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

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

Tsukiji, S.; Wang, H.; Miyagawa, M.; Tamura, T.; Takaoka, Y.; Hamachi, I.*
J. Am. Chem. Soc. **2009**, *131*, 9046–9054.

Organic Seminar 2009.06.29
Eri NISHIYAMA (Nakamura lab.)

Protein-based Fluorescent Biosensors

- **Monitoring and quantification of specific biological substances**
- **Investigating diverse biological processes**

Endocytosis

Image of endocytosis of HeLa cell

Zhang, J.; Campbell, R. E.; Ting, A. Y.; Tsien, R. Y. *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 906

- **Drug screening as powerful platforms**

QuickTimeý Ç²
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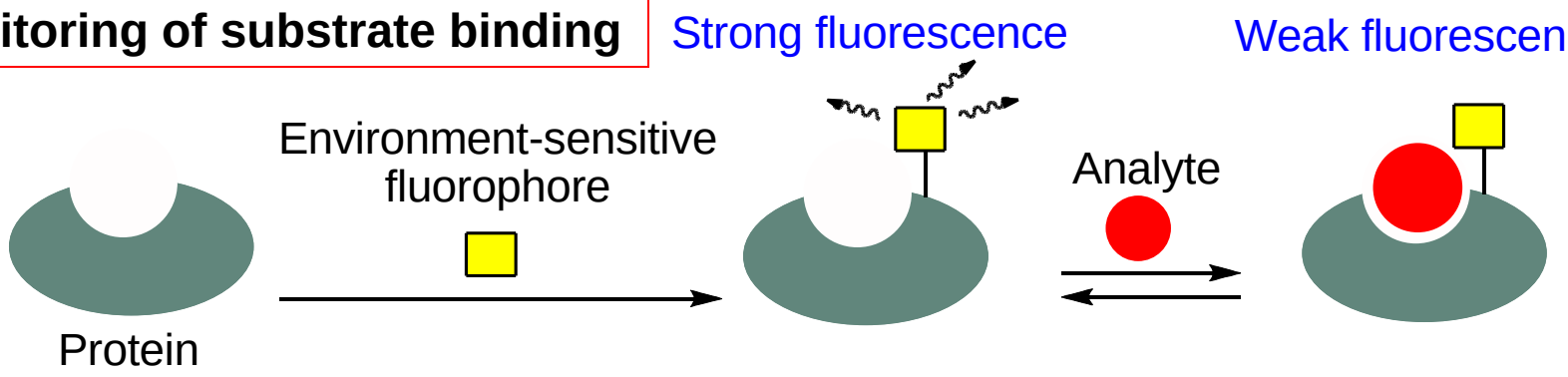
Mouse kidney imaged intravitally after intravenous injection of a nucleotide tagged with AlexaFluor680 (fluorophore)

Bullen, A *Nat. Rev. Drug Discov.* **2008**, *7*, 54.

Conversion of Proteins to Semisynthetic Biosensors via Site-specific Modification

Monitoring of substrate binding



Dynamic biological processes are detected by change in the fluorescent intensity

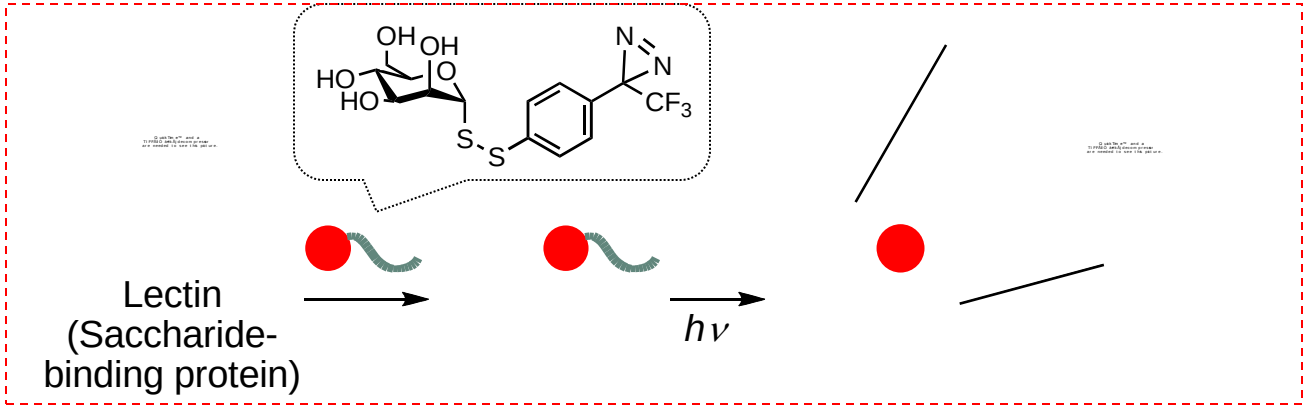
Problem 1: Limitation of protein

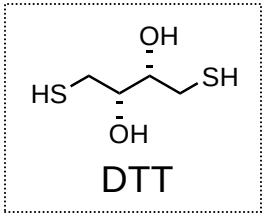
Genetic incorporation of a specific moiety (usually cysteine residue for thiol chemistry) into proteins is needed for modification with fluorophore.
=> Previous Work 1

Problem 2: The signal change is small.

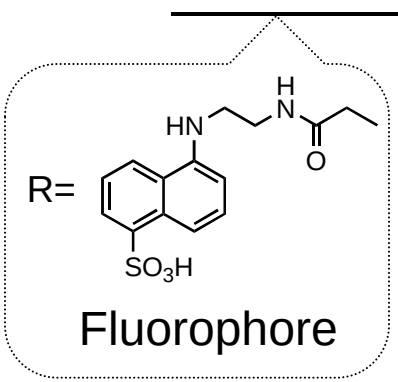
A method for construction of turn-on fluorescent biosensor is needed.
=> Previous Work 2

Previous Work 1: Ligand-directed Protein Labeling Method





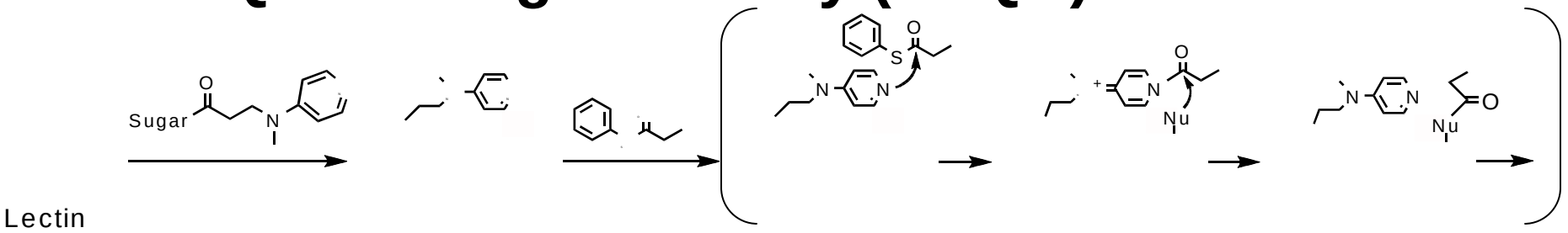
Strong fluorescence **Weak fluorescence**



J. Am. Chem. Soc. **2000**,*122*, 12065.

Labeling without genetic mutations but insignificant change in intensity of fluorescence
(Strong/weak fluorescent intensity =10/7)

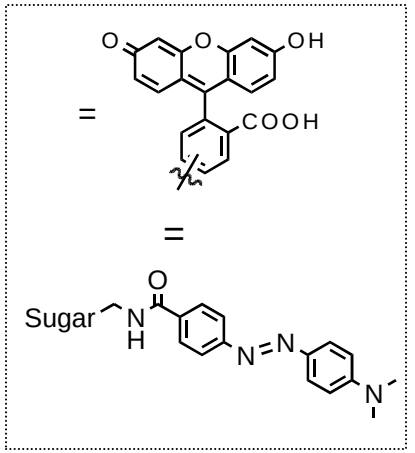
Previous Work 2: Bimolecular Fluorescence Quenching/Recovery (BFQR) Method



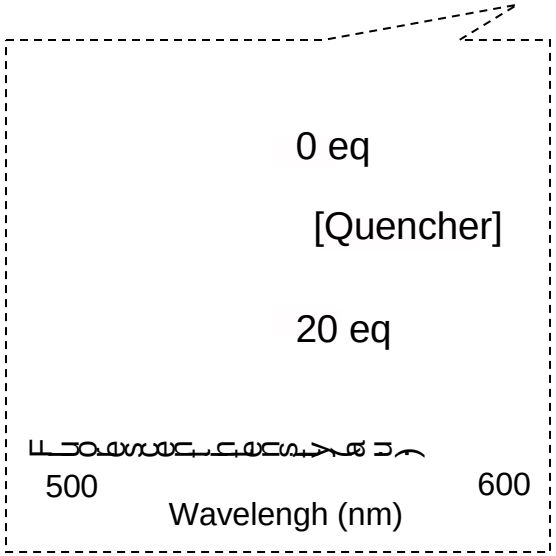
Fluorophore

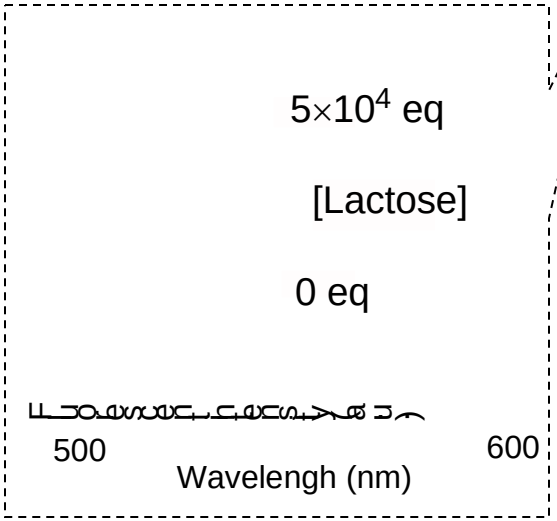
Quencher

Saccharide



J. Am. Chem. Soc. **2008**, *130*, 245.

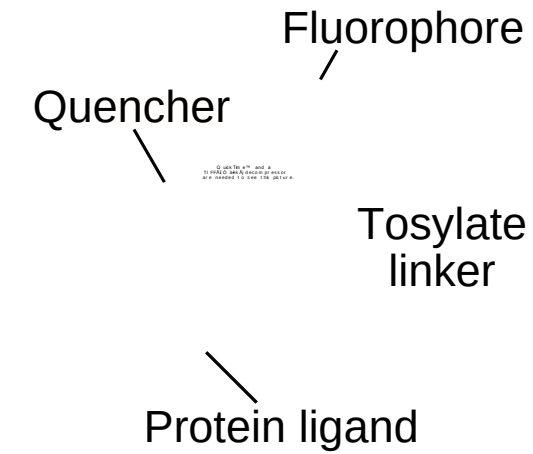


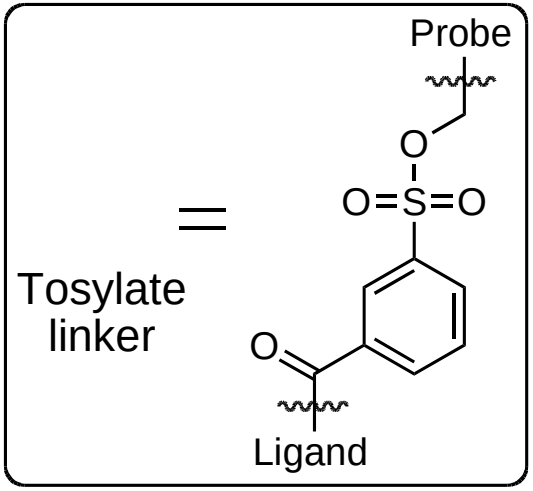


Efficient fluorescence quenching/recovery but need for multiple steps In the preparation

This Work: Quenched Ligand-directed Tosylate (Q-LDT) Method

Q-LDT reagent

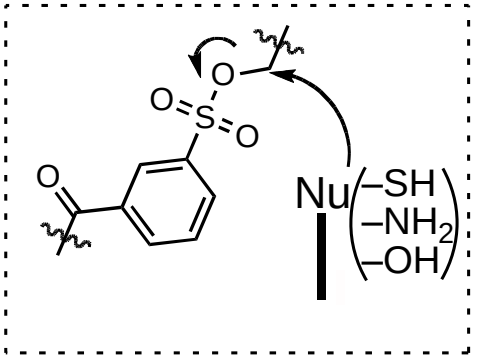




Q-LDT reagent

Protein

Quenching



BFQR scheme

Turn-on fluorescent biosensors by a single labeling step

Q-LDT Reagent for CAII

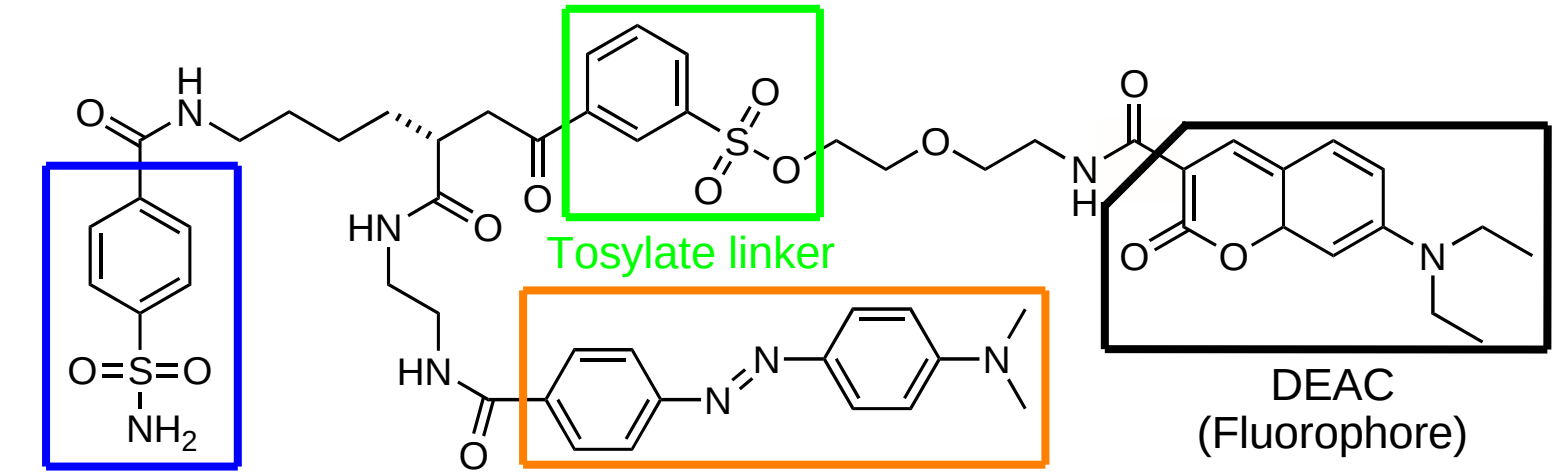
Model protein scaffold: human carbonic anhydrase II (CAII)

CAII :

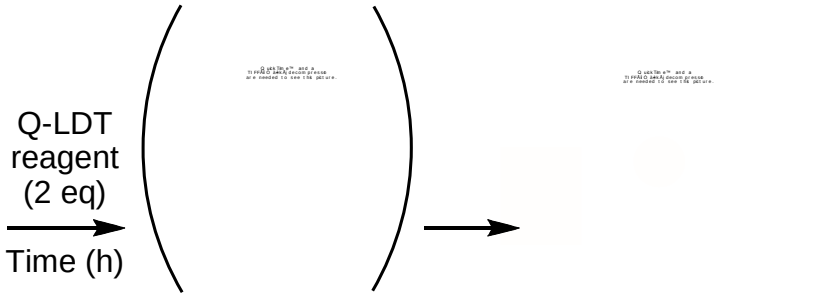
- A group of metalloenzymes involved in numerous physiological and pathological processes
- A specific ligand of CAII inhibitor: benzenesulfonamide

Conversion of CAII to a fluorescent biosensor toward its inhibitors via Q-LDT method

*Q-LDT reagent (**1**) for the biosensor based on CAII*

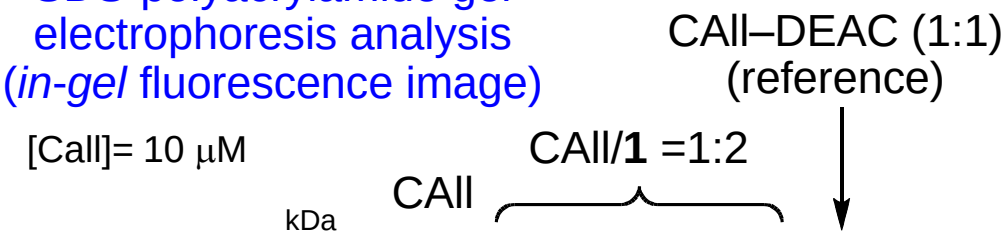


Reactivity and Site-specificity of Q-LDT reagent



Covalent modification of CAII with DEAC in a time-dependent manner

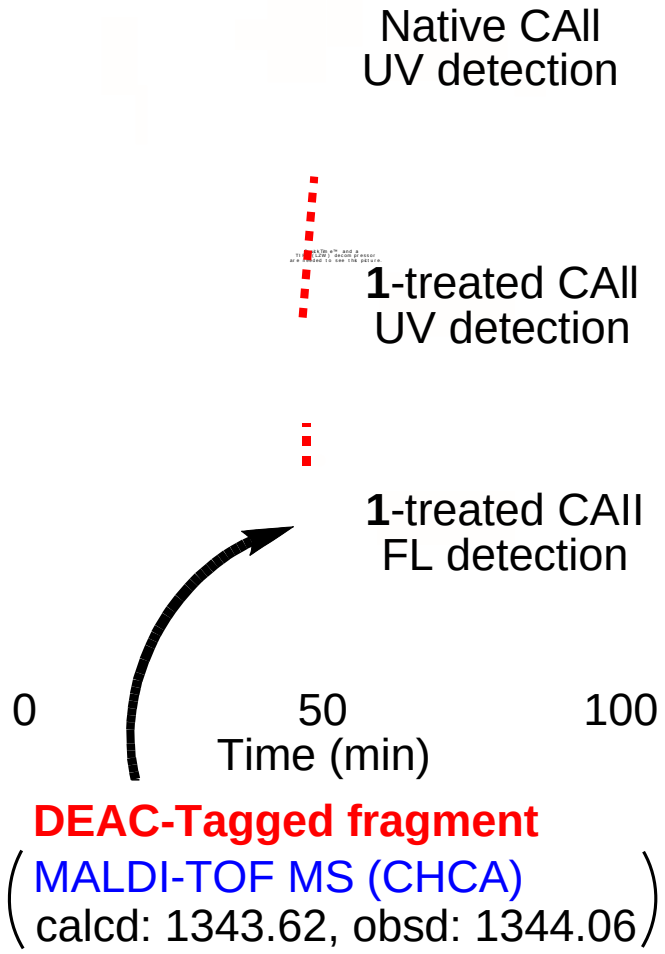
SDS-polyacrylamide gel electrophoresis analysis (*in-gel* fluorescence image)



Time (h)	48	6	24	48	–
Labeling yield (%)	0	24	37	48	100

Site-specificity

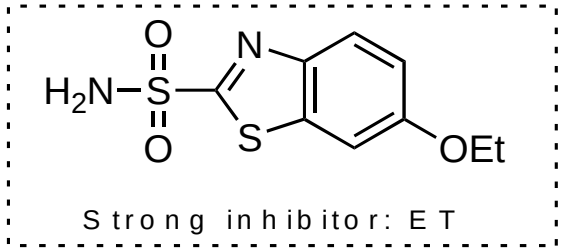
Reversed-phase HPLC of CAII fragments (digested at specific site by enzyme)



DEAC-Tagged fragment

MALDI-TOF MS (CHCA)
calcd: 1343.62, obsd: 1344.06

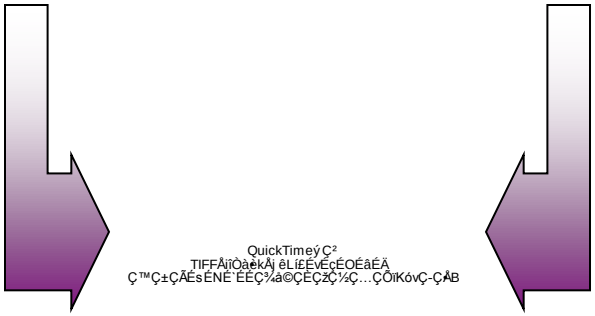
Efficient Fluorescence Quenching/Recovery

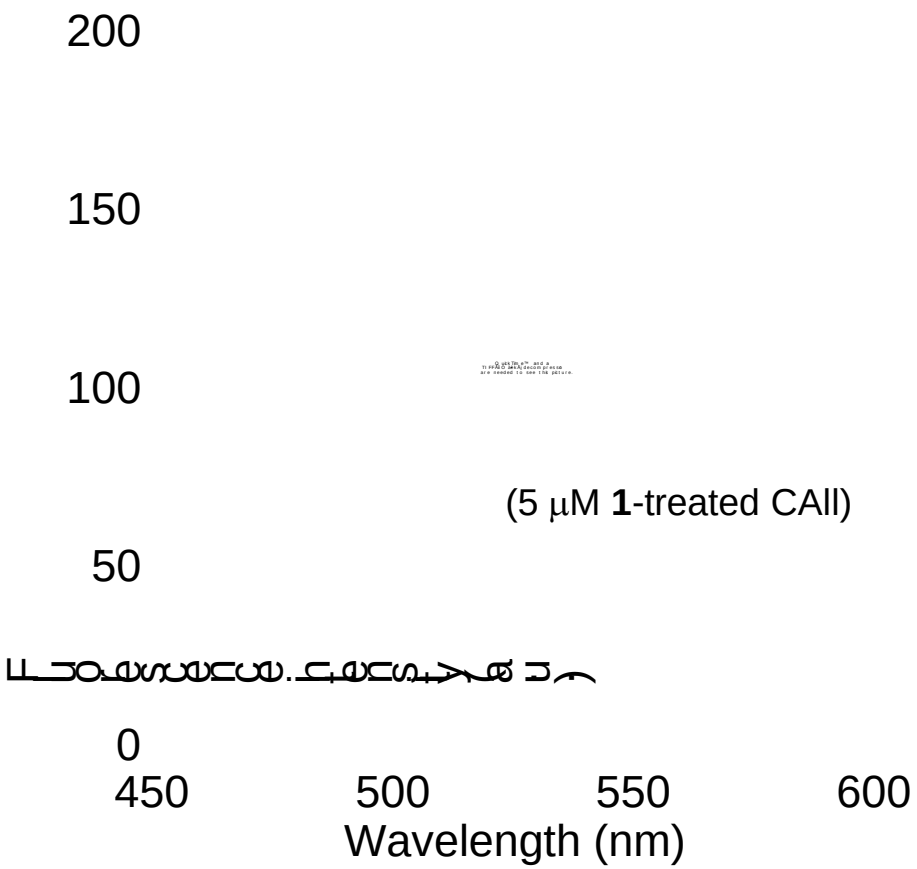


Quenching

‖

Analyte



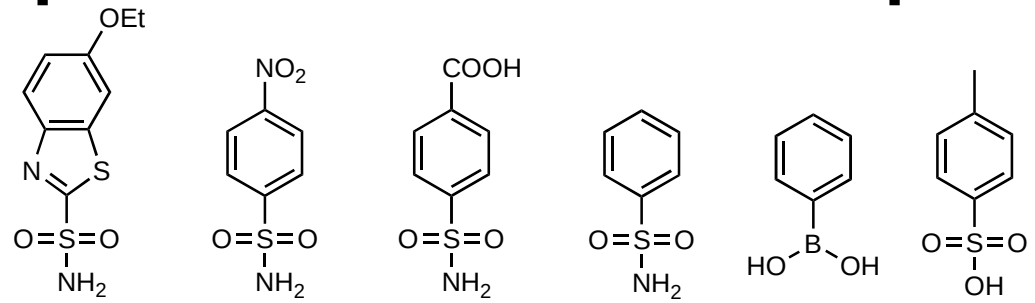


(5 μM **1**-treated CAII)

(25 μM **1**-treated CAII + 175 μM ET, λ_{ex} =365 nm)

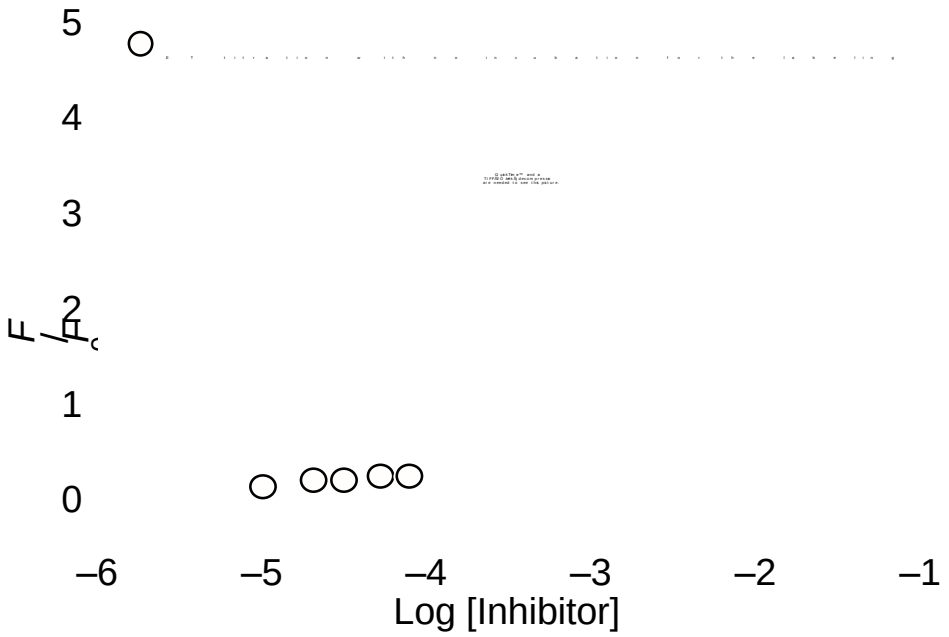
Fluorescence was recovered by 5-fold.
=> Expulsion of the quencher after adding the inhibitor(ET) was confirmed.

Fluorescence Response toward Various Species



ET NB SA BS PB TS

	ET	NB	SA	BS	PB	TS
Apparent binding constant (M^{-1})	5	8.2×10^{4}	6.5×10^{3}	9.9×10^{3}	n.d.	n.d.
Calculated binding constant (M^{-1})	3.3×10^{8}	3.4×10^{7}	2.7×10^{6}	4.1×10^{6}	n.d.	n.d.
Reported binding constant (M^{-1})	1.3×10^{8}	1.6×10^{7}	3.7×10^{6}	6.5×10^{5}	9.4×10^{1}	no value



- Fluorescence selectively recovered only after adding sulfonamide derivatives.
- Binding constant for ET: 3.3×10^{8} M^{-1} (1.3×10^{8} M^{-1}) => The binding affinity of **1**-treated CAII was retained.

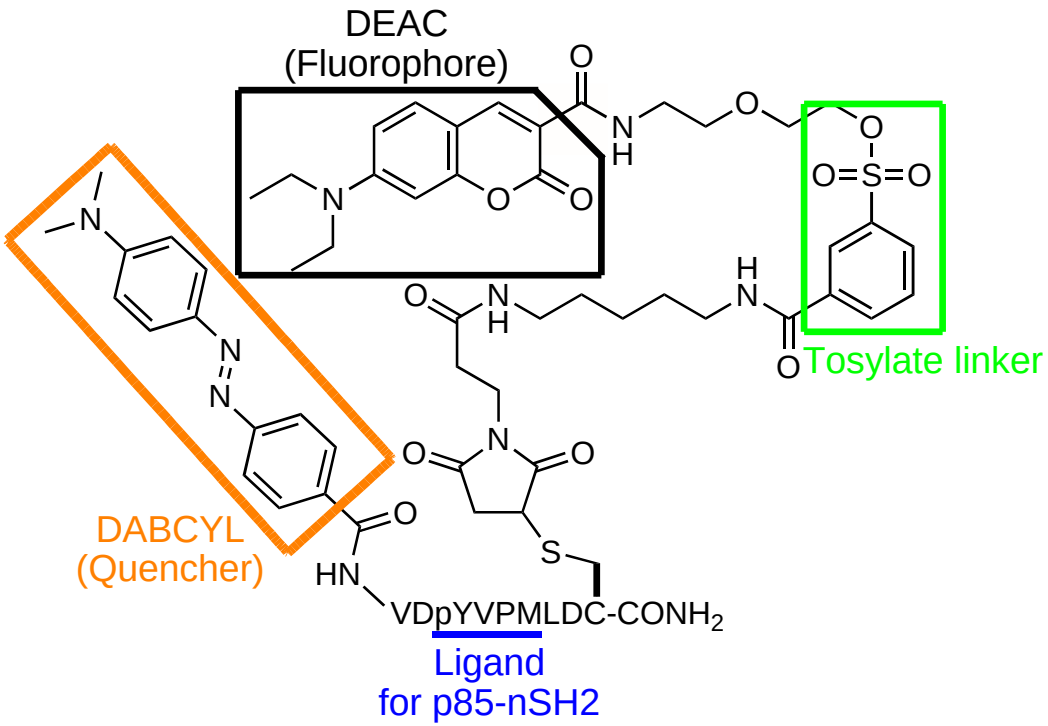
Q-LDT Biosensors toward Phosphorylated Peptides

Protein phosphorylation plays a central role in the regulation of cell function.

Model protein scaffold: N-terminal SH2 domain of the p85α subunit of human phosphatidylinositol-3-kinase (PI3K) (p85-nSH2)

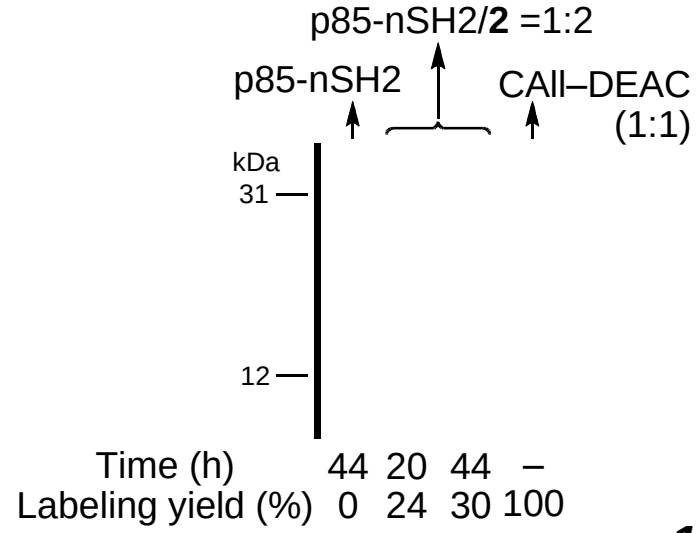
• SH2 domain recognizes peptides containing a pYZXM (pY, phosphotyrosine; Z, Met or Val; X, any amino acid)

*Q-LDT reagent containing the peptide (**2**)*



Q-LDT chemistry worked with SH2 domain of p85-nSH2

SDS-PAGE analysis
(*in-gel* fluorescence)

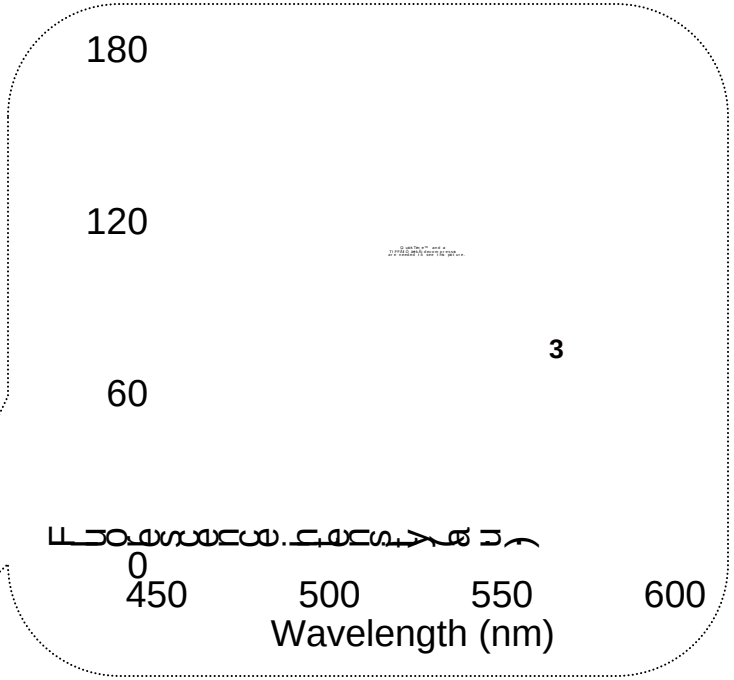


High Sensing Selectivity toward Specific Peptides

Peptides used in the analysis

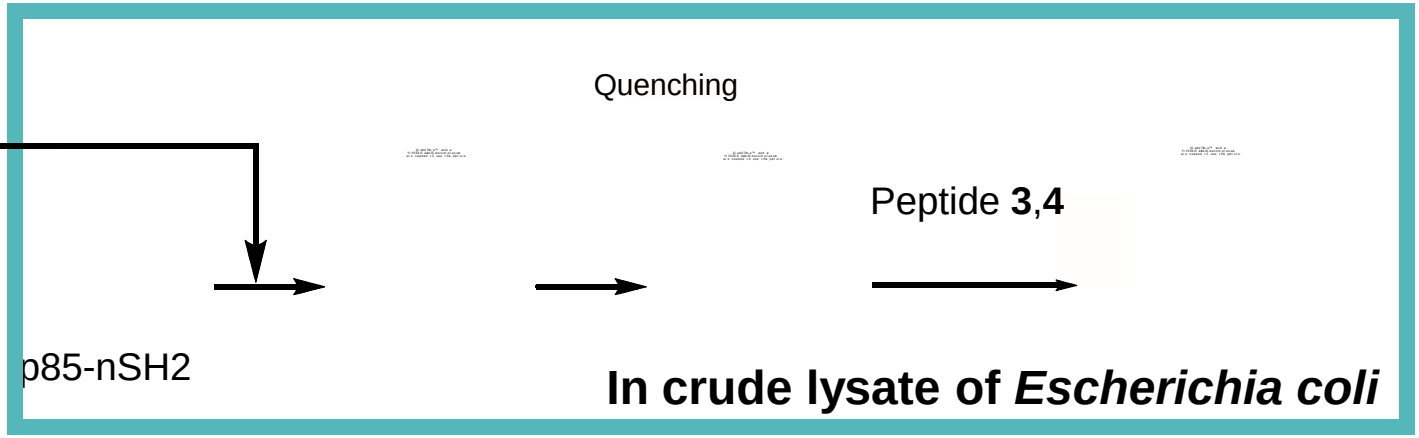
3
4
5
6
7
8
9
10

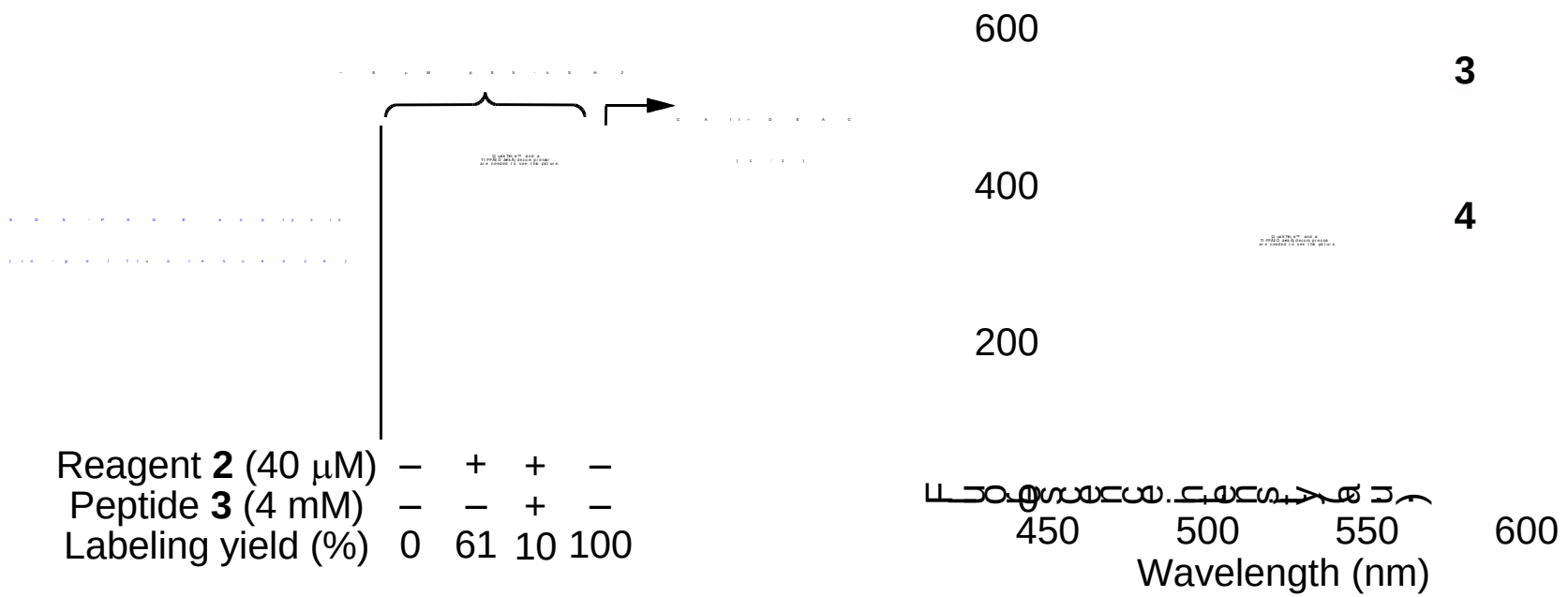
Peptides	pYZXM	F/F_0
3	+	2.7 (120 μM)
4	–	1.2 (200 μM)
5	–	1.7 (200 μM)
6	+	2.4 (120 μM)
7	+	2.6 (120 μM)
8	+	2.1 (120 μM)
9	–	1.7 (200 μM)
10	–	2.5 (150 μM)



- Fluorescence recovered selectively after adding peptides with pYZXM.
- Peptide **9**,**10**: Natural ligands for the SH2 domain family
=> Sensing selectivity agrees well with the affinity of natural p85-nSH2.

Q-LDT Biosensors in Crude Mixture





The first demonstration of semisynthetic biosensor in lysate of the cell

Conclusion

• A new powerful methodology was established to convert target proteins to fluorescent biosensors for detection of dynamic biological processes in a one-step labeling procedure without genetic mutations.

• Q-LDT method can be applied to two different protein scaffolds and used in vitro also.

• The first demonstration of the construction of a semisynthetic biosensor in a crude mixture