Bioluminescence, series 1

FIREFLY

Yoshihiro Ohmiya

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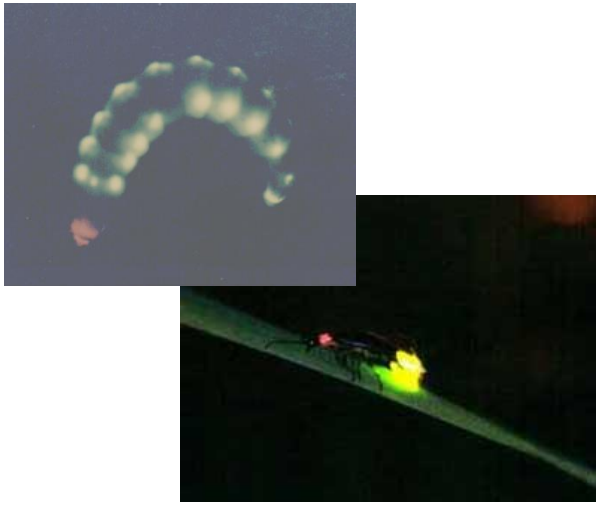
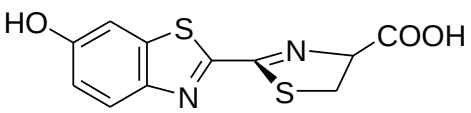
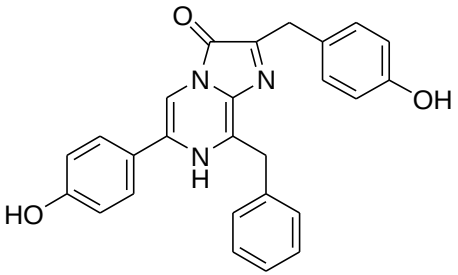
Adjunct Professor of VISTEC (Thailand)

Luminescent organism and their principle mechanism

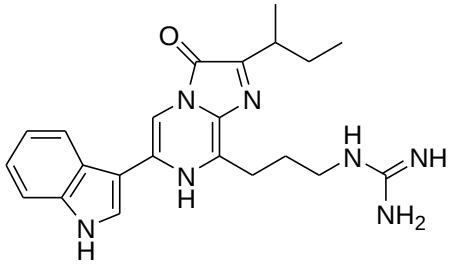




Firefly luciferin
(1961, E. H. White)



coelenterazine
(1975, S. Inoue)



Cypridina luciferin
(1965, Y. Hirata)

$$O_2 + \text{luciferin} \xrightarrow[\text{luciferase}]{} [\ \text{oxyluciferin}\ ]^* \rightarrow \text{oxyluciferin} + h\nu$$

History of Firefly in Literature

In India;

The Sanskrit word, *khadyota*, meaning firefly or grow-worm. In the Upanishadds, the part of Brahmanas probably recorded at some time before the sixth century B.C.,

Fog, smoke, sun, fire, wind,
Fireflies, lightning, a crystal, a moon
There are the preliminary appearances,
Which produce the manifestation of Brahma in Yoga.

The *Mahabharata*, of date and author unknown (200 B.C. –A.D. 200?), is probably the longest poem for firefly.

In the Buddhist scriptures, the *Dhammapada*, the Pali word, *khajjopakana*, is used firefly, who lived ca. 562-482 B.C.

History of Firefly in Literature

By ARISTOTLE in Greece;

It is with Aristotle (382-322 B.C.) that wide knowledge of **cold light** begins. He not only listed some well-known luminescence but realized that they were different from other bodies which had color and could be seen by day.

By PLINY in Roma;

Omission of luminescence has been in part balanced by the writing of Caius Plinius Secundus, Pliny the Elder (A.D. 23-79). Pliny's military career took him to all parts of the ancient world. His descriptions of luminescence were quite specific and complete as a scientific view. In the *Historia Naturalis*, written in the first century A.D., there is mention of the glowworm and others.

History of Firefly in Literature

In Japan;

Around the seventh century, the Japanese official historical book, *Nihon-shoki*, described the firefly as a devil god.

In the *Wa-Kan-Sansai-Zue*, Ryokan Terajima published in 1712 by the Japanese about natural products. He described that firefly belongs among the *kasei-rui*, insects transformed from decaying grasses.

The firefly festival on the Ugi River was an important event in the neighborhood of Kyoto from the early of eleventh century.

“HOTARU-Kari (firefly hunting)” in “Uki-yo-e”

“HOTARU-no-Sato” and “HOTARU-no-En”

Three key researchers for firefly research

Bacon (1627), Boyle (1668), Benjamin Franklin (1750), Lois Pasteur (1864) and others had been studied the bioluminescence.

Around 1900, three scientists researched the bioluminescence.

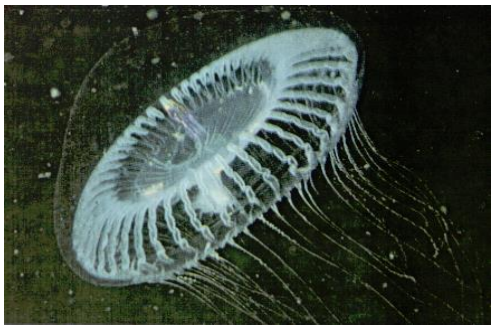
R. Dubois discovered the luciferin-luciferase reaction of luminescent click-beetle. (1884)

S. Kanda studied the Japanese fireflies and other luminescent organisms in Japan (1874-1939) and published the textbook of “HOTARU” in 1935.

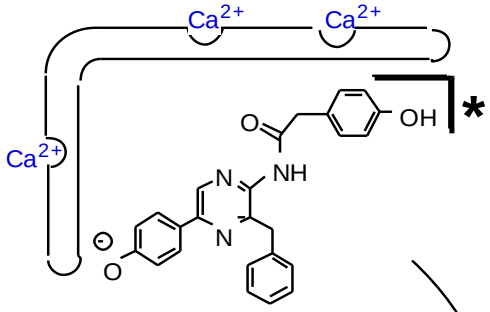
E.N. Harvey studied lots of luminescent organism, visited to Toyama, Japan to study the sea-firefly squid in 1916, and published the textbook of “BIOLUMINESCENCE” in 1953.

Prof Osamu Shimomura discovered GFP

Bioluminescence of Aequorea victoria



singlet-excited GFP

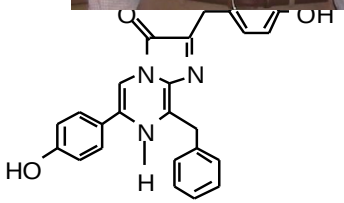


singlet-excited BFP

460 nm Light
(blue)

Energy Transfer

515 nm Light
(green)



Coelenterazine

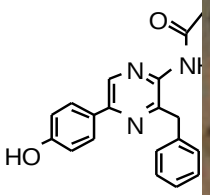
O_2, EDTA
(dithiothreitol)

Apoaequorin

- Coelenteran

+ Coelentera

189 amino acid residue
mw=21,400



Coelenteram

Fluorescent Protein
(GFP)

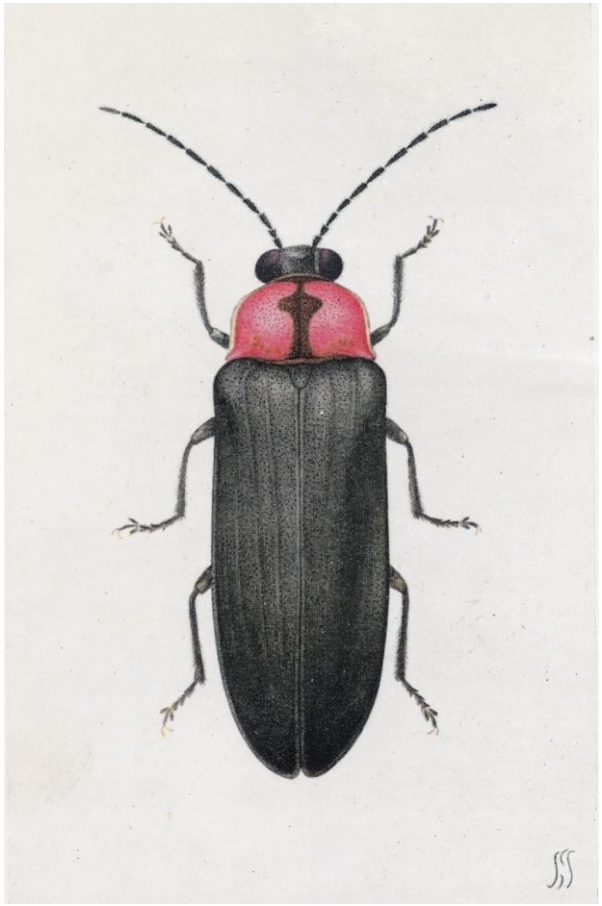
8 amino acid residue
mw=26,900

Prof Woodland Hastings named GFP

- Diversity of luminescent beetles
- Gene hunting for beetle luciferases
- Molecular mechanism of color differences
- Determining the quantum yield of bioluminescence reaction
- Biochemistry of luciferin synthesis

Diversity of luminescent beetles

Japanese firefly (Hotaru)
Genji-botaru and Heike-botaru



Luciola cruciata (male). Reproduced from Kanda (1935).

Luciola lateralis (male). Photographed by Niwa (2008).

Luminescent beetle are consisted of three genera

Drilidae: Rhagophamidae

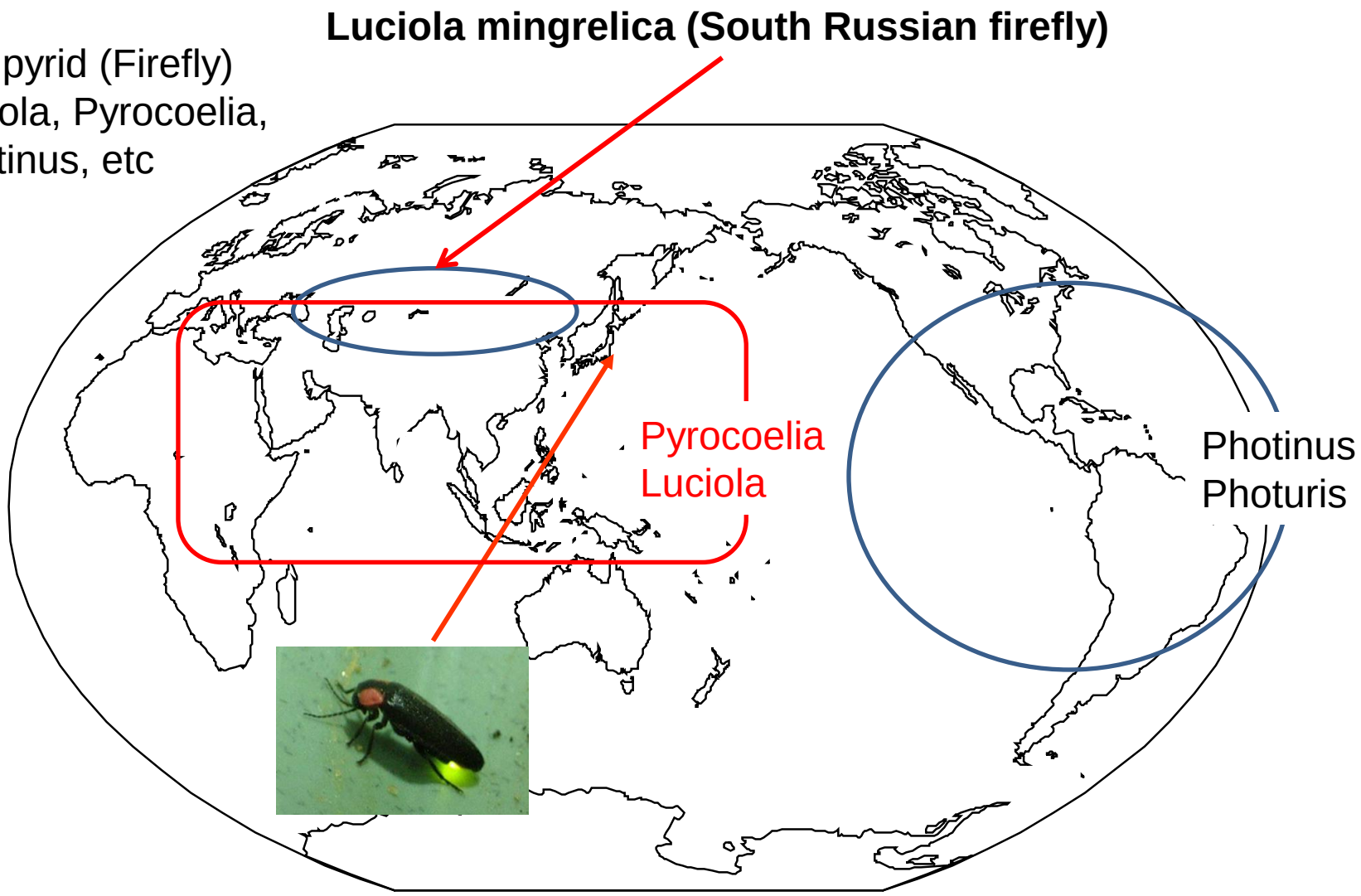
Elaterid (clickbeetle)

Lampyrid (Firefly)
Luciola, Pyrocoelia,
Photinus, etc

Drilidae: Phengodidae
(railroad worm)

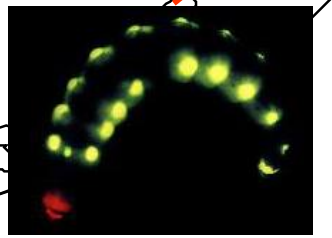
Firefly are distributed in world widely

Lampyrid (Firefly)
Luciola, Pyrocoelia,
Photinus, etc



Drilidae are distributed in the south America and the east Asia

Rhagophamidae

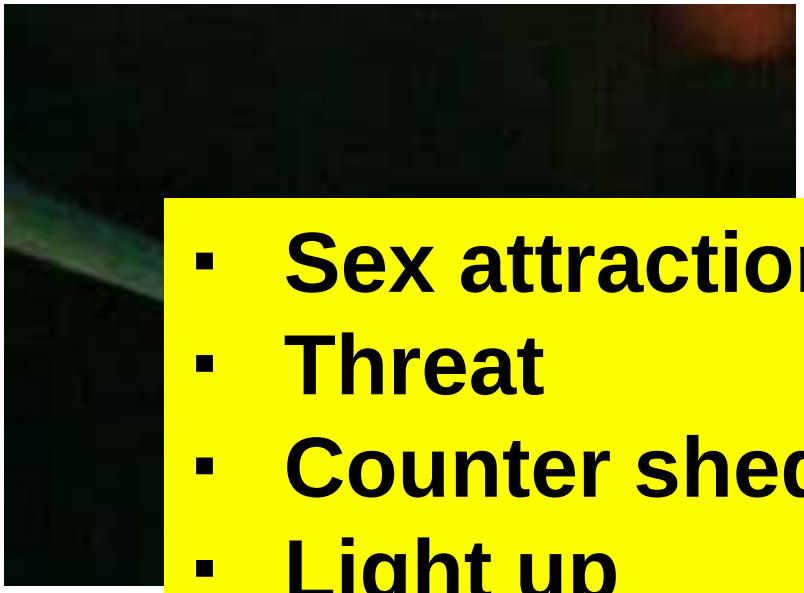


Phengodid (railroad worm)

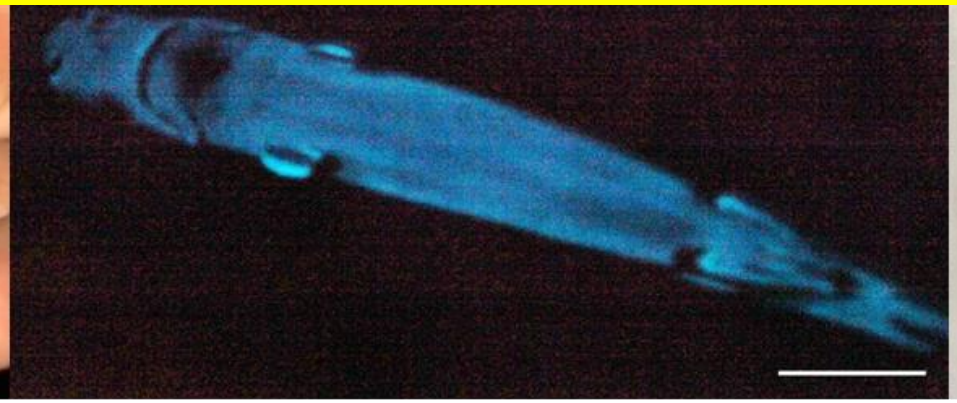
My research of bioluminescent biology; Trip in Tibet

To collect click beetle in Brazil

Diversity on bioluminescent organism



- Sex attraction (Communication)
- Threat
- Counter shedding
- Light up
- Light trap like a food



10mm

50mm

10mm

Gene hunting for beetle luciferases

Bioluminescence is a chemical reaction

- Representation of chemical reaction in vitro

Extracts by hot water

Extracts by cold water

Luciferin

Luciferase

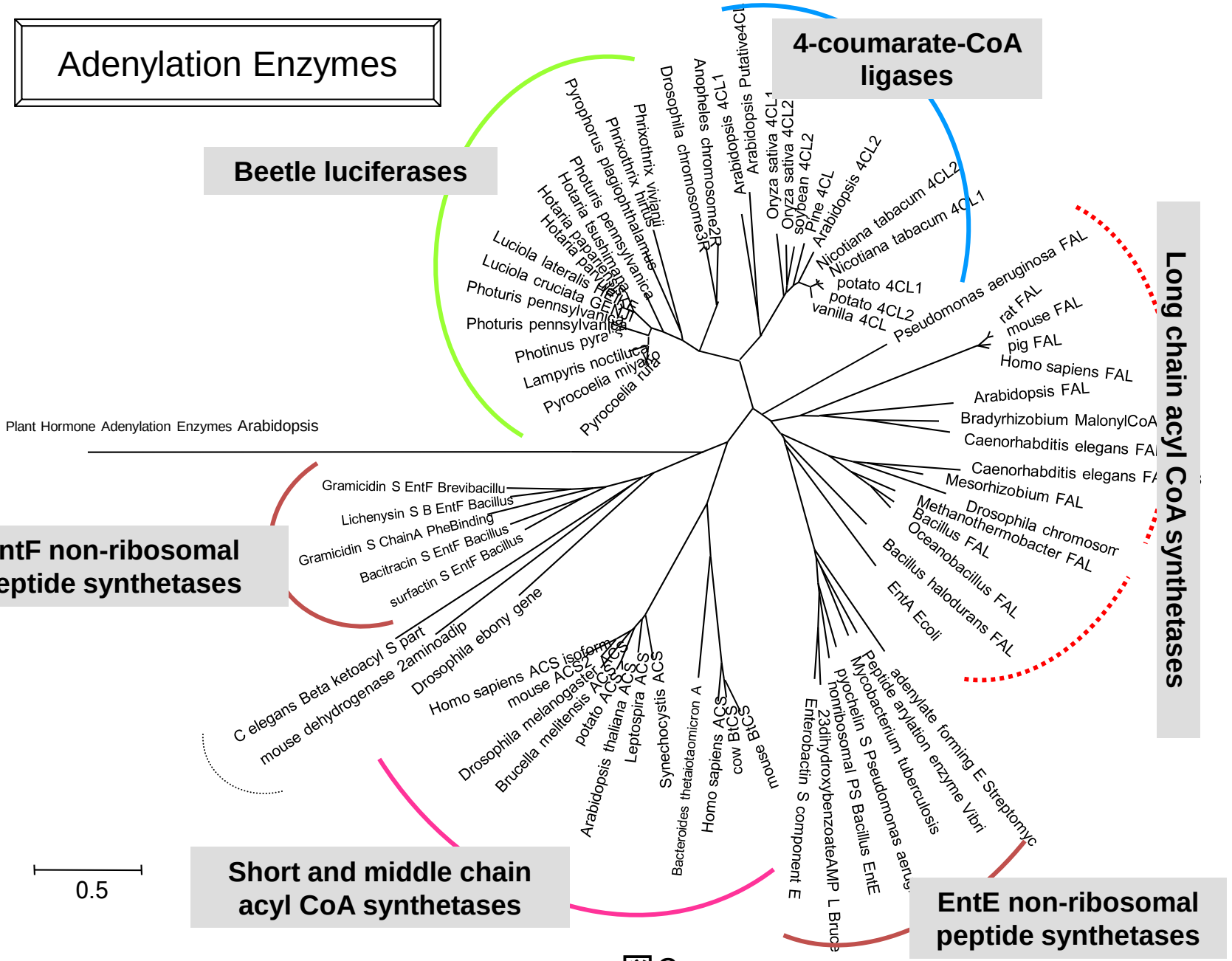

Adenylation Enzymes
4-coumarate-CoA ligases
Beetle luciferases
Long chain acyl CoA synthetases
EntF non-ribosomal peptide synthetases
Short and middle chain acyl CoA synthetases
EntE non-ribosomal peptide synthetases
Plant Hormone Adenylation Enzymes Arabidopsis
Arabidopsis Putative4CL
Arabidopsis 4CL1
Oryza sativa 4CL1
Oryza sativa 4CL2
soybean 4CL2
Pine 4CL
Arabidopsis 4CL2
Nicotiana tabacum 4CL2
Nicotiana tabacum 4CL1
potato 4CL1
potato 4CL2
vanilla 4CL
Pseudomonas aeruginosa FAL
rat FAL
mouse FAL
pig FAL
Homo sapiens FAL
Arabidopsis FAL
Bradyrhizobium MalonylCoA
Caenorhabditis elegans FA
Caenorhabditis elegans FA
Mesorhizobium FAL
Drosophila chromosom
Methanothermobacter FAL
Bacillus FAL
Oceanobacillus FAL
Bacillus halodurans FAL
EntA Ecoli
adenylate forming E Streptomyc
Peptide arylation enzyme Vibri
Mycobacterium tuberculosis
Pseudomonas aeru
pyochelin S
nonribosomal PS Bacillus EntE
23dihydroxybenzoateAMP L Bruce
Enterobactin S component E
mouse BtCS
cow BtCS
Homo sapiens ACS
Bacteroides thetaiotaomicron A
Synechocystis ACS
Leptospira ACS
Arabidopsis thaliana ACS
potato ACS
Brucella melitensis ACS
Drosophila melanogaster ACS
mouse ACS
Homo sapiens ACS isoform
Drosophila ebony gene
mouse dehydrogenase 2aminoadip
C elegans Beta ketoacyl S part
surfactin S EntF Bacillus
Bacitracin S EntF Bacillus
Gramicidin S ChainA PheBinding
Lichenysin S B EntF Bacillus
Gramicidin S EntF Brevibacillu
Photinus pyra
Lampyris noctiluca
Pyrocoelia miyako
Pyrocoelia rufa
Photuris pennsylvanica
Luciola lateralis
Luciola cruciata
Hotaria papaiensis
Hotaria tsushimana
Photuris pennsylvanica
Pyrophorus plagiophthalamus
Phrixothrix hirtus
Phrixothrix vivianii
Drosophila chromosome3R
Anopheles chromosome2R
0.5

図6

My challenge for cloning several luciferases

○ Ohmiya Y, Ohba N, Toh H, Tsuji FI: Cloning, expression and sequence analysis of cDNA for the luciferases from the Japanese firefly, *Pyrocoelia miyako* and *Hotaria parvula*. *Photochem. Photobiol*., 62, 309-313, 1995

○ Viviani VR, Bechara EJ, Ohmiya Y: Cloning, sequence analysis, and expression of active *Phrixothrix* railroad-worms luciferases: Relationships between bioluminescence spectra and primary structure. *Biochemistry* 38, 8271-8279, 1999

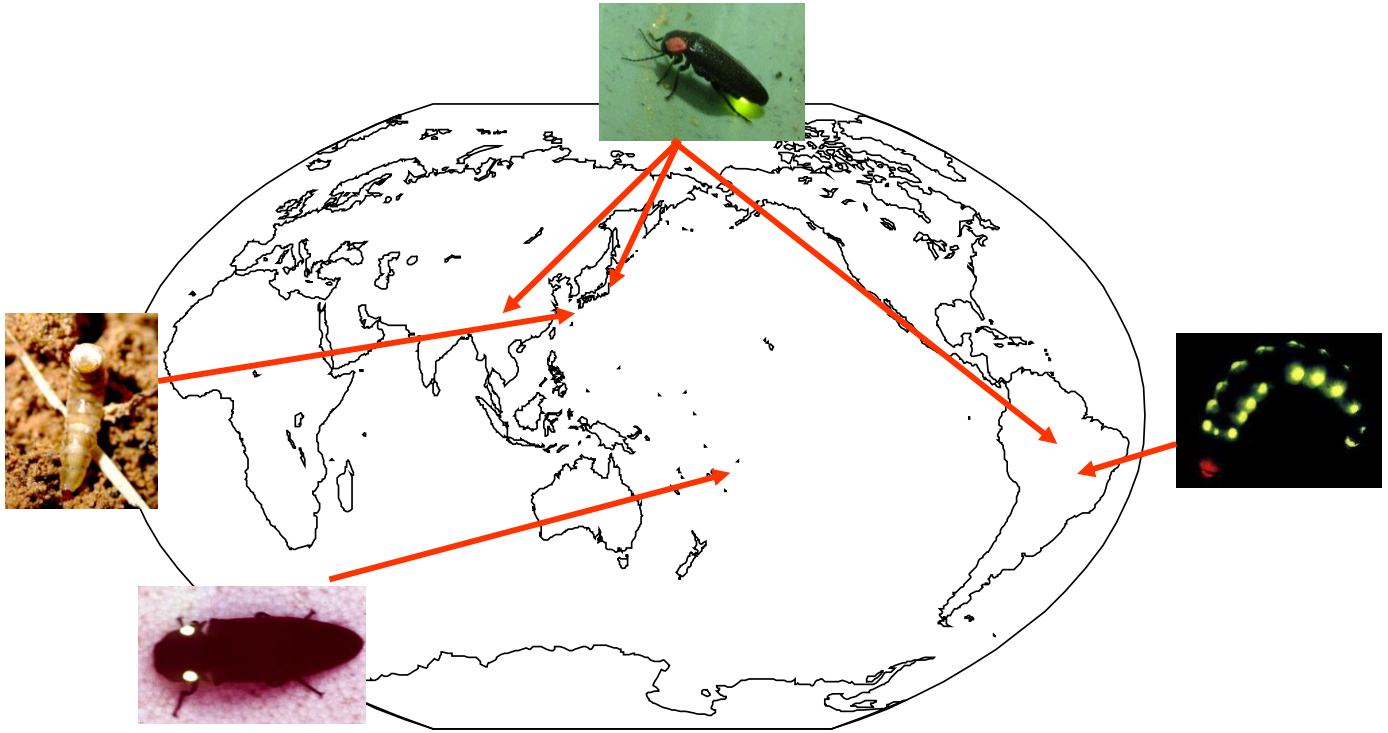
○ Ohmiya Y, Sumiya M, Viviani VR, Ohba N: Comparative aspects of a luciferase molecule from the Japanese luminous beetle *Rhagophthalmus ohba*. *Sci.Rept.Yokosuka City Mus*. 47, 31-38, 2000

○ Viviani VR, Arnoldi FG, Brochetto-Braga M, Ohmiya Y: Cloning and characterization of the cDNA for the Brazilian *Cratomorphus* distinctus larval firefly luciferase: similarities with European Lampyris noctiluca and Asiatic Pyrocoelia luciferases. *Comp Biochem Physiol B Biochem Mol Biol*. 139, 151-6, 2004

My history for cloning several luciferases

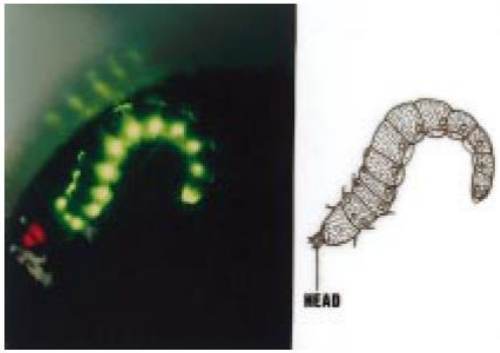
○ Mitani Y, Futahashi R, Niwa K, Ohba N, Ohmiya Y: Cloning and characterization of luciferase from a Fijian luminous click beetle. Photobiology and Photochemistry, 89(5):1163-9, 2013

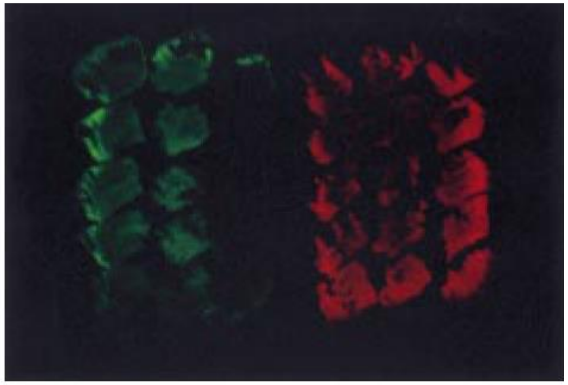
○ Mitani Y, Futahashi R, Liu Z, Liang X, Ohmiya Y: Tibetan firefly luciferase with low temperature adaptation. Photochem. Photobiol. 2016 Sep 26. doi: 10.1111/php.12643



Cloning, Sequence Analysis, and Expression of Active *Phrixothrix* Railroad-Worms Luciferases: Relationship between Bioluminescence Spectra and Primary Structures[†,‡]

Vadim R. Viviani,*[,§,||] Etelvino J. H. Bechara,[⊥] and Yoshihiro Ohmiya[§]





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PhRE   1:MEEENVVNGDRPRDLVFPGTAGLQLYQSLYKYSYITDGIIDAHTNEVISYAQIFETSCRL  60

PvGR  61:AVSIEQYGLNENNVVGVCSENNINFFNPVLAALYLGIPVATSNDMYTDGELTGHLNISKP  120
PhRE  61:AVSLEKYGLDHNNVVAICSENNIHFFGPLIAALYQGIPMATSNDMYTEREMIGHLNISKP  120

PvGR 121:TIMFSSKKALPLILRVQQNLSFIKKVVVIDSMYDINGVECVSTFVARYTDHTFDPLSFTP  180
PhRE 121:CLMFCSKKSLPFILKVQKHLDFLKRVIVIDSMYDINGVECVFSFDSRNTDHAFDPVKFNP  180

PvGR 181:KDFDPLEKIALIMSSSGTTGLPKGVVLSHRSLTIRFVHSRDPIYGTRTVPQTSILSLVPF  240
PhRE 181:KEFDPLERTALIMTSSGTTGLPKGVVISHRSITIRFVHSSDPIYGTRIAPDTSILAIAPF  240

PvGR 241:HHAFGMFTTLSYFVVGLKVVMLKKFEGALFLKTIQNYKIPTIVVAPPVMVFLAKSPLVDQ  300
PhRE 241:HHAFGLFTALAYFPVGLKIVMVKKFEGEFFLKTIQNYKIASIVVPPPIMVYLAKSPLVDE  300

PvGR 301:YDLSSLTEVATGGAPLGKDVAEAVAKRLKLPGIIQGYGLTETCCAVMITPHN-AVKTGST  359
PhRE 301:YNCSSLTEIASGGSPLGRDIADKVAKRLKVHGILQGYGLTETCSALILSPNDRELKKGAI  360

PvGR 360:GRPLPYIKAKVLDNATGKALGPGERGEICFQSEMIMKGYYNNPEATIDTIDKDGWLHSGD  419
PhRE 361:GTPMPYVQVKVIDINTGKALGPREKGEICFKSQMLMKGYHNNPQATRDALDKDGWLHTGD  420

PvGR 420:IGYYDEDGNFFIVDRLKELIKYKGYQVAPAELENLLLQHPSIADAGVTGVPDEFGGQLPA  479
PhRE 421:LGYYDEDRFTYVVDRLKELIKYKGYQVAPAELENLLLQHPNISDAGVIGFRTNLLVNYLS  480

PvGR 480:ACVVLESGKTLTEKEVQDFIAAQVTPTKHLRGGVVFVDSIPKGPTGKLIRKELREIFAQR  539
PhRE 481:ACVVLEPGKTMTEKEVQDYIAELVTTTKHLRGGVVFIDSIPKGPTGKLMRNELRAIFARE  540

PvGR 540:APKSKL  545
PhRE 541:QAKSKL  546
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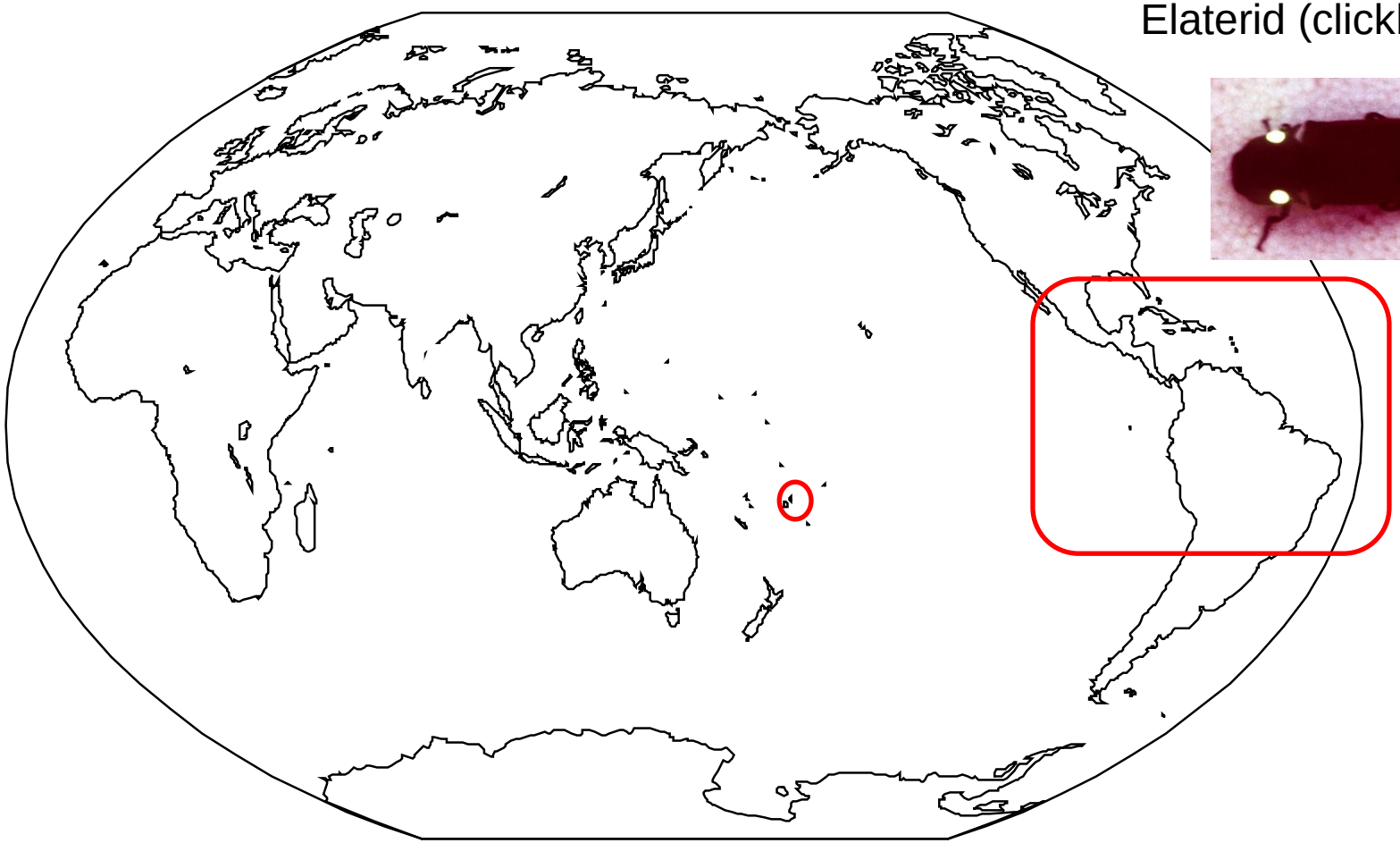
Elaterid are distributed in the middle and south America and the Fiji islands

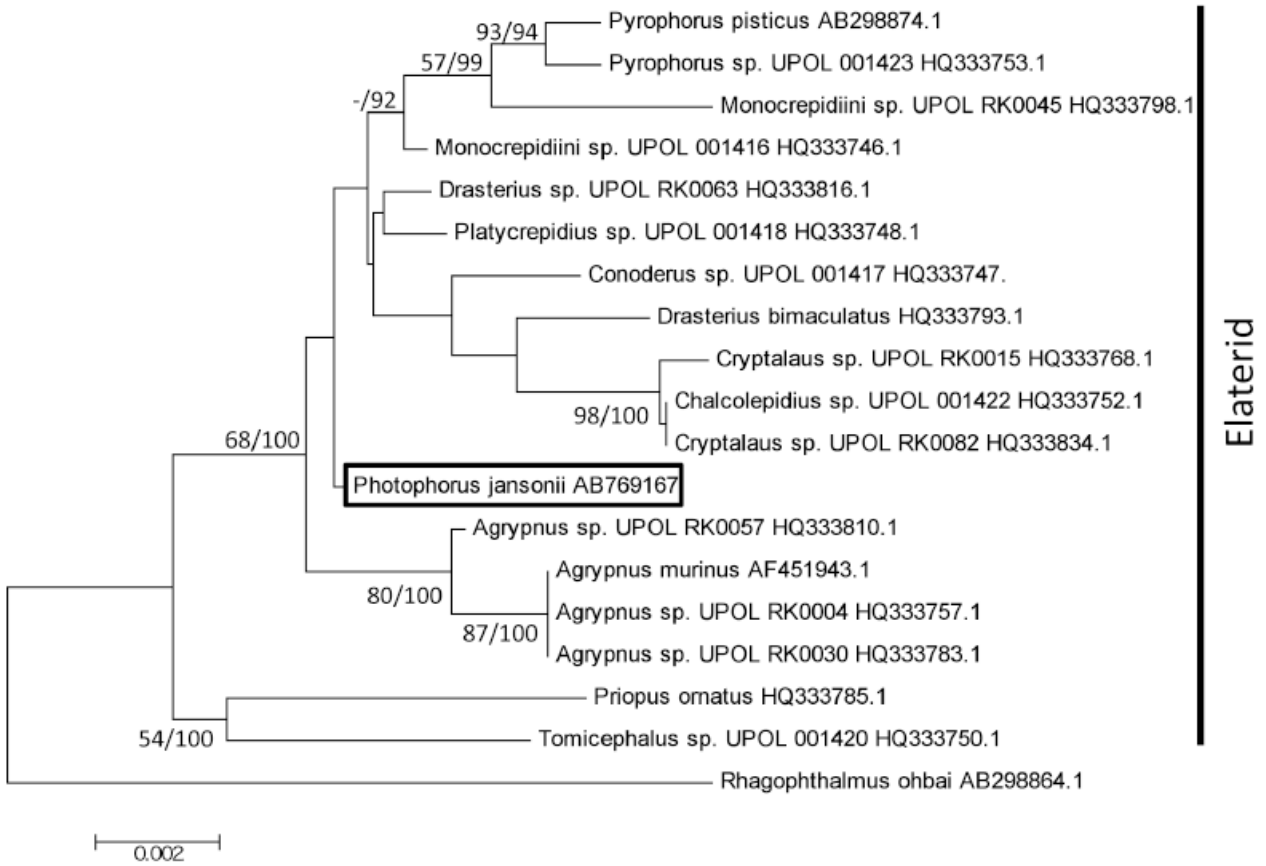


Elaterid (clickbeetle)

Photochemistry and Photobiology, 2013, 89: 1163–1169

Cloning and Characterization of Luciferase from a Fijian Luminous Click Beetle

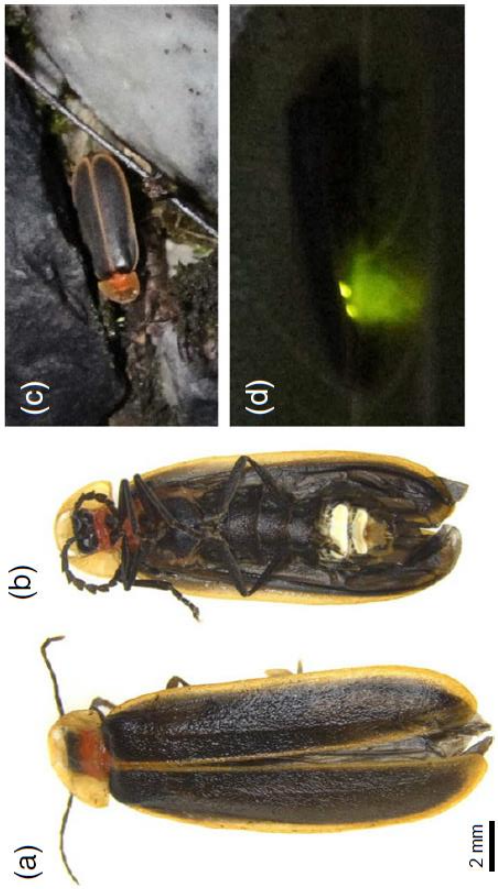
Yasuo Mitani*[1], Ryo Futahashi[1], Kazuki Niwa[2], Nobuyoshi Ohba[3] and Yoshihiro Ohmiya[4]

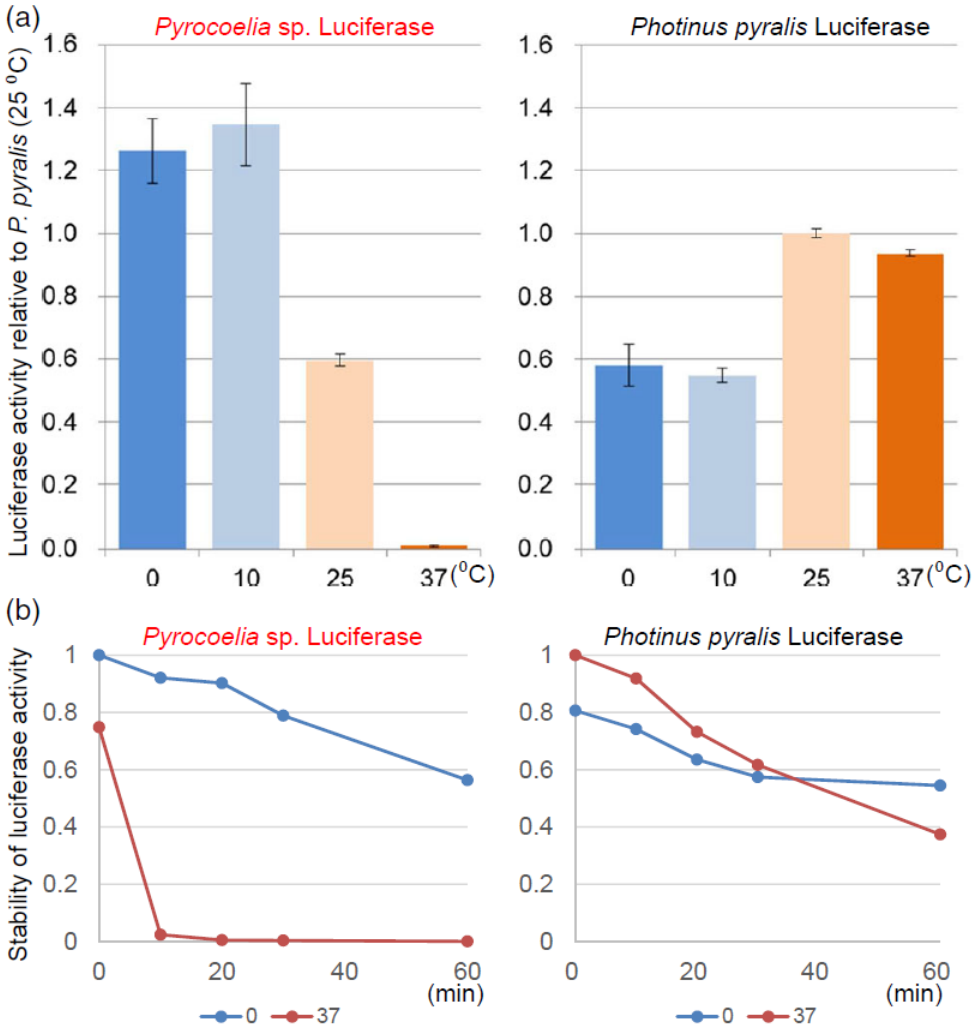


Photochemistry and Photobiology, 2017, 93: 466–472

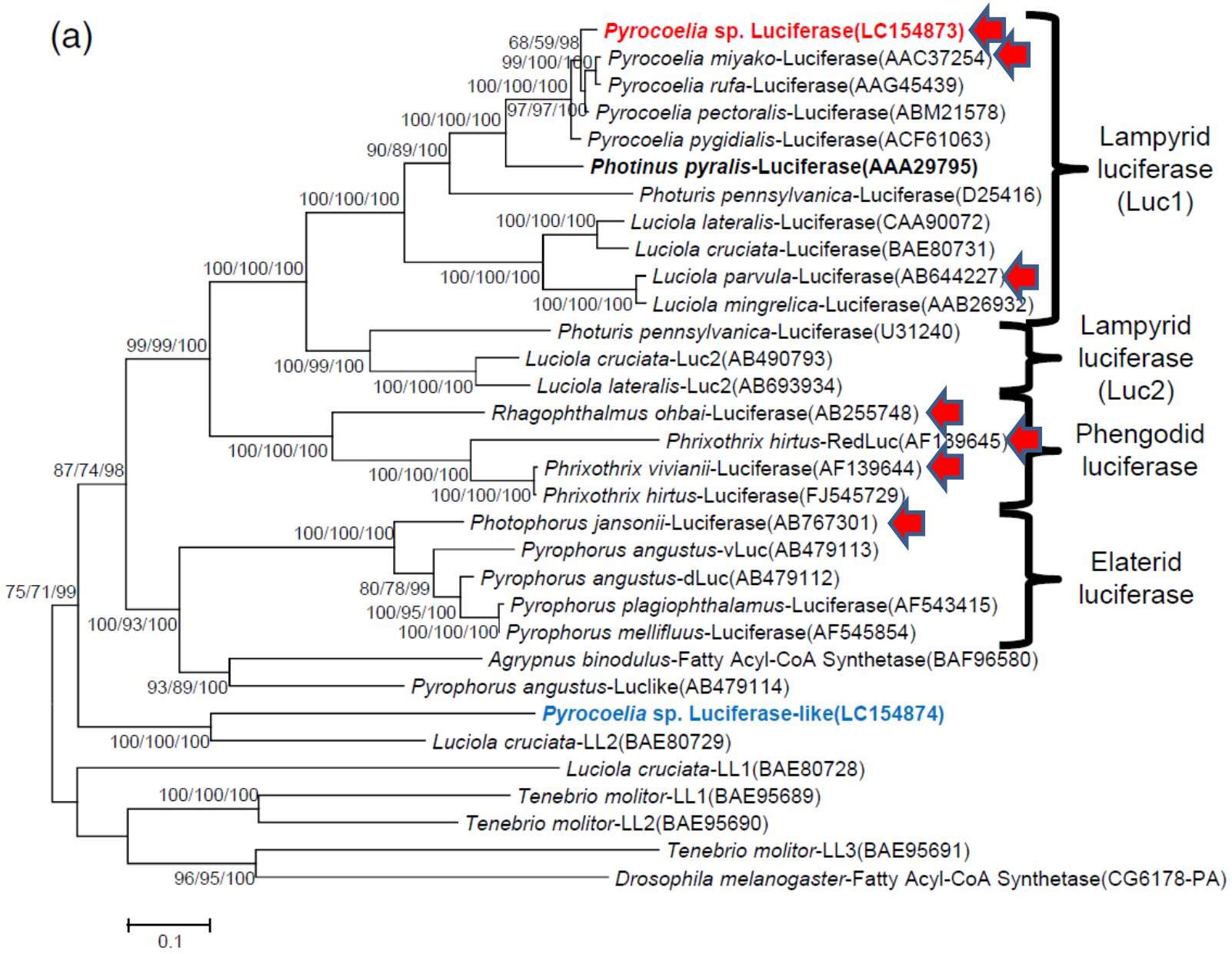
Tibetan Firefly Luciferase with Low Temperature Adaptation†

Yasuo Mitani[1], Ryo Futahashi[1], Zichao Liu[2], Xingcai Liang[3] and Yoshihiro Ohmiya[4,5]*





Phylogenetic tree of luciferase genes



Molecular mechanism of color differences

My challenge for molecular mechanism of color differences on luciferin-luciferase reaction

- Ohmiya Y, Hirano T, Ohashi M: The structural origin of the color differences in the bioluminescence of firefly luciferase. *FEBS Lett*. 384, 83-86, 1996

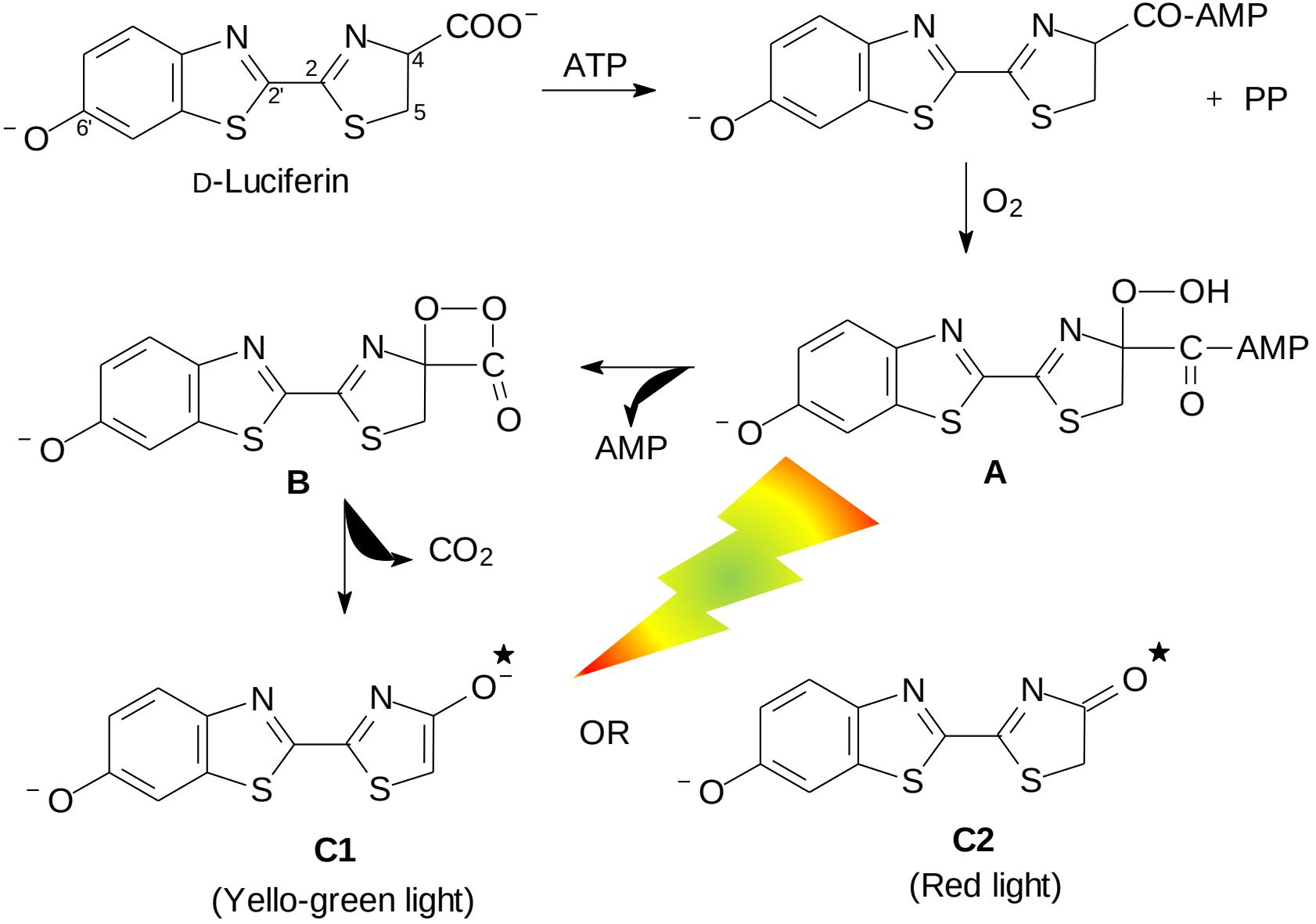
- Ohmiya Y Tsuji FI: Mutagenesis of firefly luciferase shows that cysteine residues are not required for bioluminescence activity. *FEBS Lett*. 404, 115-117, 1997

- Viviani VR, Ohmiya Y: Bioluminescence color determinants of Phrixothrix railroad-worm luciferases: Chimeric luciferases, site-directed mutagenesis of Arg 215 and Guanidine effect. *Photochem. Photobiol*.,72, 267-271, 2000

- Viviani VR, Uchida A, Suenaga N, Ryufuku M, Ohmiya Y: Thr-226 is a key-residue for bioluminescence spectra determination in beetle luciferases. *Biochem Biophys Res Commun* 280, 1286-1291, 2001

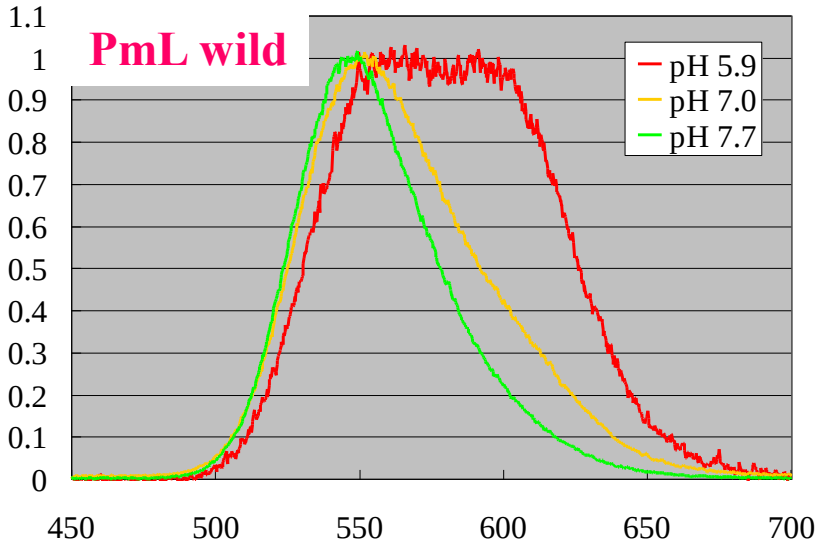
- Viviani VR, Uchida A, Viviani W Ohmiya Y: The influence of Ala243 (Gly247), Arg215 and Thr 226 (Asn230) on the bioluminescecen spectra and pH-sensitivity of Railroad worm, clicl beetle and firefly luciferases. *Photochem. Photobiol* 76, 538-544, 2002

Molecular mechanism of firefly luciferin on bioluminescence reaction

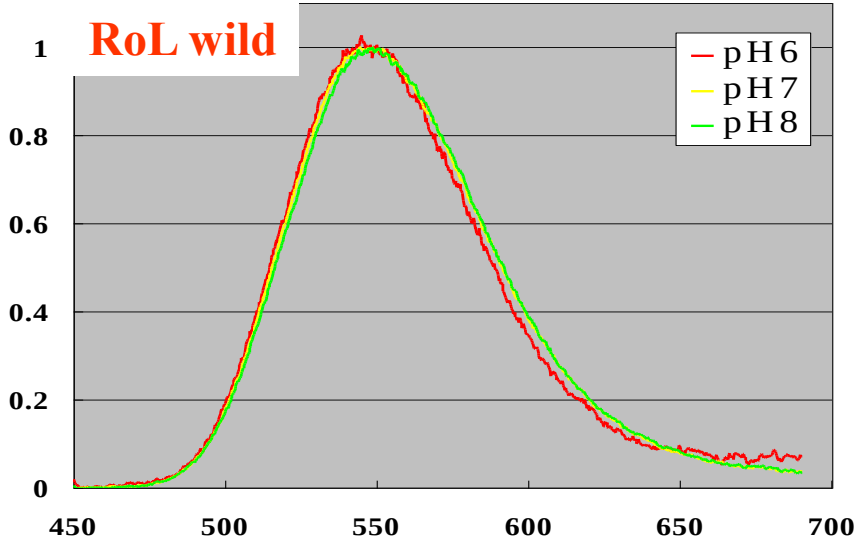


Two groups for pH-sensitivity on beetle luciferases

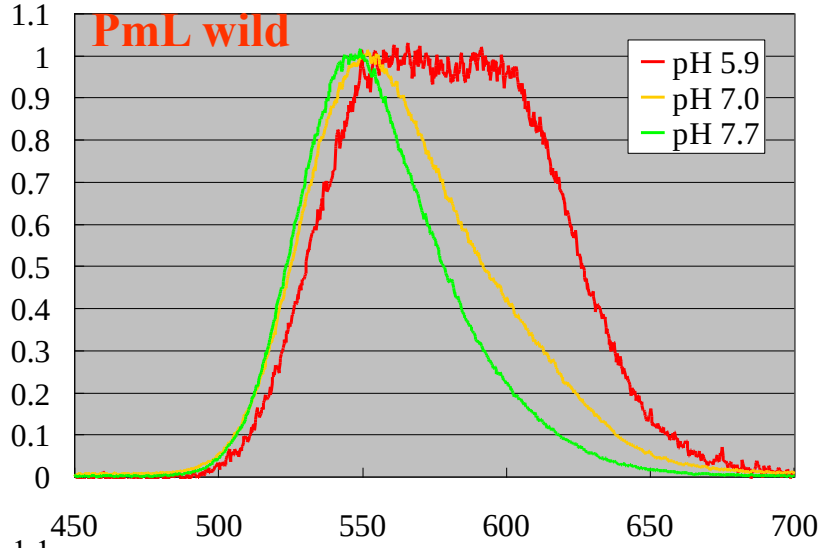
pH sensitive
(Firefly)

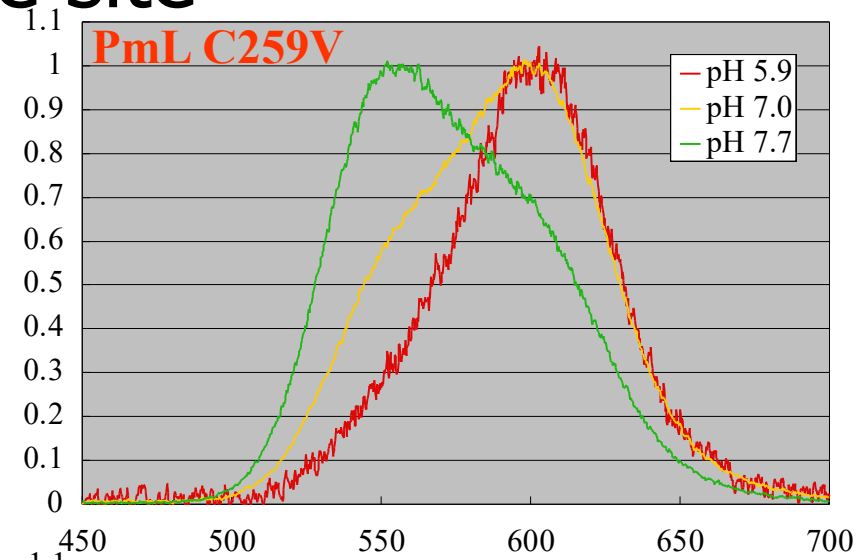


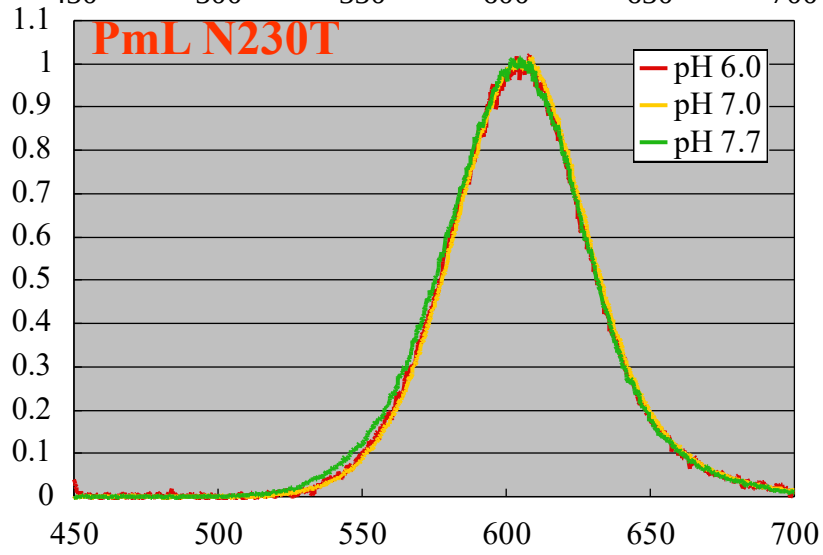
pH insensitive
(Crick beetle,
Railroadworm)

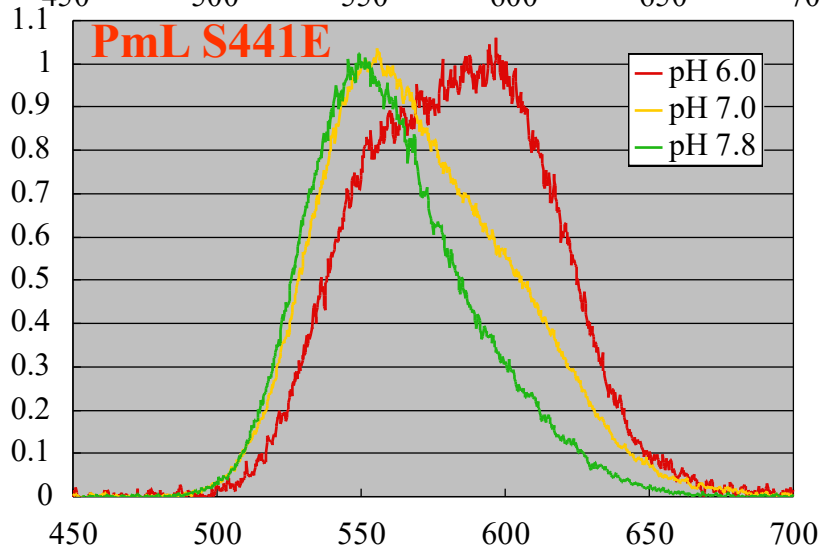


pH sensitivity could be due to fine structure of active site

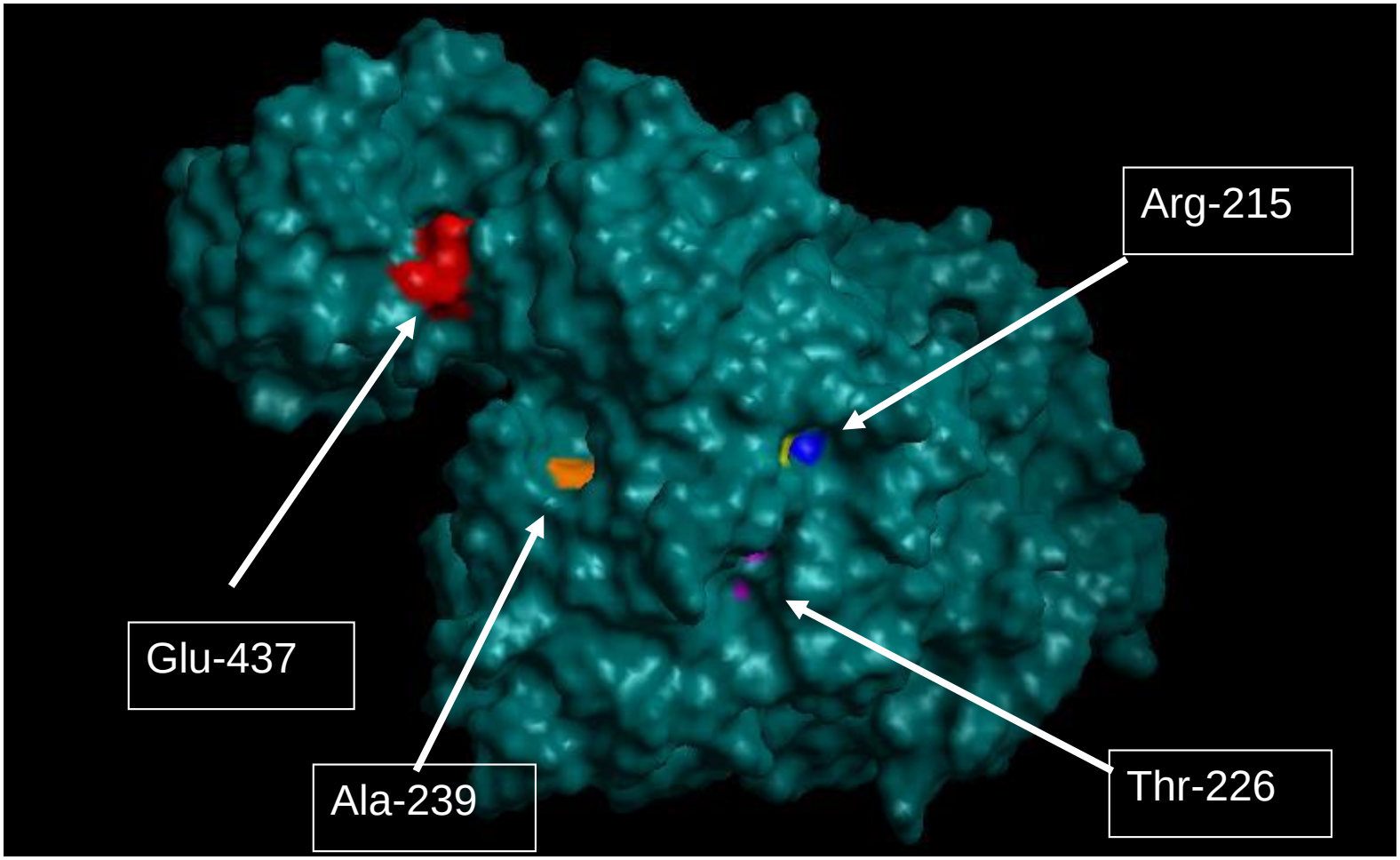






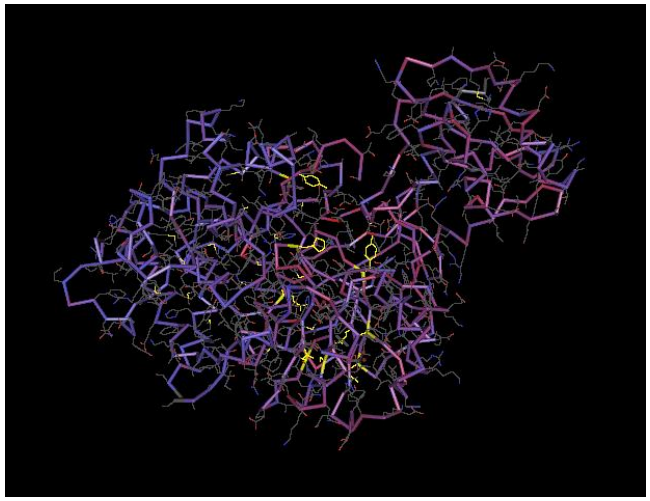


Three dimensional structure of firefly luciferase

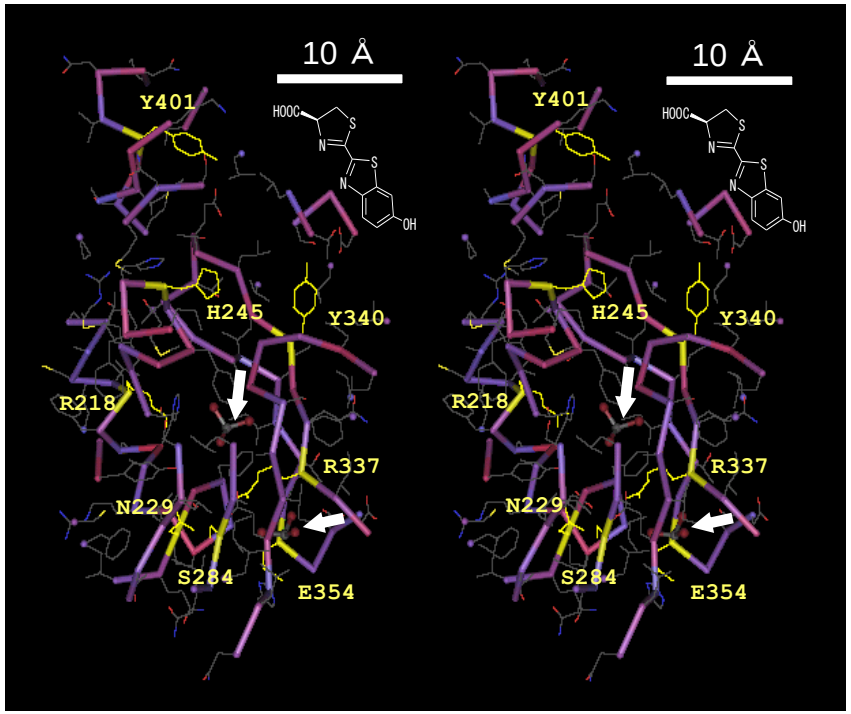


Luminescent colors determined by electric condition of active site

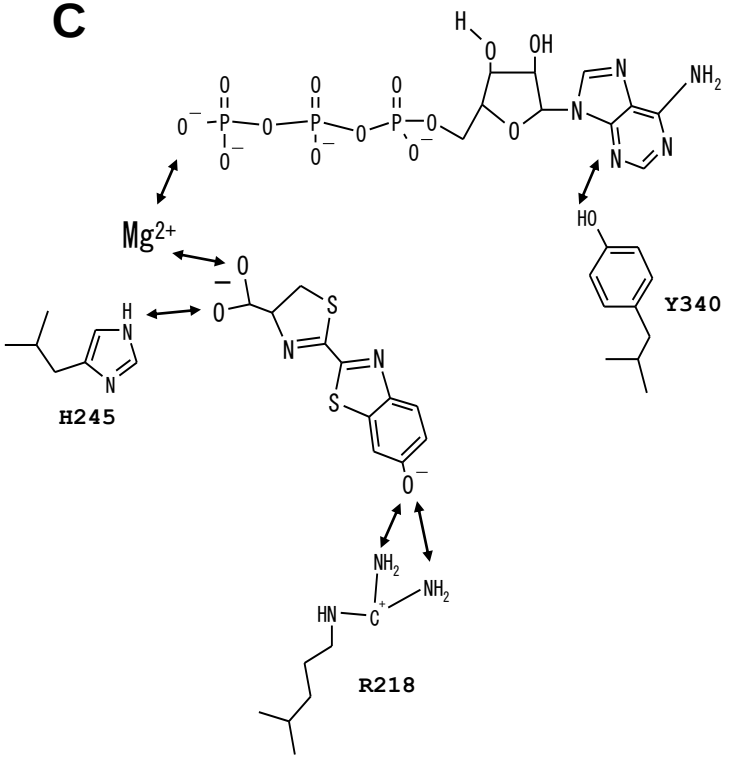
A



B



C



My challenge for novel reporter assay using color different luciferase

- Kitayama Y, Kondo T, Nakahira Y, Nishimura H, Ohmiya Y, Oyama T: An in vivo dual-reporter system of cyanobacteria using two railroad-worm luciferases with different color emissions. *Plant Cell & Physiology* 45, 109-113. 2004

- Nakajima Y, Ikeda M, Kimura T, Honma S, Ohmiya Y, Honma K: Role of orphan nuclear receptor ROR a in clock gene transcriptions demonstrated by a novel reporter assay system. *FEBS Lett* 565, 122-126, 2004

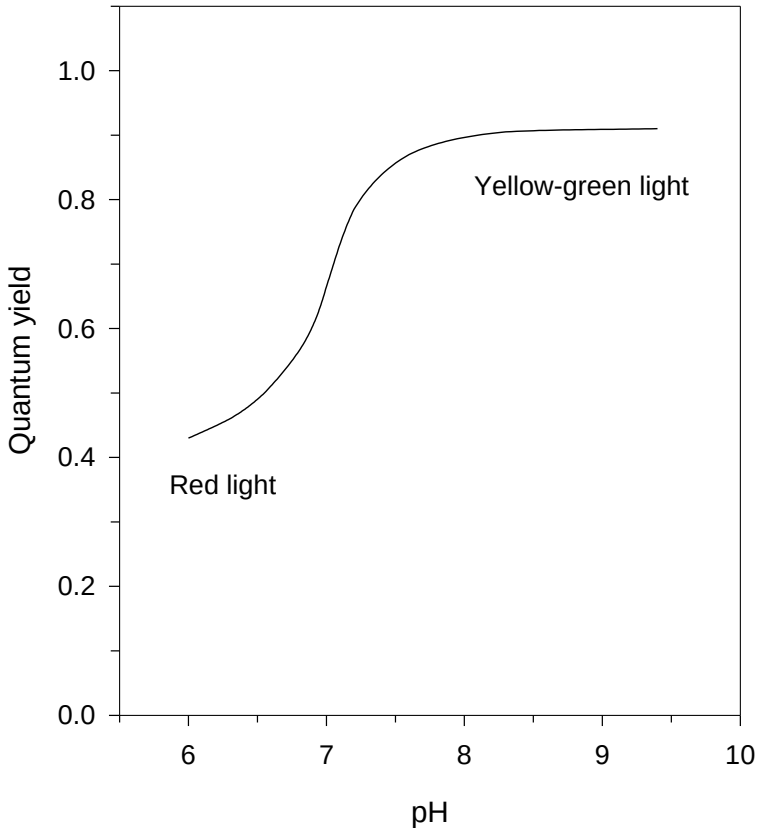
- Nakajima Y, Kimura T, Sugata K, Enomoto T, Asakawa T, Kubota H, Ikeda M, OhmiyaY: A multicolor luciferase assay system, one-step monitoring of multiple gene expressions with a single substrate. *Biotechniques* 38, 891-894, 2005

- Noguchi T, Michihata T, Nakamura W, Takumi T, Shimizu R, Yamamoto M, Ikeda M, Ohmiya Y, Nakajima Y: Dual-Color Luciferase Mouse Directly Demonstrates Coupled Expression of Two Clock Genes. *Biochemistry*, 49, 8053–8061,2010

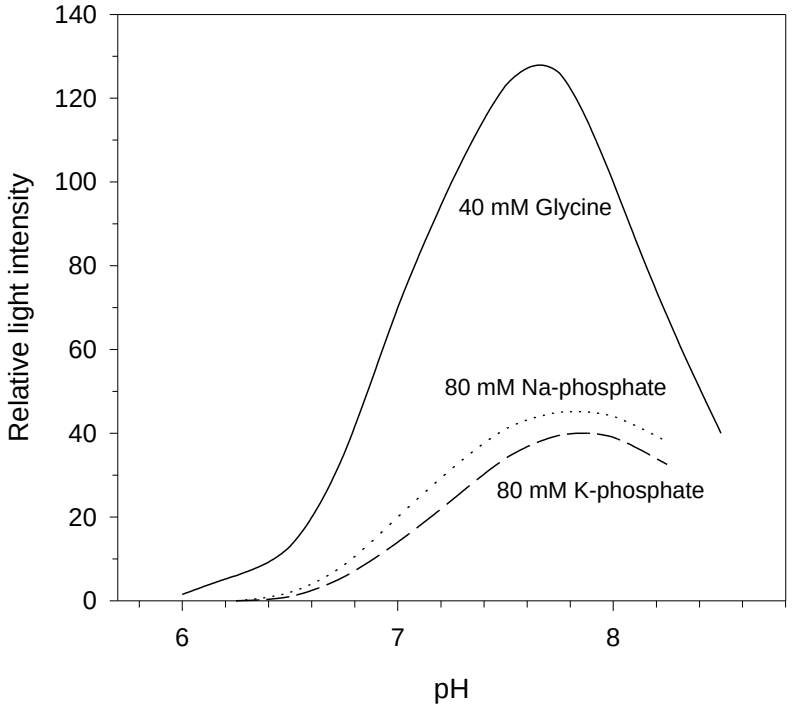
- Tarnow P, Bross S, Wollenberg L, Nakajima Y, Ohmiya Y, Tralau T, Luch A: A novel dual-colour luciferase reporter assay for simultaneous detection of estrogen and aryl hydrocarbon receptor activation. Chem Res Toxicol. 2017 30(7):1436-1447

Determining the quantum yield of bioluminescence reaction

Can the cold light produce highly efficiency?



Quantum yield of firefly bioluminescence as a function of pH (McElroy and Seliger, 1961).



Effect of pH and buffer on the activity of luciferase measured with the same concentration of luciferase (Green and McElroy, 1956).

My challenge for measuring of photon numbers on bioluminescence reaction

- Ando Y, Niwa K, Yamada N, Irie T, Enomoto T, Kubota H, Ohmiya Y, Akiyama H: Development of a Quantitative Bio/Chemiluminescence Spectrometer Determining Quantum Yields: Re-examination of the Aqueous Luminol Chemiluminescence Standard. *Photochem Photobiol*. 83, 1205-10, 2007

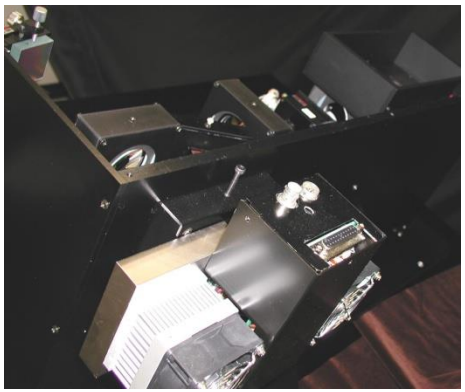
- Ando Y, Niwa K, Yamada N, Irie T, Enomoto T, Kubota H, Ohmiya Y, Akiyama H.: Quantum Yield and Colour Change of Firefly Bioluminescence. *Nature Photonics* 2, 44-47, 2008

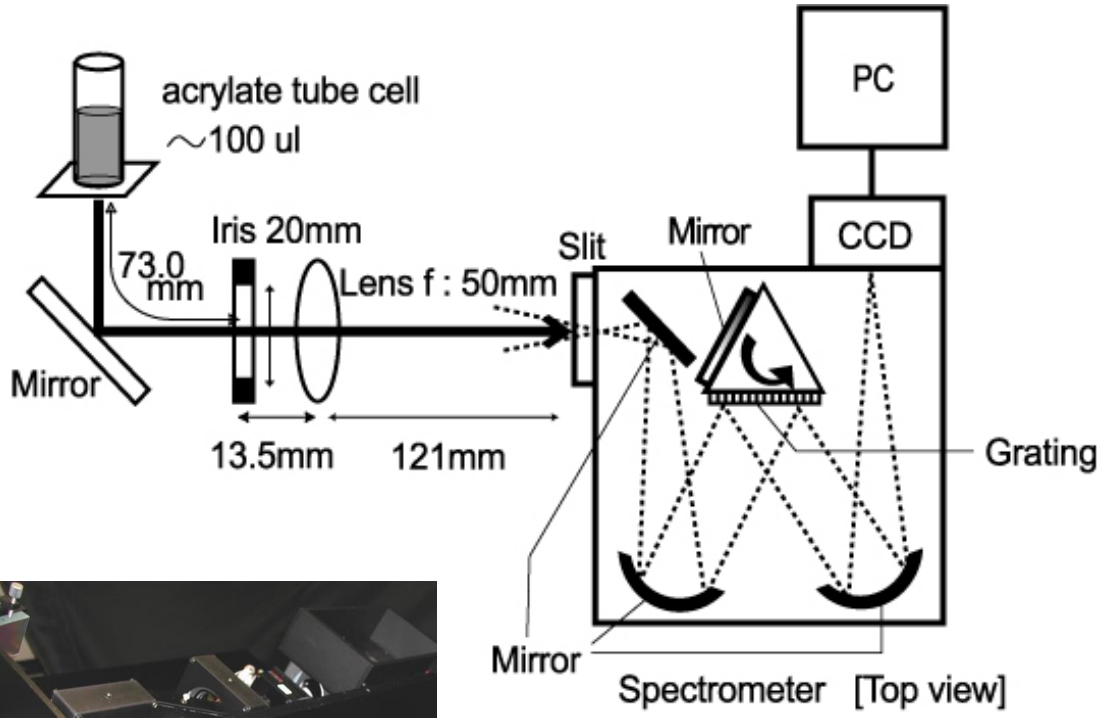
- Niwa K, Ichino Y, Ohmiya Y: Quantum yield measurements of firefly bioluminescence reactions using a commercial luminometer. *Chem.lett.* 39: 2010

- Niwa K, Ichino Y, Kumata S, Nakajima Y, Hiraishi Y, Kato D, Viviani VR, Ohmiya Y: Quantum Yields and Kinetics of the Firefly Bioluminescence Reaction of Beetle Luciferases. *Photobiology & Photochemistry*, 86, 1046-9, 2010

- Yoshita M, Kubota H, Shimogawara M, Mori K, Ohmiya Y, Akiyama H.: Light-emitting-diode Lambertian light sources as low-radiant-flux standards applicable to quantitative luminescence-intensity imaging. Rev Sci Instrum. 2017 Sep;88(9):093704. doi: 10.1063/1.5001733

Measurement system





-Calibrations-

1. Light-collection efficiency
2. Sensitivity of CCD-spectrometer system

Our measurement & calibration features

1. Light-collection efficiency

-Our system-

Using "Platelet cell" for calibration, any forms of sample cells OK!

-Before-

"Point source approximation"

2. Absolute sensitivity of detector

Semiconductor-photo-detecor technology
Light source : Laser

Thermopile
Light source : Standard lamp

3. Detector

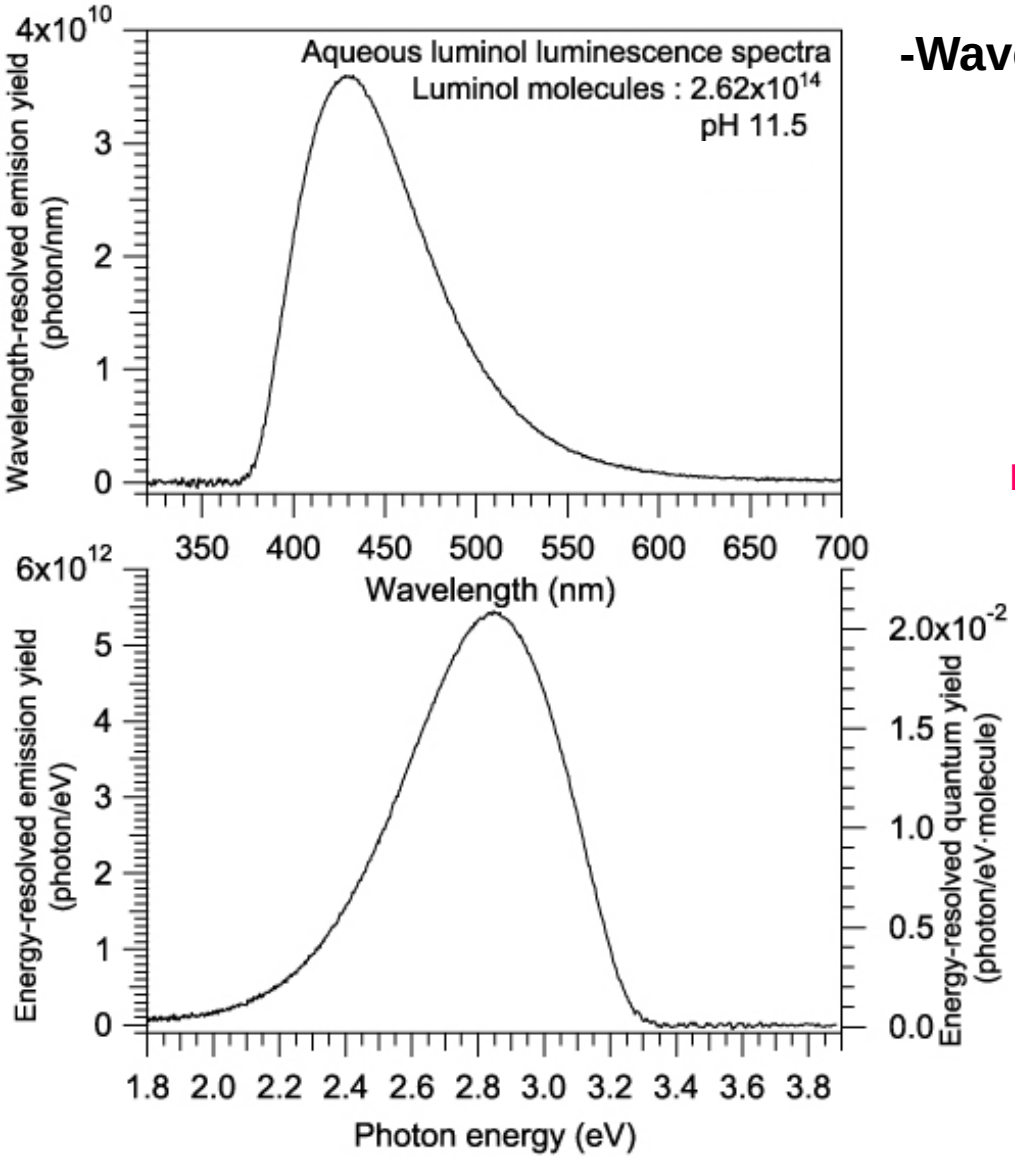
Cooled CCD camera

Photomultiplier

4. Data

Energy-resolved or wavelength-resolved emission yield
in an absolute unit of photon/eV or photon/nm
& Quantum yield

Luminol (aqueous chemiluminescence)



-Wavelength-resolved emission yield-

Reagents

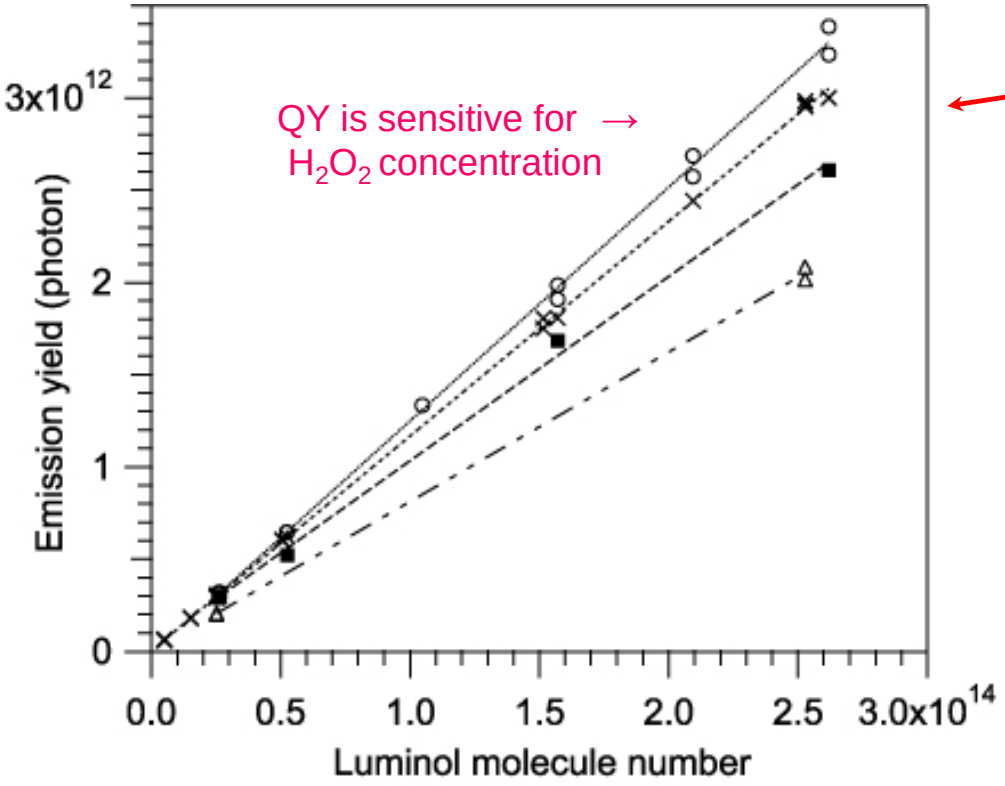
Luminol	$1x10^{-5}$ M	x 50ul
Horseradish peroxidase	$4x10^{-7}$M	x 50ul
H2O2	0.1M	x 2ul

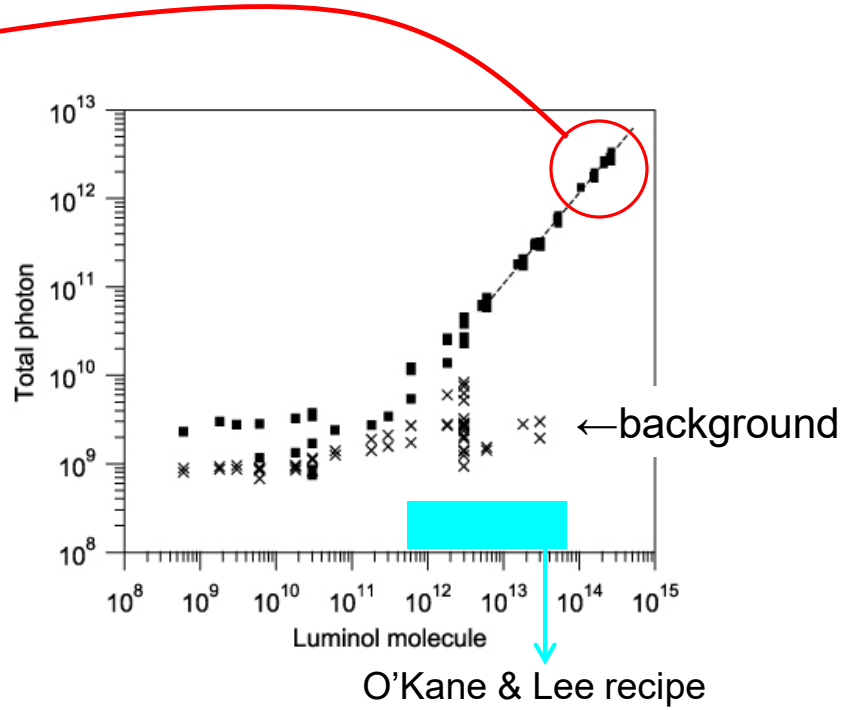
Luminol molecule number : $\varepsilon_{(347nm)}=7640M^{-1}cm^{-1}$

-Energy-resolved emission yield-

To understand or analyze the spectrum microscopically, “energy-resolved emission Yield” is better.

Emission yield vs. Luminol molecule number





Reagent	Concentration(M)	Volume(ul)	final concentration(M)	QY(%)
Liminol	$1x10^{-6}$ -$1x10^{-7}$	50	$5x10^{-7}$ -$5x10^{-6}$	
HRP	$4x10^{-7}$	50	$2x10^{-7}$	
H2O2	■ 1	0.5	$5x10^{-3}$	1.04
	○ 0.1	2	$2x10^{-3}$	1.23
	× 0.1	0.5	$5x10^{-4}$	1.18
	△ $1x10^{-2}$	1	$1x10^{-4}$	0.81

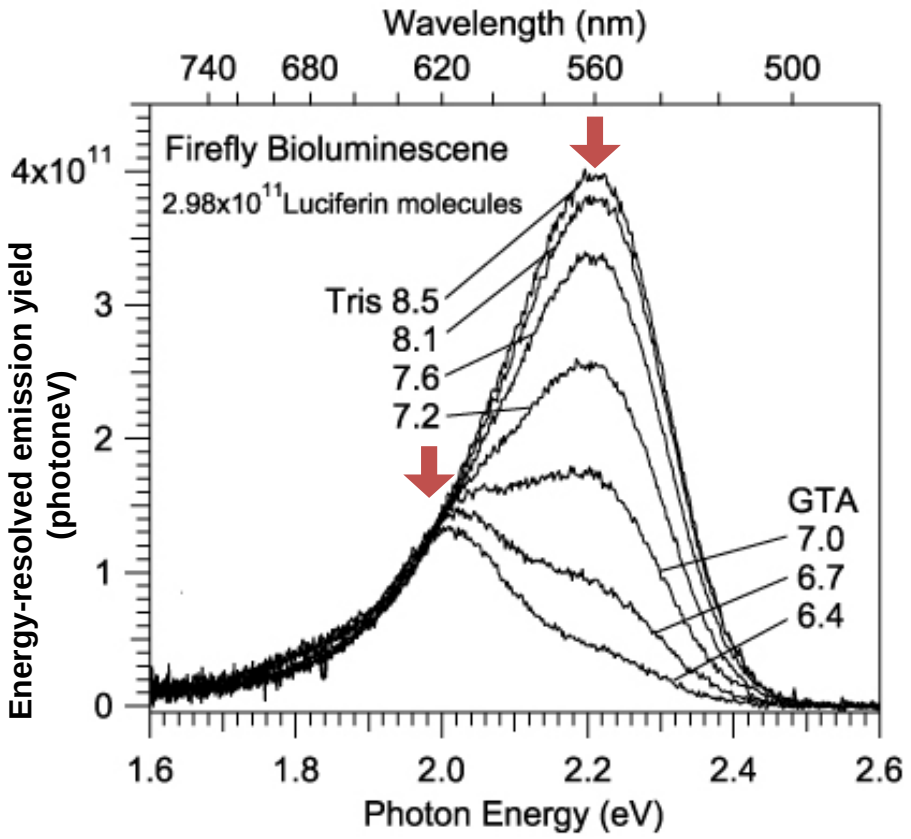
Quantum yield : 1.23±0.18%

Uncertainty of calibration(15%)

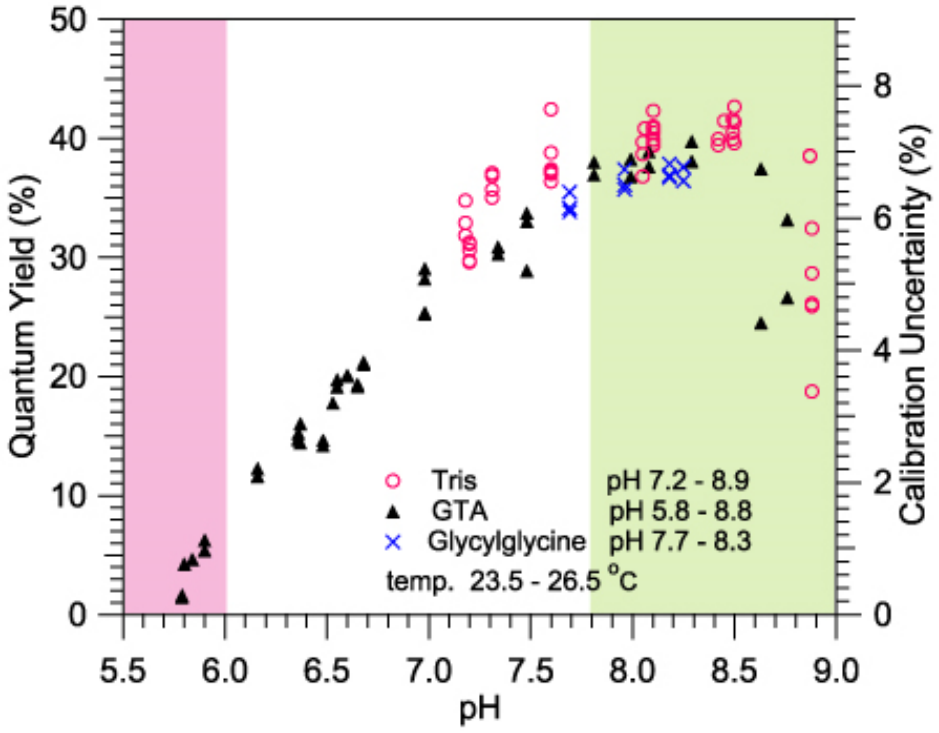
(1.24% : O'Kane, Lee et al. 1986, 2000)

Firefly

-Energy-resolved emission yield-



-Quantum yield vs. pH-

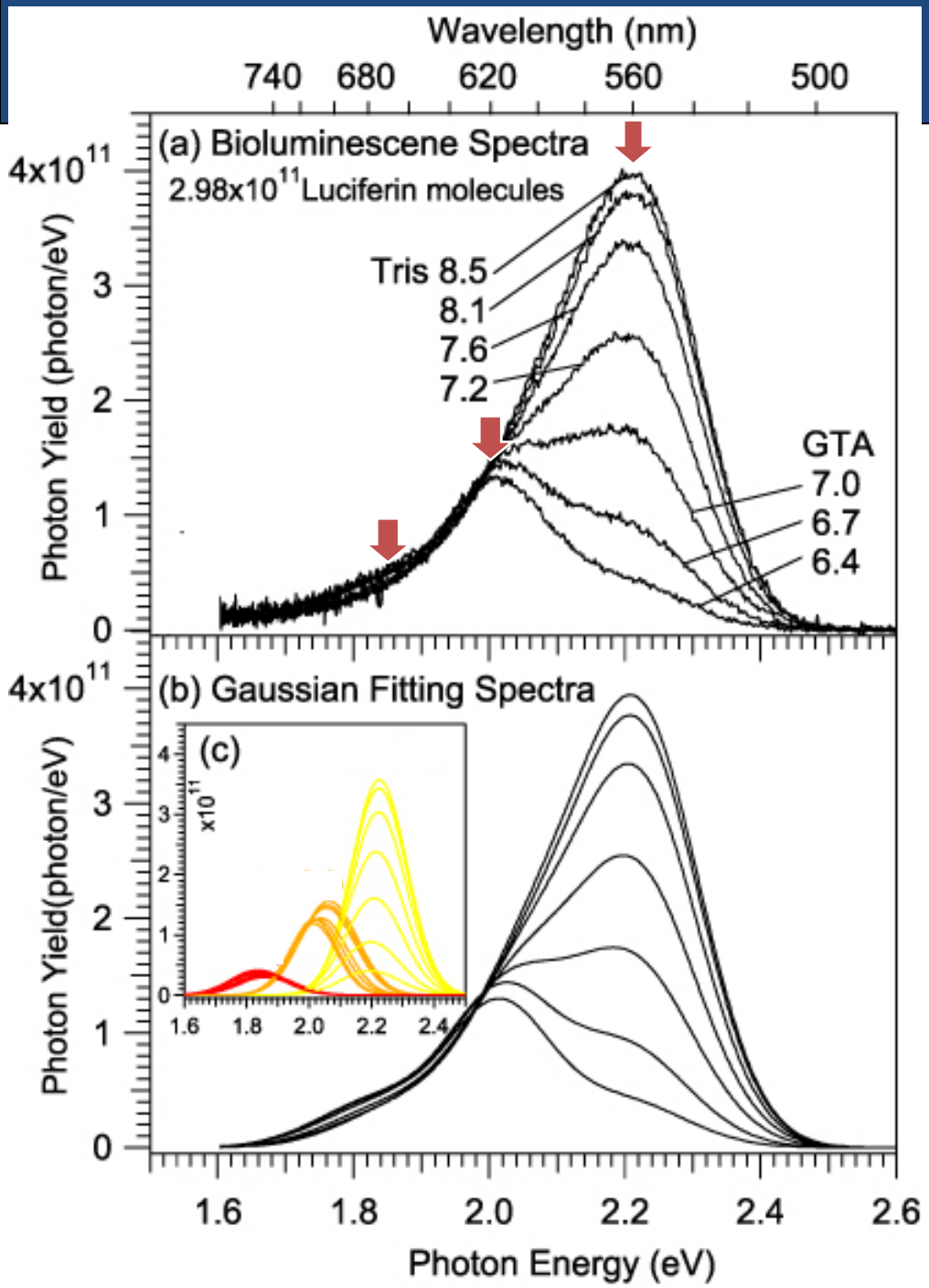


Firefly luciferase : Natural "*Photinus pyralis*"

Quantum yield : 41.0 ± 7.4 %

(pH 8.5)

Gaussian Fitting



-Energy-resolved emission yield-

Peak Energy

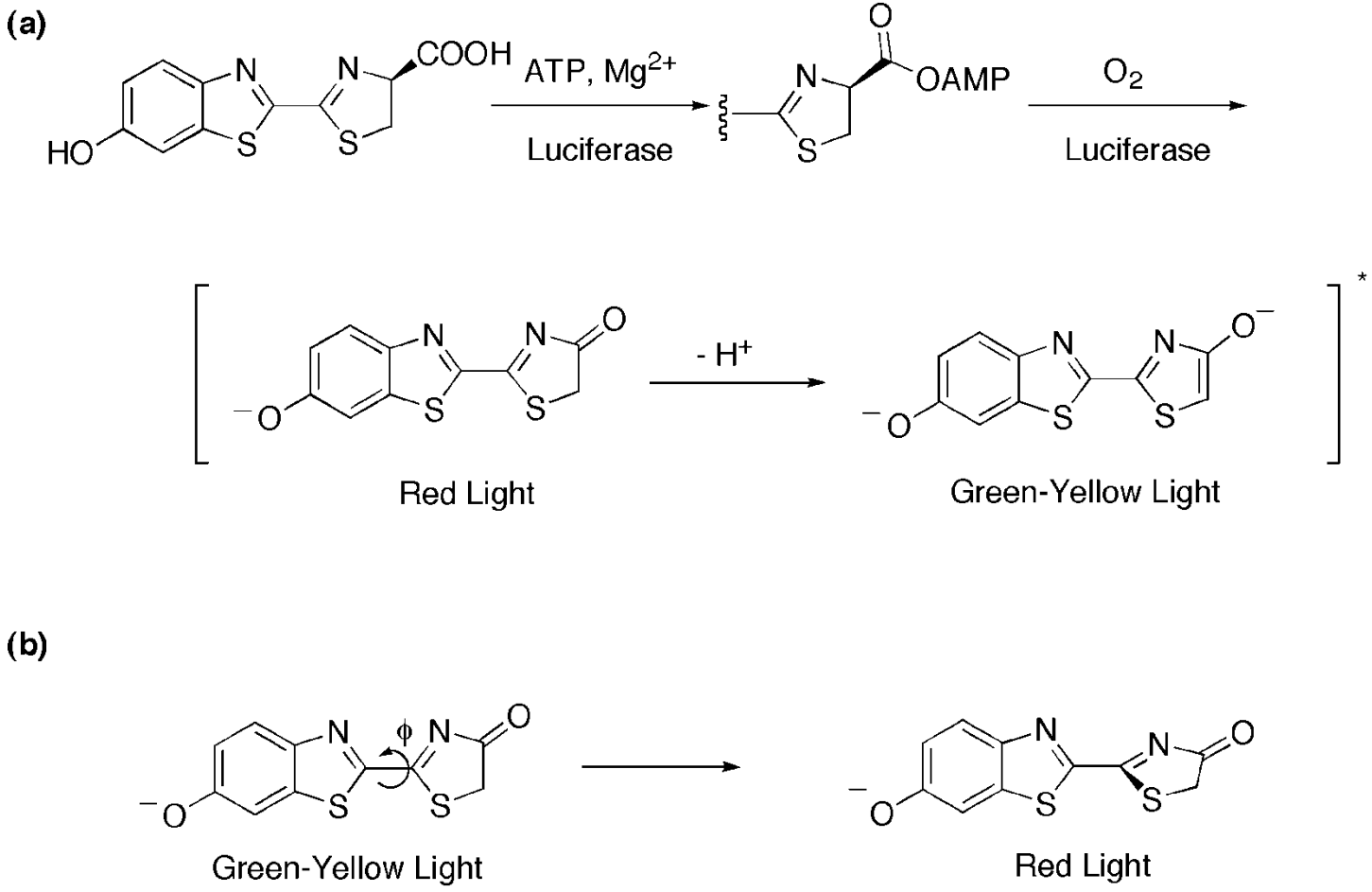
～2.2 eV (560nm)

～2.0 eV (620nm)

～1.85 eV (670nm)

Luminescence spectra of firefly can be reproduced by **three gaussian components**

Chemical Reaction on Bioluminescent Beetle

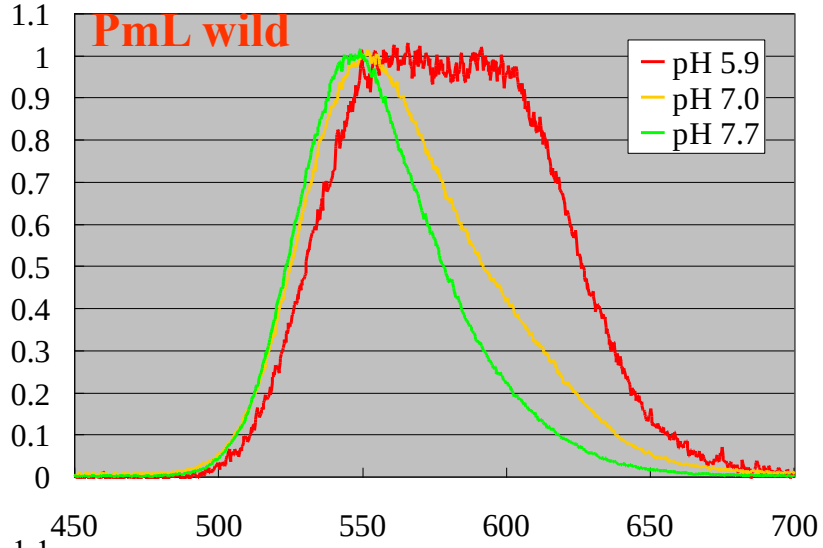


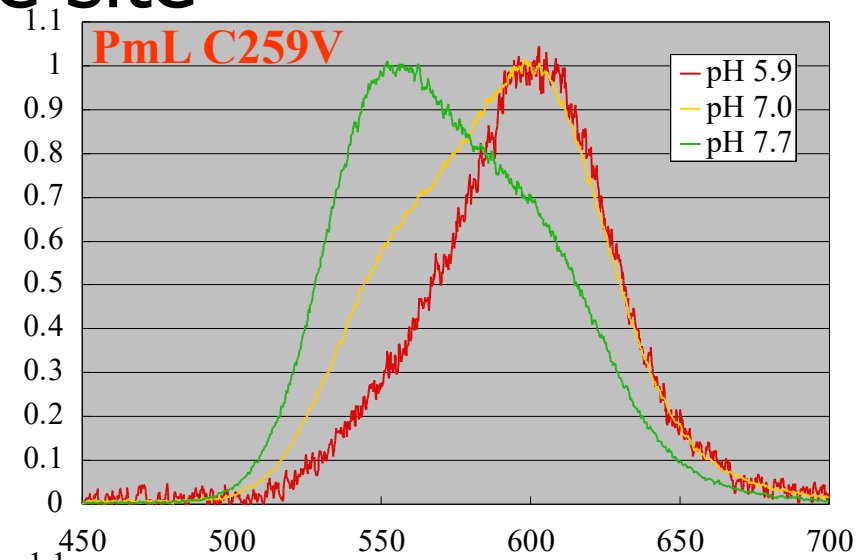
Possibility of the determination of bioluminescence color in the active-site of beetle luciferases;

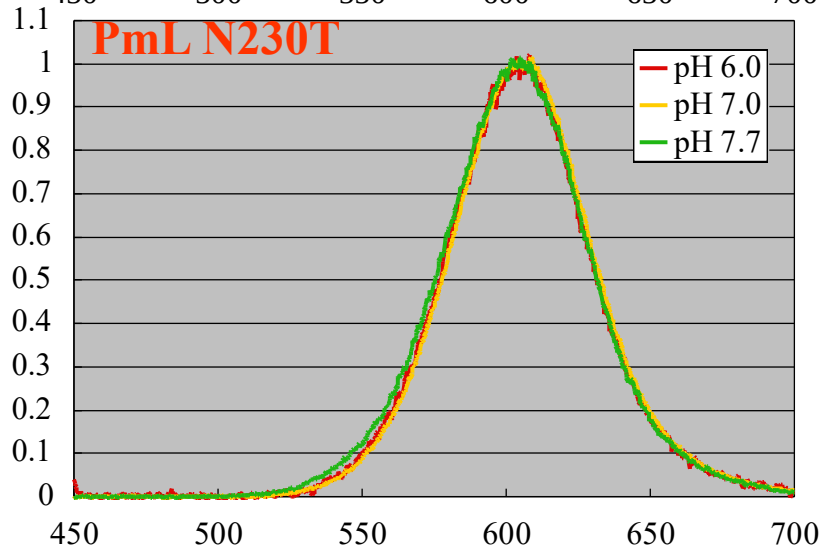
(I) the polarity of the active-site

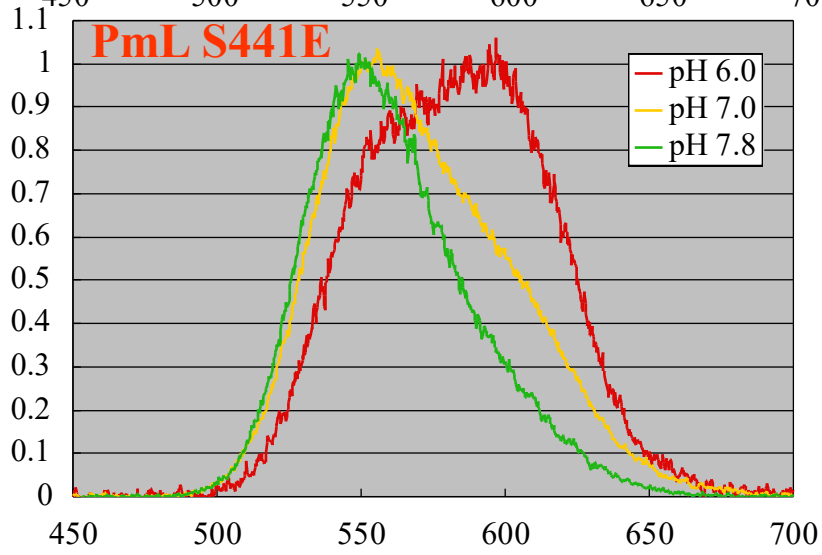
(II) presence of basic residues assisting oxyluciferin tautomerization (III) the active-site conformation

pH sensitivity could be due to fine structure of active site

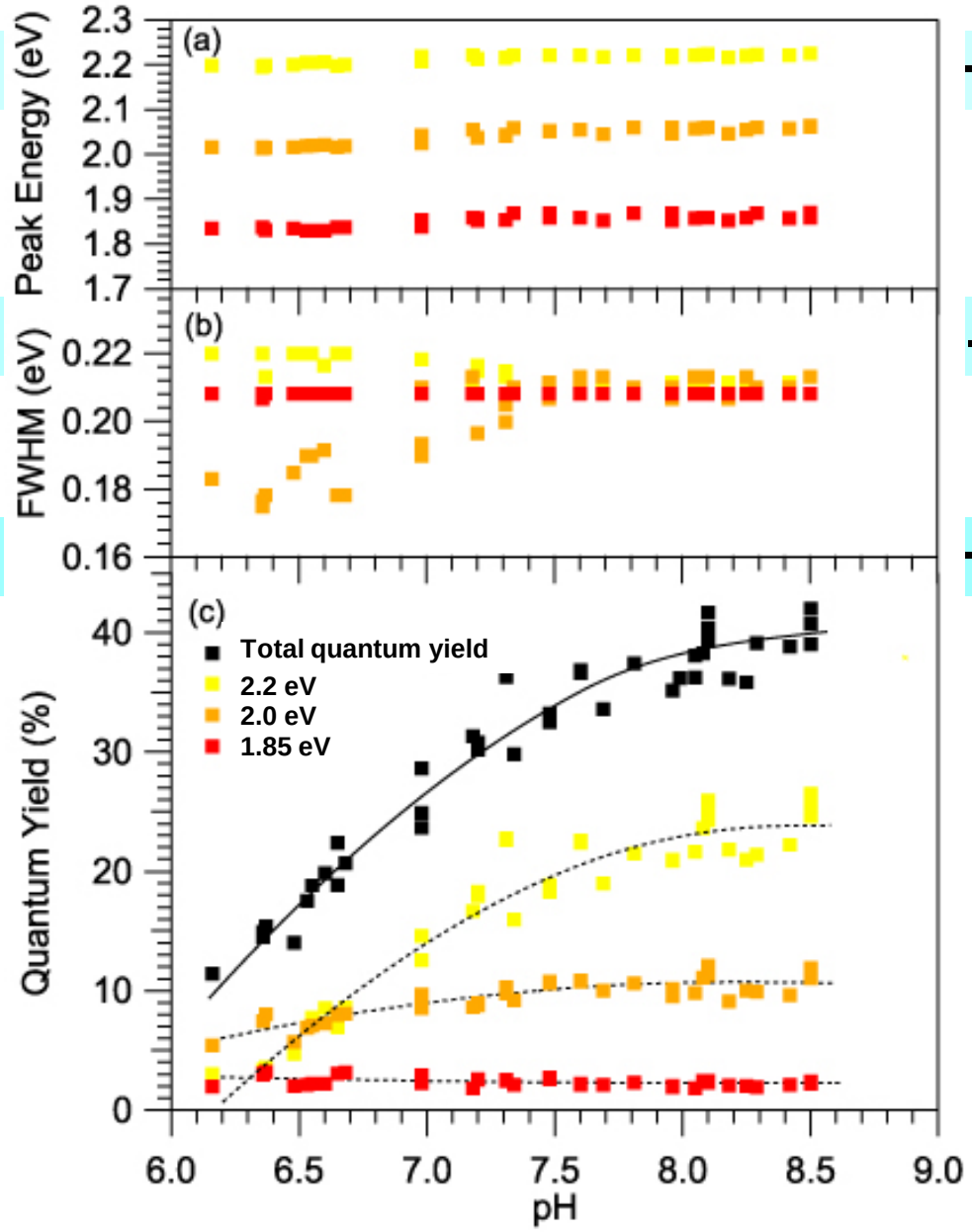








Quantitative analysis



-Peak Energy-

pH dependence : **no**

-Full width at half maximum-

pH dependence : **a little**

-Quantum yield-

Total
2.2 eV peak
} pH dependence : **yes !**

2.0 eV peak
1.85 eV peak
} pH dependence : **no**

pH dependence of total QY
is determined by that of QY of
the 2.2eV-peak component

Biochemistry of luciferin synthesis

FEBS Letters 580 (2006) 5283–5287

Stereoisomeric bio-inversion key to biosynthesis of firefly D-luciferin

Kazuki Niwa[a,b], Mitsuhiro Nakamura[c], Yoshihiro Ohmiya[a,*]

[a] *Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Japan*
[b] *National Metrology Institute of Japan, National Institute of Advanced Industrial Science and Technology (AIST), Japan*
[c] *Integrated Research Institute, Tokyo Institute of Technology, Japan*

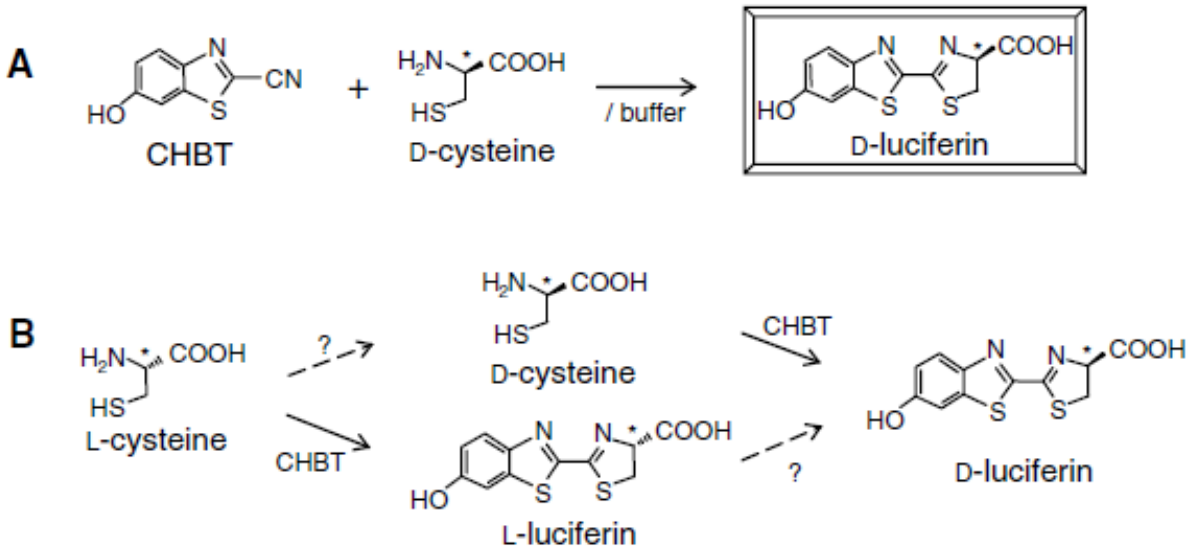
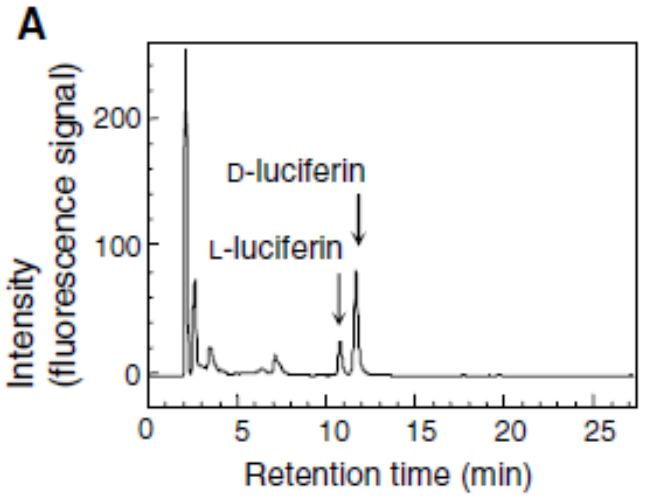


Fig. 1. Synthesis of firefly D-luciferin. (A) Chemical synthesis of D-luciferin. The asymmetric carbon of cysteine corresponds to that of luciferin. (B) Hypothetical biosynthetic pathways of D-luciferin from L-cysteine.



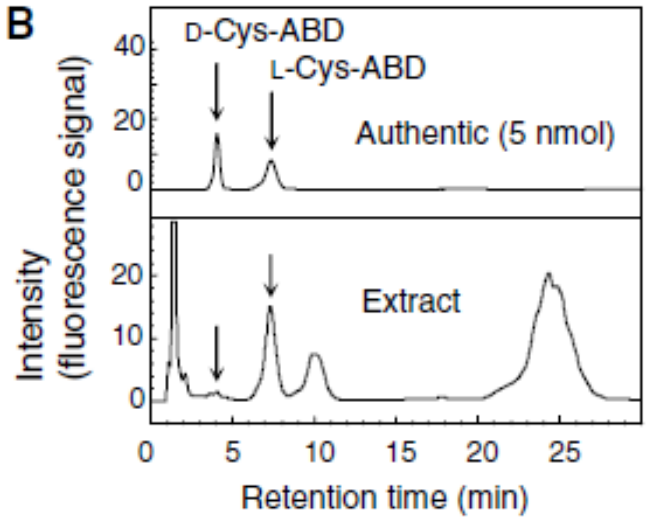


Fig. 2. Chiral analyses of in vivo luciferin and cysteine. (A) Chromatogram of luciferin in an adult firefly, obtained with a chiral column. (B) Chromatogram of ABD-labeled cysteine from four adult fireflies, obtained with a chiral column.

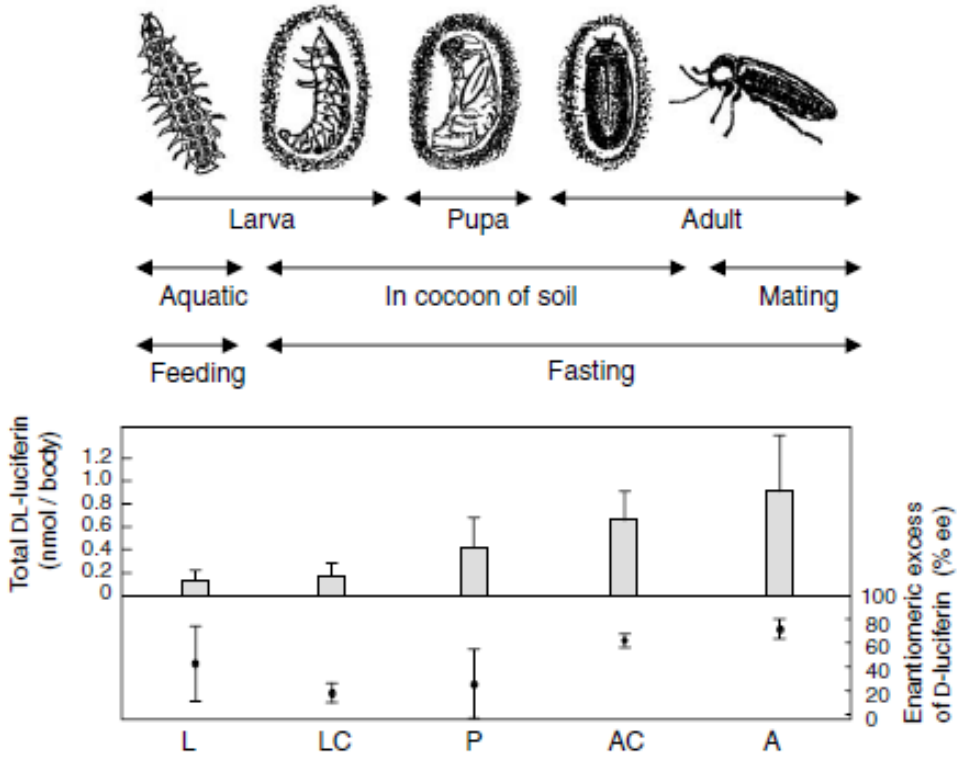


Fig. 3. Life stage, luciferin content, and D-luciferin chirality. Illustrated are the five distinctive life stages of the Japanese firefly, *L. lateralis*. L, larva; LC, larva in cocoon; P, pupa; AC, adult in cocoon; A, adult. Graphs show the total amounts of D- and L-luciferin in an individual body and the enantiomeric excess of D-luciferin (% ee). Error bars indicate standard deviations.

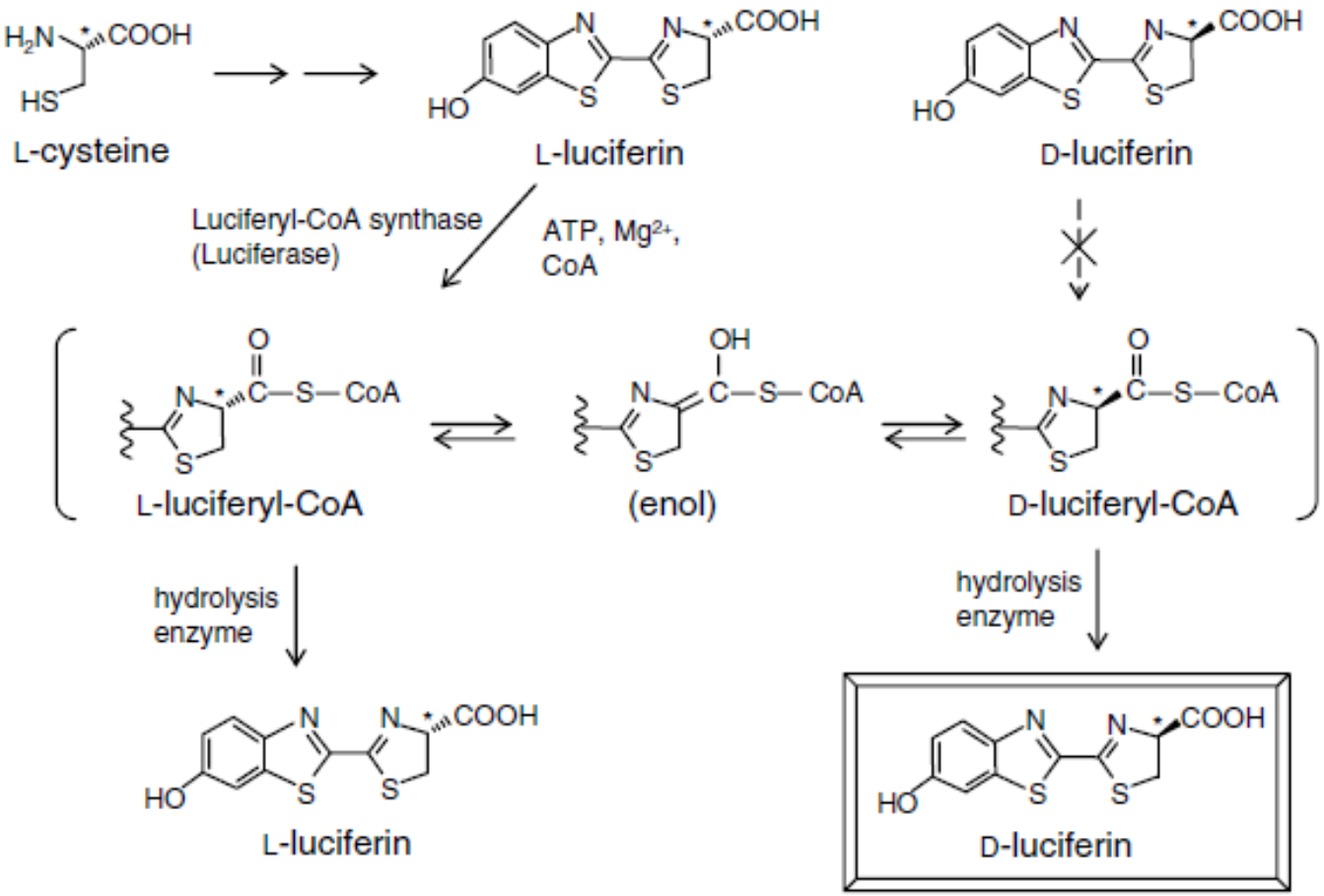


Fig. 5. Proposed biosynthetic pathway of D-luciferin. L-luciferin is produced from natural L-cysteine. L-Luciferin is converted into L-luciferyl-CoA that is easy to racemize by enolization. Hydrolysis of D-luciferyl-CoA gives the bioluminescent substrate, D-luciferin.

Application of beetle bioluminescence system

- Diversity of luminescent beetles
- Gene hunting for beetle luciferases
- Molecular mechanism of color differences
- Determining the quantum yield of bioluminescence reaction
- Biochemistry of luciferin synthesis

My challenge for novel reporter assay using color different luciferase

- Kitayama Y, Kondo T, Nakahira Y, Nishimura H, Ohmiya Y, Oyama T: An in vivo dual-reporter system of cyanobacteria using two railroad-worm luciferases with different color emissions. *Plant Cell & Physiology* 45, 109-113. 2004

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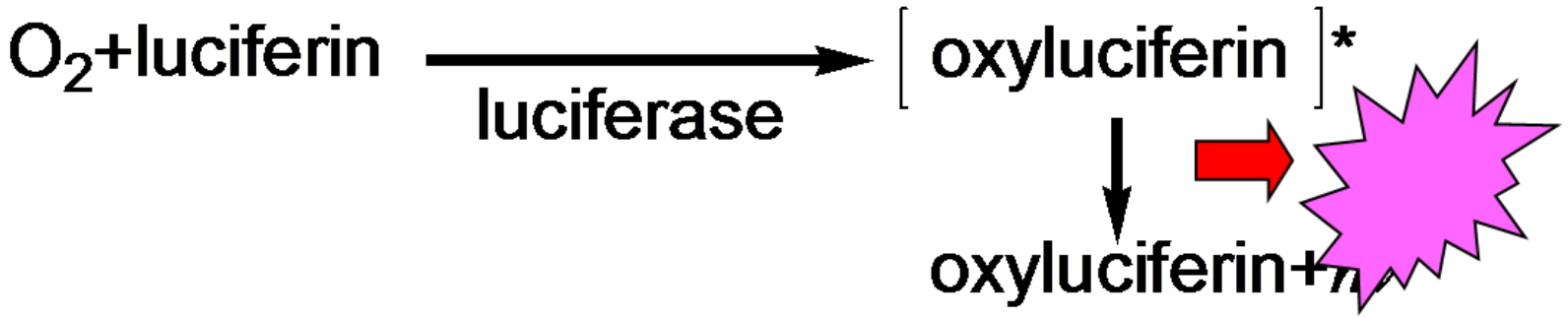
Bioluminescence is a chemical reaction between luciferin and luciferase. Light auto-put is depending to the amounts of luciferin, luciferase or cofactors (ATP etc.)



Luciferase is a powerful reporter enzyme for gene expression analysis, immune assay, and imaging!

Our group have been contributed for the basic and application of bioluminescence !!