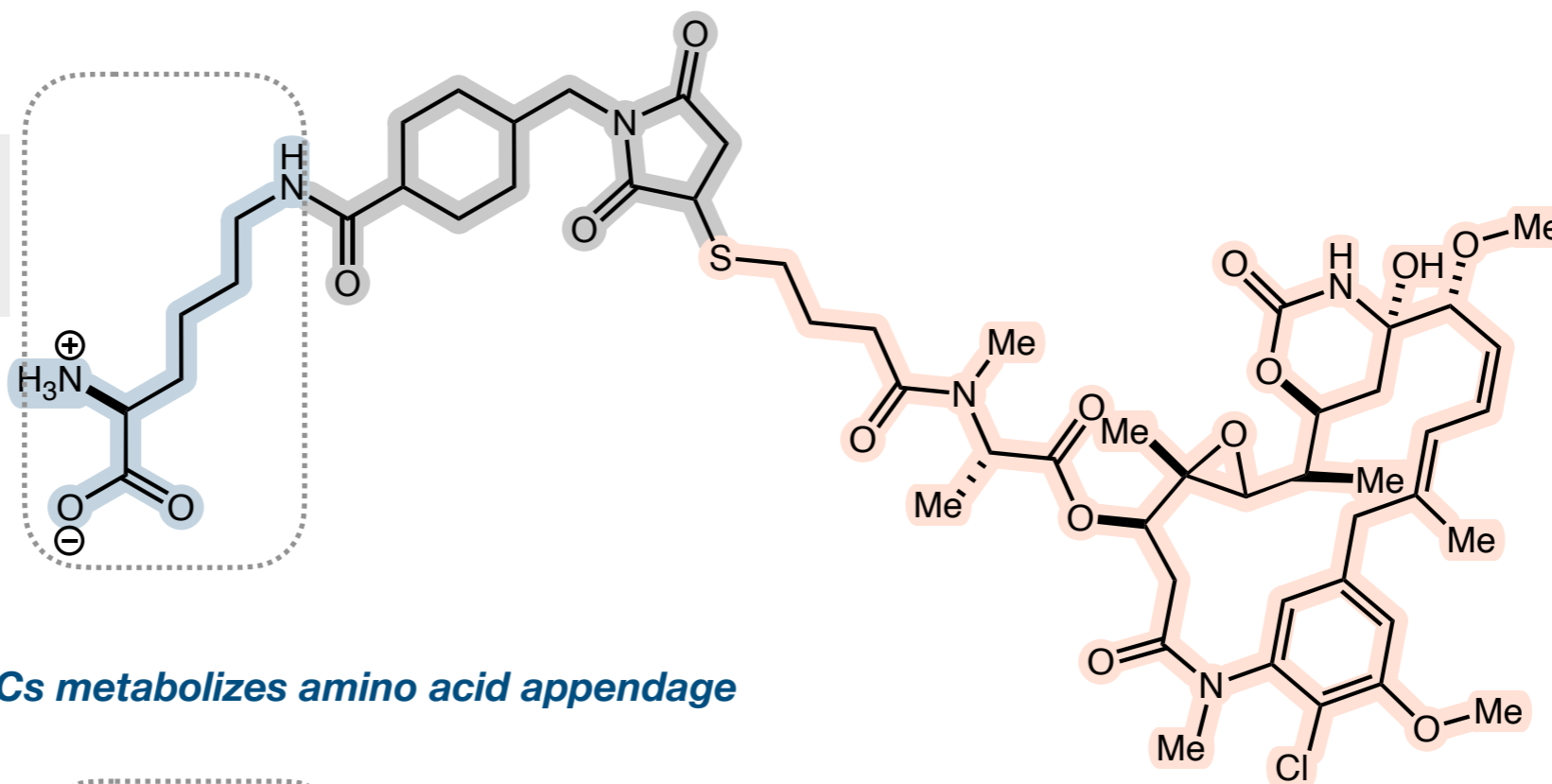
ADCs metabolizes amino acid appendage

Blenrep™
(Belantamab mafodotin)

'C' in ADC
Cleavable linker

Cleavable linkers make use of the hallmarks of cancer

- **Acidic microenvironment (~ pH 6.7)**

- **Overexpressed glutathiones (GSH)**

- **Overexpressed lysosomal protease (e.g. Cathepsin B)**

Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144* (5), 646–674.

Cleavable linker

Acidic tumour microenvironment and overexpressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Cleavable: hydrazone

Disulfide trigger

Acid hydrolysis (pH 6.4)

Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540
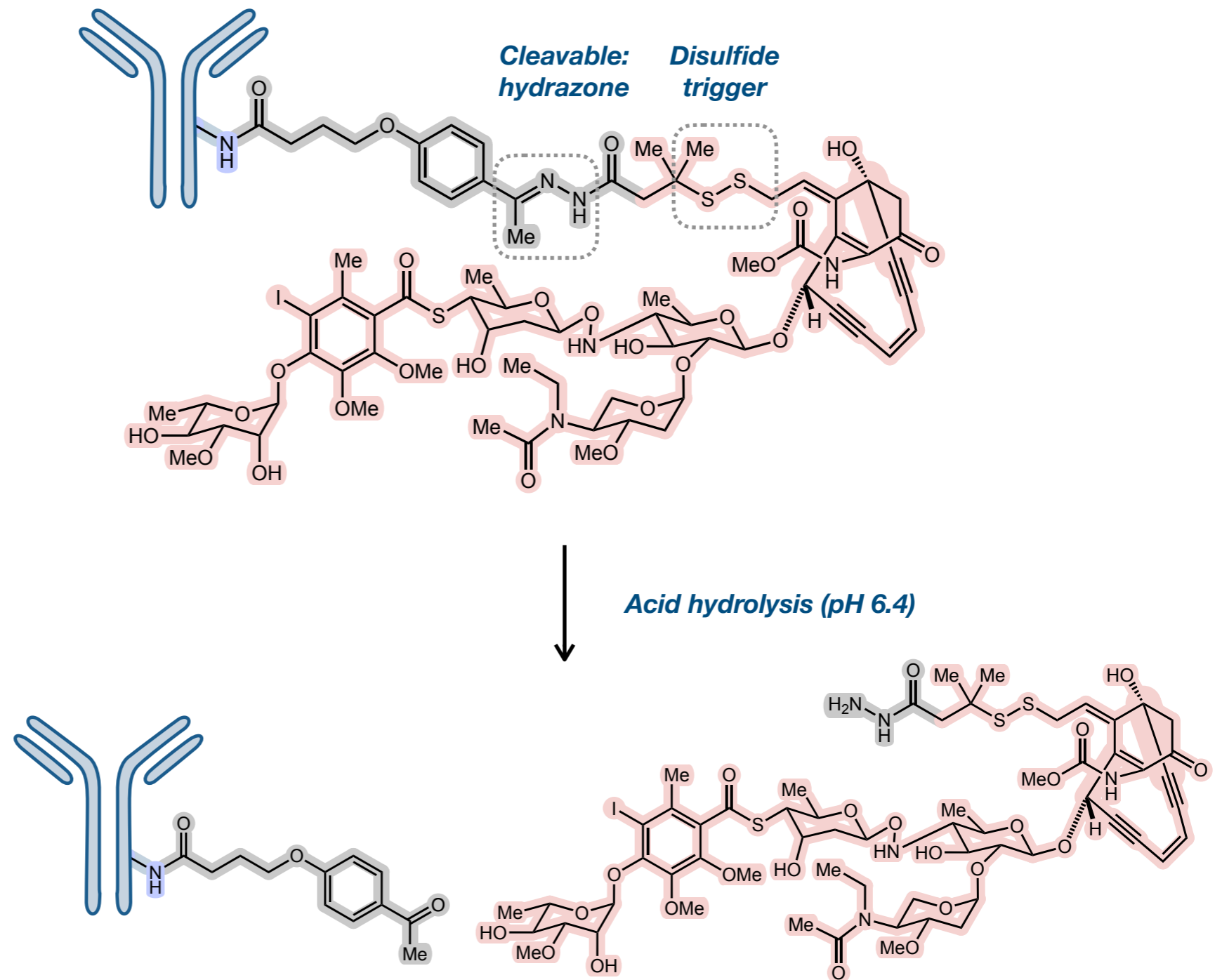
Cleavable linker

Acidic tumour microenvironment and over expressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™
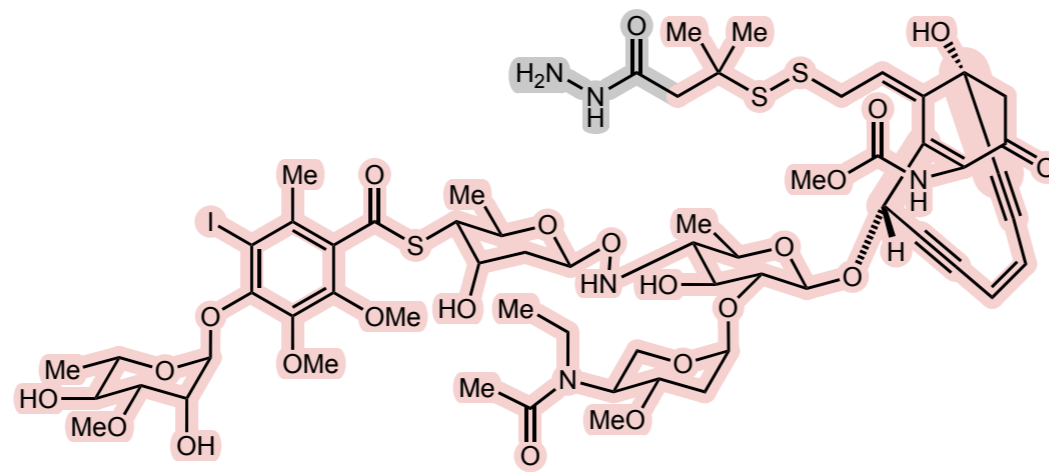
Blenrep™

Elahere™

GSH (overexpressed)
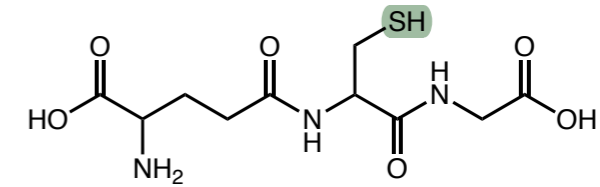
Thiol-disulfide exchange
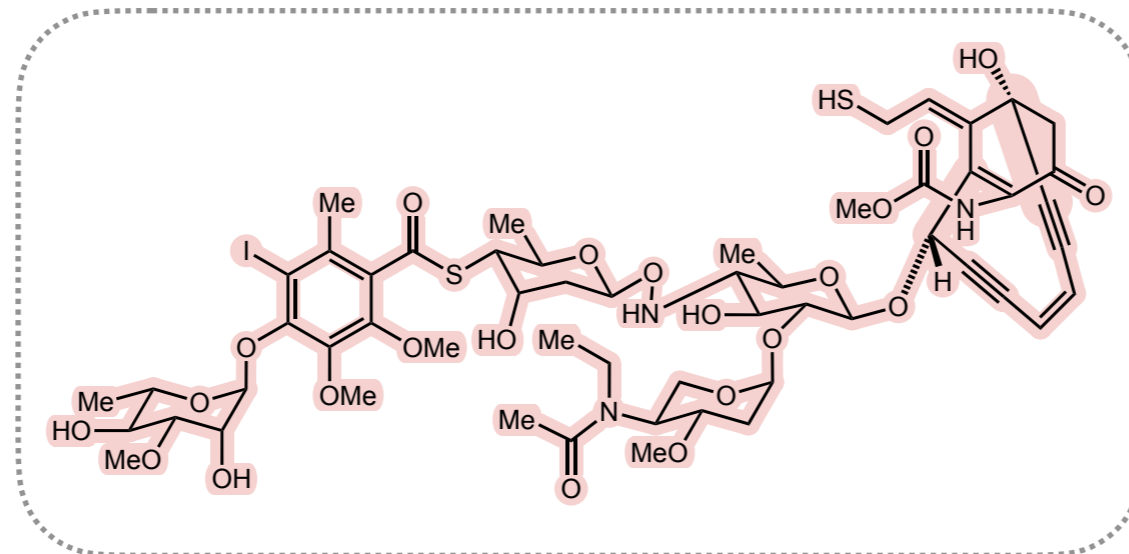
Active drug (N-Acetyl calicheamicin) released
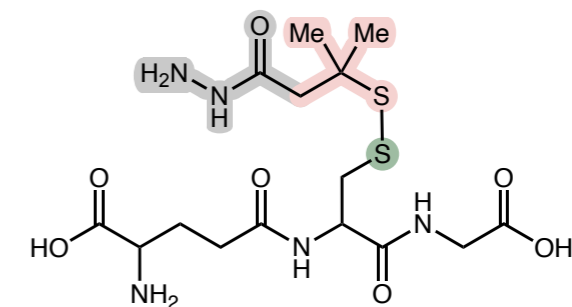
Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540

Cleavable linker

Overexpressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Disulfide trigger: thiol-disulfide exchange w/ GSH
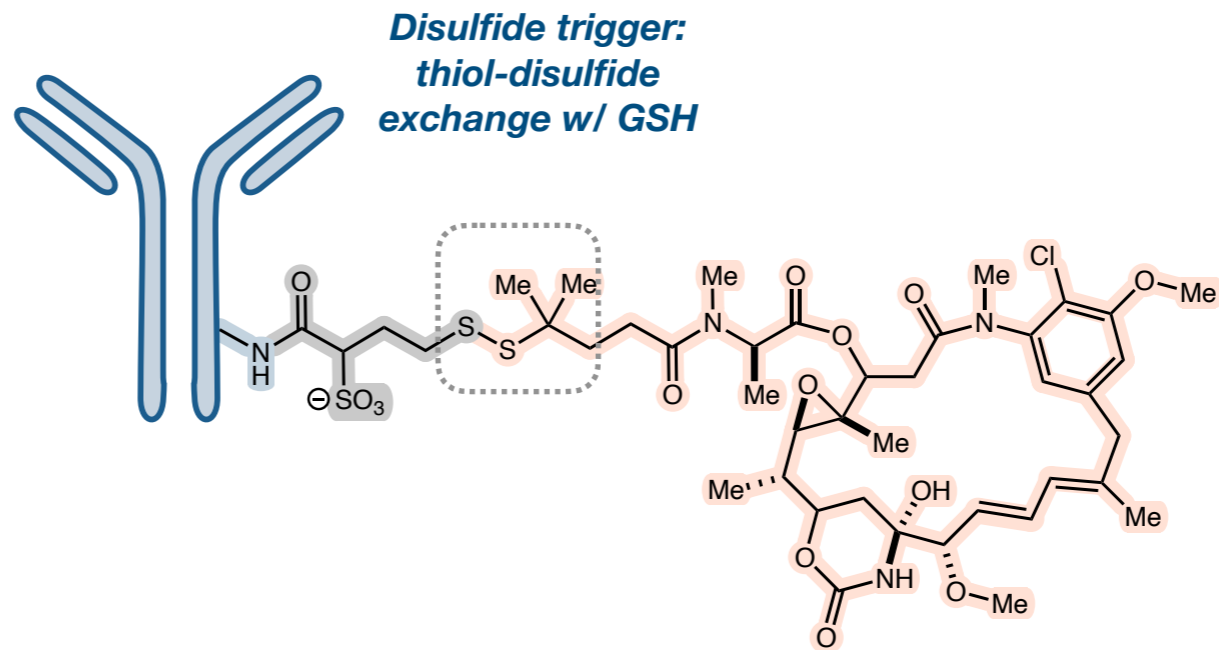
Cleavable linker

Overexpressed cathepsin B

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Tetra/Dipeptide

Val Phe Gly

Cit Ala Gly

Spacer

R_1

R_2

Cathepsin B

Proteolysis

H_2N

NH

CO_2

Active drug released

Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540
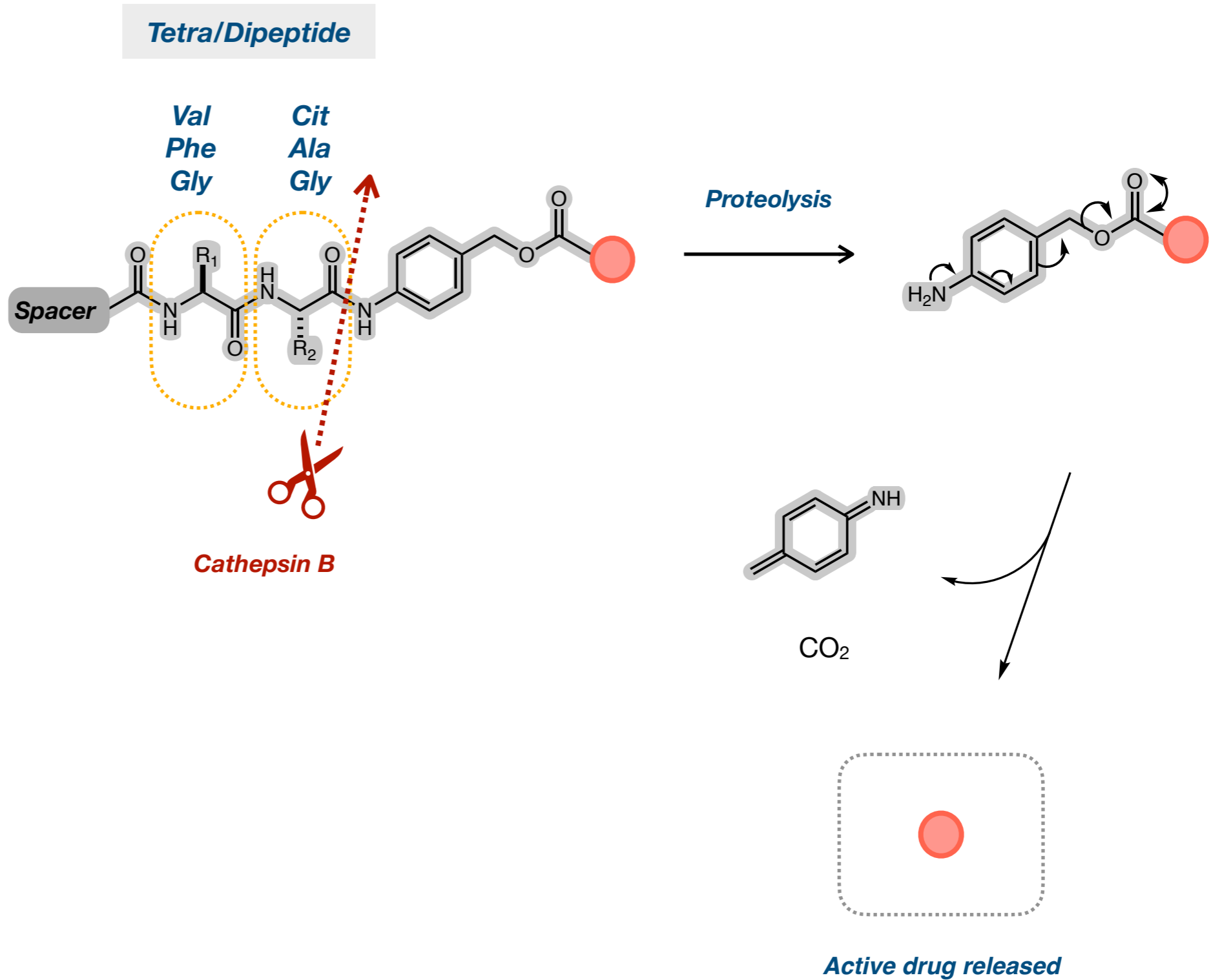
'C' in ADC

What makes a good 'C' for ADC
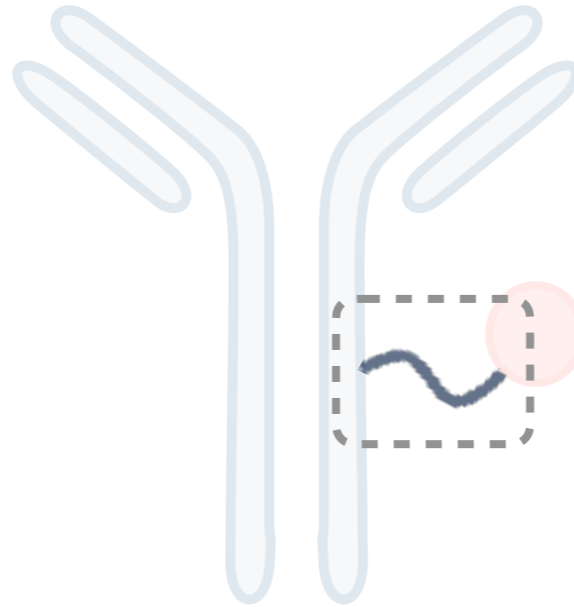
Bioconjugation

✓ Aqueous
✓ pH neutral
✓ Below 37 °C
✓ Low concentration
✓ Atom-economic
✓ Rapid

✓ No side-reactivity with 'D'

✓ Selective (chemo-, regio-, stereo-)
for **DAR control**

Cleavable/Non-cleavable

✓ Cleavable linkers should be able to reliably differentiate between normal and cancer cells

'D' in ADC
Drug
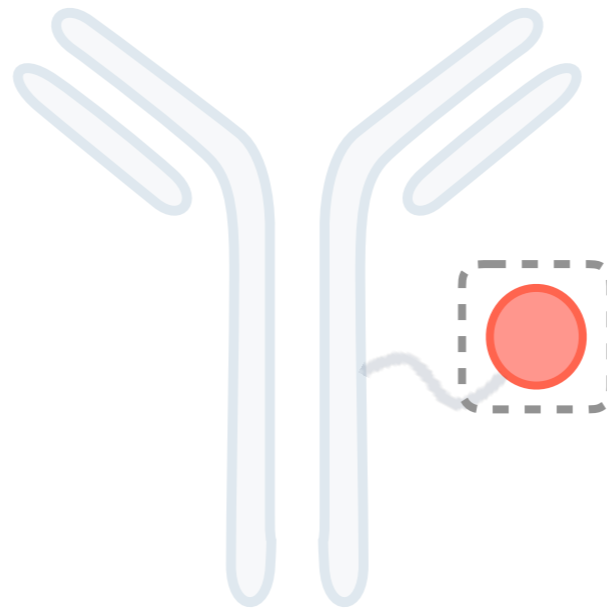
- **Types of drugs**

- **Bystander effect**

- **Other types of 'D'**

'D' in ADC
Drug
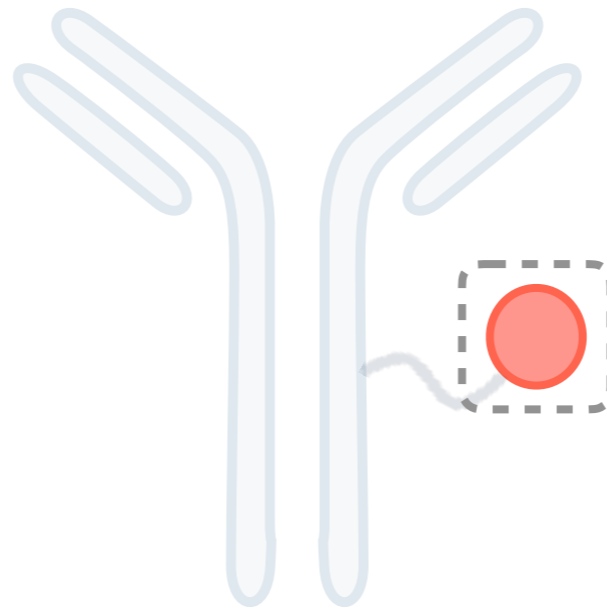
- **Types of drugs**

- *Bystander effect*

- *Other types of 'D'*

'D' in ADC
Types of drugs

Microtubule inhibitor
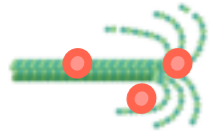
	ADC	IC$_{50}$
MMAE	*Adcteris™* *Polivy™* *Padcev™* *Tivdak™*	*1.1 nM*
MMAF	*Blenrep™*	*137 nM*
DM1	*Kadcyla™*	*30 nM*
DM4	*Elahere™*	*3.3 nM*

DNA binder
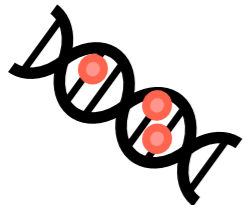
	ADC	IC$_{50}$
Calicheamicin	*Besponsa™* *Mylotarg™*	*1 nM*
PBD dimer	*Zynlota™*	*1 pM*

Topoisomerase inhibitor

	ADC	IC$_{50}$
Exatecan	*Enhertu™*	*0.31 µM*
SN-38	*Trodelvy™*	*0.17 µM*

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

'D' in ADC
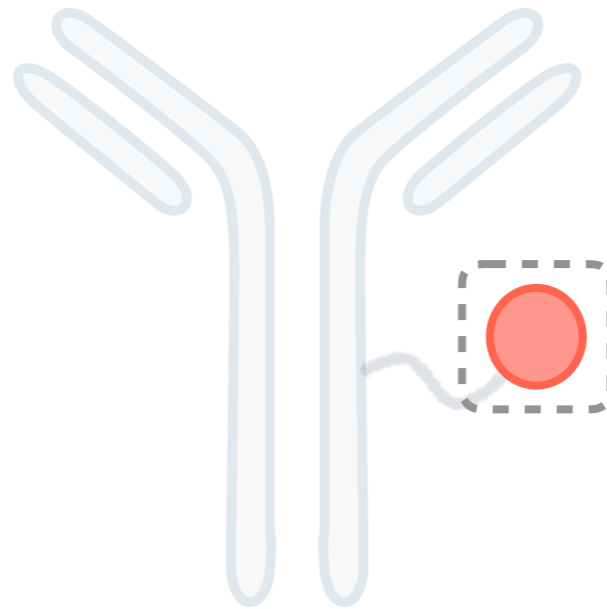
Drug

- *Types of drugs*
- **Other types of 'D'**
- *Bystander effect*

Other types of 'D'

Antibody-Radionuclide Conjugate (ARC)

ZEVALIN®
(ibritumomab tiuxetan)
INJECTION FOR INTRAVENOUS USE
3.2 mg per 2 mL

Ibritumomab

(anti-CD20 mAb)

90 Yttrium

β-particle-emitting radionuclide
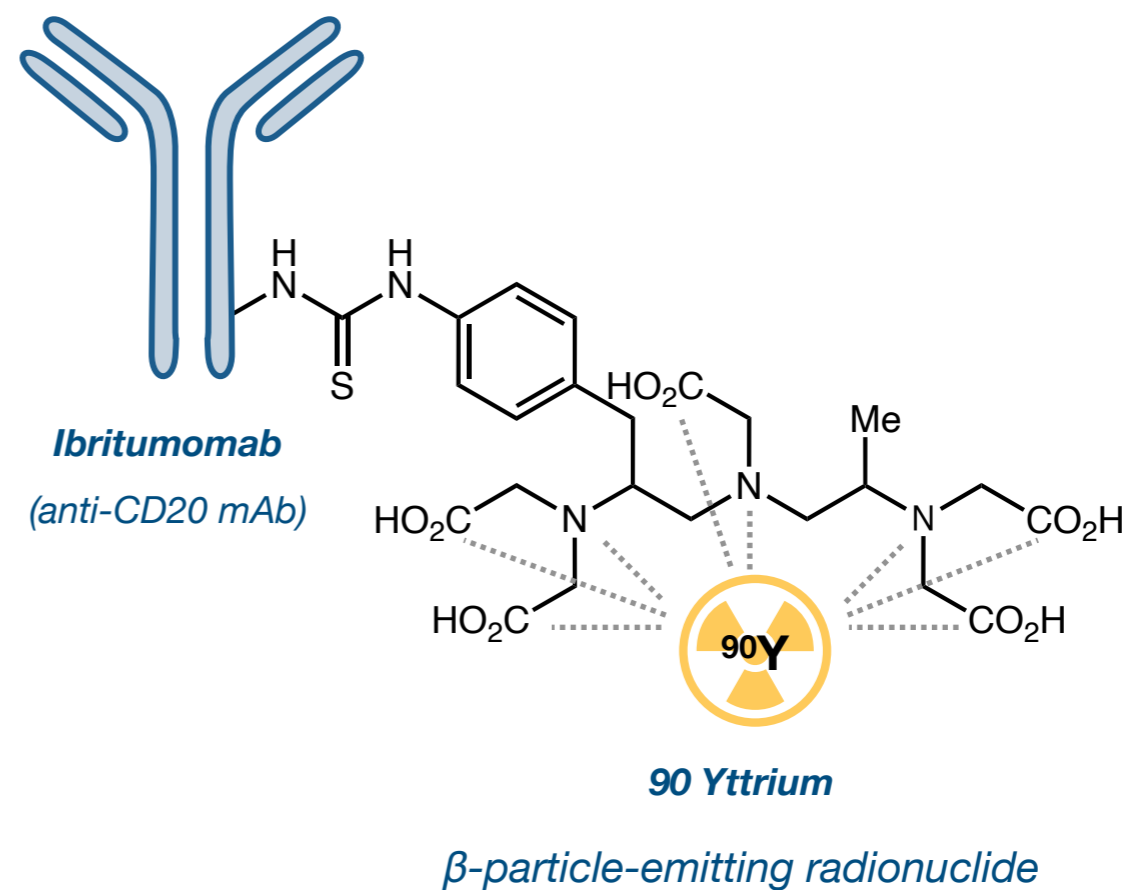
Limited number of approvals & low clinical adoptions, *due to the requirement of a multidisciplinary team of medical oncologists, radiation oncologists, and physicists to develop and deploy the molecules in the clinic*

Sgouros, G.; Bodei, L.; McDevitt, M. R.; Nedrow, J. R. *Nat. Rev. Drug Discov.* **2020**, *19* (9), 589–608

Other types of 'D'

Degrader-Antibody Conjugate (DAC) and Antibody-Oligonucleotide Conjugate (AOC)

E3 ligase recognition motif

POI ligand

Oligonucleotide

Expansion of modalities beyond ADCs

Dovgan, I.; Koniev, O.; Kolodych, S.; Wagner, A. *Bioconjug. Chem.* **2019**, *30* (10), 2483–2501

Dragovich, P. S. *Chem. Soc. Rev.* **2022**, *51* (10), 3886–3897

'D' in ADC
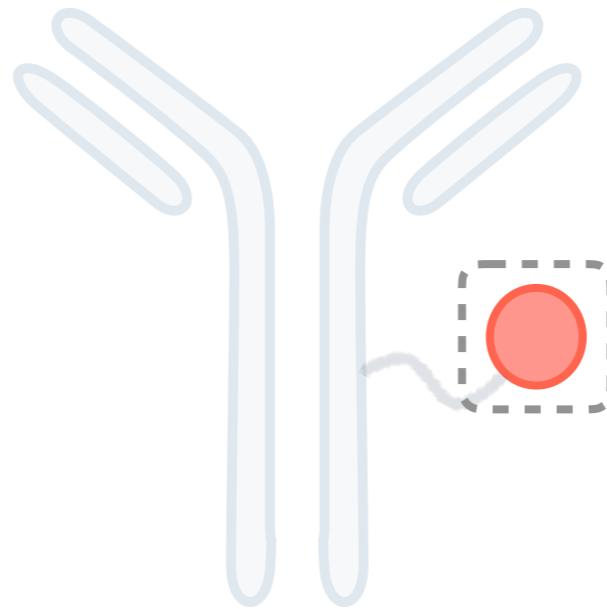
Drug

- *Types of drugs*

- *Other types of 'D'*

- **Bystander effect**

Bystander effect

Drugs tethered to non-cleavable linkers

Kadcyla™
(Trastuzumab emtansine)
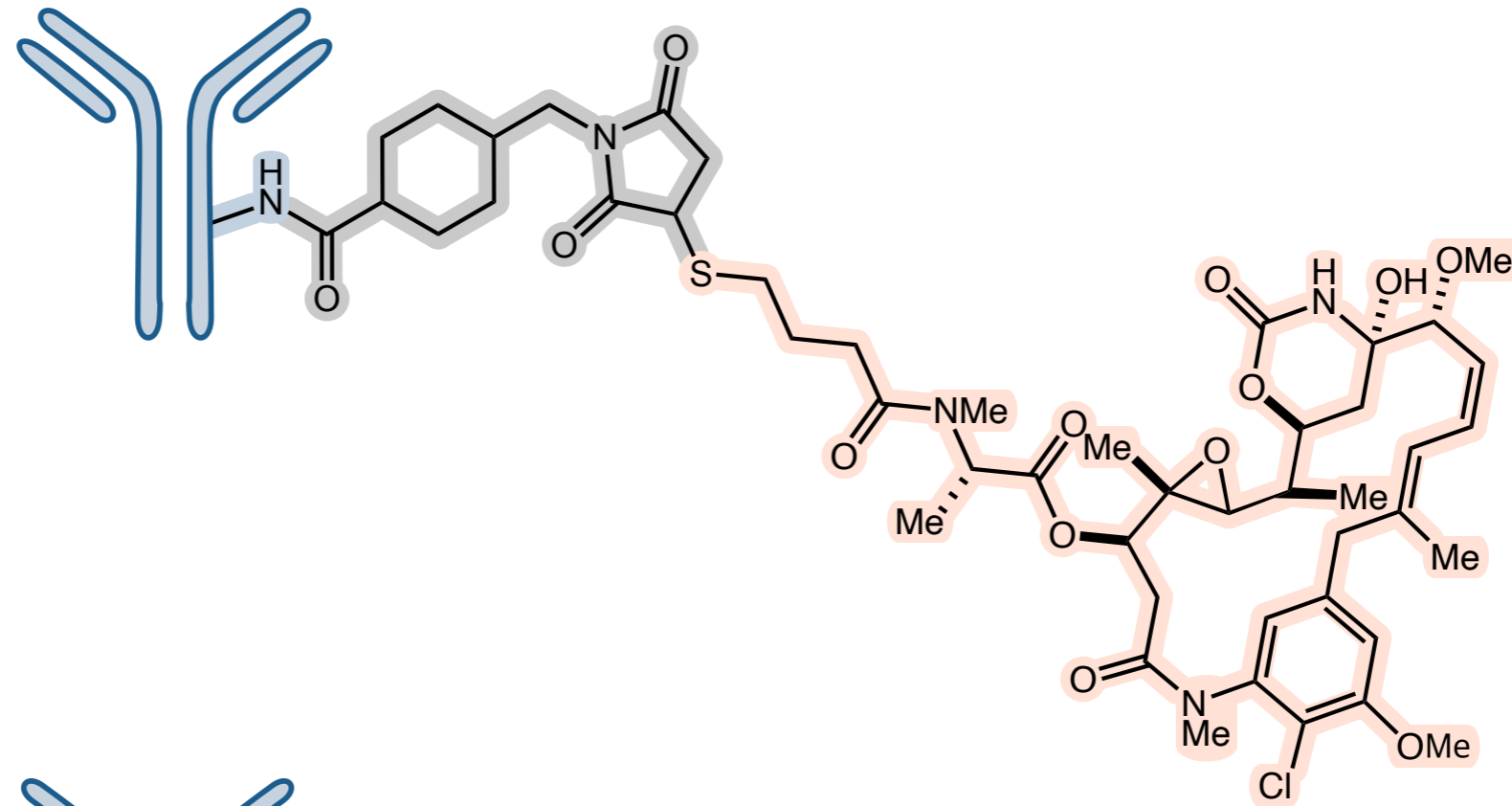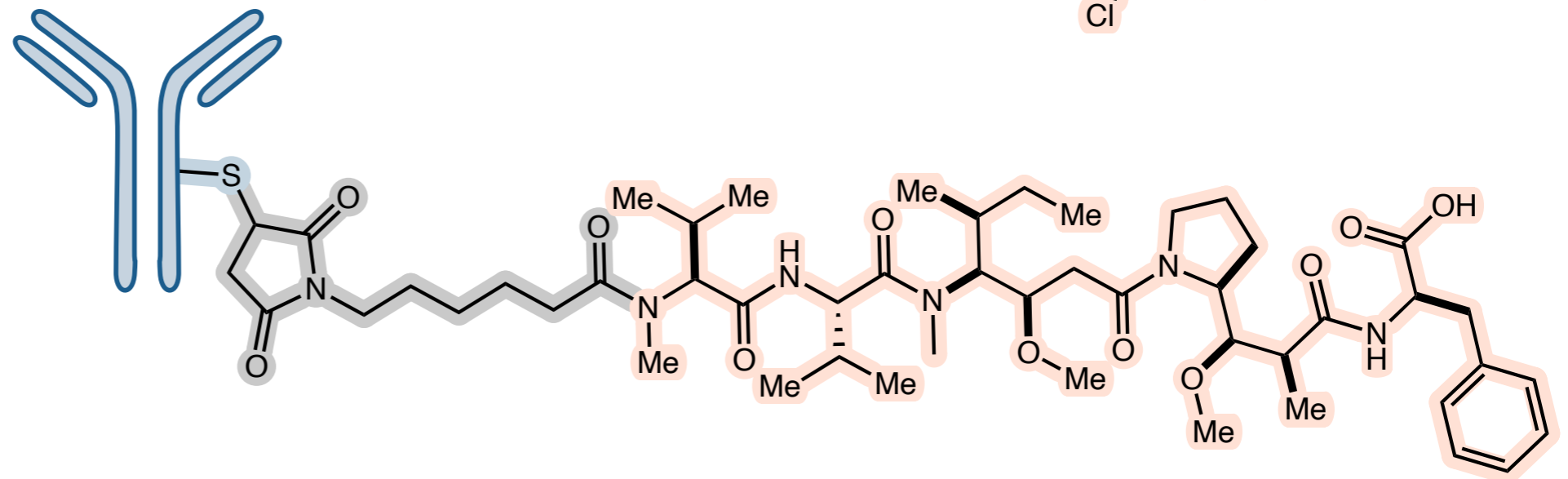
Blenrep™
(Belantamab mafodotin)

Bystander effect

Drugs tethered to non-cleavable linkers

After lysosomal degradation to release drugs

Kadcyla™
(Trastuzumab emtansine)
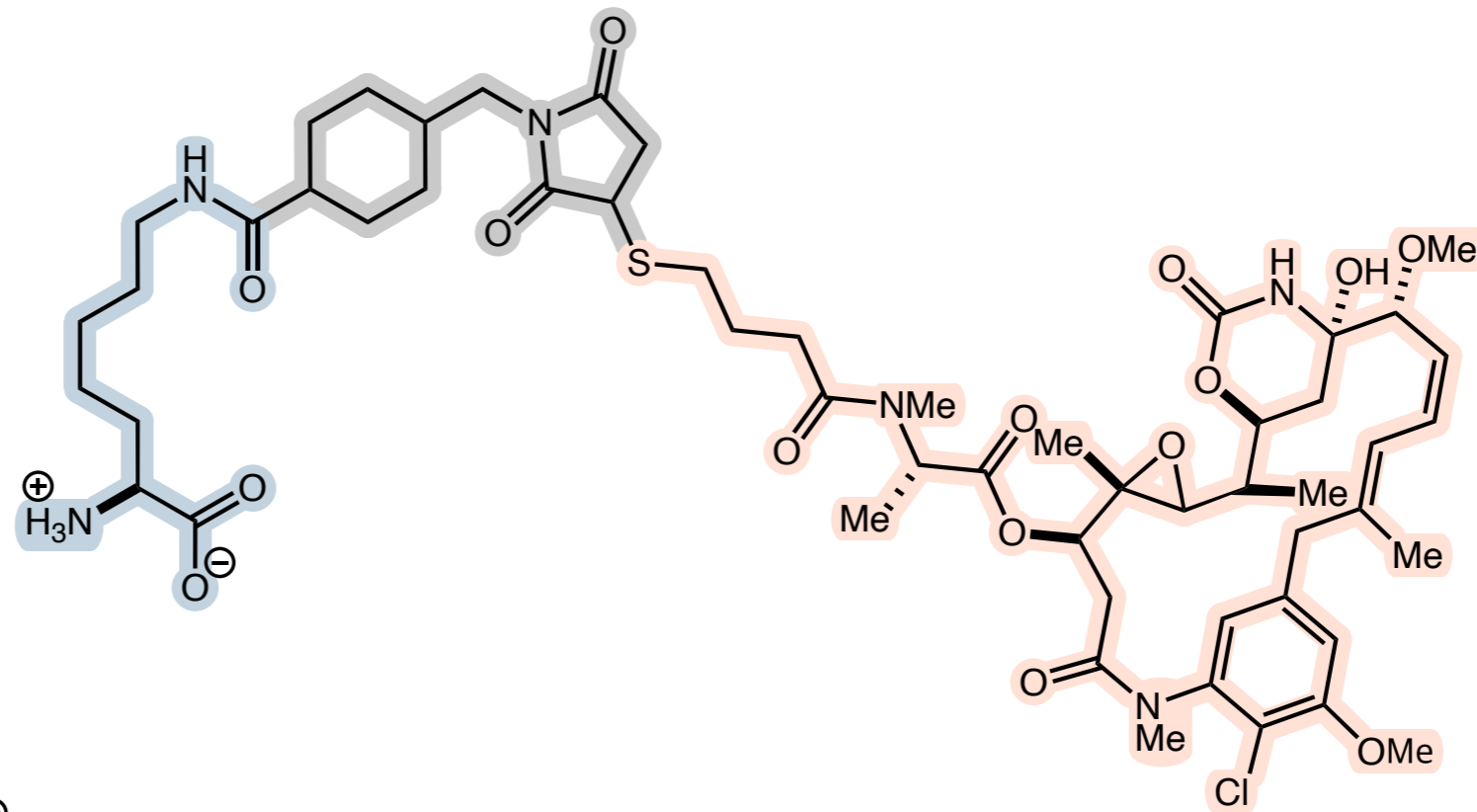
Blenrep™
(Belantamab mafodotin)
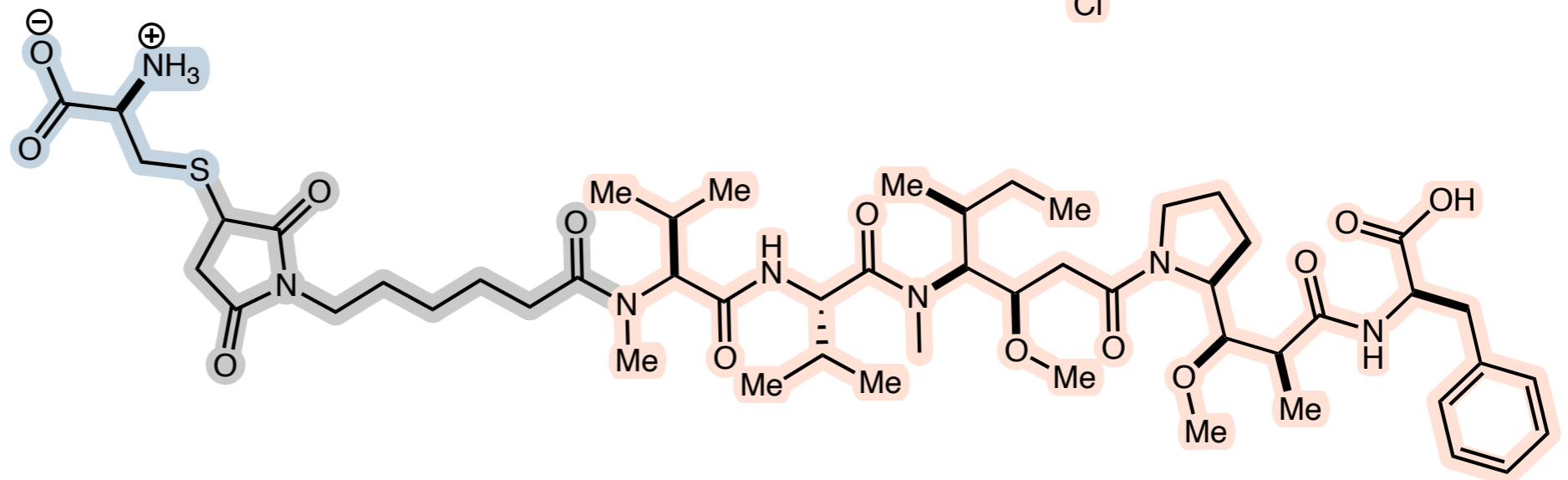
Bystander effect

Drugs tethered to non-cleavable linkers

After lysosomal degradation to release drugs

Kadcyla™
(Trastuzumab emtansine)
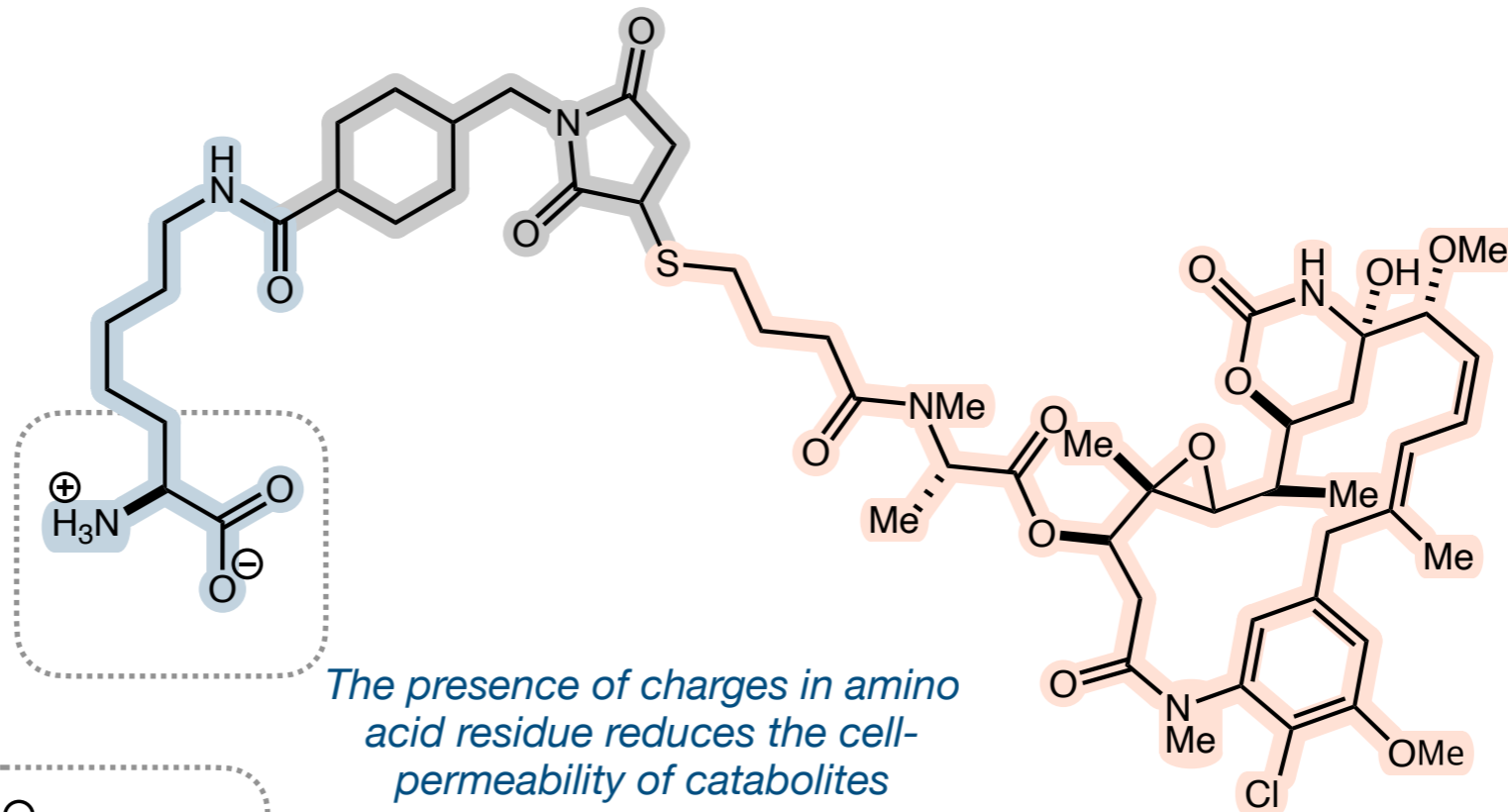
The presence of charges in amino acid residue reduces the cell-permeability of catabolites

Blenrep™
(Belantamab mafodotin)
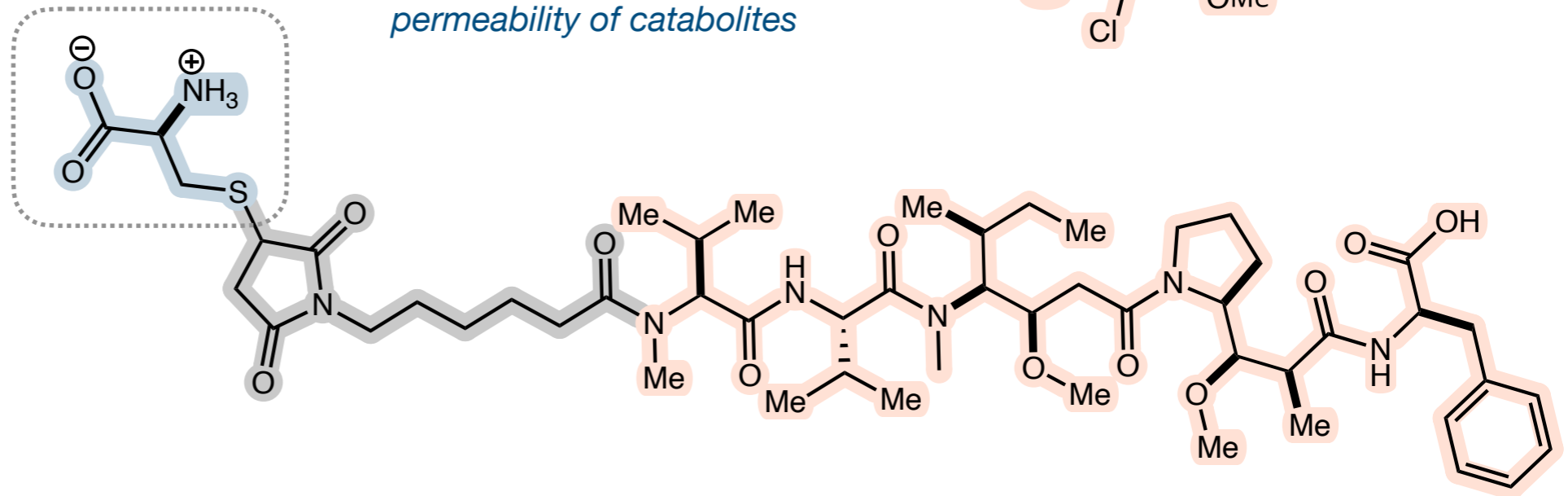
Bystander effect
Drugs tethered to non-cleavable linkers

Lower logD, lower lipophilicity,
lower cell permeability

Neighbouring antigen-
negative tumor cell
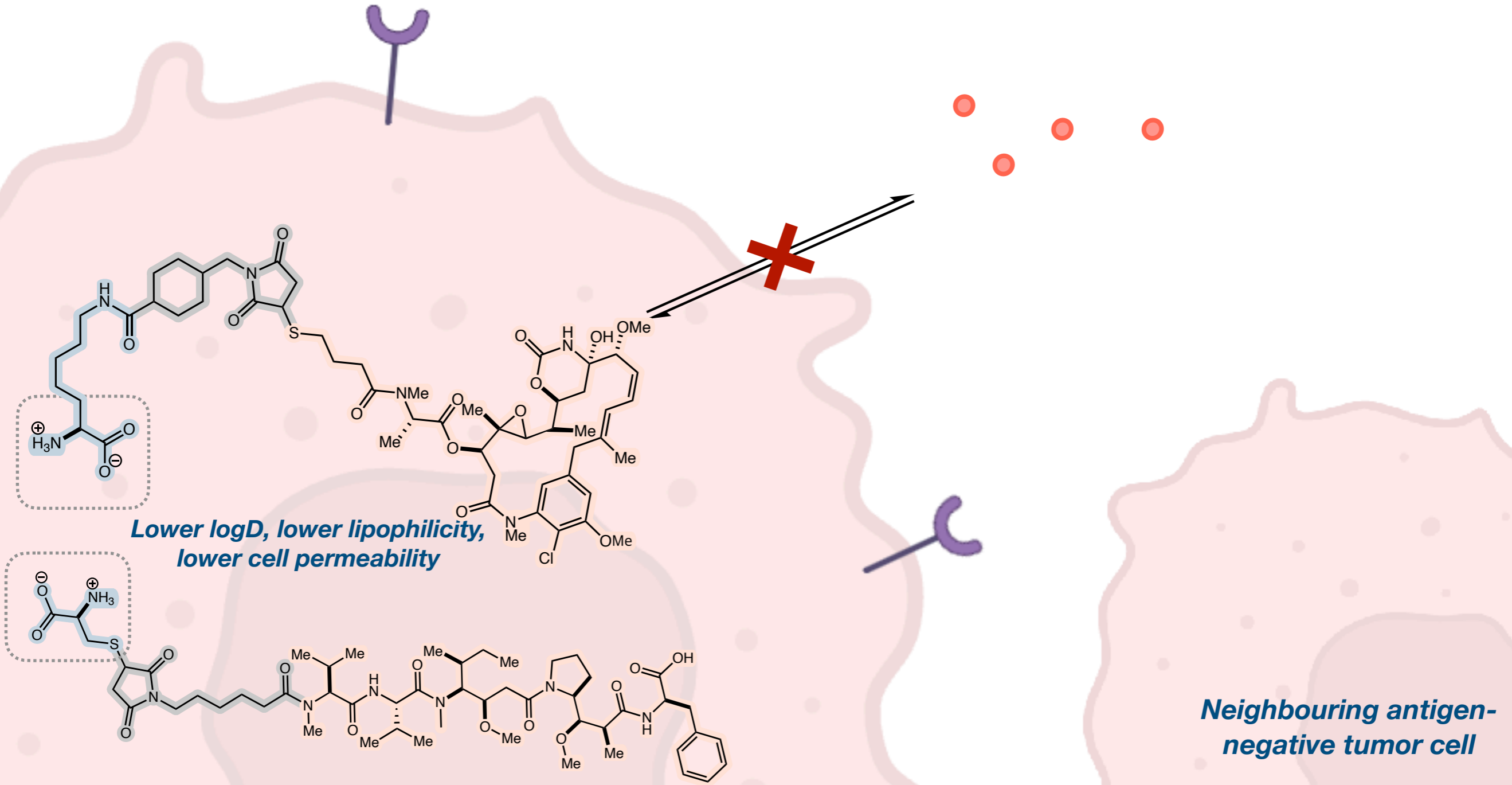
Drugs tethered to non-cleavable linkers are NOT
able to exert bystander effects

Bystander effect
Drugs tethered to cleavable linkers

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Bystander effect
Ozogamicin (Calicheamicin)-containing ADC

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Cleavable: hydrazone

Disulfide trigger

Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

After linker cleavage to release drugs
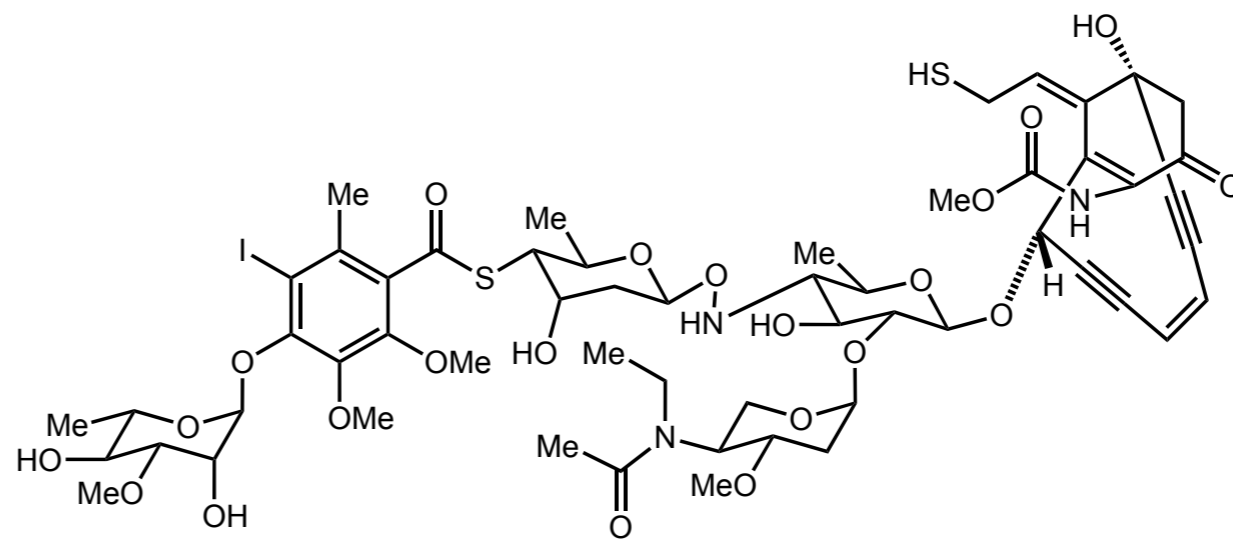
Bystander effect
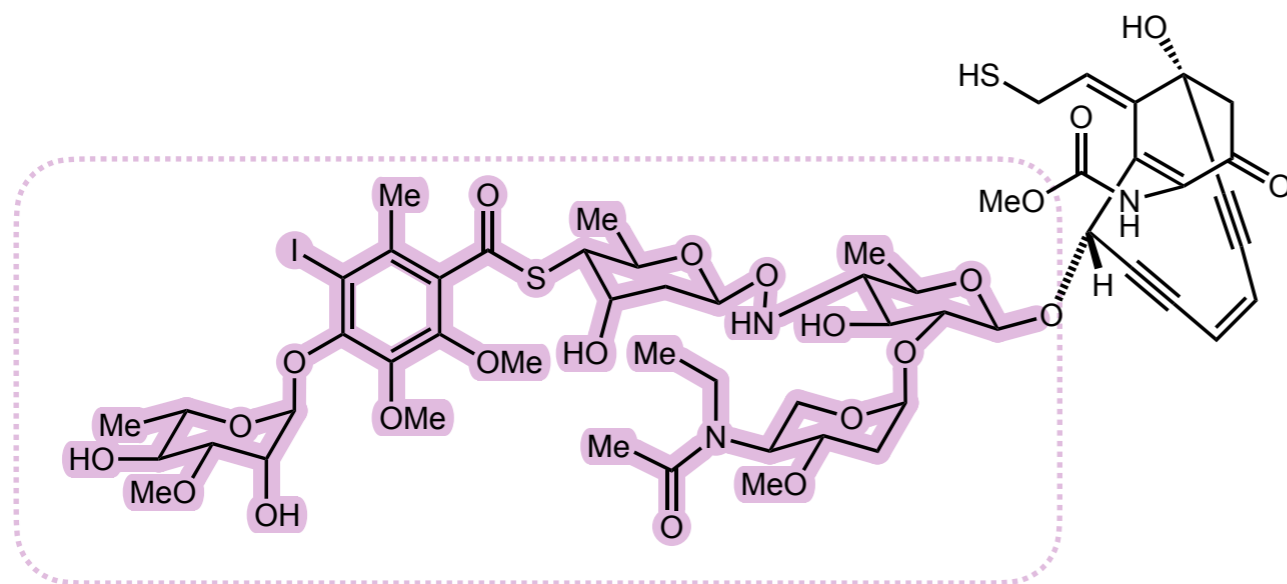
Ozogamicin (Calicheamicin)-containing ADC

Trisacchariade directs the enediyne moiety to bind specifically to the minor groove of DNA

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

Trisacchariade directs the enediyne moiety to bind specifically to the minor groove of DNA

N-Acetyl Calicheamicin

DNA minor groove

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

R = Sugar

Bergman Cyclisation

DNA

DNA diradical

Unrepaired DNA double strand cleavage

HAT

O_2

Bystander effect
Ozogamicin (Calicheamicin)-containing ADC

Short-lived nature of activated diradical

Neighbouring antigen-negative tumor cell
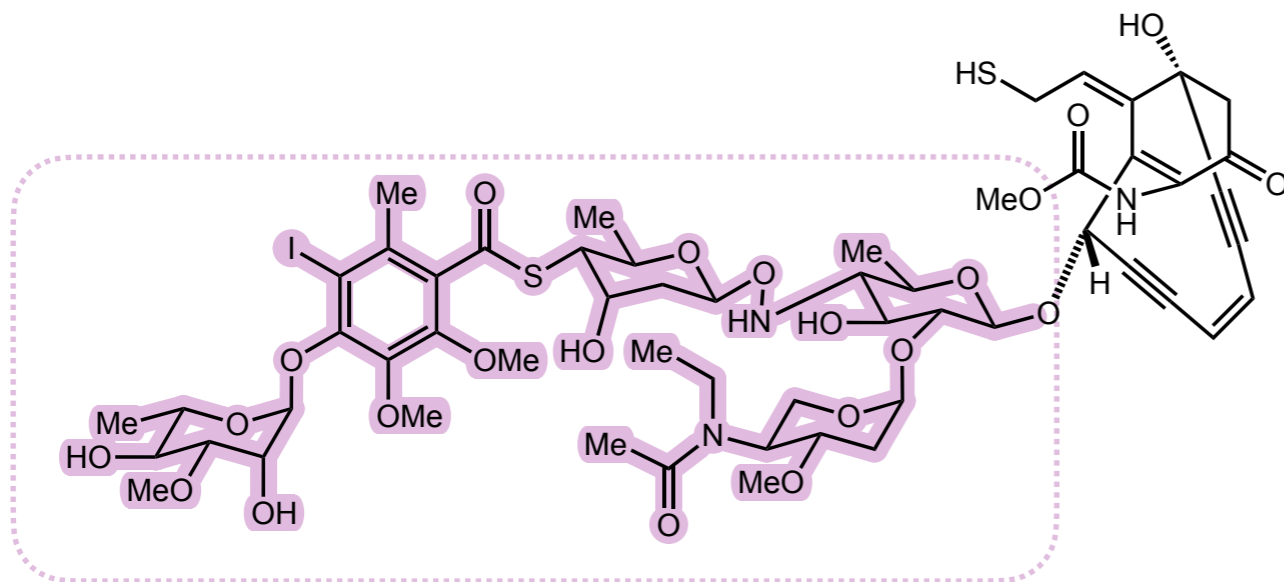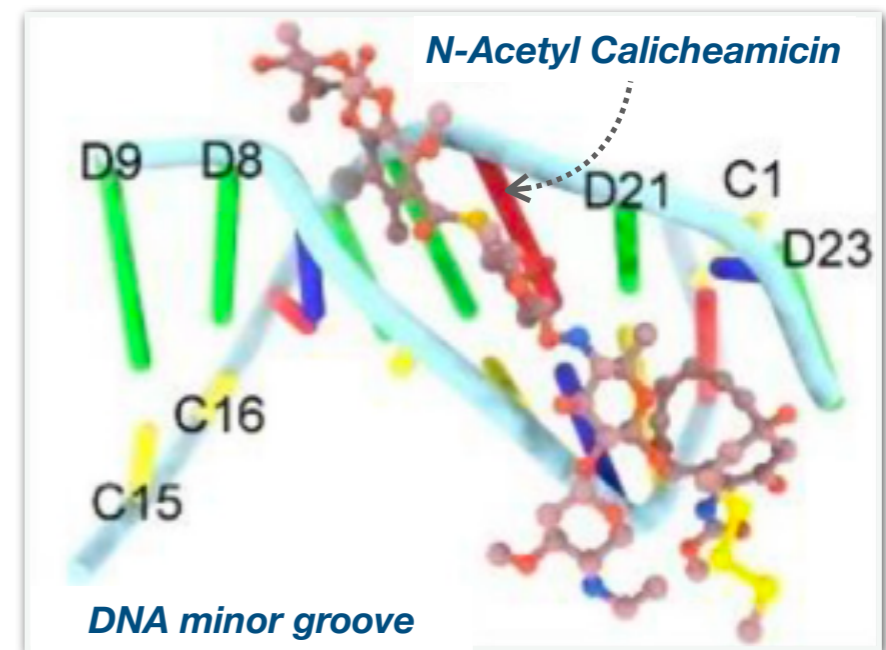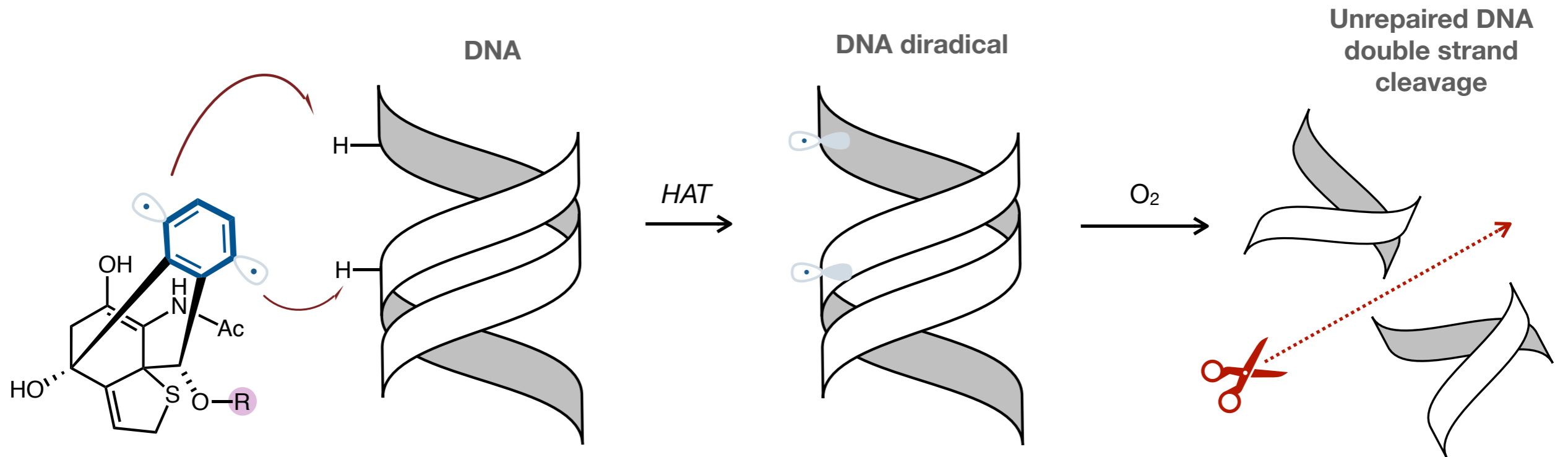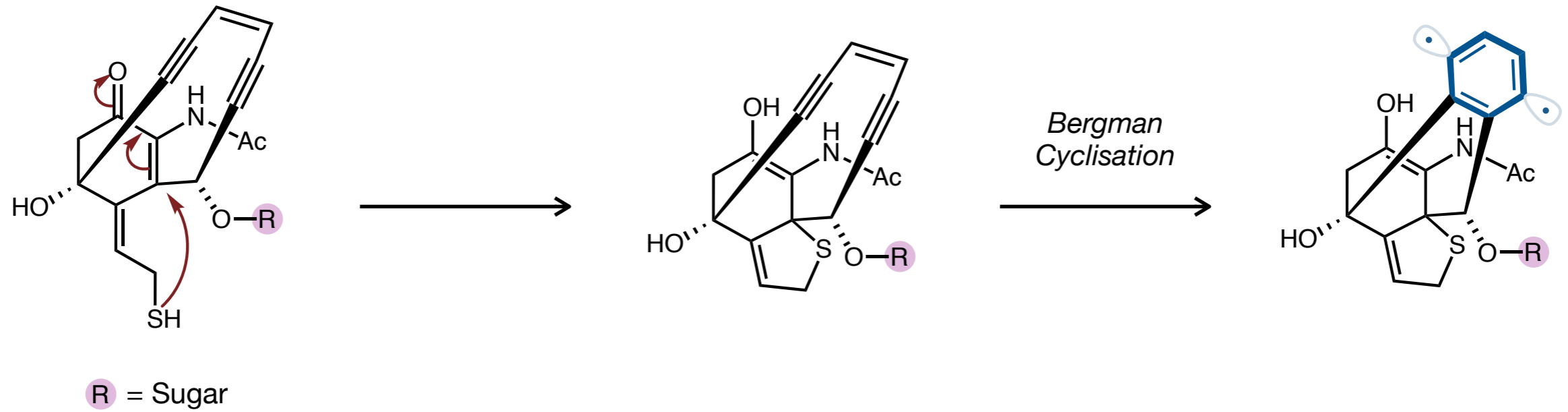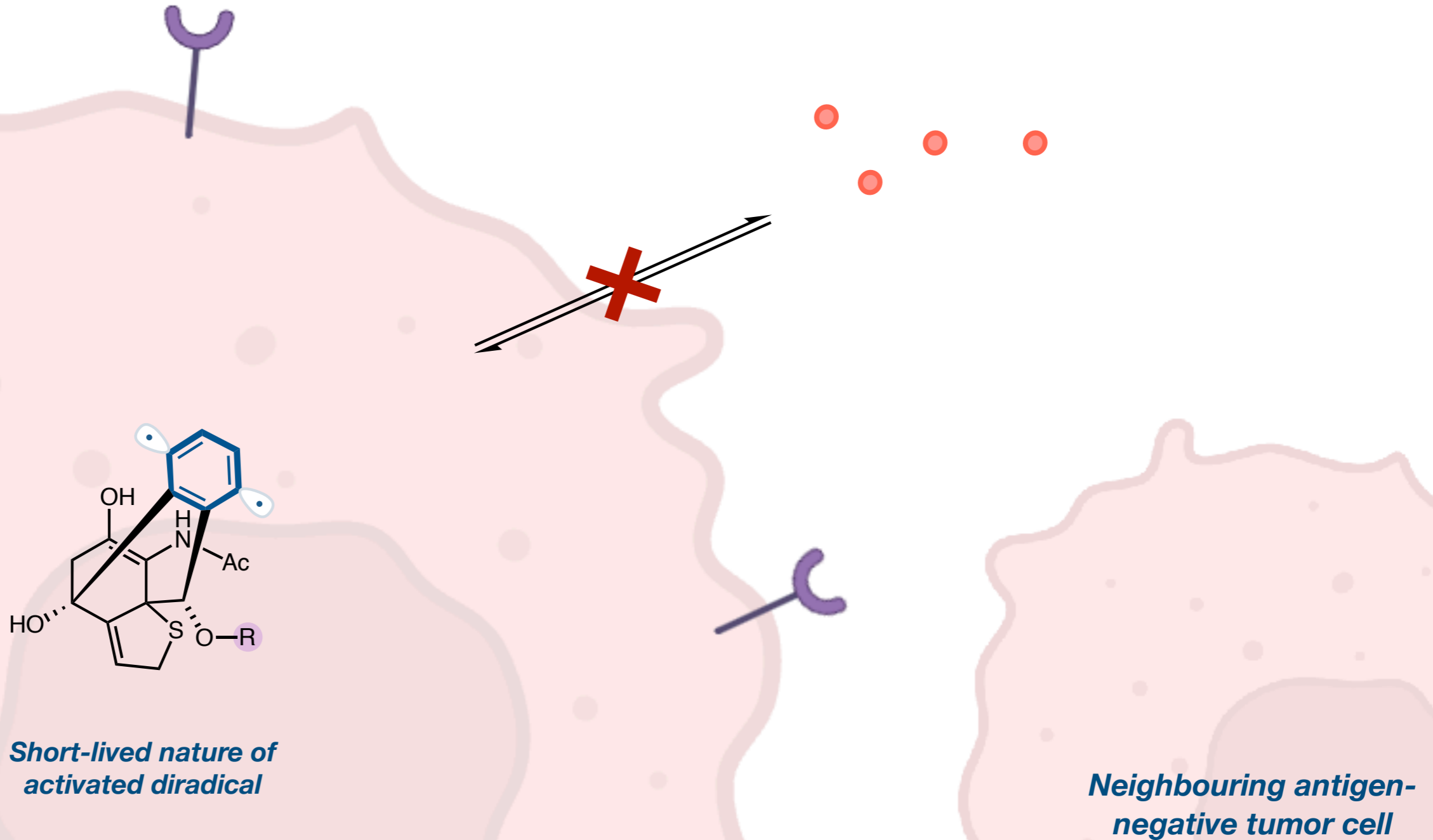
Calicheamicin-containing ADCs (Mylotarg™ and Besponsa™) do not exert bystander effects

Bystander effect

ADCs which do not exhibit bystander effect

Mylotarg™

Besponsa™

Calicheamicin-containing ADC

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Non-cleavable linker

Zynlota™

Blenrep™

Elahere™

Bystander effect

ADCs which exhibit bystander effect

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

How powerful is 'bystander effect' in the efficacy profile of ADCs?

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Kadcyla™
(Trastuzumab emtasine)
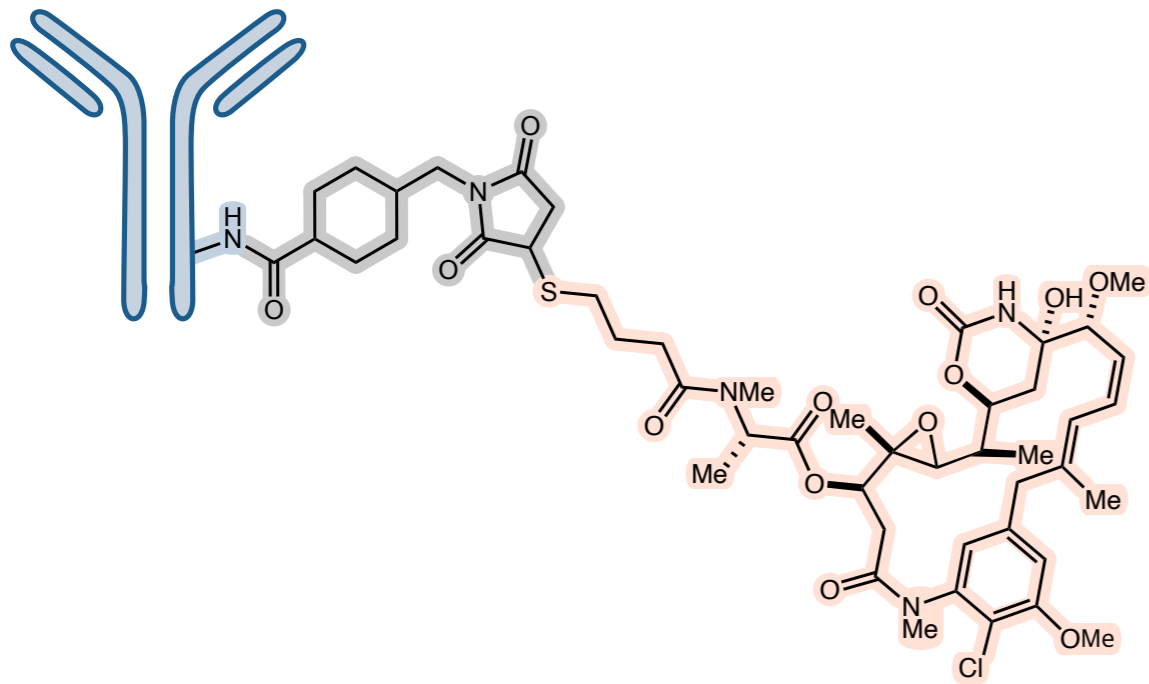
Genentech

Enhertu™
(Trastuzumab deruxtecan)

Daiichi-Sankyo

Kadcyla™ versus Enhertu™

Emtansine versus Deruxtecan
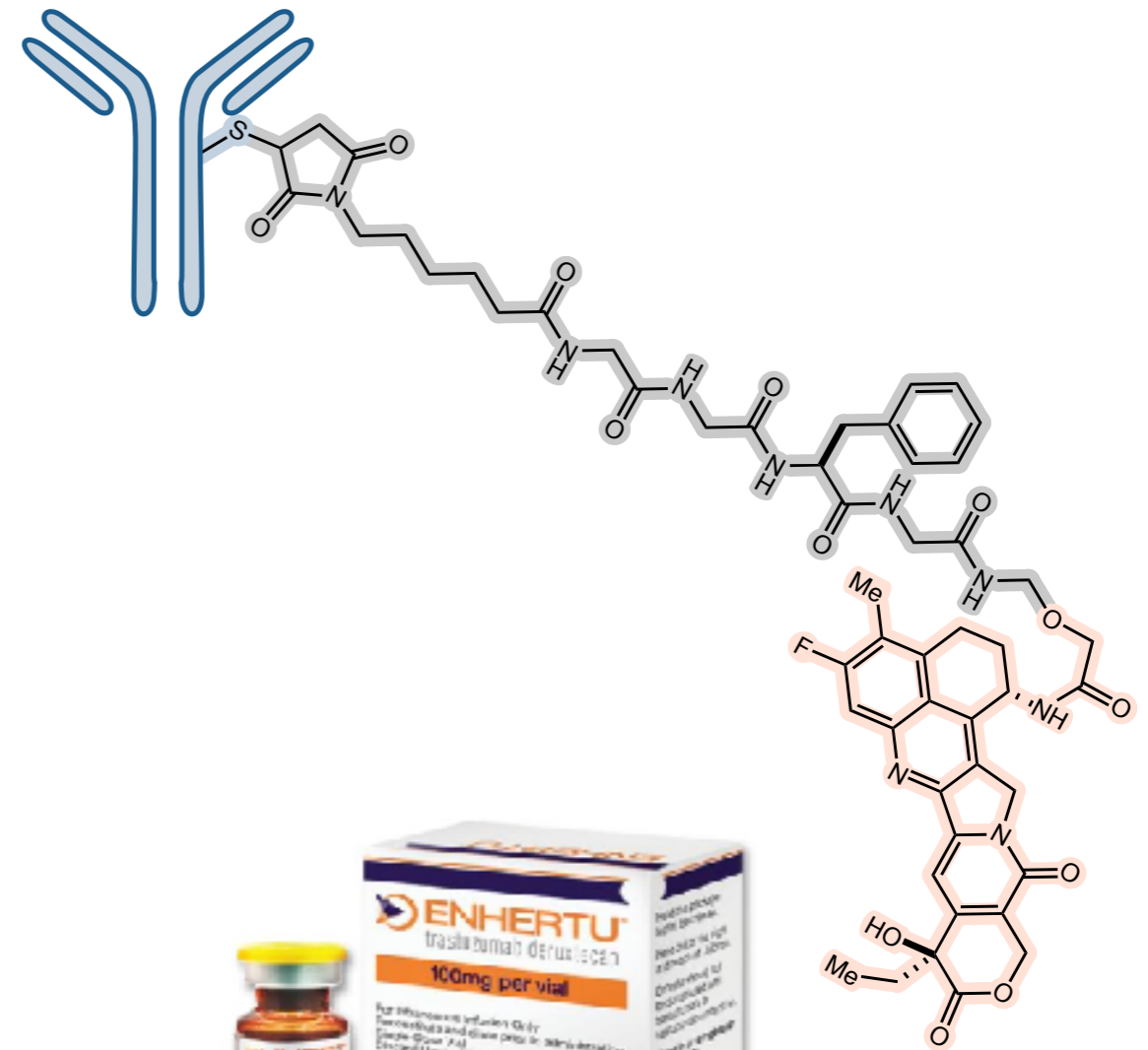
Kadcyla™
(Trastuzumab emtasine)

Genentech

Enhertu™
(Trastuzumab deruxtecan)
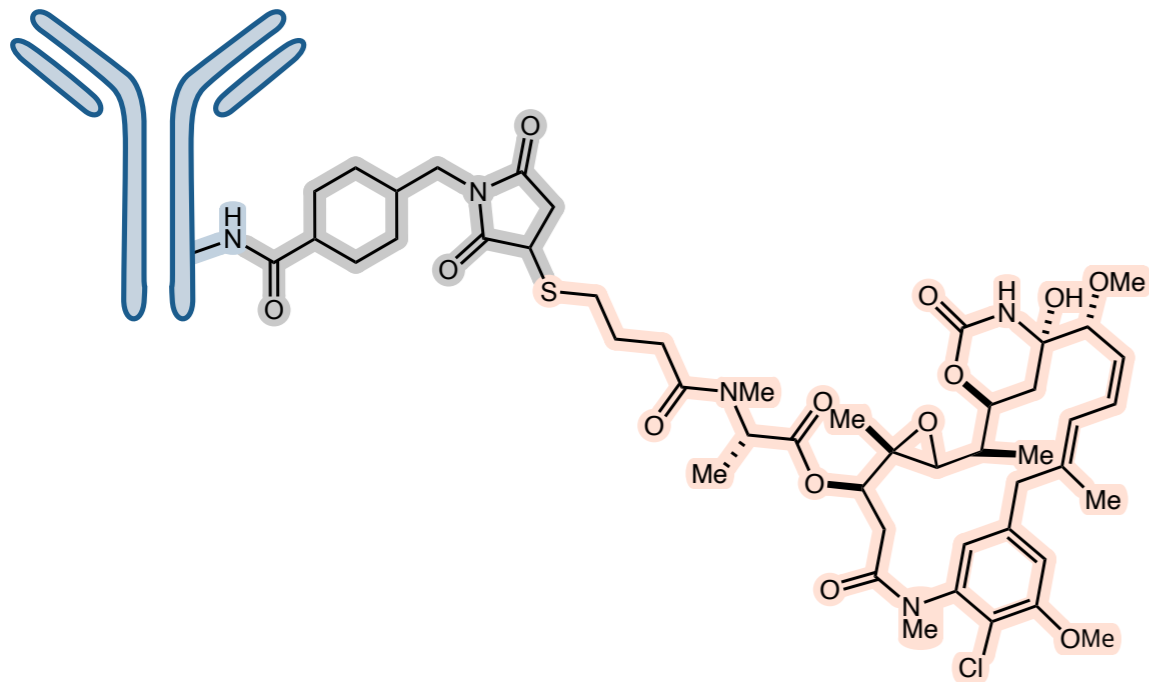
Daiichi-Sankyo

DM1 IC$_{50}$: 0.03 nM

DAR = 3.5
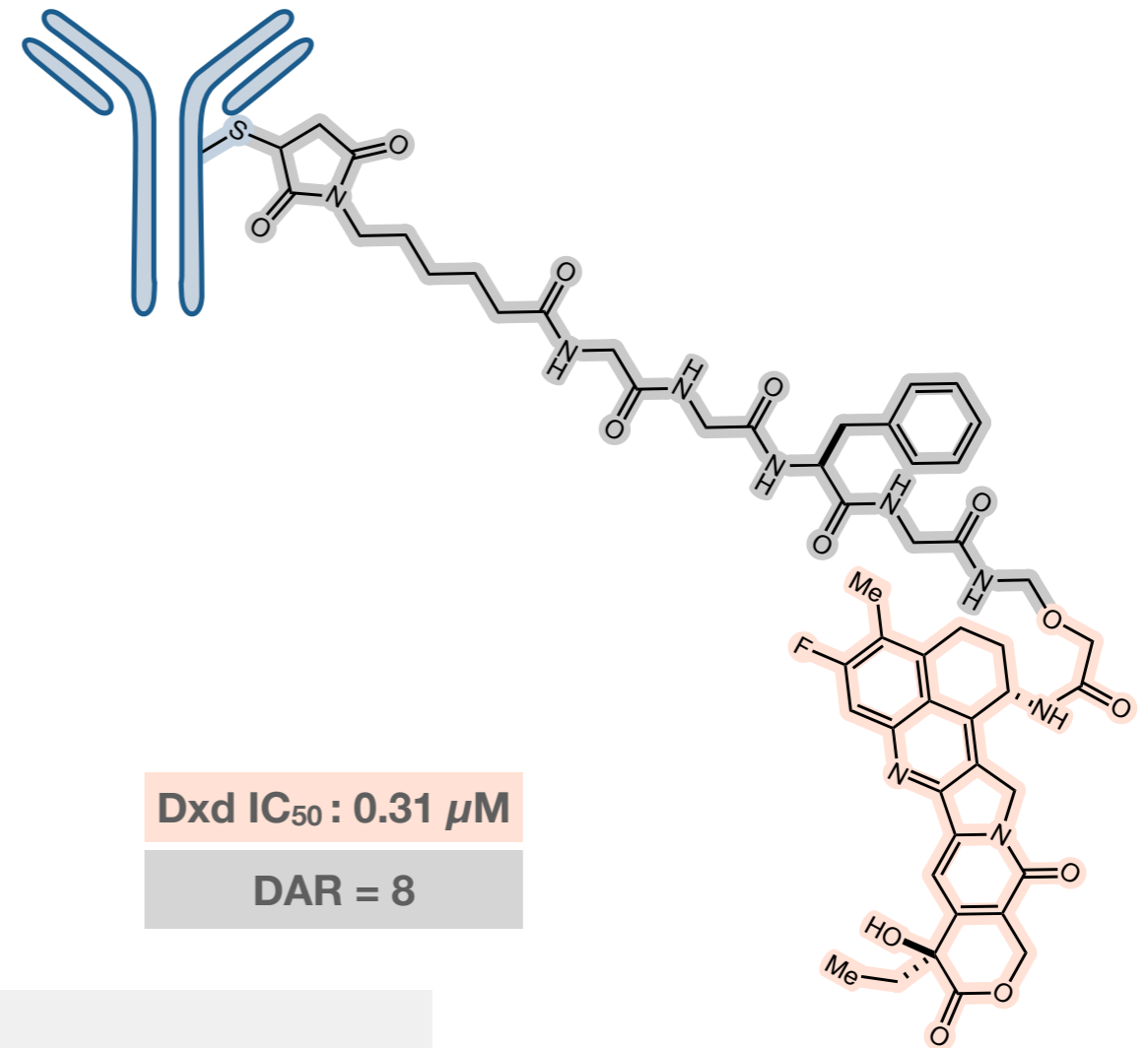
Dxd IC$_{50}$: 0.31 μM

DAR = 8

Same 'A' (trastuzumab)
Different 'DC'

Azar, I.; Alkassis, S.; Fukui, J.; Alsawah, F.; Fedak, K.; Al Hallak, M. N.; Sukari, A.; Nagasaka, M. *Lung Cancer Targets Ther.* **2021**, *Volume 12*, 103–114

Kadcyla™ versus Enhertu™

Emtansine versus Deruxtecan

Kadcyla™
(Trastuzumab emtasine)

Genentech

Enhertu™
(Trastuzumab deruxtecan)
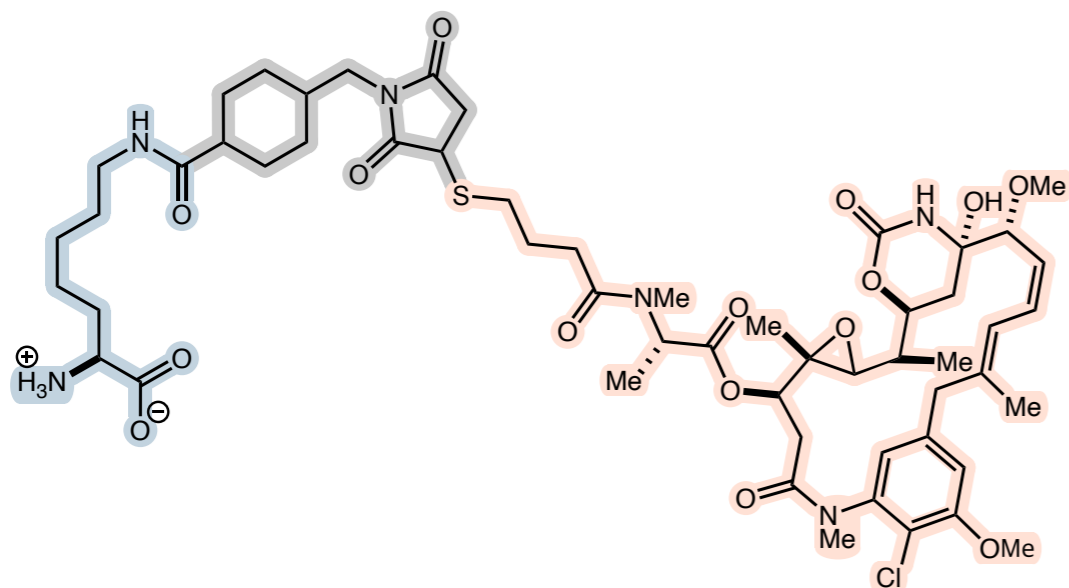
Daiichi-Sankyo

logD = 0.2
Low cell-permeability
(No bystander effect)
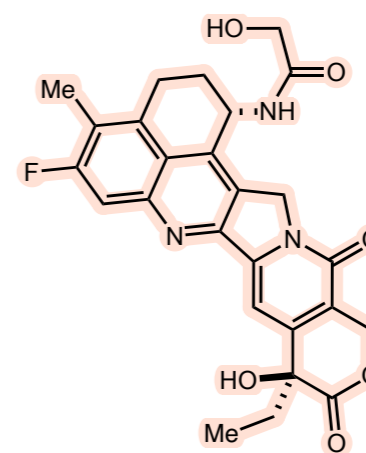
logD = 2.3
High cell-permeability
(Bystander effect)

Chen, R.; Ren, Z.; Bai, L.; Hu, X.; Chen, Y.; Ye, Q.; Hu, Y.; Shi, J. *Bioorganic Chem.* **2024**, *151*, 107697

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™
(Trastuzumab emtasine)

263 patients

Enhertu™
(Trastuzumab deruxtecan)

261 patients

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™
(Trastuzumab emtasine)

263 patients

Enhertu™
(Trastuzumab deruxtecan)

261 patients

Progression-free survival

34.1% of participants were still alive
without their cancer getting worse
after 12 months

75.8% of participants were still alive
without their cancer getting worse
after 12 months

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™
(Trastuzumab emtasine)

263 patients

Enhertu™
(Trastuzumab deruxtecan)

261 patients

Overall response rate

34.2% of participants partially or
completely responded to Kadcyla™

79.7% of participants partially or
completely responded to Enhertu™

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Phase 3, open-label, **HER2-positive patients (2L)**
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™
(Trastuzumab emtasine)

263 patients

Enhertu™
(Trastuzumab deruxtecan)

261 patients

Overall response rate

34.2% of participants partially or completely responded to Kadcyla™

79.7% of participants partially or completely responded to Enhertu™

Enhertu™ in HER2-low advanced patients
DESTINY Breast04 trial

Enhertu™ in HER2-low advanced patients
DESTINY Breast04 trial

Physician's choice

181 patients
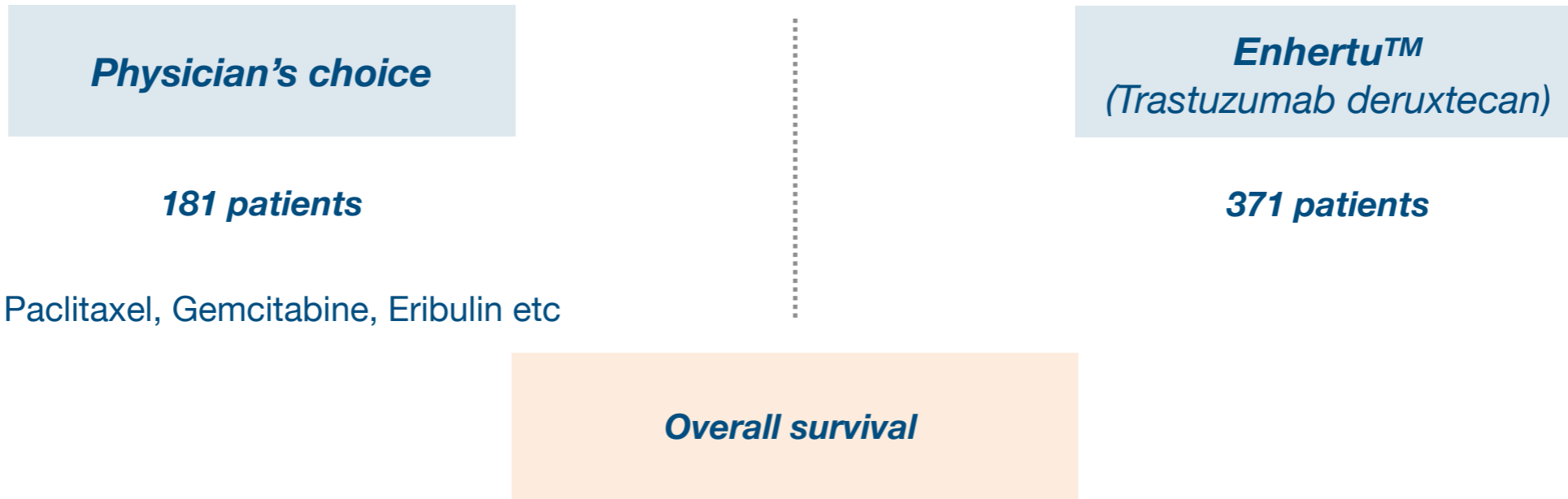
Paclitaxel, Gemcitabine, Eribulin etc
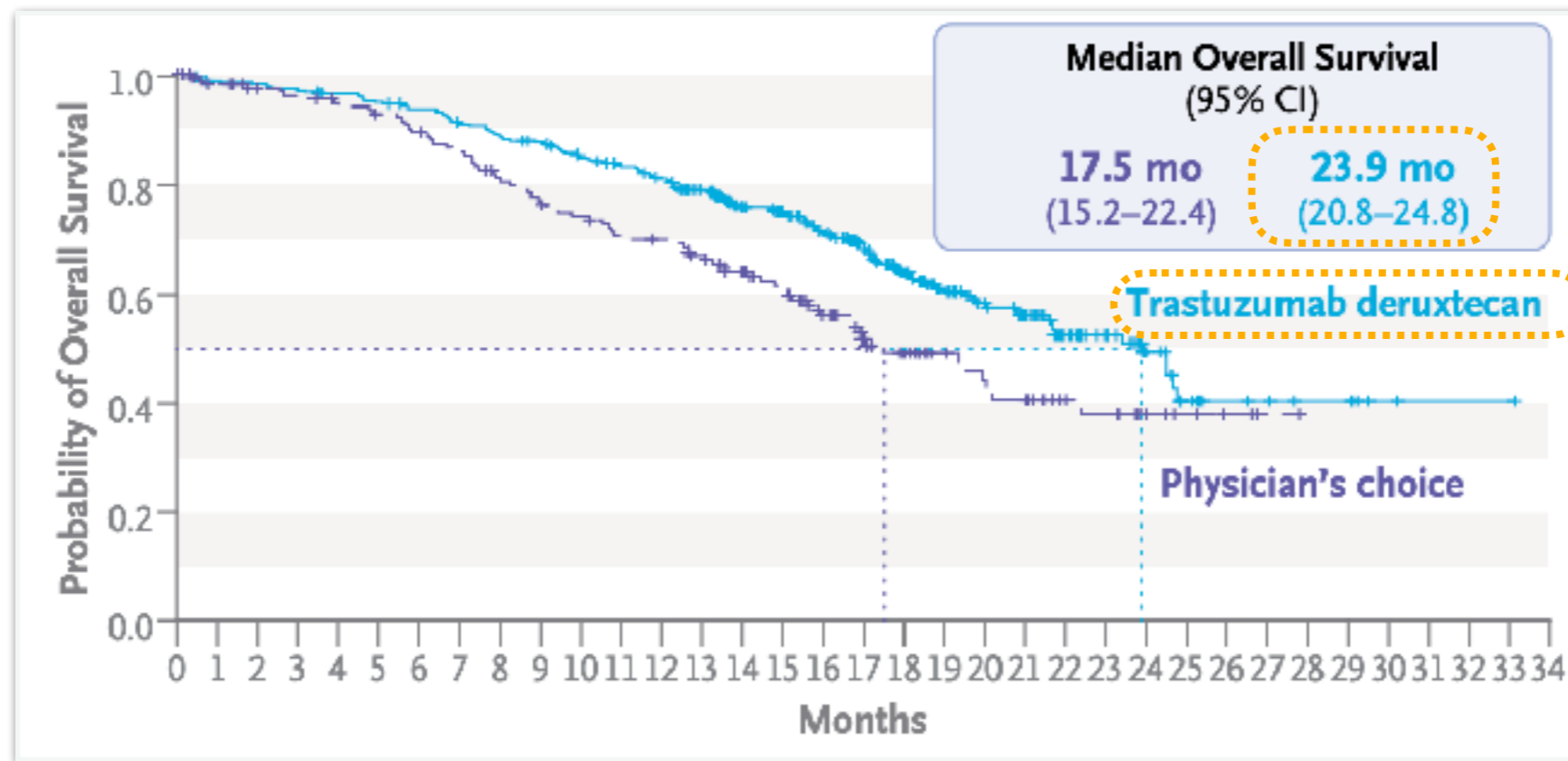
Enhertu™
(Trastuzumab deruxtecan)

371 patients

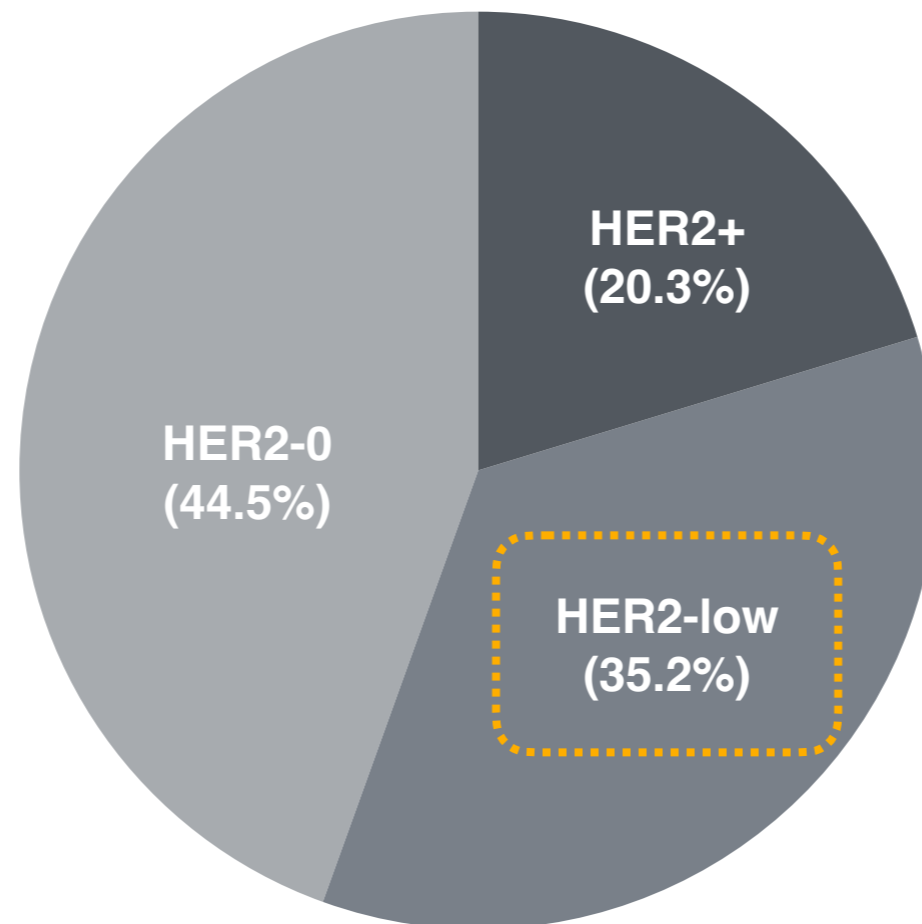# Enhertu™ in HER2-low advanced patients
DESTINY Breast04 trial

Physician's choice

Enhertu™
(Trastuzumab deruxtecan)

181 patients

371 patients

Paclitaxel, Gemcitabine, Eribulin etc

Overall survival

Enhertu™ in HER2-low patients
DESTINY Breast04 trial

Physician's choice

181 patients

Paclitaxel, Gemcitabine, Eribulin etc

Enhertu™
(Trastuzumab deruxtecan)

371 patients

Overall survival

N. Engl. J. Med. **2022**, *387* (1), 9–20

Enhertu™ in HER2-low patients
DESTINY Breast04 trial

HER2+
(20.3%)

HER2-0
(44.5%)

HER2-low
(35.2%)

Gampenrieder, S. P.; Rinnerthaler, G.; Greil, R. *Breast Cancer Res.* **2021**, *23* (1), 112

Enhertu™ in HER2-low patients
DESTINY Breast04 trial

HER2+ (20.3%)

HER2-0 (44.5%)

HER2-low (35.2%)

(ASCO 2022)

Enhertu™ in HER2-low patients
DESTINY Breast04 trial
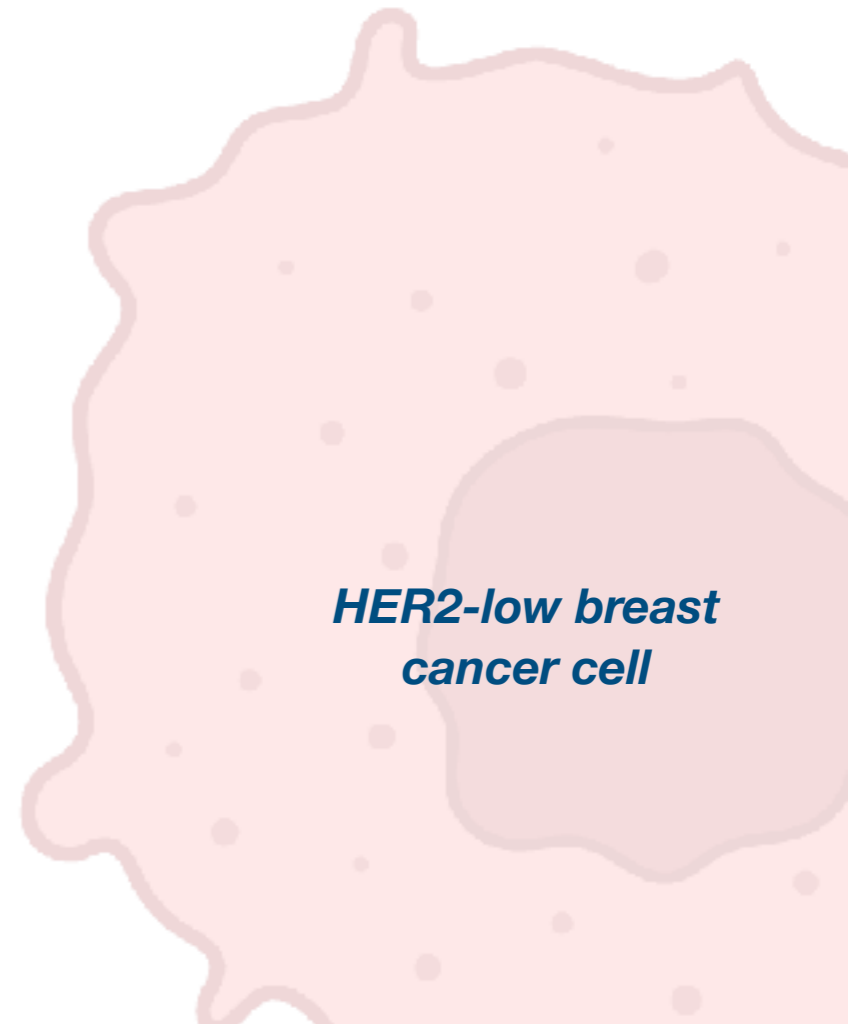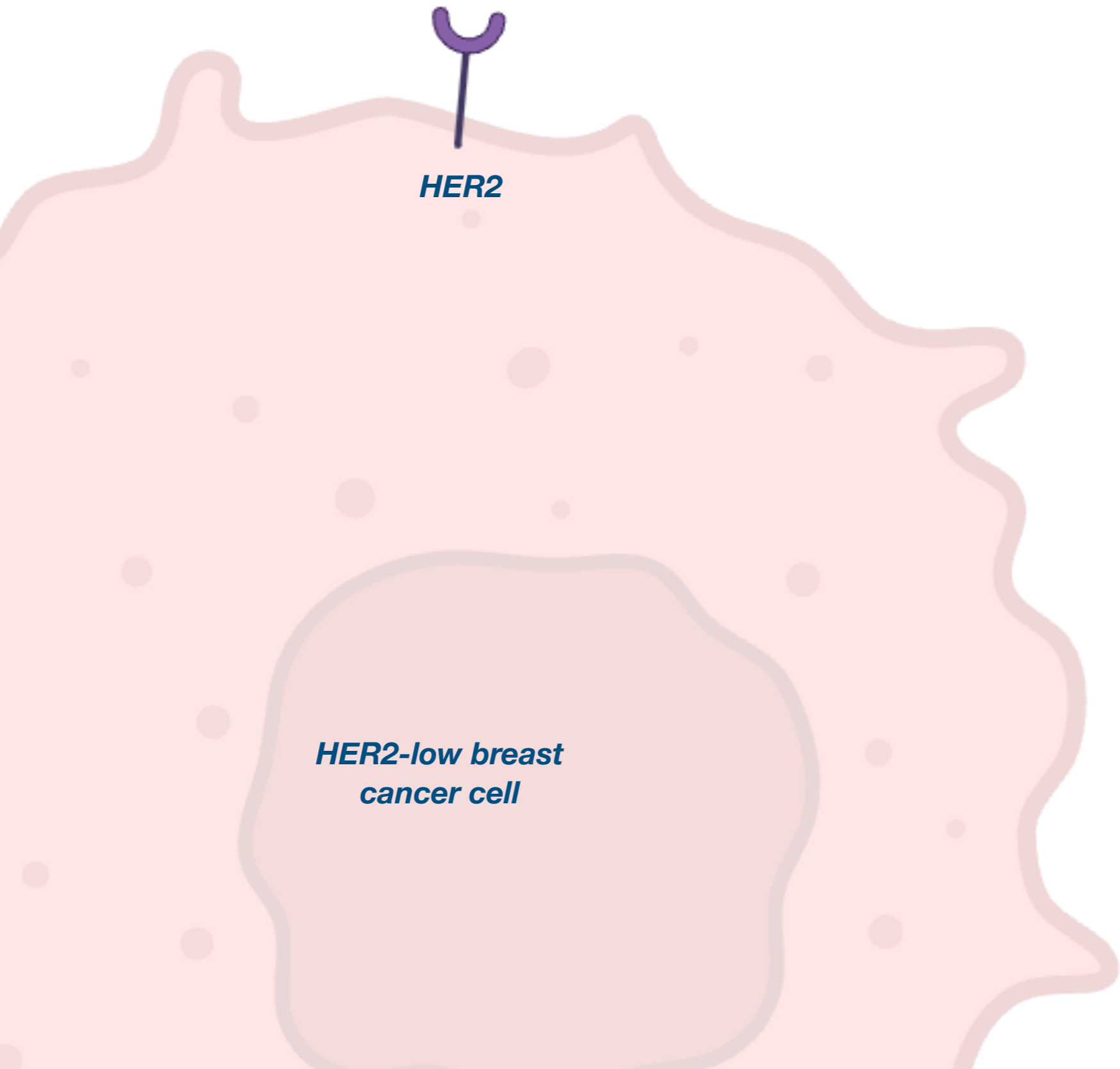
HER2

HER2-low breast
cancer cell

HER2-low breast
cancer cell

Enhertu™ in HER2-low patients
DESTINY Breast04 trial

"Bystander effect"

logD = 2.3
High cell-permeability
(Bystander effect)

HER2-low breast
cancer cell

The bystander effect of Enhertu™ has enabled its use in
tumors with low/heterogenous HER2 expression

Enhertu™ in HER2-low patients
DESTINY Breast04 trial

In interview with Breast Cancer Research Foundation, on DESTINY trial (ASCO 2022)

*"… The key feature that makes T-DXd different is the linker. T-DXd unlike T-DM1 has a cleavable linker. When the chemo is lopped off from the antibody, it leaves the chemotherapy in a membrane-permeable state. So now when the chemotherapy is released in the cancer cell, it can kill that HER2-positive cancer cell. And that's where most ADCs would stop. That's where T-DM1 would stop. But now this chemotherapy can pass through the cell membrane and can enter the microenvironment and neighboring cells and kill those cells as well. And that can include cells that have variable levels of HER2 expression—even HER2-low cells. **That's the tremendous advantage of T-DXd, not only over T-DM1, frankly, but over all of our currently available HER2-targeted therapies**"*

Dr Shanu Modi, Study Leader of DESTINY trial (Memorial Sloan Kettering Cancer Center)

'D' in ADC
What makes a good 'D' for ADC
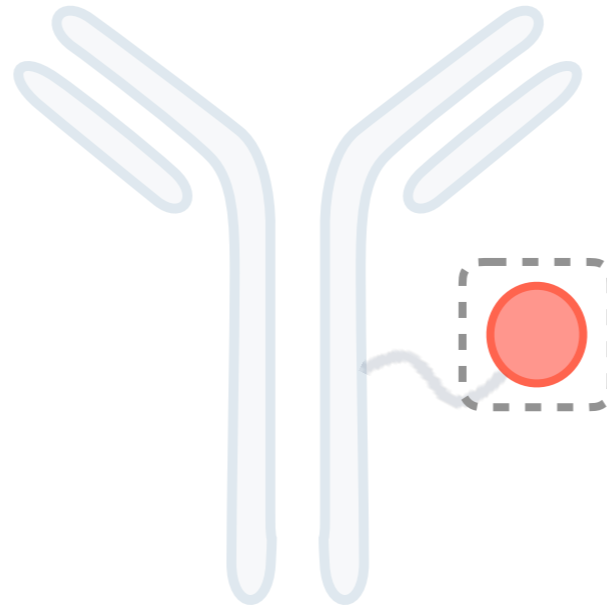
✓ *Sufficiently cytotoxic (micromolar/nanomolar)*

✓ *Bystander effect*

✓ *New class of 'D': Expansion of scope and modality*
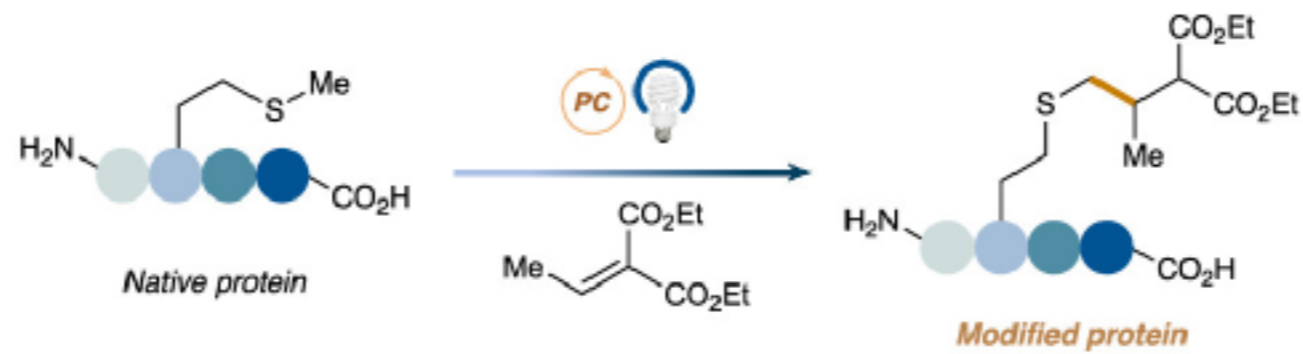
Conclusion and outlooks

Final thoughts

- **ADC: Realization of the 'Magic Bullet' concept**

- **'D' of ADC: The bystander effect plays a crucial role in enhancing ADC efficacy**

- **'C' of ADC: ADCs with heterogeneous DARs are not likely to be approved by the US FDA in the future**

- **'A' of ADC: Better elucidation of mechanism of action of 'A' (endocytosis to lysosomal degradation) will lead us to a better selection of 'A' for ADC**

Conclusion and outlooks

Final thoughts

- **'C' of ADC: A novel array of bioconjugation methods with photoredox catalysis will enable rapid access to conjugate modalities beyond ADCs**

Methionine conjugation (J. Am. Chem. Soc., 2020)

Tyrosine conjugation (Nat. Chem. 2021)
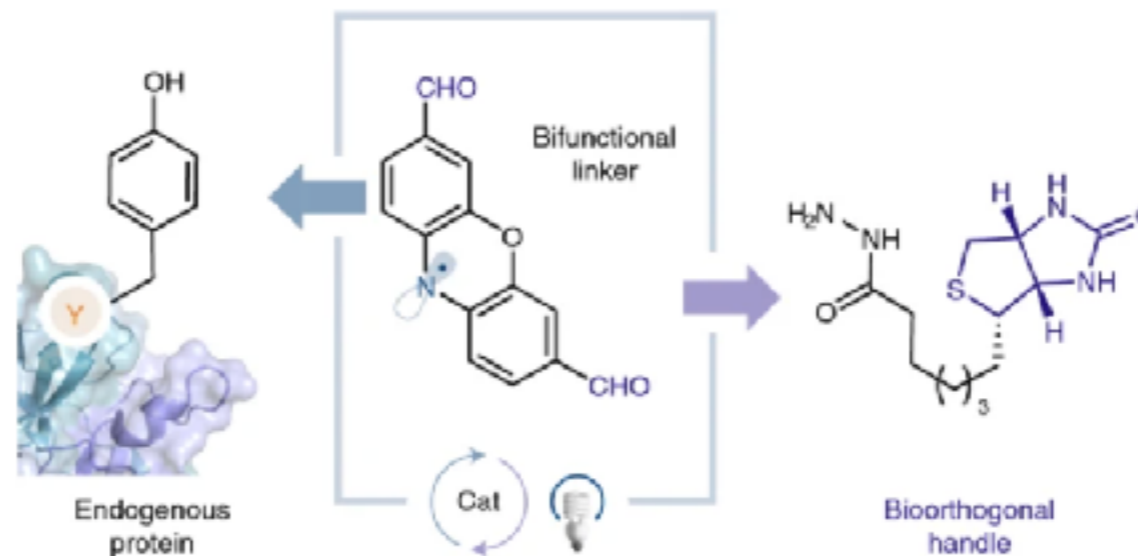
Conclusion and outlooks

Final thoughts

- *'A' of ADC: Better elucidation of mechanism of action of 'A' (endocytosis to lysosomal degradation) will lead us to a better selection of 'A' for ADC*
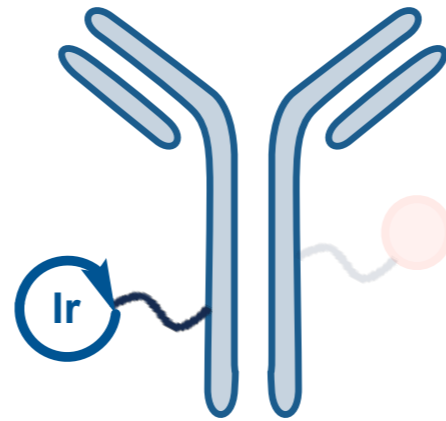
mAbs used in ADC

Time-resolved proximity labeling to elucidate the mechanism of endocytosis & its interactions with lysosome proteomes

Our own group's chemical biology tool can be used to further develop this field

Acknowledgements

ADC patent

Prof. Teck-Peng Loh

(Nanyang Technological University)

Acknowledgements

Prof. David W. C. MacMillan   **The MacMillan Group**

PRINCETON UNIVERSITY

Questions?