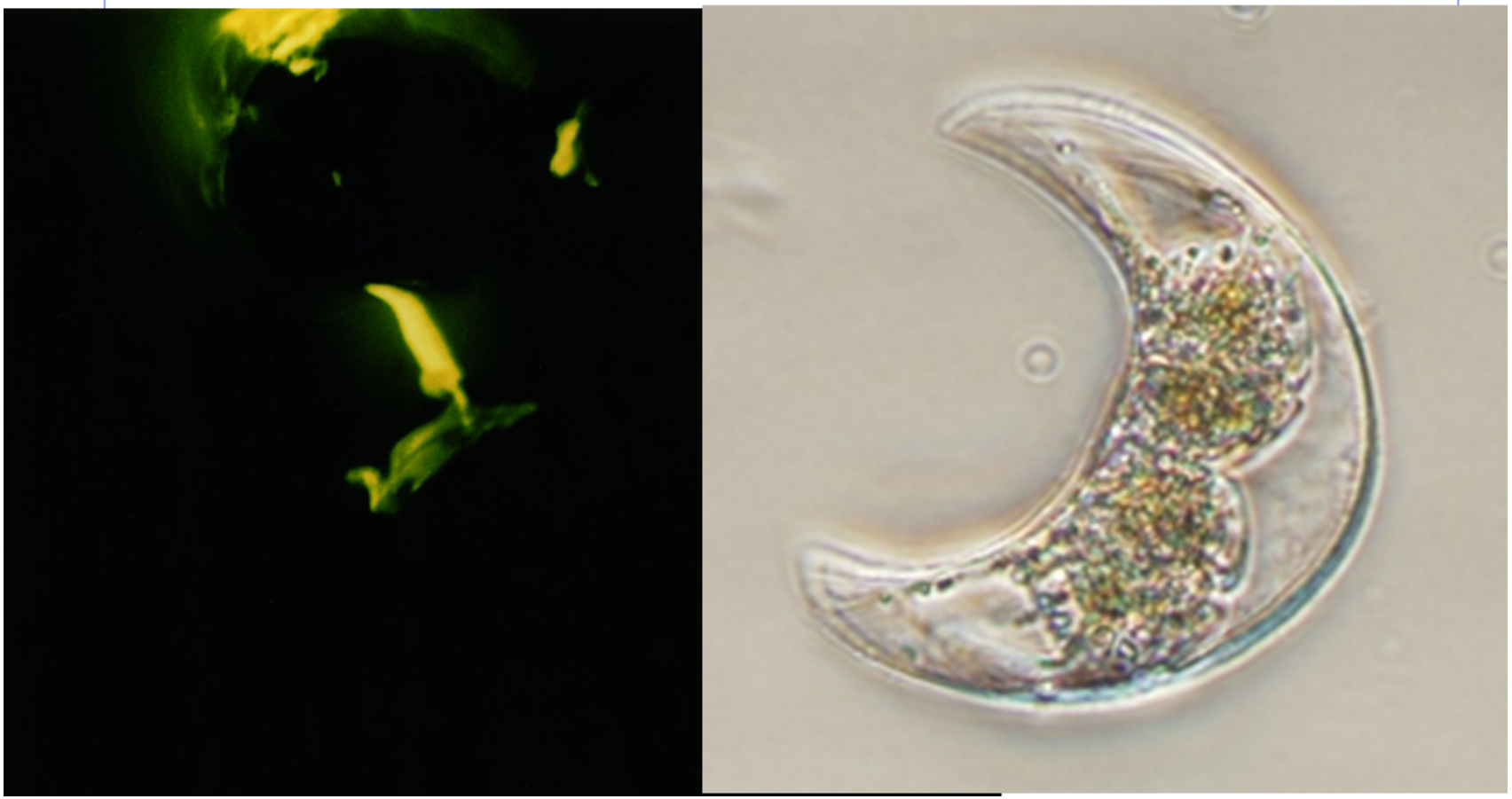
Bioluminescence-3
Latia & Dinoflagellate

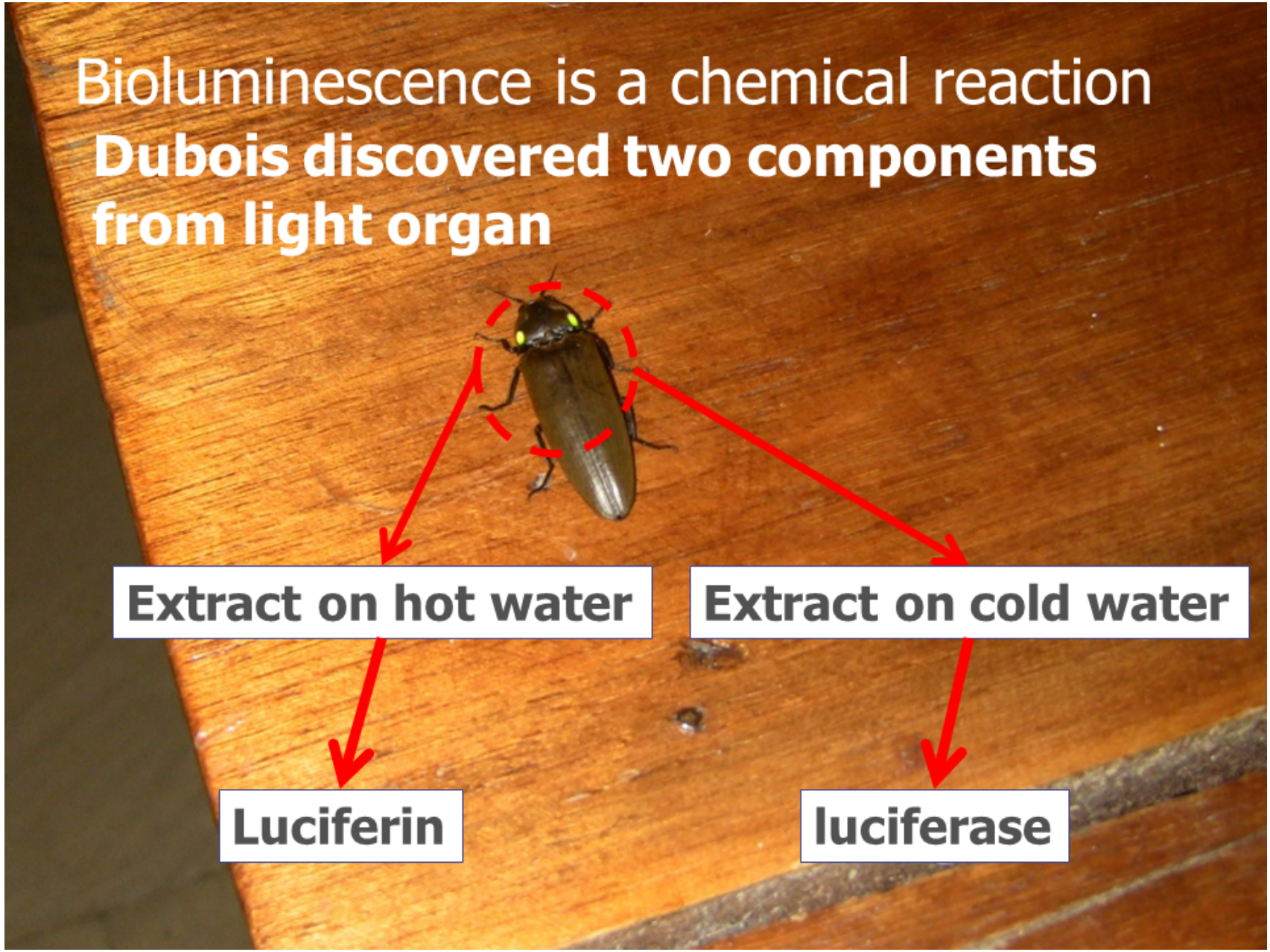
Y. Ohmiya

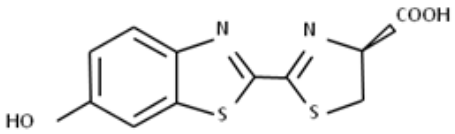


The beginning of the Chemical Study of Bioluminescence

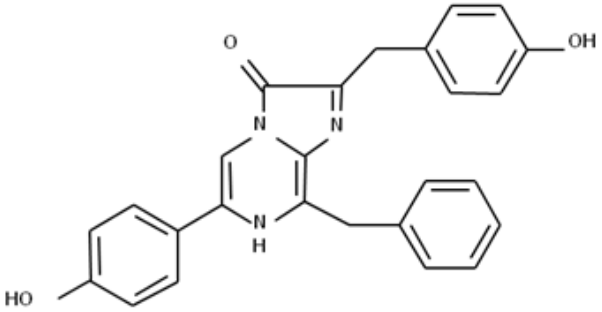
It is generally considered that the modern study of bioluminescence began when Dubois demonstrated the first example of a luciferin-luciferase reaction in 1985. He made two aqueous extracts from the luminous West Indies beetle Pyrophorus. One of the extracts was prepared by crushing the light organs in cold water, resulting in a luminous suspension. The luminescence gradually decreased and finally disappeared. The other extract was prepared by initially treating the light organs with hot water, which immediately quenched the light, and then it was cooled. The two extracts gave a luminescence when mixed together. ----------

By O Shimomura in "Bioluminescence"

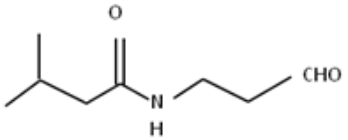

Bioluminescence is a chemical reaction
Dubois discovered two components from light organ
Extract on hot water
Extract on cold water
Luciferin
luciferase



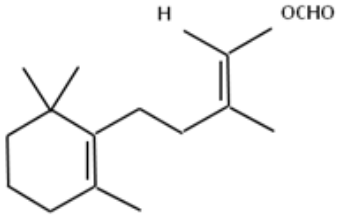
ホタル等発光甲虫
Firefly D-luciferin (D-LH_2)



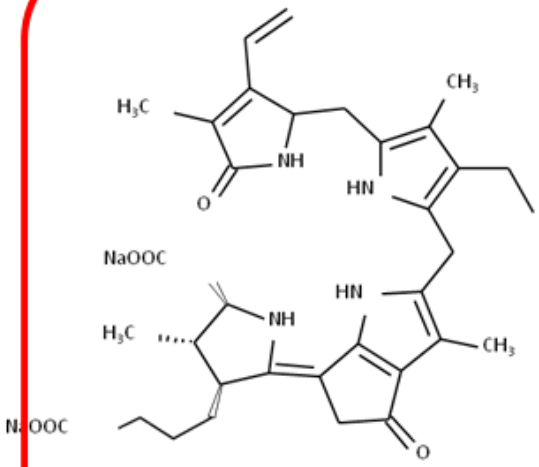
ウミシイタケ、
オワンクラゲ等腔腸動物
ヒトドシエビ、一部発光魚類
(Coelenterazine)



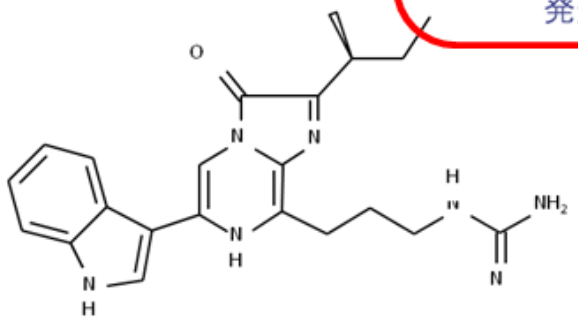
発光ミミズ



発光貝ラチア



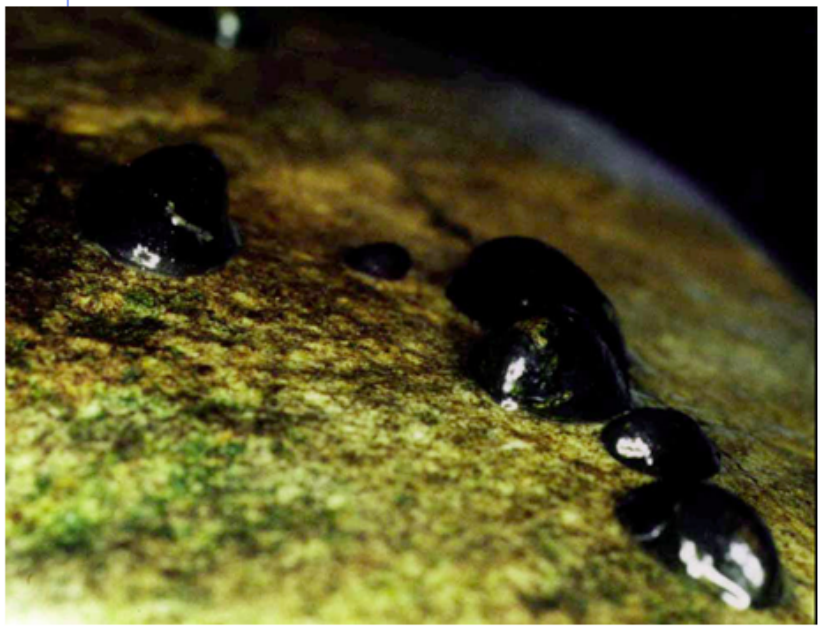
渦鞭毛藻
(Dinoflagellate luciferin)

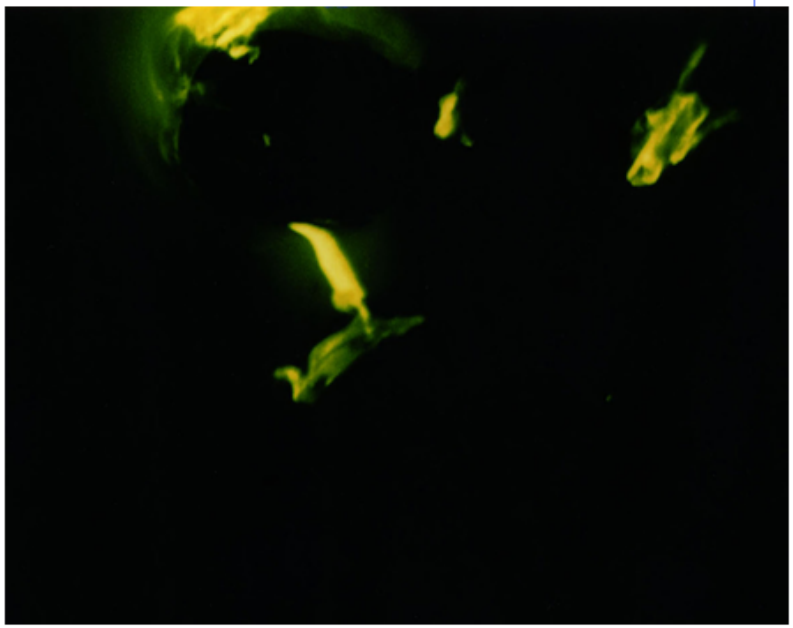


ウミホタル、一部発光魚類
(*Vargula* luciferin)

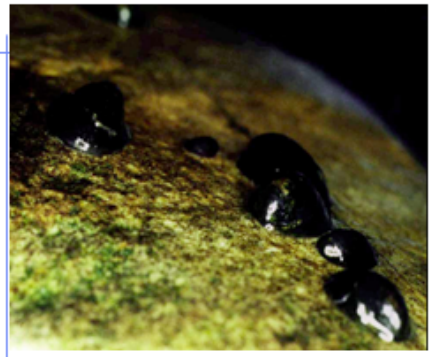
Structure of identified luciferins

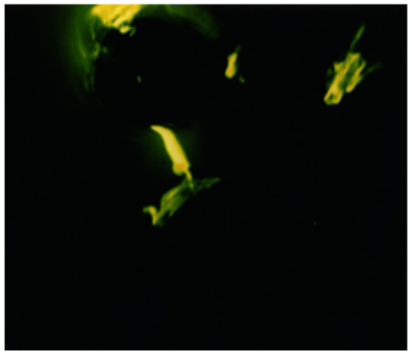
Bioluminescent limpet “Latia” in NZ

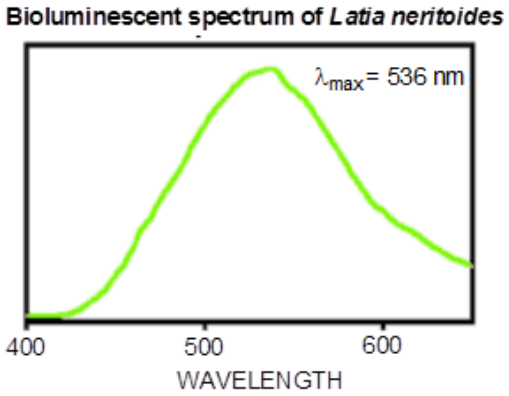


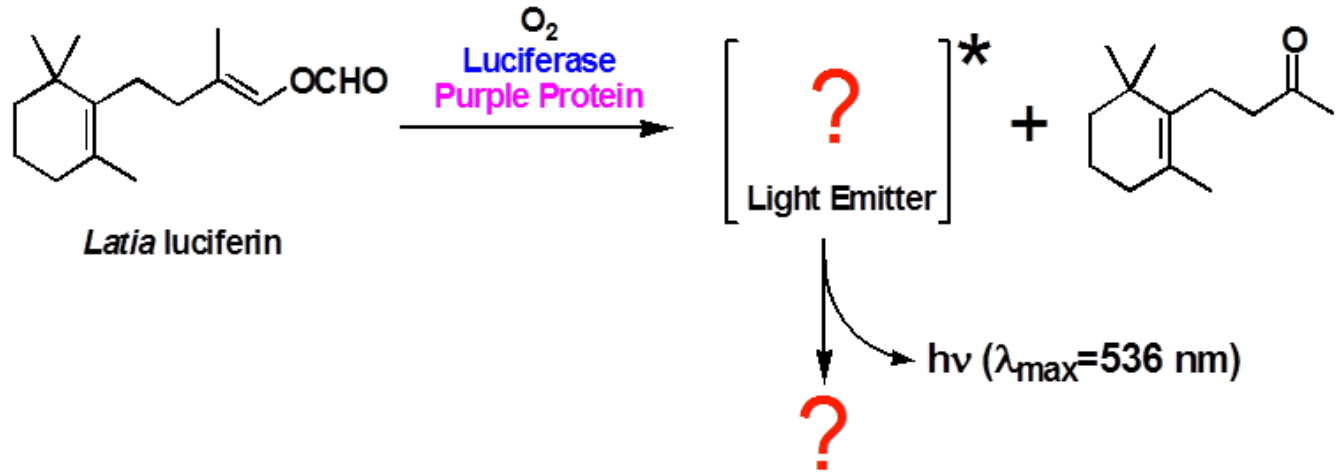


Latia *Bioluminescence*







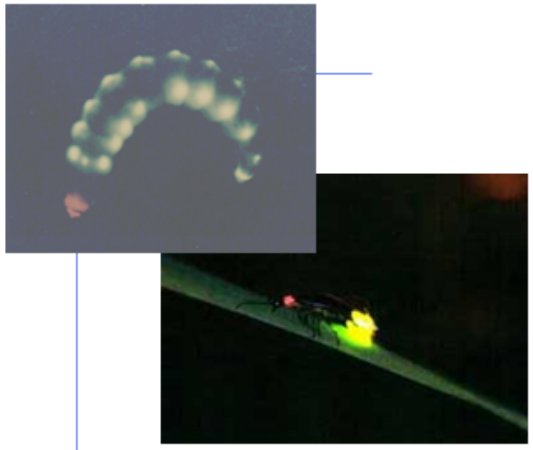


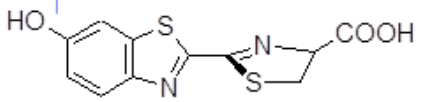
Latia luciferin

There is an exception anywhere.

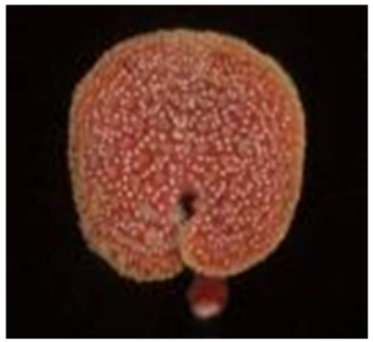
Shimomura, O., Johnson, F. H. & Kohama, Y. (1972), *Proc. Nat. Acad. Sci. USA* **8**, 2086.

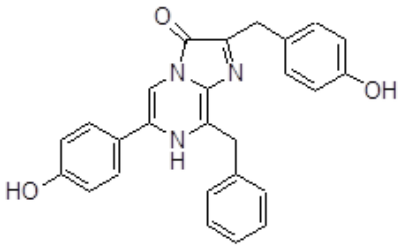
Principle of Bioluminescence



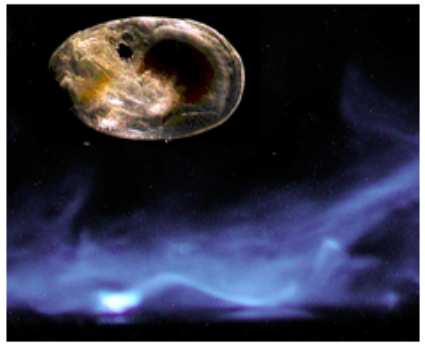


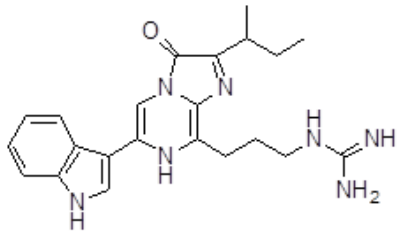
Firefly luciferin
(1961, E. H. White)



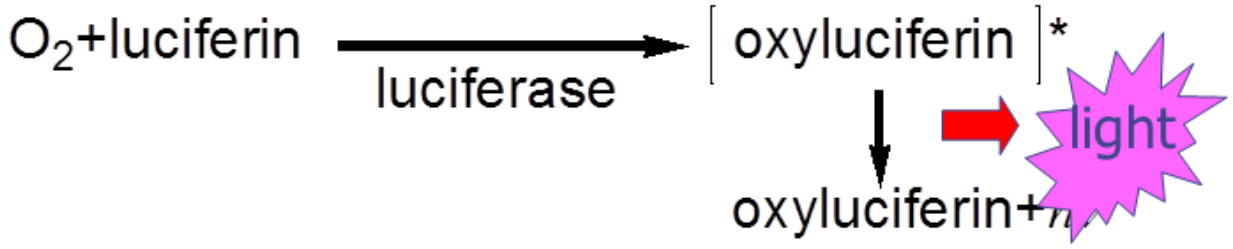


coelenterazine
(1975, S. Inoue)

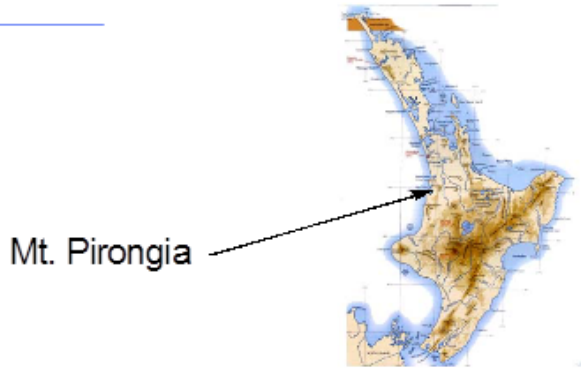




Cypridina luciferin
(1965, Y. Hirata)

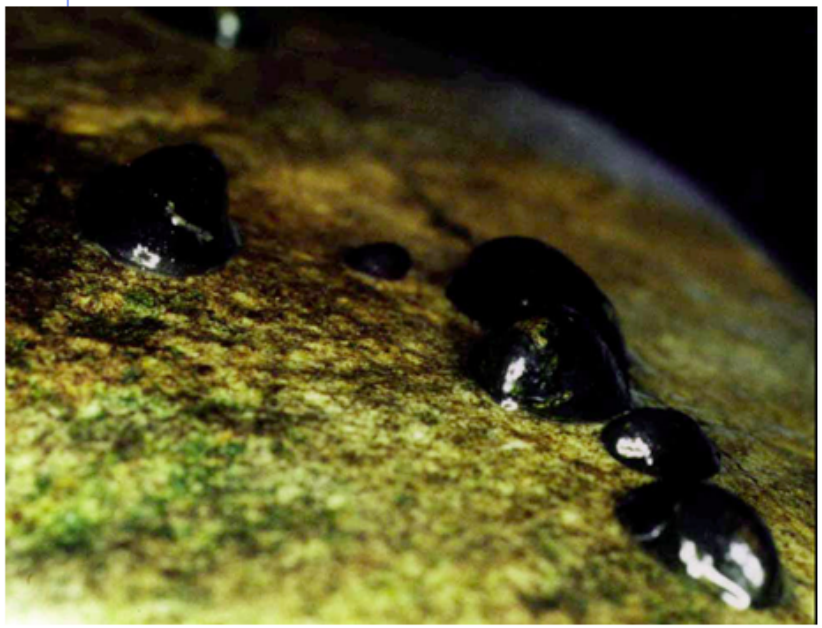


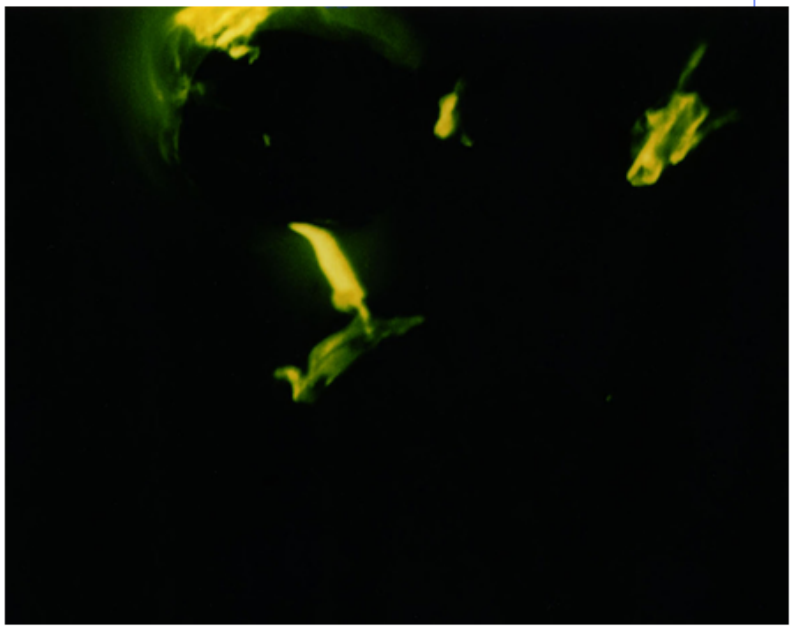
Sampling of Latia *neritoides*



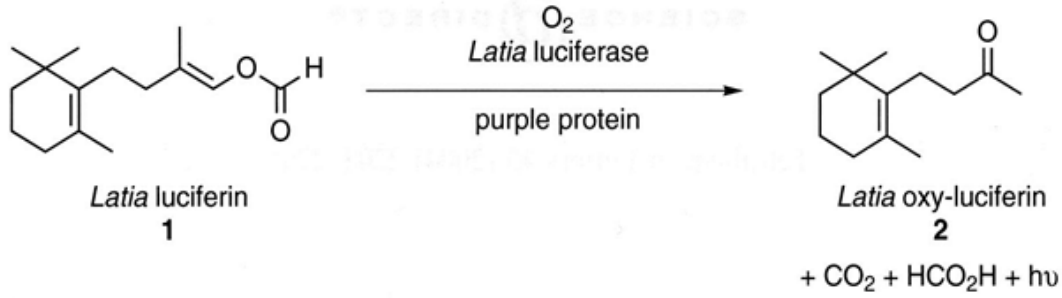
collecting 1997～1999

Bioluminescent limpet “Latia” in NZ





（電気通信大学・平野誉教授、丹羽治樹教授）



Scheme 1. Proposed bioluminescent reaction of *Latia neritoides*.

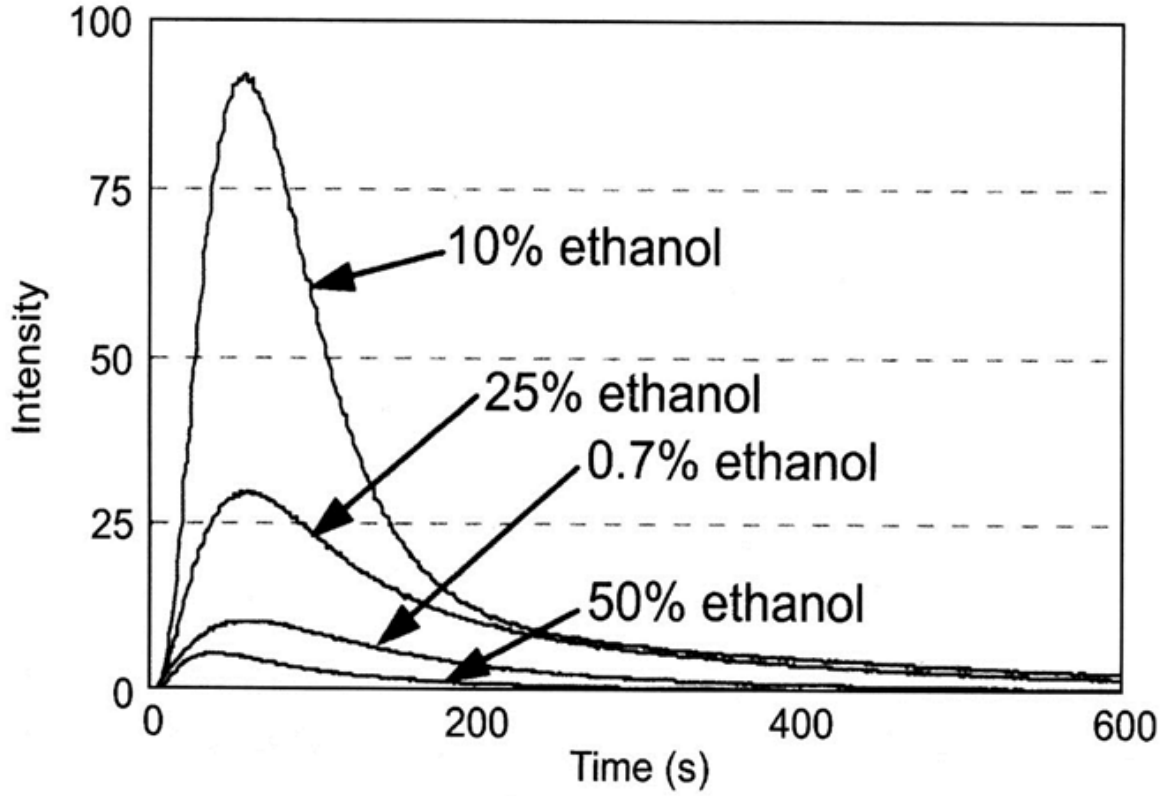
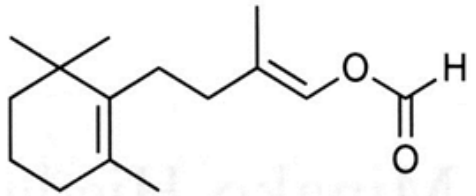
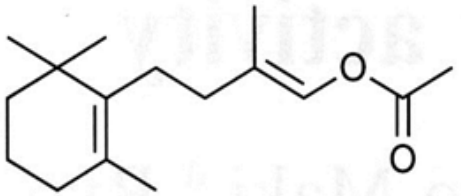


Figure 1. The bioluminescent activity of *Latia* luciferin in various ethanol concentrations. The ethanol concentration values indicate the final concentration.



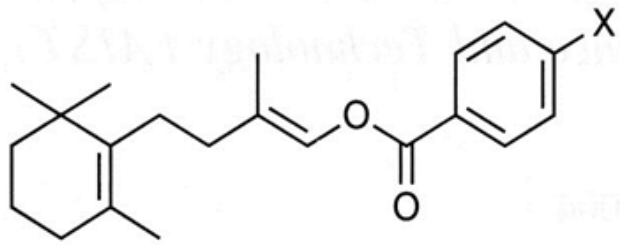
Latia luciferin

1



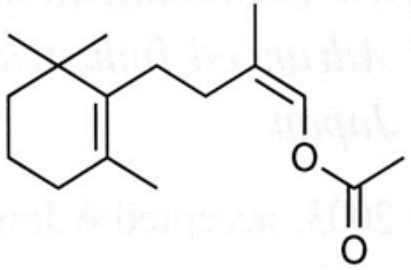
acetate analogue

E-**3**



X = H, NO_2, CN, NMe_2, OMe

4 **5** **6** **7** **8**



acetate analogue

Z-**3**

Scheme 2. The structure of *Latia* luciferin and its analogues.

Table 1. Relative bioluminescent activity of *Latia* luciferin analogues

Substrate	Luminous intensity[a]	Total luminous energy[b]	Emission max. (nm)
E-**1**	100	100	536
E-**4**	1.1	28	536
E-**5**	4.2	85	536
E-**6**	3.2	71	536
E-**7**	0.79	16	536
E-**8**	2.9	69	536

[a] The peak height was recorded as the luminous intensity.

[b] Total luminous energy was obtained by integrating the bioluminescent measured for 2 h.

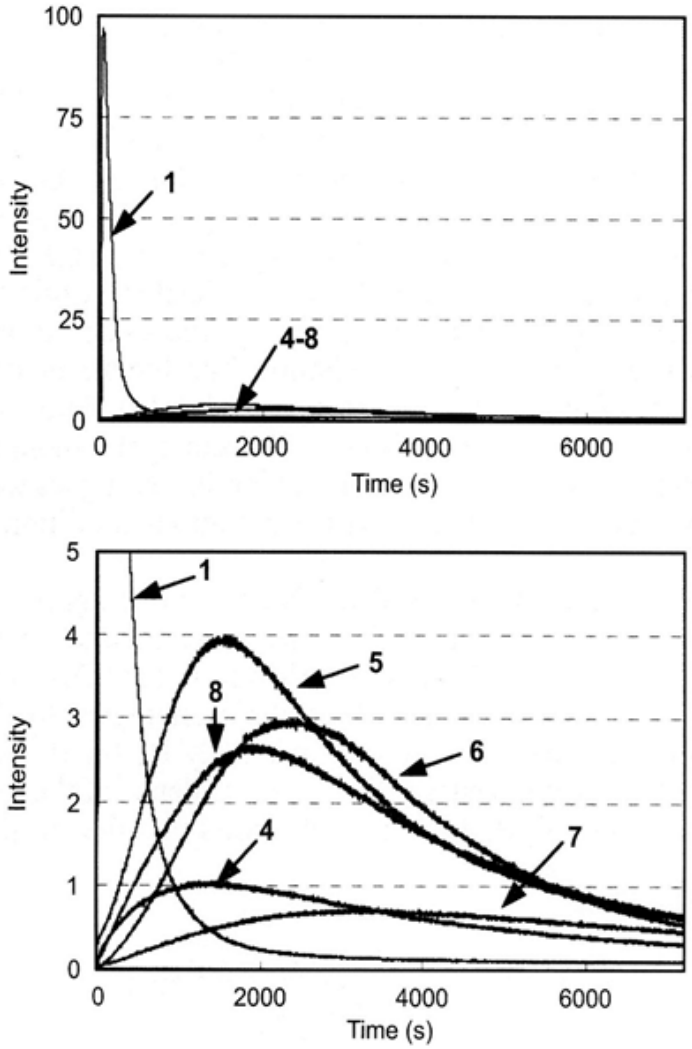
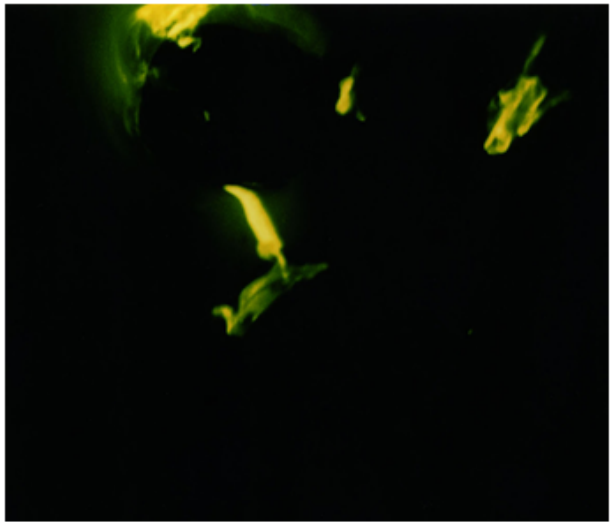


Figure 2. The bioluminescent activity of *Latia* luciferin and its analogues. Below is a magnified figure of above.

Purification Procedure of Latia *Luciferase*



Secretion of bioluminescence system

Frozen *Latia*

↓

Homogenate

↓

1) $(NH_4)_2SO_4$ Fractionation

↓

2) Size-Exclusion FPLC

↓

3) Affinity FPLC (Con A)

↓

4) Ion Exchange FPLC

↓

5) Size-Exclusion FPLC

↓

Purified Luciferase

First Size-Exclusion Chromatography

Frozen *Latia* (39 g)

Homogenate
50 mM Tris-Cl, pH 7.2

1) 33-60% $(NH_4)_2SO_4$ Fractionation (117 mg)

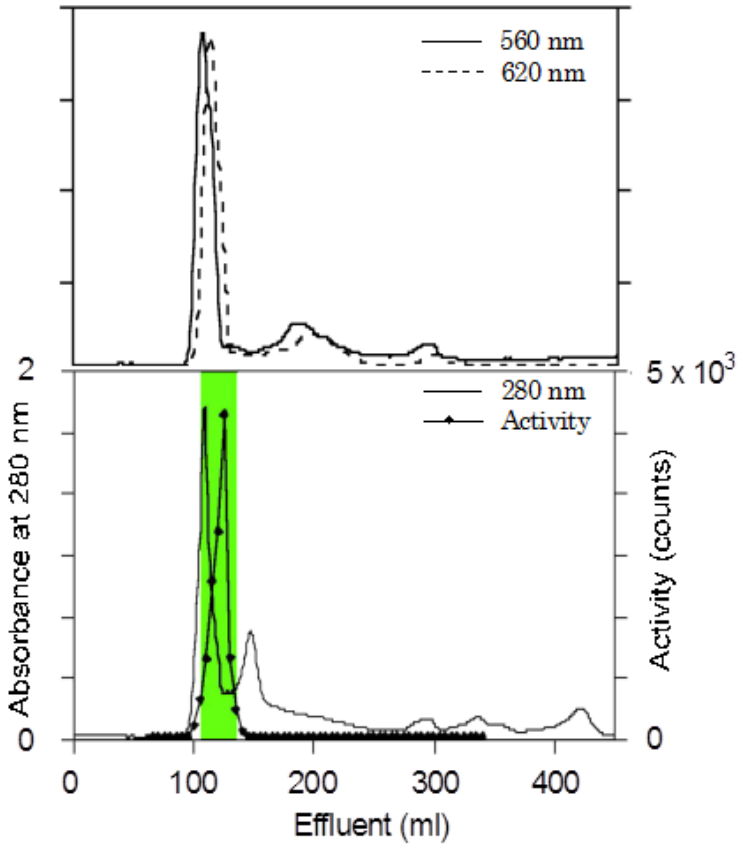
2) HiPrep Sephacryl S-200 (52.2 mg)
Effluent : 50 mM Tris-Cl, 0.2 M NaCl, pH 7.2

3) Hi Trap Con A FPLC

4) Ion Exchange FPLC

5) Size-Exclusion FPLC

Purified Luciferase



Hi-Trap Con A Chromatography

Frozen *Latia* (39 g)

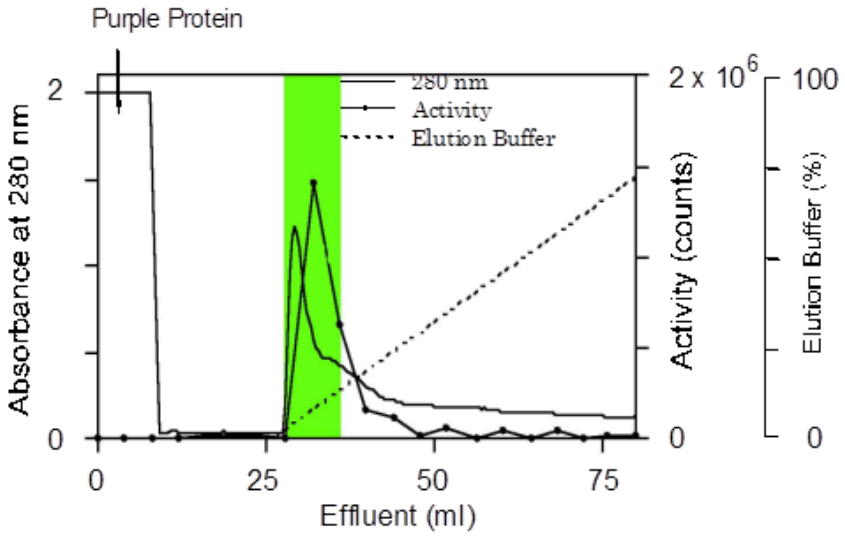
Homogenate

1) 33-60% $(NH_4)_2SO_4$ Fractionation (117 mg)

2) Hi Prep Sephacry S-200 (52.2 mg)

3) HiTrap Con A (6.60 mg)

Washing : 50 mM Tris-Cl, 0.2 M NaCl, pH 7.2
Elution : 50 mM Tris-Cl, 0.2 M NaCl,
0.5 M methyl α-D-gluco-pyranoside pH 7.2



4) Ion Exchange FPLC

5) Size-Exclusion FPLC

Purified Luciferase

Ion-Exchange Chromatography

Frozen *Latia* (39 g)

Homogenate

1) 33-60% $(NH_4)_2SO_4$ Fractionation (117 mg)

2) Hi Prep Sephacry S-200 (52.2 mg)

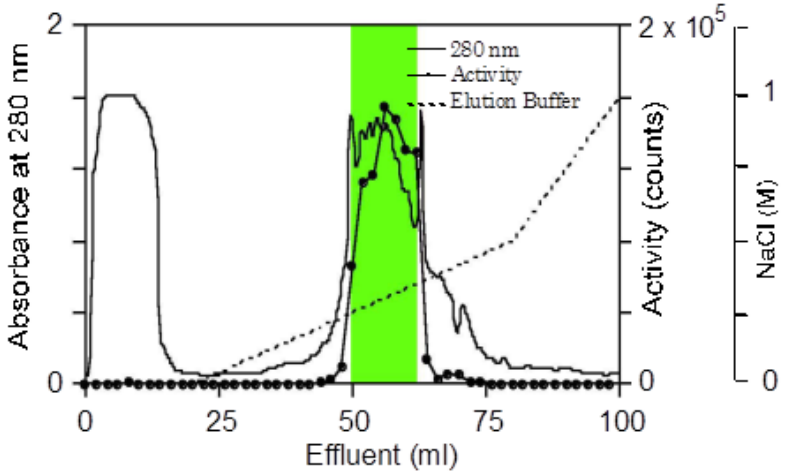
3) HiTrap Con A (6.60 mg)

4) Mono Q (2.00 mg)

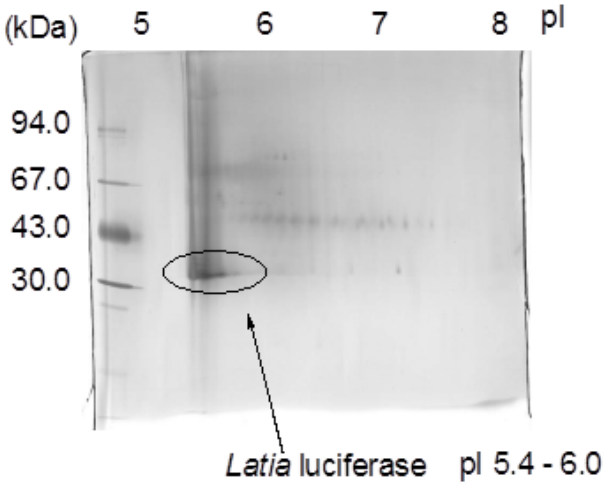
Washing : 25 mM Na-Phosphate, pH 6.4
Elution : 25 mM Na-Phosphate,
1 M NaCl, pH 6.4

5) Size-Exclusion FPLC

Purified Luciferase



2D-PAGE



Latia luciferase pI 5.4 - 6.0

Final Size-Exclusion Chromatography

Frozen *Latia* (39 g)

Homogenate

1) 33-60% $(NH_4)_2SO_4$ Fractionation (117 mg)

2) Hi Prep Sephacry S-200 (52.2 mg)

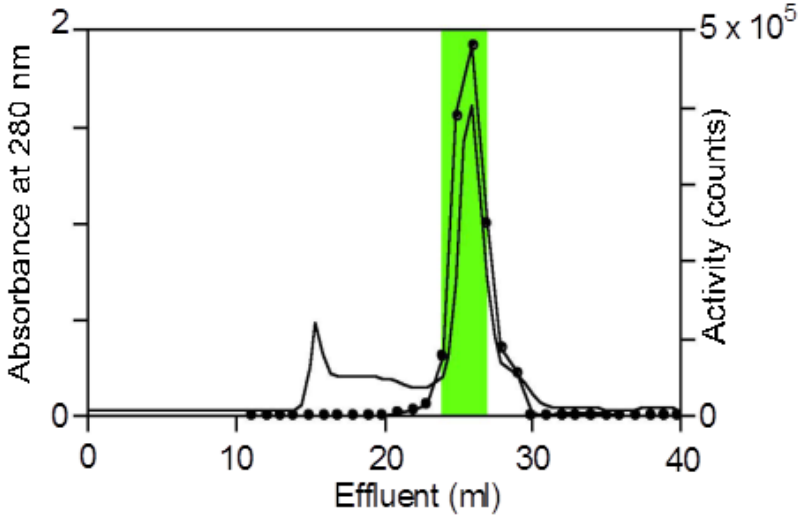
3) HiTrap Con A (6.60 mg)

4) Mono Q (2.00 mg)

5) 2 x Superdex 200

Effluent : 50 mM Tris-Cl, 0.2 M NaCl, pH 7.2

Purified Luciferase (0.52 mg)



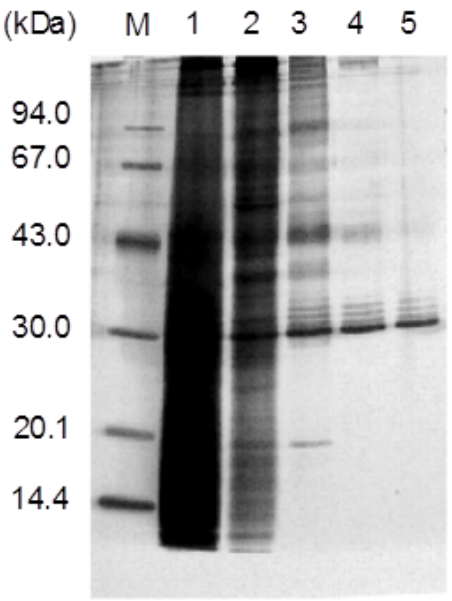
Purification of Latia *Luciferase*

Frozen *Latia* (ca. 39 g)

- Homogenate
- 1) 33-60% $(NH_4)_2SO_4$ Fractionation
- 2) HiPrepSephacryl S-200 FPLC
- 3) HiTrap Con A FPLC
- 4) Mono Q FPLC
- 5) 2 x Superdex 200 FPLC

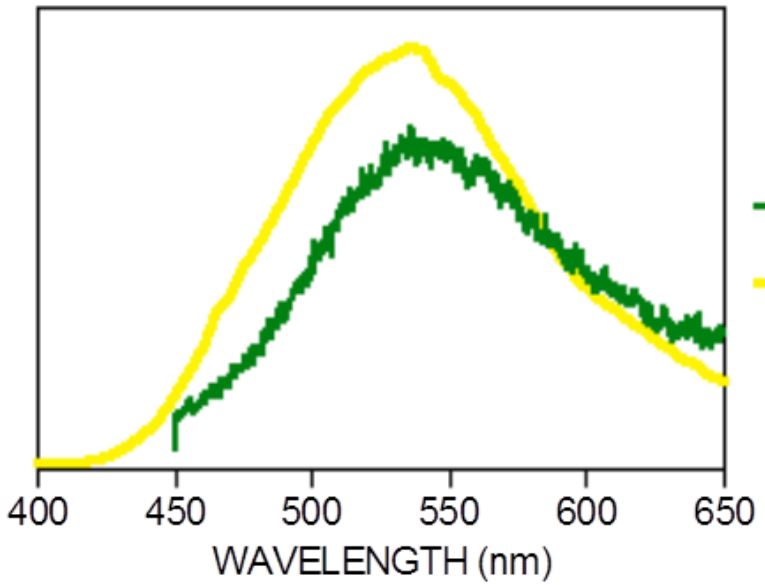
Purified Luciferase (520 μg)

12.5% SDS-PAGE (Silver Stain)



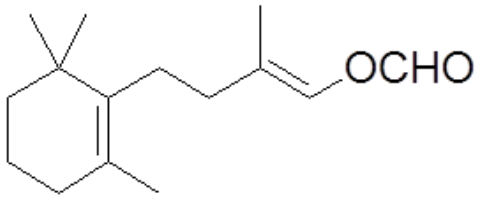
Latia (39 g)	Total Activity (counts)	Protein (mg)	Specific Activity (counts/mg)	Yield (vs. Activity)	Yield (vs. Protein)	Fold (times)
1. 33-60% $(NH_4)_2SO_4$	3.95×10^8	117	3.38×10^6	100	100	1
2. HiPrep Sephacryl S-200	2.63×10^8	52.2	5.04×10^6	67	45	1.5
3. HiTrap Con A	2.30×10^8	6.60	3.49×10^7	58	5.6	10.3
4. Mono Q	2.19×10^8	2.00	1.10×10^8	55	1.71	32.5
5. Superdex 200	1.87×10^8	0.52	3.59×10^8	47	0.45	105

Bioluminescent Spectra of Latia neritoides



Luciferin-Luciferase Reaction

Bioluminescence



Latia luciferin

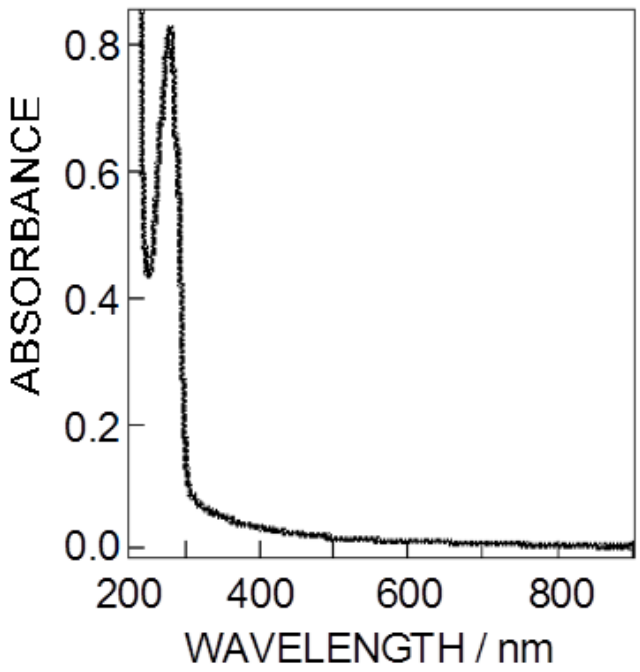
O_2

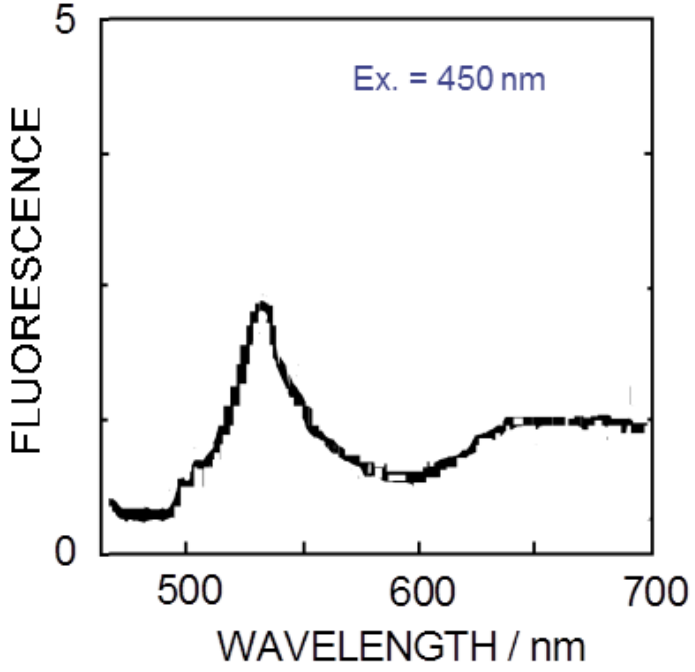
Luciferase

~~Purple Protein~~

yellow-green light (536 nm)

UV-vis Absorption and Fluorescent Spectra of Latia *Luciferase*



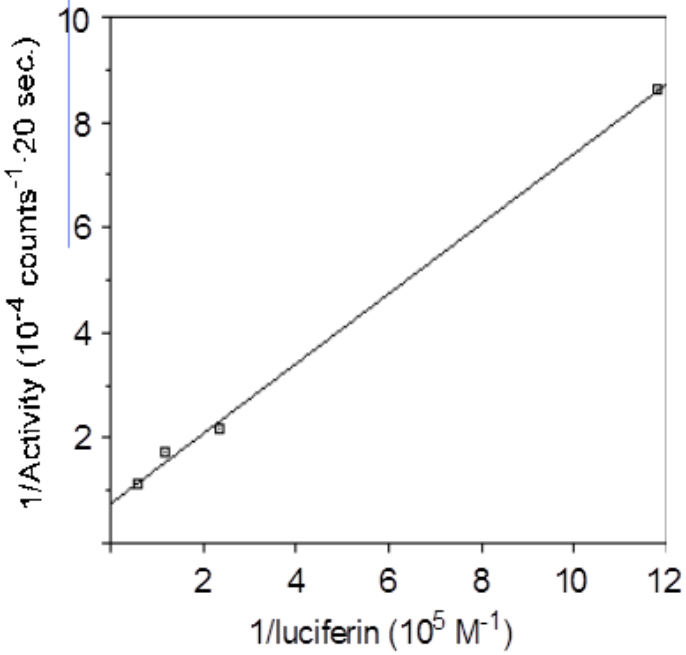


Determination of Km *Value for* Latia *Luciferin*

$$\frac{1}{v_0} = \frac{Km}{Vmax} \cdot \frac{1}{[S]} + \frac{1}{Vmax}$$

Lineweaver-Burk 式

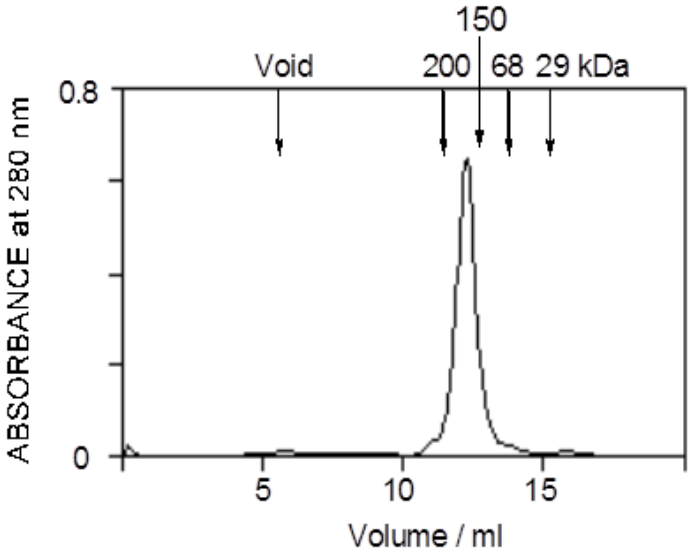
Lineweaver-Burk Plot

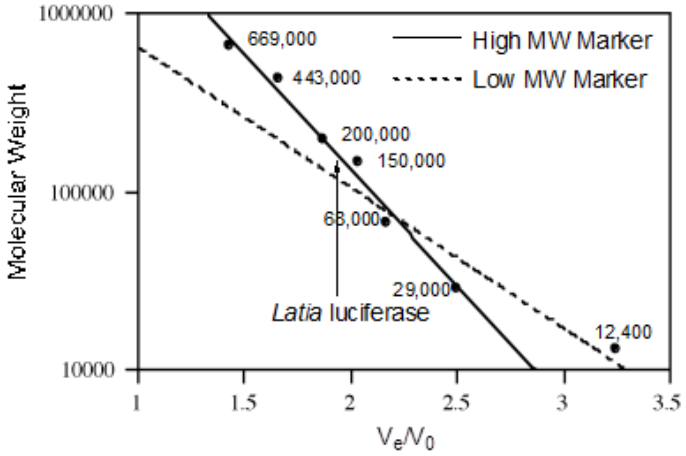


Km value（*Latia* Luciferin） 8.7 μM

Photinus pyralis Luc (for firefly luciferin)	10 μM

Molecular Mass of Latia *Luciferase by Gel Filtration*





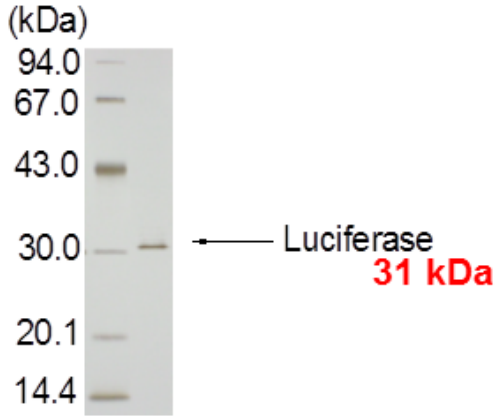
Ve : Elution volume
V_0 : Void volume

Column : Pharmacia Superdex 200
Eluent : 50 mM Tris•Cl, 0.2 M NaCl, pH 7.2
Flow rate : 0.5 ml/min

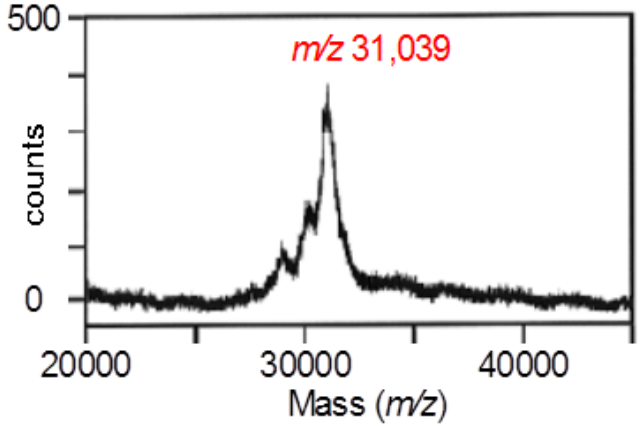
Molecular Mass **180,000 Da**

Molecular Mass of Latia *Luciferase by SDS-PAGE and MALDI-TOF*

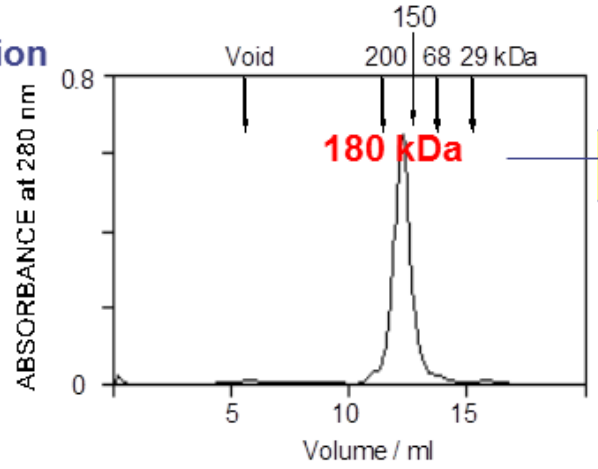
12.5% SDS-PAGE



MALDI-TOF

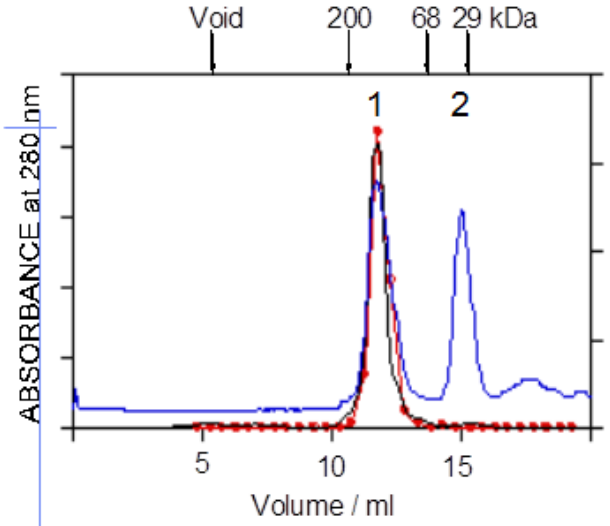


Gel Filtration



31 kDa x 6

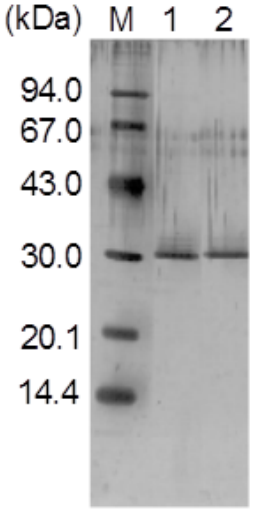
Thermal Denaturation of Latia *Luciferase*



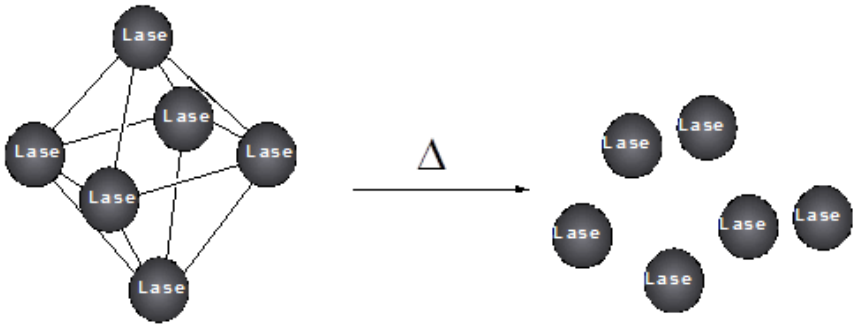
Partially Denaturated Luciferase (50 °C, 10 min)

Purified Luciferase

Activity



Column : Pharmacia Superdex 200
Eluent : 50 mM Tris•Cl, 0.2 M NaCl, pH 7.2
Flow rate : 0.5 ml/min



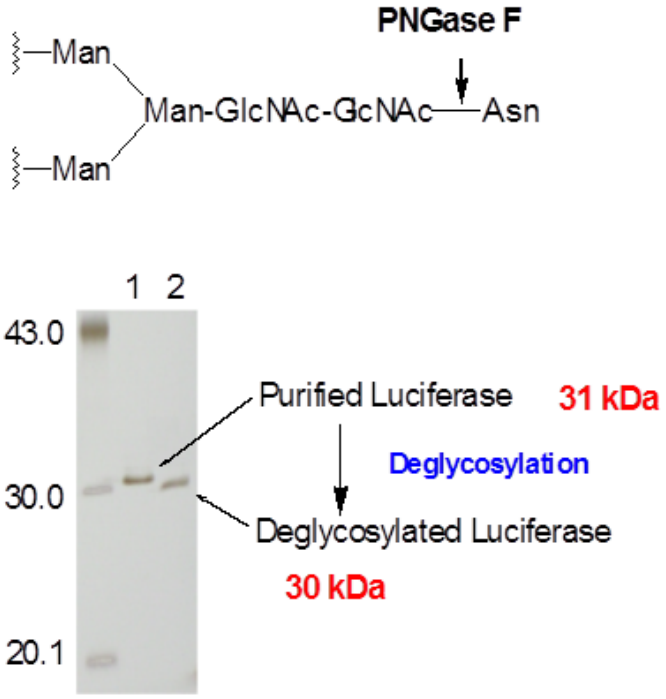
Homohexamer

(Active form)

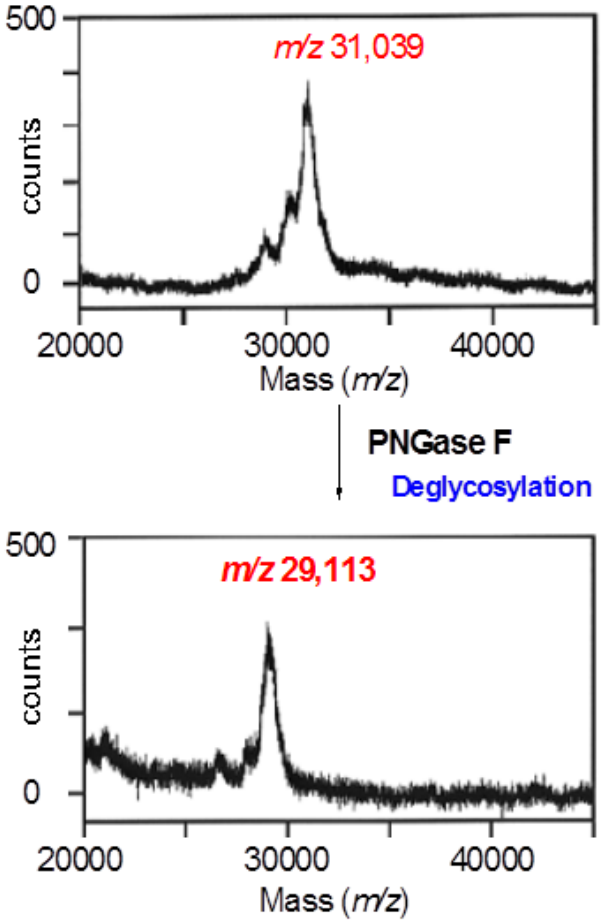
Monomer

(Inactive form)

Deglycosylation of Latia *Luciferase*

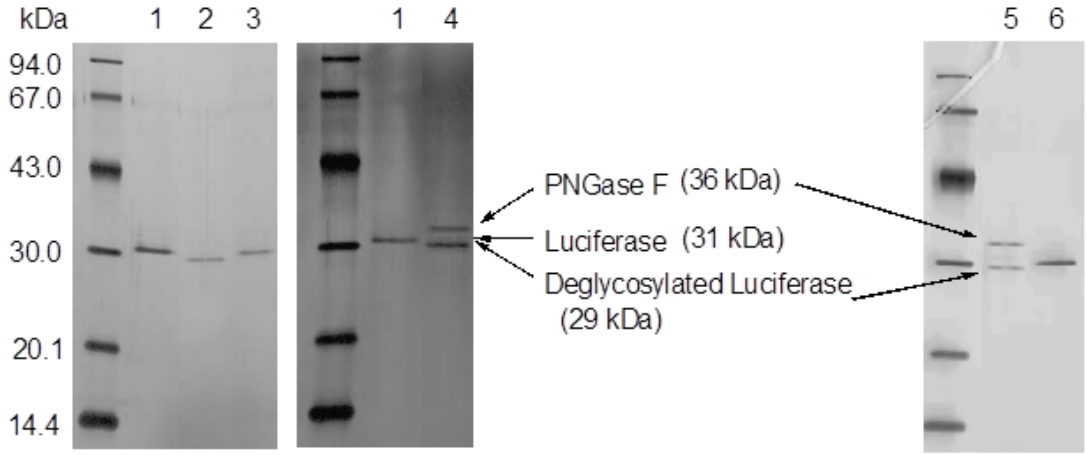


Condition ;
Luciferase (2 μg),
0.5%SDS, 1% mercaptoethanol, 1% NP-40,
PNGase F (2 unit),
37 °C, 2 hour



Glycosylation-Activities Relationship of Latia *Luciferase*

Lane	反応条件	発光活性
1	Luciferase	○
2	Luciferase (0.5% SDS, 1% mercaptoethanol, 100 °C, 10 min.) 1% NP-40, PNGase F(2 units)(37 °C , 1 h)	×
3	Luciferase, NP-40 (37 °C, 1 h)	×
4	Luciferase, PNGase F (40 units) (37 °C, 1 h)	○
5	Luciferase, PNGase F (40 units) (37 °C, 2 h)	×
6	Luciferase (37 °C, 2 h)	○

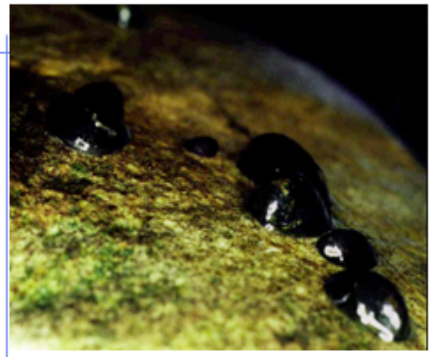


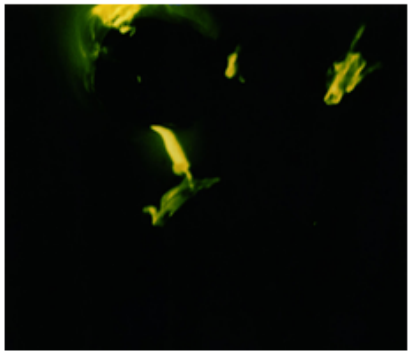
Enzymatic Properties of Latia *Luciferase*

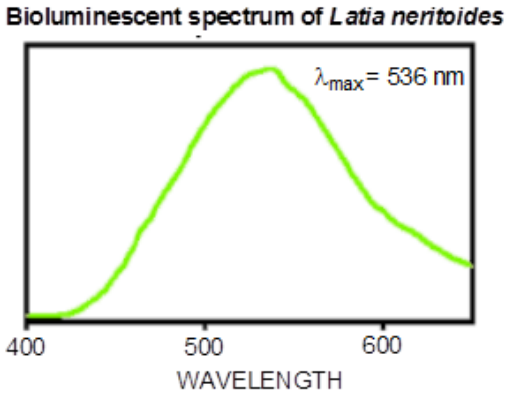
Processing	N-linked glycoprotein
Specific Activity (counts/mg)	3.59×10^8
Molecular Mass	
Gel Filtration	180 kDa
SDS-PAGE	31 kDa
MALDI-TOF	31.0 kDa
Deglycosylated Luciferase	29.1 kDa
Isoelectric Point	5.4-6.0
K_m	8.7 μM

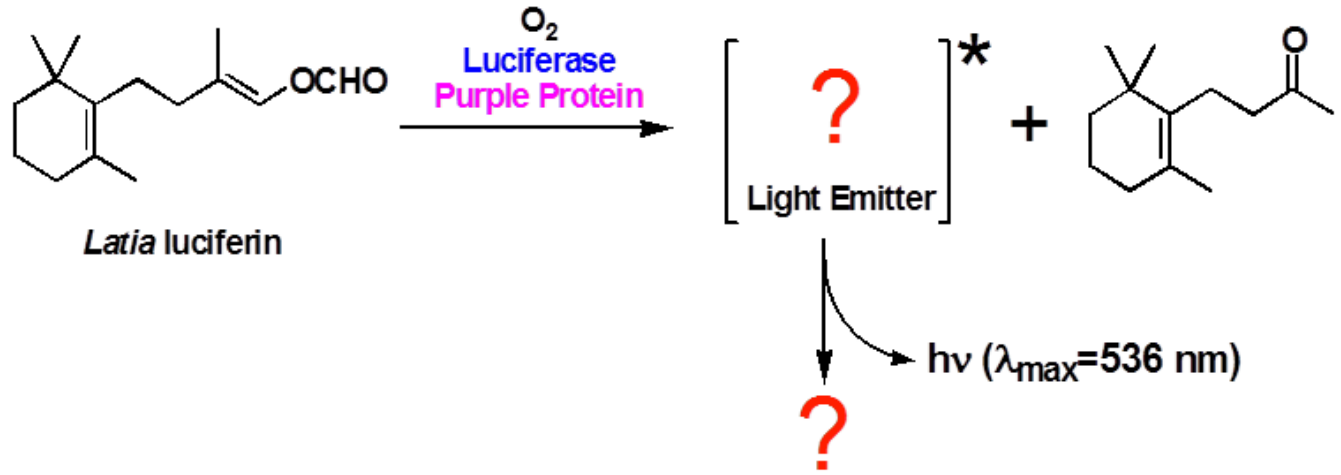
We determined the partial sequences of Latia luciferase and cloned Latia luciferase gene. However, the expressed luciferase have no potential of bioluminescence reaction.
Mystery of Latia is remained.

Latia *Bioluminescence*







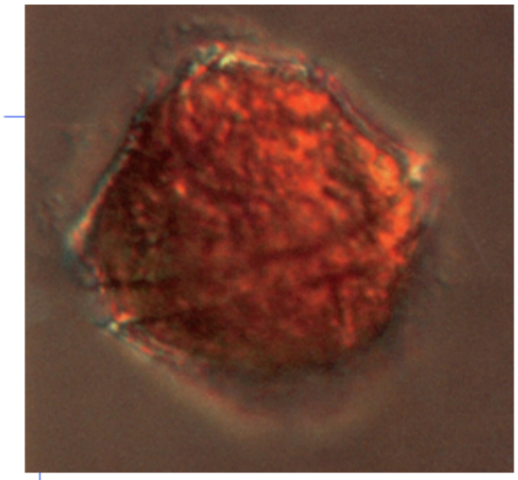


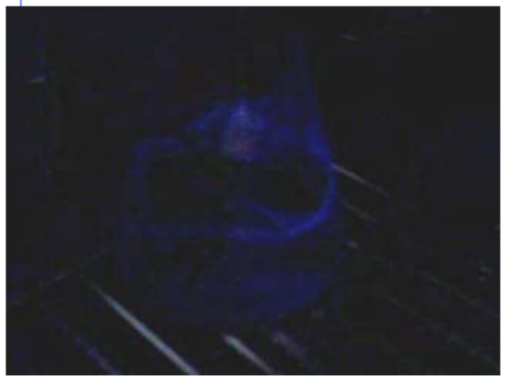
Latia luciferin

There is an exception anywhere.

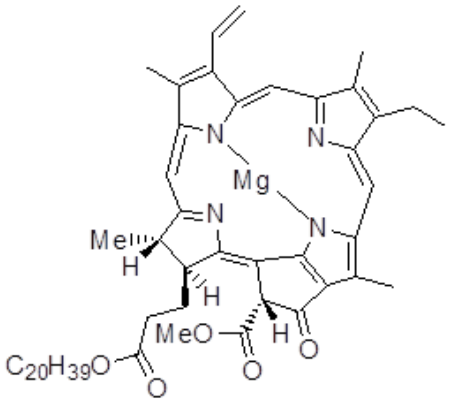
Shimomura, O., Johnson, F. H. & Kohama, Y. (1972), *Proc. Nat. Acad. Sci. USA* **8**, 2086.

Bioluminescence system in Dinoflagellate



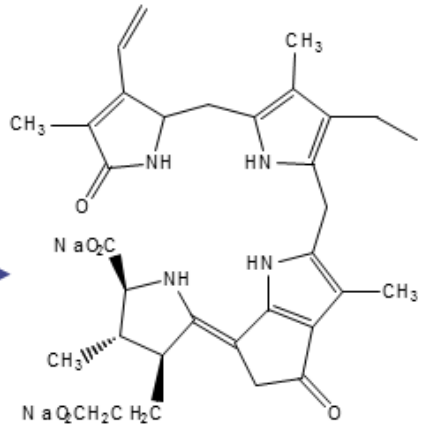


Lingulodinium polyedra



chlorophyll a

?



dinoflagellate luciferin

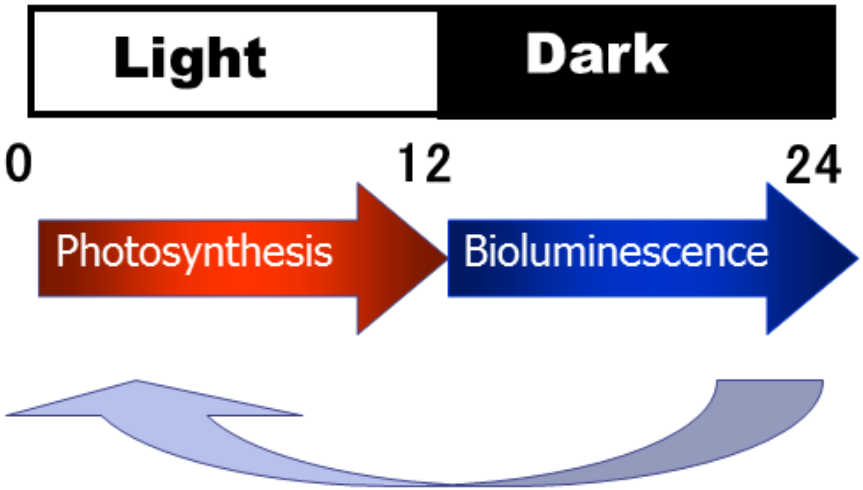
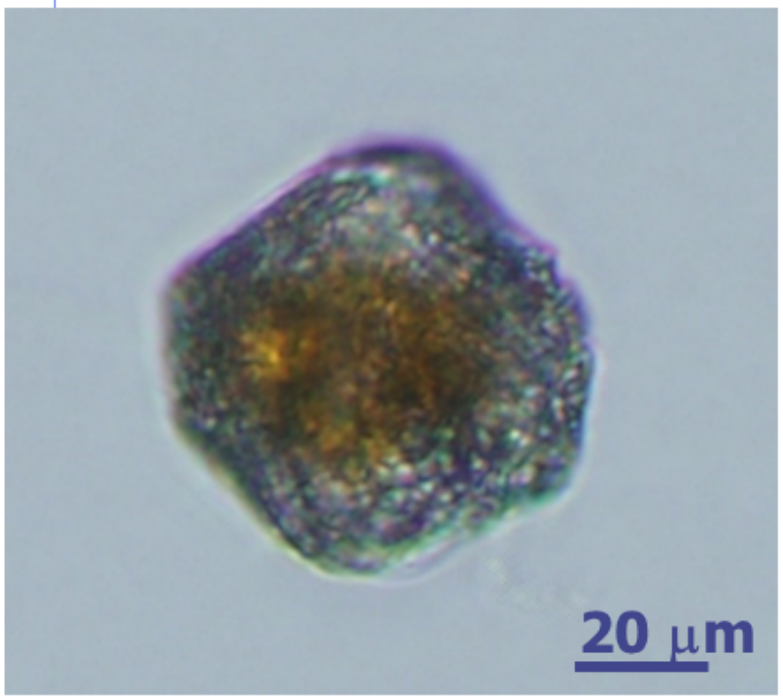
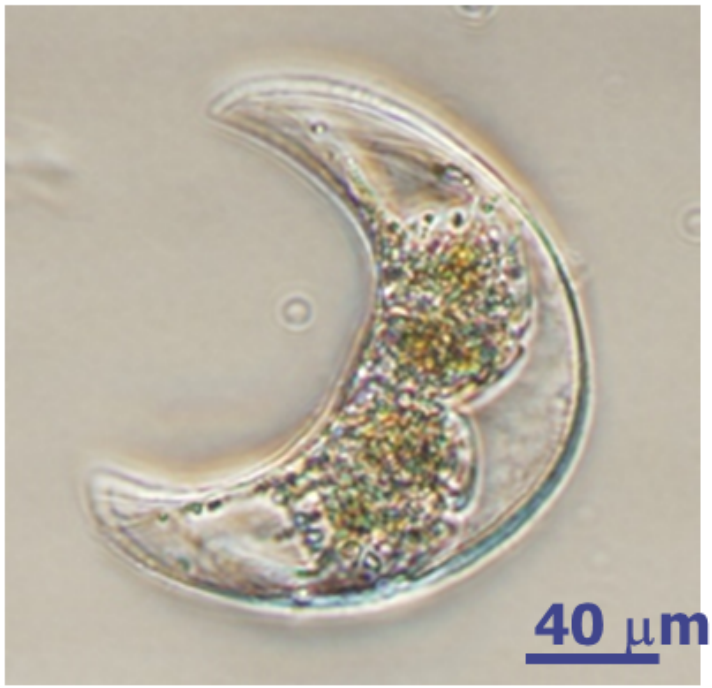


図1

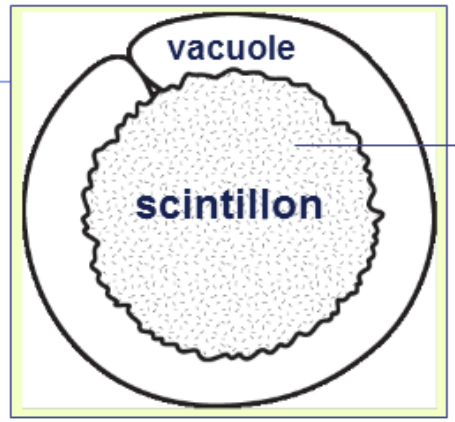


Lingulodinium polyedrum

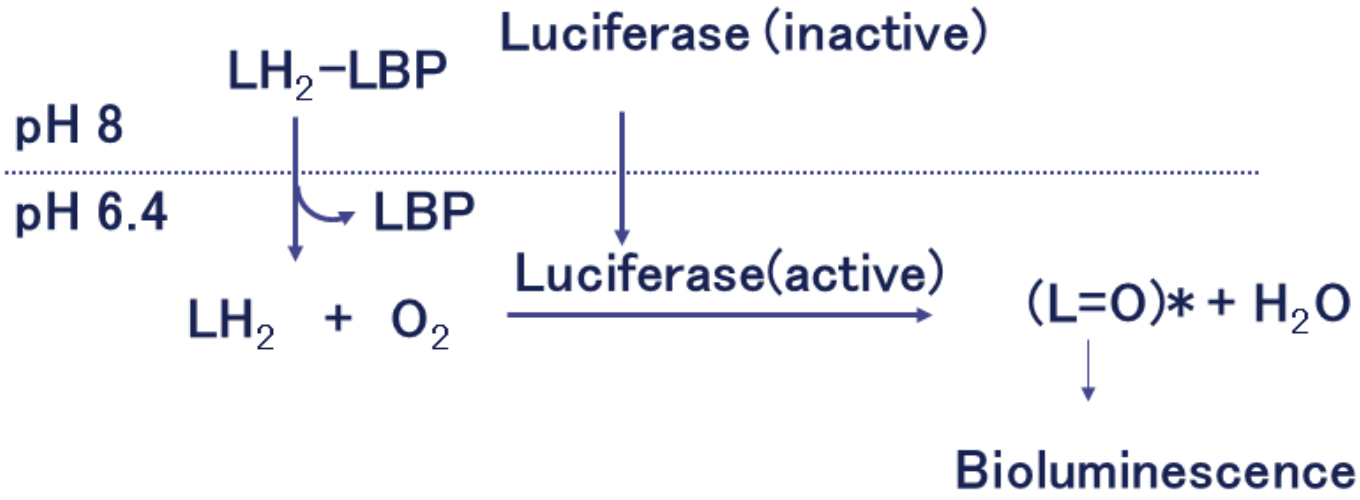


Pyrocystis lunula

Bioluminescence mechanism in Dinoflagellate



- Luciferin (LH_2)
- LBP $\longrightarrow$ LH_2–LBP
- Luciferase

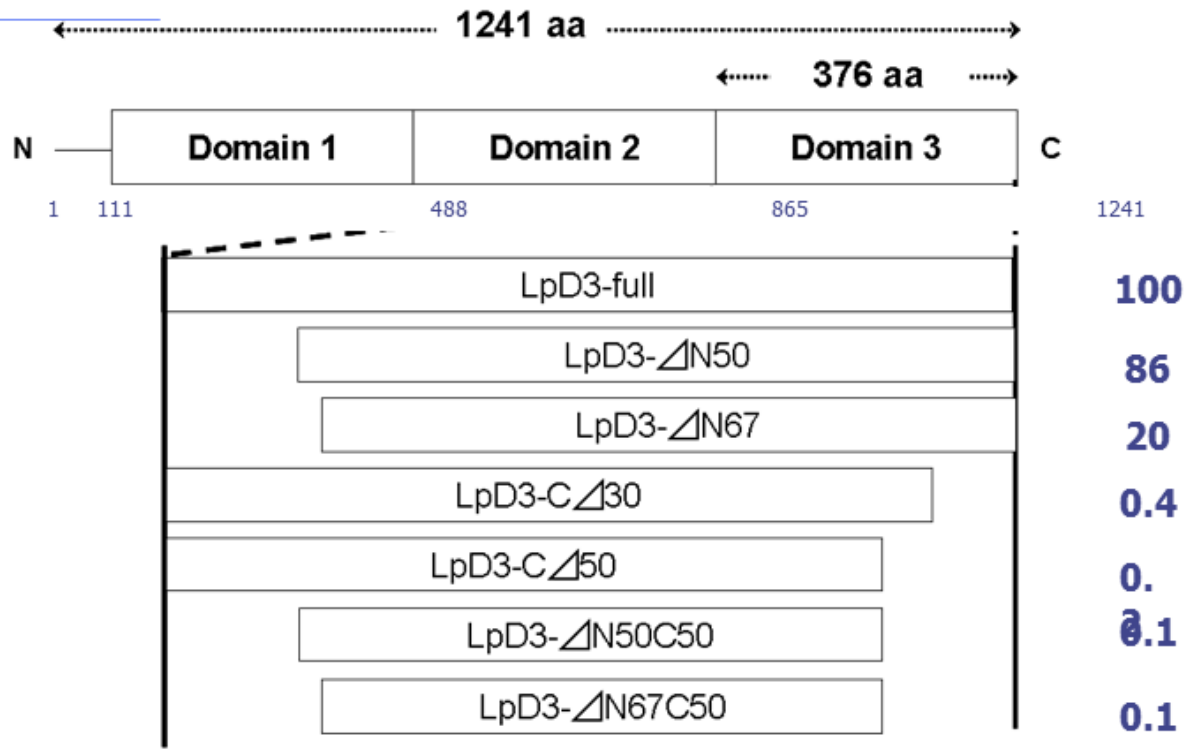


Construction and 3D-Structure of Lp Luciferase

Primary structure of third domain of Lp and Pl luciferases

Figure 1. Alignment of the amino acid sequences of the third domains of *P. lunula* and *L. polyedrum* luciferases. Amino acids are abbreviated using the standard single-letter code. Shadowed letter indicates an amino acid residue conserved between domain 3 of *P. lunula* luciferase and domain 3 of *L. polyedrum* luciferase. Boxed letter indicates an amino acid residue conserved among domain 3 of *P. lunula* luciferase and domains 1, 2 and 3 of *L. polyedrum* luciferase. Bold letters indicate an amino acid residue in the third domain of *P. lunula* luciferase, which is not conserved between the homologous amino acid residues of all the domains in *L. polyedrum* luciferase.

Lp Luciferase and Lp luciferase mutants.



Relative activities of Lp and Lp mutants under various pH

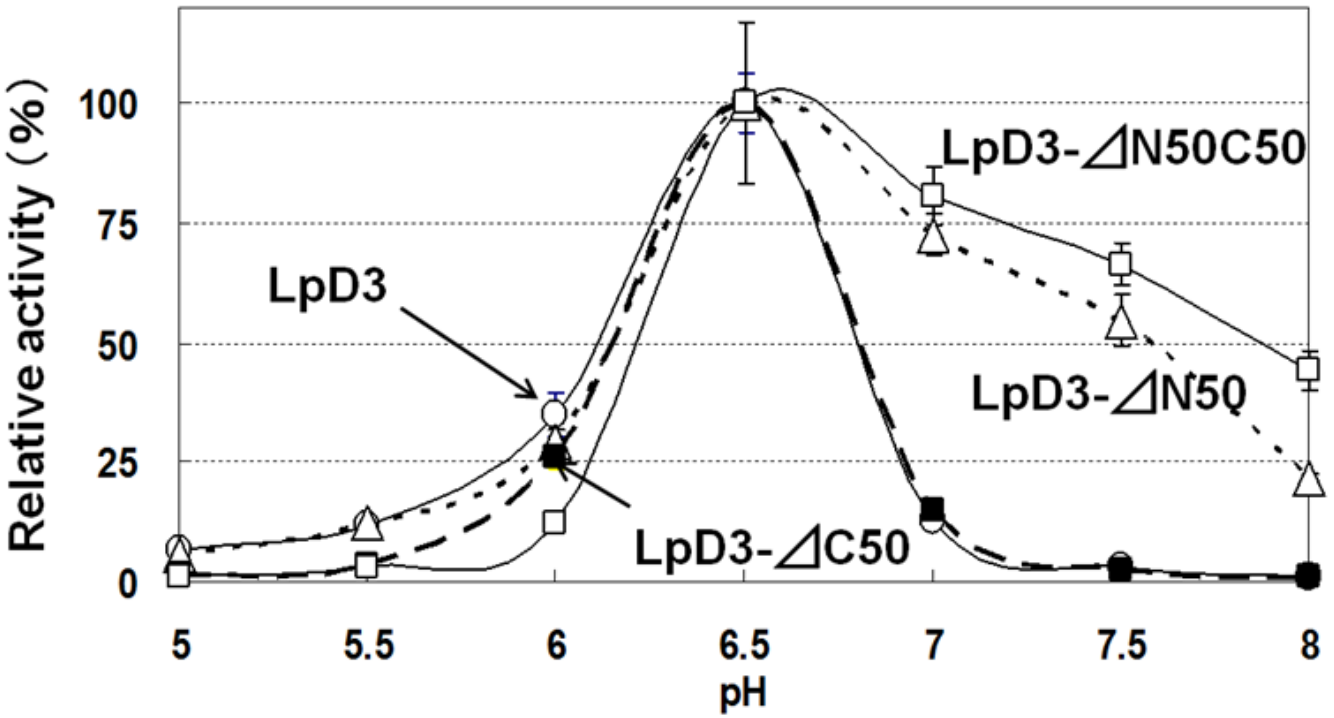
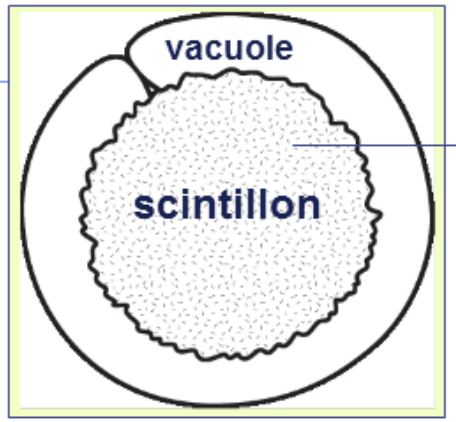
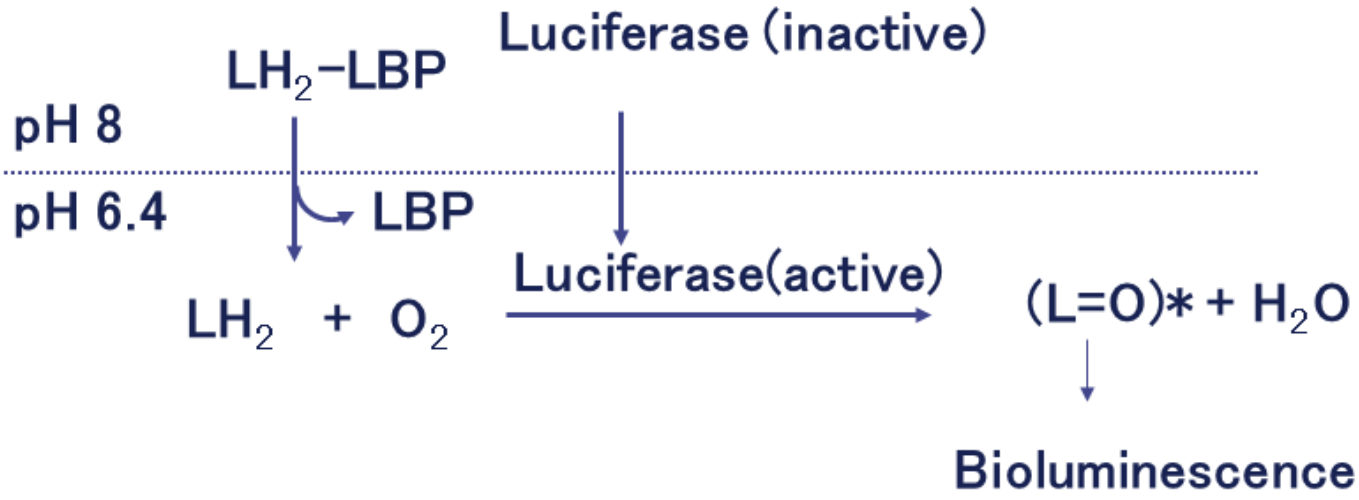


図7

Bioluminescence mechanism in Dinoflagellate



- Luciferin (LH_2)
- LBP — LH_2–LBP
- Luciferase



Photochemistry and Photobiology, 2004, 80: 31–35

Expressed Sequence Tag Analysis of the Dinoflagellate *Lingulodinium polyedrum* During Dark Phase¶

Naomi Tanikawa,[1] Hidetoshi Akimoto,[1] Katsunori Ogoh,[2] Wu Chun[2] and Yoshihiro Ohmiya*[1,2]

[1]Japan Science and Technology Agency PRESTO, Light and Control, Osaka, Japan and
[2]Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology, Osaka, Japan

Received 12 March 2004; accepted 14 May 2004

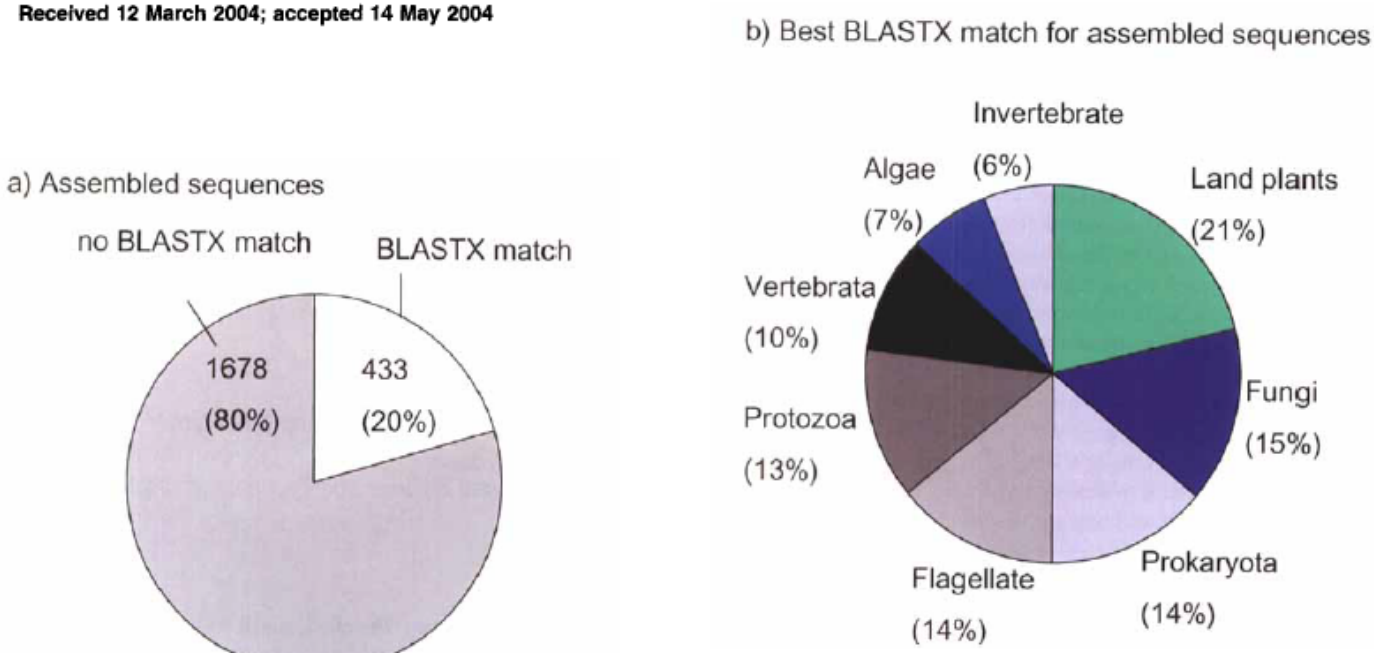


Figure 1. BLASTX results. a: The proportion of assembled sequences with and without BLASTX matches in the nonredundant protein (nr) database ($E < 10^{-13}$) is indicated for the assembled sequences. b: *Lingulodinium polyedrum* sequences with matches in the nr database (2111 total) were classified by the organism with the "best hit" protein sequence.

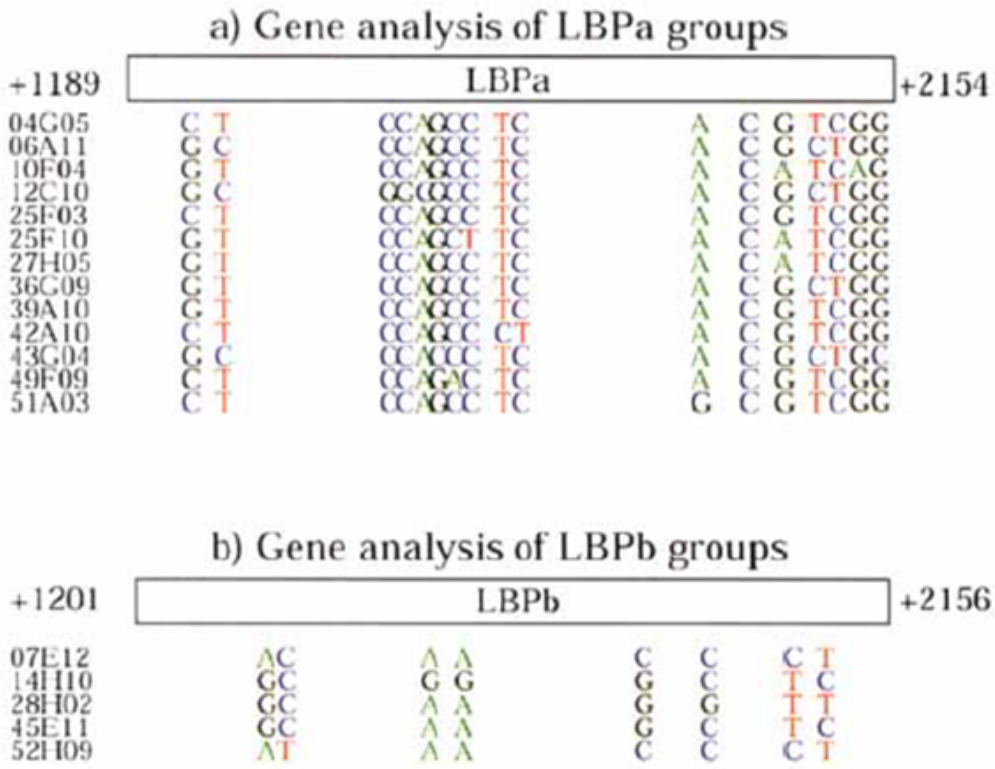
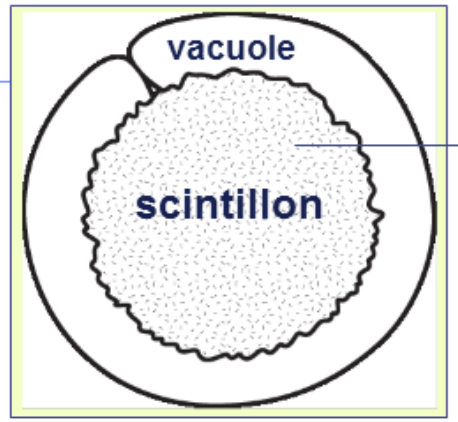
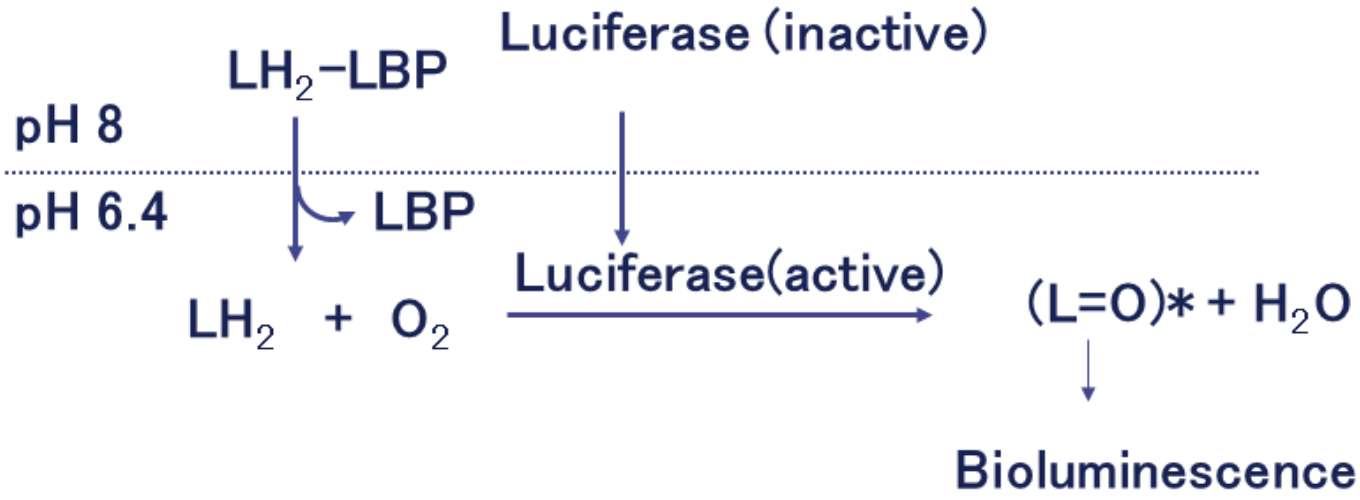


Figure 2. Analysis of the 3′ ends of variant LBP sequences. This alignment, performed with ClustalW, is of sequences entirely within the 3′ end of LBP. Nucleotides that differ in the LBP sequence are shown. a: LBPα groups, analyzed from nucleotides +1189 to +2154 (taking A of the ATG codon as the +1 position of accession number L19071) b: LBPβ groups, analysis from nucleotides +1201 to +2156.

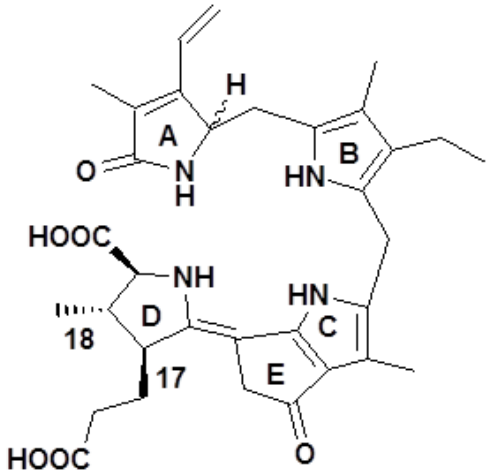
Bioluminescence mechanism in Dinoflagellate



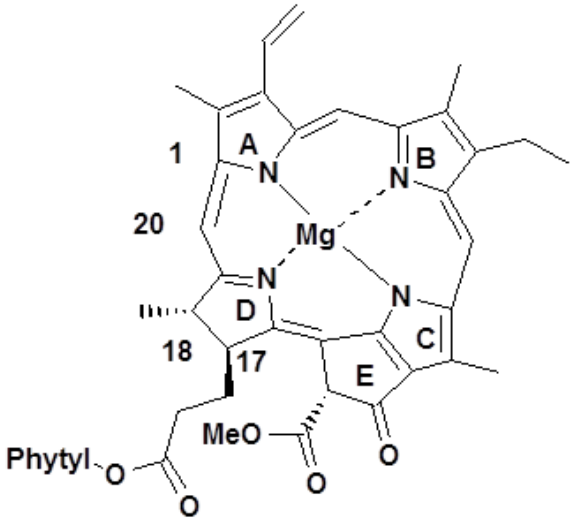
- Luciferin (LH_2)
- LBP → LH_2–LBP
- Luciferase



Structure of luciferin and Chlorophyll



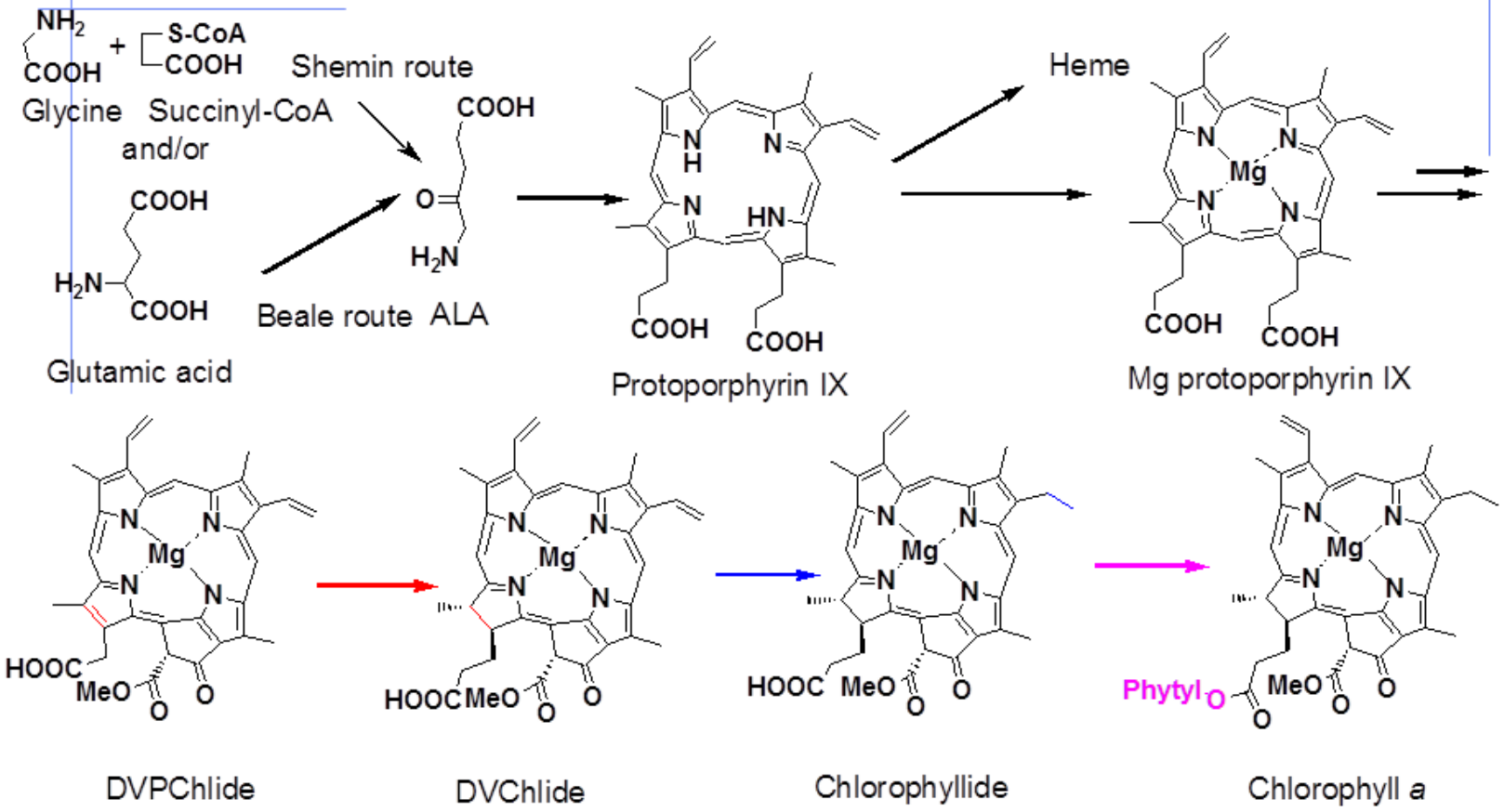
1



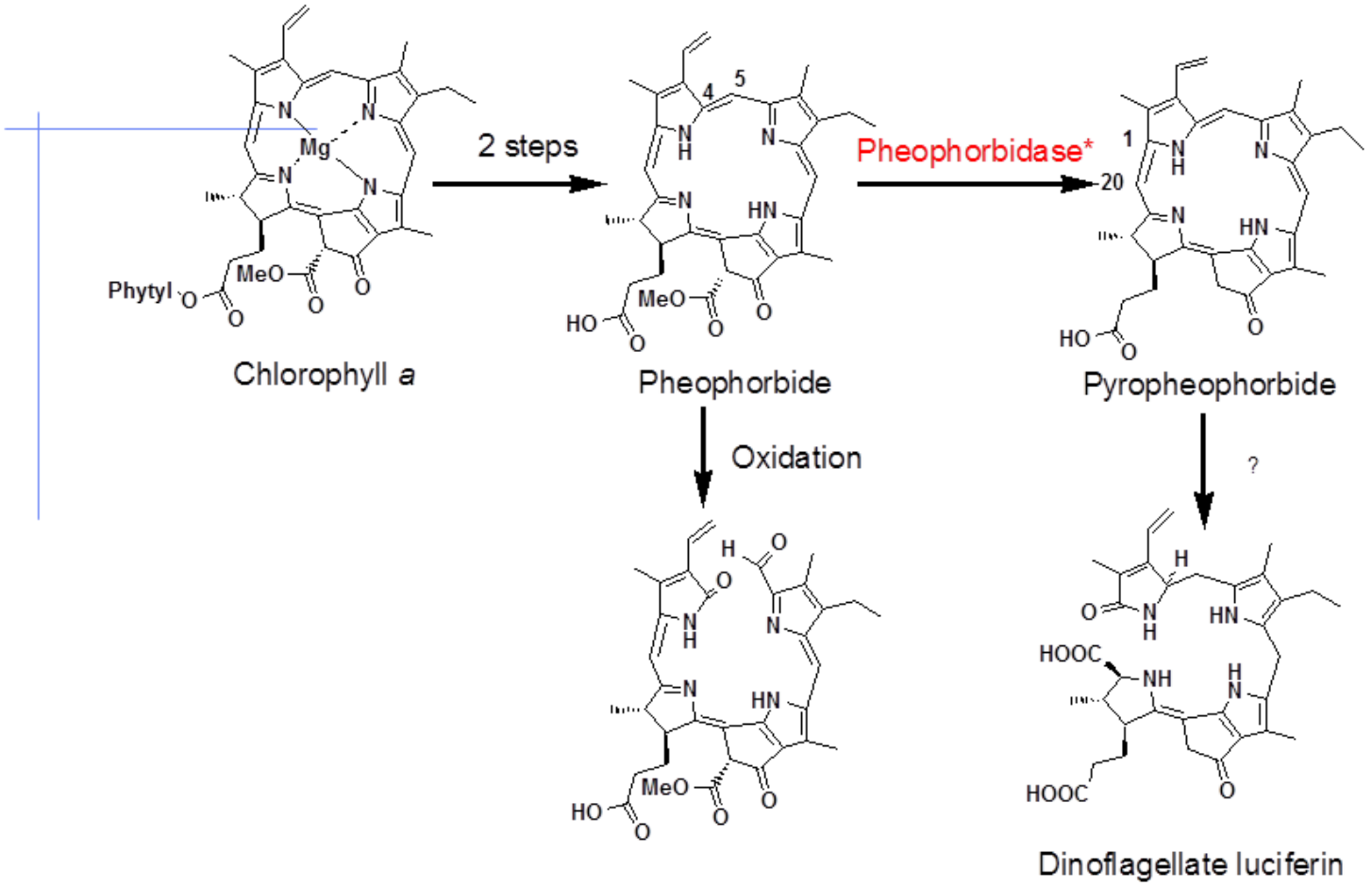
2

*Nakamura, H.; Kishi, Y.; Shimomura, O.; Morse, D; Hasting, J. W., *J. Am. Chem. Soc*. **1989**, *111*, 7607-7611.
*Nakamura, H.; Oba, Y.; Murai, A., *Tetrahedron Lett*., **1993**, *34*, 530-533.

Biosynthesis of chlorophyll



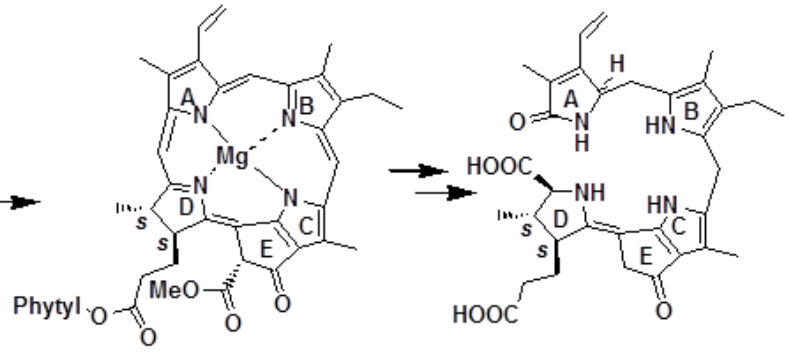
Proposal mechanism of luciferin biosynthesis



*Doi, M., Inage, T., and Shioi, Y. (2001) Chlorophyll degradation in a Chlamydomonas reinhardtii mutant: An accumulation of pyropheophorbide a by anaerobiosis. Plant Cell Physiol. 42: 469-474.

Tracer experiment for the luciferin synthesis

Glycine-^{15}N
or
$_{L}$-Glutamic-^{15}N acid



	^{15}N-Chlorophyll a	^{15}N-Luciferin
Glycine-^{15}N	0.86	0.75
$_{L}$-Glutamic-^{15}N acid	0.81	0.57

Wu, C.; Akimoto, H.; Ohmiya, Y., *Tetrahedron Lett.*, **2003**, *44*, 1263-1266.

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www.elsevier.com/locate/ybbrc

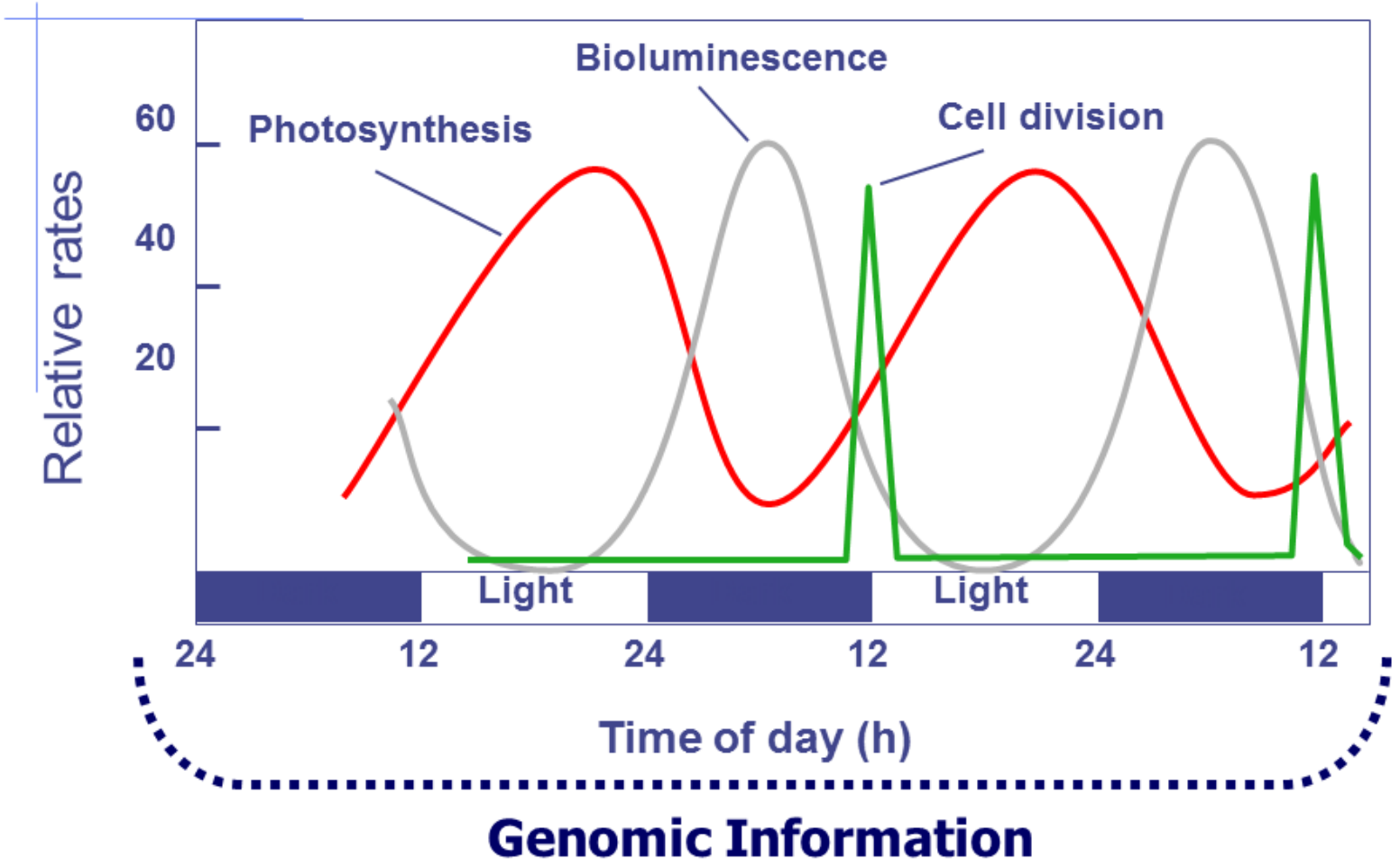
Biological rhythmicity in expressed proteins of the marine dinoflagellate *Lingulodinium polyedrum* demonstrated by chronological proteomics☆

Hidetoshi Akimoto,[a,*] Chun Wu,[b] Tomoya Kinumi,[c] and Yoshihiro Ohmiya[a,b,*]

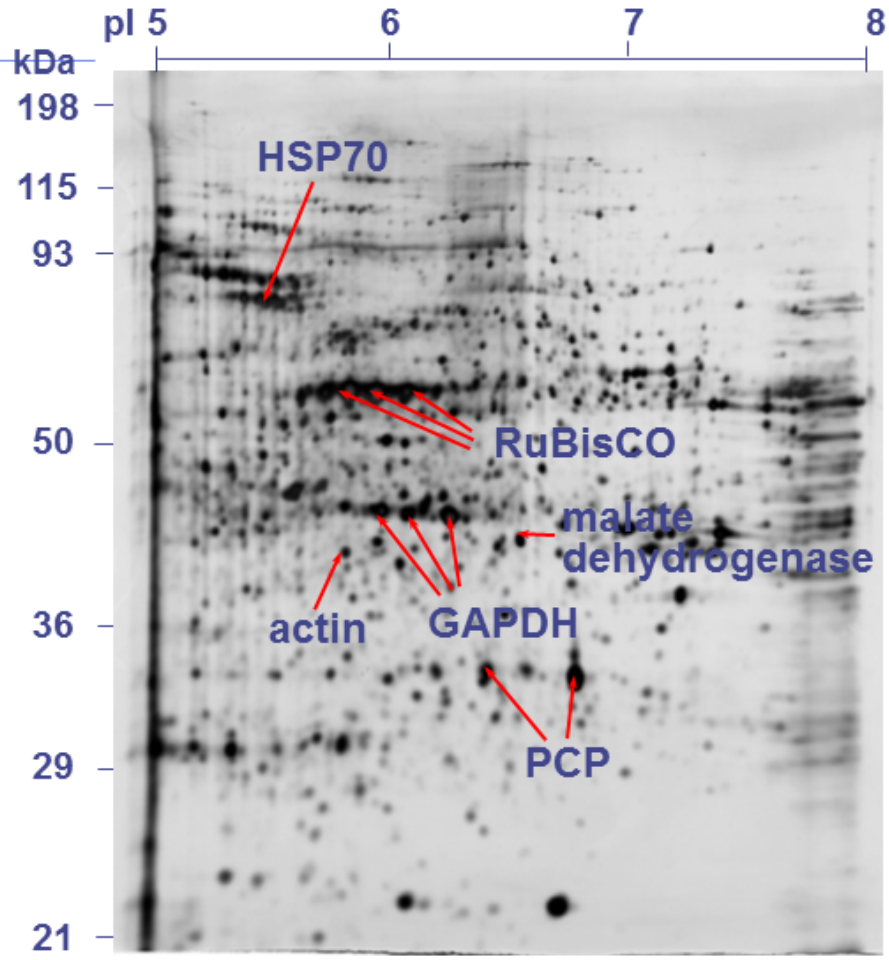
[a] *Light and Control Research Area, PRESTO, Japan Science and Technology Corporation, 1-3-1 Kashiwadaiminami, Chitose, Hokkaido, Japan*
[b] *Cell Dynamics Research Group, The Special Division for Human Life Technology, National Institute of Advanced Industrial Science and Technology, 1-8-31 Midorigaoka, Ikeda, Osaka, Japan*
[c] *Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology, 1-8-31 Midorigaoka, Ikeda, Osaka, Japan*

Received 18 December 2003

Circadian rhythms in Dinoflagellate



Chronological proteomics of Dinoflagellate



1-D = IEF sample strip: 17 cm

2-D = SDS-PAGE: 20 x 20 cm, 10 %

Akimoto H et.al ***Biochem Biophys Res Commun*** 315, 306-12, 2004

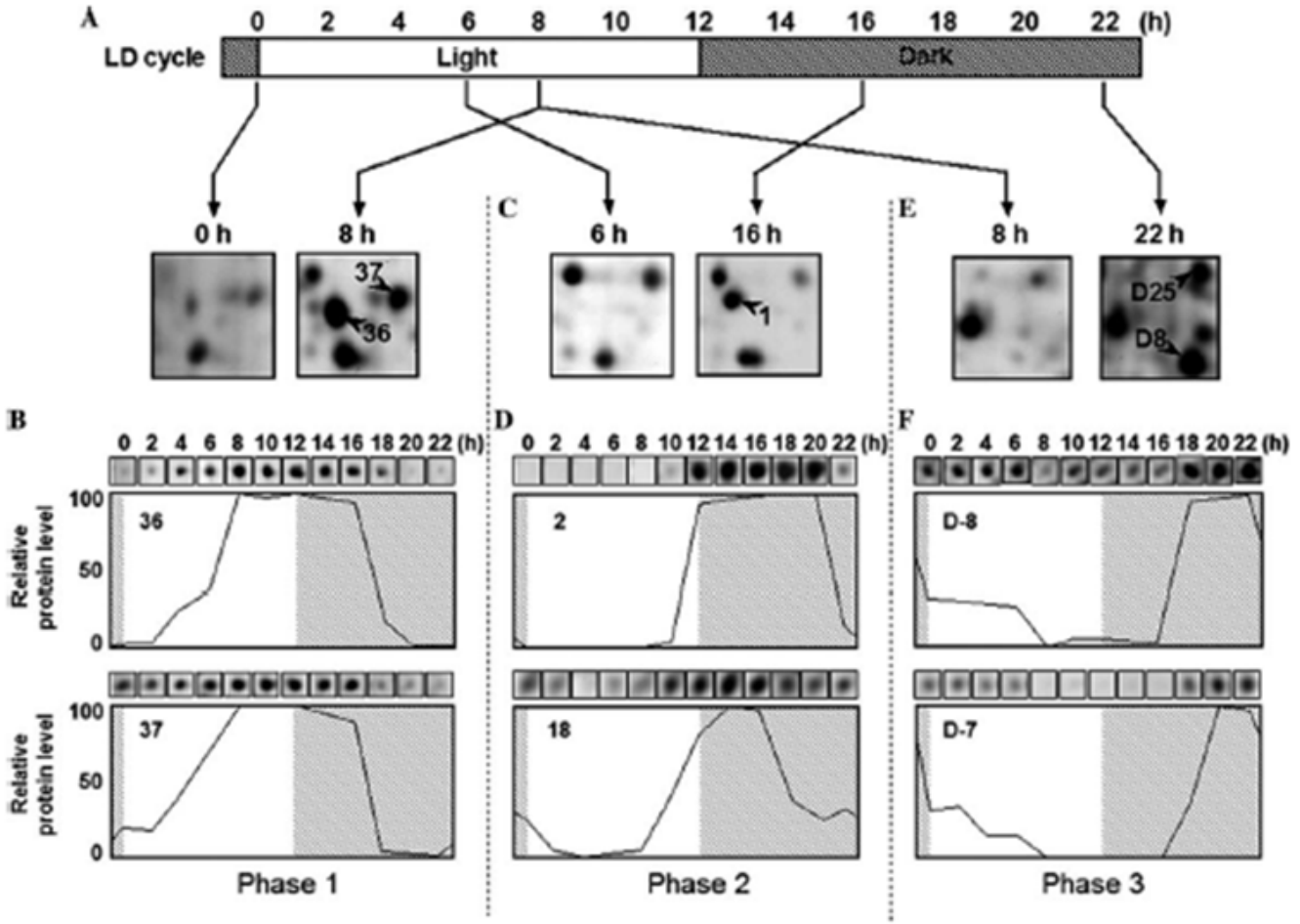


Fig. 2. Quantitative alterations of proteins in time-course 2-D PAGE at indicated times in the LD cycle (A–F). (A,B) Representative Phase 1 ('evening') profiles, (C,D) Phase 2 ('night'), and (E,F) Phase 3 ('midnight'). The numbered arrows indicate the altered spots listed in Table 1. White and hatched areas represent the light and dark phases, respectively. The peak value estimated with image analyzer was adjusted to 100. Details of the experimental procedures are described in Materials and methods.

Spots	Spot No.	MW (kDa)	pI	Result of PMF
Phase 1 (7)	36	72.5	6.7	succinate dehydrogenase (69k, 5.9)
Phase 2 (5)	90	96.3	6.3	isocitrate dehydrogenase (82k, 5.8)
	1	84.8	6.8	luciferin-binding protein (76k, 7.1)
Phase 3 (16)	D-18	55.5	5.5	ATP synthase (58k, 6.0)
	D-13	45.2	5.5	RuBisCO (58k, 5.8)
	D-12	39.4	5.6	actin (41k, 5.9)

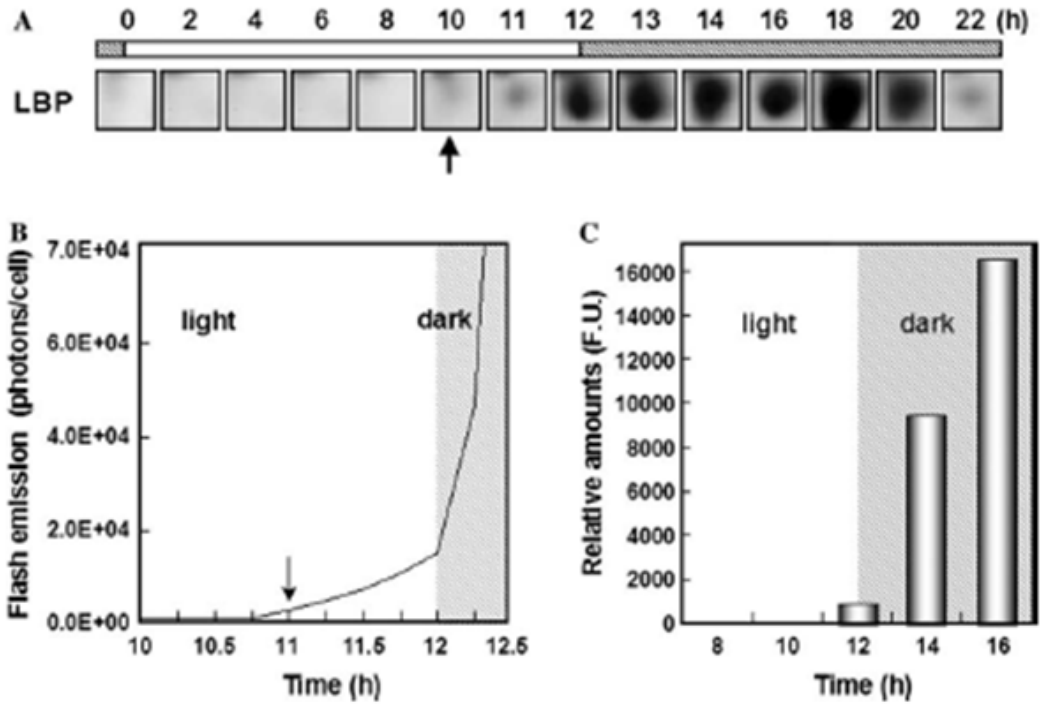


Fig. 3. The time-course of alterations in LBP and bioluminescence and luciferin contents (A–C). White and hatched areas represent the light and dark phases, respectively. (A) Time-course with 2-D PAGE of LBP concentrations. The arrow indicates the initiation of production. (B) The bioluminescence intensity represented as the absolute units of photons per organism for total light emission integrated over 1 min. The arrow indicates the initiation point. (C) The luciferin amounts in vivo were measured by HPLC every 2 h from 8 to 16 h in the LD cycle.

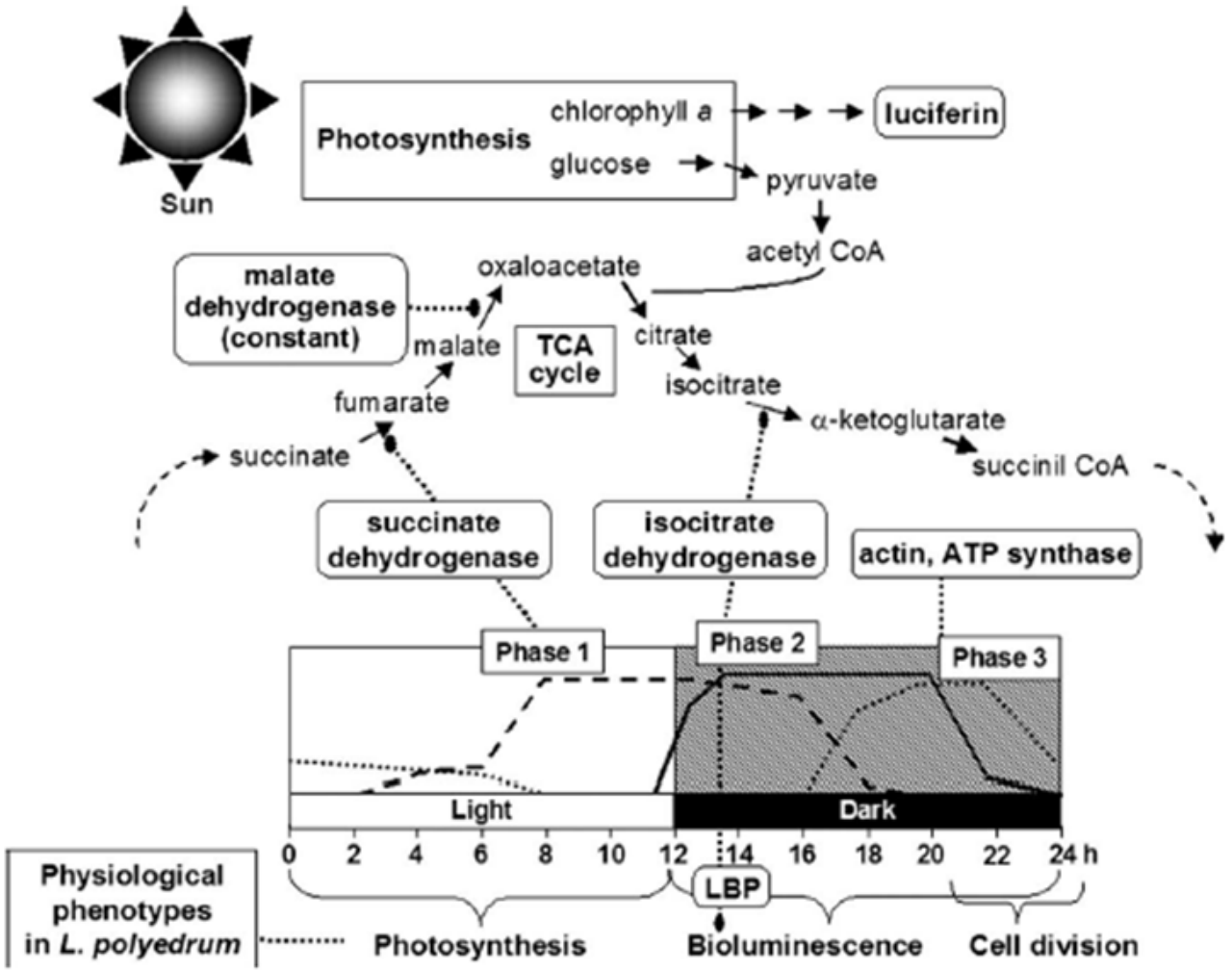


Fig. 4. Postulated scheme of the relationship between the three phases and phenotypes in *L. polyedrum*. Proteins enclosed with the square represent the proteins identified in this experiment.

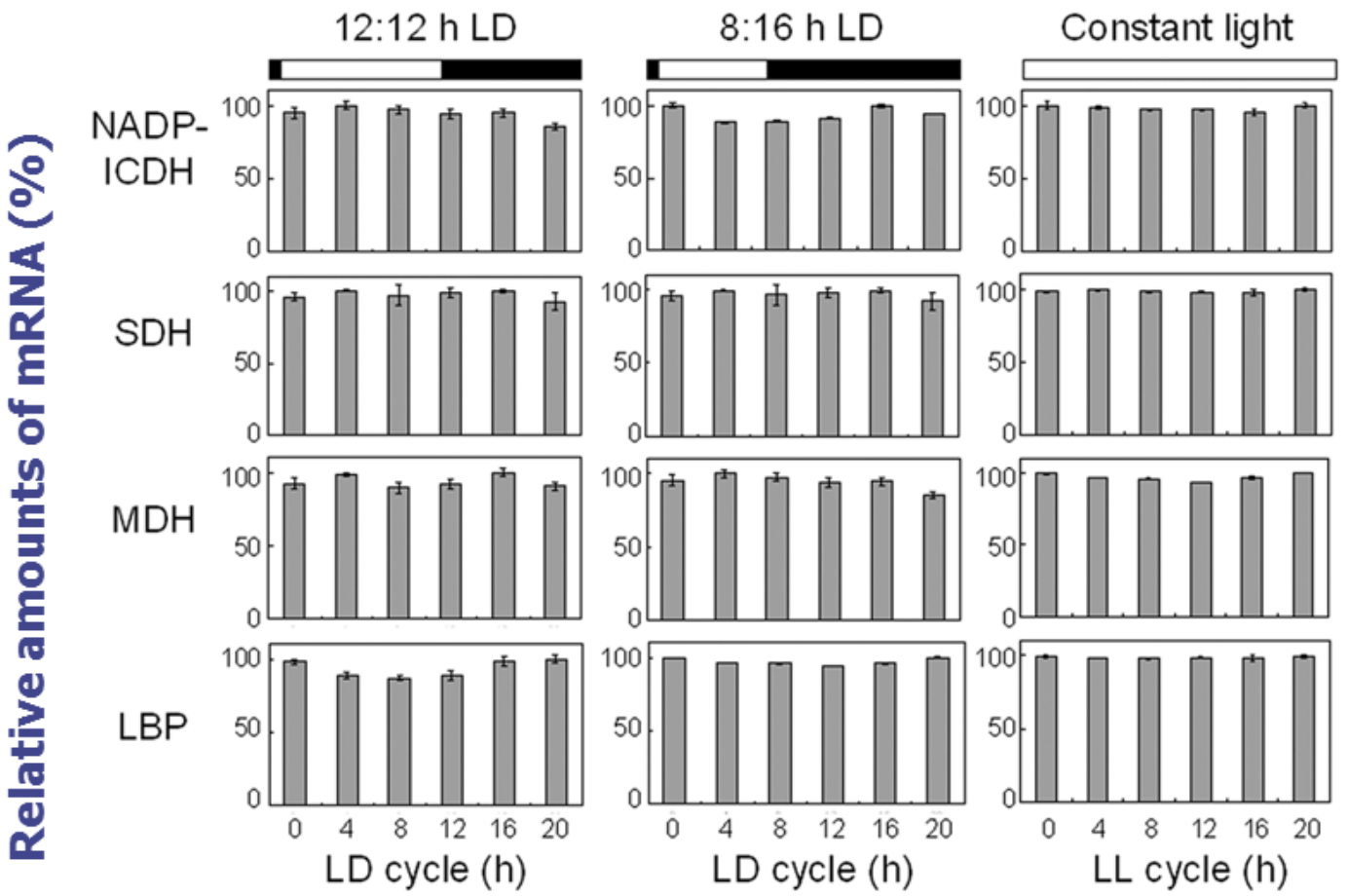
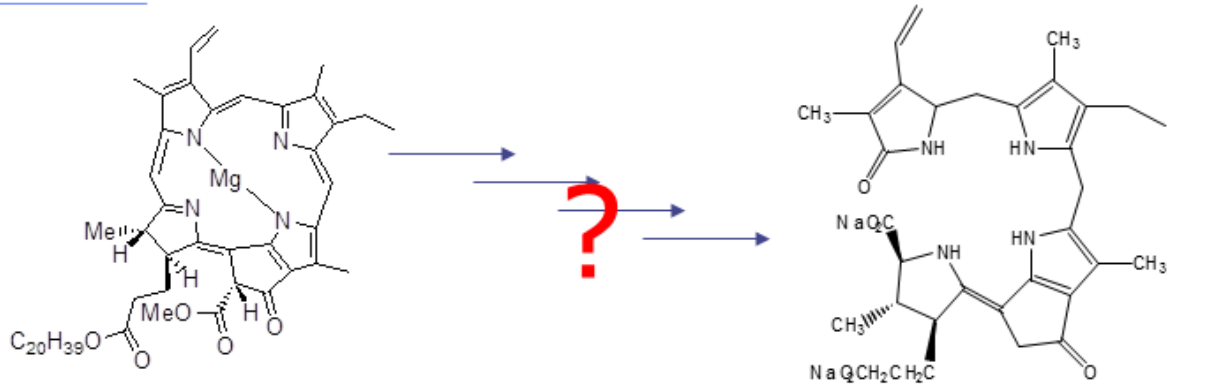

12:12 h LD
8:16 h LD
Constant light
NADP-ICDH
SDH
MDH
LBP
Relative amounts of mRNA (%)
100
50
0
0 4 8 12 16 20
LD cycle (h)
LD cycle (h)
LL cycle (h)

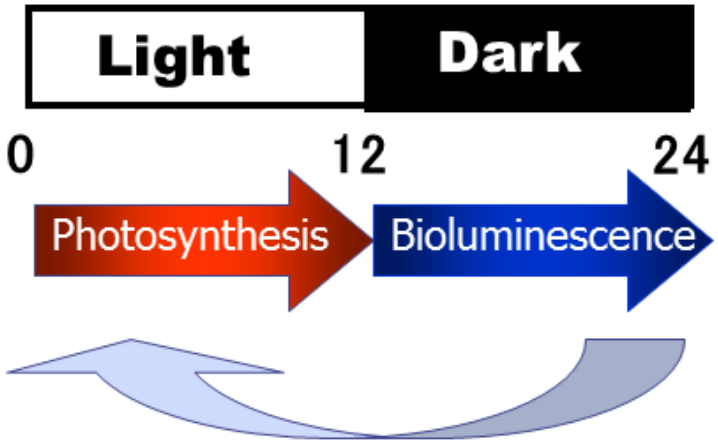
図4

If we can control of this mechanism, we can make ,,,,



chlorophyll a

dinoflagellate luciferin



Chemical Study of Bioluminescence in the Future

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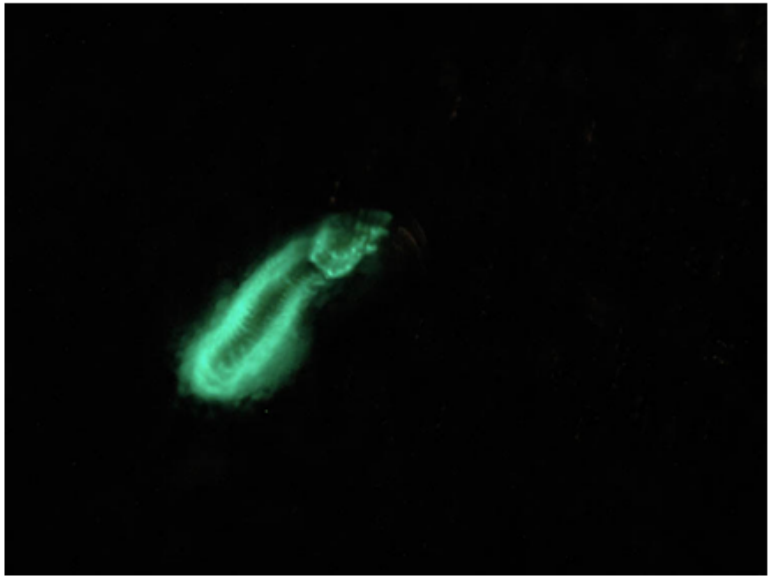
Discovery of a new luciferin and a new mechanism will provide us with enormous benefit, as it was shown in the past. The work may not be easy; however, the author believes that it can be accomplished when the researcher has a firm determination to complete it. There is no established method or protocol for studying a new type of luciferin or photoprotein; thus , the method must be worked out. ----------

By O Shimomura in "Bioluminescence"

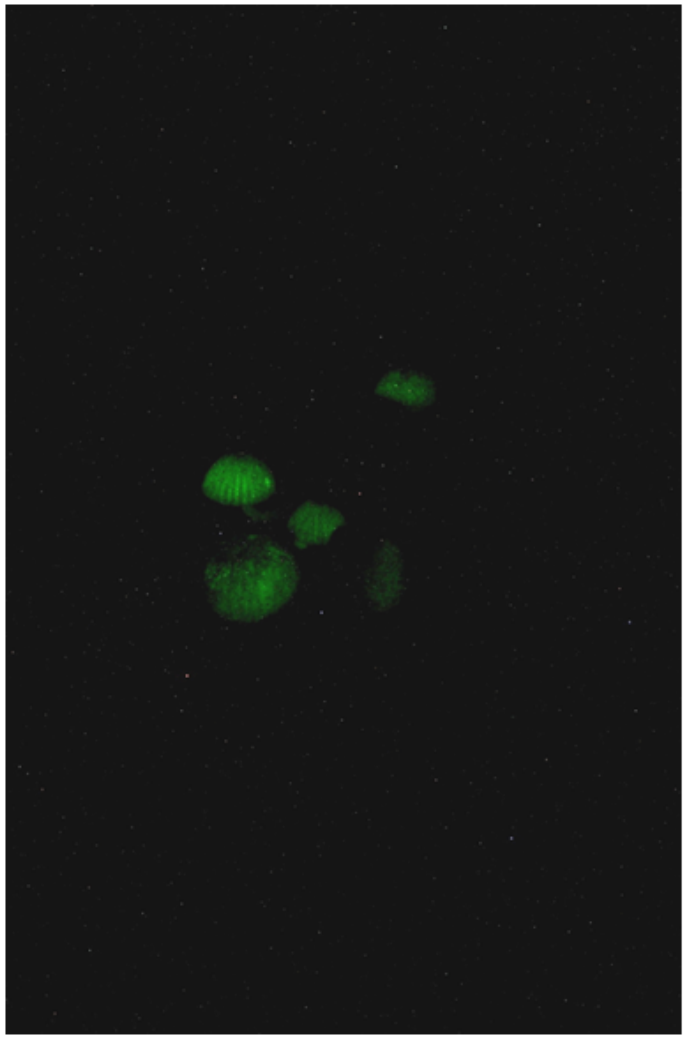
（魚津水族館、稲村修館長）

Odontosyllis in Toyama bay, Japan



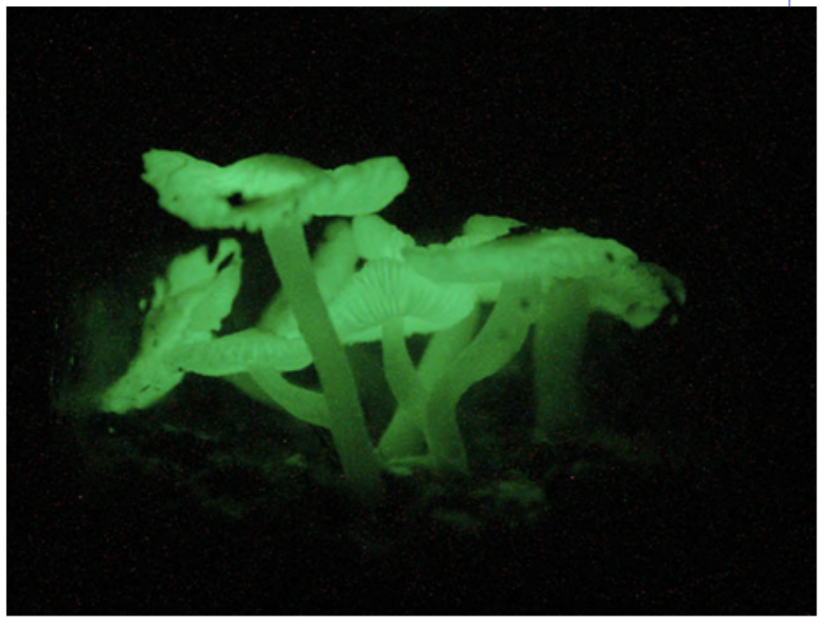
（電気通信大学・平野誉教授、丹羽治樹教授）

Fungi Shitake-tomoshibidake



（産総研・丹羽一樹研究員）

Fungi Yako-dake



（電気通信大学・平野誉教授、丹羽治樹教授）

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Please contact by mail (y-ohmiya@aist.go.jp), if you have any question about bioluminescence.

I expect and believe your success in the scientific field. Good luck !