Transition Metal Catalysis in Living Systems

Literature Seminar #3

2022/4/15 (Fri)

Wataru Atsumi (B6)

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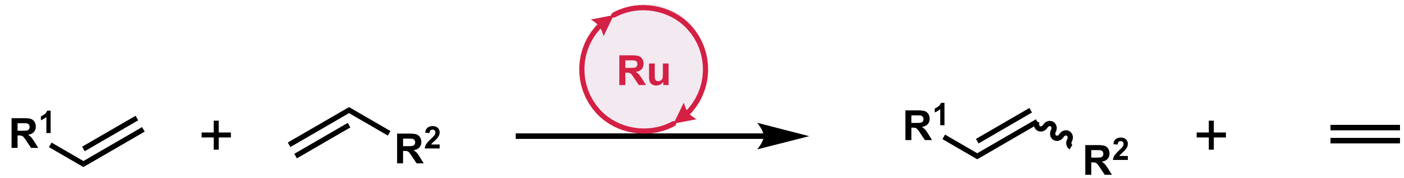
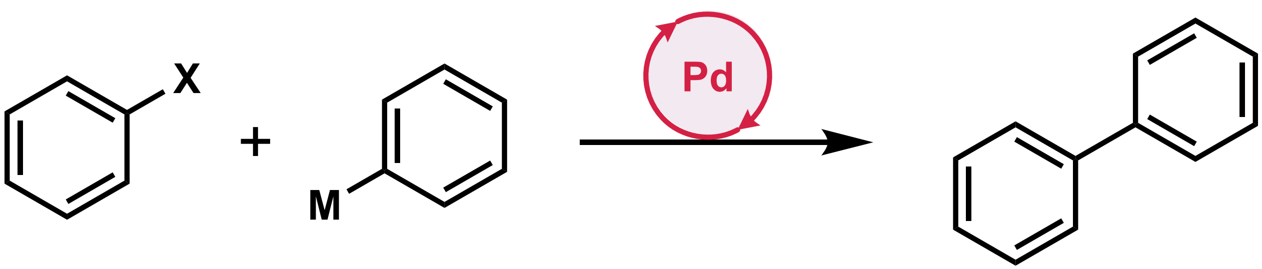
【Nobel prize in chemistry】

- **Olefin metathesis (2005)**



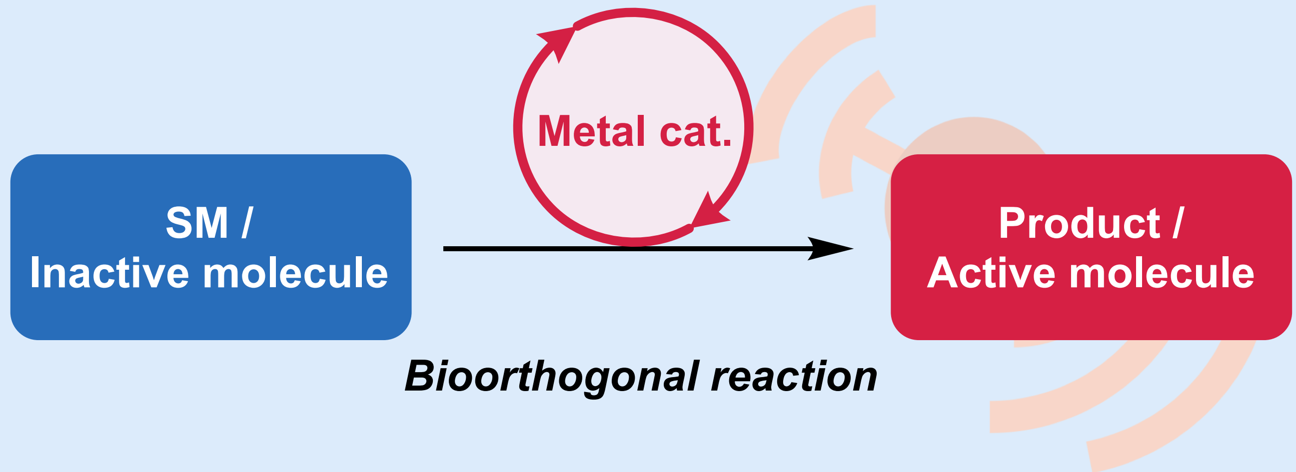
- **Pd-catalyzed cross coupling (2010)**



Nobel prize!!

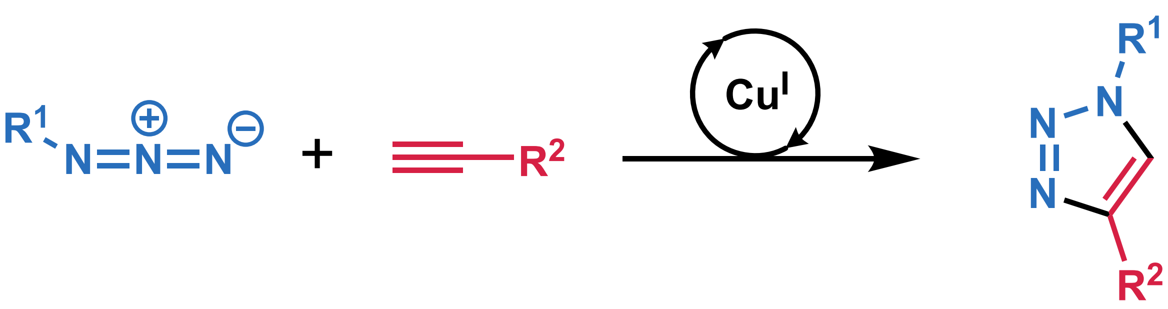
- ✓ **Transformation which has been difficult to perform by classical ways**
- ✓ **Wider range of reactions**
- ✓ **Very efficient synthesis**

TM Catalysis in Living Systems



It enables previously unfeasible chemical transformations!

【Example: Cu-catalyzed alkyne-azide cycloaddition (CuAAC)】



A powerful tool for chemical biology and pharmaceutical sciences!!

TM Catalysis in Living Systems

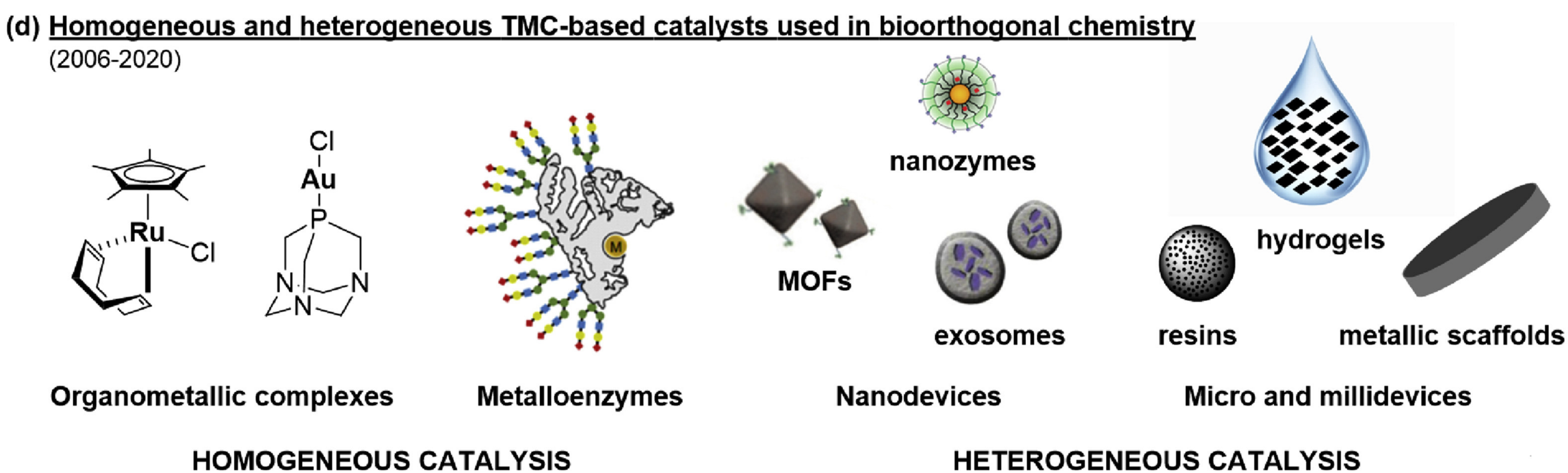
Problems

- ✗ Toxicity
- ✗ Water solubility
- ✗ Stability in water

Requirements

- ✓ Soluble in water
- ✓ Protecting active site

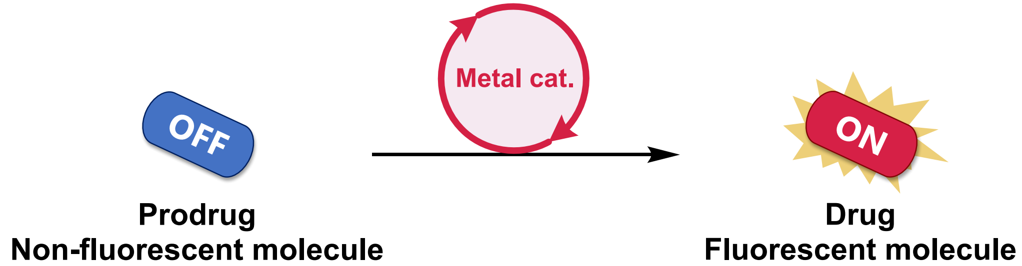
*See appendix for more information.



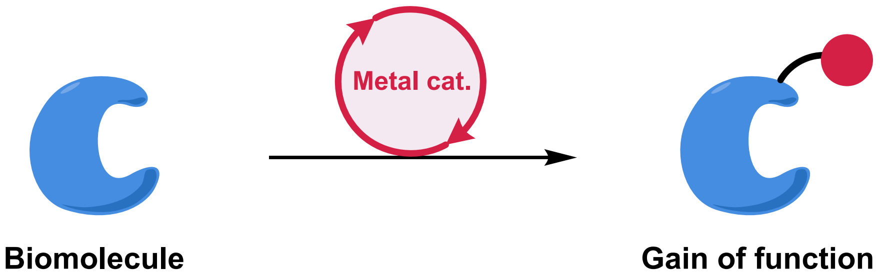
Curr. Opin. Chem. Biol. **2021**, *61*, 32–42.

Applications of TM Catalysis

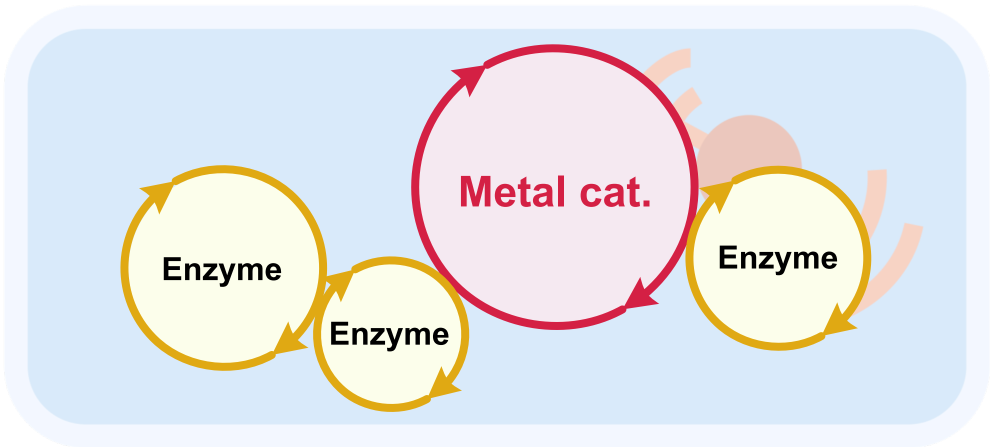
✓ **Prodrug activation / Bioimaging**



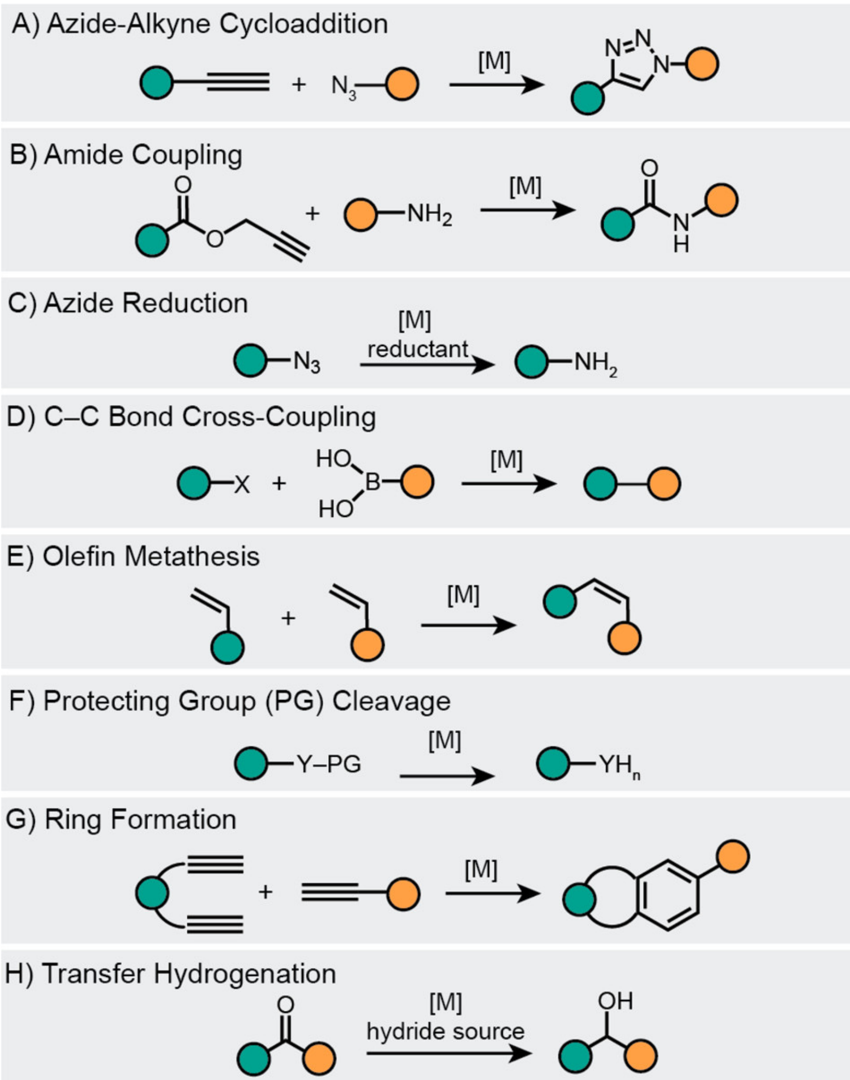
✓ **Modification of biomolecules**



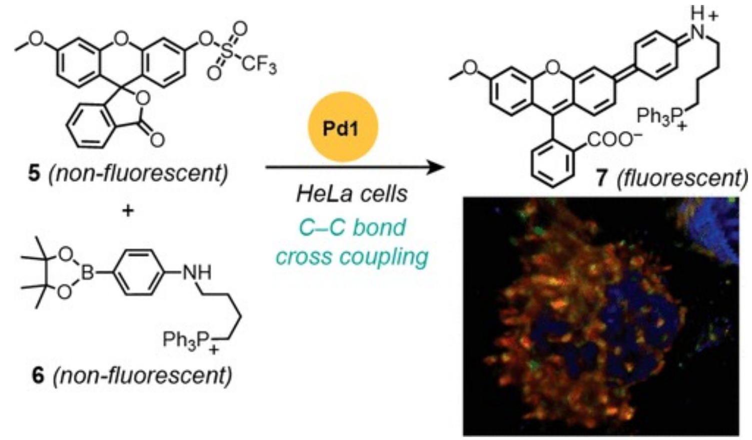
✓ **Regulating biological reactions / Creating new cascades**



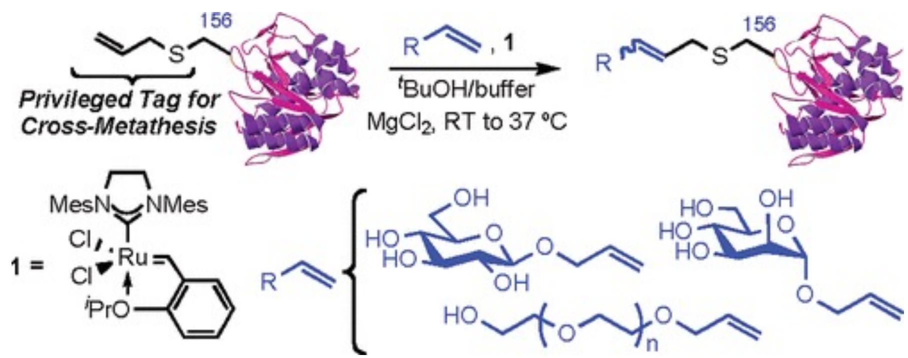
Examples of TM Catalysis in biological conditions



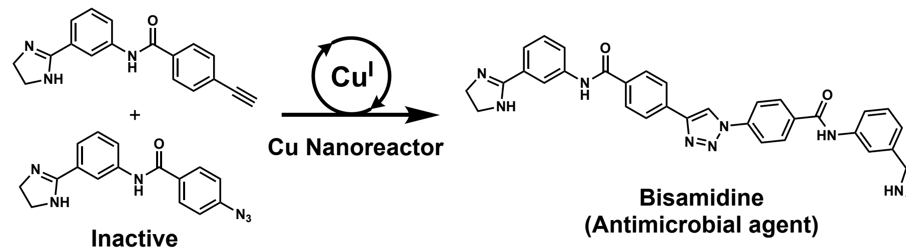
ACS Catal. **2021**, *11*, 5148−5165.



Yusop, R. M. *et al. Nat. Chem.* **2011**, *3*, 239−243.



B. G. Davis *et al. J. Am. Chem. Soc.* **2008**, *130*, 9642-9643.



Yugang Bai *et al. J. Am. Chem. Soc.* **2016**, *138*, 11077–11080.

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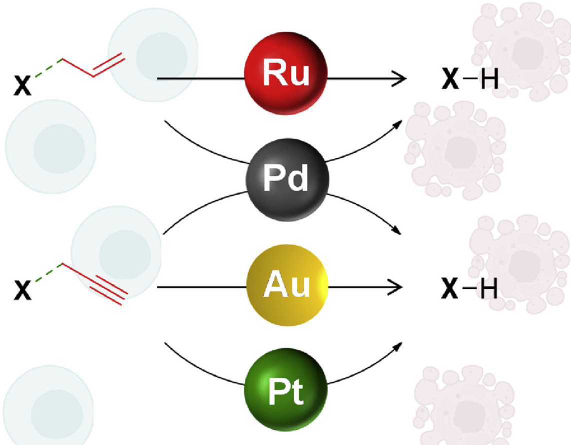
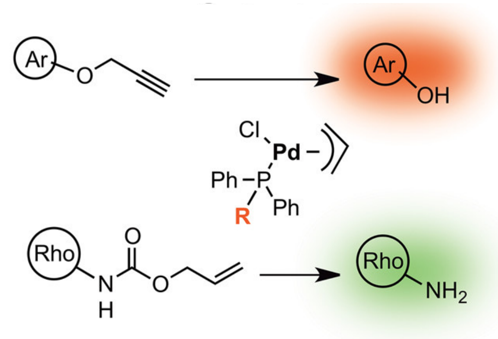
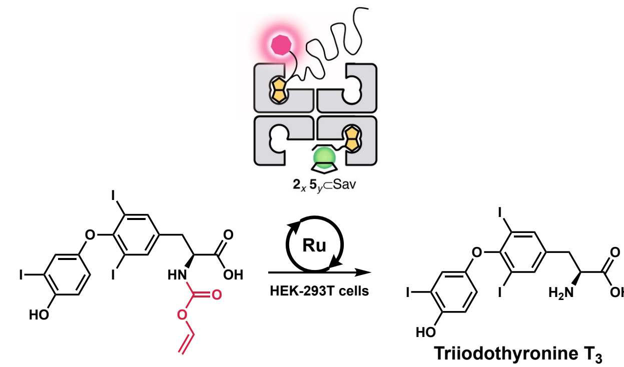
Bioorthogonal Allyl/Propargyl Cleavage

【Previous works】





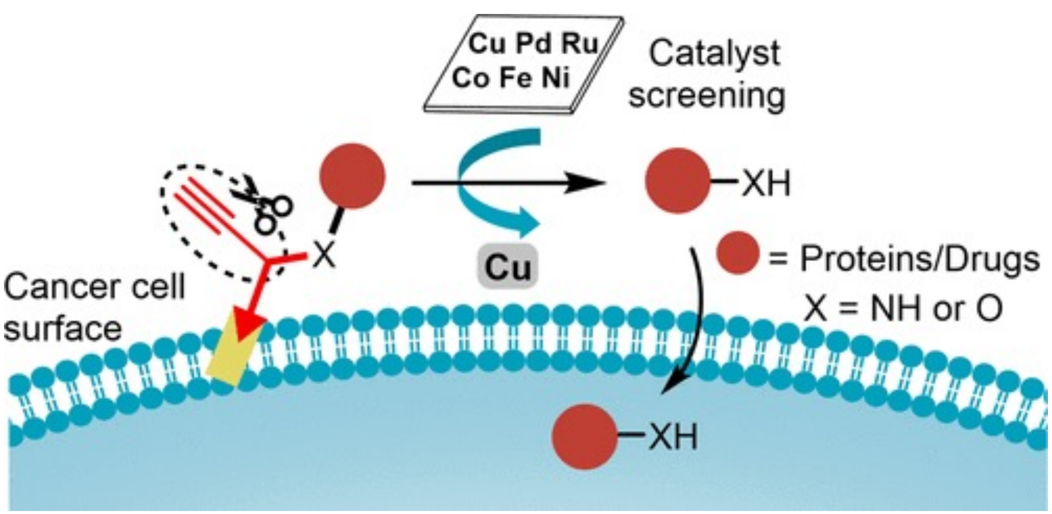
Martínez-Calvo, M. *et al. ACS Catal.* **2018**, *8*, 6055–6061.



Y. Okamoto *et al. Nat. Commun.* **2018**, *9*, 1–7.

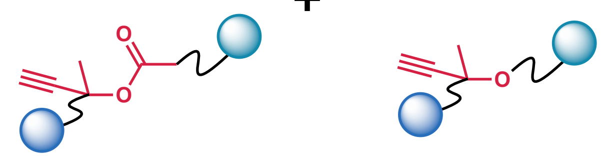
TM-catalyzed cleavage reactions have been mainly focused on terminal decaging.

【This work: Internal bond cleavage】



Cu(I) complex

+



dual-substituted propargyloxycarbonyl (dsProc)

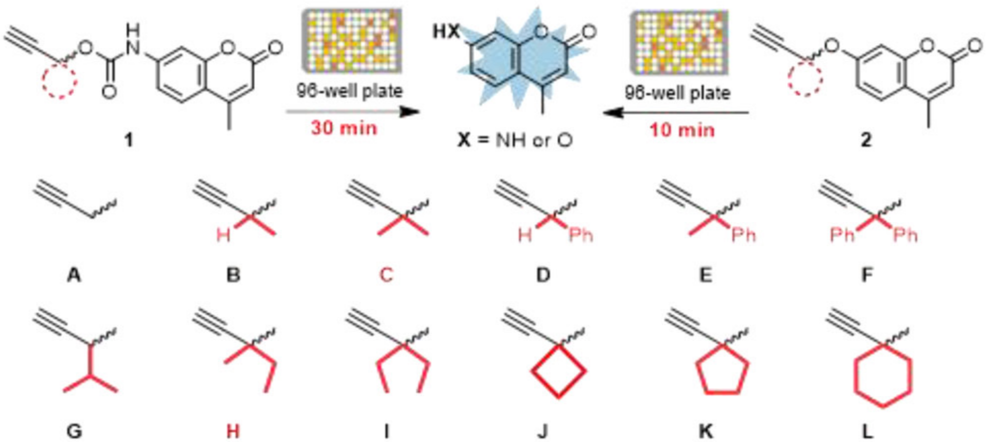
dual-substituted propargyl (dsPra)

- ✓ **ADCs linker cleavage**
- ✓ **Reversible cell modification**
- ✓ **Protein manipulation**

Curr. Opin. Chem. Biol. **2021**, *61*, 32–42. / Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Screening and Optimization

【Systematic screening】

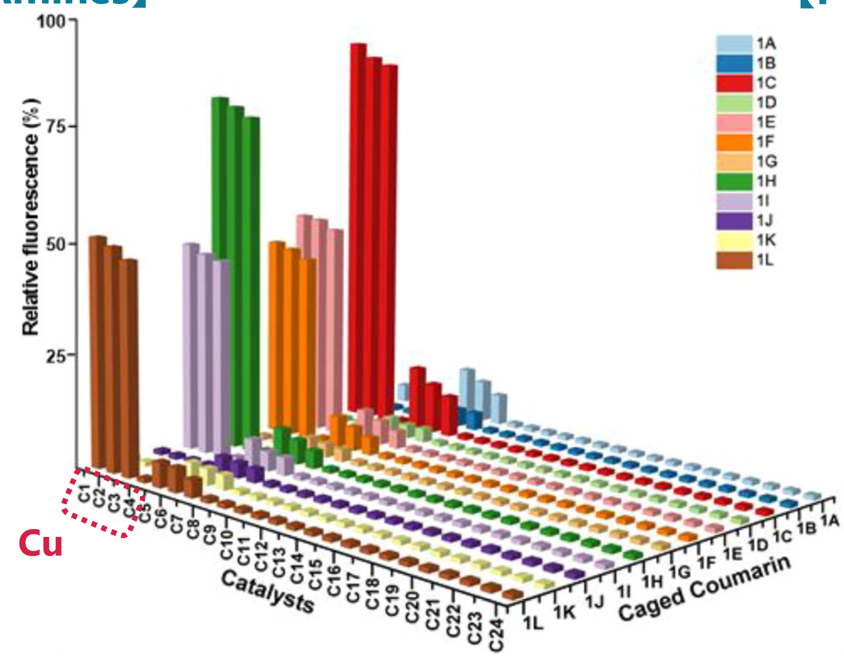


Screening using fluorogenic coumarins

- **24** different transition metal species (C1–C24)
- **12** different substrates (A–L)

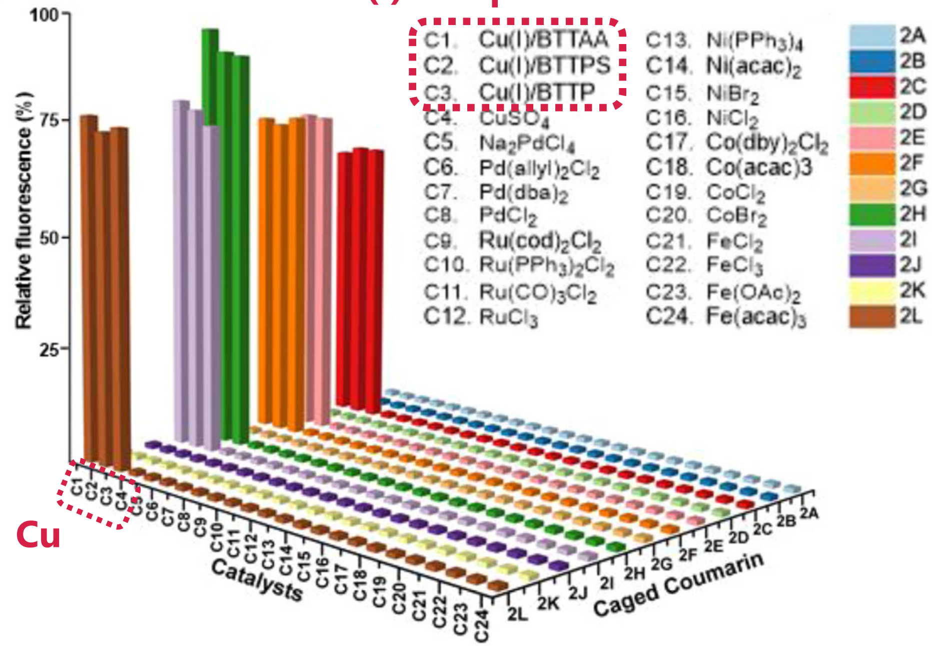
- ✓ **Cu complexes (except for $CuSO_4$) showed highly efficient cleavage.**
- ✓ **Other metals showed lower or no activity.**
- ✓ **1D and 2H gave the highest reactivity respectively.**

【Amines】



【Phenols】

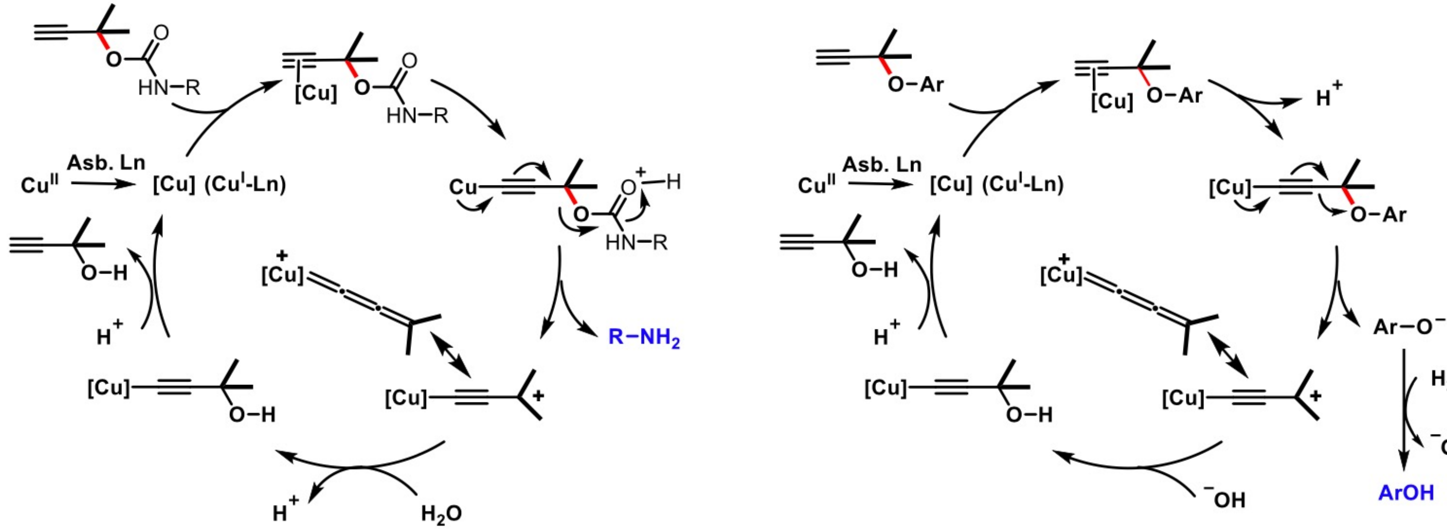
Cu(I) complexes



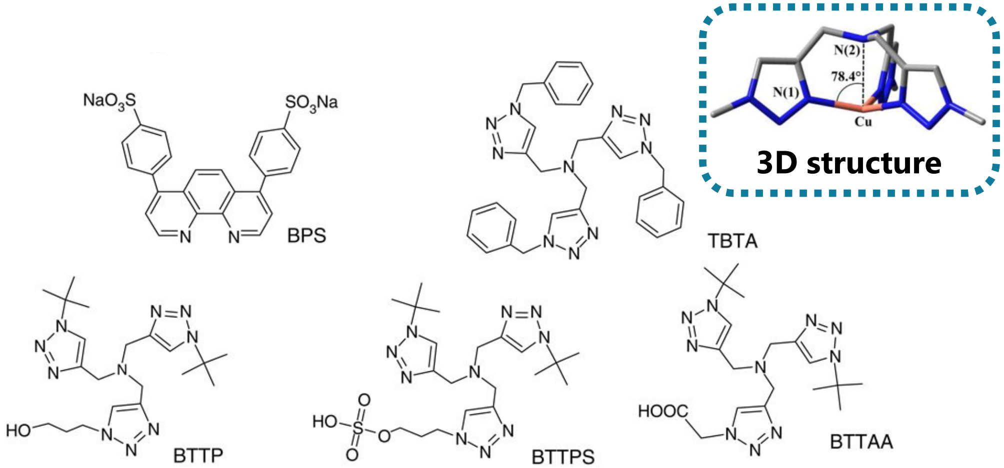
Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Action of Cu(I) Complex

【Proposed catalytic cycle】



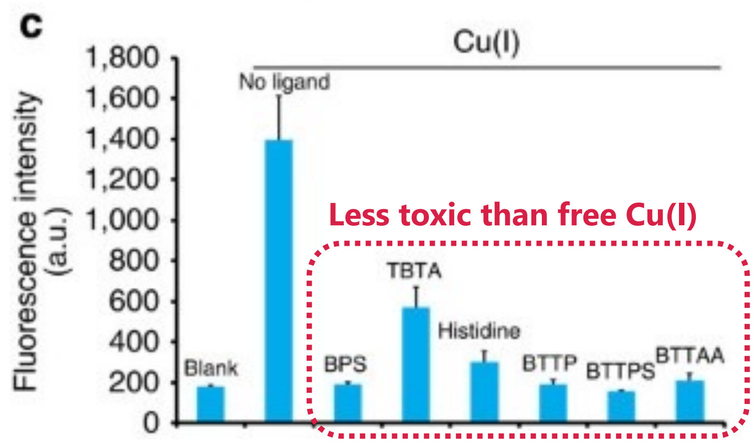
【Cu(I) stabilizing ligands】



3D structure

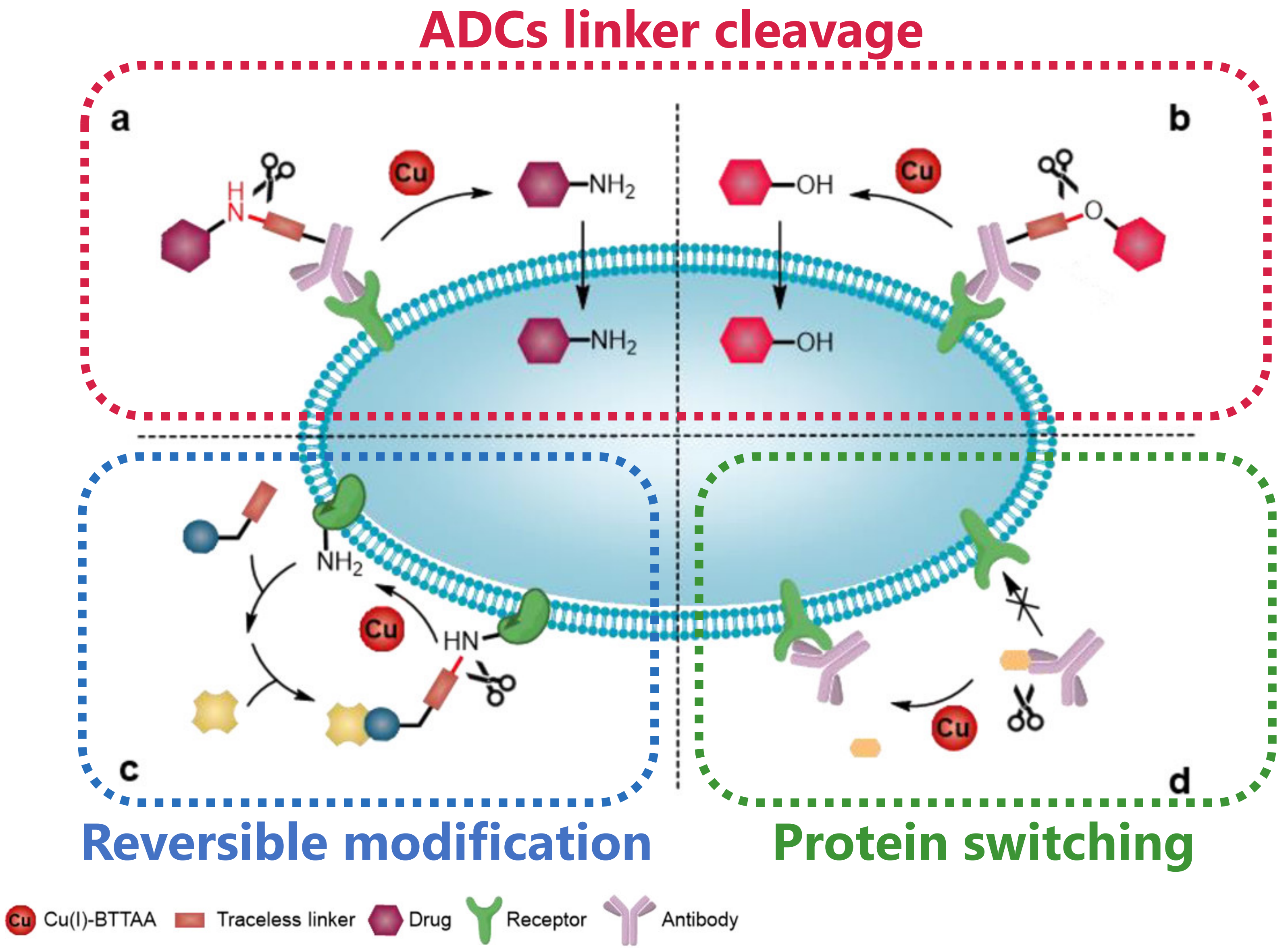
Details → See appendix

【ROS generation of Cu(I) complexes】



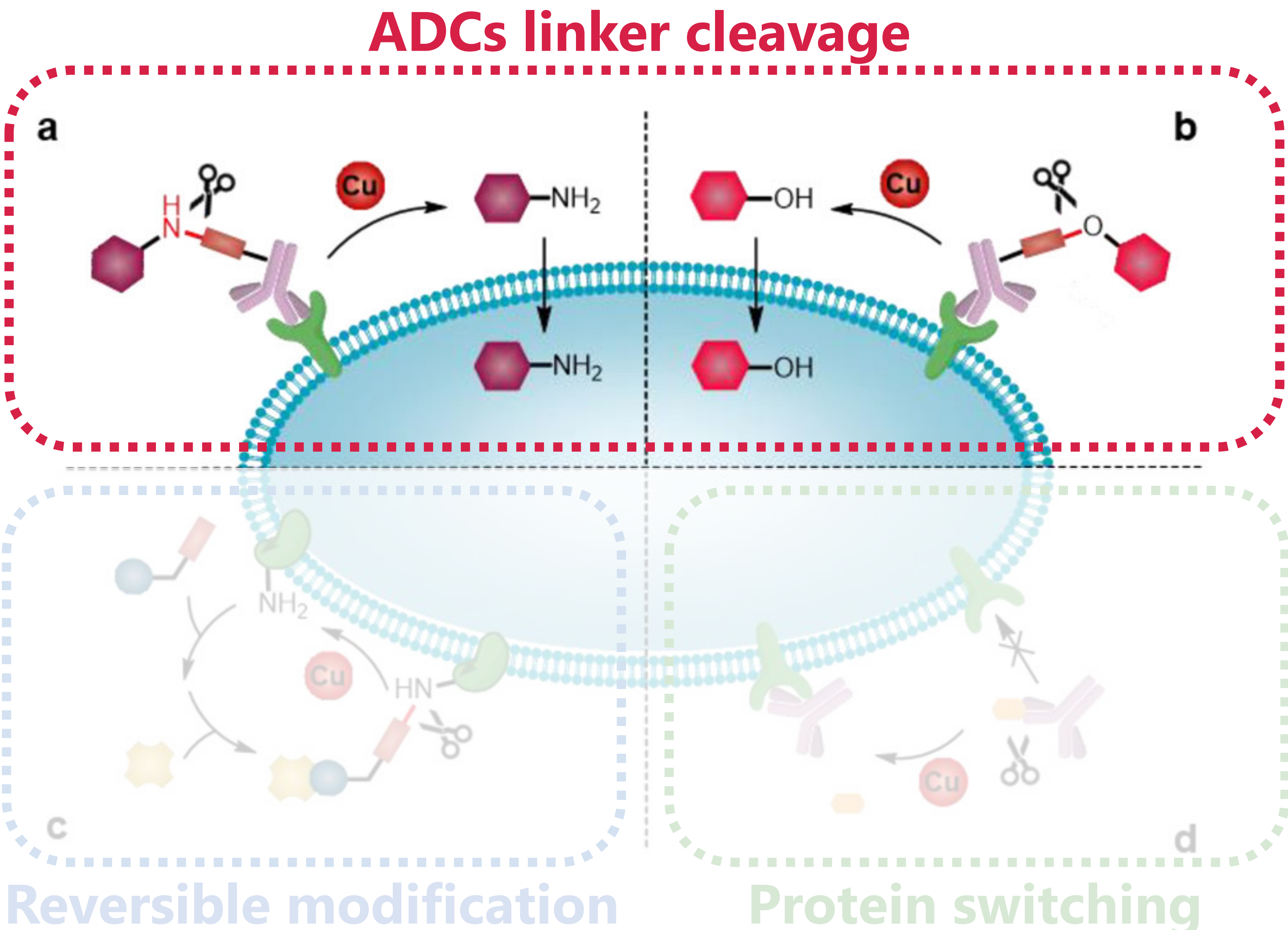
Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.
Yang M. *et al. Nat. Commun.* **2014**, *5*, 4981.

Cu-Triggered ADCs Cleavage and Modification



Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

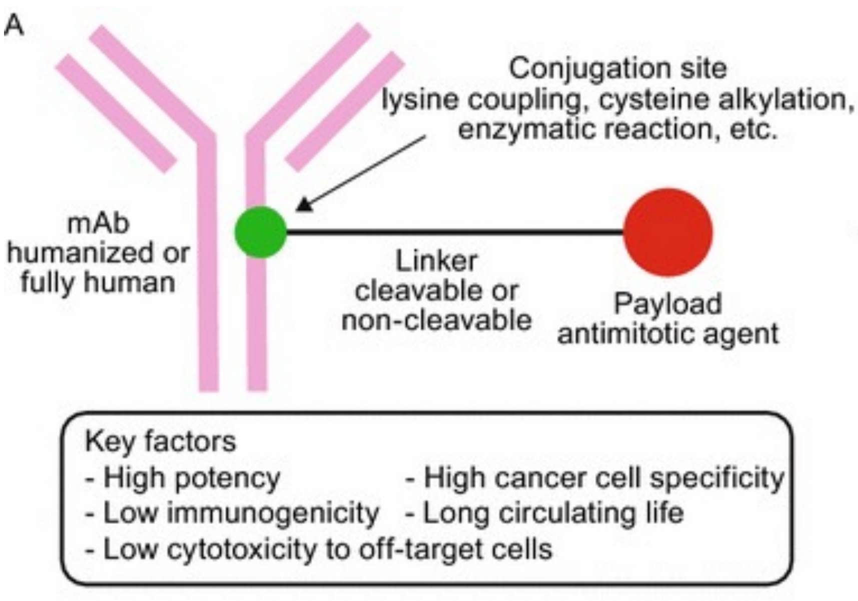
Cu-Triggered ADCs Linker Cleavage

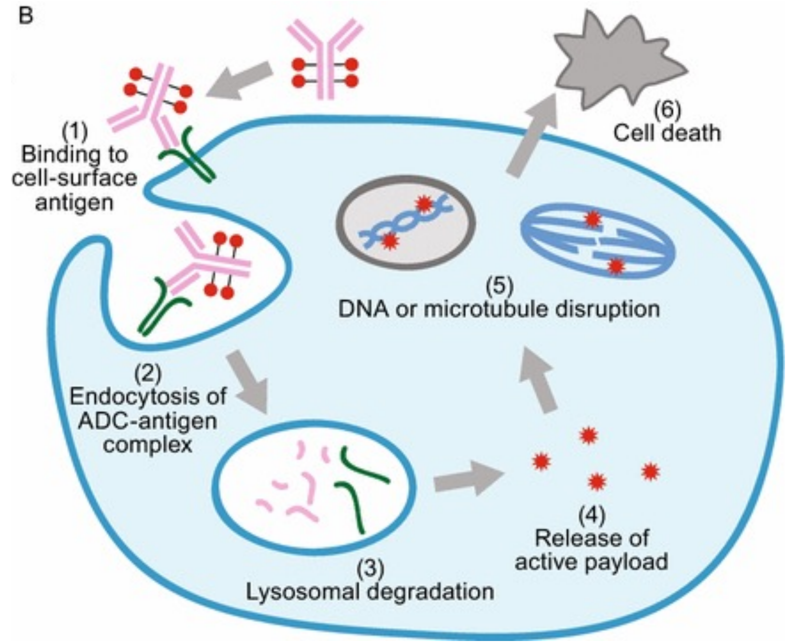


Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Antibody-Drug Conjugates (ADCs)

【Structure and mechanism of action of ADC】





Drawback of noncleavable ADCs

- ✗ Inefficient internalization
- ✗ Limited cytotoxicity

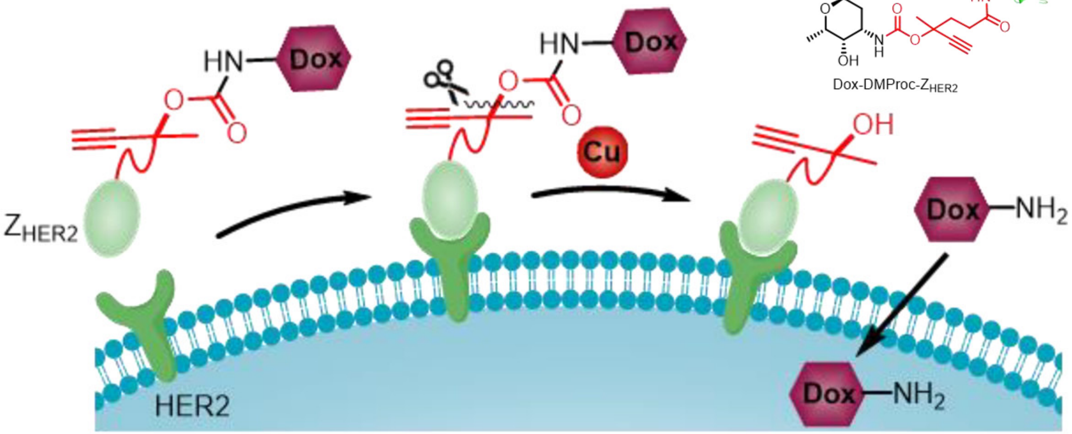
Catalytically cleavable ADCs

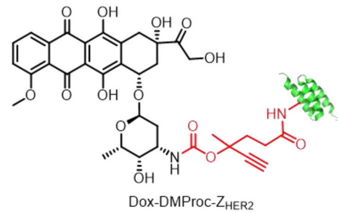
- ✓ Higher cytotoxicity
- ✓ Catalytic amount of triggers (other ways: stoichiometric)

Tsuchikama K. An Z. *Protein Cell*, **2018**, *9*, 33–46.

Cu-Triggered ADCs Linker Cleavage (Dox)

【Dox-DMProc-Z_{HER2}】





Doxorubicin (Dox)

- **Anticancer drug**
- **Forming intercalation with DNA**
- ✗ **Lack of target selectivity**
- ✗ **High level of side effect** (e.g. cardiotoxicity)

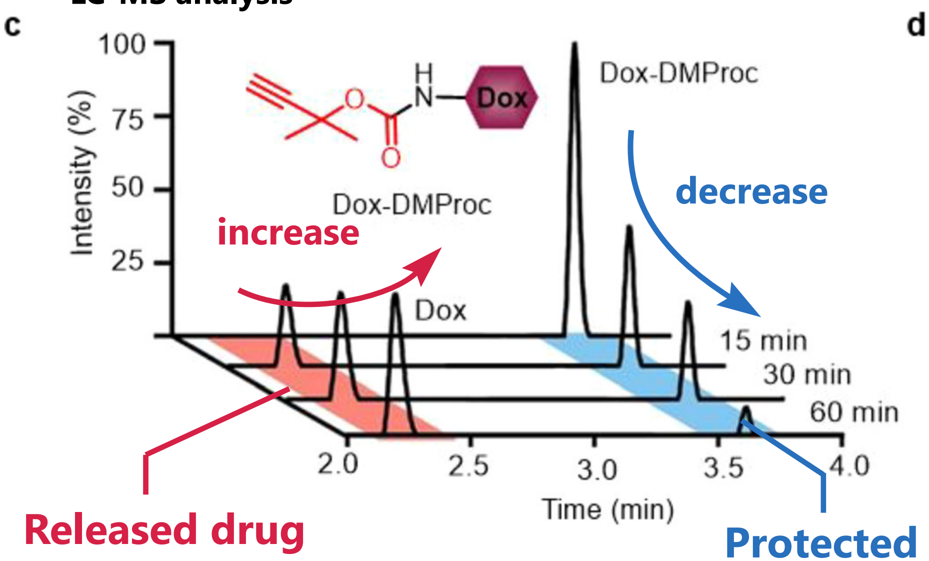
Z_{HER2}

- **Affibody targeting the HER2 receptor**
- **HER2 overexpresses in certain types of breast cancer → Targeting cancer cells**

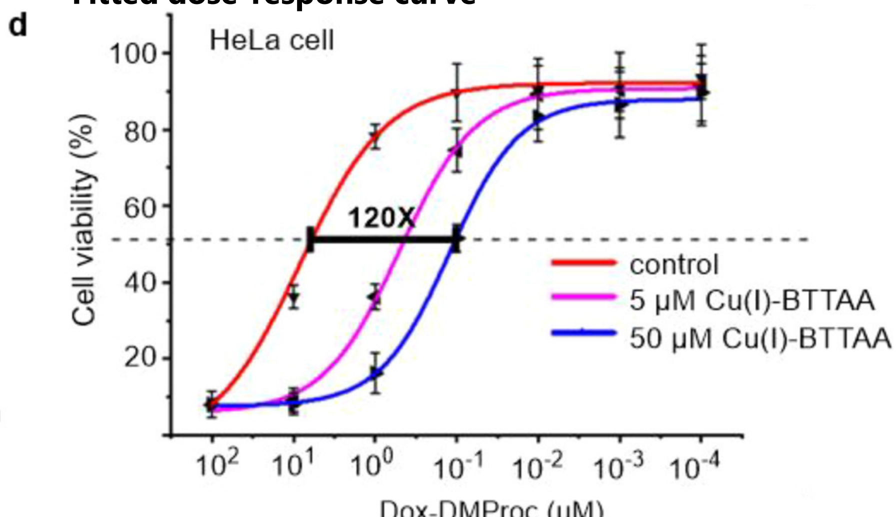
*HER2: Human Epidermal grow factor Receptor 2

【Dox-DMProc as a model substrate】

LC-MS analysis



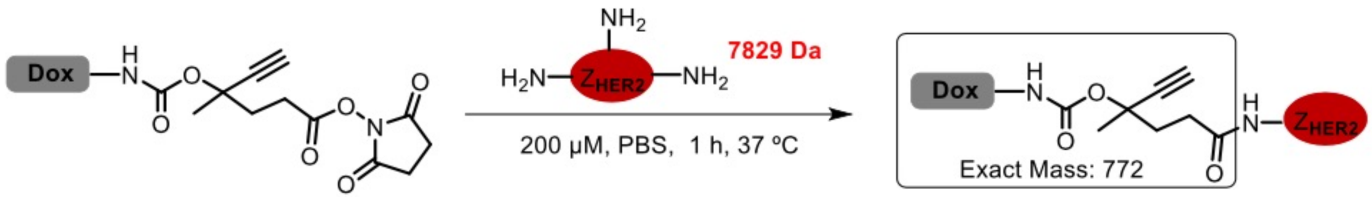
Fitted dose-response curve



Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Cu-Triggered ADCs Linker Cleavage (Dox)

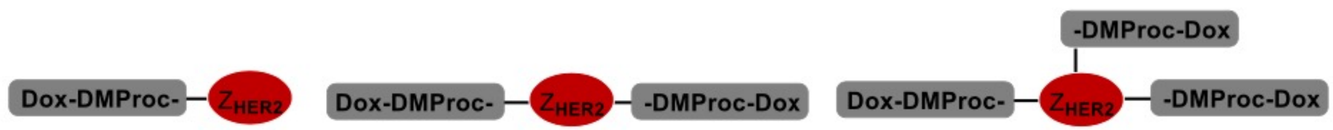
【Synthesis of Dox-DMProc-Z_{HER2}】



Dox-DMProc-NHS

React with accessible Lys residue

Dox-DMProc-Z_{HER2}

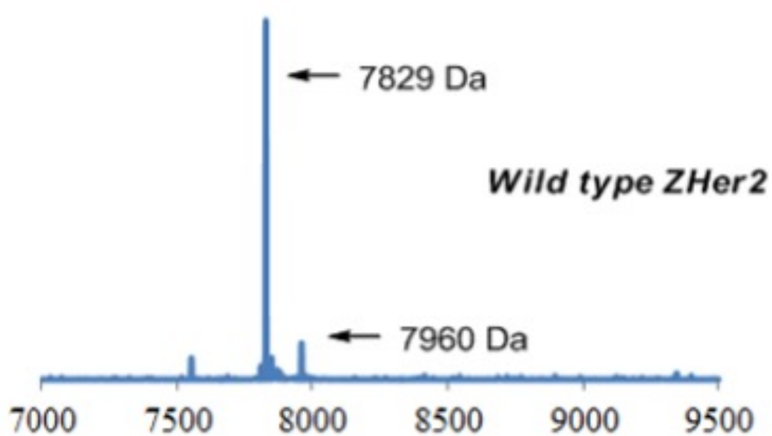


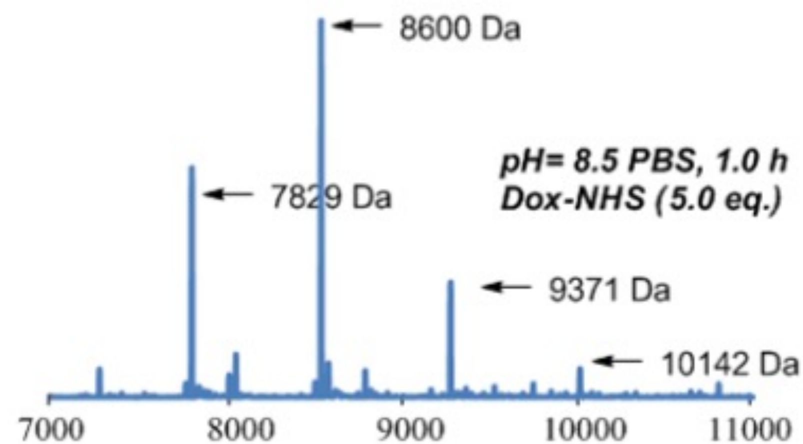
8600 Da
Mono-substituted Drug

9371 Da
Bis-substituted Drug

10142 Da
Tri-substituted Drug

【LC-MS/MS analysis】

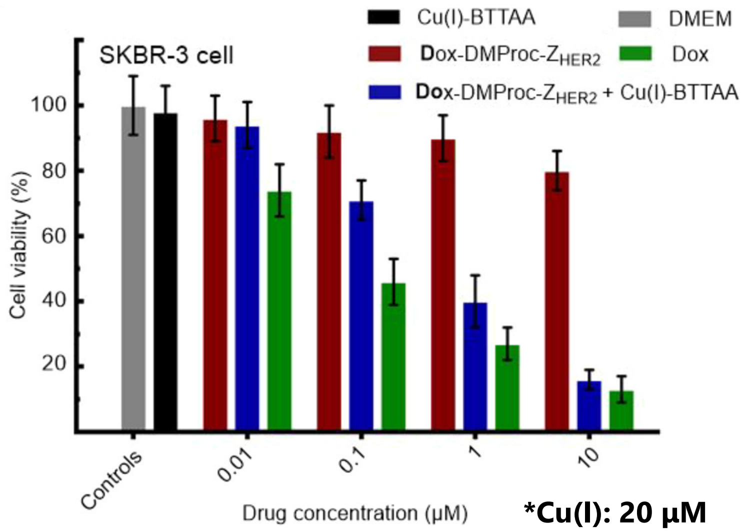




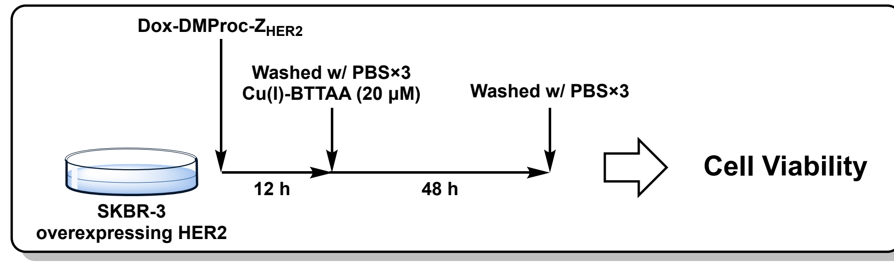
Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Cu-Triggered ADCs Linker Cleavage (Dox)

【Effective of Dox-DMProc-Z_{HER2}】

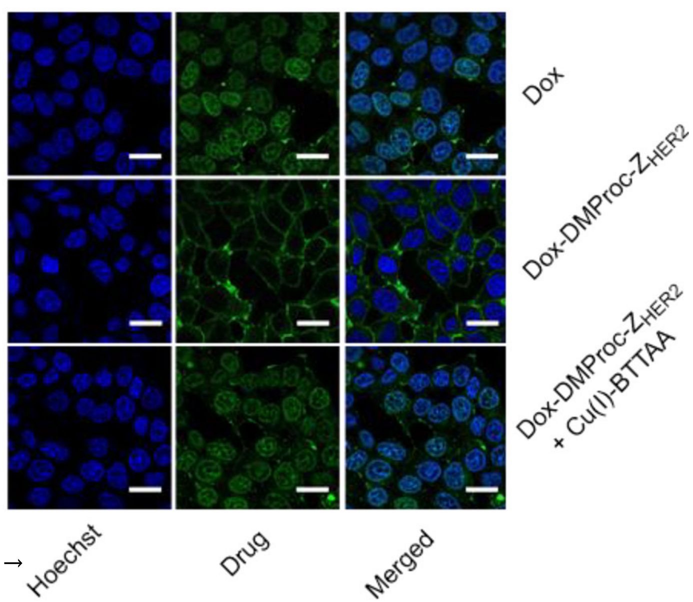


*Cu(I): 20 μM



- ✓ DMProc-Z_{HER2} conjugation effectively blocked the toxicity of Dox (▬ and ▬)
- ✓ Dox-DMProc-Z_{HER2} restored its toxicity by Cu(I) (▬ and ▬)

【Fluorescent images】



nucleus →

Free Dox
Bright fluorescent in the nucleus
(∵ Intercalation with double strand DNA)

ADCs
Fluorescence at the membrane areas
→ Accumulation at cell surface

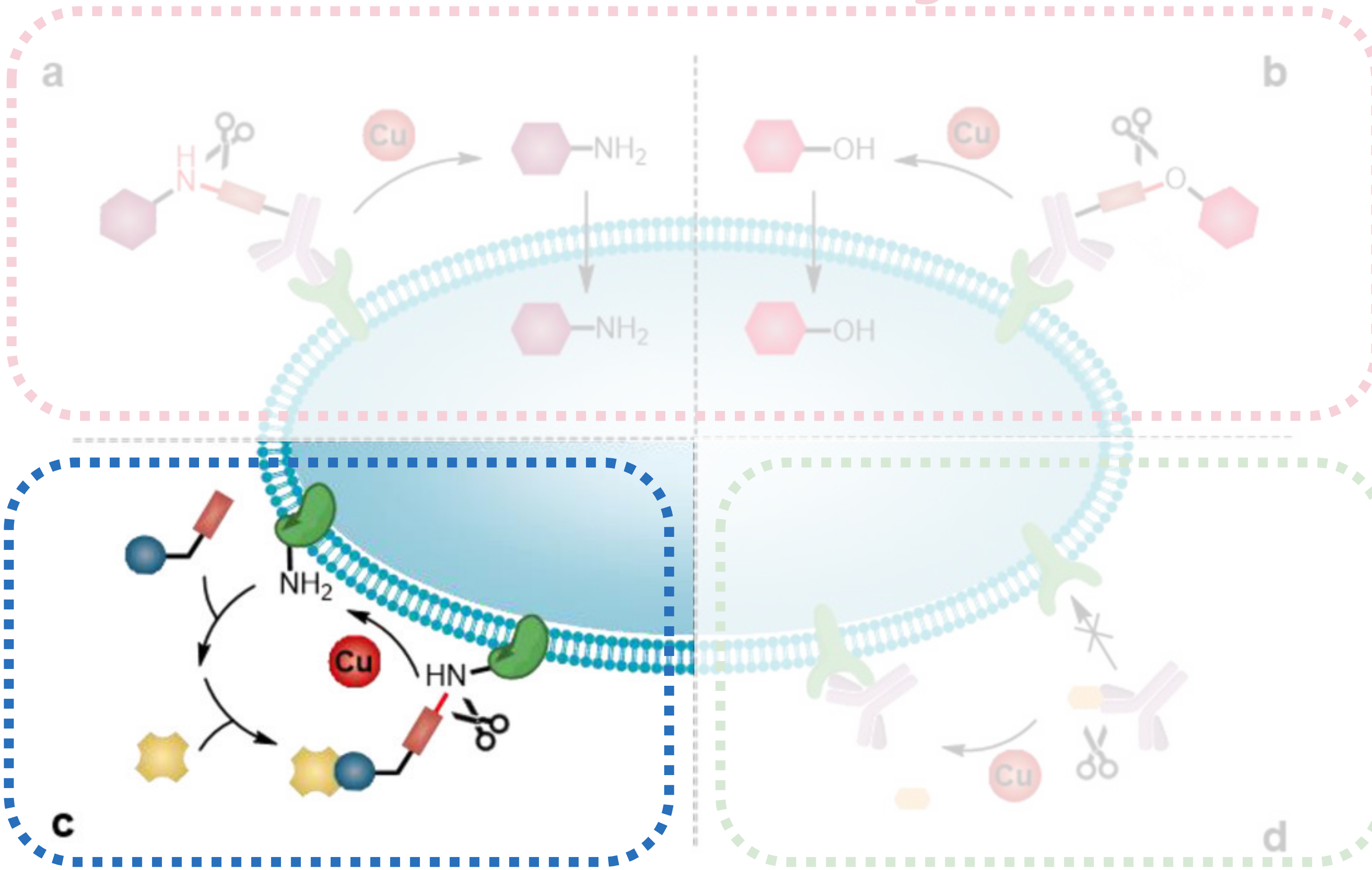
Free Dox from ADCs
Bright fluorescent within the nucleus

→ Cu-triggered cleavage was demonstrated!

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Cu-Mediated Reversible Cell Modification

ADCs linker cleavage



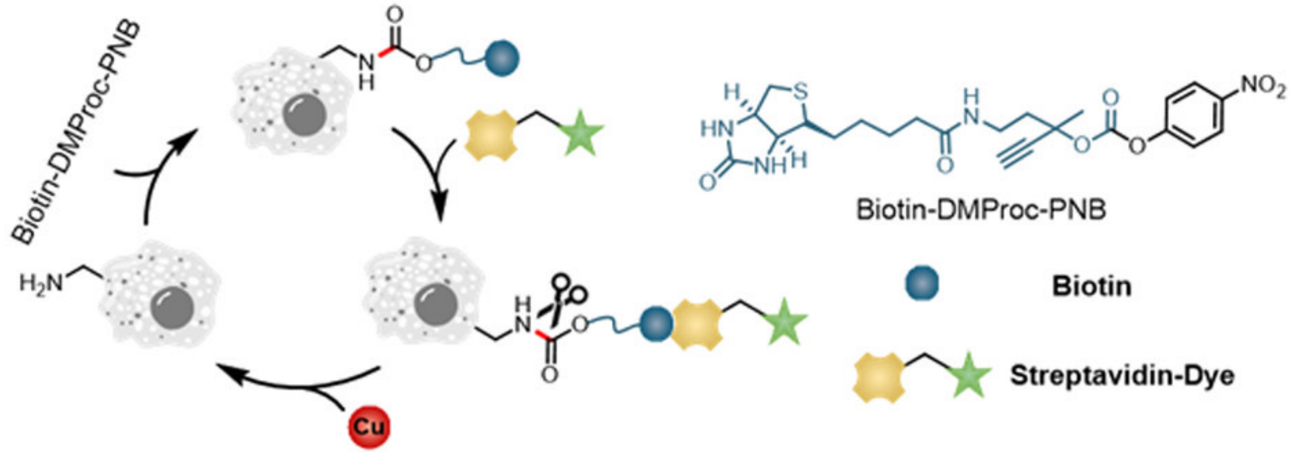
Reversible modification

Protein switching

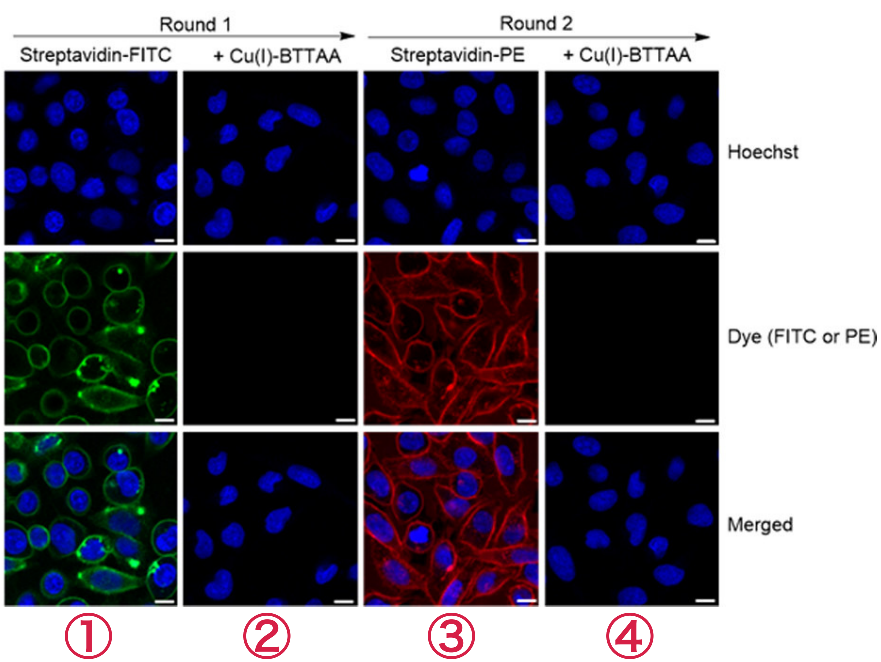
Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

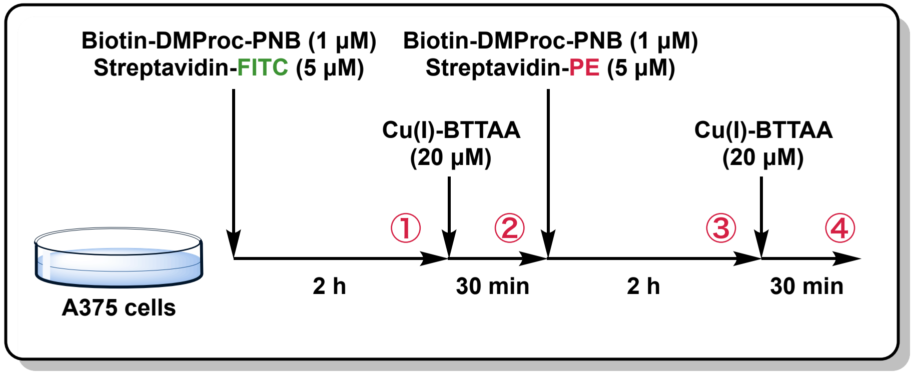
Reversible Cell Surface Modification

【Schematic view of Cu-controlled reversible cell modification】



【Fluorescent images】



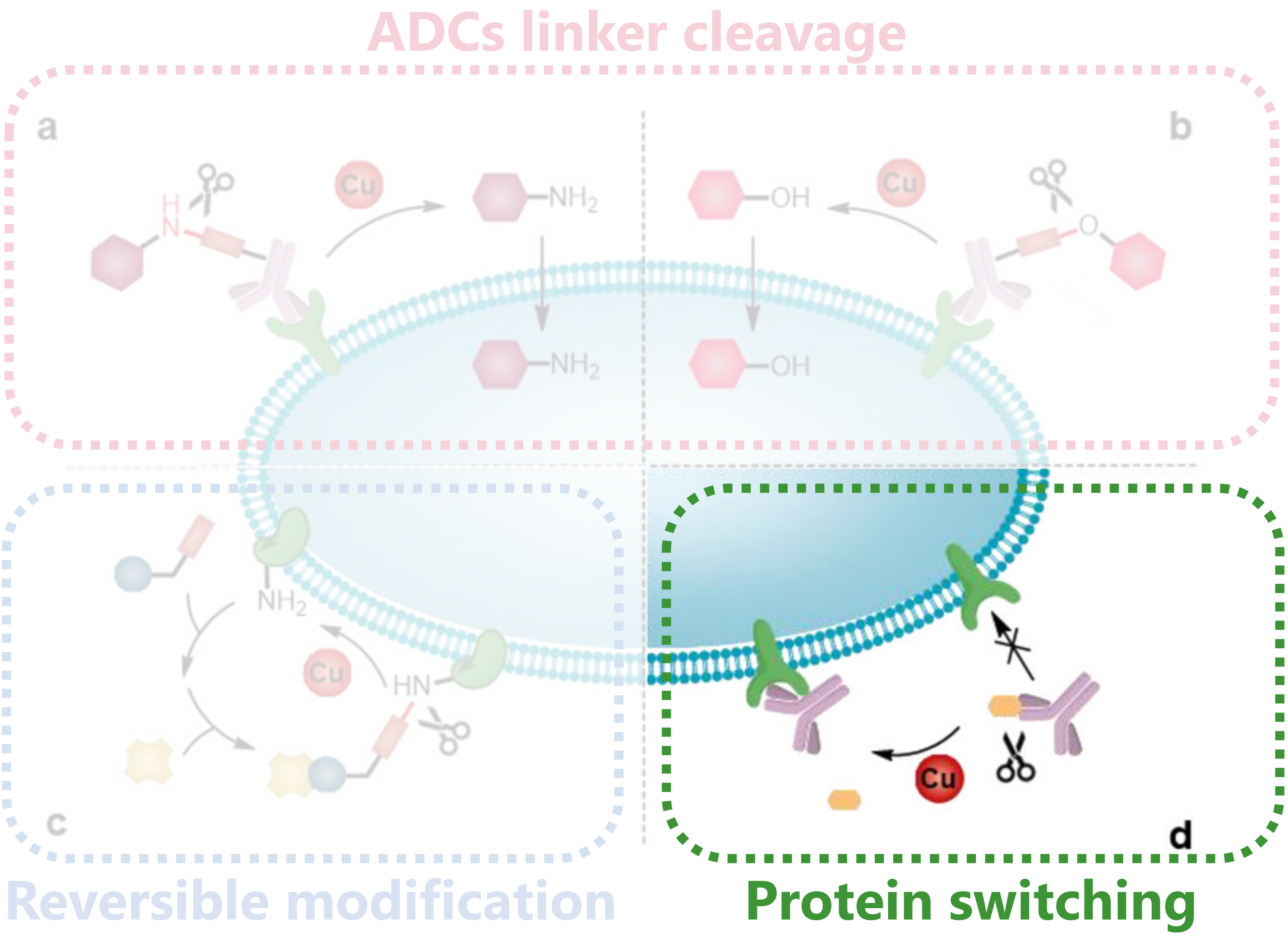


*FITC, PE: Fluorescent dye

Cu-catalyzed linker cleavage allowed the regeneration of native Lys residues on the cell surface.

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

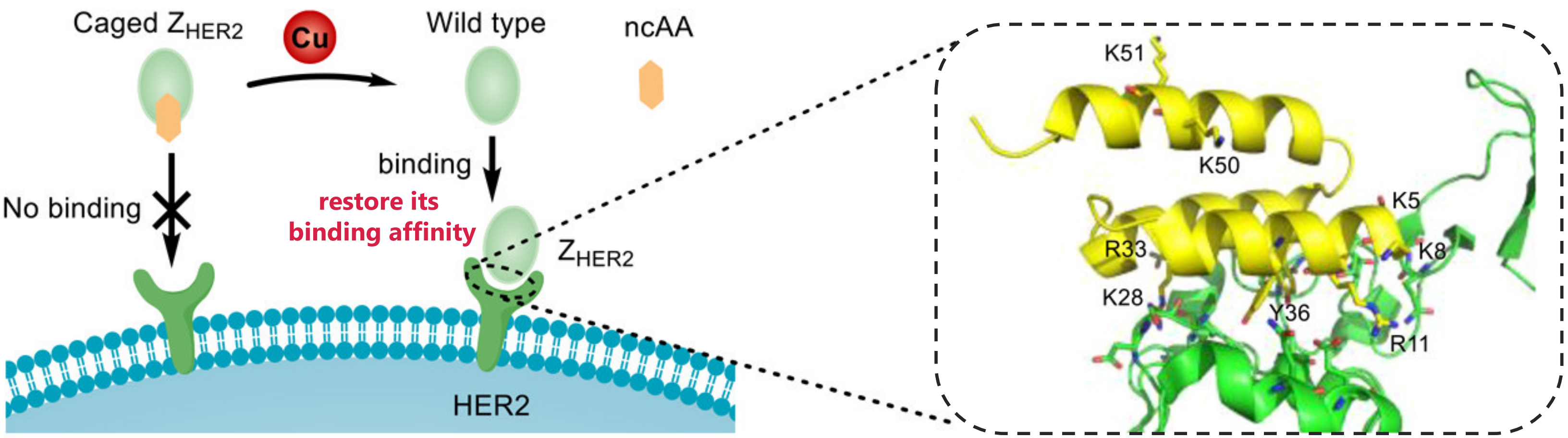
Cu-Mediated Protein Switching



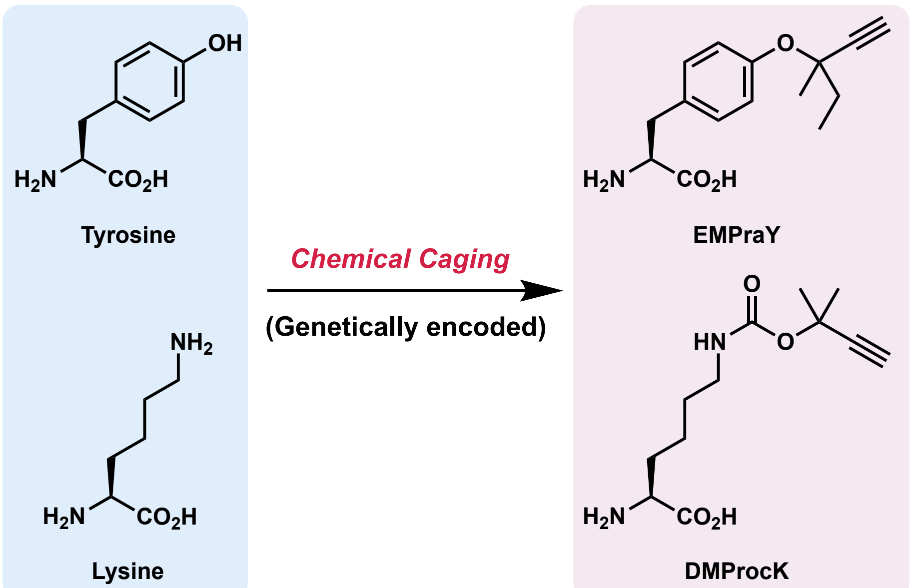
Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Cu-Mediated Protein Switching

【Schematic view of Cu-controlled protein activation】



【Chemical caged unnatural amino acids】



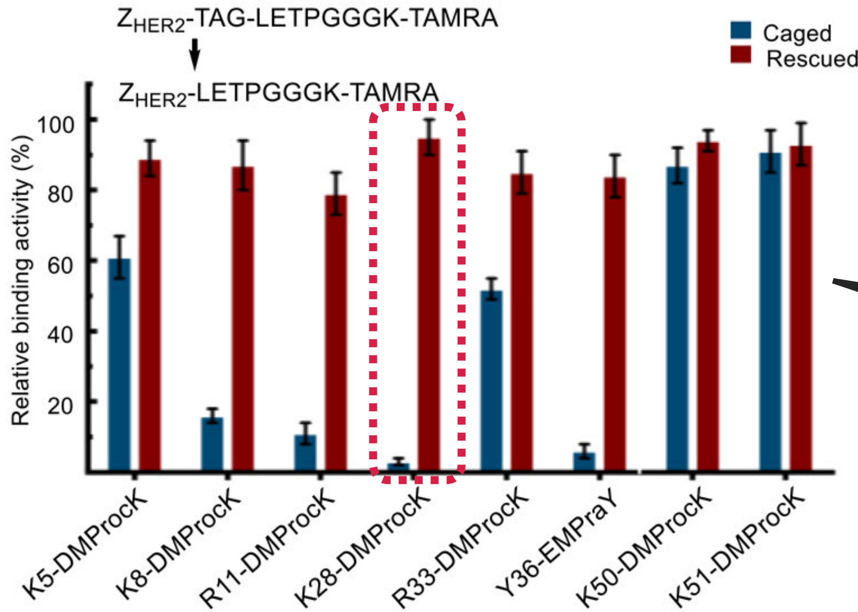
Incorporation of unnatural amino acids on the binding face of Z_{HER2}

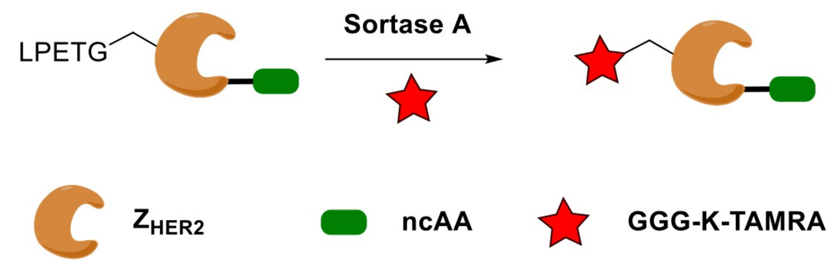
Regulation of its binding affinity by Cu-catalyzed reaction!

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

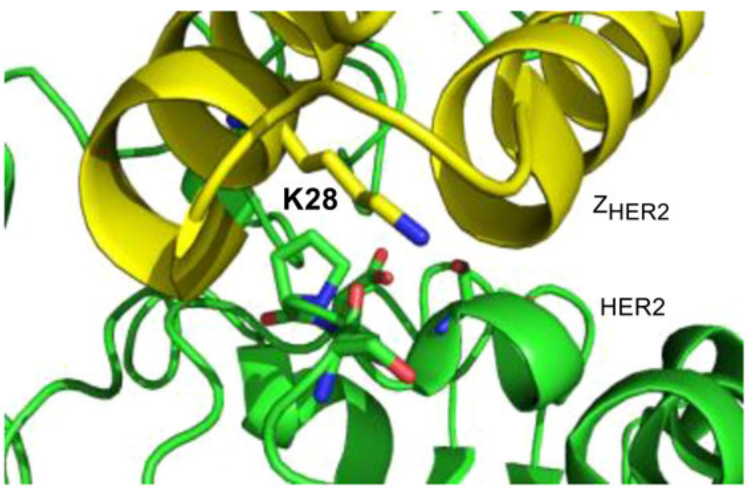
Cu-Mediated Protein Switching

【Binding activity before and after Cu catalysis】





The reduction of fluorescence signal → The blockage of interaction



✓ Chemical caging through K28 almost completely blocked the interaction between Z_{HER2} and HER2

Manipulation of protein-protein interaction *in situ*.

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Short Summary & Perspective

- **ADC linker cleavage**

  It allows extracellular release of payloads that can **overcome the drawback of noncleavable ADCs**.

- **Reversible cell surface modification**

  It can be applied for the cell capture, which could be quite useful in **cancer cell diagnosis**.

- **Protein manipulation**

  It has the potential to facilitate the development of **protein-based prodrug therapy**.

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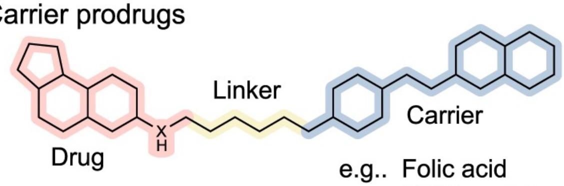
TM-Catalyzed Prodrug Activation

【Types of prodrug】

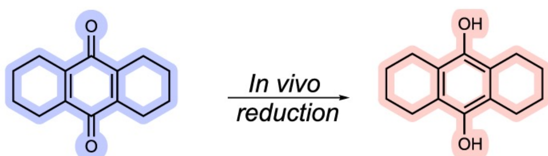
- Physiologically activated

Carrier prodrugs



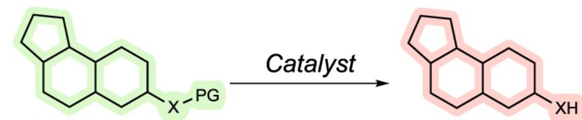
e.g.. Folic acid
RGD peptide
Sugars etc.

Bioprecursor prodrugs

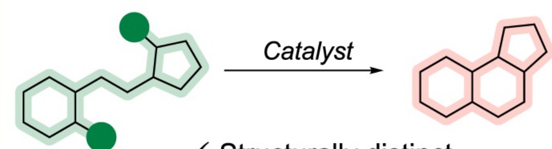


- Externally activated

Decaging Prodrug



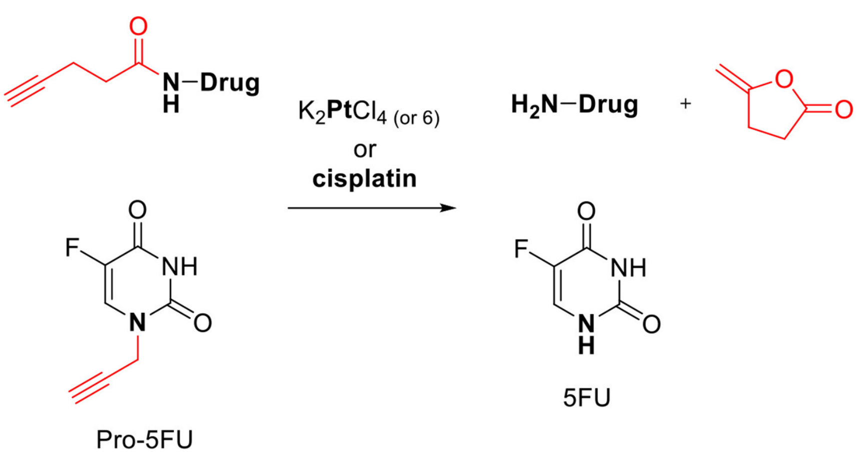
Synthetic Prodrug (*This work*)



✓ Structurally distinct
✓ Increased differences in bioactivities

Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

【Decaging prodrug by TM catalysis】

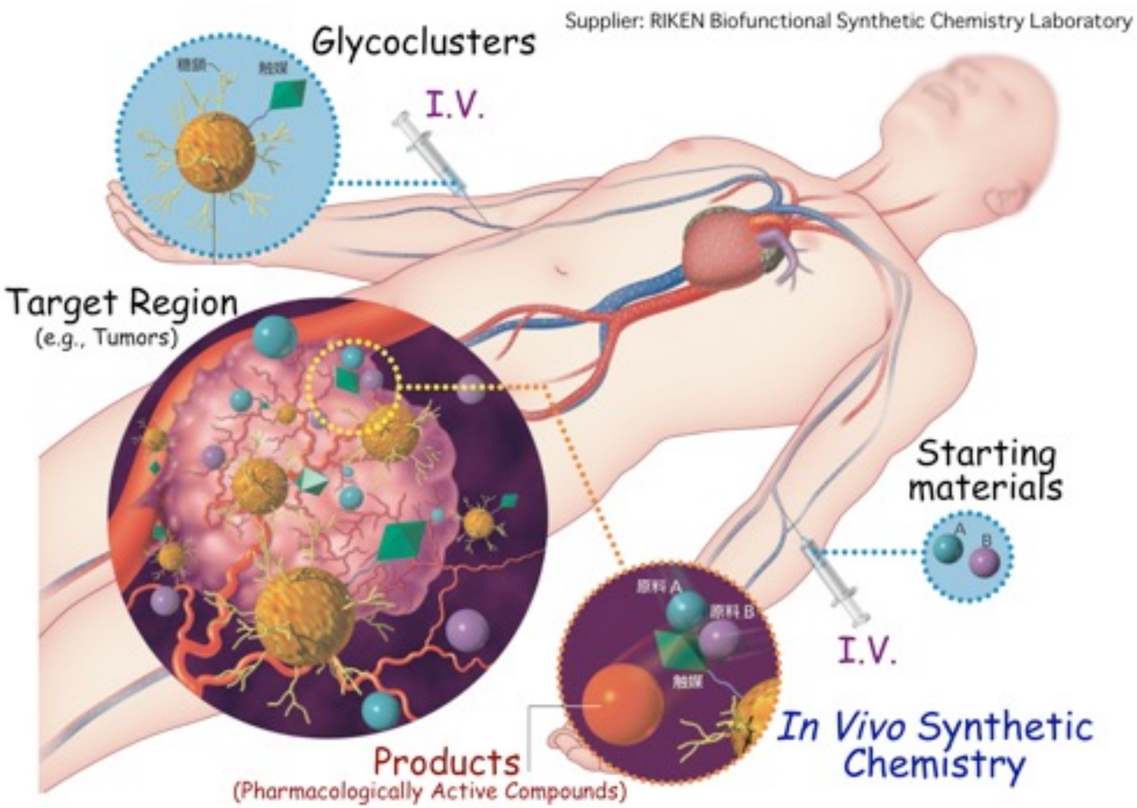


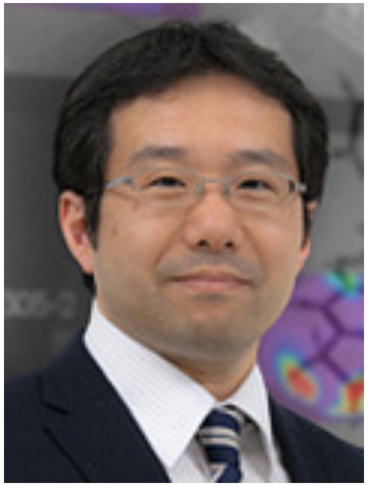
Decaging prodrug activation strategy by TM catalysis has been demonstrated in many papers.

How about **synthetic prodrug**?

B. L. Oliveira *et al. J. Am. Chem. Soc.* **2020**, *142*, 10869–10880.

Therapeutic *In Vivo* Synthetic Chemistry





Prof. Katsunori Tanaka
Tokyo Tech & RIKEN

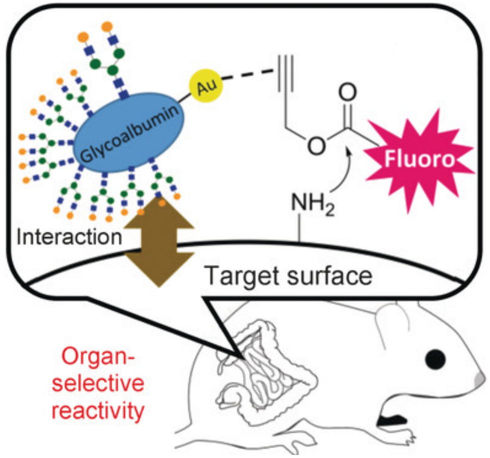
Project

The development of a model system
where bioactive compounds can be synthesized within living animals.

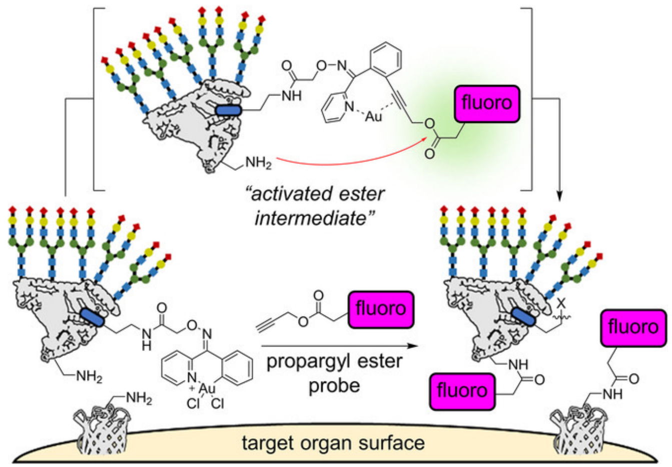
From the website of Katsunori Tanaka Laboratory

Previous Researches

【Au catalysis at specific organs (*in vivo*)】

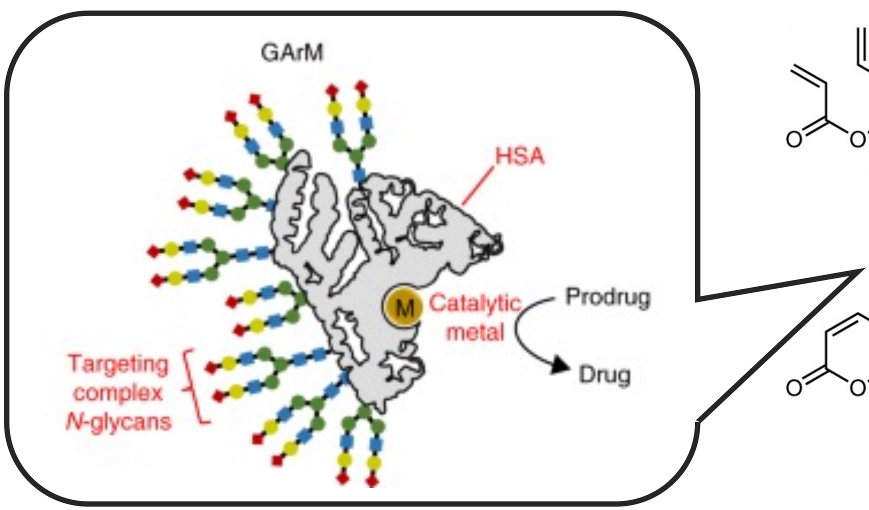


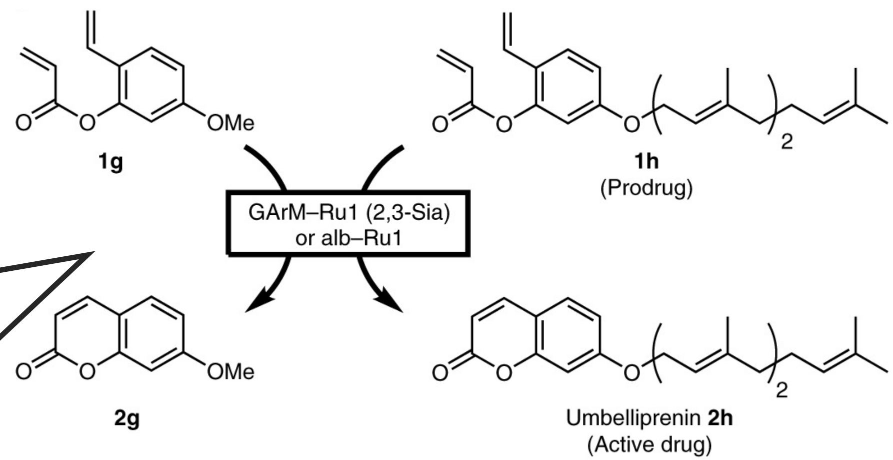
Tsubokura K., Tanaka K. *et al. Agew. Chem., Int. Ed.* **2017**, *56*, 3579–3584



Lin Y., Tanaka K. *et al. Chem. Eur. J.* **2018**, *24*, 10595–10600.

【Ru catalysis the biological condition (in cell)】

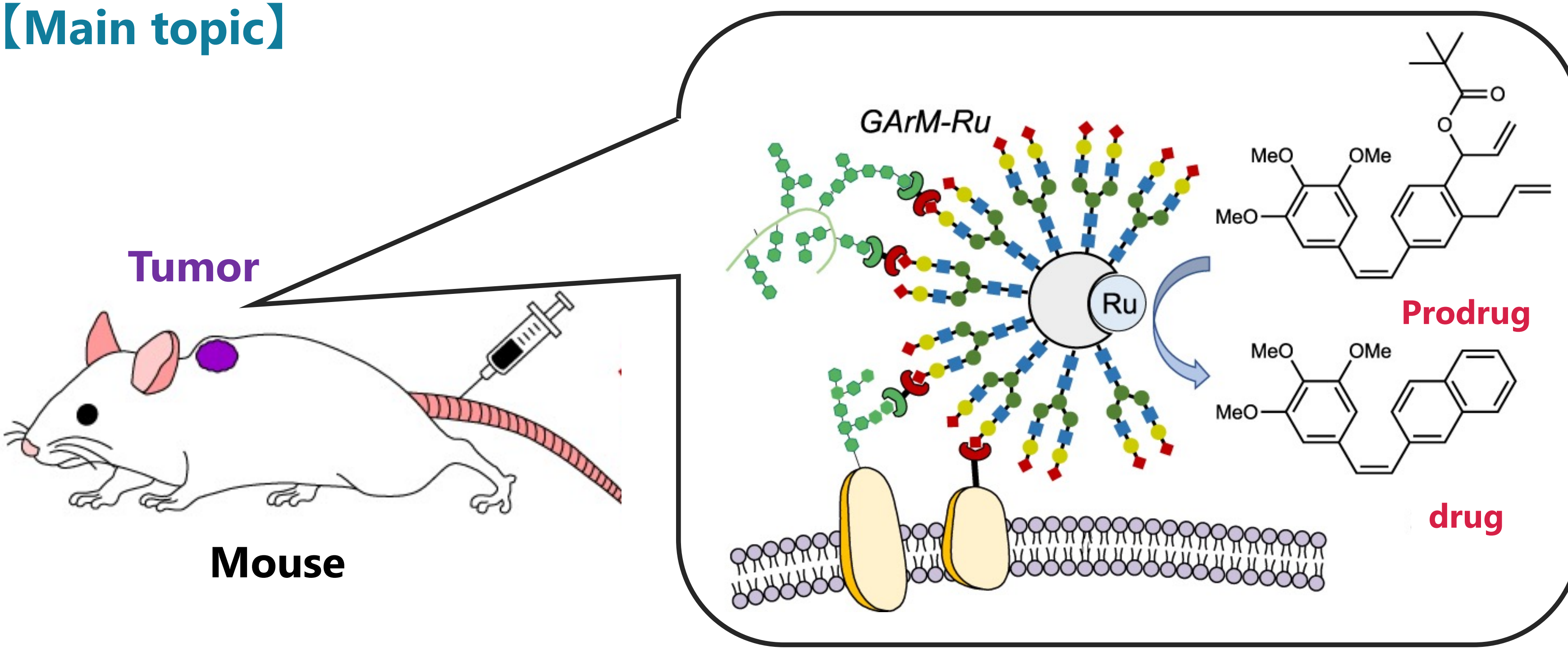




Eda S., Tanaka K. *et al. Nat. Catal.* **2019**, *2*, 780–792.

Synthetic Prodrug Strategy

【Main topic】

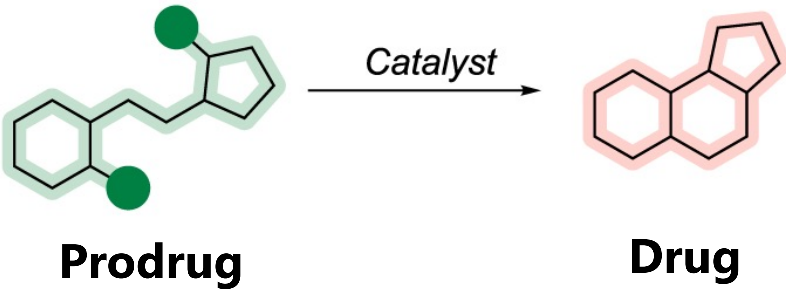


- ✓ **Design of synthetic prodrug to maximize its activation**
- ✓ **Catalytic activation of prodrug in mice**
- ✓ **Albumin-based artificial metalloenzyme**
- ✓ **N-glycosylation to target cancer cells**

Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Retrosynthetic Prodrug Design

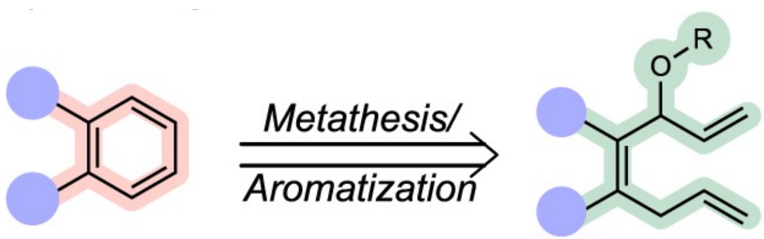
【Advantage of synthetic prodrug】



- ✓ **Activation via bond forming reactions to construct a pharmacophore (drug's backbone)**
- ✓ **No pharmacophore in the prodrug structure → Less adverse effect**

【Design and optimization】

1) Finding a suitable reaction

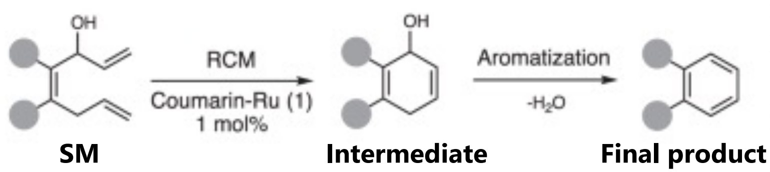


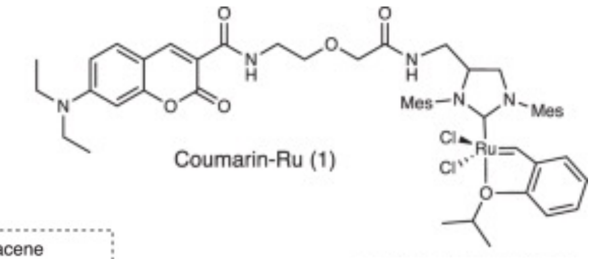
2) Structure optimization

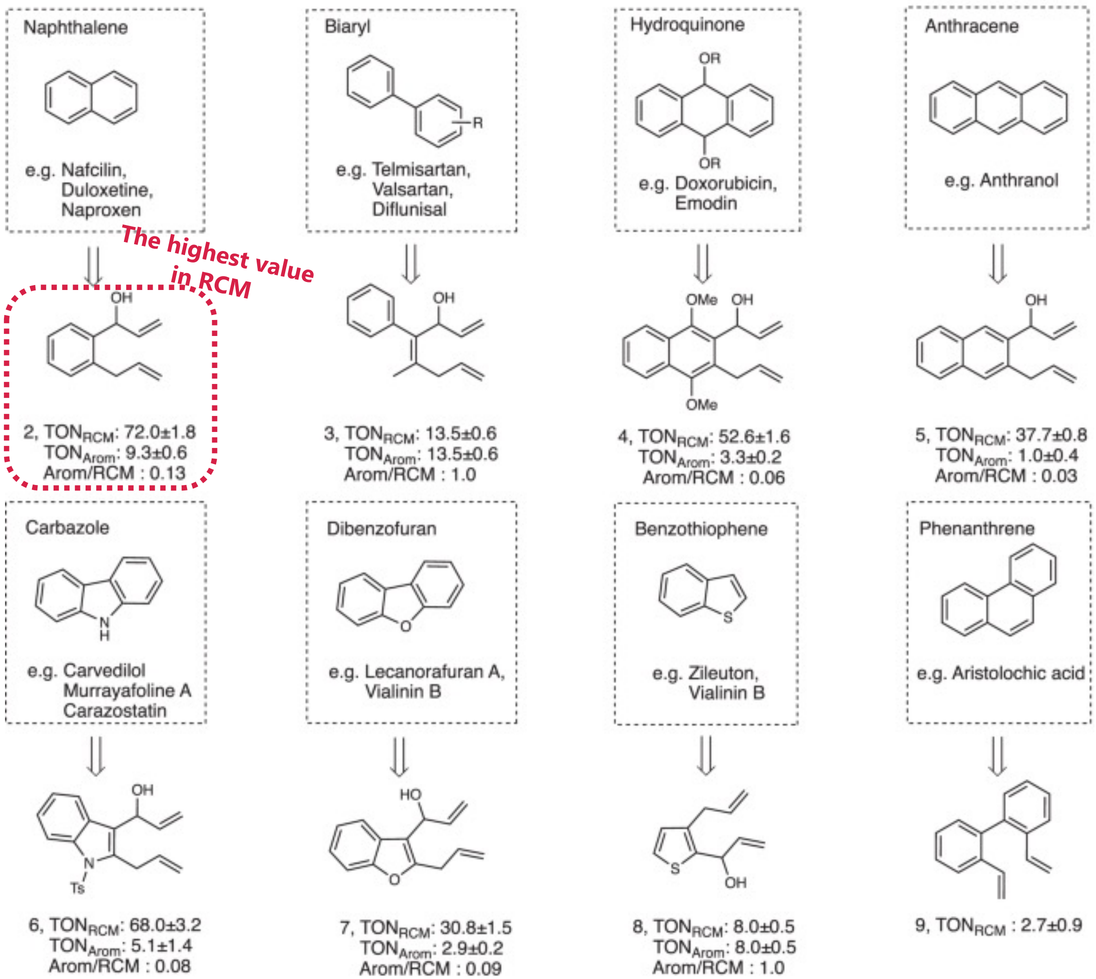
- Increase cascade reactivity
- Increase activity with biocatalyst
- Decrease prodrug effects
- Increase hydrolytic stability

Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Pharmacophore Backbone Screening







✓ **High activity in RCM**

✗ **Lower activity in aromatization**

(∵ the dependency of slightly acidic pHs (<6) to drive the final 1,4-elimination)

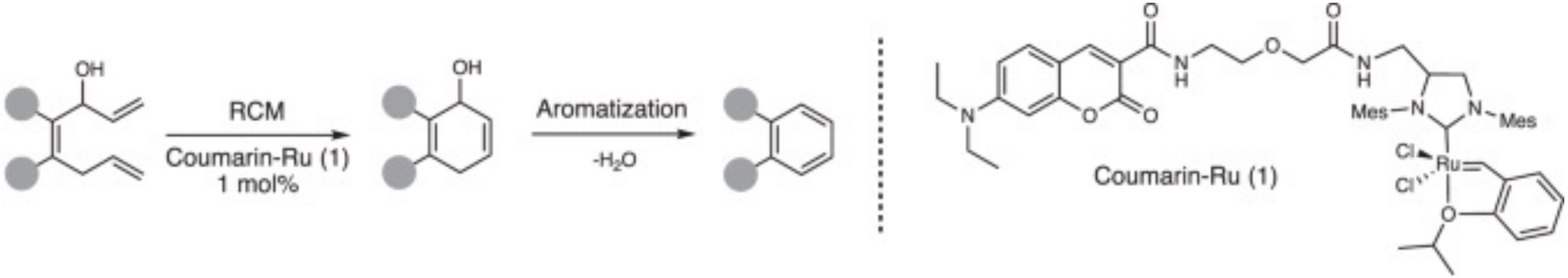
***RCM: Ring Closing Metathesis**

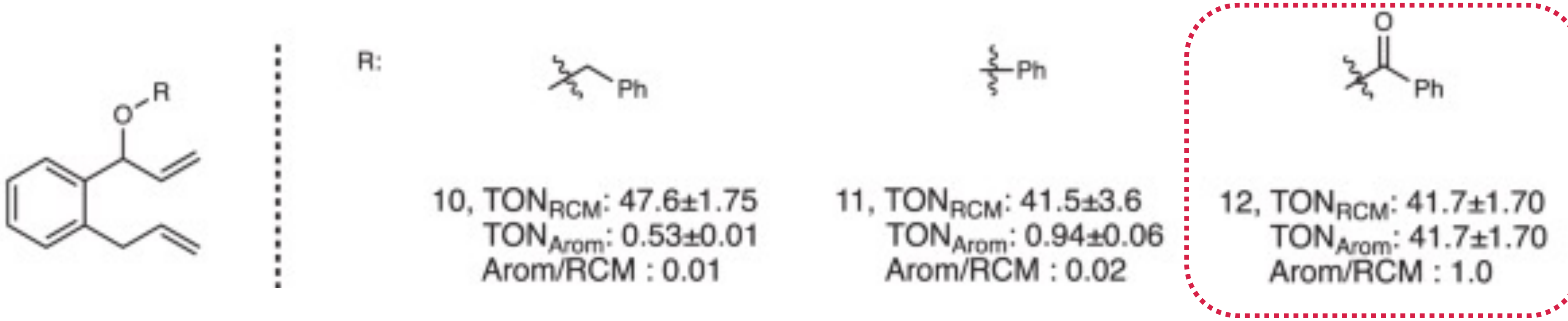
Optimization to further push aromatization yields higher

TON: Product yield determined by HPLC
TON_{RCM}: Value for the intermediate
TON_{Arom}: Value for the final product

Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Optimization of Leaving Group





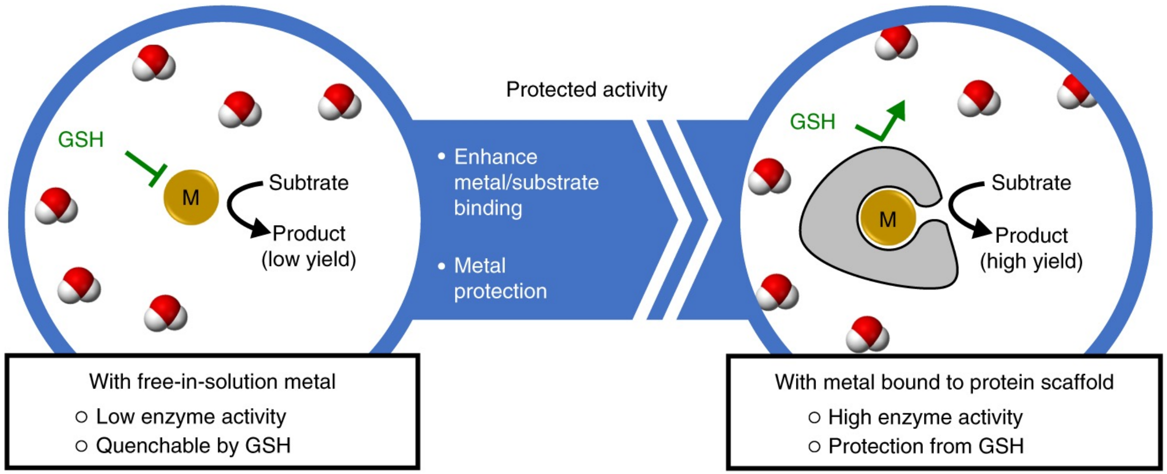
The highest yield!

The most acidic leaving group (ester-containing precursor 12) showed both excellent RCM activity and full aromatization.

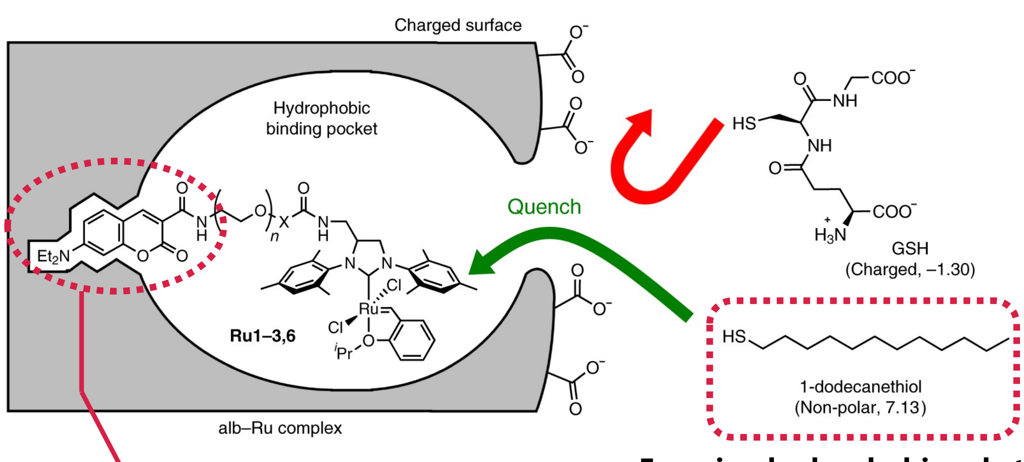
Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Strategy for RCM Reaction *In Vivo*

【Albumin-based artificial metalloenzyme (ArM)】



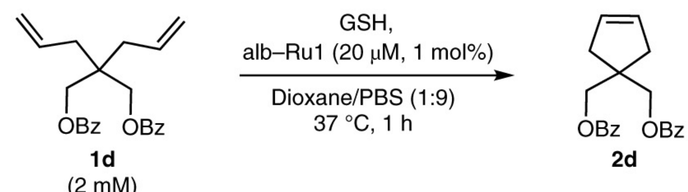
【Structure of metalloenzyme】

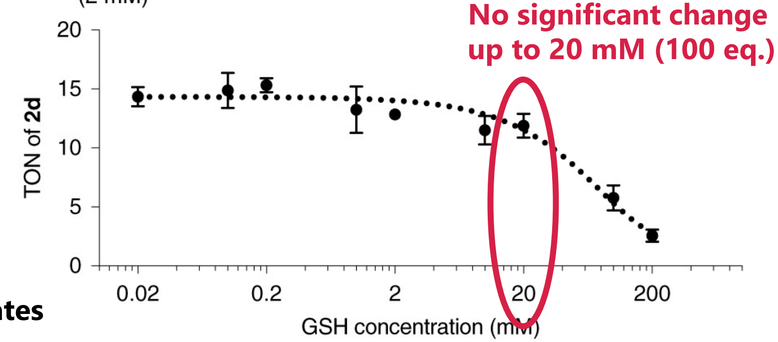


Favoring hydrophobic substrates

This coumarin moiety binds to the hydrophobic pocket of albumin.

【Deactivation by glutathione】





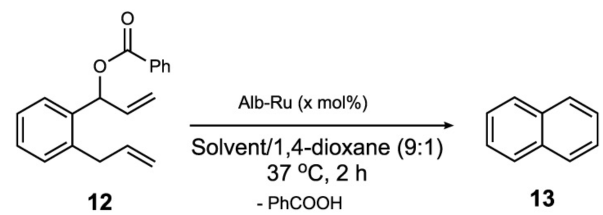
Eda S., Tanaka K. *et al. Nat. Catal.* **2019**, *2*, 780–792.

Hoveyda-Grubbs Type ArM Activity

【Reactivity in physiological conditions】

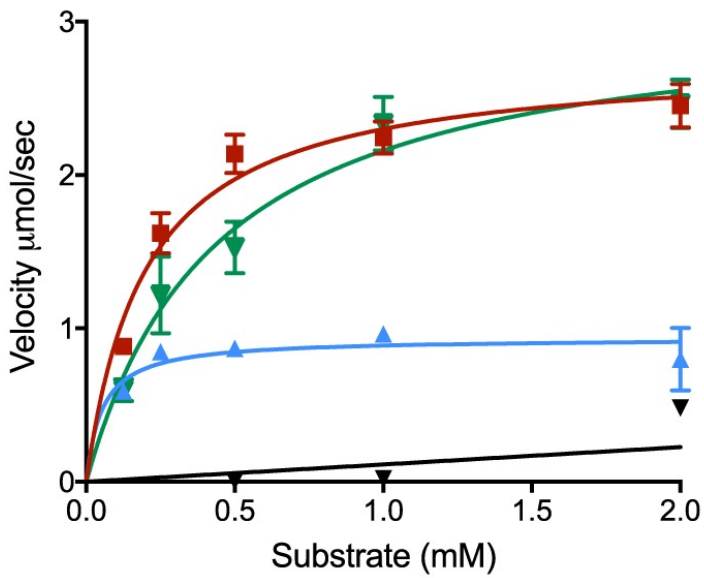
Entry	Solvent	12 conc. (mM)	Alb-Ru (mol%)	TON
1	PBS	4	1	31.1±1.1
2[a]	PBS	4	1	30.8±0.1
3	PBS	2	1	34.2±0.5
4	PBS	1	1	36.3±3.7
5	D-MEM media	4	1	12.0±0.4
6	D-MEM media	2	1	5.6±0.4
7	D-MEM media	1	1	3.4±0.2

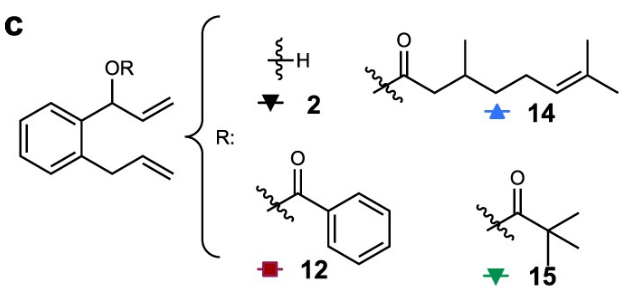
[a] 20 mol% of GSH



- ✓ Relatively high yield in the presence of GSH
- ✓ No adverse effect under lower conc. of substrate
- △ Lower yield in the cell growth media

【Substrate specificity and reactivity】





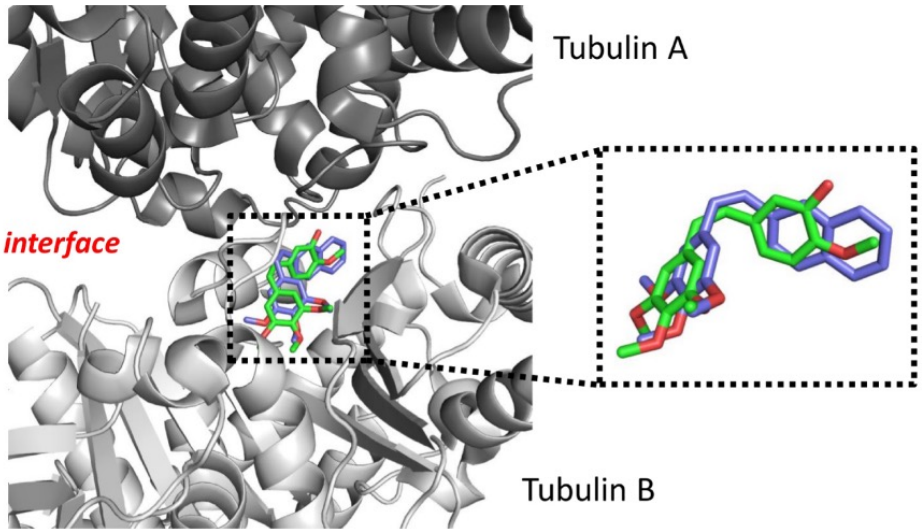
14 showed the highest catalytic efficiency.

↓

Due to the introduction of C10 terpene chain, which mimics fatty acid (good albumin ligand).

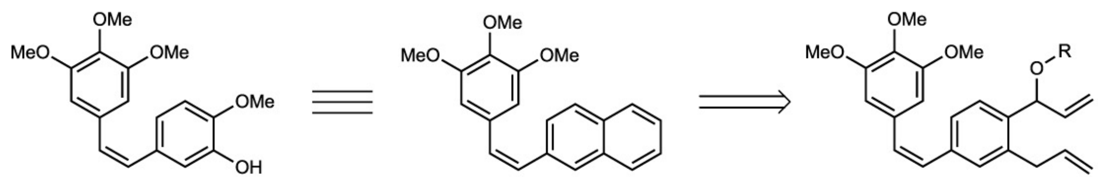
Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Prodrug Design



Combretastatin derivatives

- ✓ Binding into colchicine site of β-tubulin
- ✓ Microtubule polymerization inhibitor
- ✓ Disruption of tumor growth



Combretastatin A-4

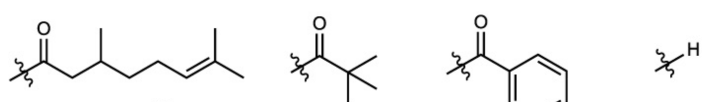
Natural compound
- Tubulin polymerization inhibitor
- Anti-vascular

20

Synthetic analogue
- Similar binding affinity with tubulin
- nmol level of cytostatic activity

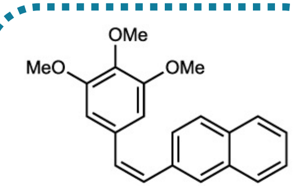
16-19

Retrosynthetic Prodrug



Calc. avg. binding energy (kcal/mol)	**16**	**17**	**18**	**19**
	-1.15	-6.16	-6.81	-8.53

Binding affinity with β-tubulin



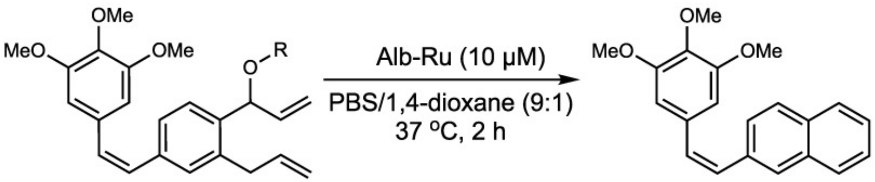
20

-9.2 kcal/mol

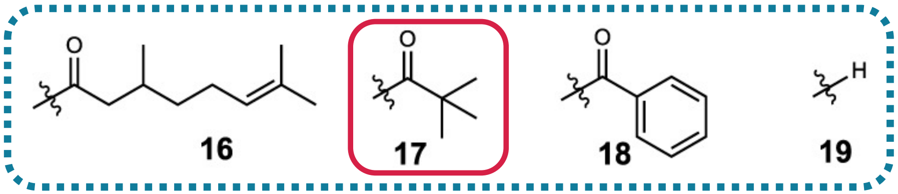
Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Prodrug Reactivity and Stability

【Prodrug reactivity】

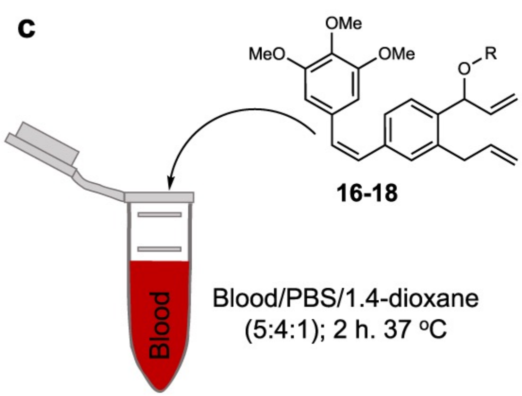


16-19 **20**



Substrate conc. (μM)	Product yield (%) 19	16	17	18
5	0	28.0±1.4	12.8±0.4	4.6±0.9
10	0	35.5±0.1	37.5±2.9	28.5±1.0
50	3.3±0.2	47.4±0.3	57.0±0.6	44.1±2.3
100	38.2±1.6	58.3±2.3	69.7±1.7	50.4±0.8
250	36.7±0.5	54.6±0.7	77.0±0.1	31.4±1.6

【Hydrolytic stability】



Substrate	Hydrolysis (%)
16	22.3±1.3
17	9.8±0.7
18	31.5±1.7

From these results, prodrug 17 (pivalate) is the best candidate moving forward.

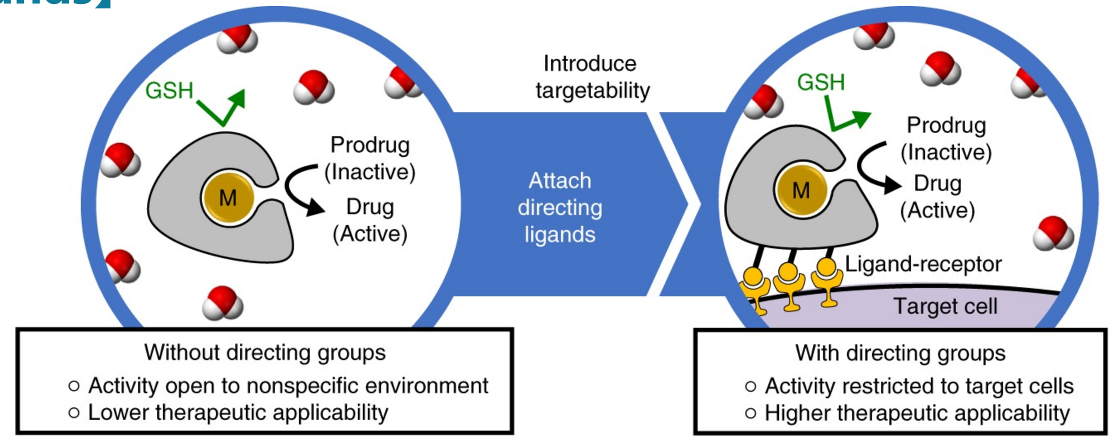
Next

Evaluation of therapeutic effect *in cellulo* and *in vivo*.

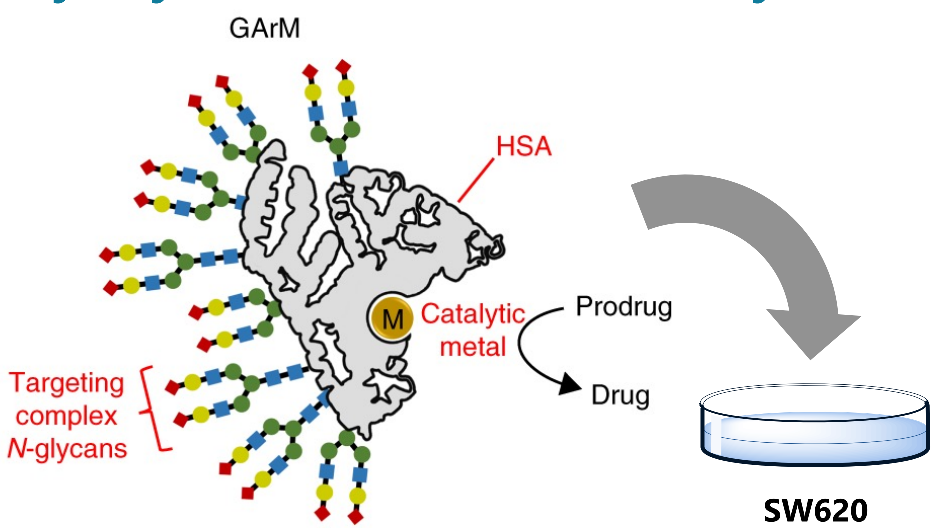
Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Targeting Cancer Cells

【Directing ligands】

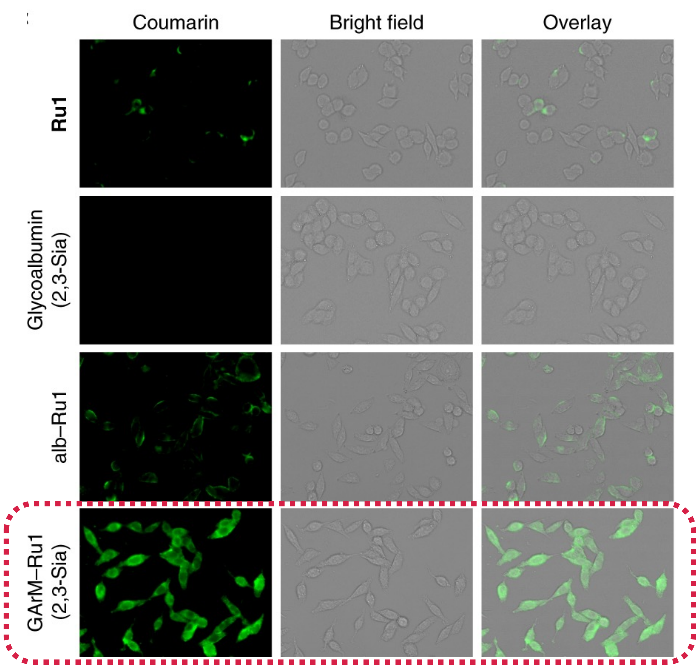


【Glycosylated artificial metalloenzyme (GArM)】



α(2,3)-linked sialic acid

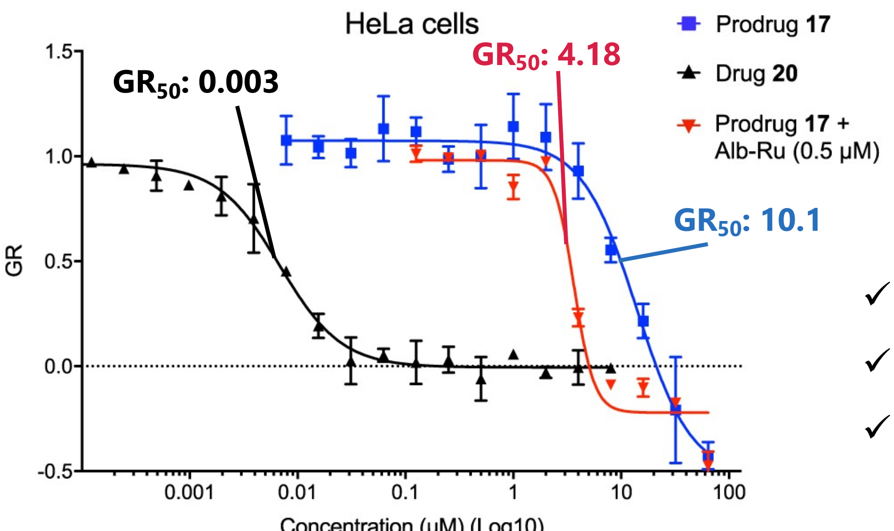
SW620
Overexpressing galectin-8
(α(2,3)-linked sialic acid receptor)



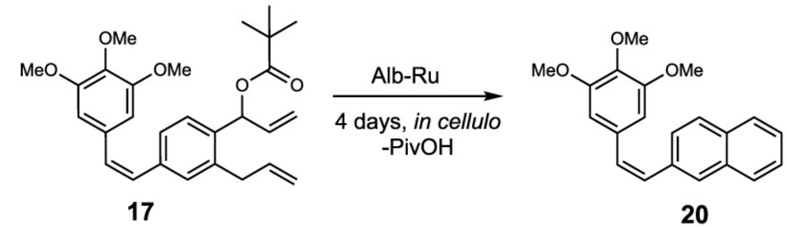
Eda S., Tanaka K. *et al. Nat. Catal.* **2019**, *2*, 780–792.

In Cellulo Study

【Prodrug efficacy】

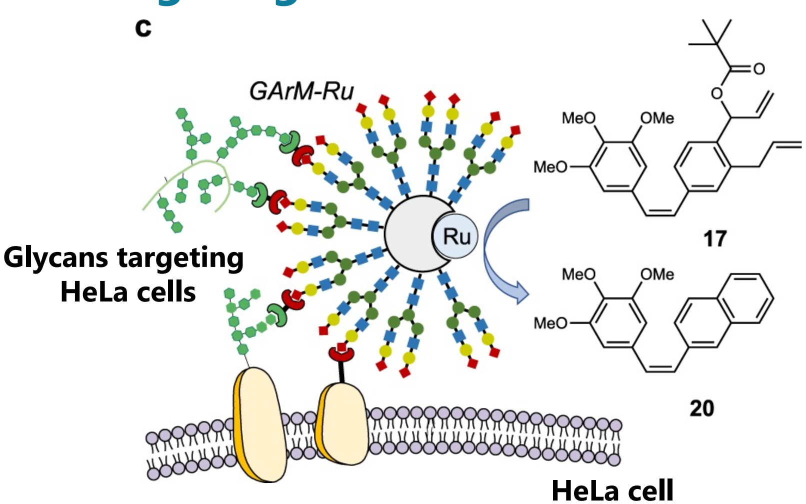


*GR: Growth rate

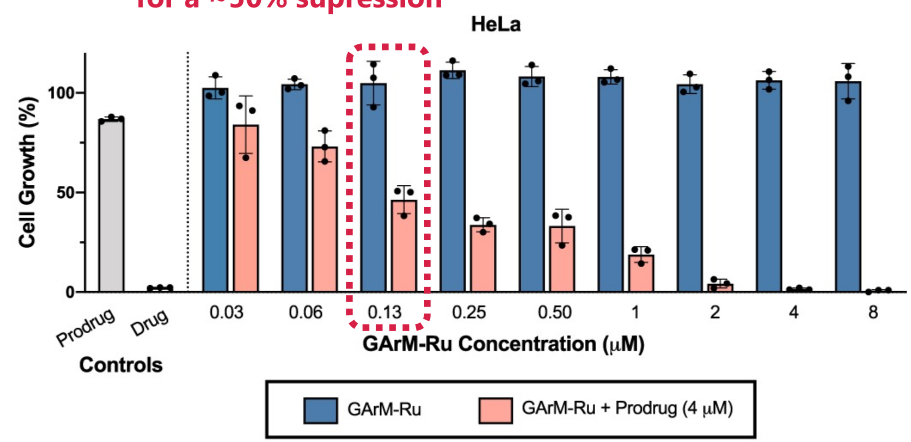


- ✓ **Drug 20 showed excellent cytotoxicity (nM range)**
- ✓ **Prodrug 17 was less toxic (mM range)**
- ✓ **Prodrug activation by GArM was observed**

【Targeting HeLa cells】



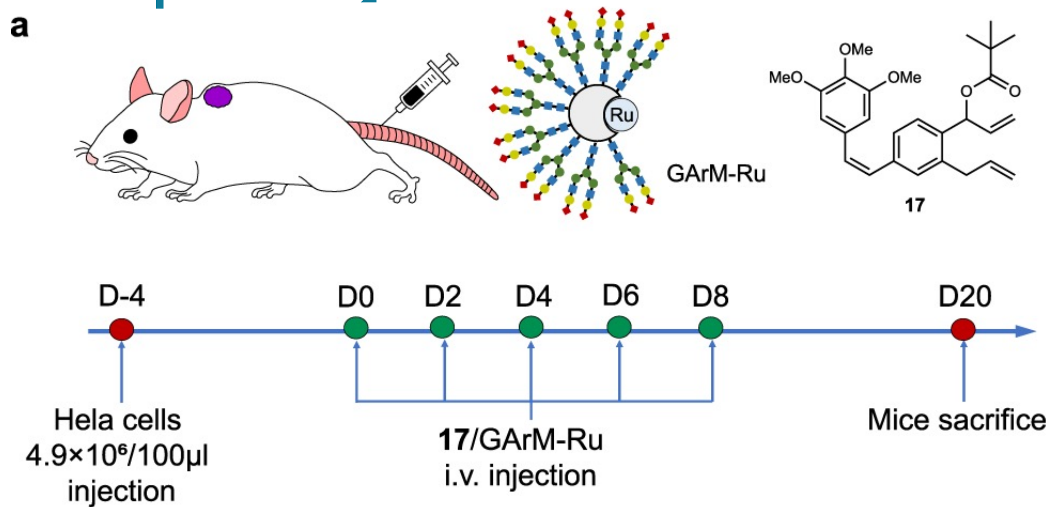
4 μM of 17 and 130 nM of GArM for a ~50% supression



Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

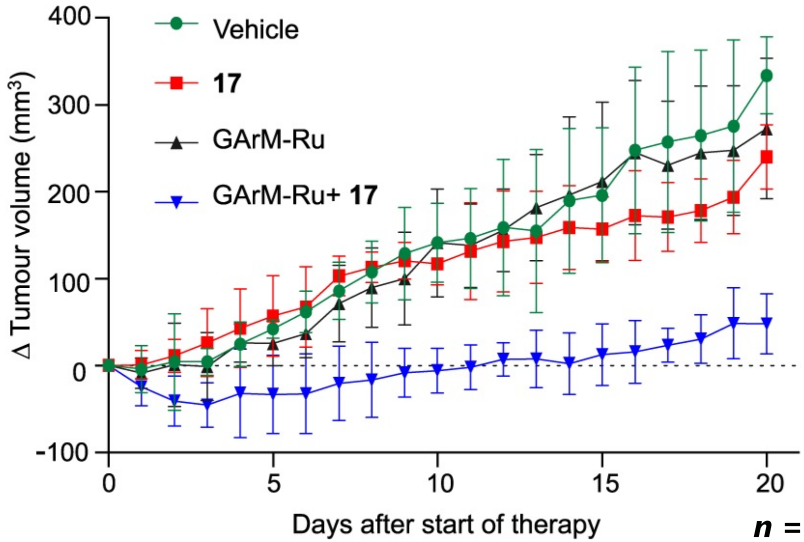
Prodrug Activation in Mice

【Protocol of animal experiment】



Pictures and the weight of tumor tissue → See appendix

【Tumor size in mice】



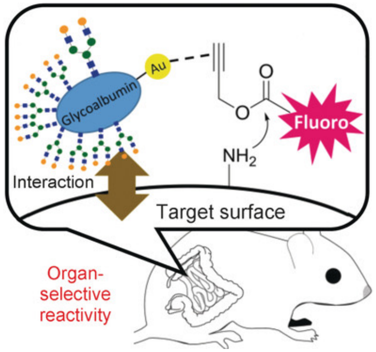
n = 5

- ✓ **17 or GArM-Ru: No activity**
- ✓ **GArM-Ru + 17: Suppression of tumor growth**

Prodrug activation via Ru-catalyzed RCM was achieved in mice!

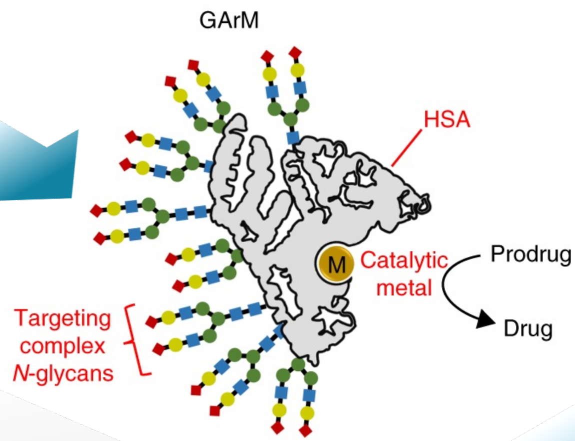
Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

Short Summary



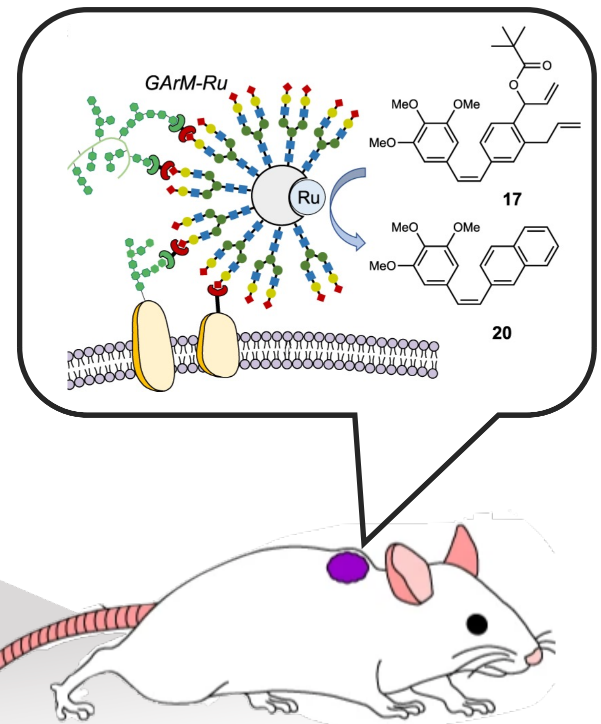
***In vivo* Au catalysis**
Targeting specific organs

Angew. Chem., Int. Ed. **2017.**
Chem. Eur. J. **2018.**



Ru-catalyzed RCM in cells
GArM targeting cancer cells

Nat. Catal. **2019.**



Tumor suppression
via prodrug activation *in vivo*!!

Nat. Commun. **2022.**

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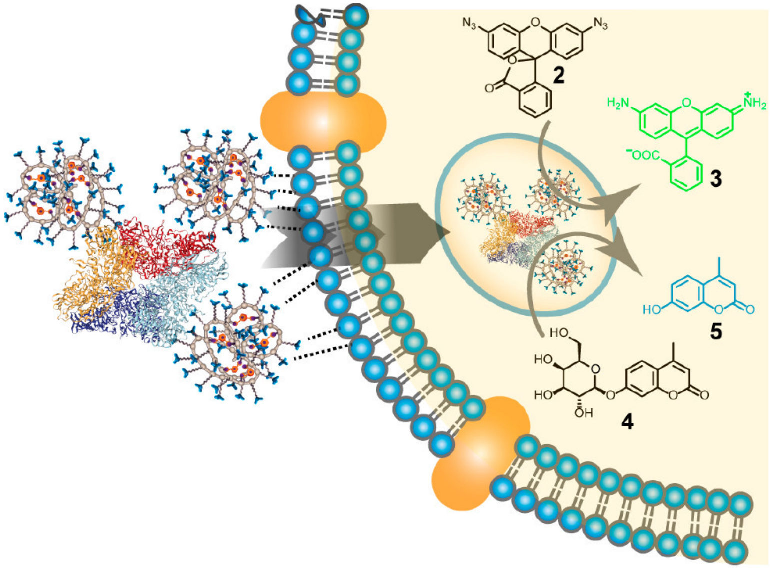
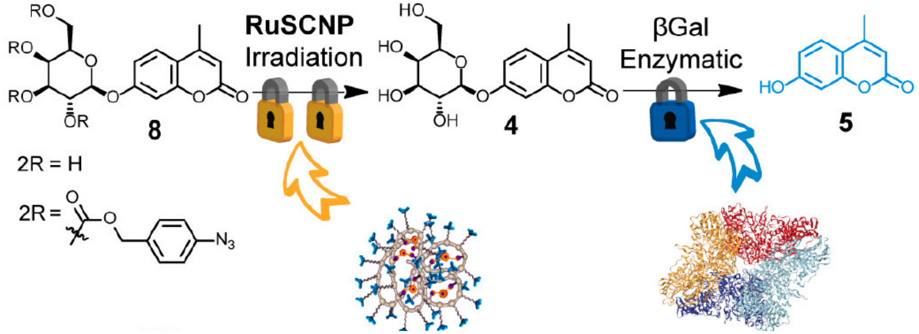
【Creating new tandem reactions】

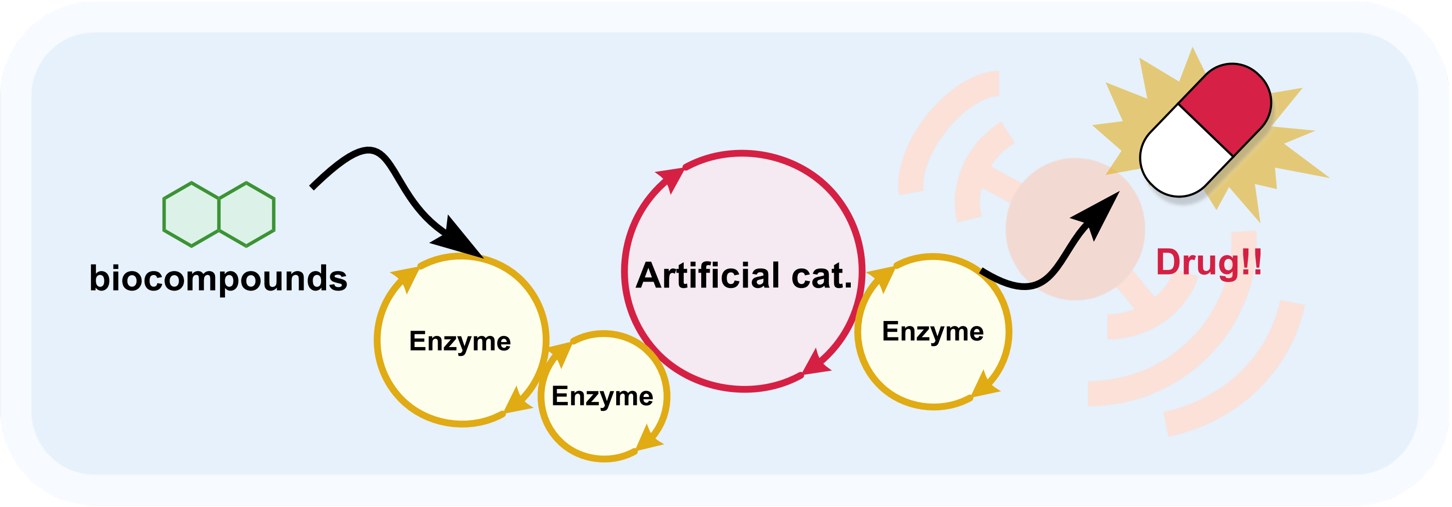




Tandem catalysis by Ru cat. and enzyme to provide bioactive agents in cells

J. Chen. *et al. J. Am. Chem. Soc.* **2020**, *142*, 4565–4569.

【TM catalysis *in vivo* in the future…】



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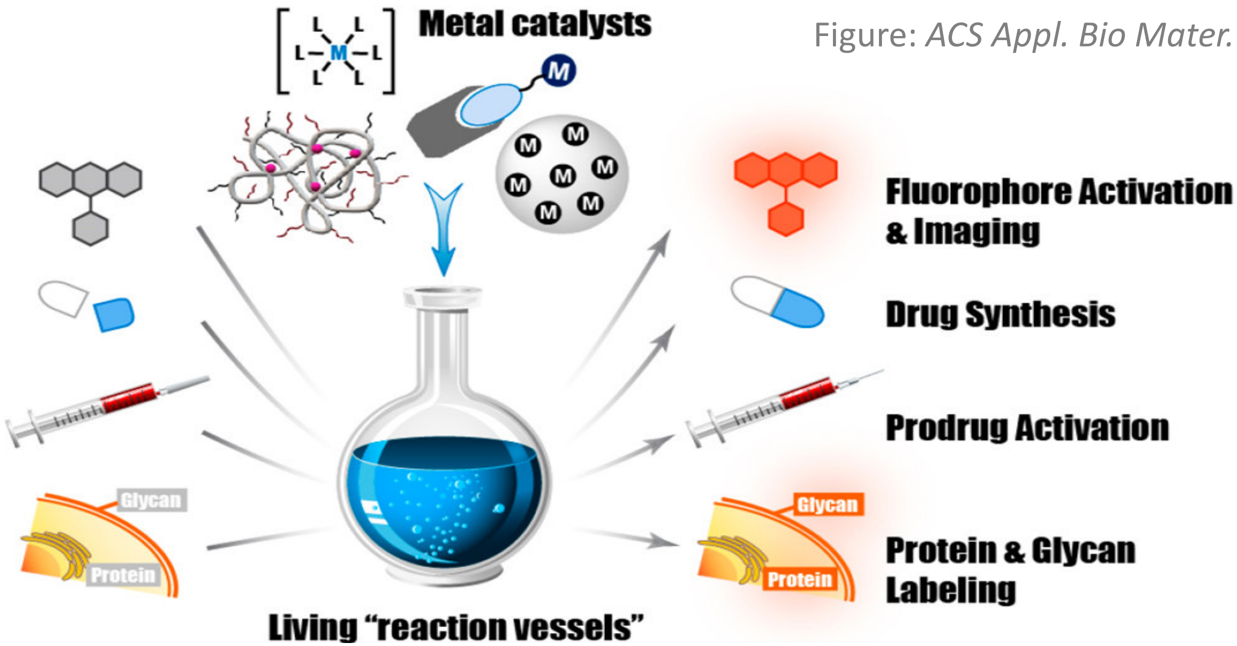
Summary

Figure: *ACS Appl. Bio Mater.* **2020**, *3*, 4717–4746.



TM catalysis allows a great variety of reactions *in vivo*.

It leads to the further development of biological tools and the creation of new therapeutic modalities.

Thank you for your attention!

Appendix

Toxicity of Metal Compounds

【Myths of metal compounds】

- **Heavy metals correspond to more toxic compounds in comparison to lighter metals**
  →This brief is ungrounded as toxic features of a metal depend on its oxidation state, ligands, etc.

- **Toxicity can be directly correlated with the structure of metal compounds.**
  →It is hardly possible to draw a direct rule for correlating the structure with toxicity.

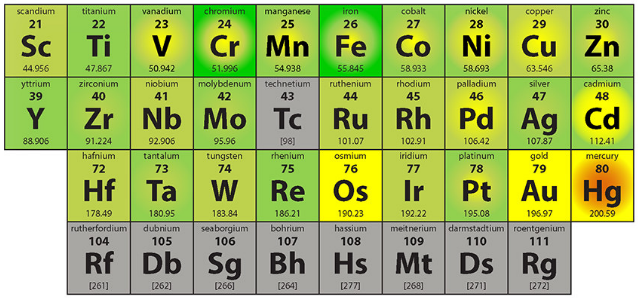
- **All nanoparticles are toxic.**
  →As their transformations in the environment are often intricate and difficult to predict, it is hard to establish the rule of their toxicity.

***For detailed information, please see the review cited below.**

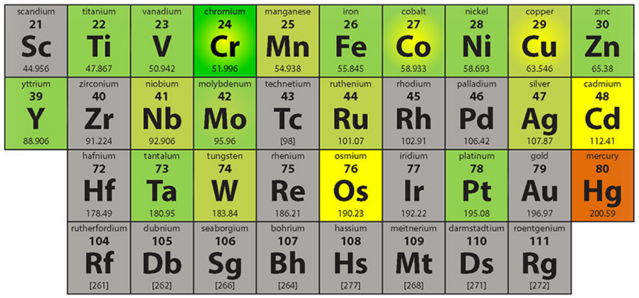
Egorova K. S., Ananikov V. P., *Organometallics*, **2017**, *36*, 4071–4090.

Toxicity of Metal Compounds

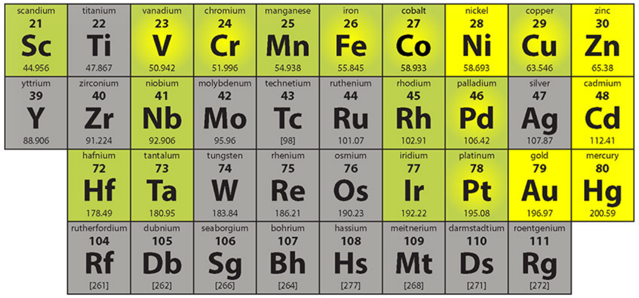
A **All available compounds**



C **Oxides**



B **Chlorides**



D

Rating	Common description	LD_{50} (single oral dose for rats, mg kg^{-1})
1	Extremely toxic	≤1
2	Highly toxic	1-50
3	Moderately toxic	50-500
4	Slightly toxic	500-5000
5	Practically non-toxic	5000-15000
6	Relatively harmless	>15000

Compound	Oral LD_{50}, mg×kg^{-1} (rat)
CrO_3	52
$CrCl_3$	440
$CrCl_2$	1870
$Cr(NO_3)_3 \times 9H_2O$	3250
$Cr(acac)_3$	3360
Cr_2O_3	>15000

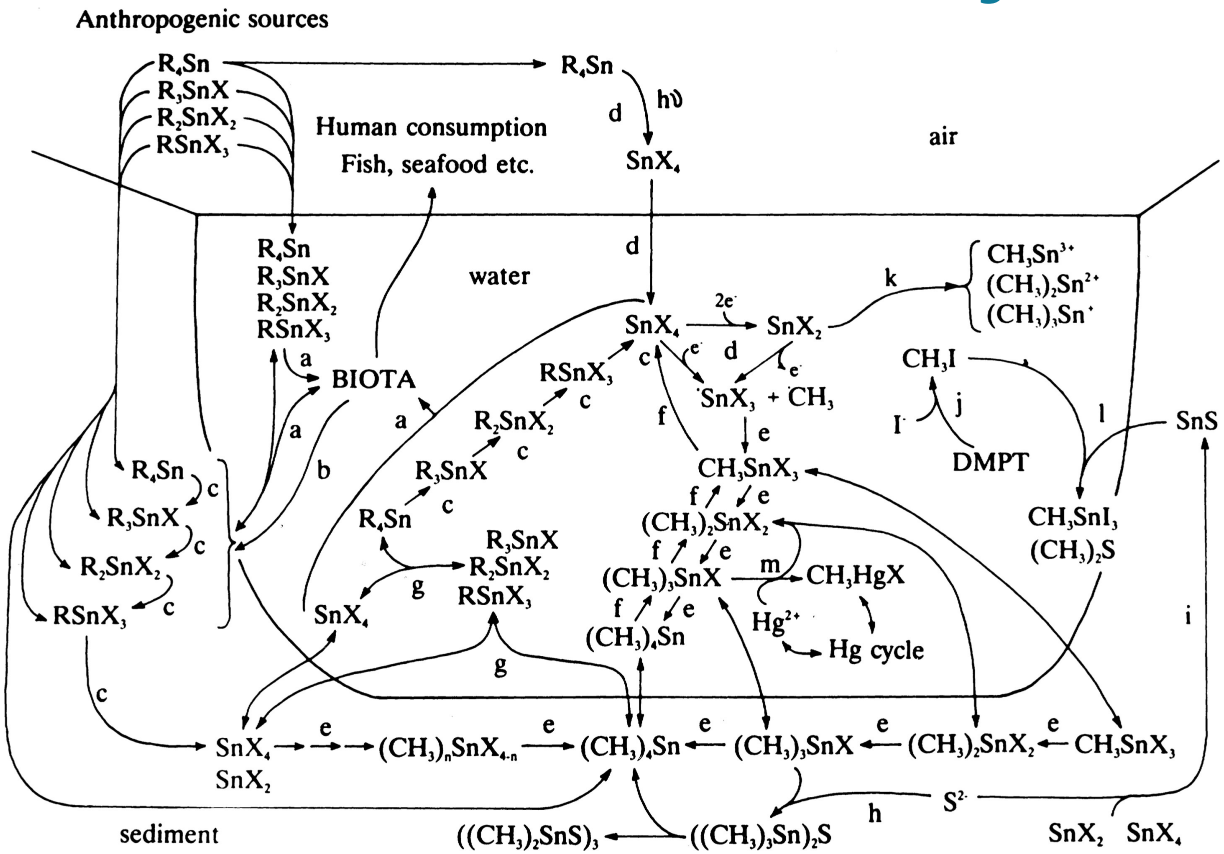
The toxicity of metal compounds can differ greatly because of differences in **solubility, oxidation state, ligands** etc.

Heavy metals are not necessarily toxic than light metals.

Egorova K. S., Ananikov V. P., *Organometallics*, **2017**, *36*, 4071–4090.

Toxicity of Metal Compounds

【An example: Possible biochemical transformations of organotin compounds】

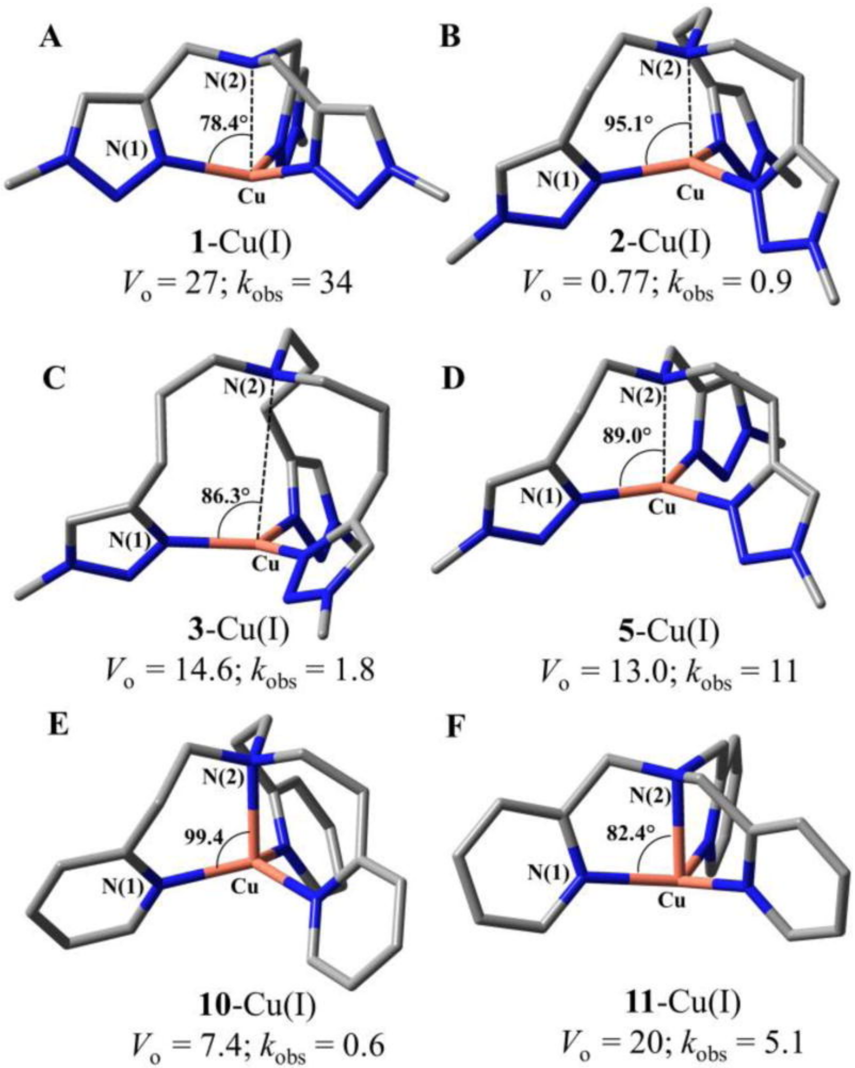


Due to liability and facile reactivity of metal-containing compounds, they can undergo profound structural transformations before affecting living organisms.

→ **It is difficult to identify the structure that causes the most pronounced toxicity.**

Egorova K. S., Ananikov V. P., *Organometallics*, **2017**, *36*, 4071–4090.

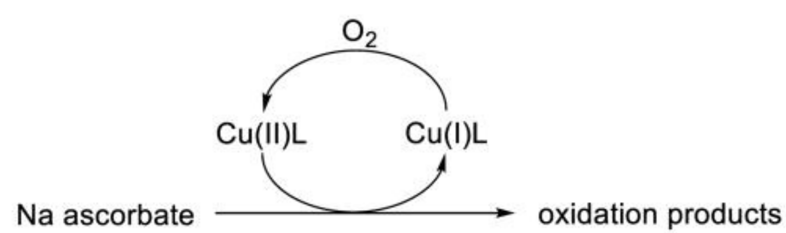
Tripodal Amine Ligands for Cu(I) Complexes



V_o: oxidation rate
k_{obs}: CuAAC second-order rate constant

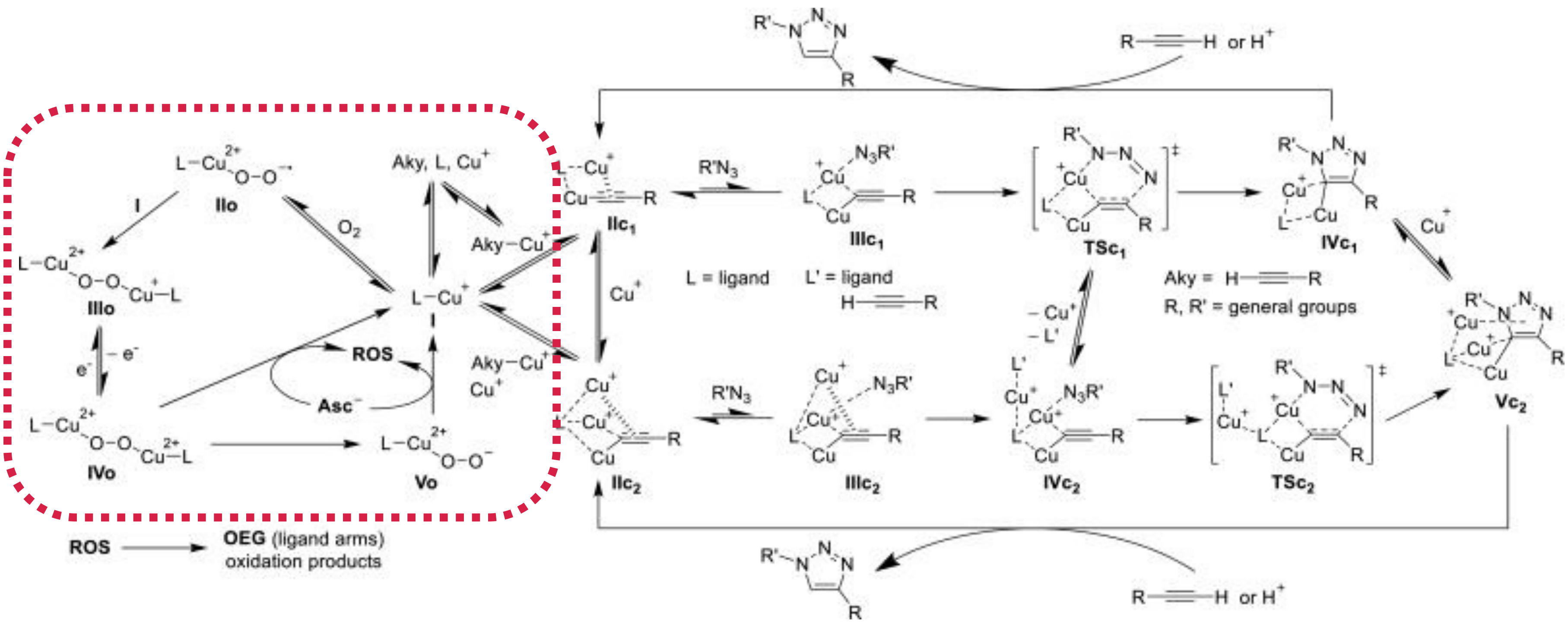
Chelate arms length of Cu(I) complexes mainly influences the angles between **N(1)-Cu(I)-N(2).**

Larger N(1)−Cu(I)−N(2) angle gives better protection of Cu(I) against oxidation .



Zhu Z *et al. Catal. Sci. Technol.* **2017**, *7*, 2474–2485.

Cu(I)-Triggered ROS Production



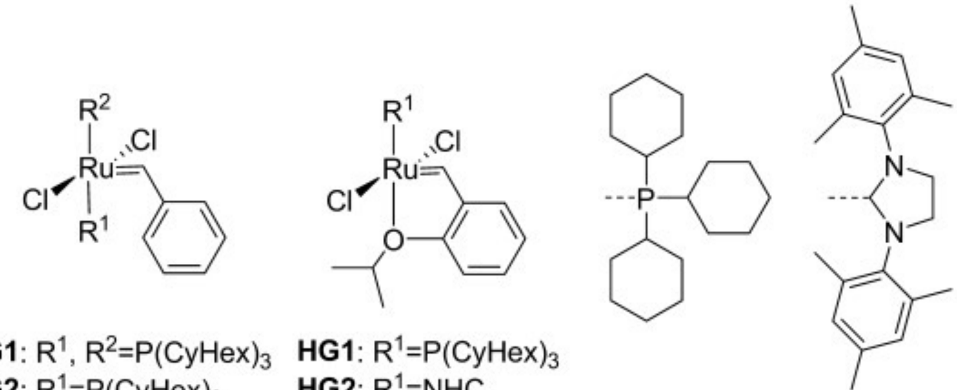
The toxicity of Cu(I)

→ Due to the production of reactive oxygen species (ROS)

(Fenton's reaction is also a plausible mechanism of its toxicity.)

Zhu Z *et al. Catal. Sci. Technol.* **2017**, *7*, 2474–2485.

Biological Effects of Grubbs Catalysts



G1: R1, R2=P(CyHex)$_3$
G2: R1=P(CyHex)$_3$, R2=NHC

HG1: R1=P(CyHex)$_3$
HG2: R1=NHC

P(CyHex)$_3$ NHC

Table 1. Inhibition of TrxR, GR, trypsin, and catB by Grubbs-type catalysts.[a]

Compd	EC_{50} [μM] TrxR	GR	trypsin	catB
G1	>100 (53±5%)	>100 (78±7%)	>100 (83±3%)	>100 (75±2%)*
G2	16.4±2.3	>100 (79±5%)	>100 (87±3%)	>100 (62±3%)*
HG1	3.4±0.9	>100 (71±2%)	>100 (90±4%)	29.3±5.2*
HG2	2.5±0.3	>100 (80±4%)	>100 (96±2%)	8.0±1.2*

[a] Results are expressed as mean (±SD) of three independent experiments. If no EC_{50} value could be calculated, the residual enzymatic activity at 100 μM is given in parentheses. *: insufficient solubility at 100 μM.

Table 2. Antiproliferative effects of Grubbs-type catalysts in MCF-7 and HT-29 cells.[a]

Compd	IC_{50} [μM] MCF-7	HT-29
Cisplatin[b]	2.0	7.0
G1	54.8±2.1	>100 (69±4%)
G2	>100 (60±3%)	>100 (94±2%)
HG1	27.8±1.4	>100 (76±3%)
HG2	9.9±3.7	13.4±4.4

[a] Results are expressed as mean (±SD) of three independent experiments. If no IC_{50} value could be calculated, the percentage of cell biomass (compared with an untreated control) at 100 μM of the compound is given in parentheses. [b] IC_{50} values for cisplatin in the same assay are given as a reference (data taken from Ref. [25,30]).

Grubbs-type catalysts have potential as inhibitors of tumor-relevant enzymes, exhibit antiproliferative effects in cultured tumor cells, and influence cell metabolism.

L. Oehninger, *et al. ChemMedChem*, **2011**, *6*, 2142–2145.

【Cleavable linkers so far】

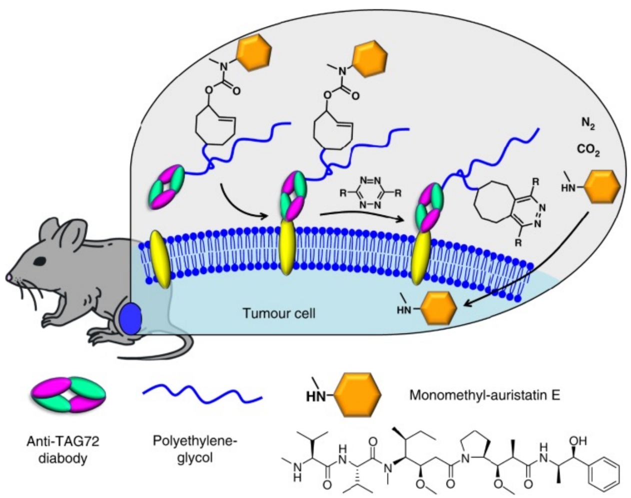
Current cleavable linkers rely on…

- Redox cleavage
- Enzyme-mediated cleavage
- photocleavage

These are not amendable for bioorthogonal control over the activation process.

- Transcyclooctene (TCO)-based linker

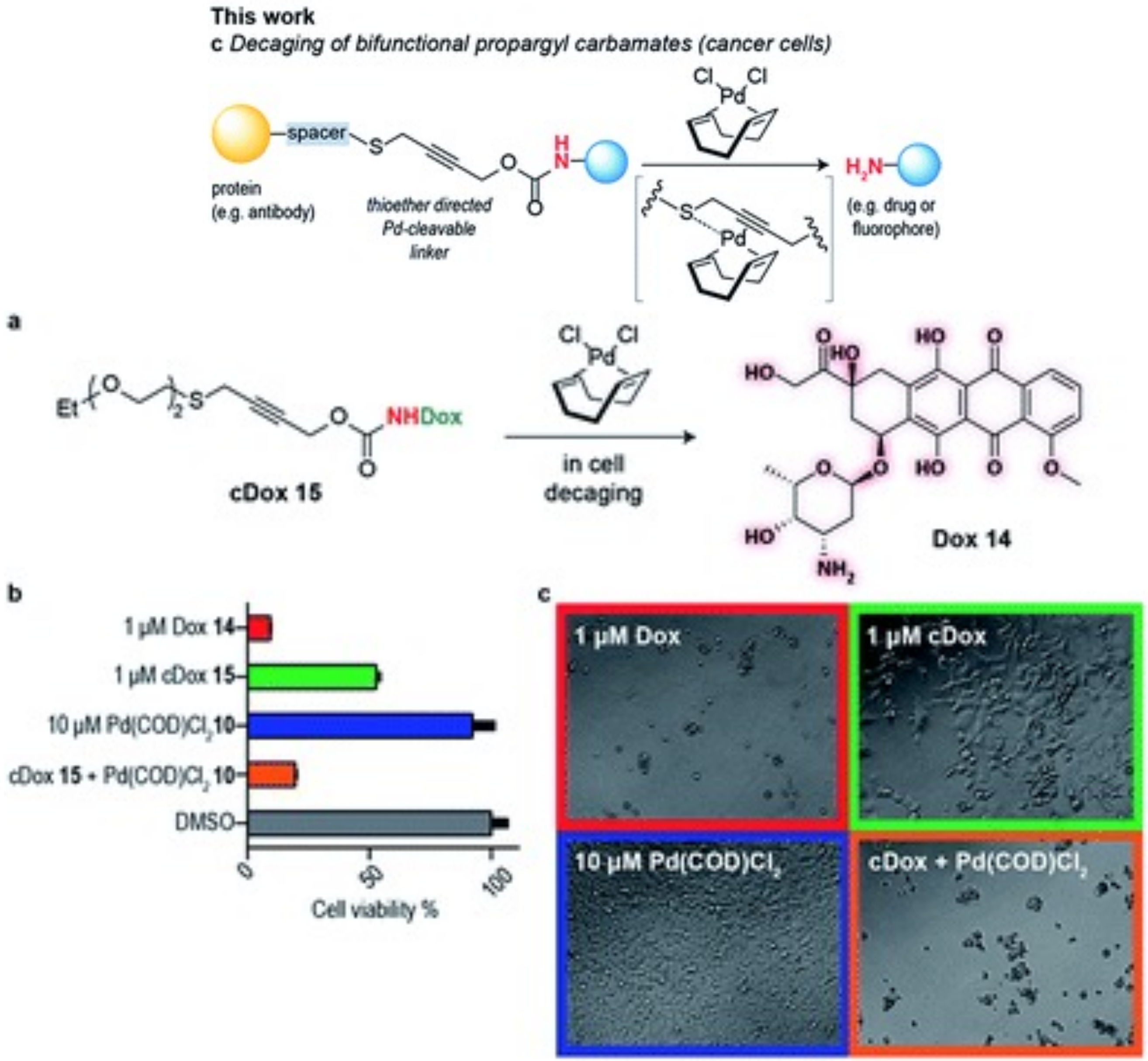
→It requires a sophisticated synthesis procedure, which hampers large-scale production.



(TCO)-based linker

R. Rossin, *et al. Nat. Commun.* **2019**, *9*, 1484.

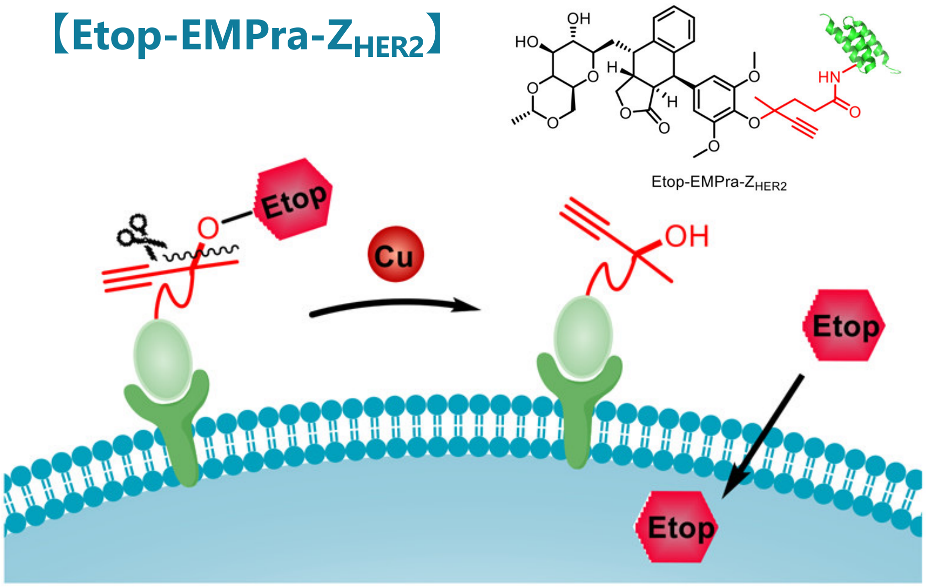
Another Example of ADC Cleavage by TM



B. J. Stenton, *et al. Chem. Sci.* **2019**, *9*, 4185–4189.

Cu-Triggered ADCs Linker Cleavage (Etop)

【Etop-EMPra-Z_{HER2}】

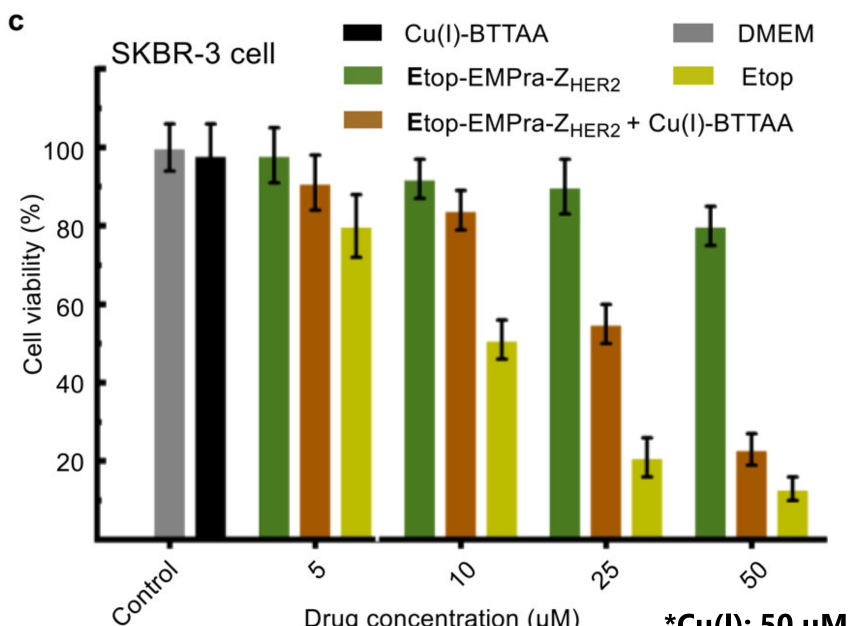


Etoposide

- **Anticancer drug**
- **Topoisomerase Ⅱ inhibitor → DNA damage**

✗ **Side effects due to the lack of selectivity**

Cu-mediated release of phenols instead of amines



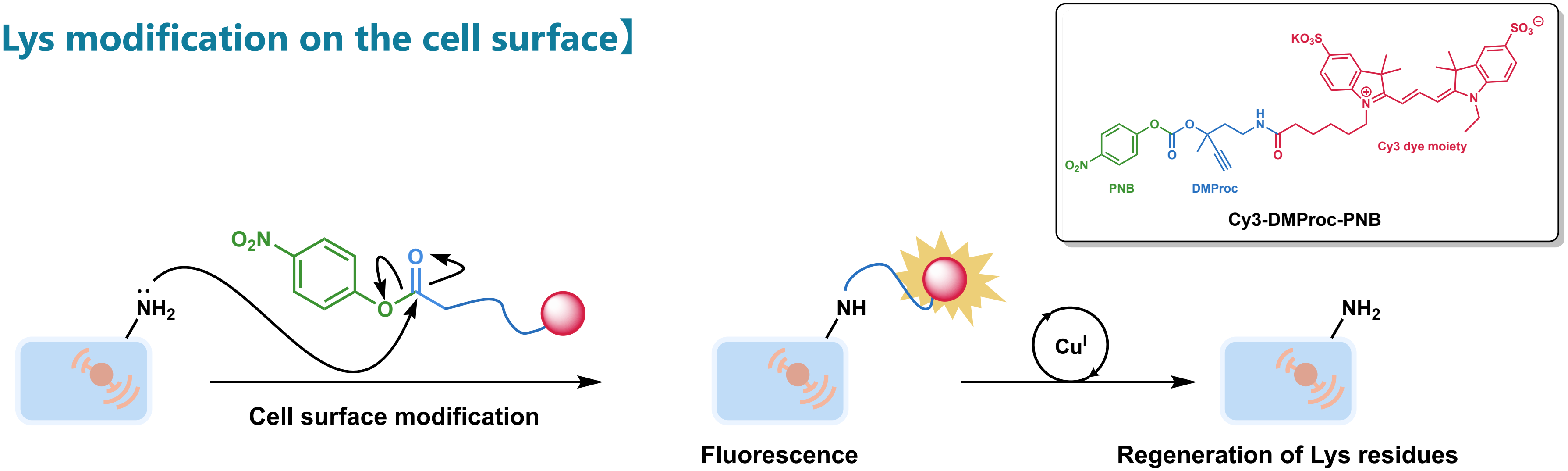
***Cu(I): 50 μM**

Cu-catalyzed, on demand and on target ADC activation strategy was also demonstrated in Etop-EMPra-Z_{HER2}.

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

Cell Surface Modification

【Lys modification on the cell surface】



【Fluorescent images】

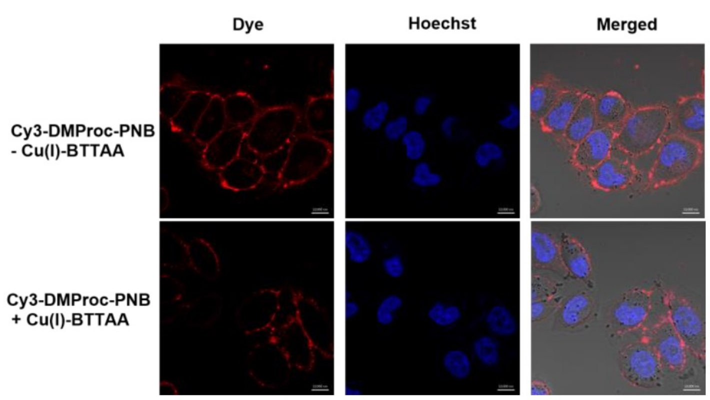
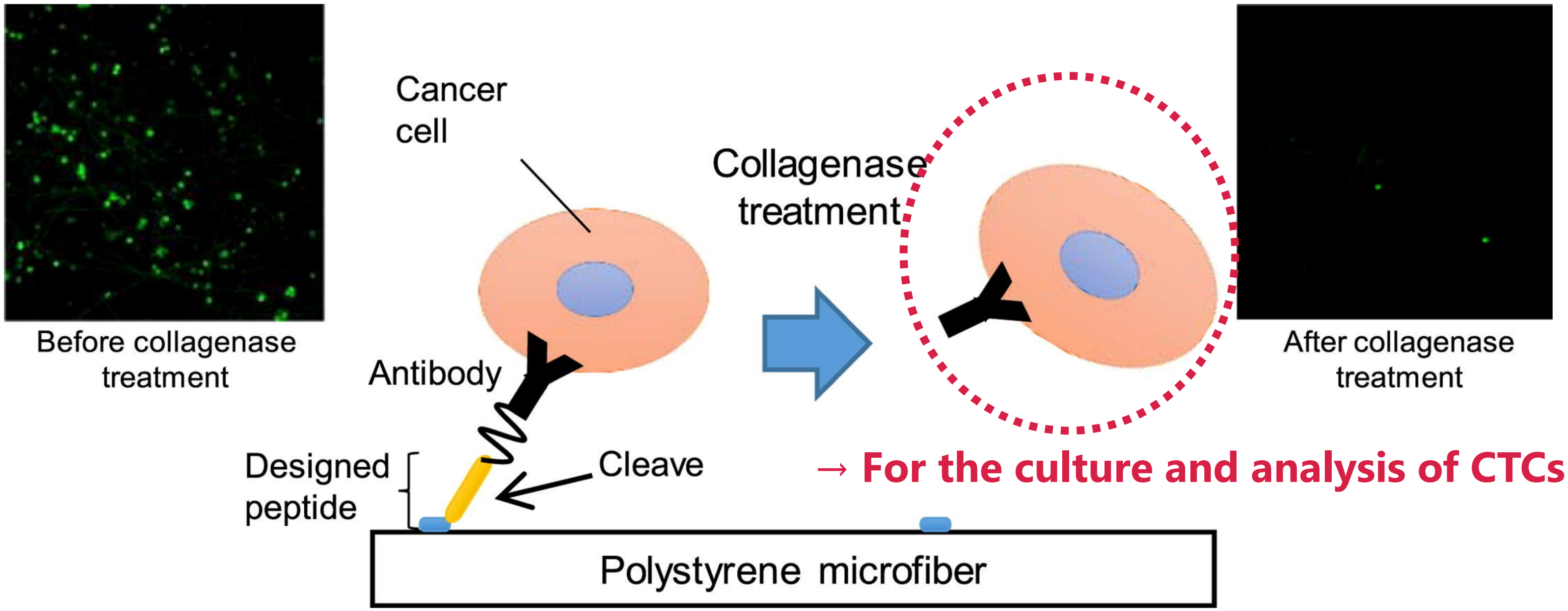


Figure S8. Fluorescent imaging of the release of Cy3 by Cu(I)-BTTAA on living cells. The copper-triggered cleavage of the internal linker resulted in the decreased fluorescence of Cy3. Scale bar: 100 µm

Xin Wang *et al. J. Am. Chem. Soc.* **2019**, *141*, 17133–17141.

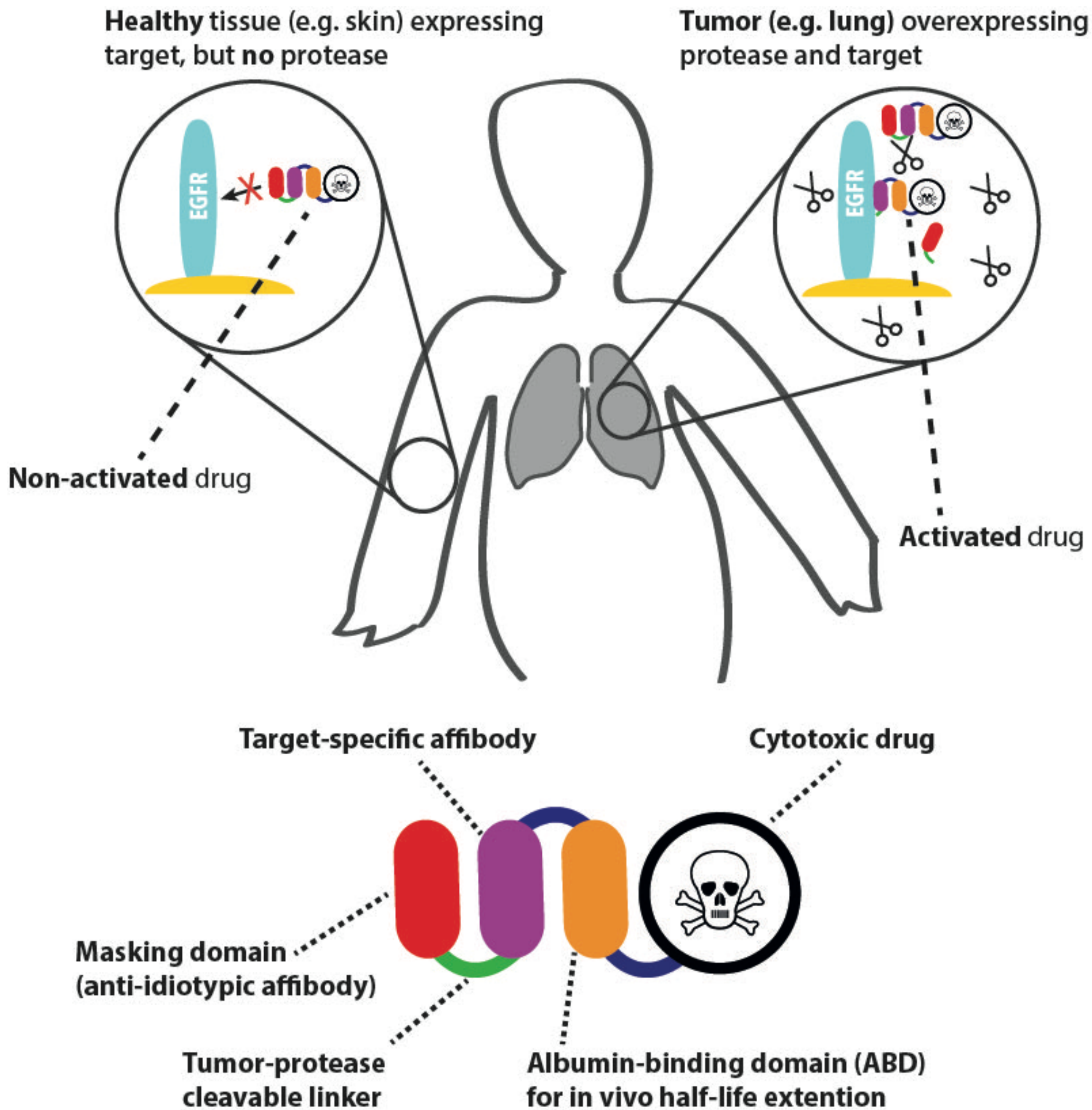
Capture and Release of CTCs



- ✓ CTCs (<u>C</u>irculating <u>T</u>umor <u>C</u>ells), which are tumor cells present in the blood, have attracted much attention as **a new tumor marker**.
- ✓ The capture and release system will be useful for **further CTCs analysis**.
- ✓ This system can be applicable for **more accurate cancer diagnosis**.

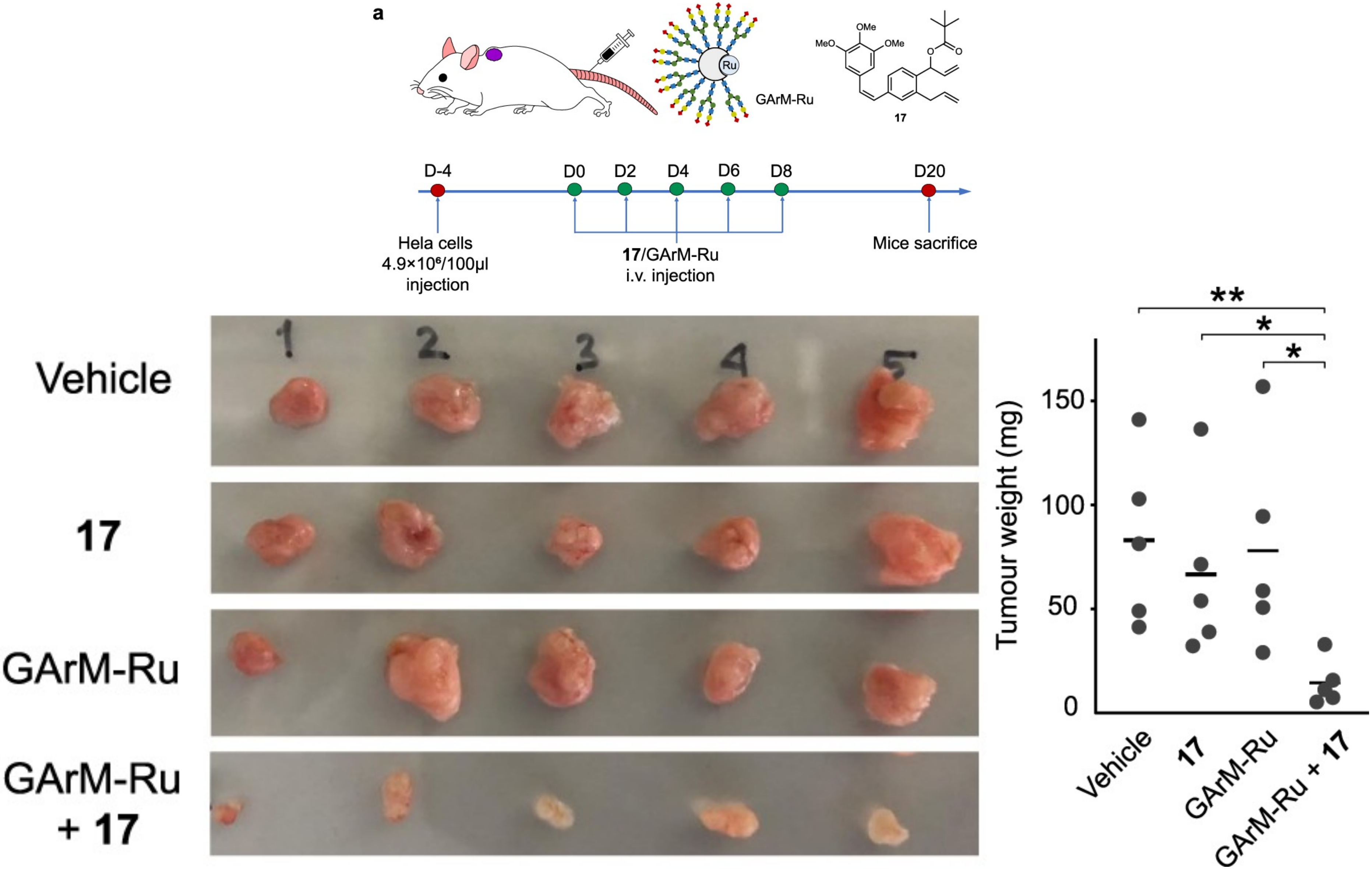
A. Yoshihara *et al. Acta biomater.* **2018**, *67*, 32–41.

Protein-Based Prodrug



https://www.kth.se/profile/lofblom/page/protein-based-prodrugs

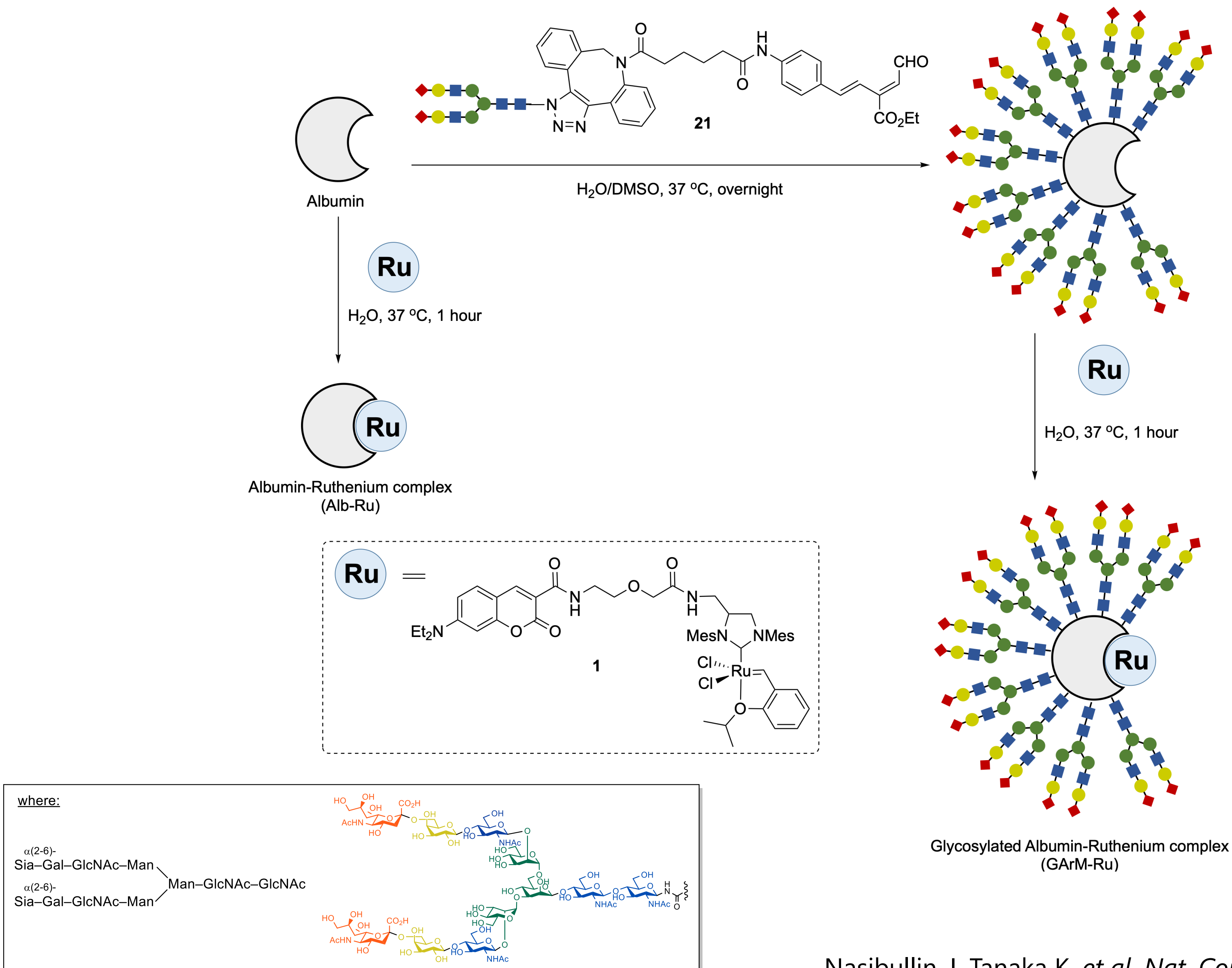
Tumor Size



Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

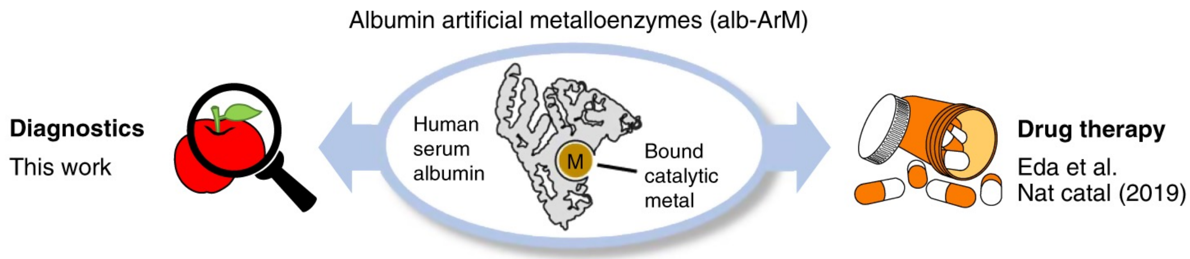
Preparation of ArM

Supplementary Figure 1. Structure of the α(2,6)-Sia terminated glycan-aldehyde probe **21**



Nasibullin, I, Tanaka K. *et al. Nat. Commun.* **2022**, *13*, 1–12.

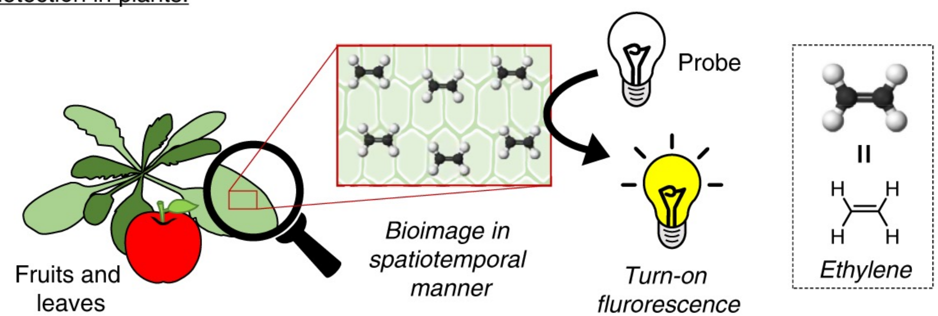
ArM as an Ethylene Sensor



ArM-based ethylene detection in plants:

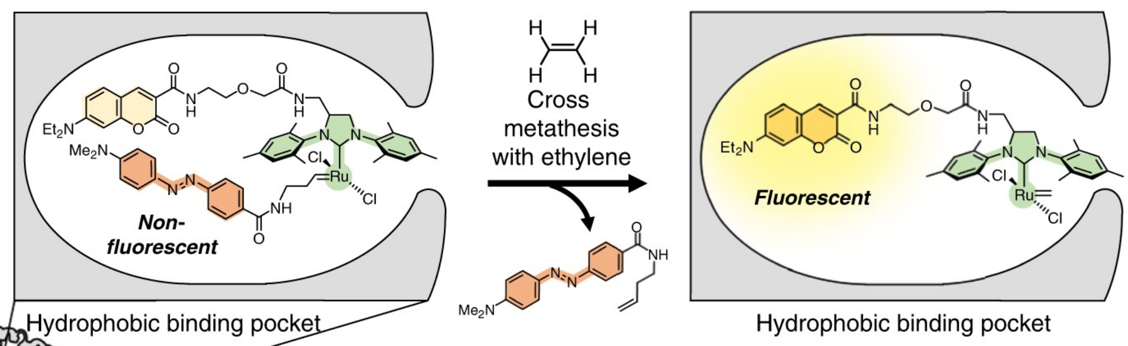
GOAL

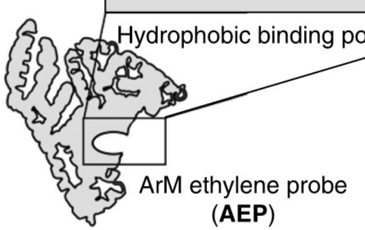
- To create an enzyme biosensor that can detect ethylene in a spatiotemporal manner



STRATEGY

- Utilize FRET-based detection based on ruthenium-catalyzed, cross metathesis with ethylene gas



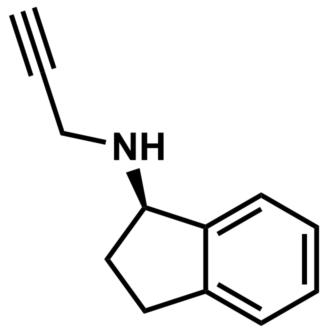


Advantages of alb-ArM usage:

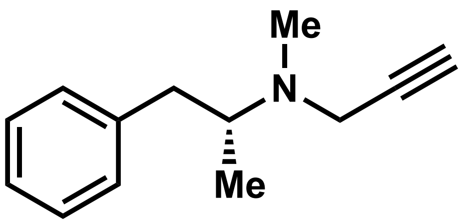
- Metal complexes becomes **water-soluble**
- Protein scaffold protects metal catalyst

Bong, K., Tanaka K. *et al. Nat. Commun.* **2019**, *10*, 1–15.

Drugs Possessing Propargyl Group



Rasagiline
For Perkinson's disease



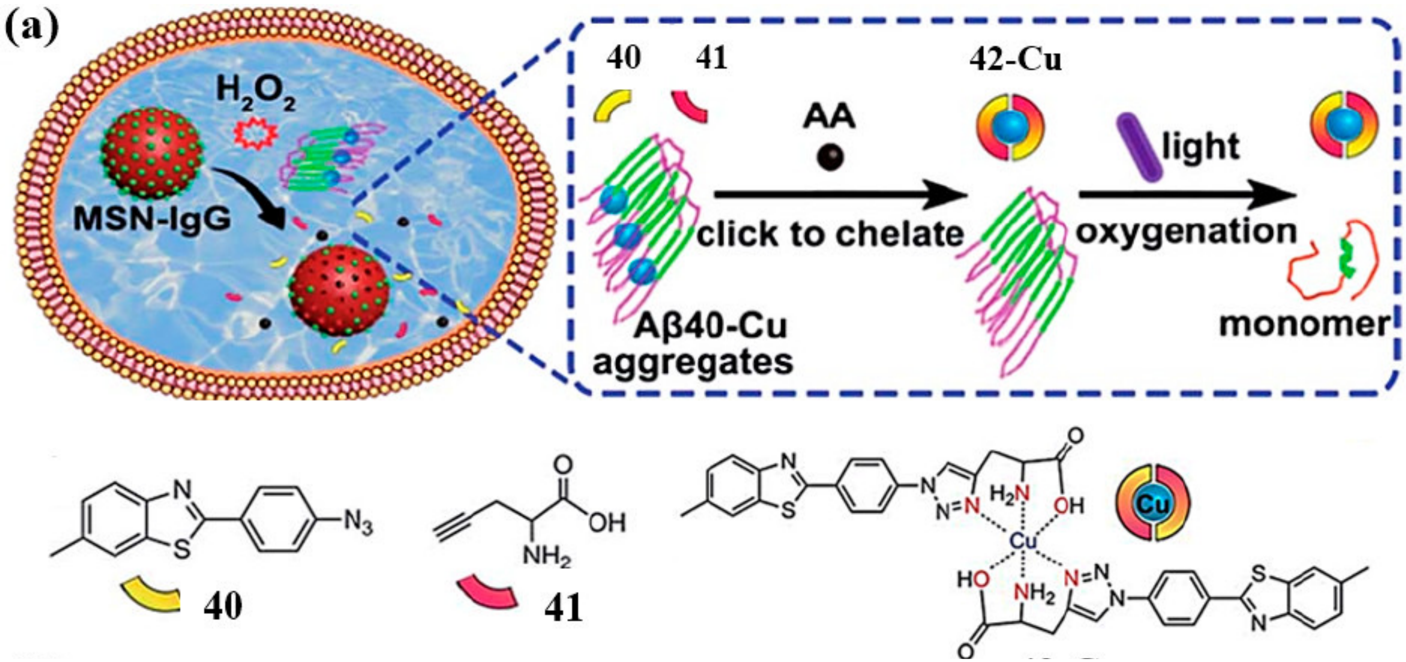
Selegiline
For Perkinson's disease

+

Transition metal catalyst

Causing a new drug interaction(?)

Cu catalysis in Aβ plaques



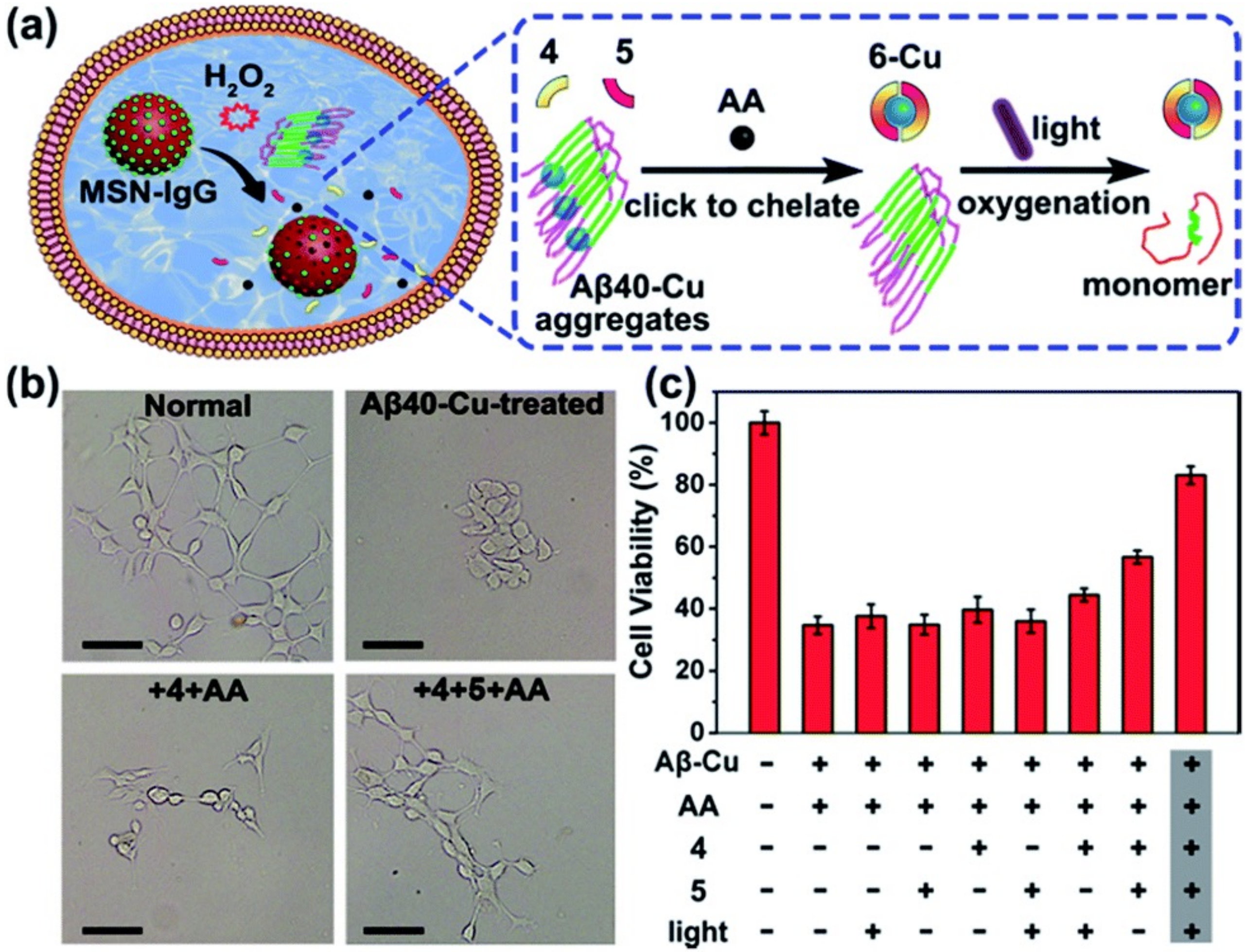
Azide-alkyne cycloaddition catalyzed by
endogenous copper accumulated in Aβ plaques.

Aβ photo-oxygenation & Cu extraction from Aβ-Cu aggregates

→**The synergistic effects promoting the disassembly of Aβ-Cu aggregates!!**

Du Z. *et al. Chem. Sci.* **2019**, *10*, 10343–10350.

Cu catalysis in Aβ plaques



✓ 40+41+ascorbic acid(AA)→ cell morphology was restored.

✓ After UV irradiation (photo-oxygenation), cell viability further improved.

Du Z. *et al. Chem. Sci.* **2019**, *10*, 10343–10350.