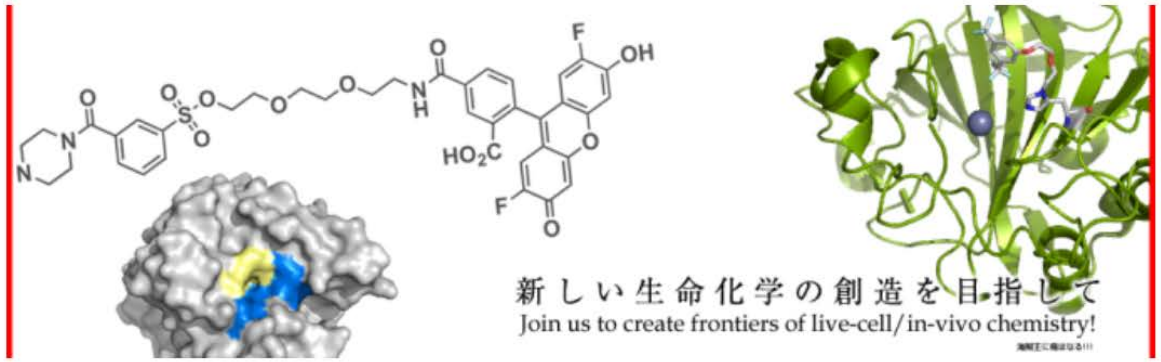
Protein Modification and Function Study

Liu Wenjuan

2020.12.24



- Received Ph.D. in 1988 from Kyoto University under the guidance of the late Professor Iwao Tabushi.
- Worked as an assistant professor for 3 years in Kunitake laboratory and became an associate professor in Shinkai laboratory in 1992 at Kyushu University.
- Became a full professor of IFOC, Kyushu University in 2001, and then moved to Kyoto University in 2005.

- live-cell organic chemistry
- chemical biology
- bioorganic and bioinorganic chemistry
- supramolecular biomaterials

➢ **P-PALM method: post-photoaffinity labeling modification**

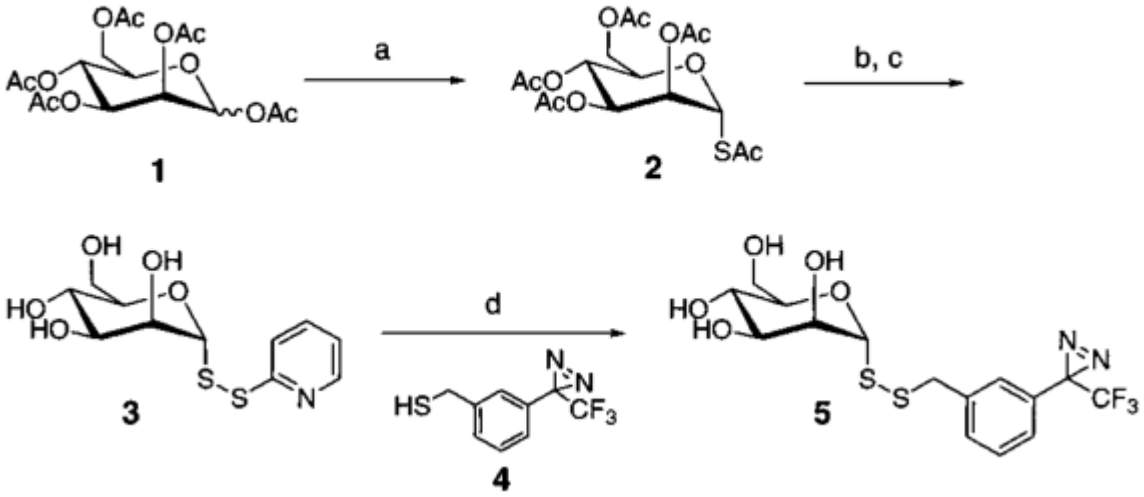
A General Semisynthetic Method for Fluorescent Saccharide-Biosensors Based on a Lectin

Itaru Hamachi,*,† Tsuyoshi Nagase, and Seiji Shinkai

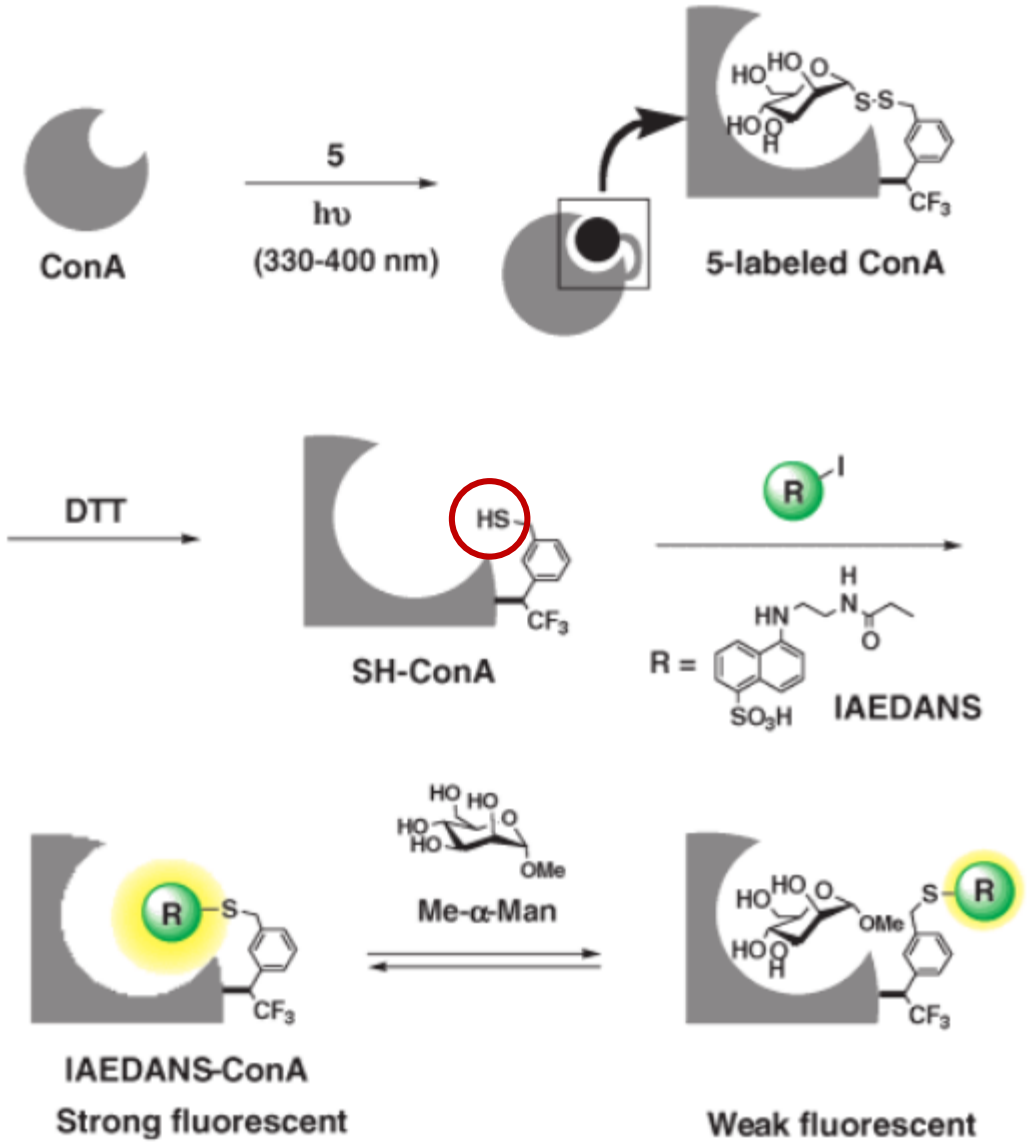
Department of Chemistry and Biochemistry
Graduate School of Engineering
Kyushu University, Fukuoka, 812-8581, Japan

Received June 8, 2000

Scheme 1. Synthetic Scheme for the Photoaffinity Ligand **5**[a]



Scheme 2. Schematic Illustration for Semisynthesis of IAEDANS-ConA

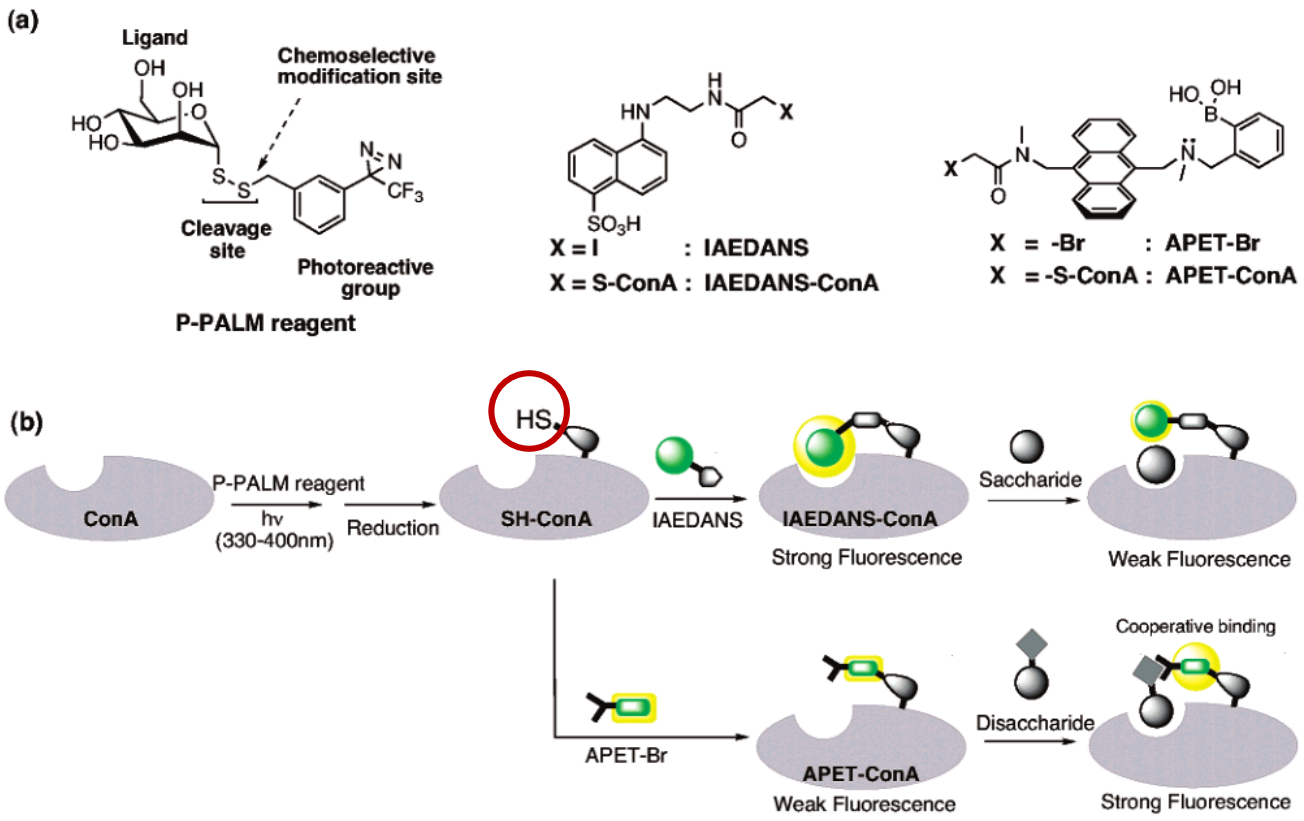


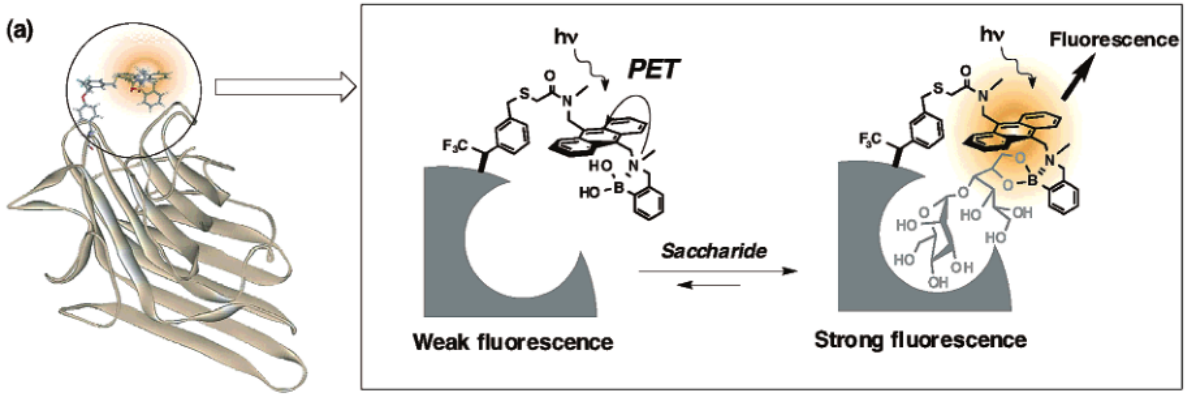
JACS_2000_12065

➢ P-PALM method: post-photoaffinity labeling modification

Coupling a Natural Receptor Protein with an Artificial Receptor to Afford a Semisynthetic Fluorescent Biosensor

Eiji Nakata,† Tsuyoshi Nagase,† Seiji Shinkai,† and Itaru Hamachi*,†,‡,§



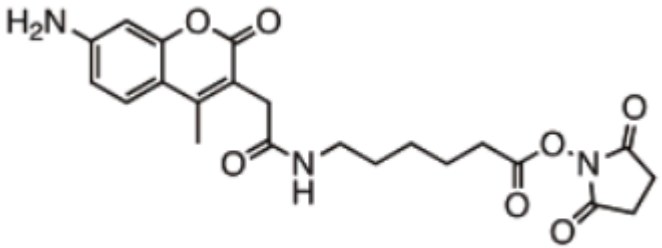


JACS_2004_490

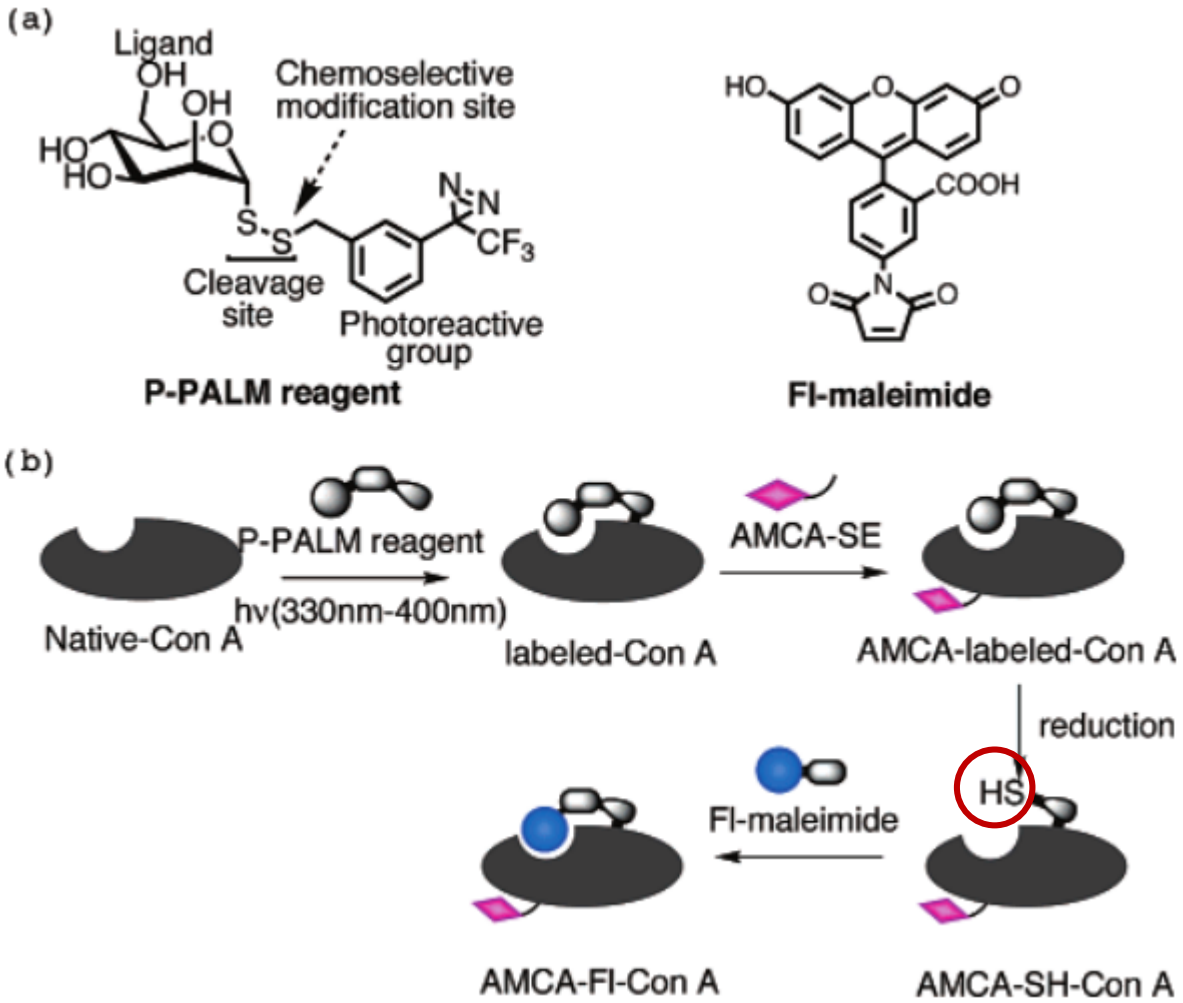
➢ P-PALM method: post-photoaffinity labeling modification

Double-Modification of Lectin Using Two Distinct Chemistries for Fluorescent Ratiometric Sensing and Imaging Saccharides in Test Tube or in Cell

Eiji Nakata,[†,‡] Yoichiro Koshi,[†] Erina Koga,[‡] Yoshiki Katayama,[‡] Itaru Hamachi*,[†]



AMCA-SE

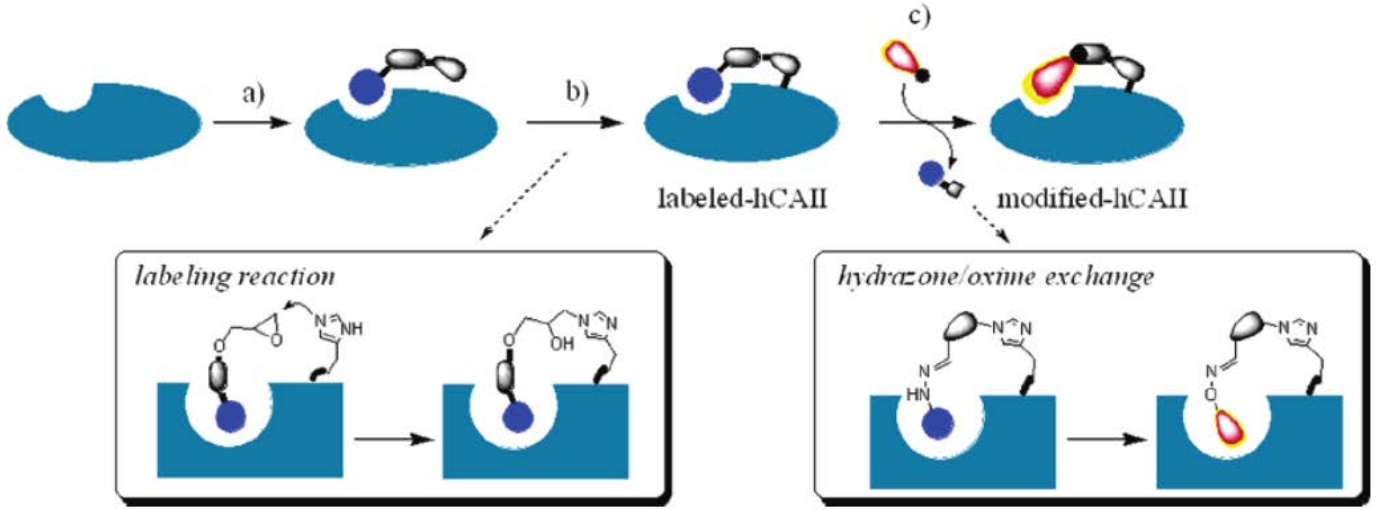


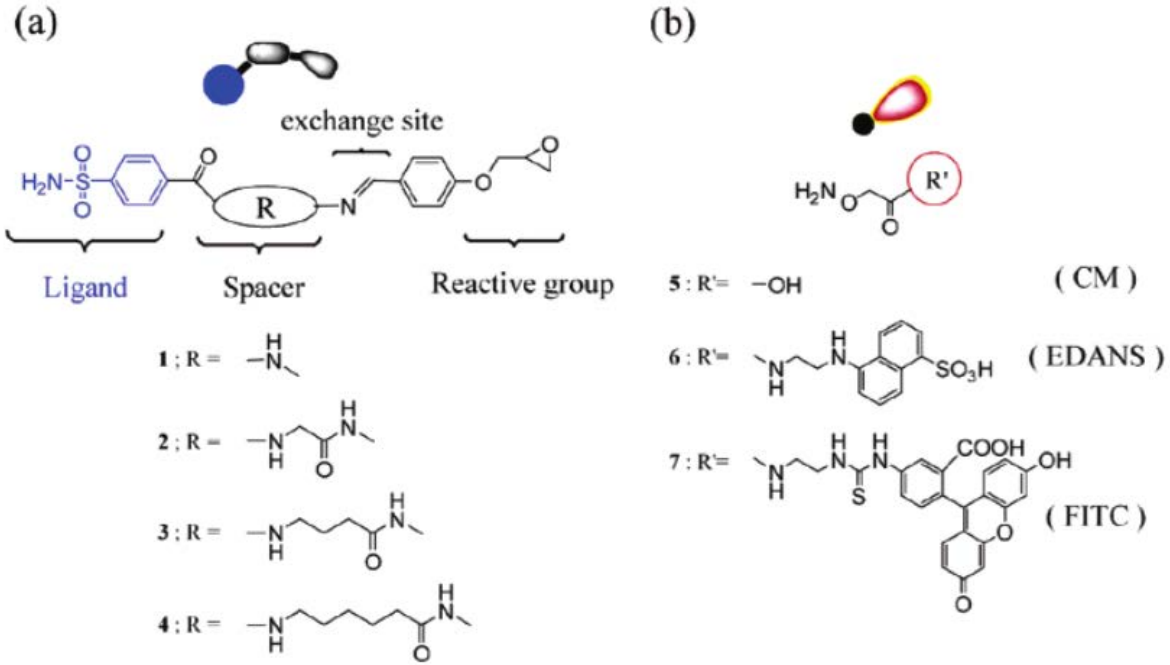
JACS_2005_13253

➢ P-ALM: Post-Affinity Labeling Modification

One-Pot and Sequential Organic Chemistry on an Enzyme Surface to Tether a Fluorescent Probe at the Proximity of the Active Site with Restoring Enzyme Activity

Yousuke Takaoka,§ Hiroshi Tsutsumi,‡ Noriyuki Kasagi,§ Eiji Nakata,† and Itaru Hamachi*,†,‡



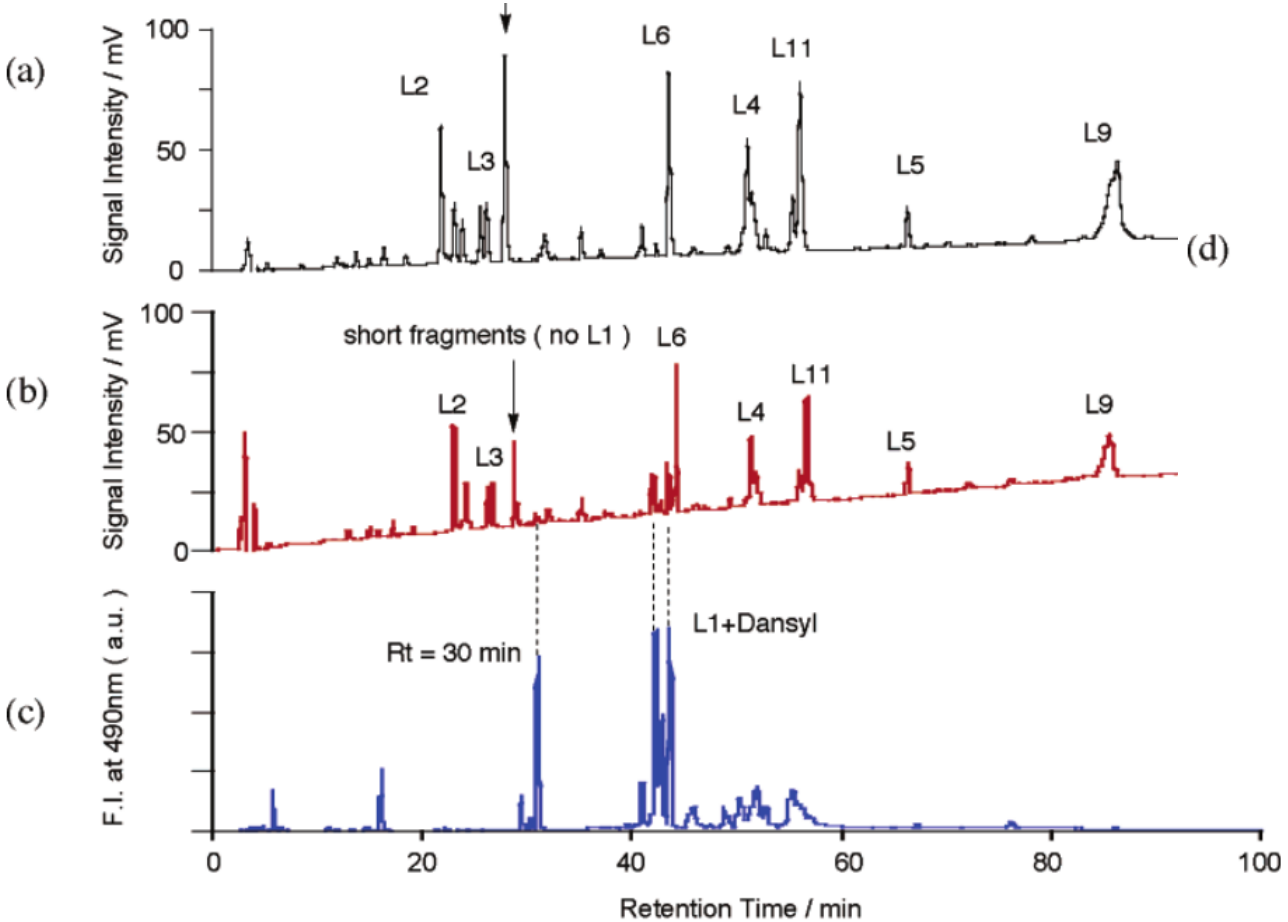


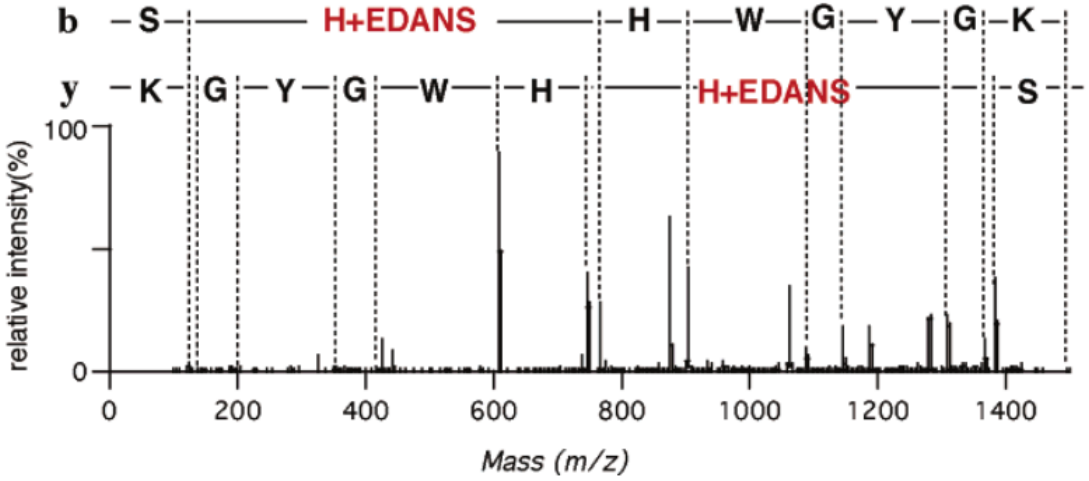
JACS_2006_3273

➢ P-ALM: Post-Affinity Labeling Modification

One-Pot and Sequential Organic Chemistry on an Enzyme Surface to Tether a Fluorescent Probe at the Proximity of the Active Site with Restoring Enzyme Activity

Yousuke Takaoka,§ Hiroshi Tsutsumi,‡ Noriyuki Kasagi,§ Eiji Nakata,† and Itaru Hamachi*,†,‡



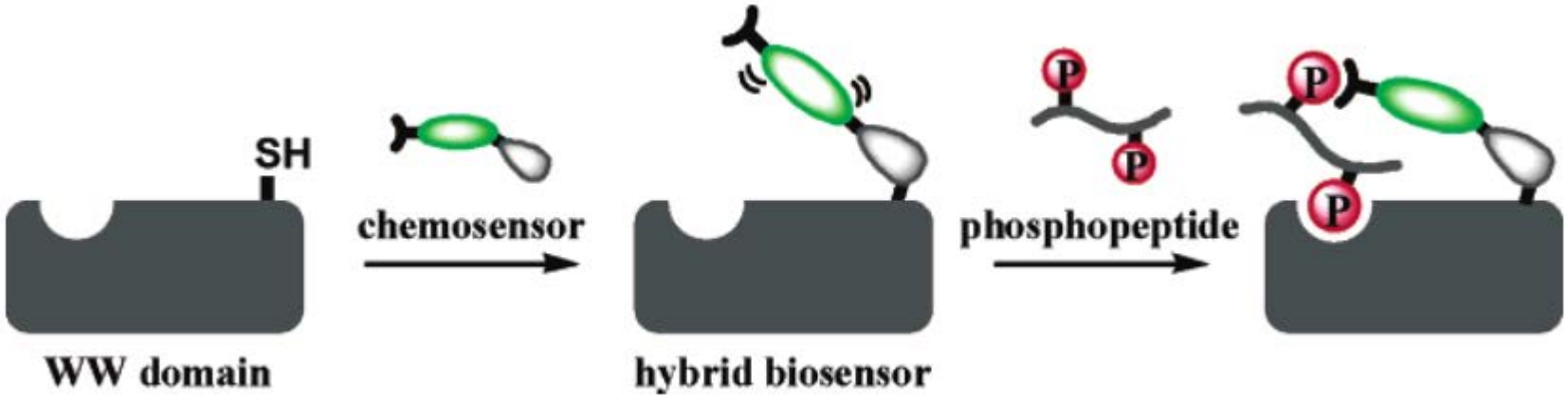


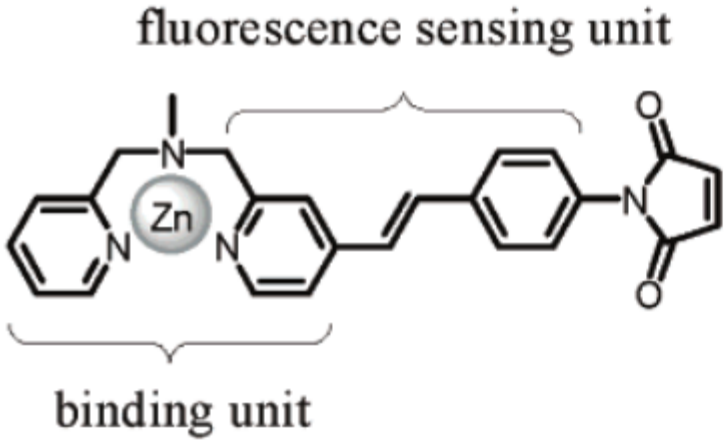
JACS_2006_3273

➢ **P-ALM: Post-Affinity Labeling Modification**

Design of a Hybrid Biosensor for Enhanced Phosphopeptide Recognition Based on a Phosphoprotein Binding Domain Coupled with a Fluorescent Chemosensor

Takahiro Anai, Eiji Nakata, Yoichiro Koshi, Akio Ojida, and Itaru Hamachi*



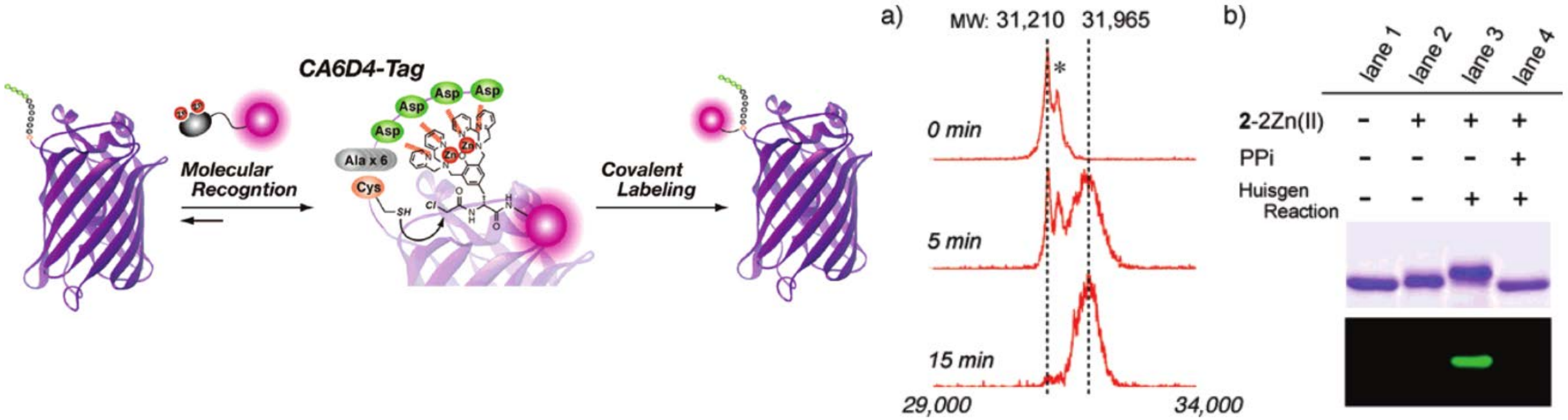


chemosensor : 1-Zn(II)

JACS_2007_6232

Non-enzymatic Covalent Protein Labeling Using a Reactive Tag

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



(i) Various artificial molecules can be permanently incorporated into the tag-fused protein

(ii) Postlabeling analyses such as SDS-PAGE or blotting can be performed conveniently

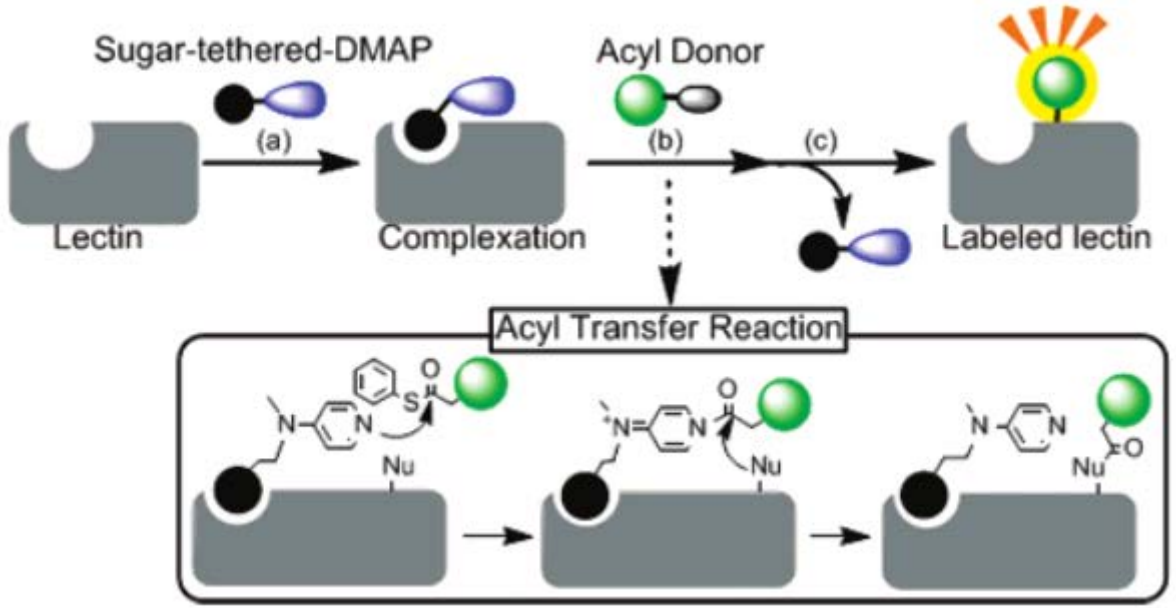
(iii) The labeling can be performed incrude conditions such as protein mixtures, cell lysate, and inside of cells

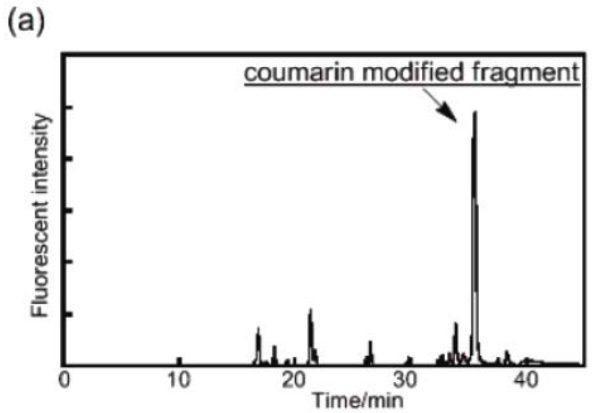
JACS_2007_15777

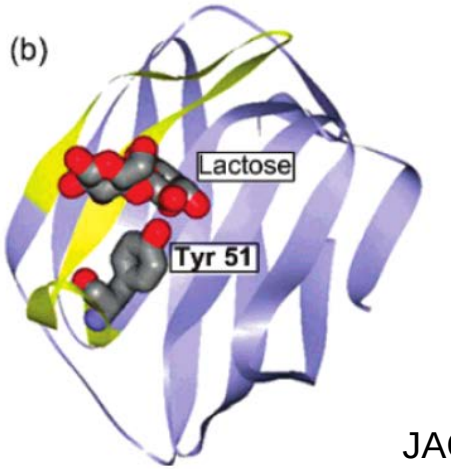
Target-Specific Chemical Acylation of Lectins by Ligand-Tethered DMAP Catalysts

Yoichiro Koshi,† Eiji Nakata,† Masayoshi Miyagawa,† Shinya Tsukiji,† Tomohisa Ogawa,‡ and Itaru Hamachi*,†

Scheme 1. Scheme of Acyl Transfer Reaction for Lectin[a]

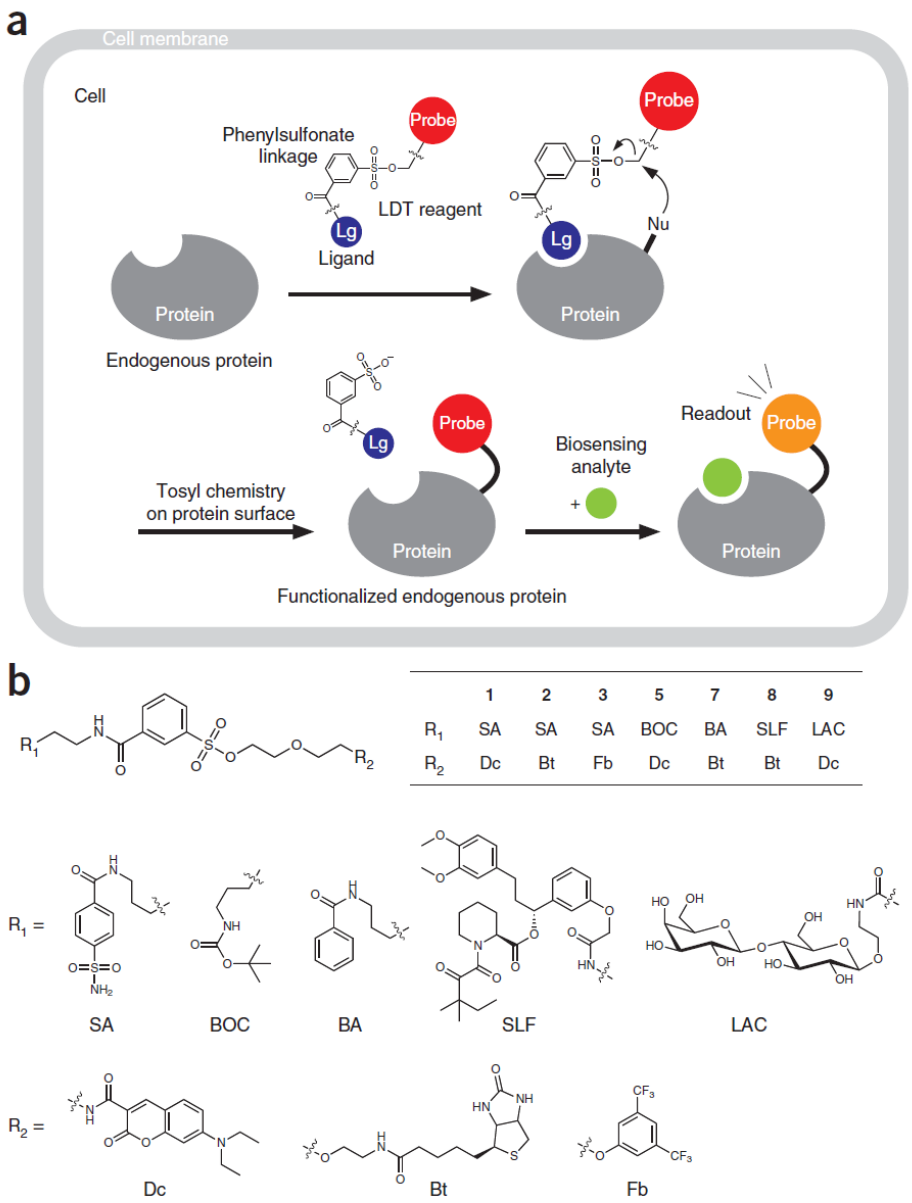






JACS_2008_245

Ligand-directed tosyl chemistry for protein labeling in vivo



	1	2	3	5	7	8	9
R_1	SA	SA	SA	BOC	BA	SLF	LAC
R_2	Dc	Bt	Fb	Dc	Bt	Bt	Dc

Nature Chemical Biology_2009_341

Quenched Ligand-Directed Tosylate Reagents for One-Step Construction of Turn-On Fluorescent Biosensors

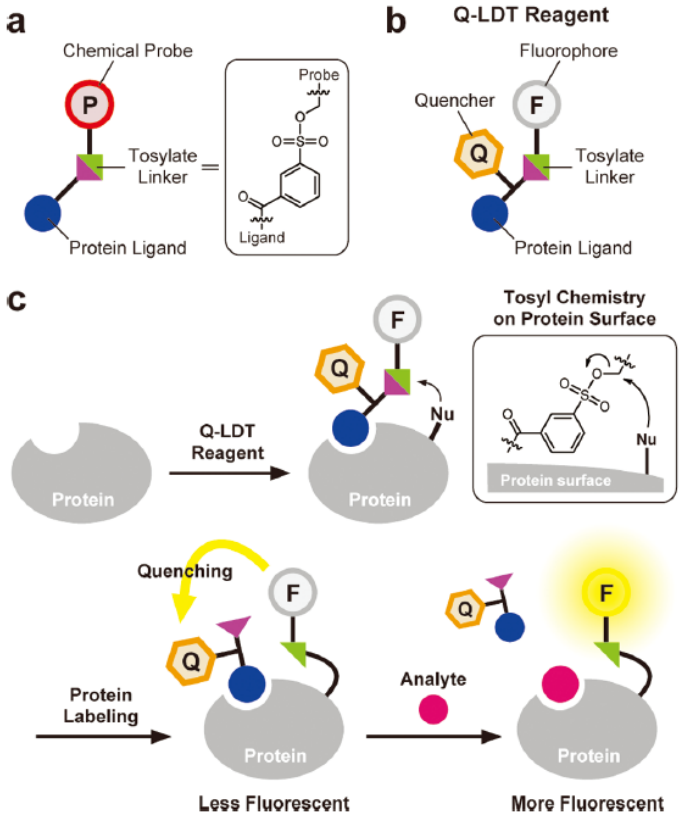


Figure 1. Molecular configurations of (a) original ligand-directed tosylate (LDT) reagent and (b) quenched ligand-directed tosylate (Q-LDT) reagent. (c) Schematic illustration of the strategy for the Q-LDT-mediated construction of turn-on fluorescent biosensors.

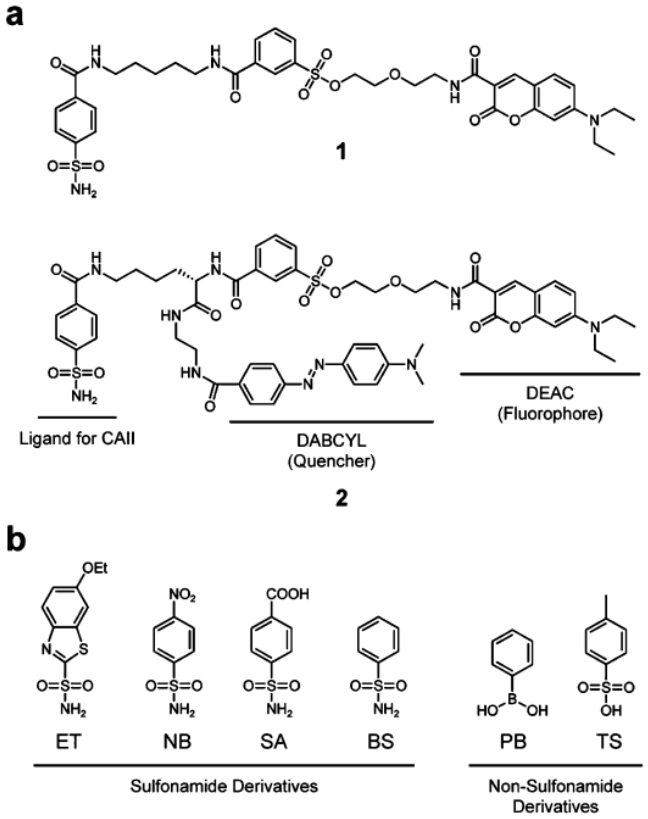
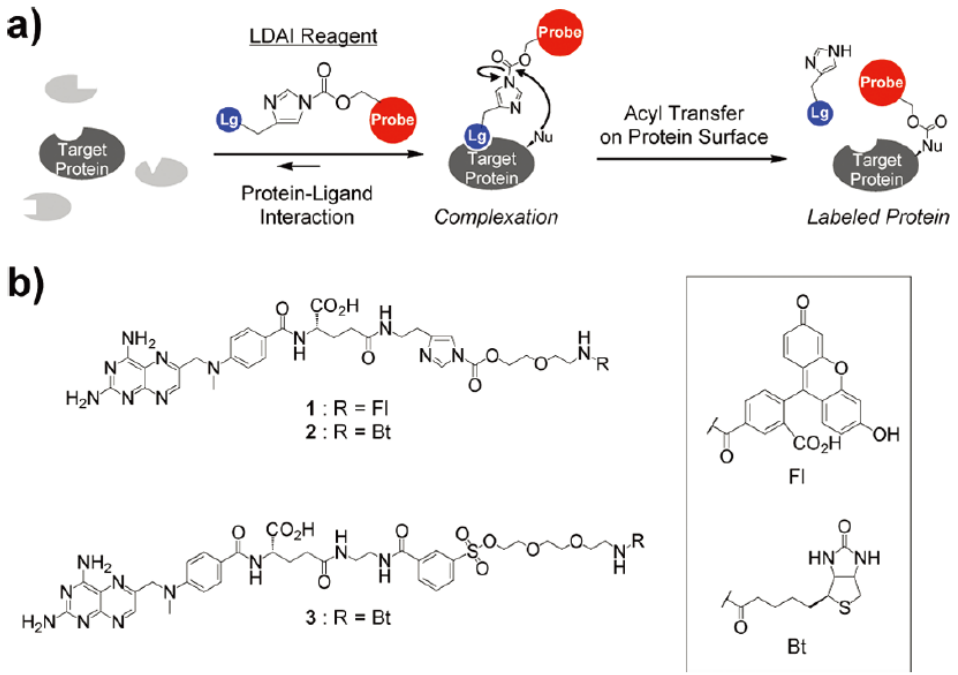


Figure 2. (a) Molecular structures of LDT reagent **1** and Q-LDT reagent **2** designed for CAII labeling. (b) Sulfonamide and nonsulfonamide derivatives used in this study. ET, ethoxzolamide; NB, *p*-nitro-benzenesulfonamide; SA, 4-sulfamoylbenzoic acid; BS, benzenesulfonamide; PB, phenylboronic acid; TS, *p*-toluenesulfonic acid.

JACS_2009_9046



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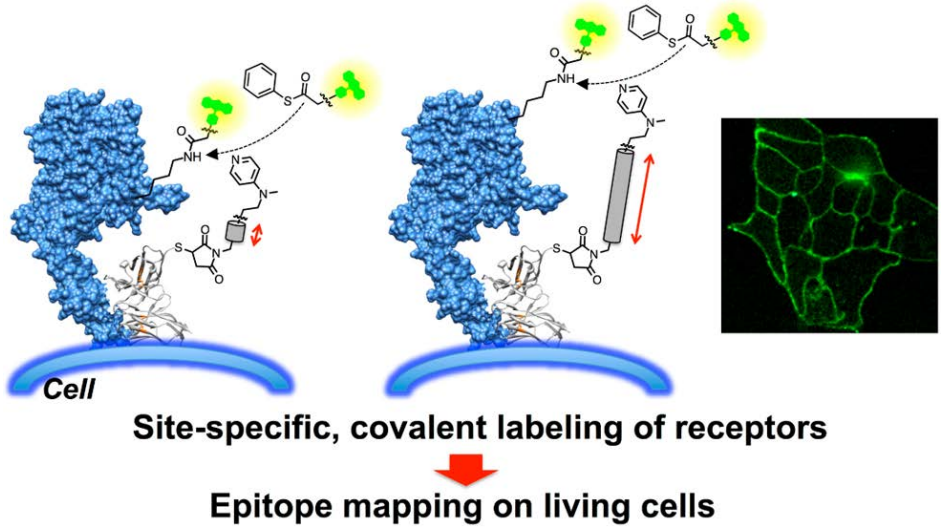
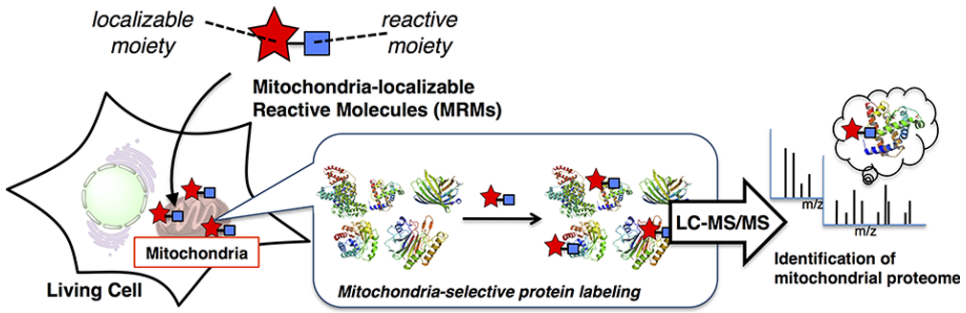
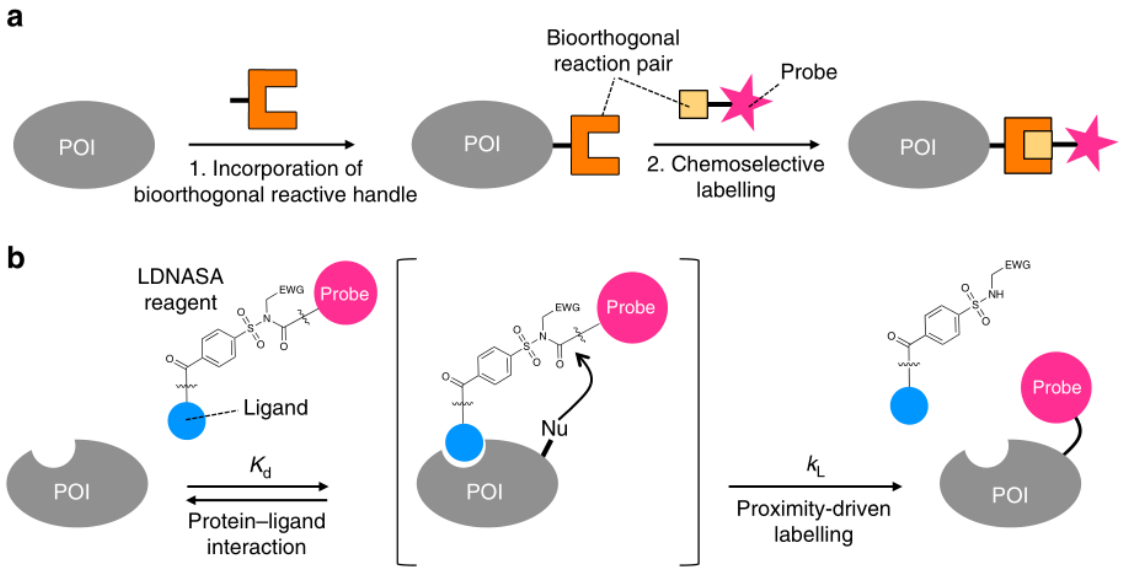


JACS_2014_5374



JACS_2016_7592

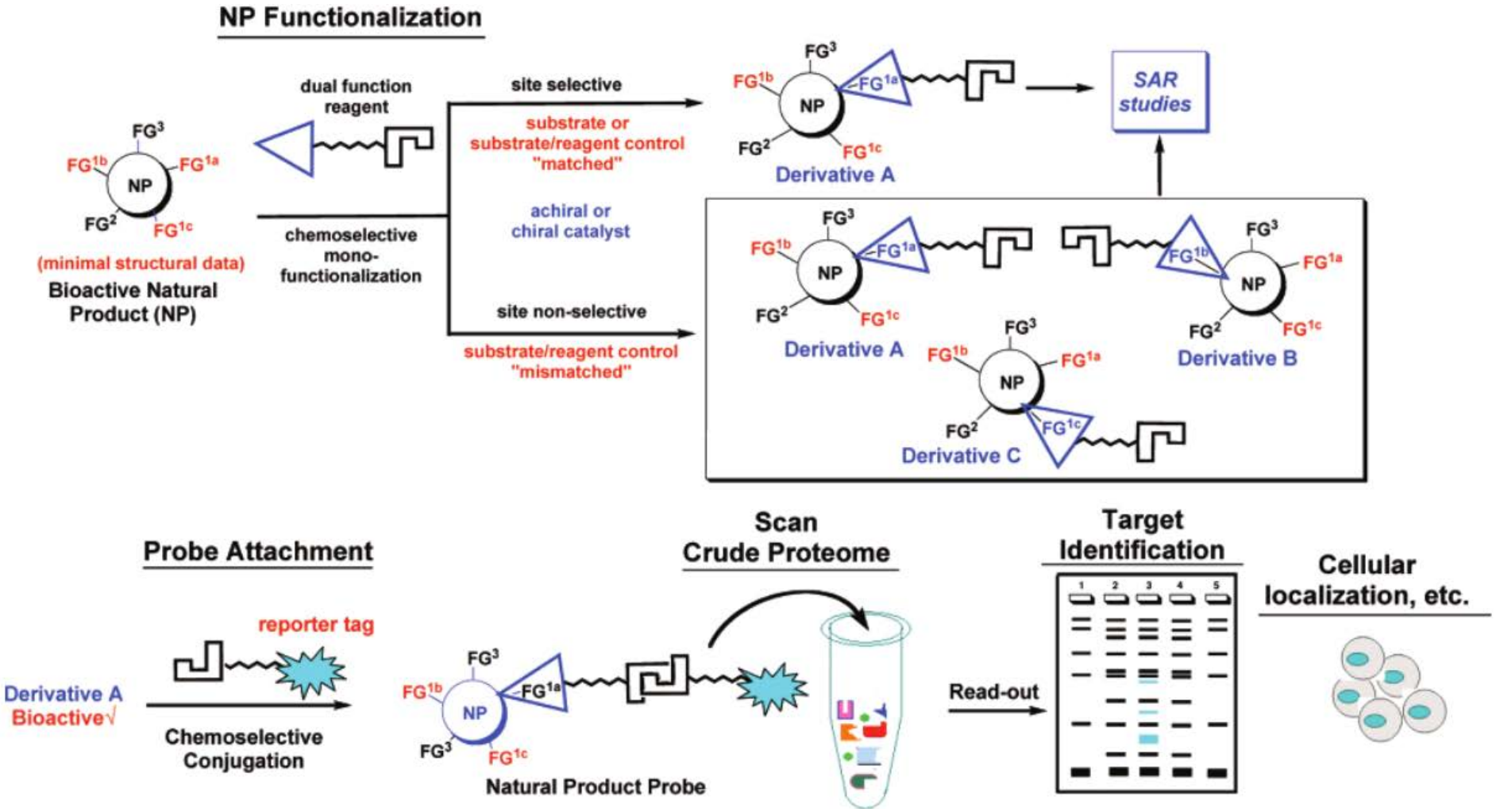


Nature Communications_2018_1870

Simultaneous Arming and Structure/Activity Studies of Natural Products Employing O-H Insertions: An Expedient and Versatile Strategy for Natural Products-Based Chemical Genetics

Satyamaheshwar Peddibhotla,† Yongjun Dang,‡ Jun O. Liu,‡ and Daniel Romo*,†

Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77842-3092, and Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland 21205

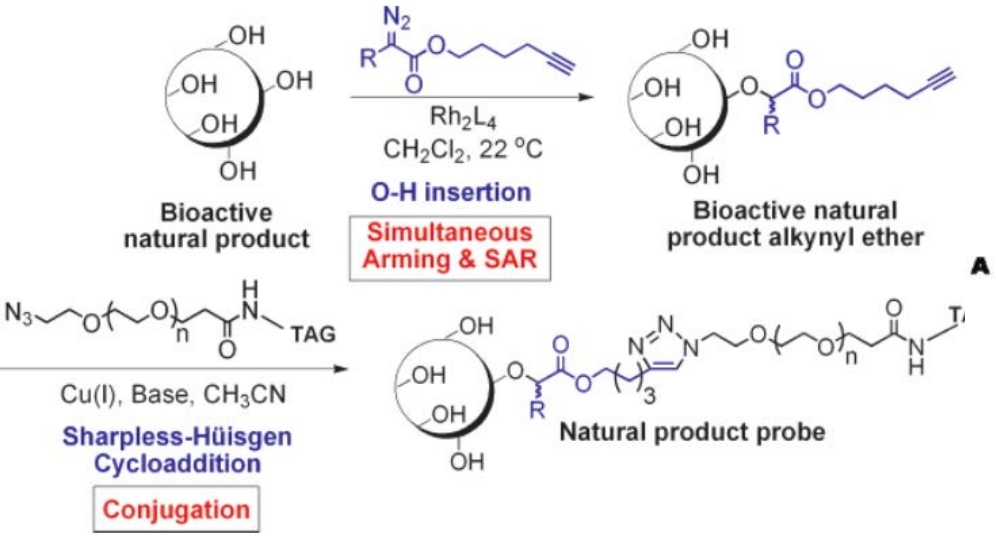


JACS, 2007, 12222

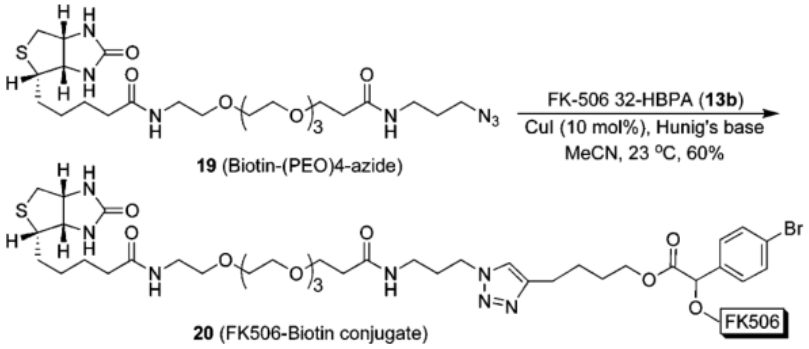
Simultaneous Arming and Structure/Activity Studies of Natural Products Employing O-H Insertions: An Expedient and Versatile Strategy for Natural Products-Based Chemical Genetics

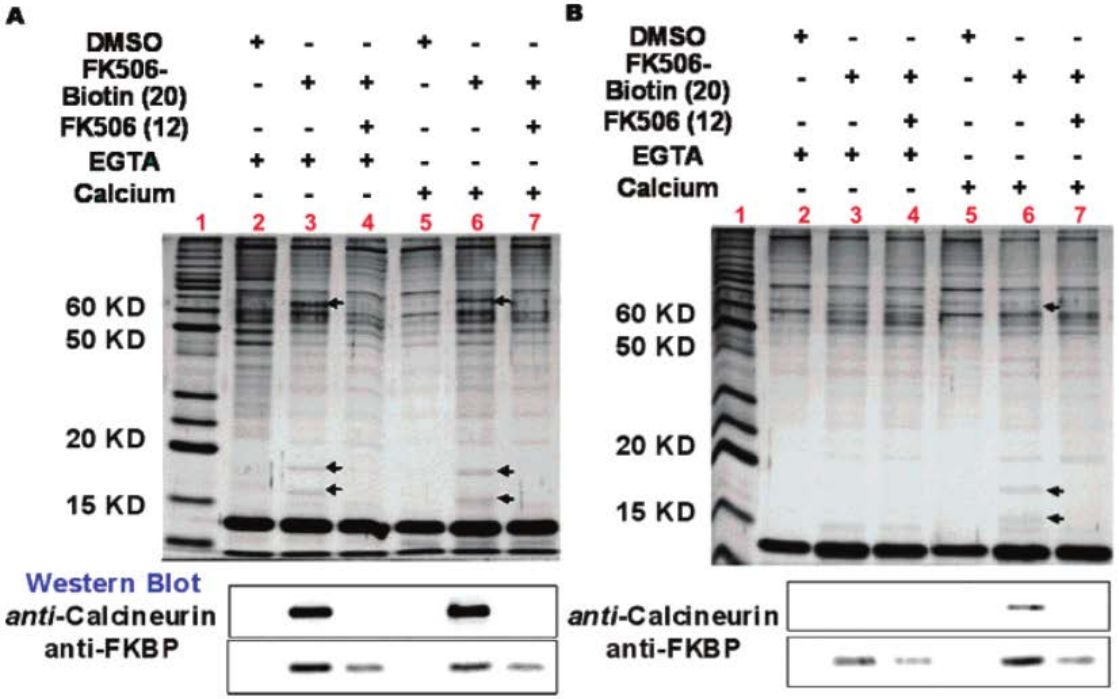
Satyamaheshwar Peddibhotla,† Yongjun Dang,‡ Jun O. Liu,‡ and Daniel Romo*,†

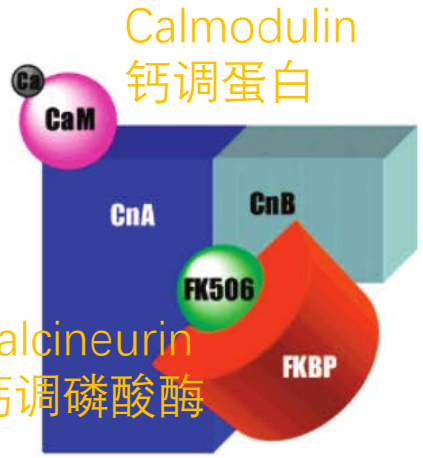
Contribution from the Department of Chemistry, Texas A&M University, College Station, Texas 77842-3092, and Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland 21205



Scheme 2. Synthesis of FK506–Biotin Conjugate **20** via Sharpless–Hüisgen [3 + 2] Cycloaddition



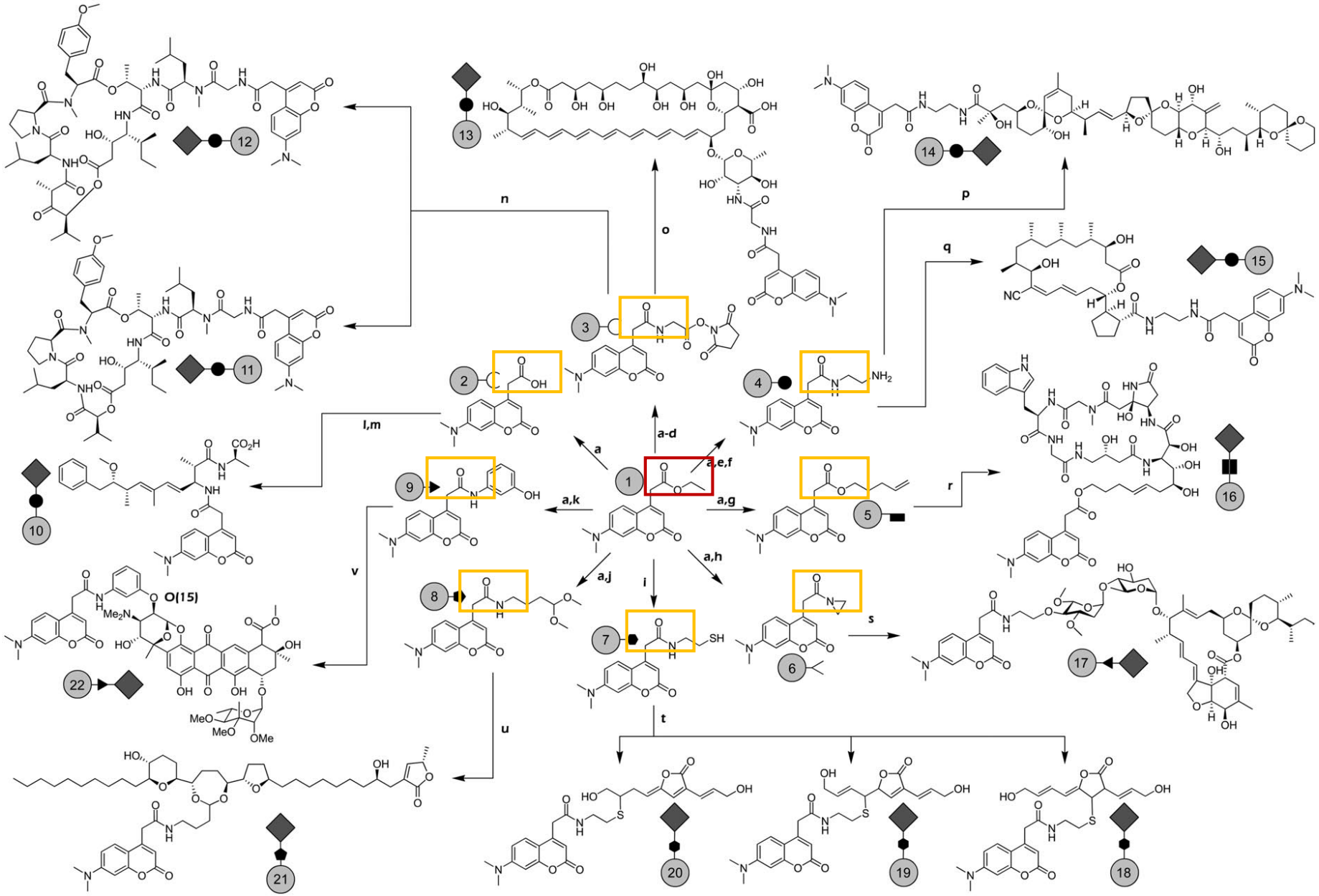




抗钙调磷酸酶

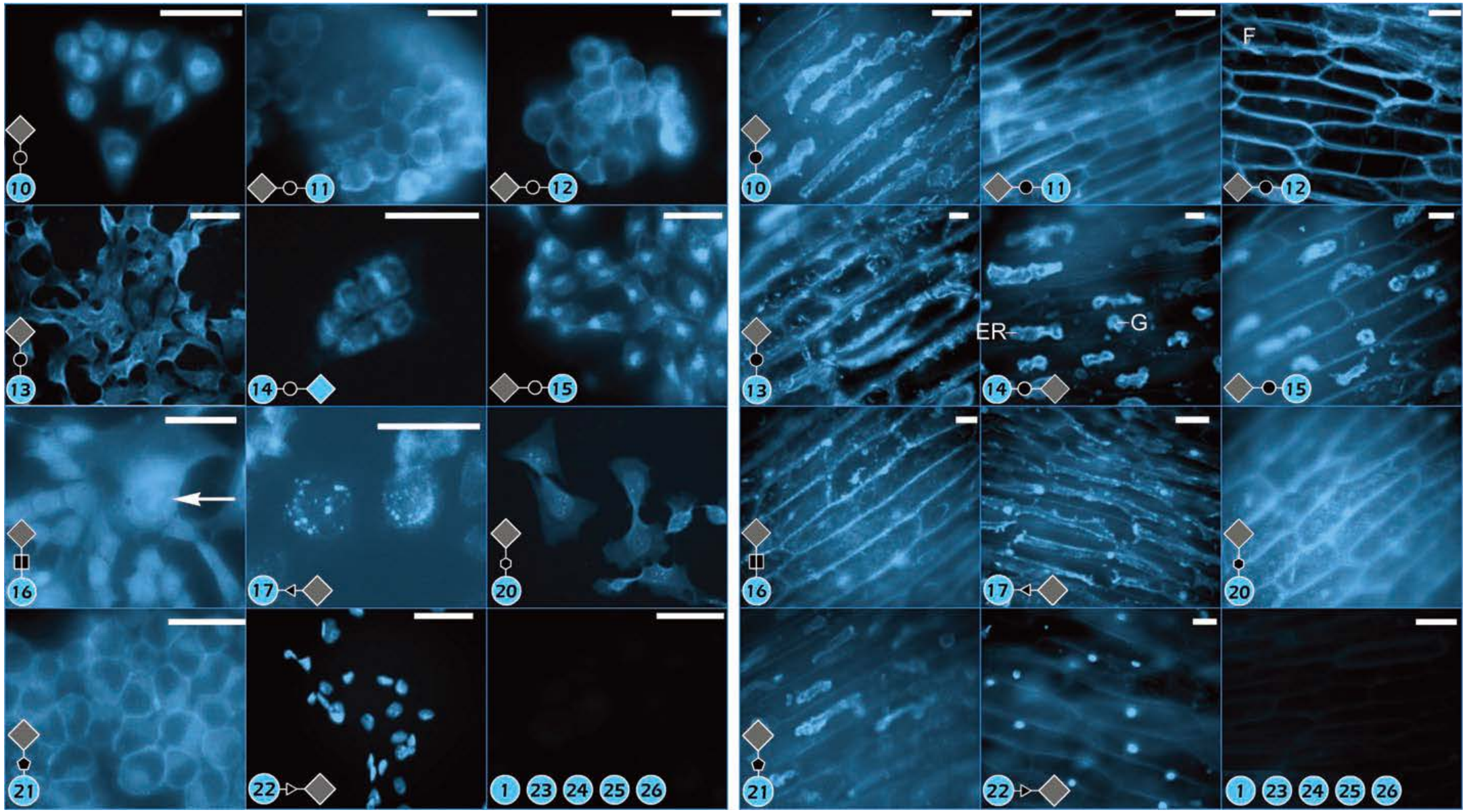
JACS, 2007, 12222

A Central Strategy for Converting Natural Products into Fluorescent Probes



ChemBioChem, 2006, 409

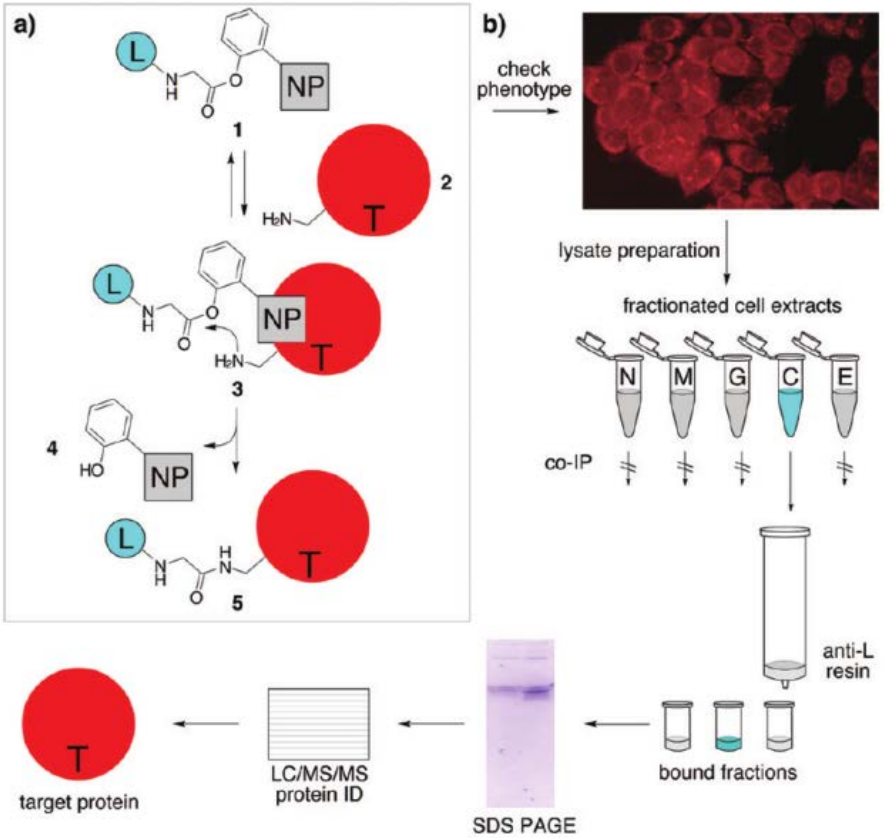
A Central Strategy for Converting Natural Products into Fluorescent Probes

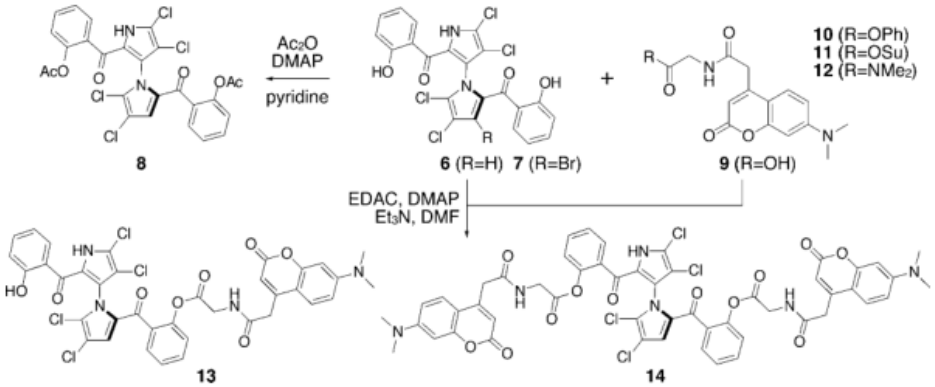


ChemBioChem, 2006, 409

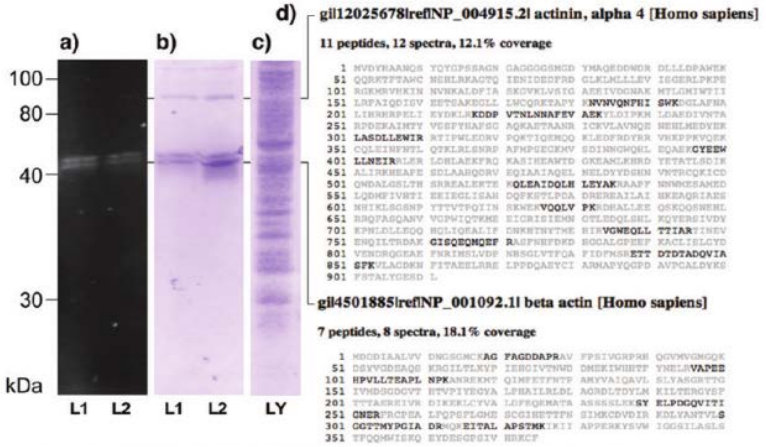
Marinopyrrole A Target Elucidation by Acyl Dye Transfer

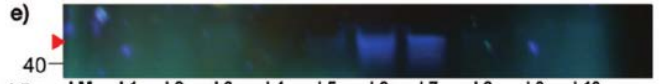
Chambers C. Hughes,† Yu-Liang Yang,‡ Wei-Ting Liu,‡ Pieter C. Dorrestein,*,‡ James J. La Clair,*,§ and William Fenical*,†



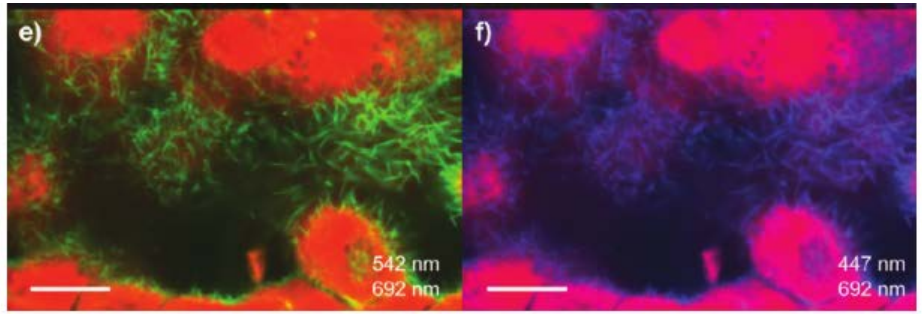


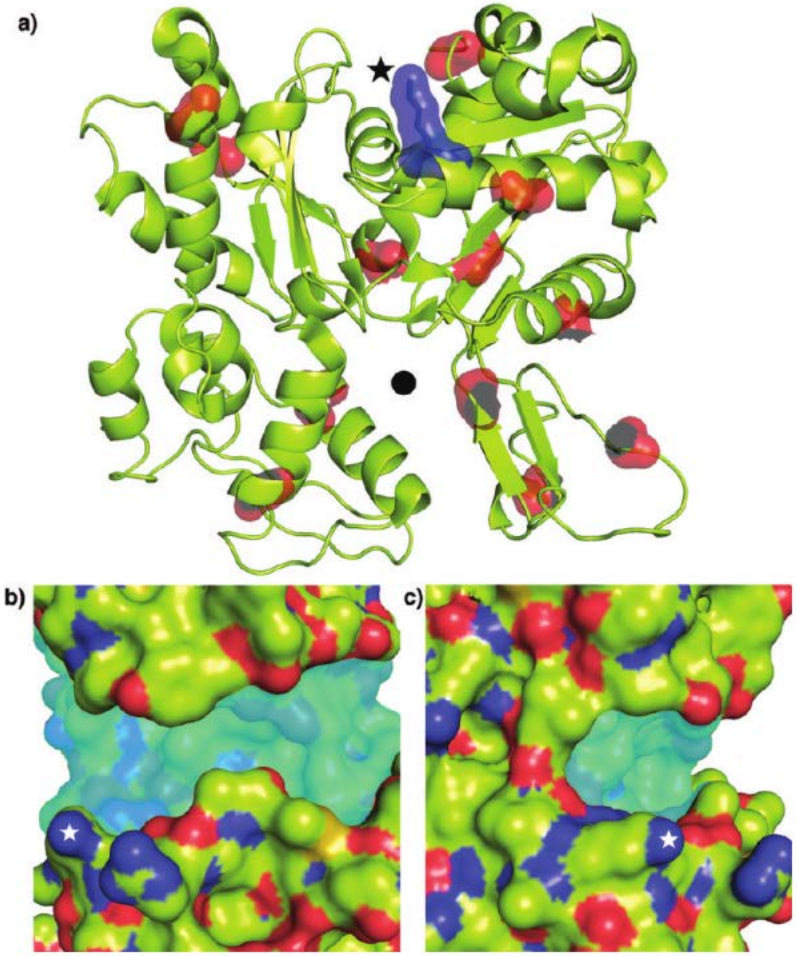
- 双标记导致靶标扩大，影响活性
- 已有的交联方法产率低，选择性差，增加了下游分析的难度
- 天然产物的存在可能参与代谢过程，导致多产物的形成





LM	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	
lm	a	a	a	a	a	a	a	a	a	a	protein
0	0	0	0	0	0	0	0	50	0	0	μM **6**
0	500	500	500	0	0	0	0	0	0	0	μM **10**
0	0	0	0	0	0	0	0	0	500	500	μM **11**
10	0	0	0	0	10	0	10	10	0	0	μM **13**
0	0	0	0	10	0	10	0	0	0	0	μM **14**
12	1	6	12	1	1	6	6	6/6	1	6	time (h)





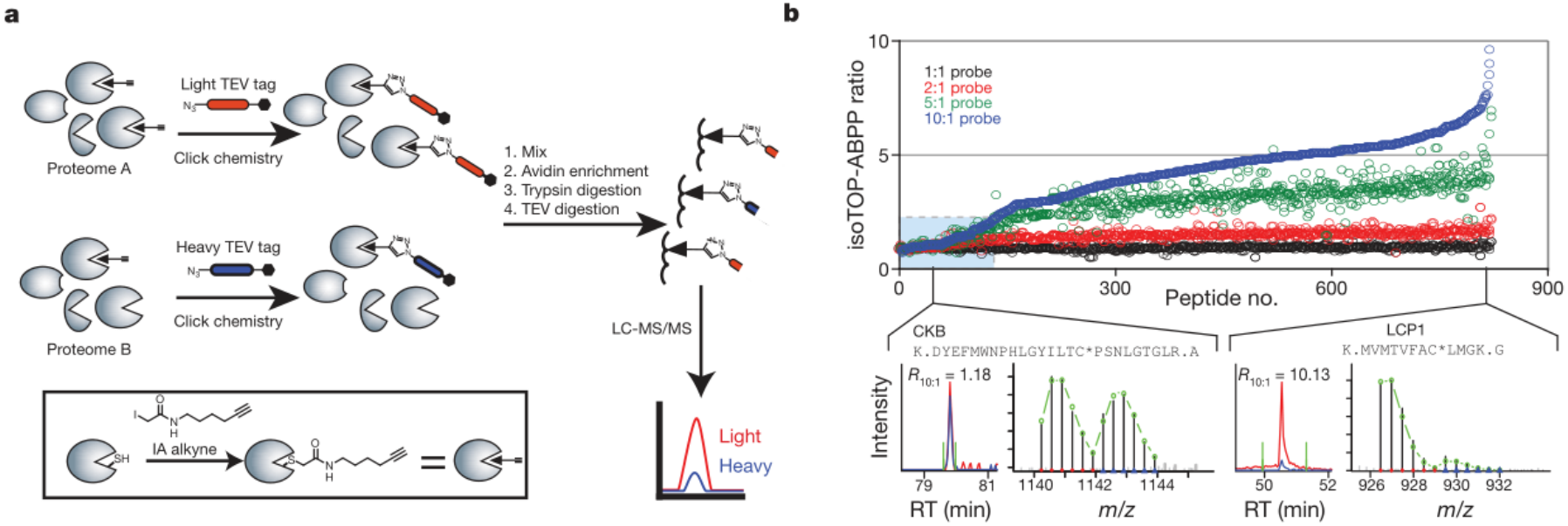
JACS, 2009, 12094

ARTICLE

doi:10.1038/nature09472

Quantitative reactivity profiling predicts functional cysteines in proteomes

Eranthie Weerapana[1,2]*, Chu Wang[1,2]*, Gabriel M. Simon[1,2], Florian Richter[3,4], Sagar Khare[3,5], Myles B. D. Dillon[2], Daniel A. Bachovchin[1,2], Kerri Mowen[2], David Baker[3,4,5] & Benjamin F. Cravatt[1,2]



Nature_2010_790

➢ Summary

Interests：

- 天然产物作用靶点
- 蛋白质活性基团功能、数量等
- 蛋白组学

Modification Methods：

↓ 配体导向

↓ 活性基团引入：SH，Tag，正交基团

↓ 原位反应

Characterization Methods：

↓ SDS-Page/液相-光谱联用

↓ LC/MS/MS

↓ 荧光成像