Methods for Site-selective Protein Modification

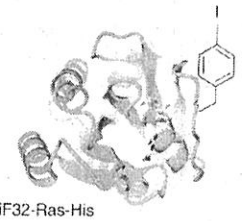
2007. 12. 12
Rie MOTOKI (D2)

in post-genome era...

DNA → RNA → protein (primary structure) → function ? manipulation ?

well-known

folding
higher-order architecture
posttranslational modification



iF32-Ras-His

Site-selective incorporation of unnatural amino acid is possible.
(see Motoki's Lit. seminar (M1))

Is site-selective protein modification possible ?

Following conditions are necessary.

- mild condition (temp=rt, pH=neutral)
- H_2O, salt compatible
- functional group specific (OH, NH_2, SH etc.)

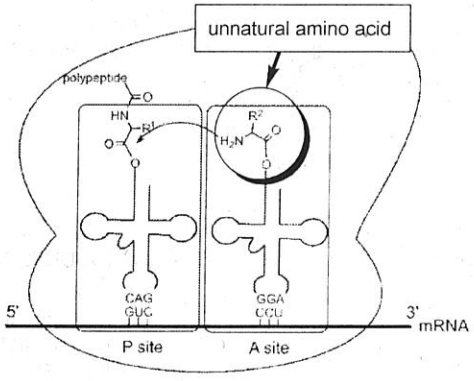
review: Antos, J M.; Francis, M. J. *Curr. Opin. Chem. Biol.* **2006**, *10*, 253.

contents

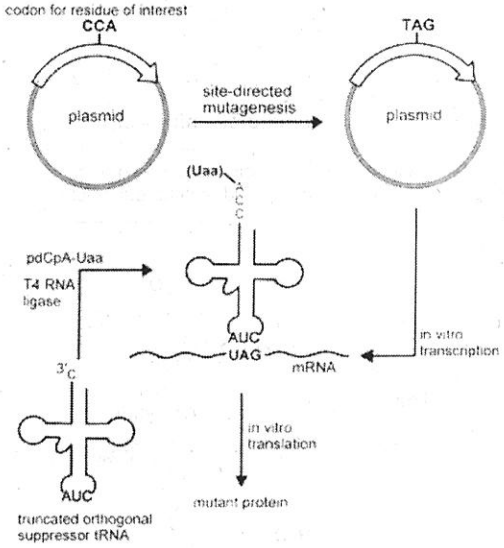
1. Site-specific modification of artifical protein
2. Site-selective modification of "natural" protein

1. Site-specific Modification of Artificial Protein

1.1 Unnatural amino acid containing protein



"Expanding the Genetic Code"
Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 34.

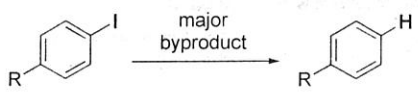


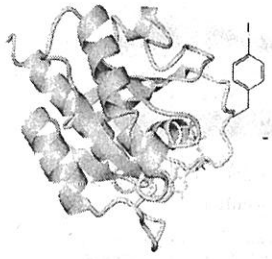
transition metal catalyzed C-C bond-forming reaction

Regioselective Carbon–Carbon Bond Formation in Proteins with Palladium Catalysis; New Protein Chemistry by Organometallic Chemistry

Koichiro Kodama,[a, c] Seketsu Fukuzawa,*[b] Hiroshi Nakayama,[d] Takanori Kigawa,[c] Kensaku Sakamoto,[a, c] Takashi Yabuki,[c] Natsuko Matsuda,[c] Mikako Shirouzu,[c] Koji Takio,[c] Kazuo Tachibana,*[b] and Shigeyuki Yokoyama*[a, c, e]

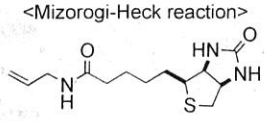
ChemBioChem **2006**, *7*, 134



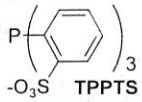


(WT: Y32) **iF-Ras protein**

<Mizorogi-Heck reaction>

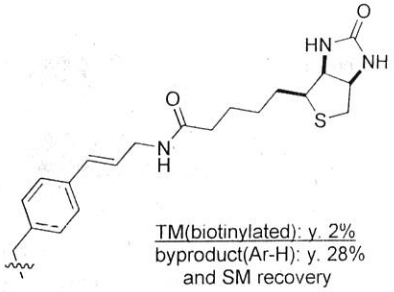


Pd, 12% DMSO in H_2O buffer (pH=8.3)



5 °C, 50 hr

TPPTS
(water soluble ligand)

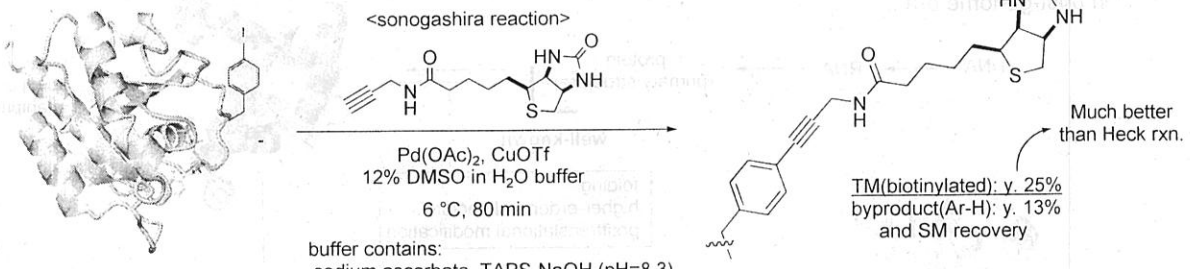


TM(biotinylated): y. 2%
byproduct(Ar-H): y. 28%
and SM recovery

Site-Specific Functionalization of Proteins by Organopalladium Reactions**

Koichiro Kodama,[a, b, c] Seketsu Fukuzawa,*[b] Hiroshi Nakayama,[d] Kensaku Sakamoto,[a, c] Takanori Kigawa,[c] Takashi Yabuki,[c] Natsuko Matsuda,[c] Mikako Shirouzu,[c] Koji Takio,[c] Shigeyuki Yokoyama,*[a, c] and Kazuo Tachibana*[b]

ChemBioChem **2007**, *8*, 232.



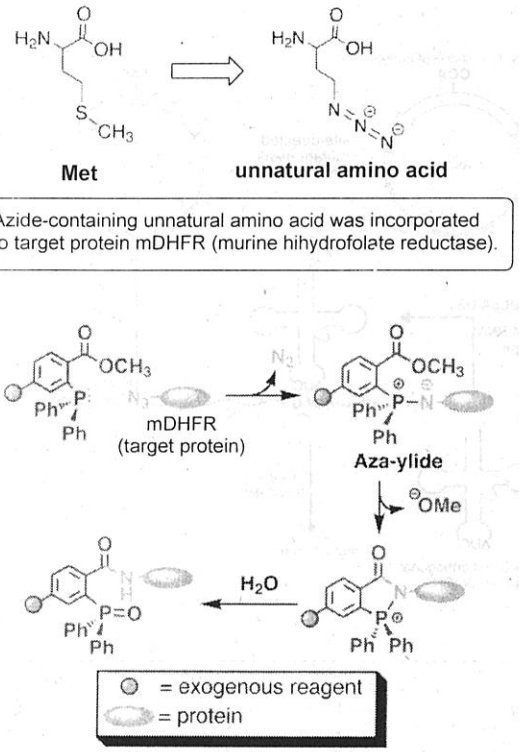
azide chemistry

Staudinger Reaction

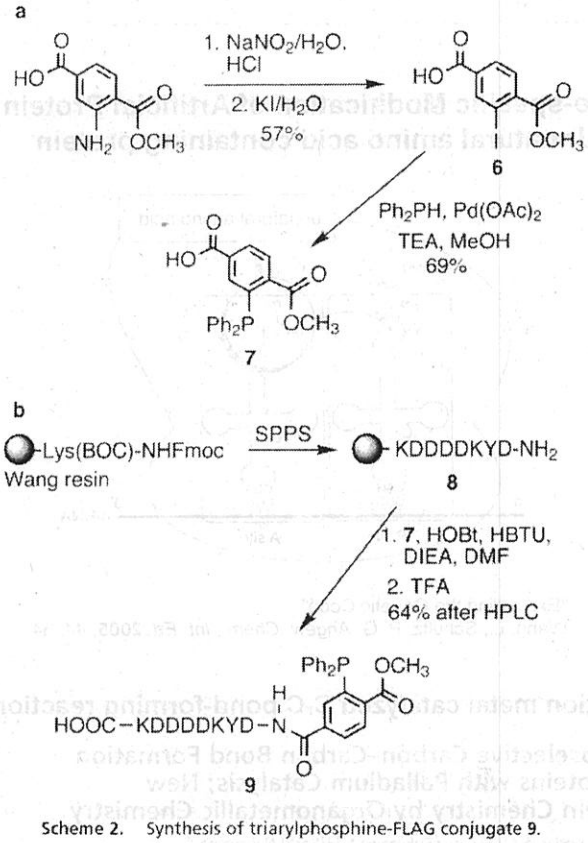
Incorporation of azides into recombinant proteins for chemoselective modification by the Staudinger ligation

Kristi L. Kiick*†, Eliana Saxon*‡, David A. Tirrell§¶, and Carolyn R. Bertozzi‖

PNAS | January 8, 2002 | vol. 99 | no. 1 | 19–24



Scheme 1. The Staudinger ligation between a protein containing azide functionalized amino acid side chains and a phosphine reagent.



Scheme 2. Synthesis of triarylphosphine-FLAG conjugate 9.

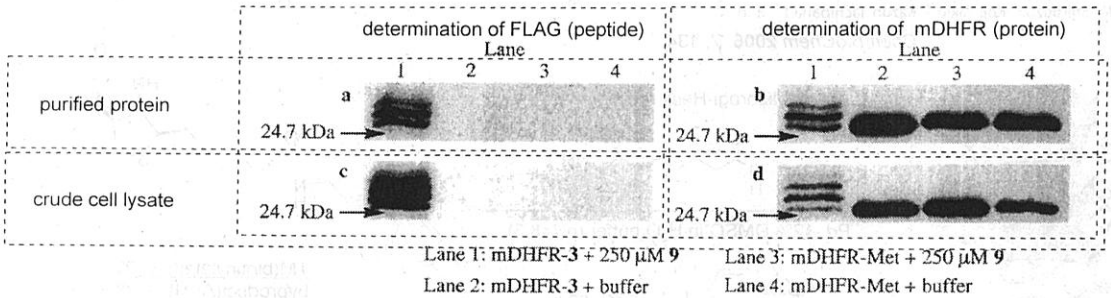
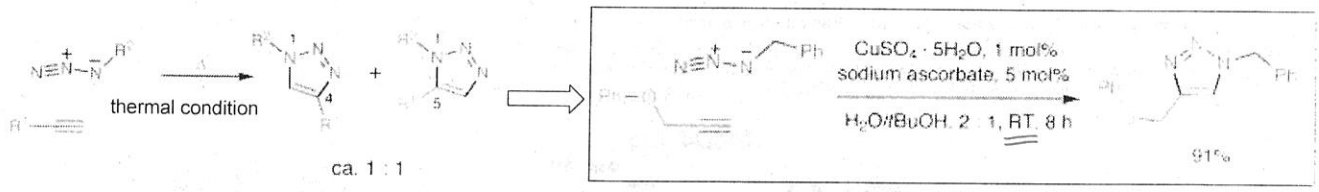


Fig. 6. Western blot analysis of the products of Staudinger ligation. (*a*) Purified protein (mDHFR-3 or mDHFR-Met) was used in the ligation, and the blot was labeled with anti-FLAG M2 mAb followed by HRP-rat anti-mouse IgG1. (*b*) Similar to a but labeled with India HisProbe-HRP. (*c*) Crude cell lysate (containing either mDHFR-3 or mDHFR-Met) was used in the ligation, and the blot was labeled with anti-FLAG M2 mAb followed by HRP-rat anti-mouse IgG1. (*d*) Similar to c but

Cu(I)-catalyzed (3+2) cycloaddition

Fokin, V. V.; Sharpless, K. B. *et al. Angew. Chem., Int. Ed.* **2002**, *41*, 2596.



> rxn proceeds at rt in regioselectively !!
> pH range from 4 to 12

copper source//

- Cu(II) ⟶ Cu(I)

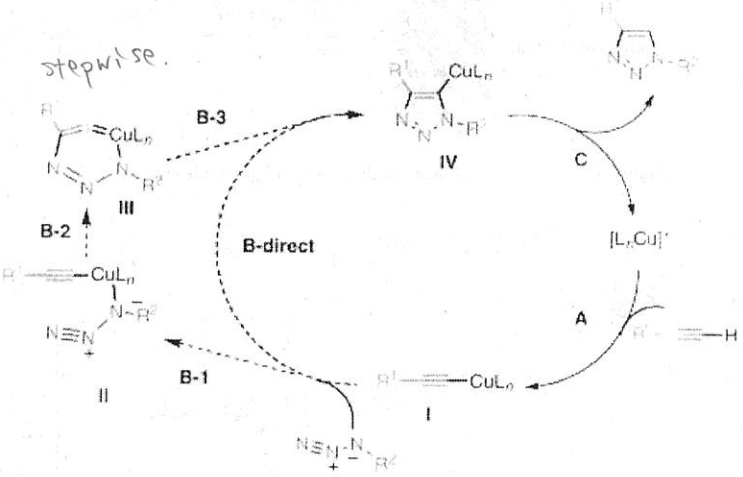
  in situ reduction with sodium ascorbate/ ascorbic acid

- Cu(I) can be also used. (less satisfactory)

  CuI, CuOTf 1/2benzene, $[Cu(NCCH_3)_4][PF_4]$

proposed catalytic cycle

stepwise mechanisme was proposed
(12-15 kcal favor than B-direct<concerted>)

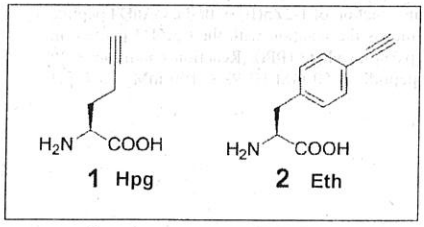


Selective Dye-Labeling of Newly Synthesized Proteins in Bacterial Cells

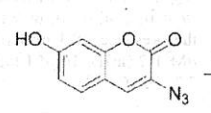
Kimberly E. Beatty,† Fang Xie,‡ Qian Wang,‡ and David A. Tirrell*,†

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, and Department of Chemistry and Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, South Carolina 29208

J. AM. CHEM. SOC. 2005, *127*, 14150−14151



Hpg: homopropargylglycine
Eth: ethynylphenylalanine



fluorescent coumarin

3

after [3+2] cycloaddition

tetracycline: inhibitor of protein synthesis

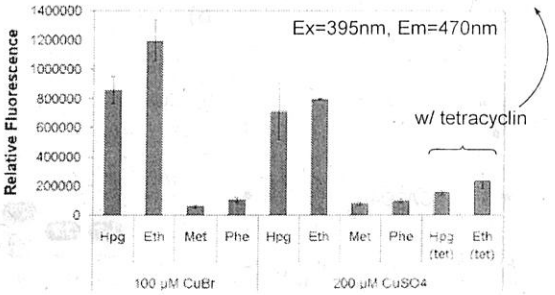
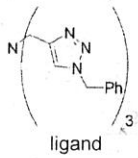


Figure 2. Fluorescence of induced *E. coli* cells after reaction with **3**. Fluorescence was also measured for uninduced cells in media supplemented with tetracycline (tet).

Met ⟶ Hpg
Phe ⟶ Eth

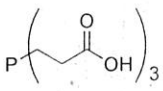
recombinant protein (barstar) was prepared.

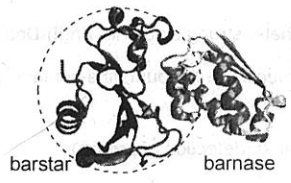


ligand

conditions
CuBr, ligand, PBS buffer(pH=7.9), 4C, 15hr
or
$CuSO_4$, ligand, TCEP, PBS buffer(pH=7.9), 4C, 15hr

TCEP = tris(carboxyethyl)phosphine





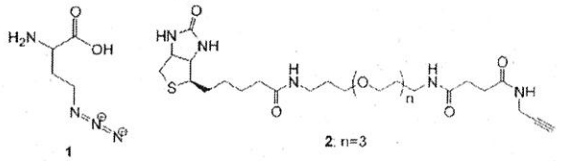
...its function is to inhibit the ribonuclease activity of its binding partner barnase...

Cell Surface Labeling of *Escherichia coli* via Copper(I)-Catalyzed [3+2] Cycloaddition

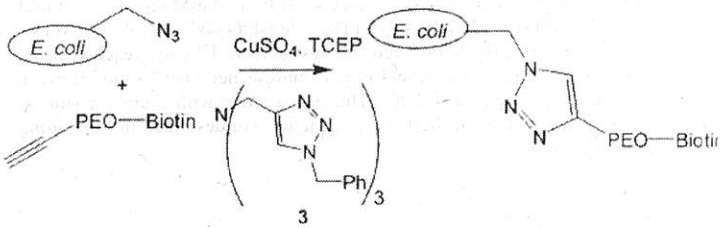
A. James Link and David A. Tirrell*

J. AM. CHEM. SOC. 2003, *125*, 11164−11165

Scheme 1. Structure of Azidohomoalanine **1** and Biotin-PEO Propargylamide **2**; Biotinylation Reaction of Whole *E. coli* via [3+2] Cu-Mediated Azide-Alkyne Cycloaddition



Similar strategy mentioned above, but azide was incorporated into outer membrane protein C (OmpC) of *E. Coli.*



1.2 Tag-Fused Protein

Non-enzymatic Covalent Protein Labeling Using a Reactive Tag

Hiroshi Nonaka,† Shinya Tsukiji,† Akio Ojida,†,‡ and Itaru Hamachi*,†

Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Katsura Campus, Nishikyo-ku, Kyoto, 615-8510, Japan, and PRESTO (Life Phenomena and Measurement Analysis, JST), Sanbancho, Chiyodaku, Tokyo, 332-0012, Japan

JACS asap

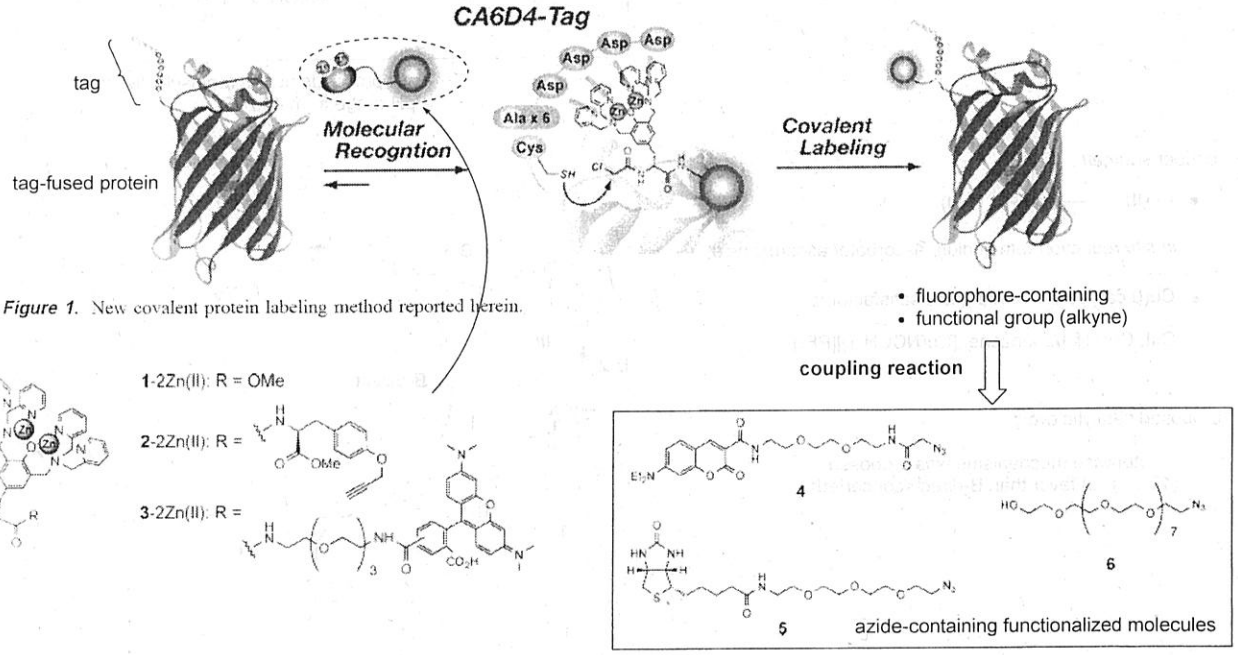


Figure 1. New covalent protein labeling method reported herein.

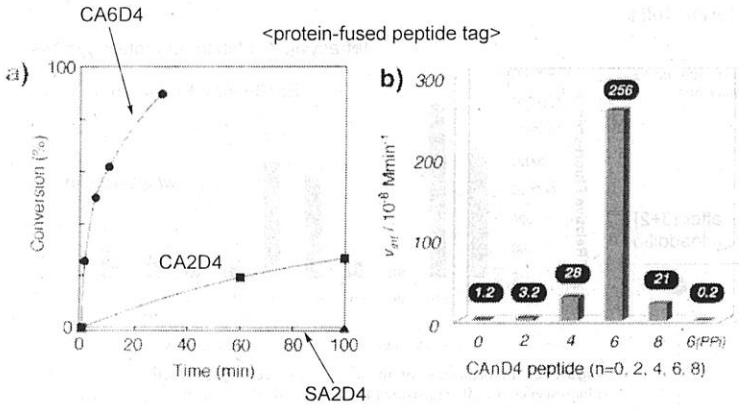
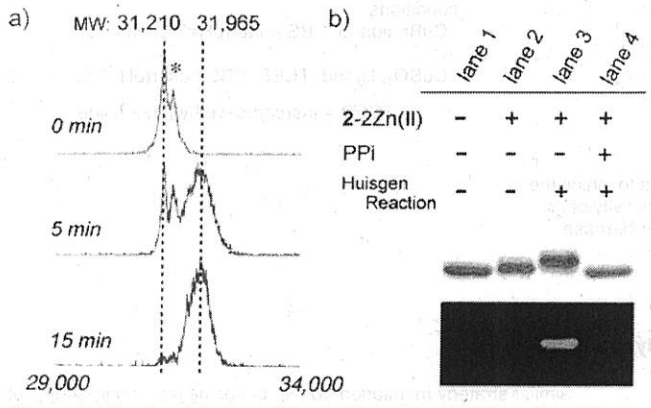


Figure 2. (a) Time trace of the labeling reaction of **1**-2Zn(II) with CA6D4 (●), CA2D4 (■), and SA6D4 peptide (▲). (b) Summary of the initial rate (v_{ini}, M min^{-1}) of the labeling reaction of **1**-2Zn(II) with the CAnD4 peptide (n = 0, 2, 4, 6, 8); 6(PPi) means the reaction with the CA6D4 peptide in the presence of 3 mM of pyrophosphate (PPi). Reaction conditions: 20 μM **1**-2Zn(II), 10 μM tag peptide in 50 mM HEPES, 100 mM NaCl, pH 7.2, 20 °C.



PPi=pyrophosphate; strong binder for Zn(II)-DpaTyr

azide+alkyne (coupling with coumarine-containing azide **4**)

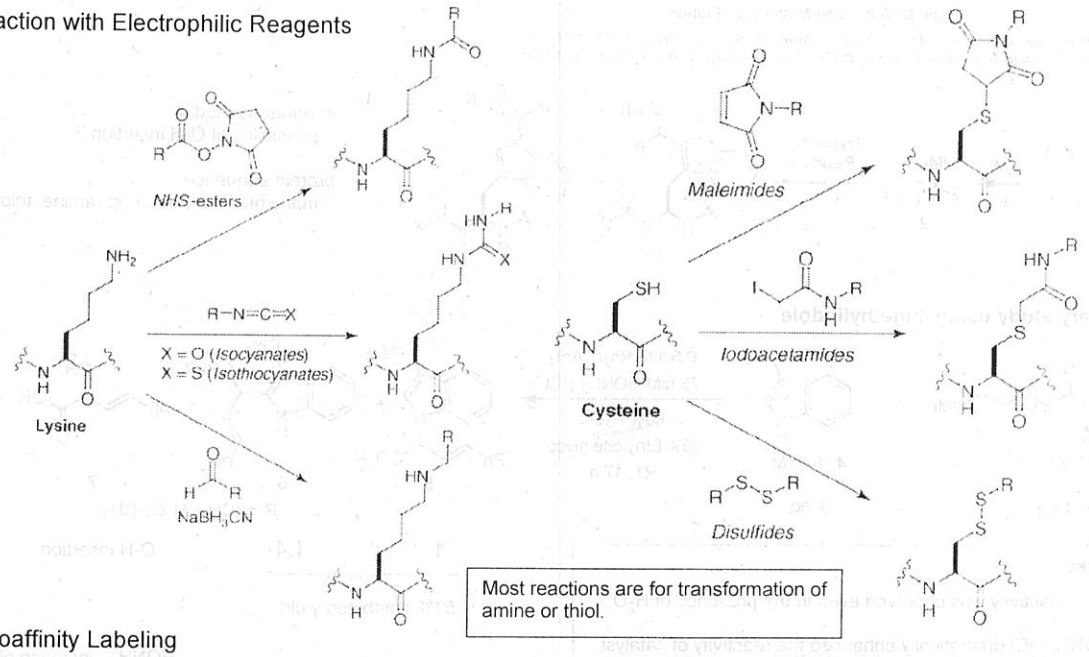
coomassie staining (detection of protein)

fluorescence visialization (detection of coumarine)

Figure 3. Covalent labeling of the CA6D4-tagged EGFP with **2**-2Zn(II). (a) MALDI-TOF mass analysis of the labeling reaction. Reaction conditions: 20 μM **2**-2Zn(II), 5 μM CA6D4-EGFP in 50 mM HEPES, 100 mM NaCl, 1 mM DTT, pH 7.2, 20 °C. (The asterisk (*) is the peak of CA6D4-EGFP + matrix) (b) SDS-PAGE analysis of the labeling reaction using Coomassie staining (upper) and in-gel fluorescence visualization (lower). The analysis was performed after Huisgen reaction with coumarin azide **4**. Experimental details of the Huisgen reaction are described in Supporting Information.

2. Site-specific Modification of "natural" Protein precedents

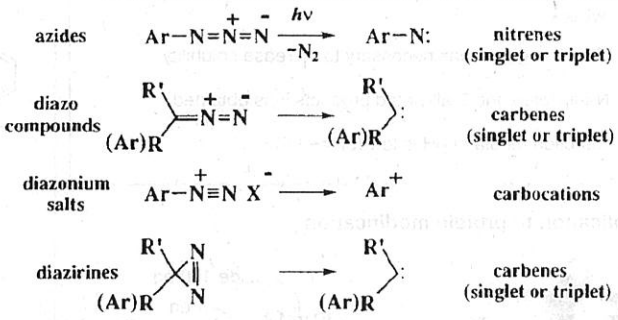
- Reaction with Electrophilic Reagents



Most reactions are for transformation of amine or thiol.

- Photoaffinity Labeling

review: *Angewandte Chemie., Int. Ed. Engl.* **1995**, *34*, 1296.

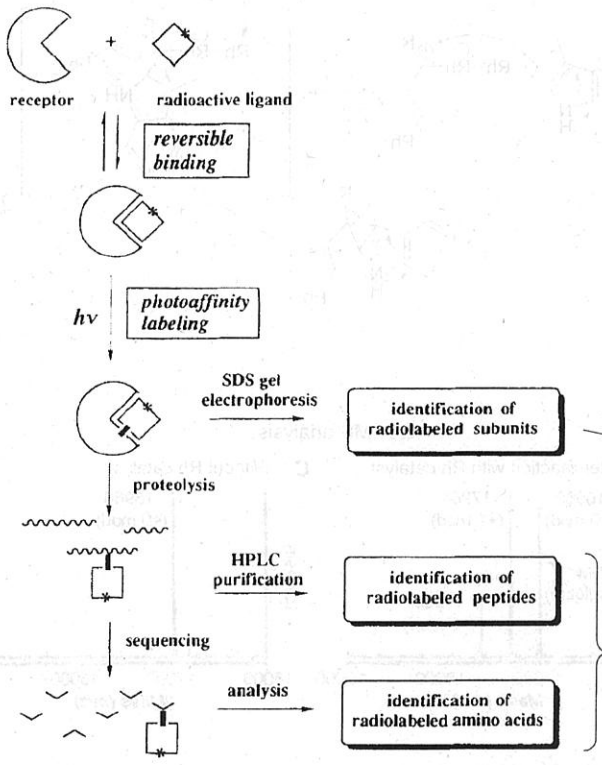


Scheme 1. Typical compounds used as precursors of reactive species in photoaffinity labeling.

reactive species

⇩

C-H insertion, radical reaction rearrangement



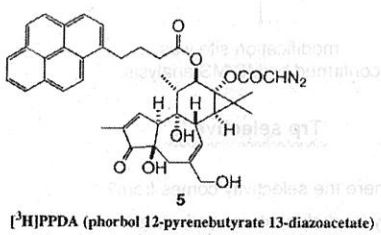
Usually photoaffinity label was used by this stage.

Only a few example led to the identification of the amino acid residues labeled by the photosenseitve probes.

(due to non-specific binding?)

Fig. 1. Photoaffinity labeling: principle and successive steps for the identification of amino acids.

non specific binding quenched by H_2O or ... ⇨ low yield, although useful method

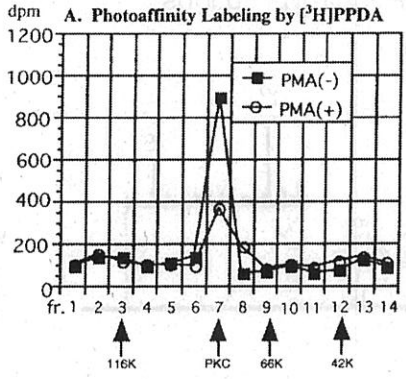


5

[^{3}H]PPDA (phorbol 12-pyrenebutyrate 13-diazoacetate)

Yield of PKC cross-linking was 1.1 %.

Shibasaki, M. *et al. BMC* **1998**, *6*, 1117.



One way for the site selective modification of protein is...

To target less common residue in the presence of abundant residue (Lys, Cys etc.)

Transition metal catalyzed reaction

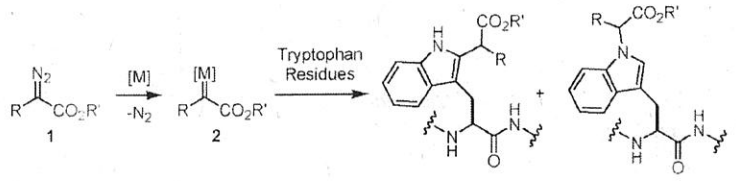
- Rh carbenoid (Trp)
- reductive amination (Lys)
- mannnich-type reaction (Tyr)
- Tsuji-Trost reaction (Tyr)
- oxidative cross-linking (Tyr)

Modification of Trp

Selective Tryptophan Modification with Rhodium Carbenoids in Aqueous Solution J. AM. CHEM. SOC. 2004, 126, 10256

John M. Antos and Matthew B. Francis*

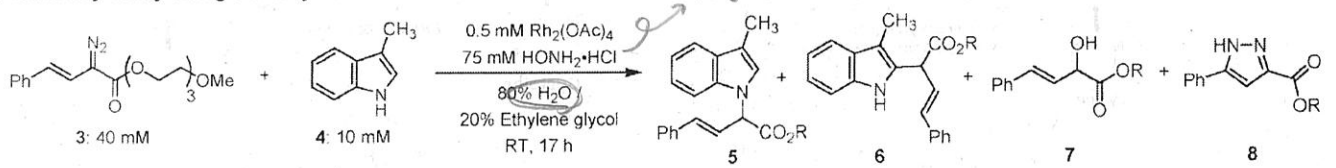
Department of Chemistry, University of California, Berkeley, California 94720-1460, and Material Science Division, Lawrence Berkeley National Labs, Berkeley, California 94720



in aqueous media
possibility of O-H insertion ?

protein sequence
many nucleophilic group (amine, thiol, OH) exist

prelimilary study using 3-methylindole



1 : 1.4

51% combined yield

O-H insertion

pyrazole without carbenoid

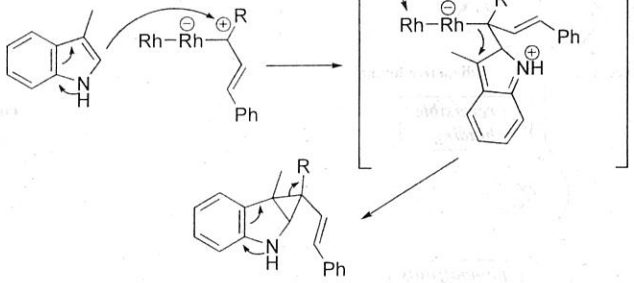
remarks

- High reactivity was observed even in the presence of H_2O.
- $HONH_2$·HCl dramatically enhanced the reactivity of catalyst.

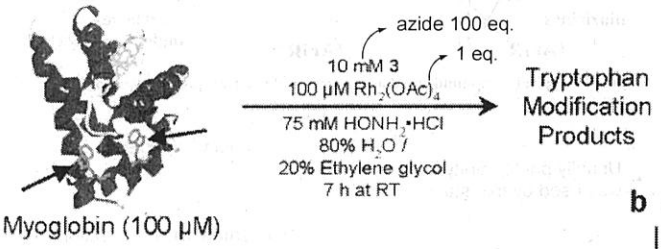
drawback

- Ethylene glycol was necessary to increase solubility.
- N-alkylated and 2-alkylated products was obtained.
- Reaction media of pH is low (pH=~3.5)

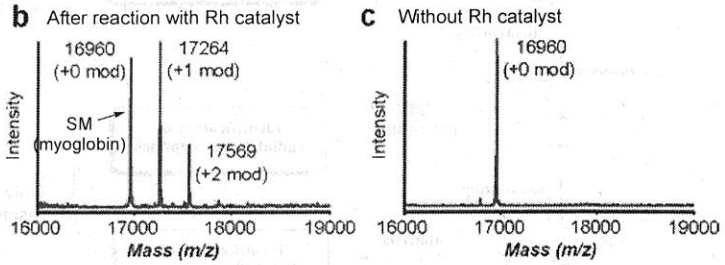
$HONH_2$ increase electron density?



applicaiton to protein modification

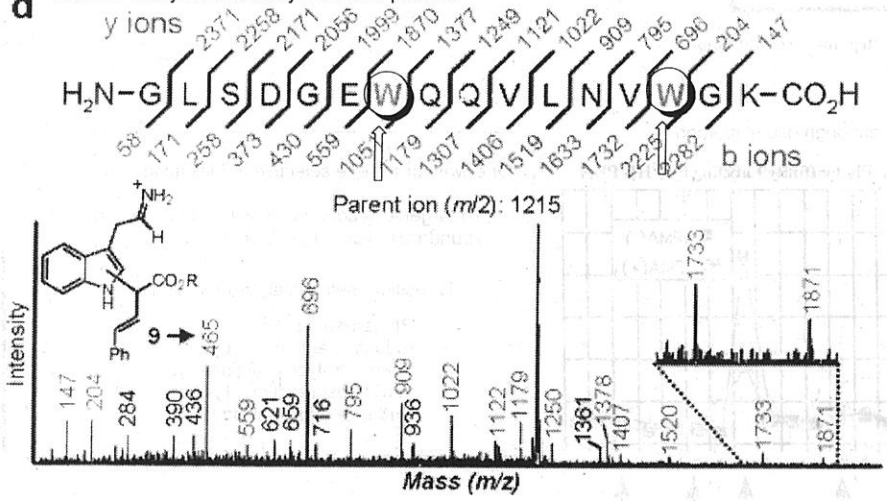


ESI-MS analysis



~60% conversion was observed by ESI-MS.

d MS/MS analysis of doubly modified protein



reaction mixture

↓

digested with tripsin

↓

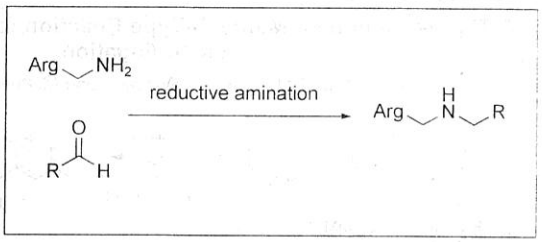
modification site was confirmed by MS/MS analysis.

Trp selective !!

Where the selectivity comes from?

Hydrophobic interacion between aromatic carbenoid intermediate and indole ring might facilitate the rxn...

Modification of Lys (-NH_2)

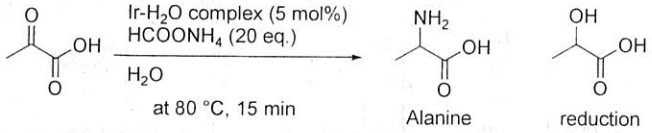


pH-Dependent Chemoselective Synthesis of α-Amino Acids. Reductive Amination of α-Keto Acids with Ammonia Catalyzed by Acid-Stable Iridium Hydride Complexes in Water

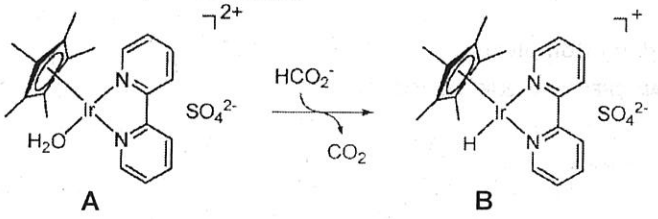
Seiji Ogo,* Keiji Uehara, Tsutomu Abura, and Shunichi Fukuzumi*

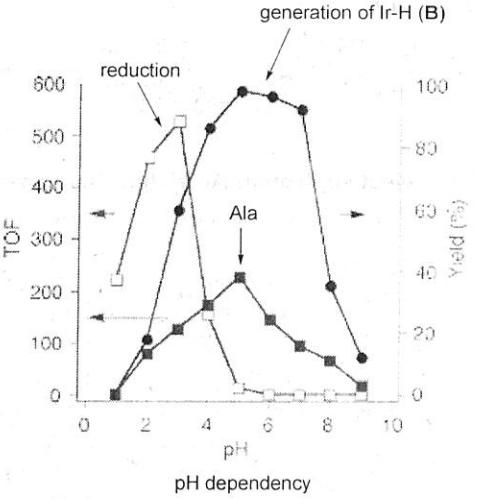
JACS **2004**, *126*, 3020.

target reaction



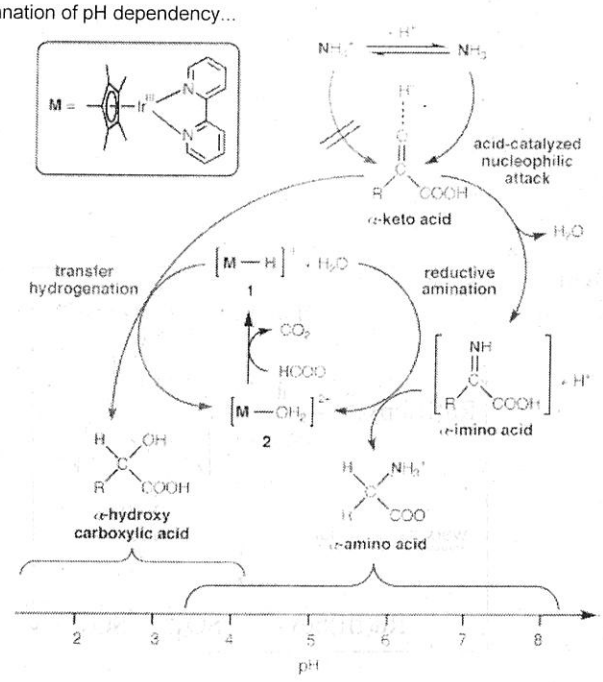
Water-stable Ir compex was reported.





in pH>5 reductive amination is dominant compared with reduciton.

Explanation of pH dependency...



drawback:

elevated temperature is necessary (80 °C)

only works well at slightly acidic condition (pH 5.0-6.5)

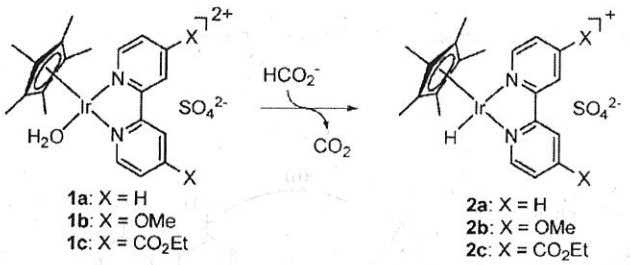
for the application to protein modification

rt, neutral buffered condion

Reductive Alkylation of Proteins Using Iridium Catalyzed Transfer Hydrogenation

Jesse M. McFarland and Matthew B. Francis* *JACS* **2005**, *127*, 13490

Modification of the bipuridine ligand was the key.



Only in the case of **2b** (X=H, CO_2Et), rxn can proceed at rt.

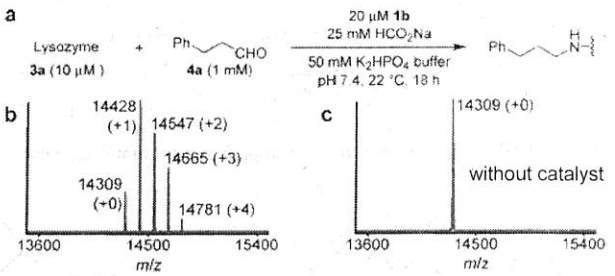


Figure 1. Modification of lysozyme using reductive alkylation. Under the reaction conditions summarized in (a), a distribution of alkylated products results (b). (c) Control experiments lacking catalyst yielded no reaction products. Spectra shown are reconstructed from charge ladders obtained using ESI-MS analysis.

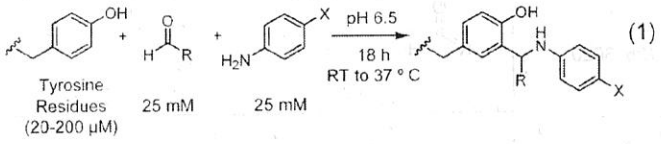
Site selectivity was confirmed using model substrate (peptide containing Lys).

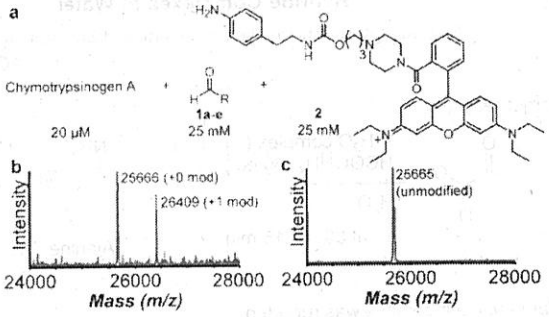
Modification of Tyr

A Three-Component Mannich-Type Reaction for Selective Tyrosine Bioconjugation

JACS **2004**, *126*, 15942.

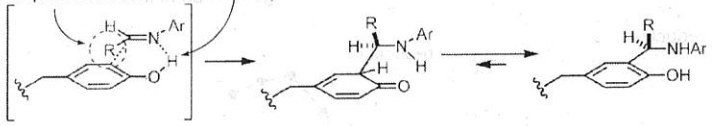
Neel S. Joshi, Leanna R. Whitaker, and Matthew B. Francis*





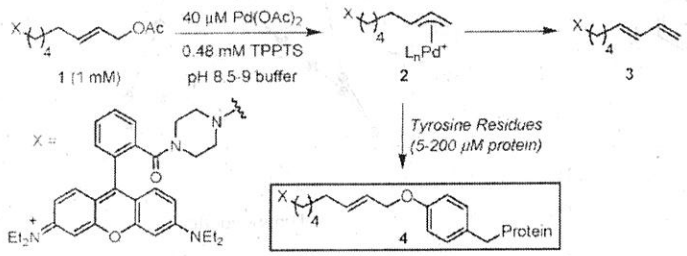
slightly acidic condition is necessary ?

Hydrophobic effect ?



Tyrosine-Selective Protein Alkylation Using π-Allylpalladium Complexes

S. David Tilley and Matthew B. Francis* **J. AM. CHEM. SOC. 2006**, *128*, 1080–1081



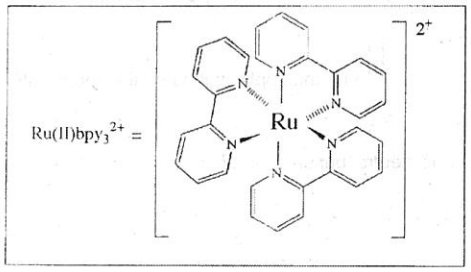
Oxidative Protein Cross-linking

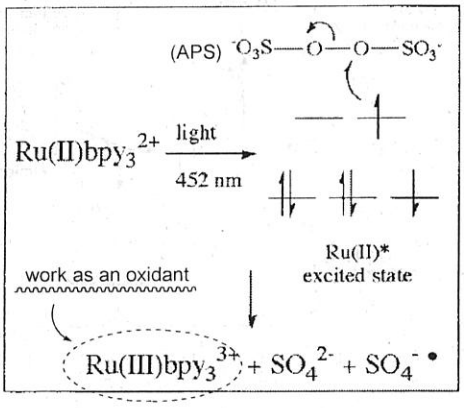
Chemistry for the analysis of protein–protein interactions: Rapid and efficient cross-linking triggered by long wavelength light

DAVID A. FANCY AND THOMAS KODADEK†

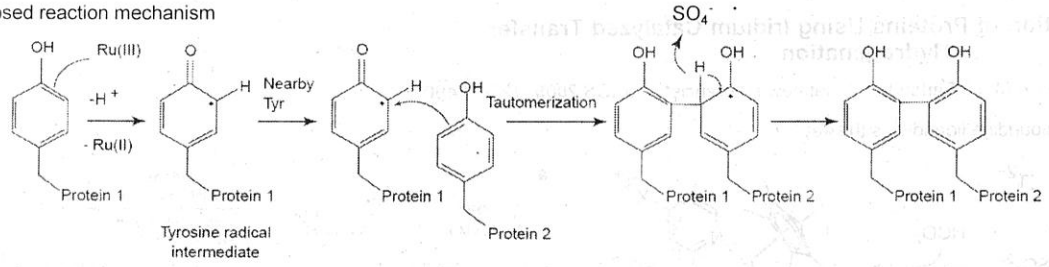
Departments of Internal Medicine and Biochemistry, Center for Biomedical Inventions, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8573

PNAS **1999**, *96*, 6020.

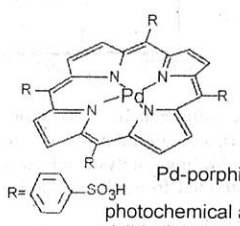




proposed reaction mechanism

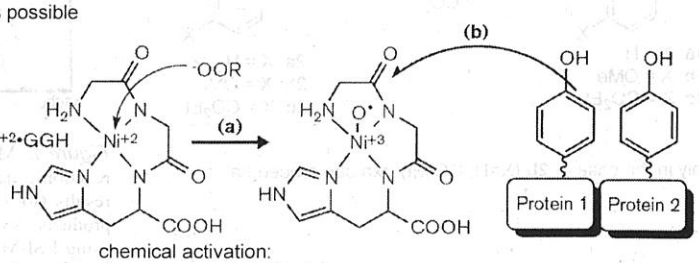


other combination of trandition metal complex and oxidant is possible



Pd-porphiline

photochemical activation:
visible light irradiation and
ammonium persulfate (APS)



chemical activation:
magnesium monoperoxyphthalate
(MMPP) or oxone($KHSO_5$)

Crosslinking Photosensitized by a Ruthenium Chelate as a Tool for Labeling and Topographical Studies of G-Protein-Coupled Receptors

Isabelle Duroux-Richard,[1,3] Philippe Vassault,[1,3] Guy Subra,[1] Jean-François Guichou,[1] Eric Richard,[1] Bernard Mouillac,[2] Claude Barberis,[2] Jacky Marie,[1] and Jean-Claude Bonnafous[1,*]

Chemistry & Biology, Vol. 12, 15–24, January, 2005

about bradykinin

9-amino acid peptide chain
(Arg - Pro - Pro - Gly - Phe - Ser - Pro - Phe - Arg)
involved in the mechanism of pain

kininogen → bradykinin
↑
kallikrein

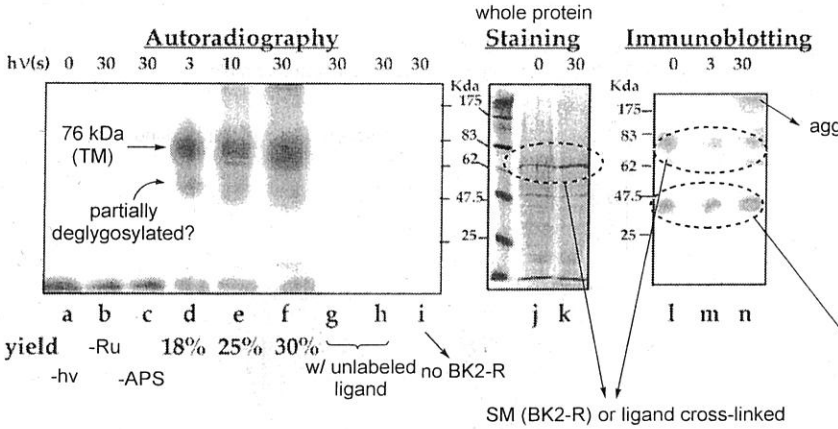
target: human B2 bradykinin receptor (GPCR) expressed on COS-7 cell surface

Receptor		Ligand name	Peptide sequence	
B_2	L_1	[^{125}I]HPP-HOE 140	HO-(4-^{125}I-phenyl)-$(CH_2)_2$-CO-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg	BK antagonist
	L_2	[^{125}I]Tyr^0-BK	Tyr(^{125}I)-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	BK agonist
	L_3	[^{125}I](Tyr^8)-BK	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Tyr(^{125}I)-Arg	
	L_4	[^{125}I]Lys^0-(Tyr^8)-BK	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Tyr(^{125}I)-Arg	
	L_{10}	[^{125}I]Tyr^0-HOE 140	Tyr(^{125}I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg	
	L_{12}	[^{125}I]GGHY-HOE 140	Gly-Gly-His-Tyr(^{125}I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg	
	L_{13}	[^{125}I]Bio-Tyr^0-HOE 140	Biotinesulfone-6-Ahx-Tyr(^{125}I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg	

functionalized ligand

A Antagonist cross-linking

[^{125}I]HPP-HOE 140 (L_1)



a: before irradiation
b: without Ru catalyst
c: without APS oxidant
d: irradiate for 3 sec
e: irradiate for 10 sec
f: irradiate for 30 sec
} labeled product was obtained
g: with ex. unlabeled HPP-HOE
h: with ex. unlabeled HOE
i: nontranfected COS-7 cell

l-n: stained by anti-c-Myc antibody
(B_2-R possesses a C-Myc tag at N-terminal)

l: before irradiation
m: irradiate for 3 sec
n: irradiate for 30 sec

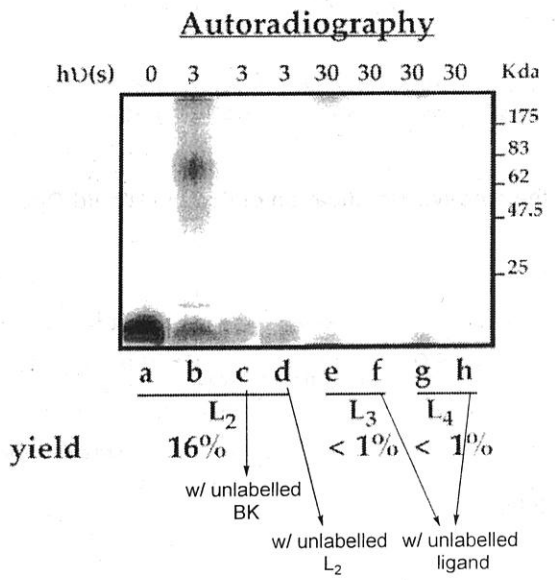
B Agonist cross-linking

Tyr positioned at N-terminal [^{125}I]Tyr^0-BK (L_2)

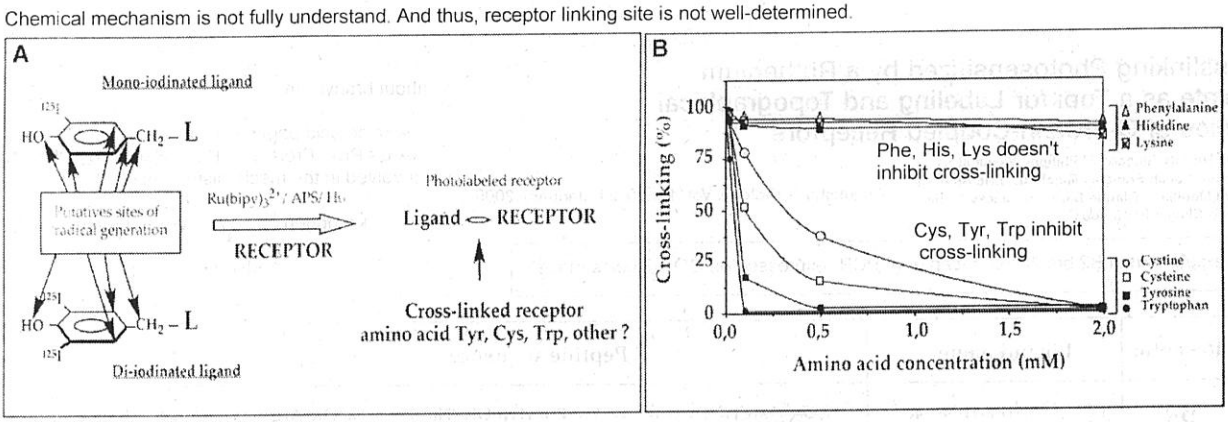
Tyr located at internal position
- [^{125}I](Tyr^8)-BK (L_3)
- [^{125}I]Lys^0-(Tyr^8)-BK (L_4)

a: no irradiation
b: irradiate 3 sec
c: with ex. unlabelled BK
d: with ex. unlabelled L_2

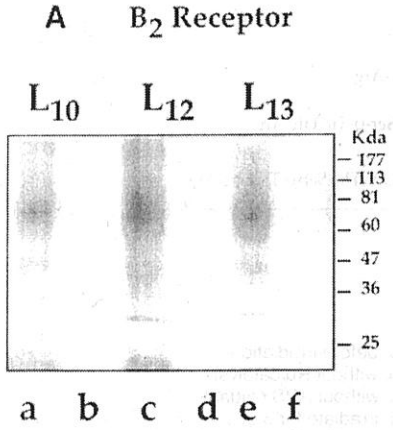
The presence of a phenol moiety at the ligand N-terminal ends was required for efficient covalent labeling.



Chemical mechanism is not fully understand. And thus, receptor linking site is not well-determined.



Potential Applications of Oxidative Cross-linking to GPCR Structual and Signalling Mechanism Studies



L10 Tyr(125I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg

L12 Gly-Gly-His-Tyr(125I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg

L13 Biotinesulfone-6-Ahx-Tyr(125I)-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg

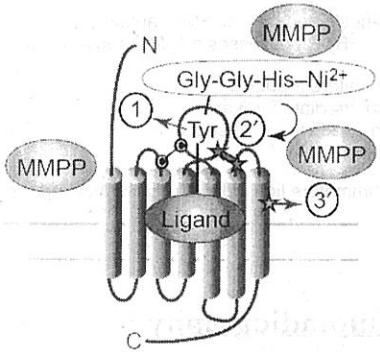
Using Ru/APS crosslinking system

a, c, e: photolabelling for 5s
b, d, f: with unlabeled ligand

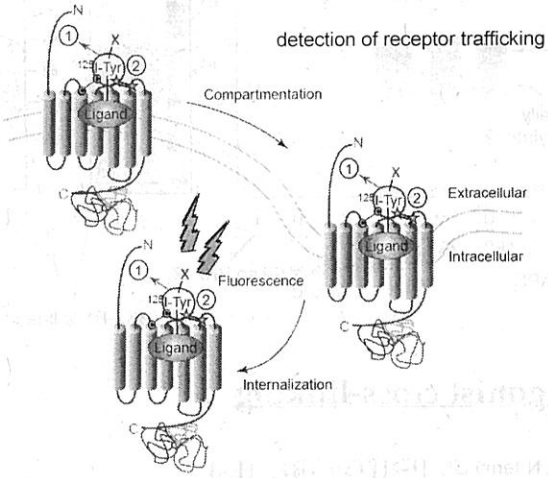
funtionalized ligand = Gly-Gly-His or biotin-containing ligand

(1) selective cross-linking
(increase specificity and efficiency with auto-photo-sensisized process)
(2) detection and purification of receptor-ligand complex
(3) stabilization of complex
(provide topographical information)

(1) Targeted cross-linking



(2) spatio-temporal detection of receptor-ligand complex



review: Kodadek, T.; Duroux-Richard, I.; Bonnafous, J.-C.
Trends in Pharmacological Sciences **2005**, *26*, 210.

Site-selective Modification of Protein Would Open Up a New Field in Pharmaceutical Science