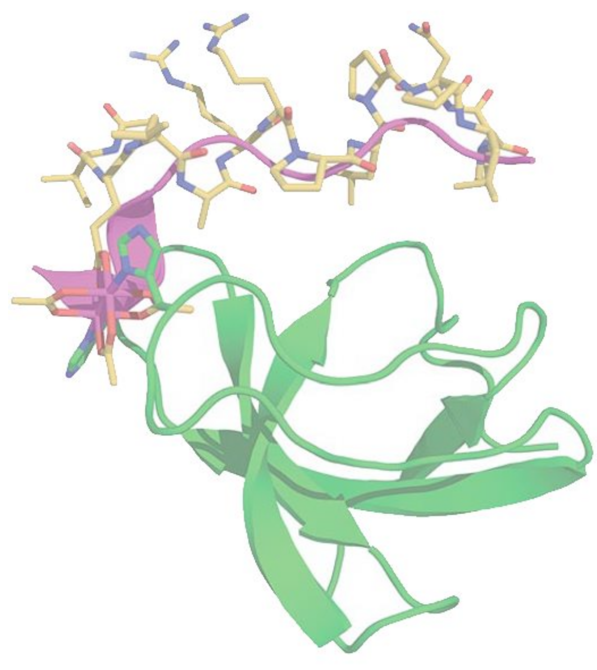
Chemical Protein Modification with Rhodium Metallopeptide Catalysts



B4 Furuta

2018/02/10

Contents

1. Introduction

2. Advances in chemical protein modification

    2-1. Ionic reaction

    2-2. Radical reaction

    2-3. Pericyclic reaction

    2-4. Transition metal reaction

3. Rhodium metallopeptide

4. Conclusion

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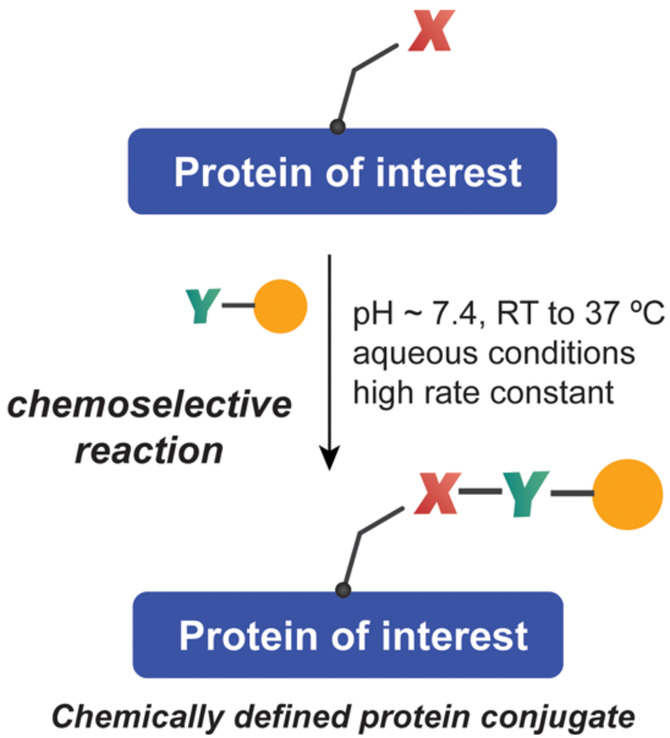
3. Rhodium metallopeptide

4. Conclusion

1. Chemical Protein Modification

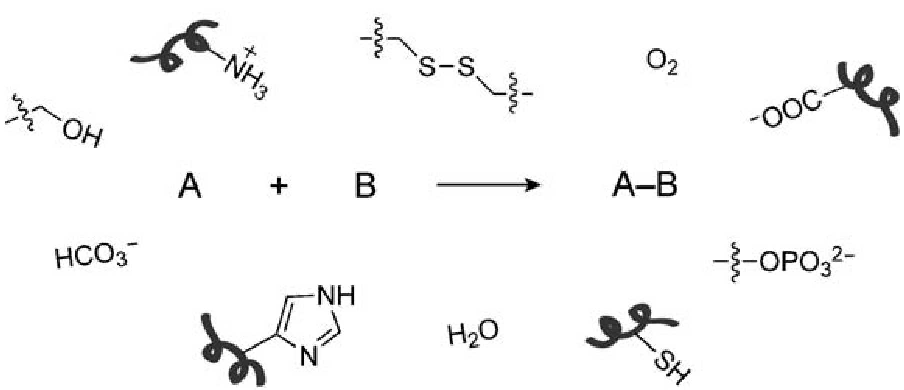
- Purpose
  - study effects of post-translational protein modifications
  - interrogate and intervene biological systems
  - confer desired properties (affinity probes, fluorophores, reactive tags, increasing the circulation half-time,…)



Omar B, and Gonçalo J. L.B., *Chem. Rev.* **2015**, *115*, 2174

1. Chemical Protein Modification

- Requirements for the reactions
  - functional group tolerance or compatibility
  - selectivity (site- or regio-)
  - water as a reaction media
  - near neutral pH and room temperature (or up to 40℃)
  - high reaction rates and high reaction efficiency
  - low reactant concentrations
  - nontoxic reagents



Ellen M. S., and Carolyn R. B., *Angew. Chem. Int. Ed*. **2009**, *48*, 6974

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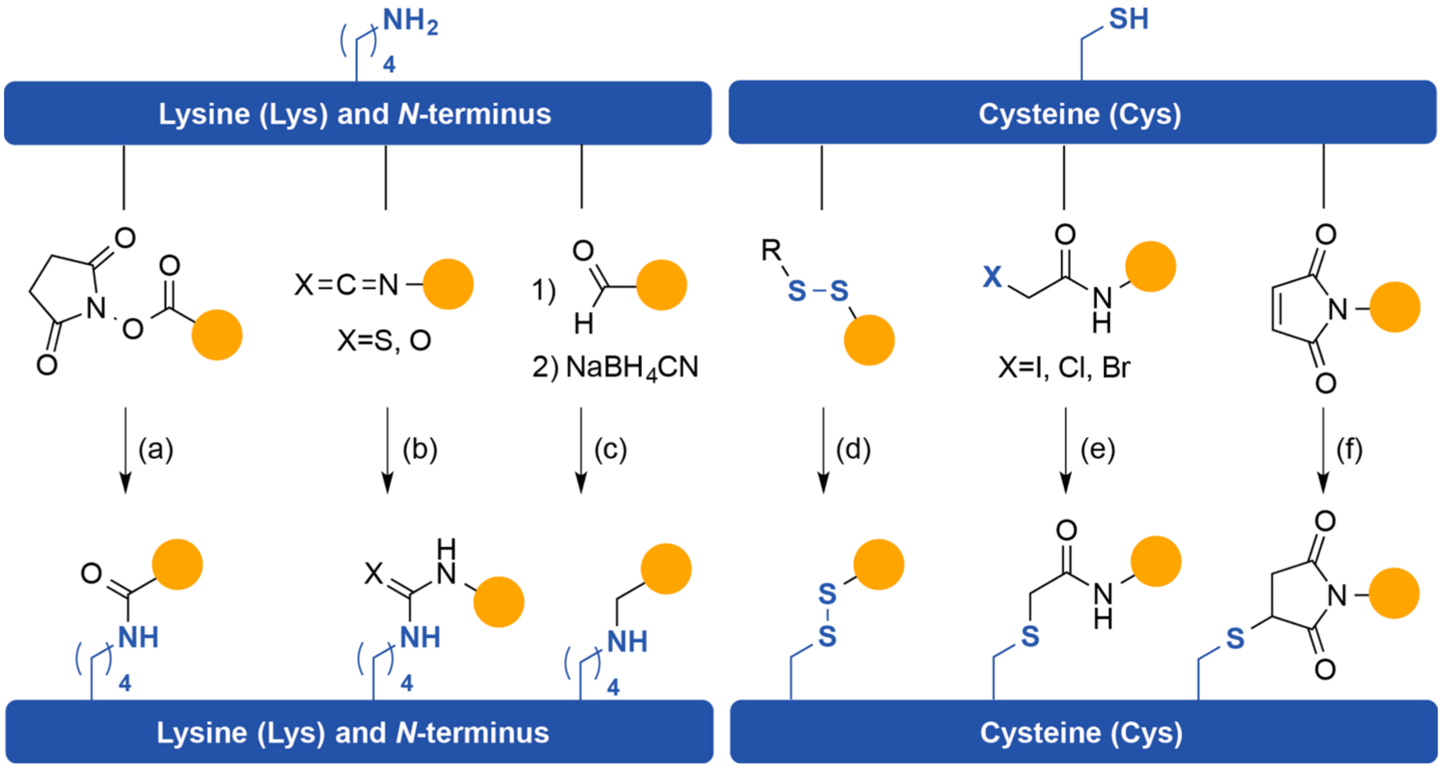
2-1. Ionic reaction

- Classical methods have relied on reactions at nucleophilic amino acids.

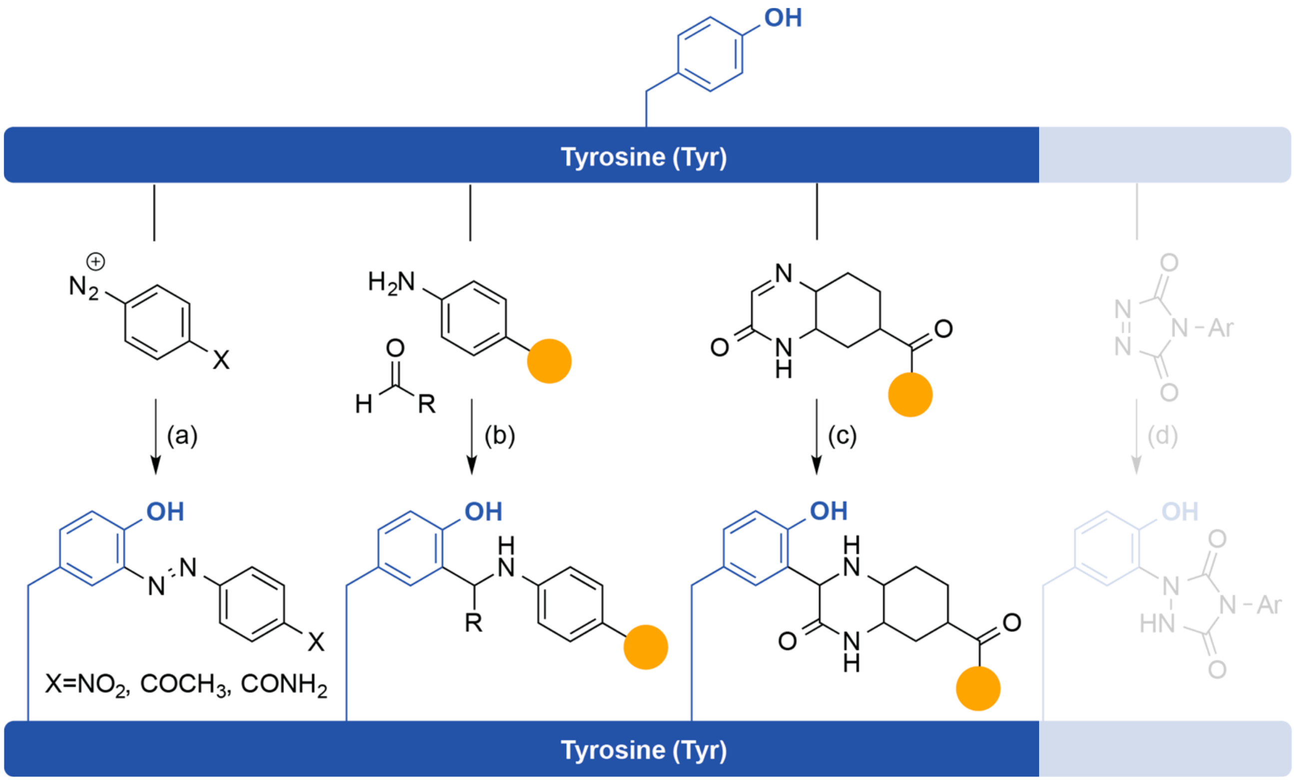


(a) Amide Formation, (b) Urea and Thiourea Formation, (c) Reductive Amination, (d) Cys-Specific Disulfide Exchange, (e) Alkylation, and (f) Conjugate Addition to a Representative Maleimide Michael Acceptor

Omar B, and Gonçalo J. L.B., *Chem. Rev.* **2015**, *115*, 2174

2-1. Ionic reaction

- Electrophilic aromatic substitution is utilized in the modification of Tyr.



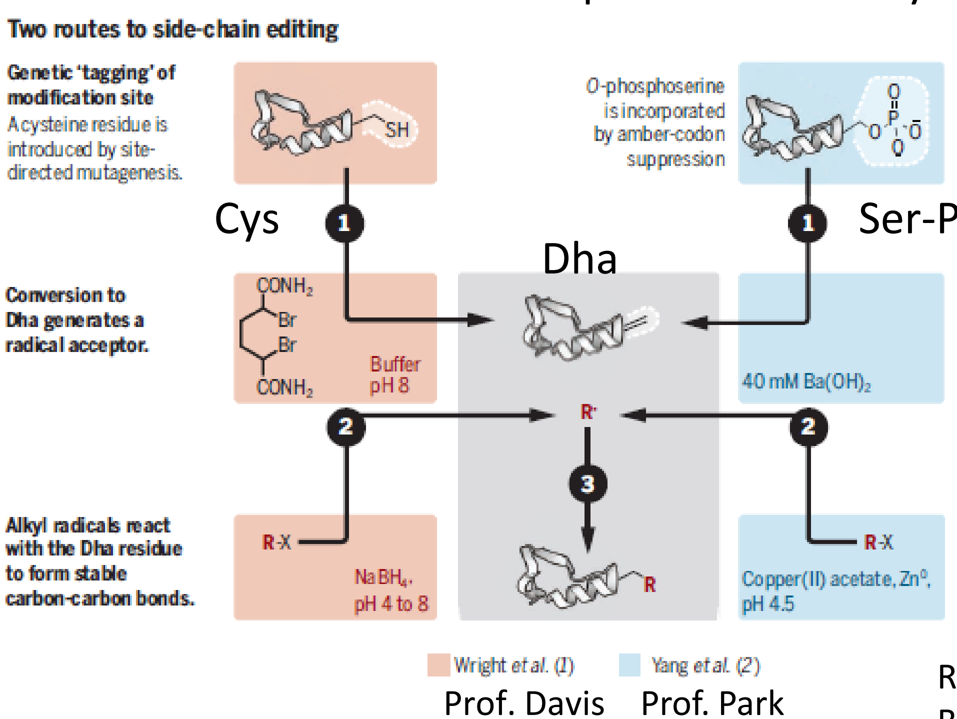
(a) Reaction with Diazonium Salts, (b) Three-Component Mannich Reaction, (c) Reaction with Preformed Imines, and (d) Ene-type Reaction with Diazodicarboxylate Reagents

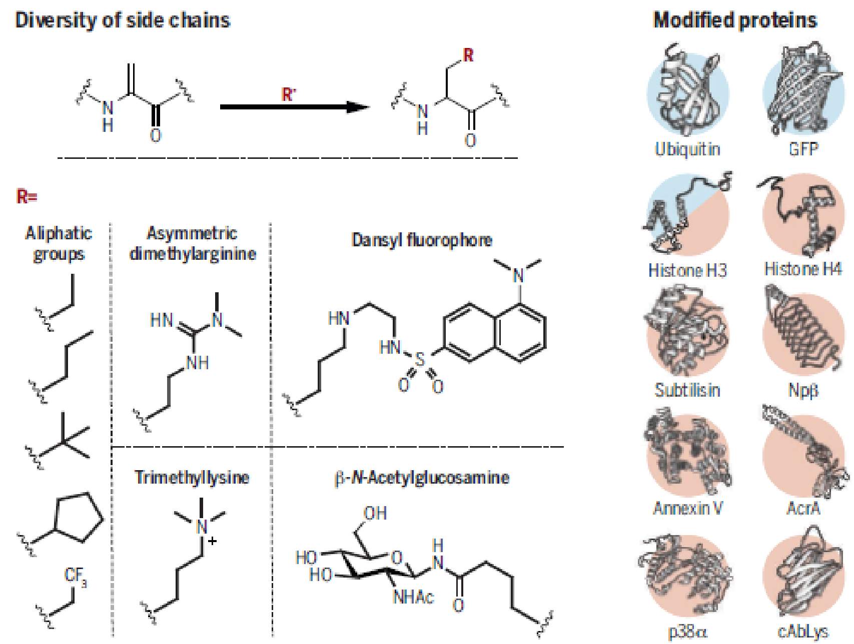
Omar B, and Gonçalo J. L.B., *Chem. Rev.* **2015**, *115*, 2174

2-2. Radical reaction

- Radicallic C(sp^3)-C(sp^3) bond formation
  - tolerant of aqueous conditions
  - unreactive with the majority of the functionality

→ Free radical chemistry would be a powerful method for protein chemistry.

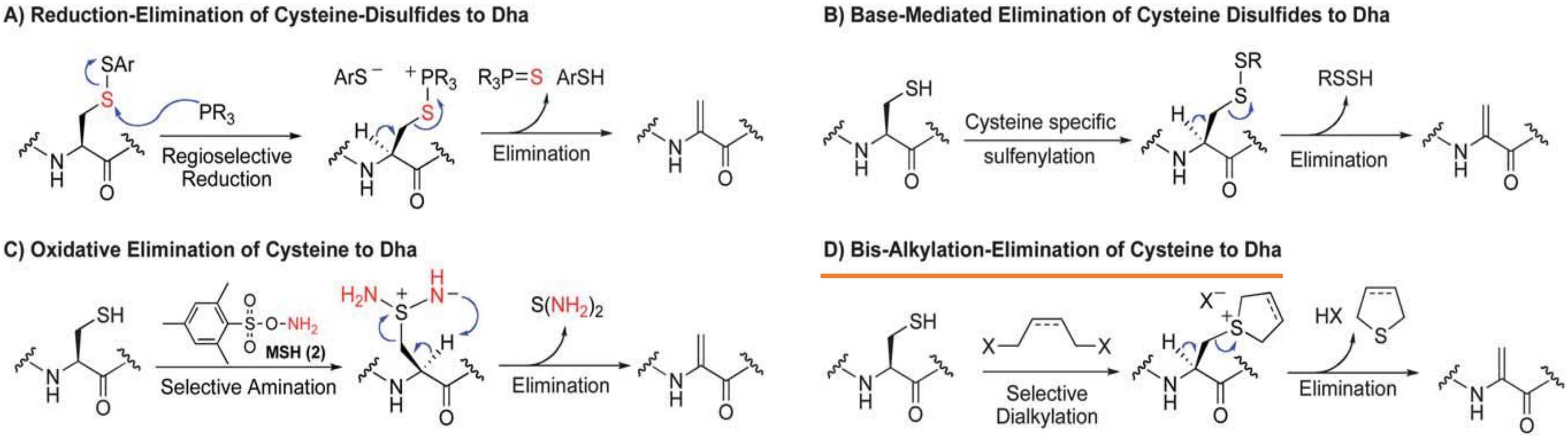




Raphael H., and Jeffrey W. B., *Science*, **2016**, *354*, 553
Benjamin G. D.,*et al., Science*, **2016**, *354*, aag1465
Hee-Sung P., *et al., Science*, **2016**, *354*, 623

2-2. Radical reaction

- Four complementary modes of elimination of cysteine to dehydroalanine

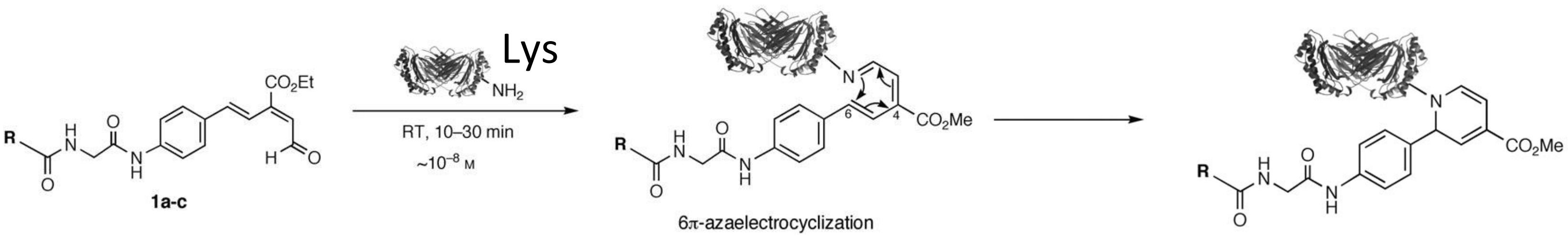


Benjamin Davis's method

Benjamin G. D., *et al., Chem. Sci.*, **2011**, *2*, 1666

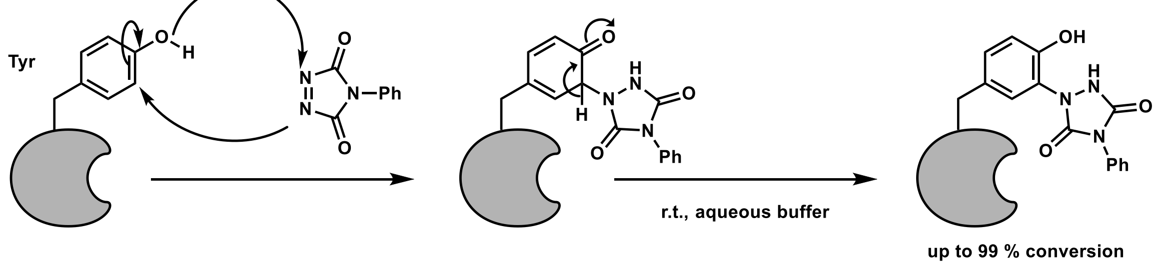
2-3. Pericyclic reaction

1. 6π-azaelectrocyclization



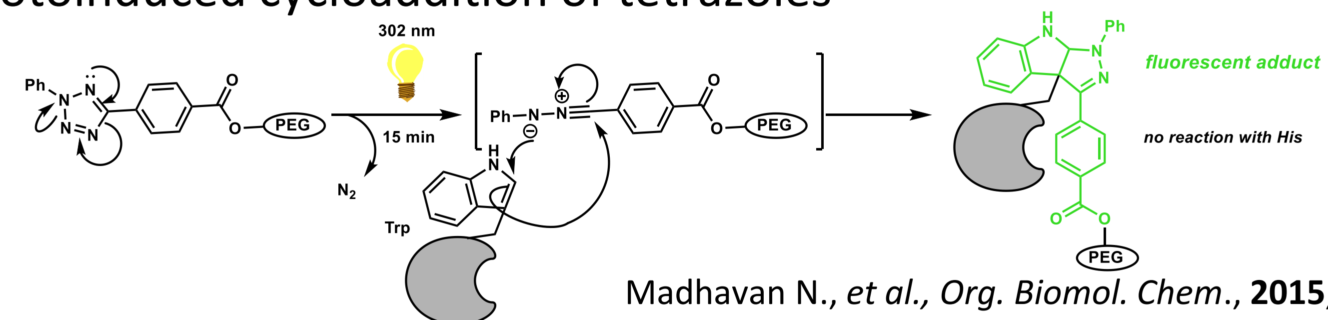
Fukase K., *et al., ChemBioChem* **2008**, *9,* 2392

2. Ene-like reaction with triazoline-dione



Carlos F. B. III., *et al., J. Am. Chem. Soc.* **2010**, *132*, 1523

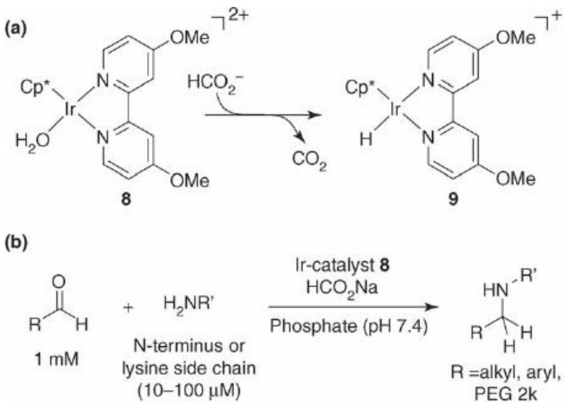
3. Photoinduced cycloaddition of tetrazoles



Madhavan N., *et al., Org. Biomol. Chem.*, **2015**, *13*, 3202
Qing L., *et al., Angew. Chem. Int. Ed.* **2008**, *47*, 2832

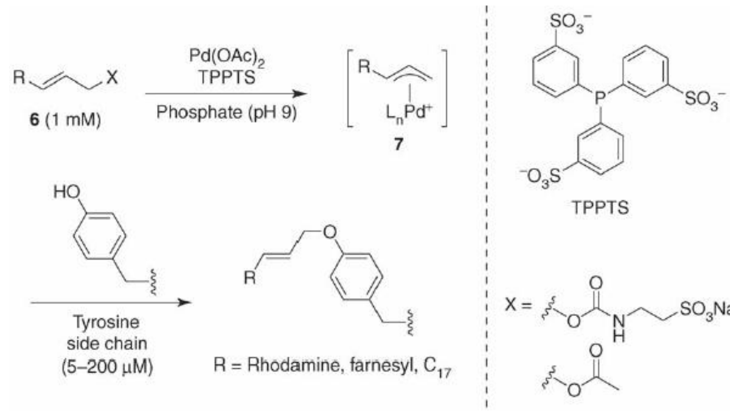
2-4. Transition metal reaction

1. Reductive alkylation reaction of Lys with *Ir* complex



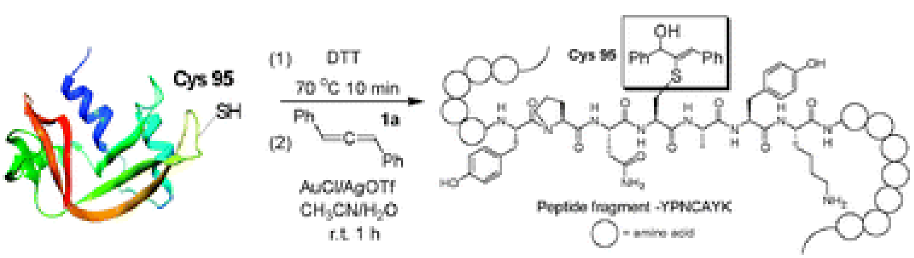
McFarland, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 13490.

2. Tsuji-Trost reaction with *Pd* (π-allylpalladium complex)



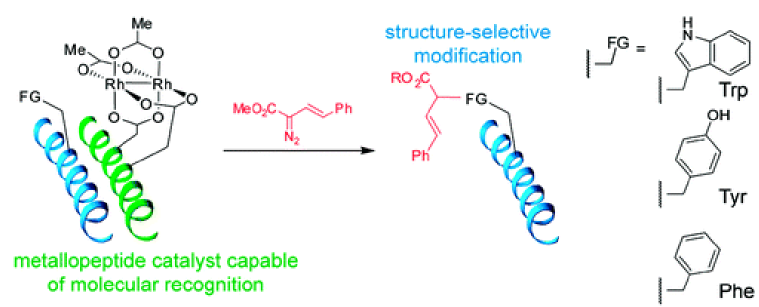
Tilley, S. D.; Francis, M. B. *J. Am. Chem. Soc.* **2006**, *128*, 1080.

3. Oxidative coupling reaction with *Au*, *Pd*, …



Che, C.-M., *et al., Chem. Commun.* **2013**, *49*, 1428.

4. C-H activation reactions with *Rh* carbenoid, *Pd* complex,…



Ball, Z. T., *et al., J. AM. CHEM. SOC.* **2010**, *132*, 6660

2-4. Transition metal reaction

1. Reductive alkylation reaction of Lys
 with ***Ir*** complex

McFarland, J. M.; Francis, M. B. *J. Am. Chem. Soc.* **2005**, *127*, 13490.

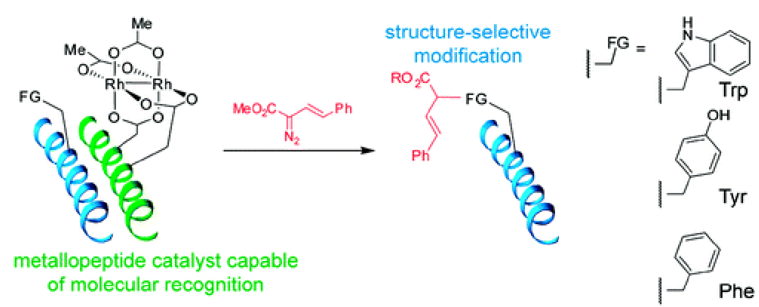
2. Tsuji-Trost reaction
 with ***Pd*** (π-allylpalladium complex)

Tilley, S. D.; Francis, M. B. *J. Am. Chem. Soc.* **2006**, *128*, 1080.

3. Oxidative coupling reaction
 with ***Au***, ***Pd***, ...

Che, C.-M., *et al.*, *Chem. Commun.* **2013**, *49*, 1428.

4. C-H activation reactions
 with ***Rh*** carbenoid, ***Pd*** complex,...



Ball, Z. T., *et al.*, *J. AM. CHEM. SOC.* **2010**, *132*, 6660

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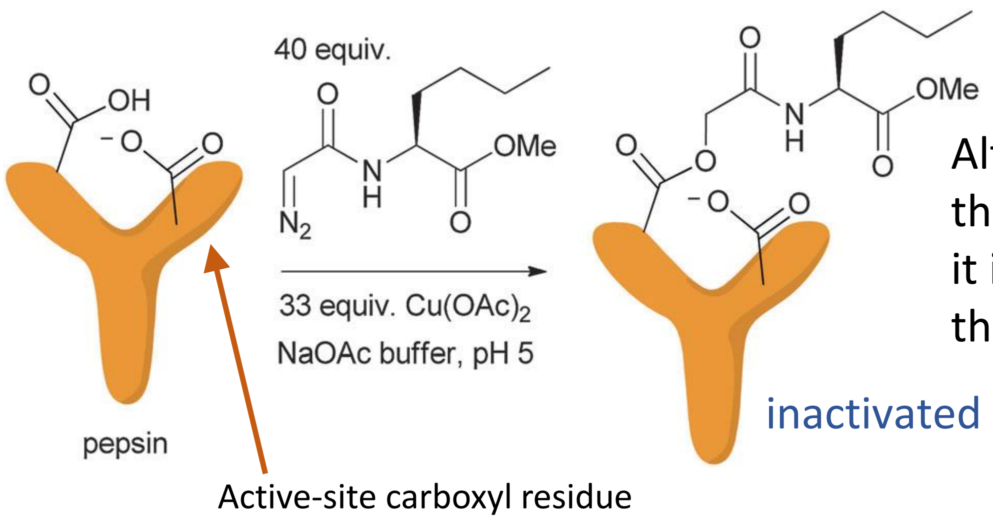
3. Earliest examples of carbenoid chemistry for protein modification

- Copper-carbenoid chemistry



Although mechanistic underpinnings of the reaction were unknown at the time, it is reasonable that this is ***catalytic O-H insertion reaction***.

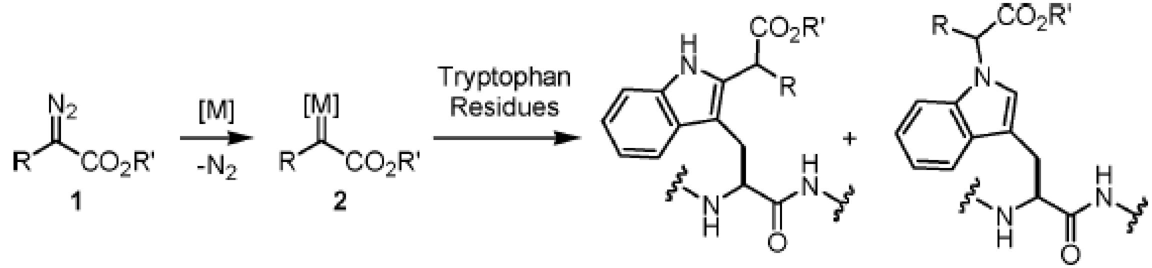
Moore S. *et al., J. Biol. Chem.*, **1966**, *241*, 4295
Delpierre G. R., Fruton J. S., *Proc. Natl. Acad. Sci. U. S. A.*, **1966**, *56*, 1817

38 years later …

Rh(II) complexes promote catalytic X-H insertion reactions for chemical biology.

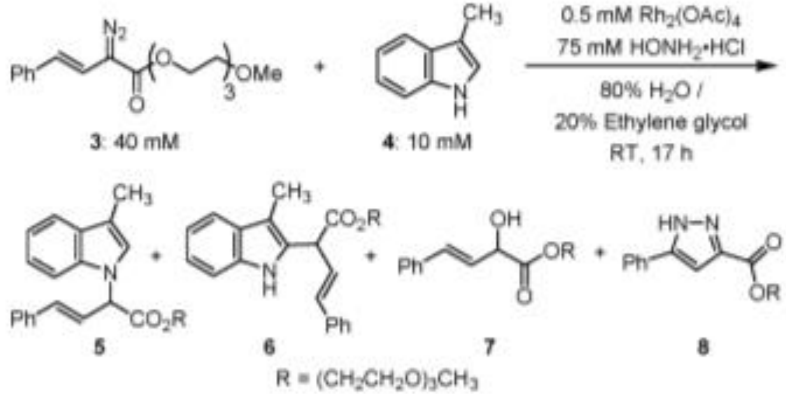
3. Selective Trp Modification with Rh carbenoids in aqueous solution

Scheme 1. Covalent Modification of Tryptophan Residues on Proteins Using Metallocarbenes



- Preliminary study with model compound

Scheme 2. Modification of 3-Methylindole with Metallocarbenes in Aqueous Media

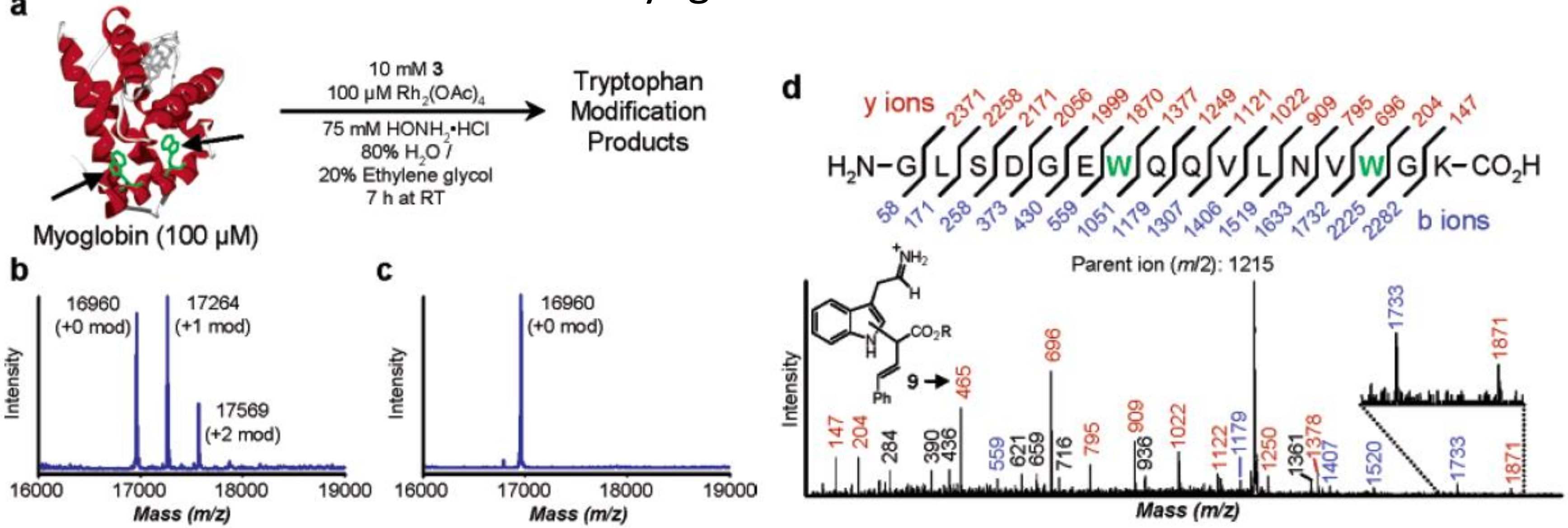


- $HONH_2$ … a necessary additive (presumably by binding to the metal center and stabilizing the reactive intermediates)
- compound 7 … reaction with H_2O
- compound 8 … electrocyclization pathway

Antos, J. M.; Francis, M. B., *et al., J. Am. Chem. Soc.* **2004**, *126*, 10256.

3. Selective Trp Modification with Rh carbenoids in aqueous solution

- Reaction with horse heart myoglobin



$Rh_2(OAc)_4$(+) **$Rh_2(OAc)_4$(-)**

Clean conversion to the singly and doubly modified products utilizing rhodium carbenoid species with very high selectivity.

- Problem

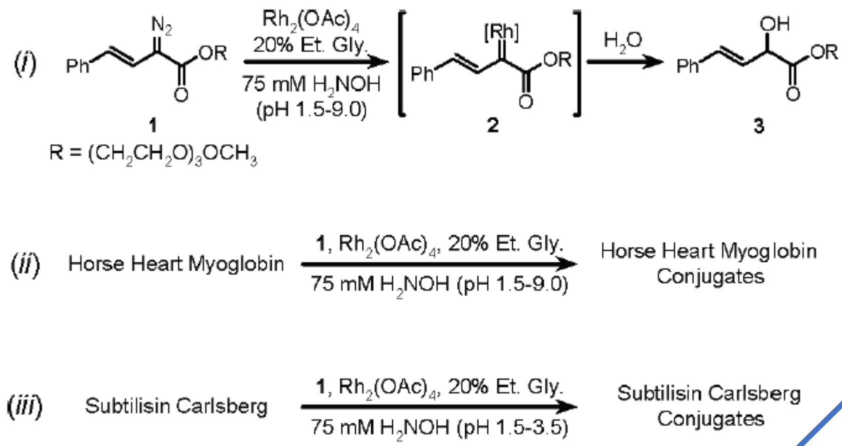
Using **$HONH_2$** causes the low pH of the reaction medium (<3.5)

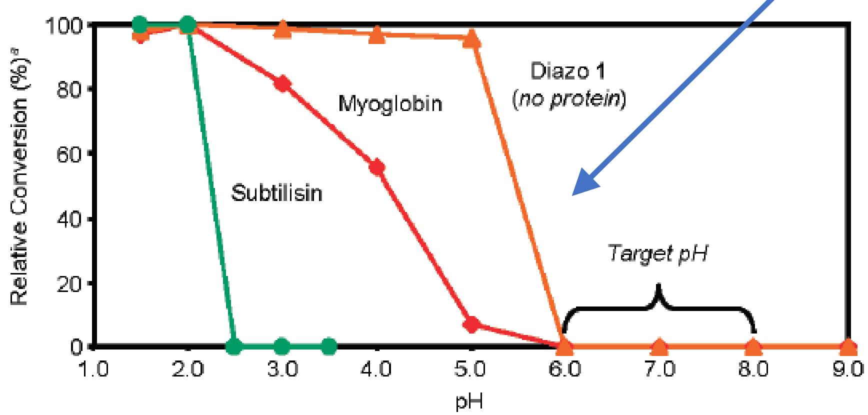
→ The scope of this methodology is limited.

Antos, J. M.; Francis, M. B., *et al., J. Am. Chem. Soc.* **2004**, *126*, 10256.

3. Overcoming the drawbacks

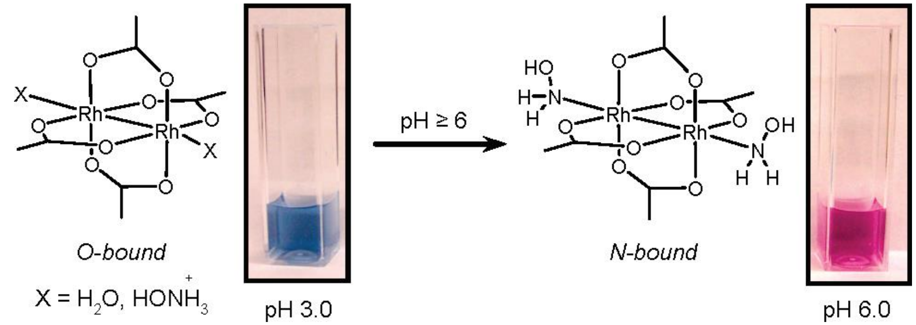
- Coordination sites of rhodium





This dramatic drop in catalytic activity around pH 6.0 can be explained by considering the pKa of $HONH_2$ (pKa = 5.97) .

pH-dependent binding of $HONH_2$ to $Rh_2(OAc)_4$ in aqueous solution



This ligand exchange process effectively deactivates the Rh catalyst.

Francis, M. B., *et al.*, *J. Am. Chem. Soc.* **2009**, *131*, 6301.

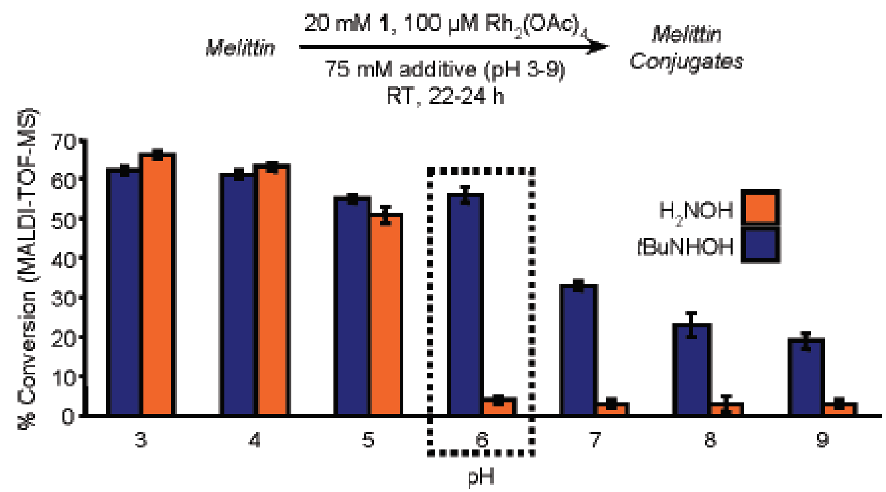
3. Overcoming the drawbacks

- Optimization of additives

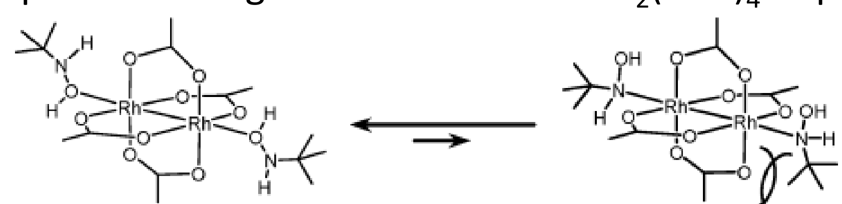
Table 1. Modification of Melittin in the Presence of Various Solution Additives[a]

Buffer/Additive	+0 mod (%)	+1 mod (%)	+2 mod (%)
—	94	6	0
NaCl	95	5	0
phosphate	90	10	0
borate	92	5	3
TRIS	73	21	6
triethanolamine	96	4	0
HEPES	92	8	0
H_2NOH	96	4	0
*t*BuNHOH	49	43	8
NH_4Cl	91	8	1
Me_3NO	90	9	1
AcNHOH	97	3	0
$H_2NOCH_2CO_2H$	81	17	2
H_2NNH_2	75	22	3

[a] Conditions: 100 μM melittin, 100 μM $Rh_2(OAc)_4$, 20 mM **1**,
*t*BuOH (v/v), RT, 24 h. Product ratios estimated by MALDI-TOF-M
Values represent the average of three independent MALDI-TOF-M
analyses of the same sample.



Proposed binding of *t*BuNHOH with $RH_2(OAc)_4$ at pH 6.0

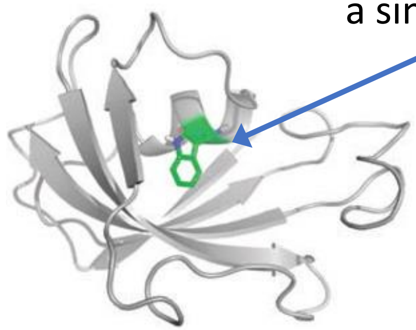


Francis, M. B., *et al., J. Am. Chem. Soc.* **2009**, *131*, 6301.

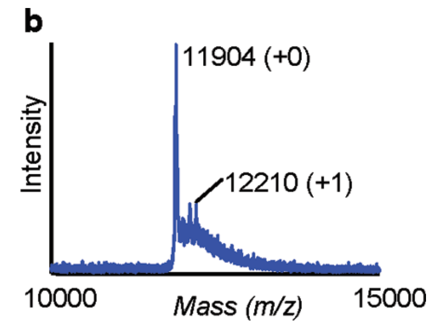
3. Overcoming the drawbacks

- Addition of tryptophan containing tags

a single ***buried*** Trp

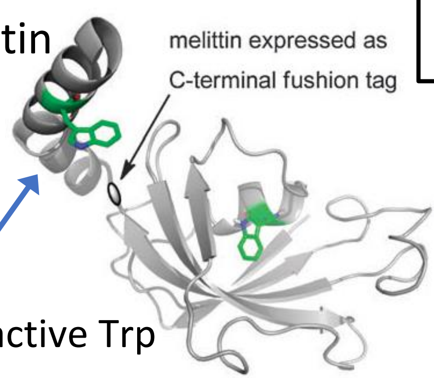


FKBP

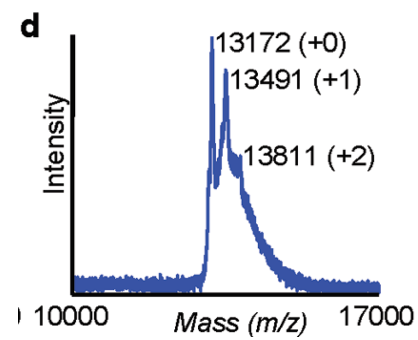


condition:
for 15 h, at room temperature,
pH 6.0, add $tBuNH_2OH$-HCl

Melittin



more reactive Trp



>40 %
modification

A general protein labeling strategy with Trp was established based on the expression of Trp-containing tags.

Francis, M. B., *et al., J. Am. Chem. Soc.* **2009**, *131*, 6301.

3. Different approach

However, in the Francis's method, there are many points to be improved.

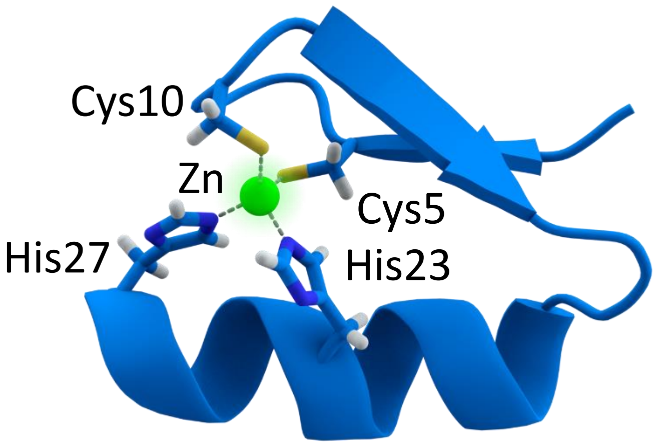
- require large amounts of Rh complex
- modification is limited to Trp
- may be necessary to denature with pH or temperature
- conversions were only moderate

Different approach : ***Rhodium Metallopeptide***

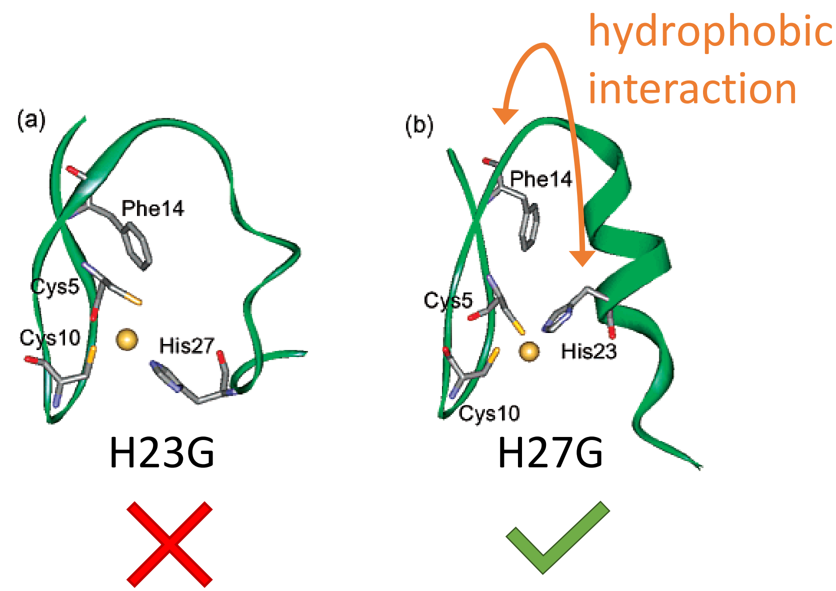
- catalytic amount of Rh complex
- modification of a variety of amino acid side-chain including unreactive ones
- site-selectivity controlled by catalyst
- in a mild reation condition
- high conversion

3. Dawn of the metallopeptide chemistry

- Contribution of zinc-liganding amino acid residues for α-helix formation in the zinc finger peptide



Zinc finger peptide



By choosing an appropriate amino acid sequence, small metallopeptide with the desired coordination geometry can be synthesized.

Nomura A., Sugiura Y., *Inorg. Chem.* **2002**, *41*, 3693

3. Zachary Ball's Chemistry

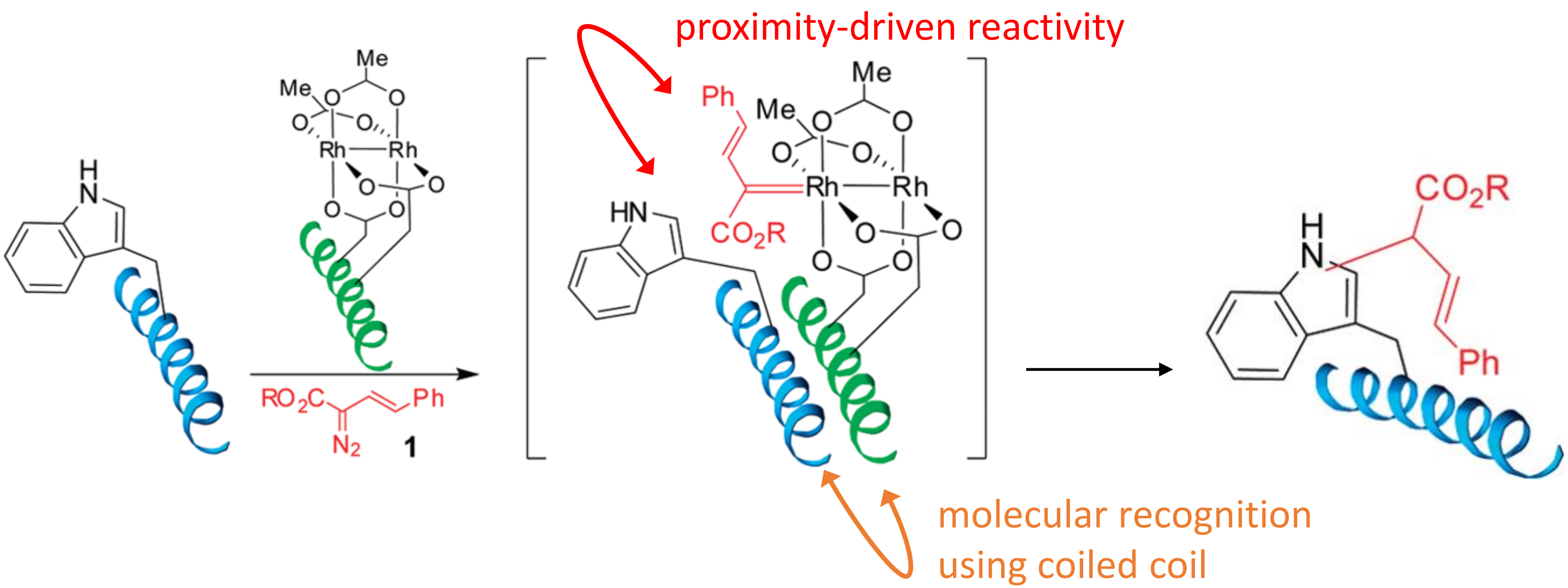
- Zachary T. Ball

Associate Professor
Department of Chemistry, Rice University

Research themes

1. **Rhodium metallopeptides (synthesis and structure)**
2. **Rhodium metallopeptides that interact with proteins**
3. **Small-molecule catalysis**
4. **Copper catalysis and other research**

3. Concept of the rhodium metallopeptide



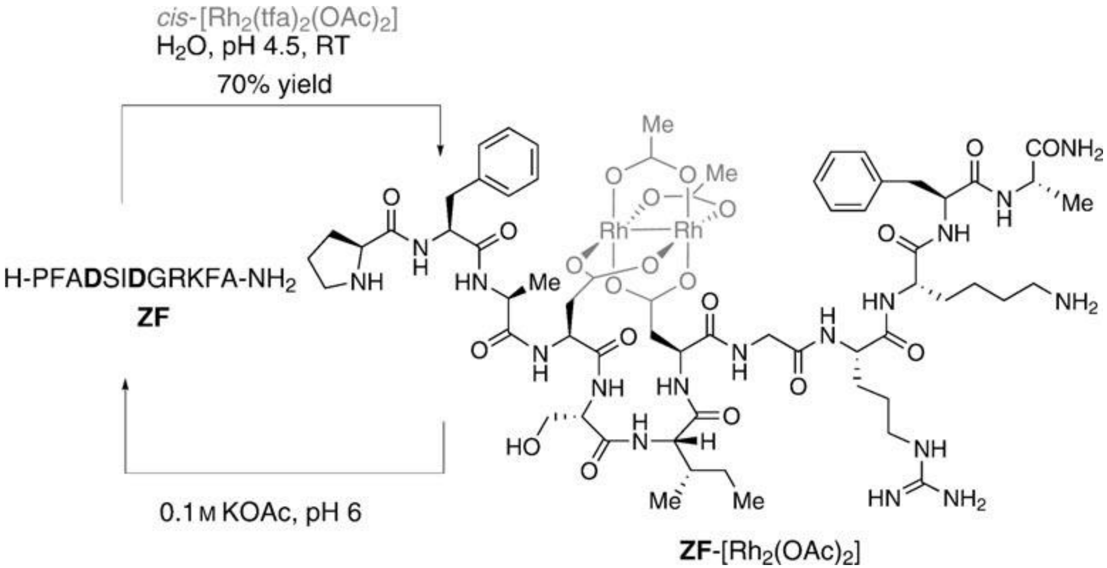
dirhodium metallopeptide bound to side chain carboxylate

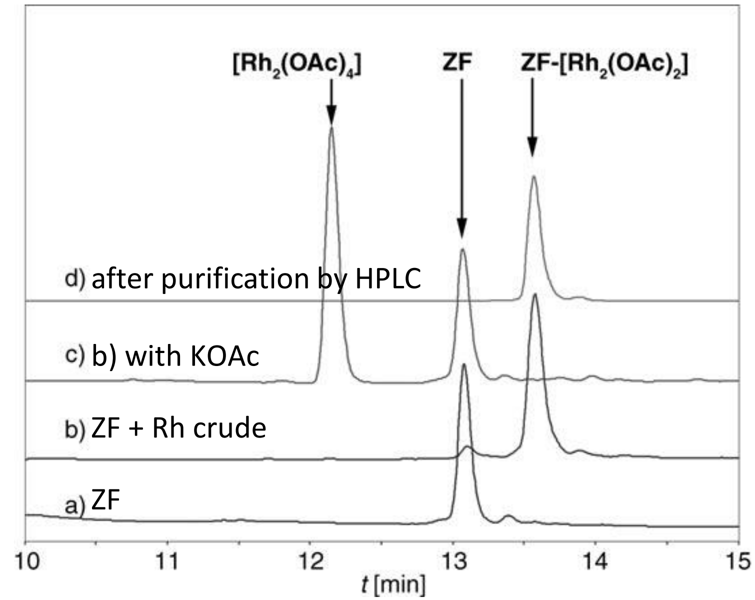
- stable with respect to ligand exchange in water at pH ≤ 7
- catalyze X-H and C-H insertion in water

Ball, Z. T. et al., *J. Am. Chem. Soc.* **2010**, *132*, 6660

3. Synthesis and structure of the rhodium metallopeptide

- Reversible synthesis of a peptide-dirhodium adduct





In the presence of reactive amino acids, yield is low….

with Ac-His-NHMe (2 eq.) … > 50 % yield

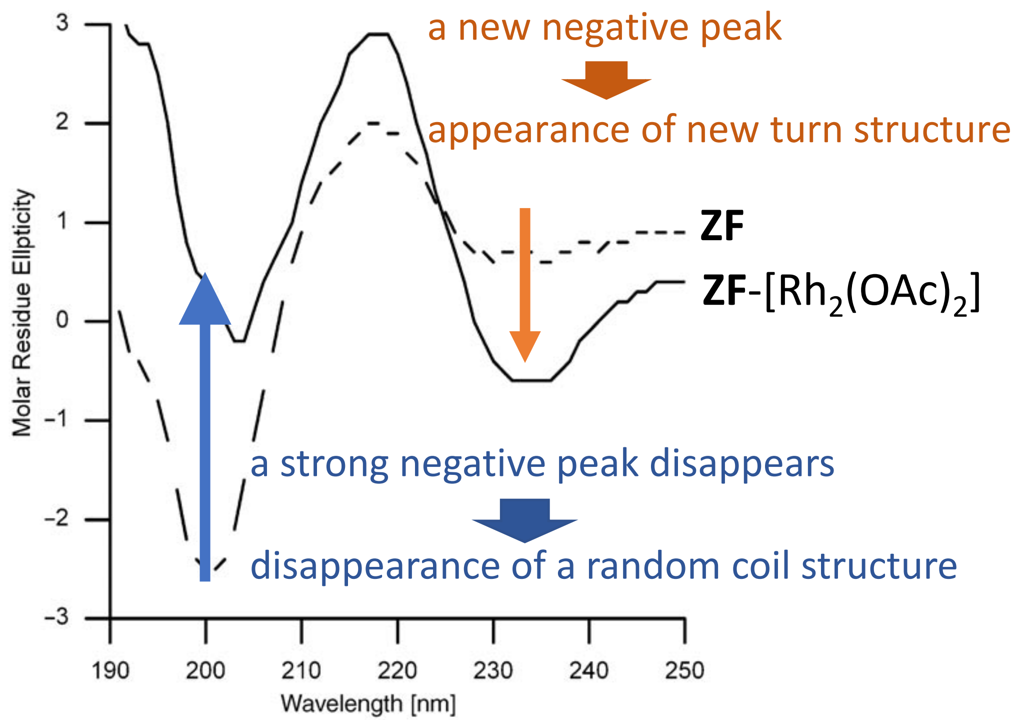
with Ac-Met-OMe (2 eq.) … ca. 40 % yield

Ball, Z. T. et al., *Chem. Eur. J.* **2009**, *15*, 8961

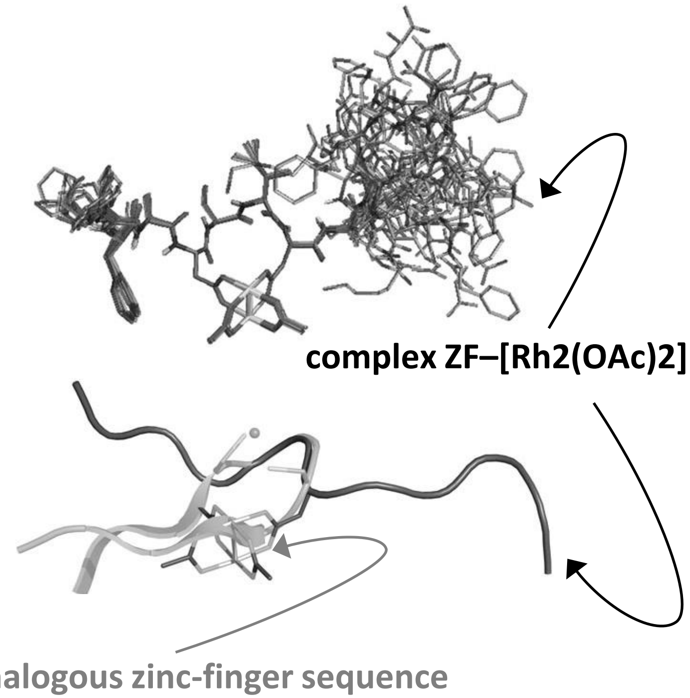
3. Synthesis and structure of the rhodium metallopeptide

- Structure of the rhodium metallopeptide

CD spectra



molecular dynamics calculations



Dirhodium binding can be used to control the conformation of the bound peptide.

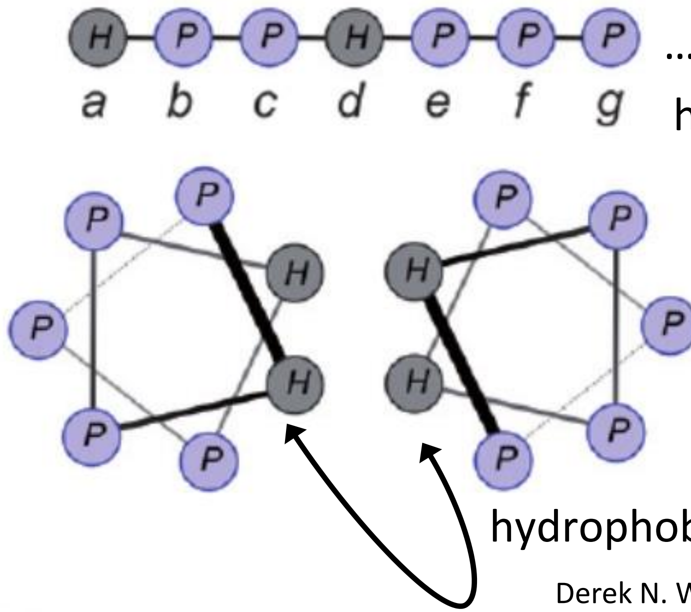
Ball, Z. T. et al., *Chem. Eur. J.* **2009**, *15*, 8961

3. Coiled coil interaction

- Coiled coil … a simple and robust peptide assembly

A

B *<u>Helical wheel diagram</u>*



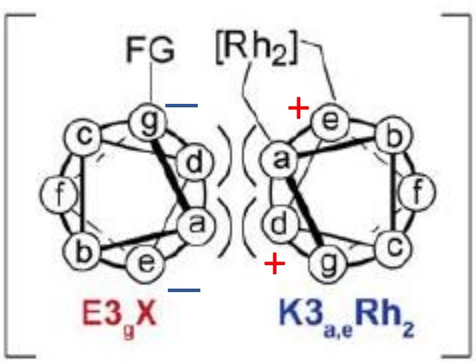
… heptad repeat

H … hydrophobic
P … polar

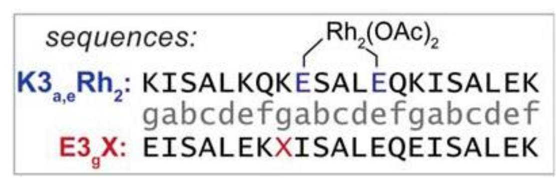
hydrophobic interfaces

Derek N. W., *et al., Faraday Discuss.*, **2009**, *143*, 1

e.g. E3/K3 pair



at the position of ***e*** and ***g***,
K3 … lysine-rich
E3 … glutamate-rich

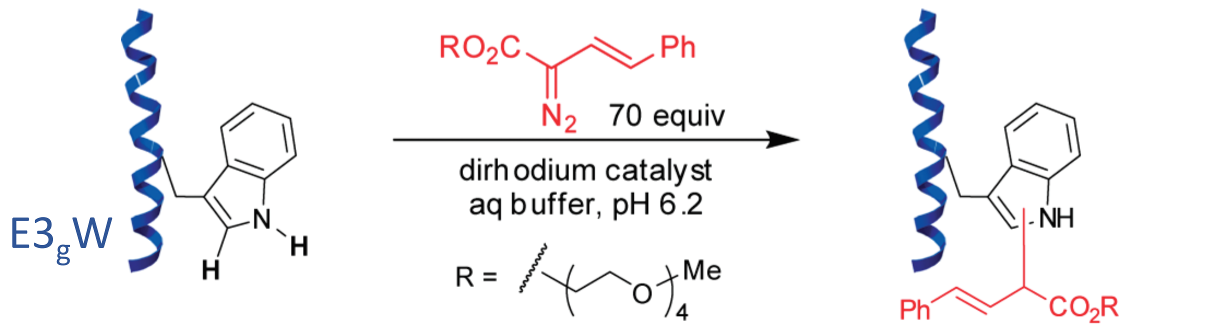


electrostatic interaction

Ball, Z. T. et al., *J. Am. Chem. Soc.* **2010**, *132*, 6660

3. Site-specific protein modification

- comparison of rhodium metallopeptide to $Rh_2(OAc)_4$



entry	catalyst (mol %)	conv. of **E3gW** 0.3 h	2 h	20 h	$t_{1/2}$
1	$Rh_2(OAc)_4$ (100)	10	8	17	>20
2	**$K3_{a,e}Rh_2$** (10)	64	>95	>95	0.25
3	**$K3_{a,e}Rh_2$** (2)	30	74	>95	1
4[b]	**$K3_{a,e}Rh_2$** (10)	7	22	35	–

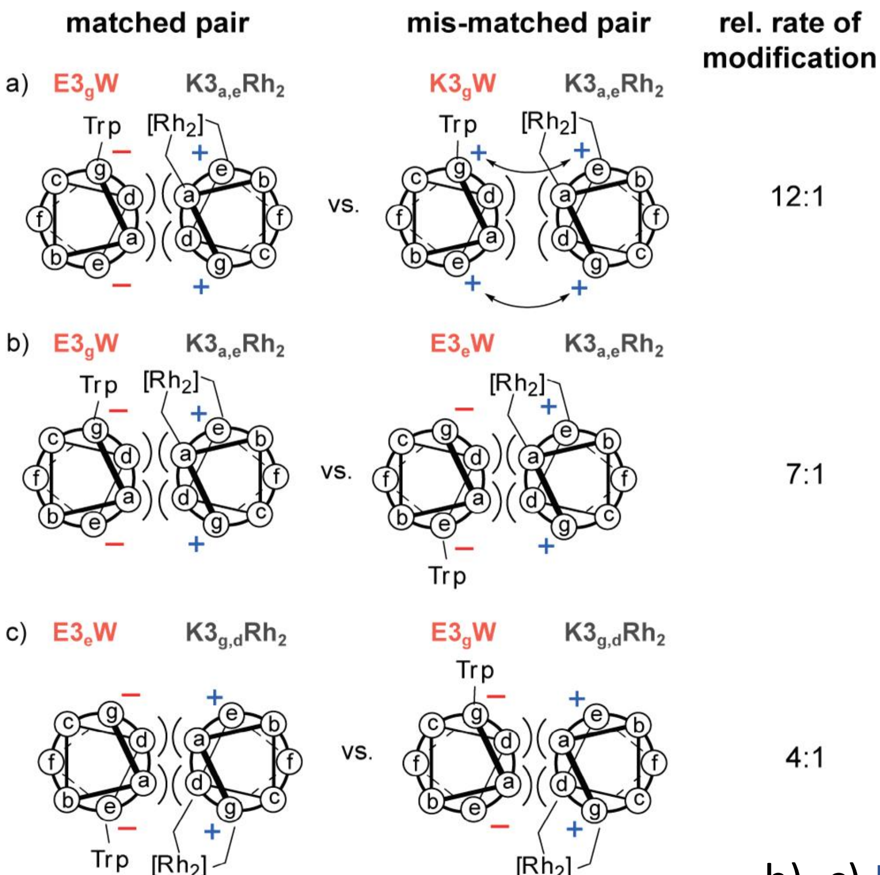
[a] All of the reactions used 50 μM substrate at room temperature. Conversion data are based on MALDI–TOF MS analysis of the reaction mixture. [b] Using 1 equiv of diazo reagent.

- Only 2 mol % of $K3_{a,e}Rh_2$ shows ***>10³ rate enhancement*** compared with 100 mol % of $Rh_2(OAc)_4$ due to substrate binding.

Ball, Z. T. et al., *J. Am. Chem. Soc.* **2010**, *132*, 6660

3. Site-specific protein modification

- Effect of the coiled coil interaction on the rate of modification



noncomplementary charges in the flanking ***e*** and ***g*** positions

Trp residue at the opposite flank of the coiled coil interface (position ***e***)

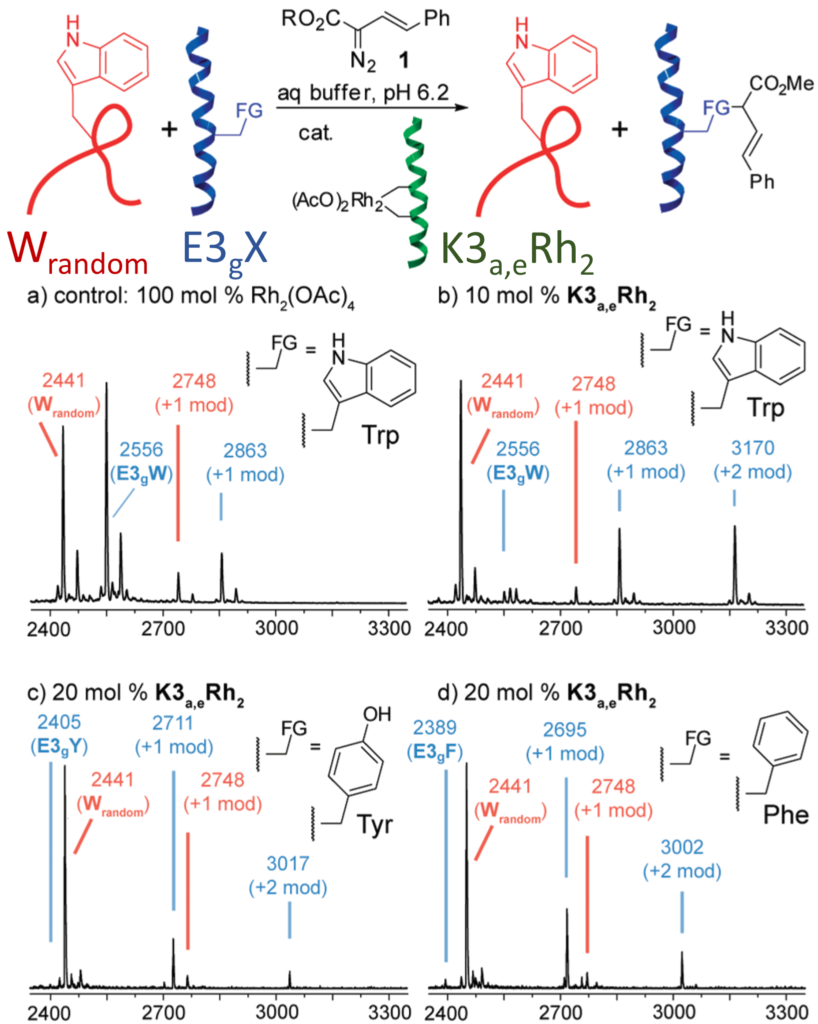
noncomplementary charges in the flanking ***e*** and ***g*** positions

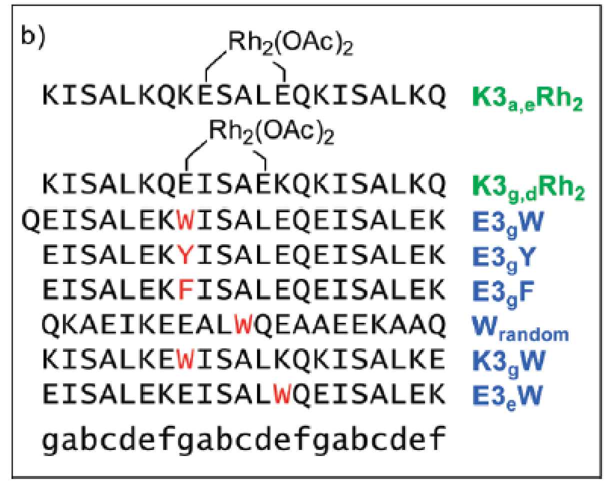
b), c) → parallel > antiparallel

Ball, Z. T. et al., *J. Am. Chem. Soc.* **2010**, *132*, 6660

3. Site-specific protein modification

- Expansion of the scope of dirhodium-catalyzed side-chain modification





Proximity-driven reactivity promotes the site-specificity even in **Tyr** or **Phe** residue.

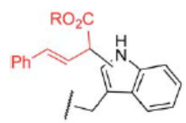
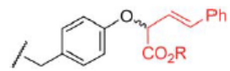
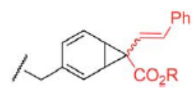
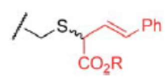
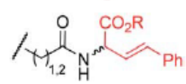
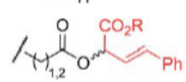
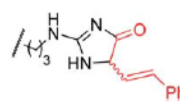
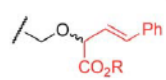
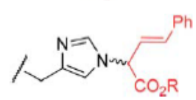
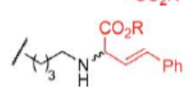
Unreactive residues (aliphatic residues) can be modified with this rhodium metallopeptide?

Ball, Z. T. et al., *J. Am. Chem. Soc.* **2010**, *132*, 6660

3. Site-specific protein modification

- Expansion of the scope of dirhodium-catalyzed side-chain modification

Table 1 Covalent modification of $E3_gX$ peptides.[a]

Entry	$E3_gX$ X =	Mol % $K3_{a,e}Rh_2$	Proposed product bond connectivity[b]	% Conv at 24 h[c]	k_{rel}[d]
1	Trp	1		> 95 (1 : 1.3)[e,f]	3000
2	Tyr	10		> 95 (7 : 1)[g]	100
3	Phe	10		> 95 (8 : 1)[g]	87
4	Cys	10		78 (5 : 1)	560
5	Gln	10		> 95[f]	280
6	Asn	10		70	110
7	Glu	20		76 (9 : 1)[g]	130
8	Asp	20		53 (5 : 1)[g]	100
9	Arg	50	 [h]	73 (3 : 1)[e,g]	97
10	Ser	50		28[g]	32
11	His	50		17[g]	18
12	Lys	50		8[g]	11
13	Thr	50	—	NR	—
14	Val	50	—	NR	—
15	Ala	50	—	NR	—
16	Met	50	—	NR	—

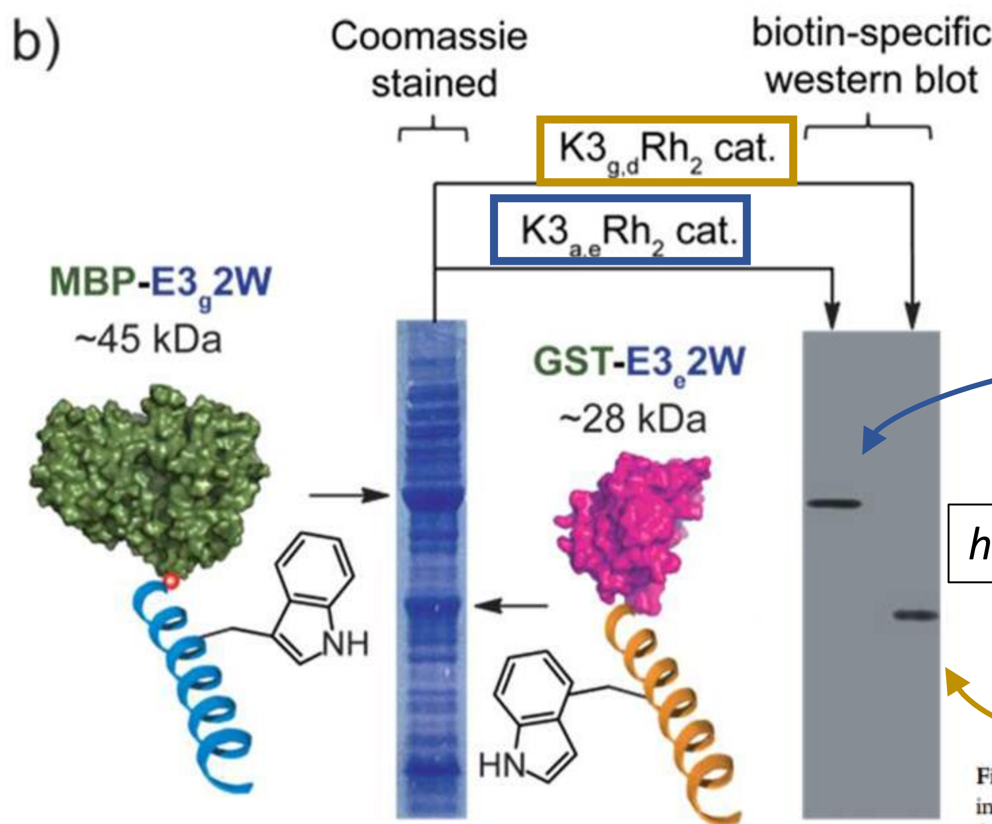
>50% conversion for **X = Trp, Tyr, Phe, Asn, Gln, Asp, Glu, Arg, Cys** (in order of reactivity)

comprising >40% of natural protein space!

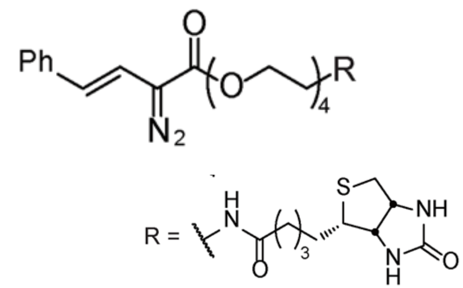
Ball, Z. T. *et al.*, *Chem. Sci.*, **2011**, *2*, 690

3. Application of rhodium metallopeptide (1)

Orthogonal protein modification in lysate



biotin-diazo reagent



high specificity and orthogonality !

1 : 1 mixture of lysates from expression of MBP-E3$_g$2W and GST-E3$_e$2W

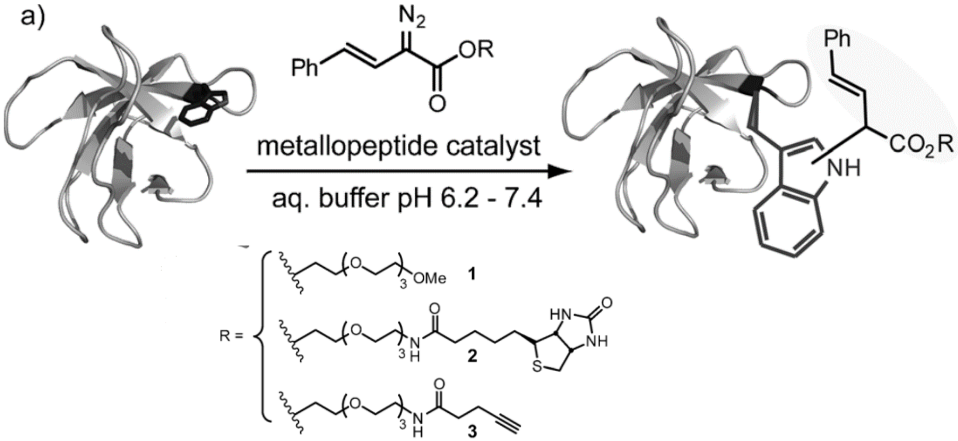
Figure 4. Orthogonal chemical biotinylation of two different proteins in cell lysate with designed metallopeptide catalysts. Recombinant fusions MBP-E3$_g$2W and GST-E3$_e$2W were expressed in *E. coli*. Reaction conditions: proteins, ~1.0 μM for each; metallopeptide, 2.0 equiv, 2.0 μM; biotin diazo **1b** (100 μM) in aq PBS buffer (0.10 M, pH = 7.2); total reaction volume, 20 μL; 4°C; 16 h. MBP = Maltose-binding protein, GST = glutathione *S*-transferase.

Ball, Z. T. *et al.*, J. Am. Chem. Soc. **2012**, *134*, 10138

3. Application of rhodium metallopeptide (2)

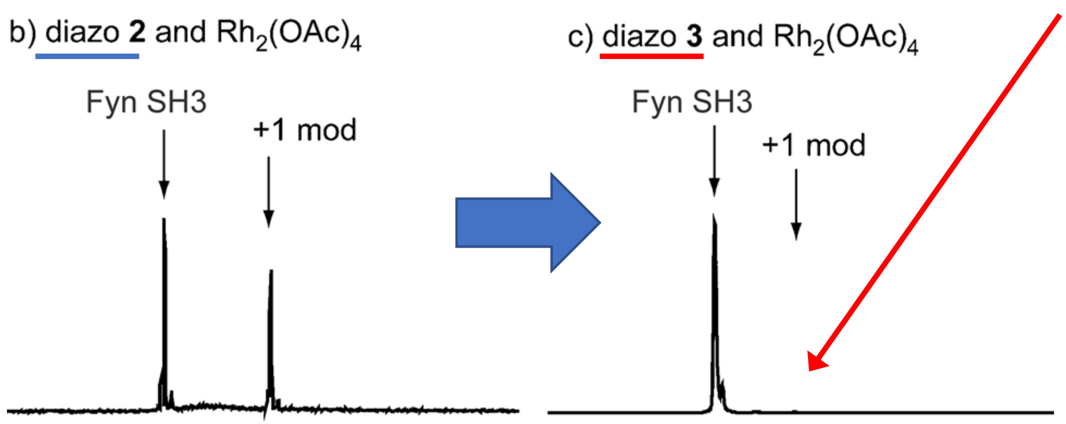
SH3 domain site-selective modification

SH3 domain protein (Fyn)



SH3 binding peptide VSL12(VSLARRPLPPLPP) is used as ligand of Rh_2

- Improvement of efficiency and realization of useful functionalization①



Negative control doesn't show +1 modification peak.

diazo **3** can be bound to fluorophore by Cu-mediated azide-alkyne cycloaddition.

Non-selective reactivity diazo **2** is due to interaction of the thiolester group with a metallocarbene intermediate.

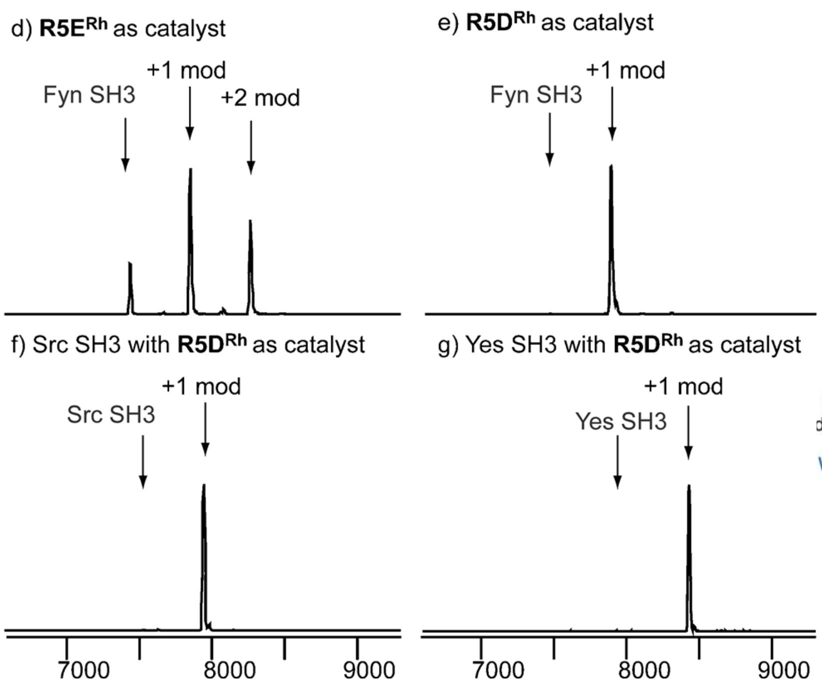
Ball, Z. T. *et al.*, *Angew. Chem. Int. Ed*. **2015**, *54*, 4587

3. Application of rhodium metallopeptide (2)

SH3 domain site-selective modification

- Improvement of efficiency and realization of useful functionalization②

R5E^{Rh} → R5D^{Rh} … decrease conformational freedom (aspartate < gultamate)



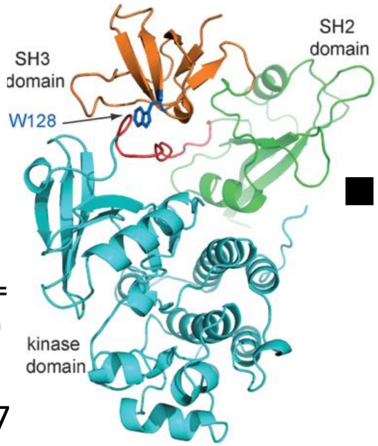
Ball, Z. T. *et al.*, *Angew. Chem. Int. Ed*. **2015**, *54*, 4587

mixture of +1 mod and +2 mod

↓

only +1 mod

c) Yes kinase

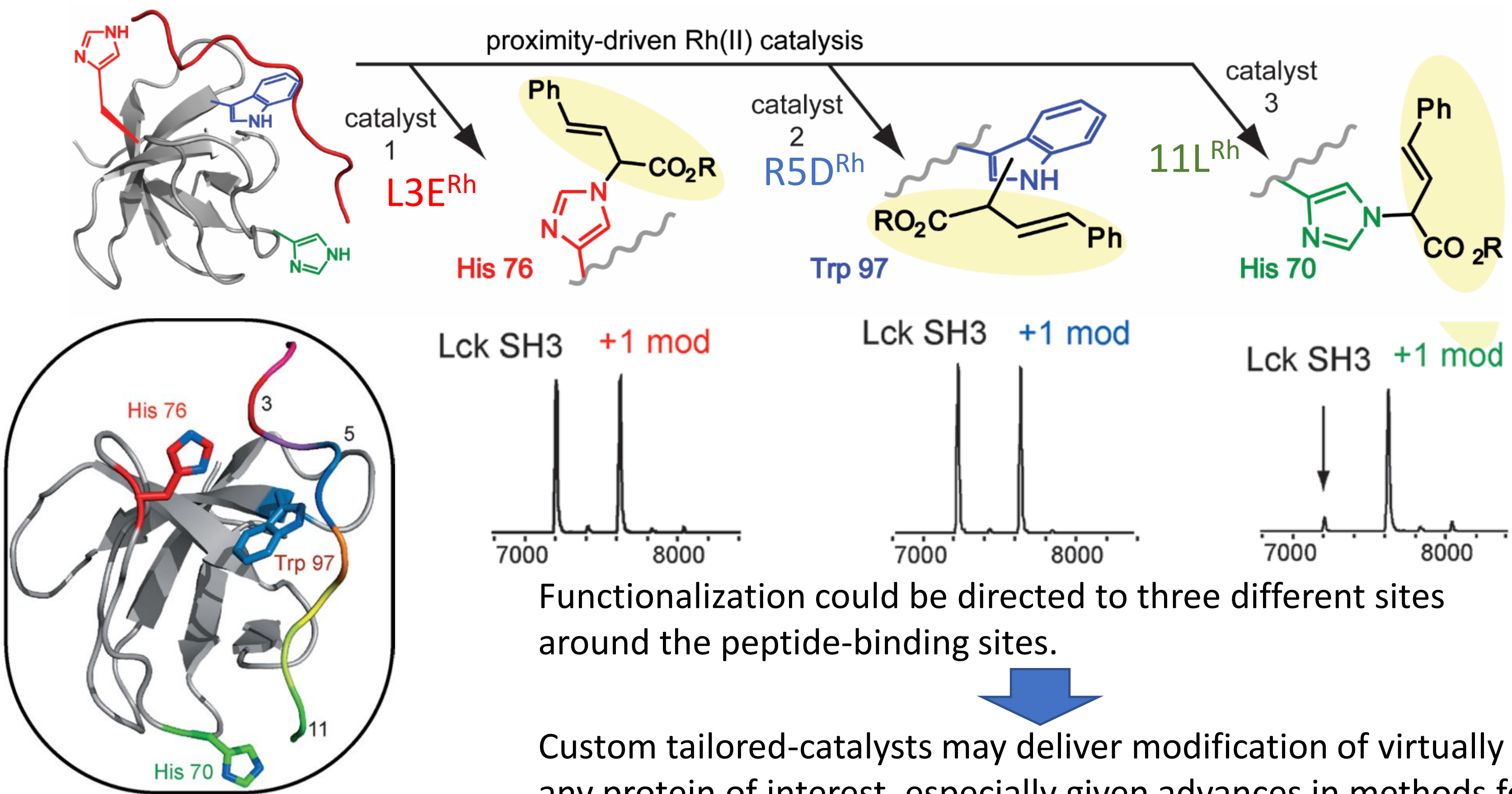


- Selectivity is retained across of the other members of the Src SH3 family.
- Selectivity is retained even in Yes SH3 (containing buried SH3 and Trp).

3. Application of rhodium metallopeptide (2)

SH3 domain site-selective modification

- A wide range of Src-family SH3 domains modification



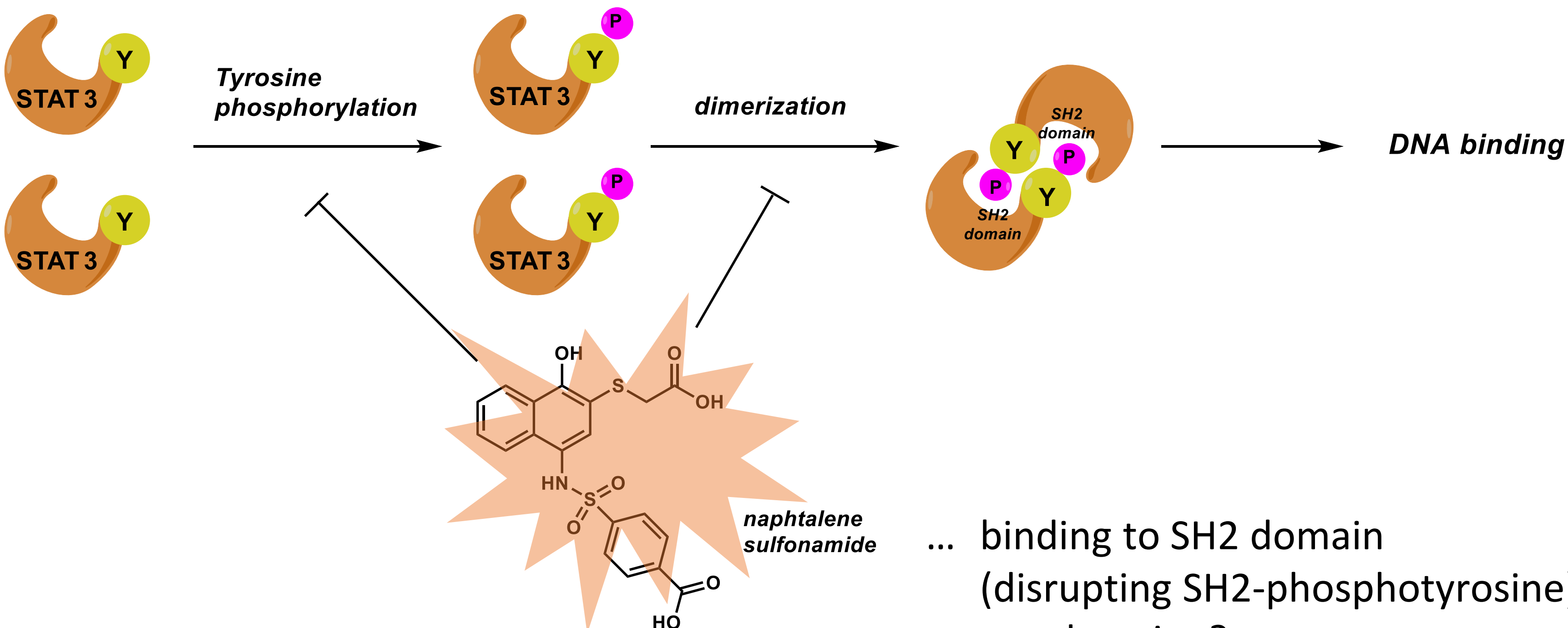
Functionalization could be directed to three different sites around the peptide-binding sites.

Custom tailored-catalysts may deliver modification of virtually any protein of interest, especially given advances in methods for ligand discovery.

Ball, Z. T. *et al*., *Angew. Chem. Int. Ed*. **2015**, *54*, 4587

3. Application of rhodium metallopeptide (3)

Identification of naphthalene-sulfonamide-binding site of STAT3

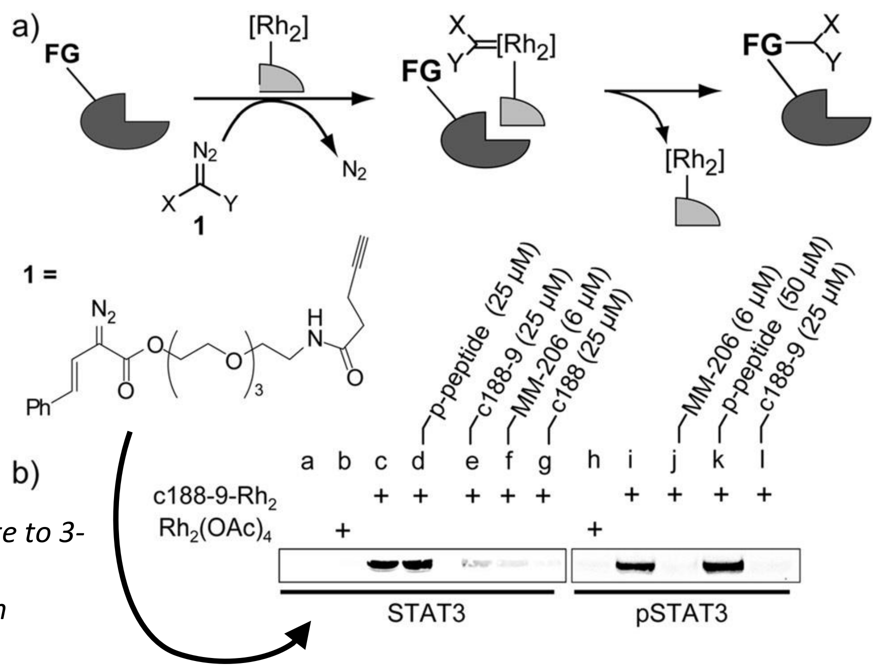


... binding to SH2 domain
(disrupting SH2-phosphotyrosine)
or other site ?

Ball, Z. T. *et al., Angew. Chem. Int. Ed.* **2015**, *54*, 13085

3. Application of rhodium metallopeptide (3)

Identification of naphthalene-sulfonamide-binding site of STAT3



Conjugate to 3-azide-7-coumarin

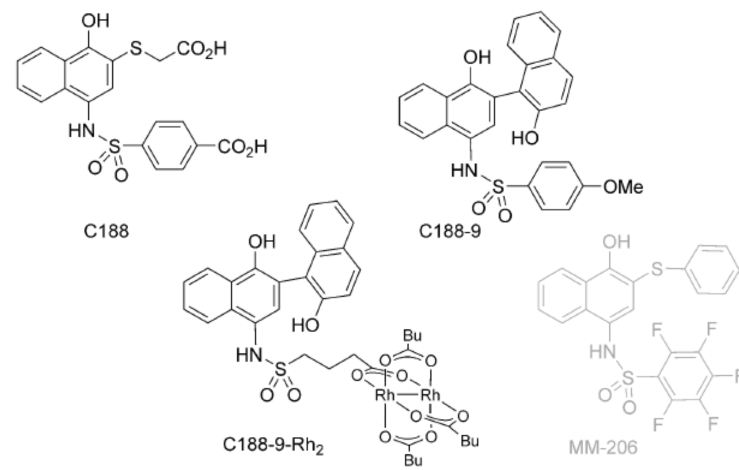


Figure 1. Structures of selected STAT3 inhibitors.

- lane d, k … p-peptide doesn't inhibit C188-9-Rh_2 binding.
  - ➡ naphthalene sulfonamide doesn't bind to SH2 domain.
- lane e, g, l … Other naphthalene sulfonamides inhibit C188-9-Rh_2 binding.
  - ➡ Other naphthalene sulfonamides are bound to the same site.

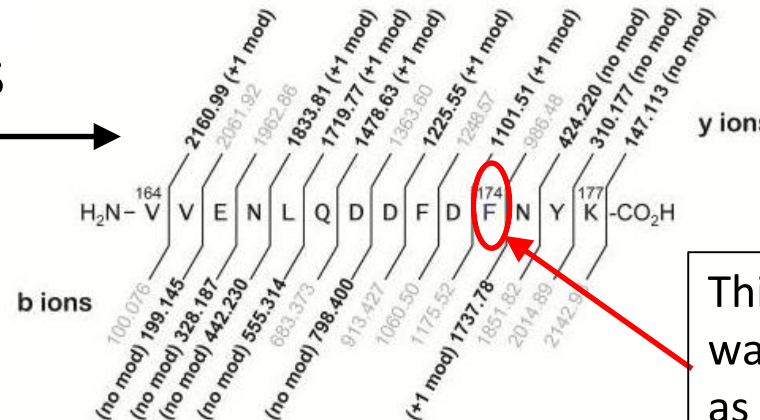
Ball, Z. T. *et al., Angew. Chem. Int. Ed.* **2015**, *54*, 13085

3. Application of rhodium metallopeptide (3)

Identification of naphthalene-sulfonamide-binding site of STAT3

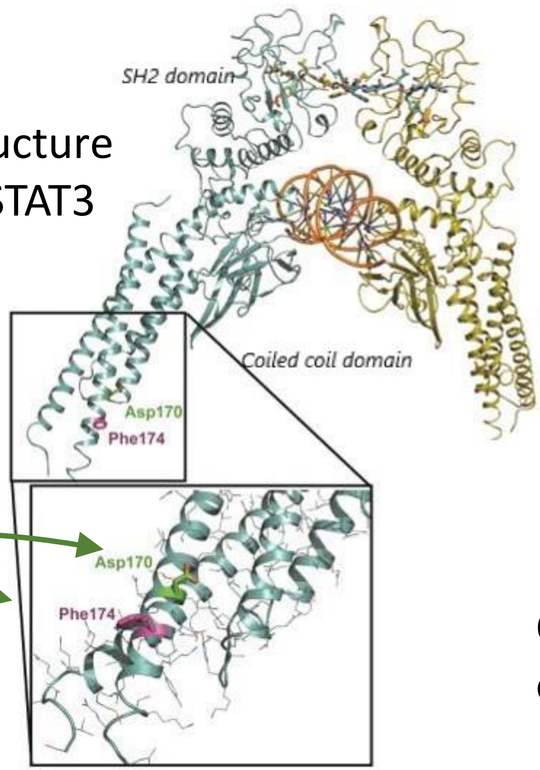
Tryptic digestion of the modified STAT3

LC-MS/MS



This *Phe174* was identified as the site of modification!

Structure of STAT3



One helix turn away

- ***Phe174*** lies in a coiled coil domain.

This is consistent with the fact that the key residue in the CCD, a point mutant of which exerted the largest effect on STAT3 activity specially SH2 affinity, was ***Asp170***.

Coiled coil region was identified as the targeting site other than SH2.

Ball, Z. T. *et al., Angew. Chem. Int. Ed*. **2015**, *54*, 13085

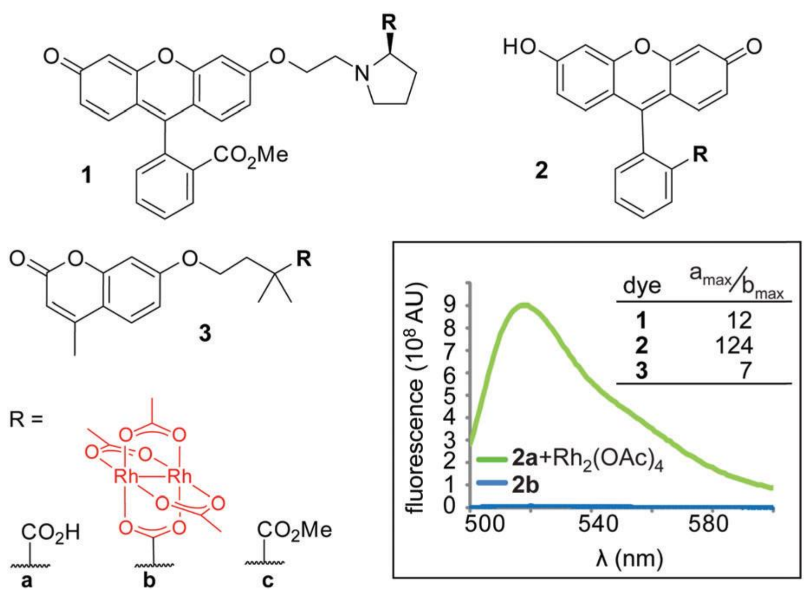
3. Application of rhodium metallopeptide (4)

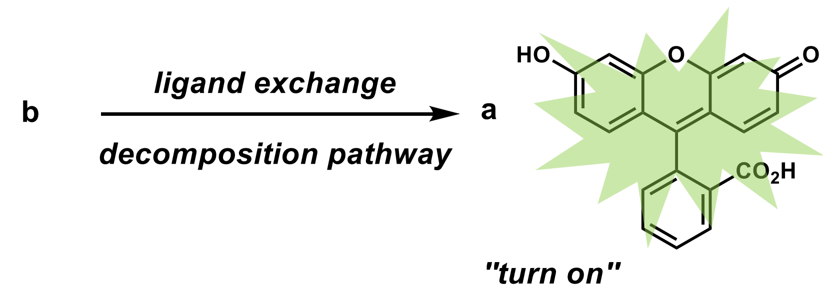
Assessing intracellular fate of rhodium complex

In utilizing of rhodium complexes in living cells,

- toxicity … inherently low (able to design with the proper ligand choice)
- stability … **limited stability under biologically relevant conditions** (ligand exchange, irreversible reduction, …)

- Improvement of the stability of rhodium complex





Ball, Z. T. *et al., Chem. Commun.*, **2016**, *52*, 11685

3. Application of rhodium metallopeptide (4)

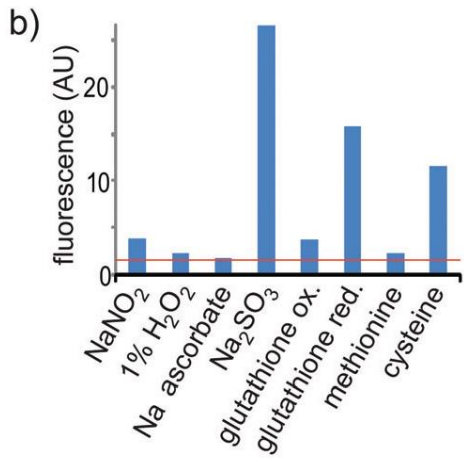
Assessing intracellular fate of rhodium complex

- Improvement of the stability of rhodium complex

a)

entry	additive	concn (mM)	$t_{1/2}$ (h)
1	cysteine	0.2	0.2
2	TCEP	40	1
3	trypsin	2	5
4	BSA	0.15	8
5	medium		9
6	Gly-OEt	10	13
7	N-Ac-Gly	10	19
8	Glycine	10	31
9	MOL-M cells		66

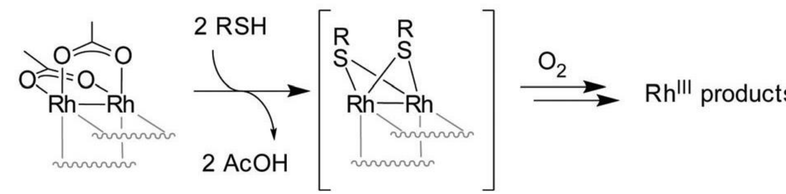
half-life of **1b** with additive

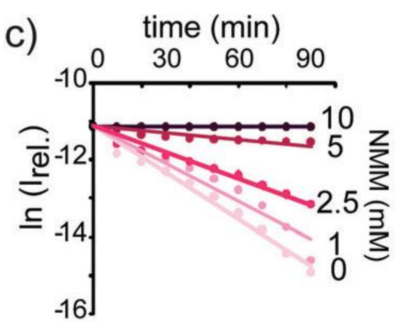


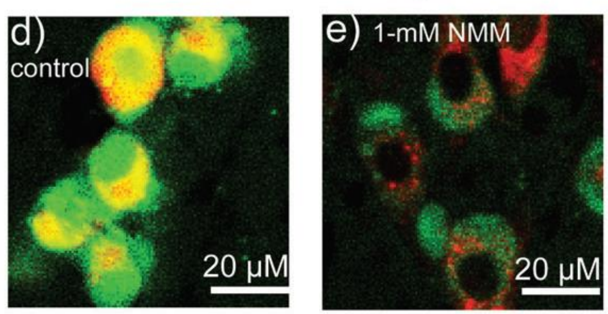
3b was treated with a redox mediators.

- faster decomposition in containing surface-exposed thiols

Proposed mechanism







3b (15 uM) in 1x PBS with GSH (10 mM)

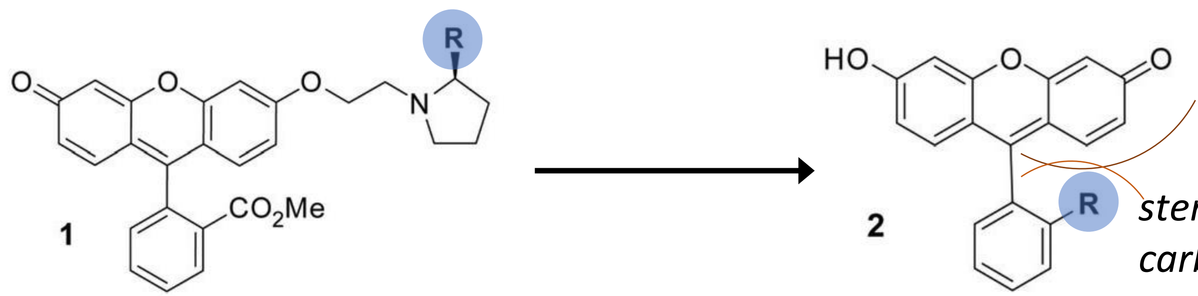
- N-methylmaleimide (NMM), a common thiol blocking reagent, retards decomposition of **3b** and slows the rate of fluorescence turn-on.

Ball, Z. T. *et al.*, *Chem. Commun.*, **2016**, *52*, 11685

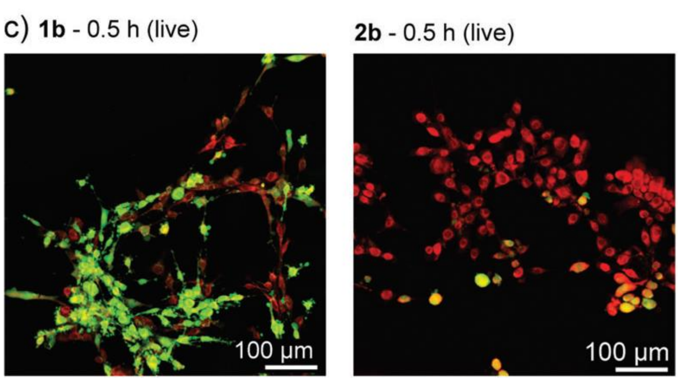
3. Application of rhodium metallopeptide (4)

<u>Assessing intracellular fate of rhodium complex</u>

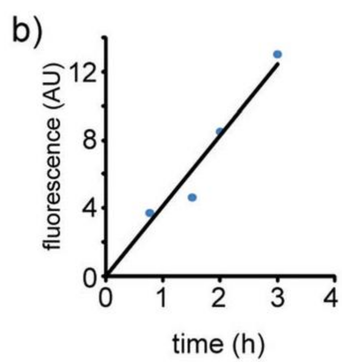
- Improvement of the stability of rhodium complex



sterically demanding carboxylate



Fluorescent image of cells (NIH 3T3) Fluorescent probe (488 nm, green) is shown overlaying cytosol stain (561 nm, red).



Fluorescence of **2b** in MOL13-M cells (1×10^5) treated with 2b (30 mM, 0.5 h) aliquots taken at different times were analyzed by flow cytometry.

1b ... maximally fluorescent within 30 min
2b ... mostly dark after 30 min, but continued to grow in brightness over time

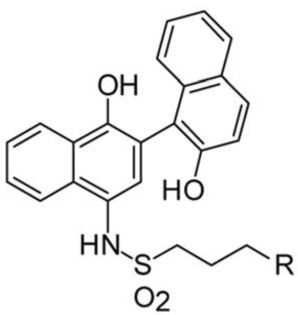
A long-lived rhodium complex inside cells was successfully prepared.

Ball, Z. T. *et al., Chem. Commun.*, **2016**, *52*, 11685

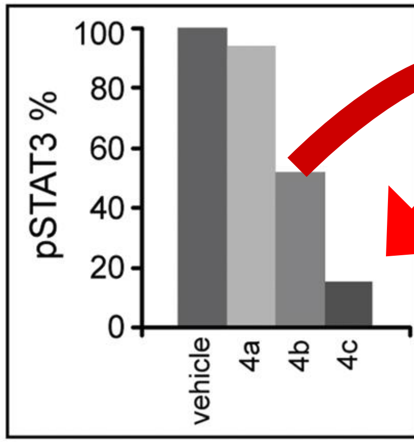
3. Application of rhodium metallopeptide (4)

Assessing intracellular fate of rhodium complex

- Improvement of the stability of rhodium complex

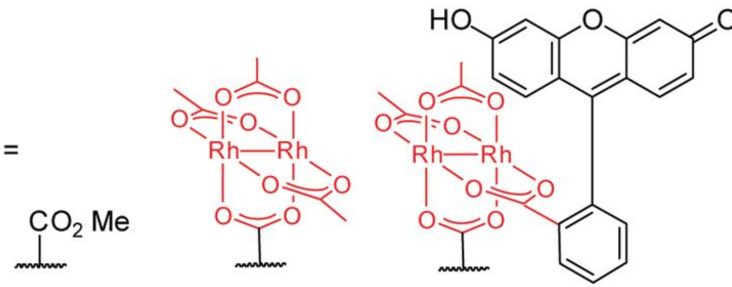


4



Improvement of ***cellular potency*** *to inhibit STAT3 activation (phosphorylation)!*

R =



SPR	a	b	c
IC_{50} (μM)	20	1.0	1.0

in vitro potency does not change

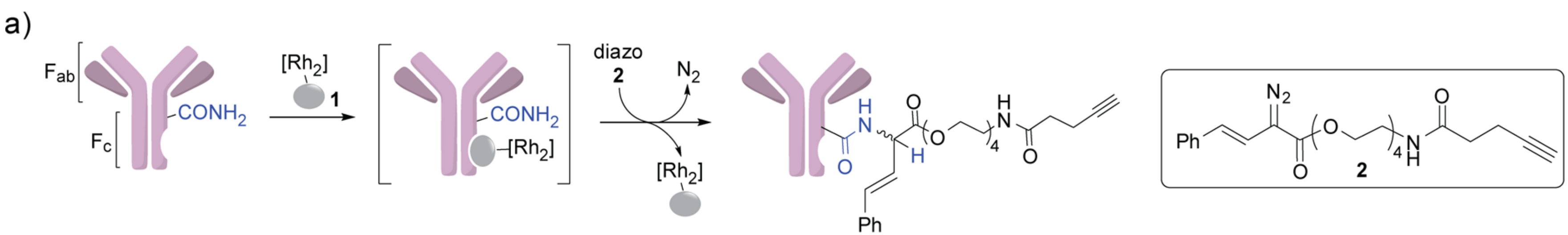
- Intracellurar stability studies can be used to optimize rhodium complex structure.

Ball, Z. T. *et al., Chem. Commun.*, **2016**, *52*, 11685

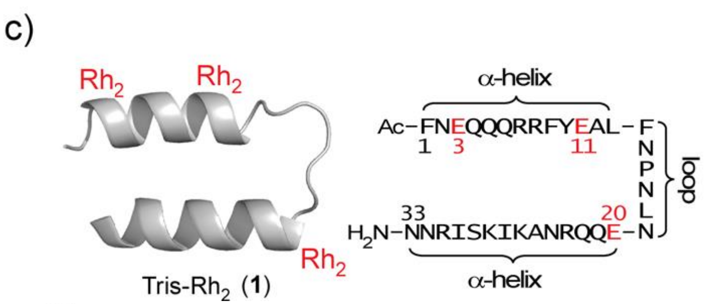
3. Application of rhodium metallopeptide (5)

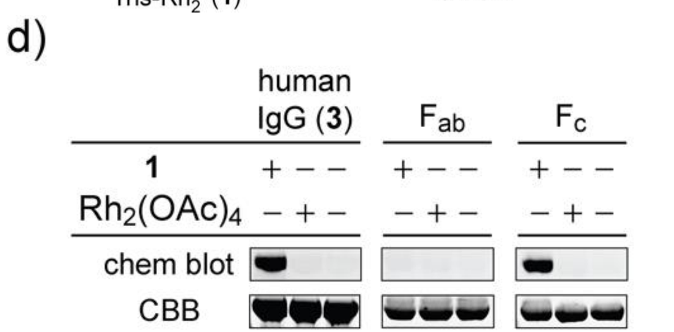
Antibody modification using hexa-rhodium metallopeptide

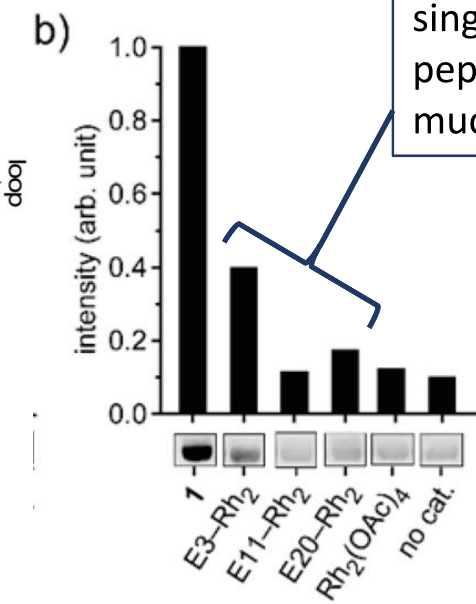
Assumed scheme



hexa-rhodium metallopeptide (triply metalated peptide)







singly metalated peptides displays much lower reactivity

- *The reactivity of triply metalated peptide may stem from* ***synergetic action of the dirhodium cores****.*
  - *one Rh acts as* ***a catalytic site***
  - *other Rhs act as* ***Lewis acids*** *to anchor the catalyst at the binding site on the Fc region*

Ball, Z. T. *et al.*, J. Am. Chem. Soc. **2017**, *139*, 12617

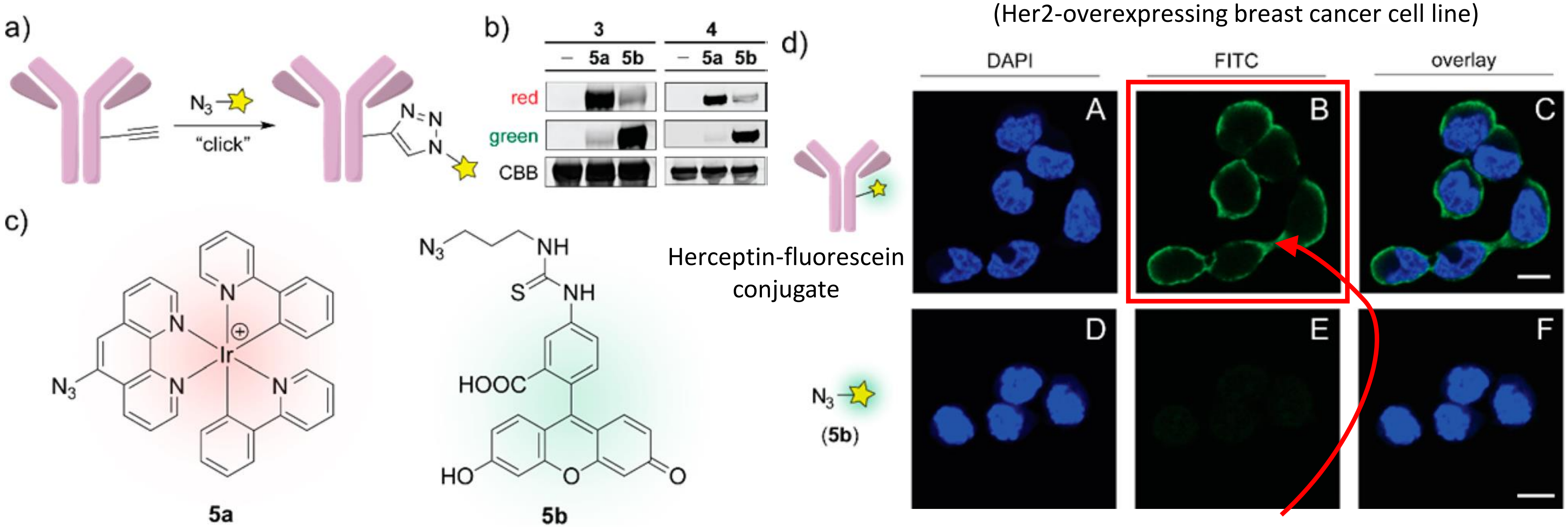
3. Application of rhodium metallopeptide (5)

<u>Antibody modification using hexa-rhodium metallopeptide</u>

- Antibody's functionalization using azide-alkyne cycloaddion①

<u>Fluorescently labeling</u>

SK-BR3 cells
(Her2-overexpressing breast cancer cell line)



- localization of the fluorescein fluorescence on the surface of the cells (**B**),
- absent in control experiment (**E**)

→ Modified antibodies retain antigen-binding properties.
So, it can be used as a ***fluorescently labelled antibody***.

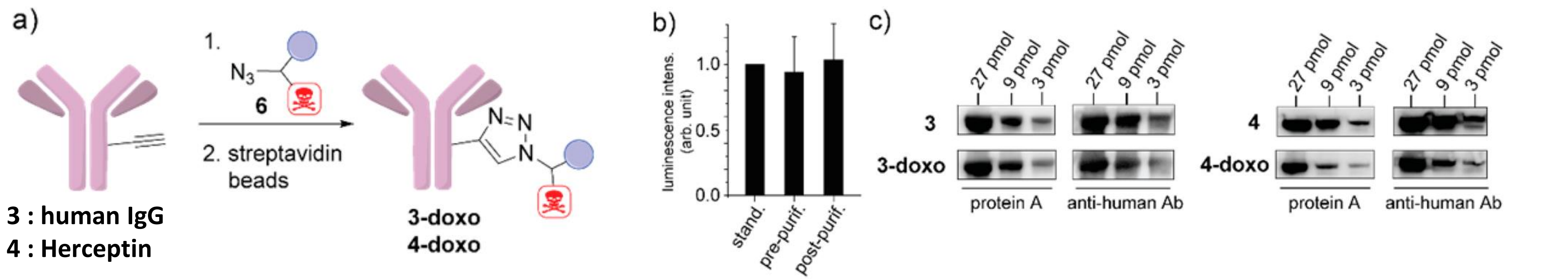
Ball, Z. T. *et al.*, J. Am. Chem. Soc. **2017**, *139*, 12617

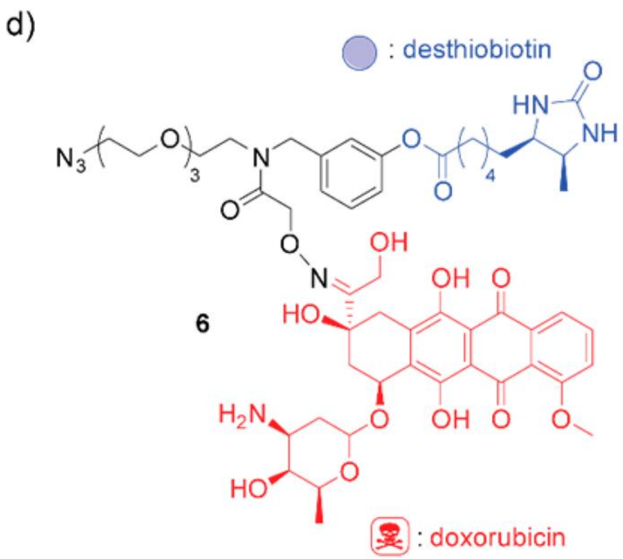
3. Application of rhodium metallopeptide (5)

Antibody modification using hexa-rhodium metallopeptide

- Antibody's functionalization using azide-alkyne cycloaddion②,③

Desthiobiotinylation for facile purification and Preparation of antibody-drug conjugates (ADCs)





- Chemiluminescence determined a ***1:1 herceptin/ doxorubicin ratio (DAR, drug antibody ratio)***.

- ADCs bind protein A *with efficacy comparable to the unmodified antibodies*.
- Secondary antibodies show binding ability to $F(ab')_2$ of ADCs as well as that of unmodified ones.

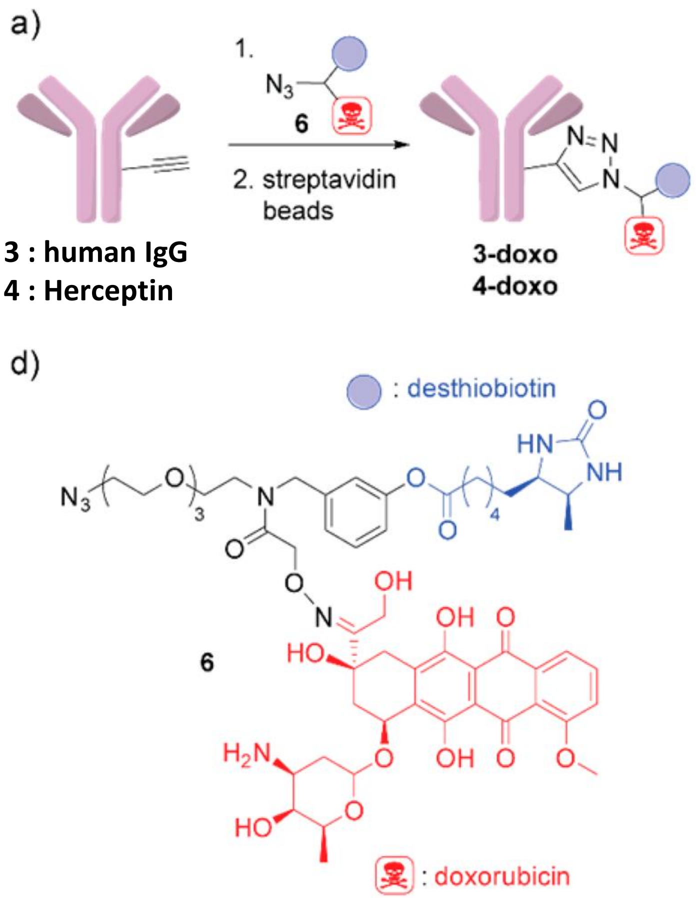
Ball, Z. T. *et al.*, J. Am. Chem. Soc. **2017**, *139*, 12617

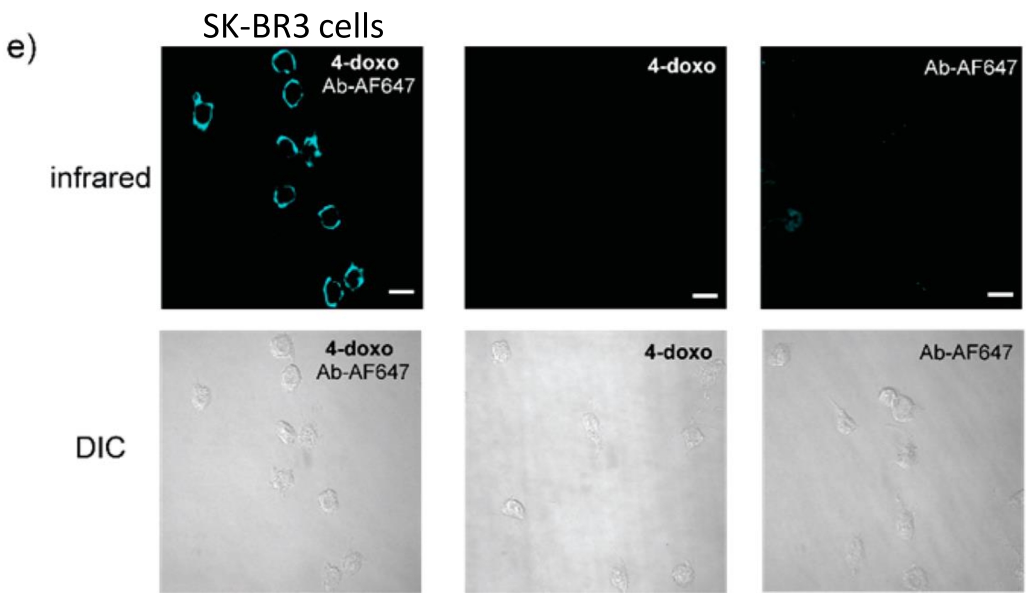
3. Application of rhodium metallopeptide (5)

Antibody modification using hexa-rhodium metallopeptide

- Antibody's functionalization using azide-alkyne cycloaddion②,③

Desthiobiotinylation for facile purification and Preparation of antibody-drug conjugates (ADCs)





- 4-doxo retains affinity for both Her2 receptor and the antihuman antibodies.

→ the function of antibody can be maintained in presence of modification when modified site was chosen with tactful design.

Ball, Z. T. *et al.*, J. Am. Chem. Soc. **2017**, *139*, 12617

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    2-2. Radical reaction

    2-3. Pericyclic reaction

    2-4. Transition metal reaction

3. Rhodium metallopeptide

4. Conclusion

4. Conclusion

- Rhodium carbenoid is the approach of chemical modification of Trp through C-H activation.

- Directing with the ligand peptide (rhodium metallopeptide) enables catalytic and residue-selective reaction.

- Directing with the ligand peptide amino acids comprising >40% of natural protein other than Trp. (over 50% conversion)

- Many applications of rhodium metallopeptide are possible even in cellular reaction or preparation of ADCs.