SibFU-1
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BIOLUMINESCENCE

A graduate course for the Siberian Federal University,
Krasnoyarsk, Russian Federation

John Lee: "My First 50 Years"

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Course Outline

Lecture 1. **Bioluminescence: A 3000-year old Science.**

Bioluminescence is defined as the emission of light from a living organism that performs some biological function.
Bioluminescence is one of the oldest fields of scientific study.

Lecture 2. **Chemiluminescence**
130 years of mystery

Bioluminescence is an enzyme catalyzed chemiluminescence reaction. Model chemiluminescence reactions and the mechanisms of chemi-excitation will be reviewed:

Chemiluminescent reactions: excitation, Marcus theory, luminol mechanism, spectra , quantum yields, dioxetanes, acridines, singlet oxygen, CIEEL postulate, coelenterazine models, NMR studies, firefly spectra and chemical models, the “glow stick puzzle”.

Lecture 3. **Bioluminescence Biophysics**

Firefly, bacteria, and coelenterazine bioluminescence, are the ones investigated in most detail over the last 50 years.

Careful observations and availability of recombinant proteins, makes these the most amenable to biophysical investigation.

Lecture 4. **Quantitative Spectroscopy**

Quantitative spectroscopy has revealed that even within one type of system there are variations of spectral properties, often having to do with biological function. This lecture will outline simple and appropriate principles of modern molecular spectroscopy, sufficient for the understanding of the literature:

Measuring photons, Theory of fluorescence, inhomogeneous broadening, transition probabilities, band analysis, spectrographs and measurement of fluorescence and chemiuminescence.

Lecture 5. **Structural Biology**

The “Holy Grail” of biochemists is the three-dimensional structures of their favorite protein. The spatial structures of many bioluminescence proteins have been determined.

Principles of methods of NMR and crystallography. The Protein Data Bank holds structures of many bioluminescence proteins.

Bioluminescence Systems

The Insects

ATP (Mg^{++}) and oxygen are common requirements for both coleoptera and diptera (glowworm) bioluminescence. Firefly biochemistry produces CO_2 and oxyluciferin via a **dioxetanone** intermediate.

Bacterial Bioluminescence

- A deceptively simple system: bacterial luciferase, reduced flavin, oxygen, and aldehyde, all easily obtained.
- Flavin peroxide intermediate identified by NMR.
- Some types produce shifted colors via a separate “antenna” protein involving electronic coupling by **FRET**.

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Coelenterazine and other marine systems

Many coelenterate marine bioluminescences involve "**coelenterazine**", as the substrate and also GFP, the **Green-fluorescent protein**, maybe the most famous protein in modern biological research!

Renilla, aequorin and obelin, other types. Structural origin of color shifts. Structure and mechanism.

Antenna Proteins: LumP and GFP

- Green-fluorescent proteins (GFP) and lumazine proteins, interact with the luciferase system to modulate bioluminescence color.

- Electronic coupling and protein-protein interactions are identified by time-resolved fluorescence and anisotropy decay, and by NMR.

PREFACE **Some Definitions**

Bioluminescence

Bioluminescence is the efficient generation of visible light from an animal that benefits its survival and propagation.

Chemiluminescence

Emission of light as the result of a chemical reaction.

GLOW STICK

PREFACE **Other Luminescences**

Fluorescence
Light emission following the absorption of radiant energy.

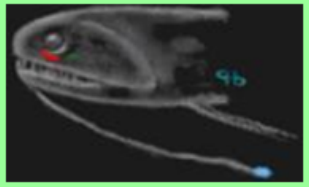
Phosphorescence
Delayed light emission following radiant energy absorption.

Iridescence
Combination of reflectance and interference resulting in appearance of colors.

Bioluminescent organisms

Terrestrial (rare)
1. Insects: beetles, gloworms (diptera)
2.Fungi
3.Worms

<u>Marine</u>
1. Bacteria
2. Fish
3. Cnidaria: octopi, coelenterates, copepods, etc.
4. Many others



Luciferin-Luciferase

• **Dubois** (Paris, 1886) made extracts from the luminous tissue of the mollusc, *Pholas*.
• **hot** water extract was dark; **cold** water extract allowed to become dark.
Mixing **hot** extract with **cold** extract □
LUMINESCENCE!

Dubois named the hot water extract "**luciferine"** and the cold water, **luciferase.**

Pholas dactylus
(piddock)

Origin of Nomenclature

Genus *Pyrophorus*, from Greek *pyro* Fire; *phero* to bear.

Phosphor, Greek *phos* light, *phosphoros* the morning star.

Lucifer “light bearing”, Latin *lux, lucis* light; *lucifer,* the morning star

Luciferase, luciferin, are ***generic*** terms used today.

Insect Bioluminescence

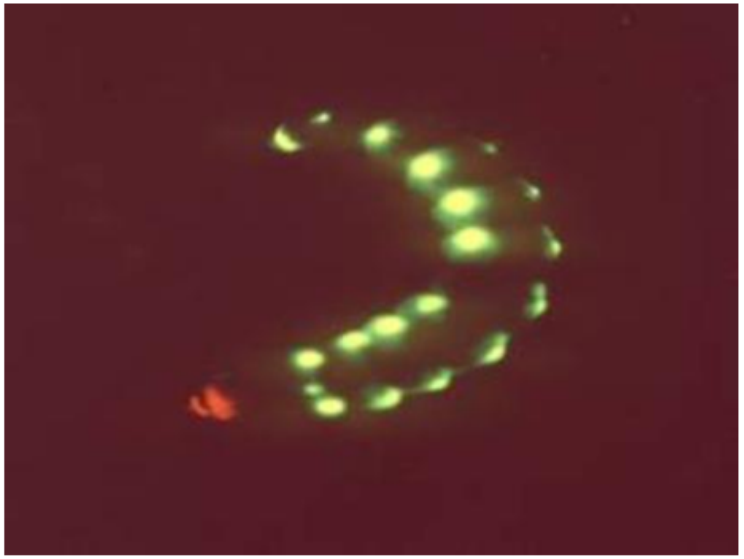
Bioluminescence in insects is mainly found in the coleoptera (beetles) and diptera (flies).

"Fireflies" are not flies, but **beetles**

NOT These Beatles!

Beetle Bioluminescence

Click beetle
Railroad worm automobile bug



Firefly Colors

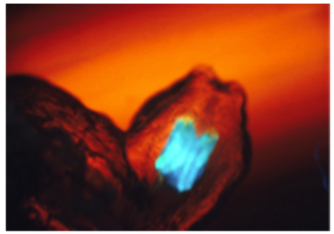
- Most fireflies flashes a yellow color.

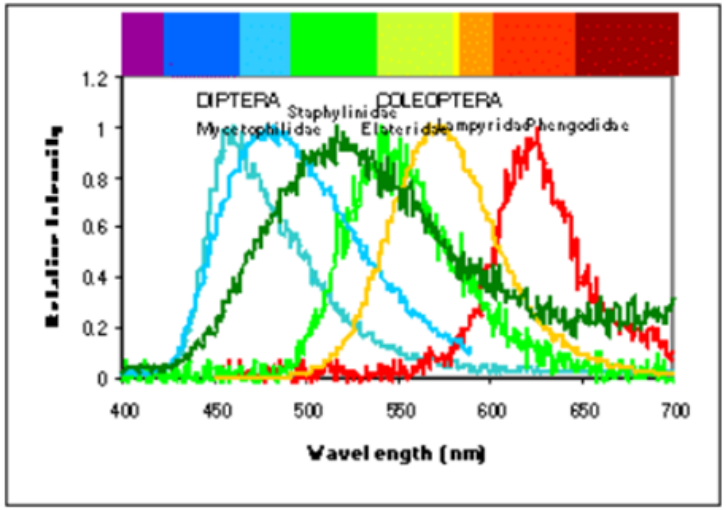
- Macair (1821) found that a red emission occurred from **warmed** up fireflies.

- The “railroad worm” (Brazil) has both red and yellow lights.

- The “click beetle” (Jamaica) has both yellow front lights and an orange tail light.

Colors of Insect Bioluminescence





- Blue for glow-worm, green□red for the beetles
- **Visible light** is 400 nm – 650 nm.

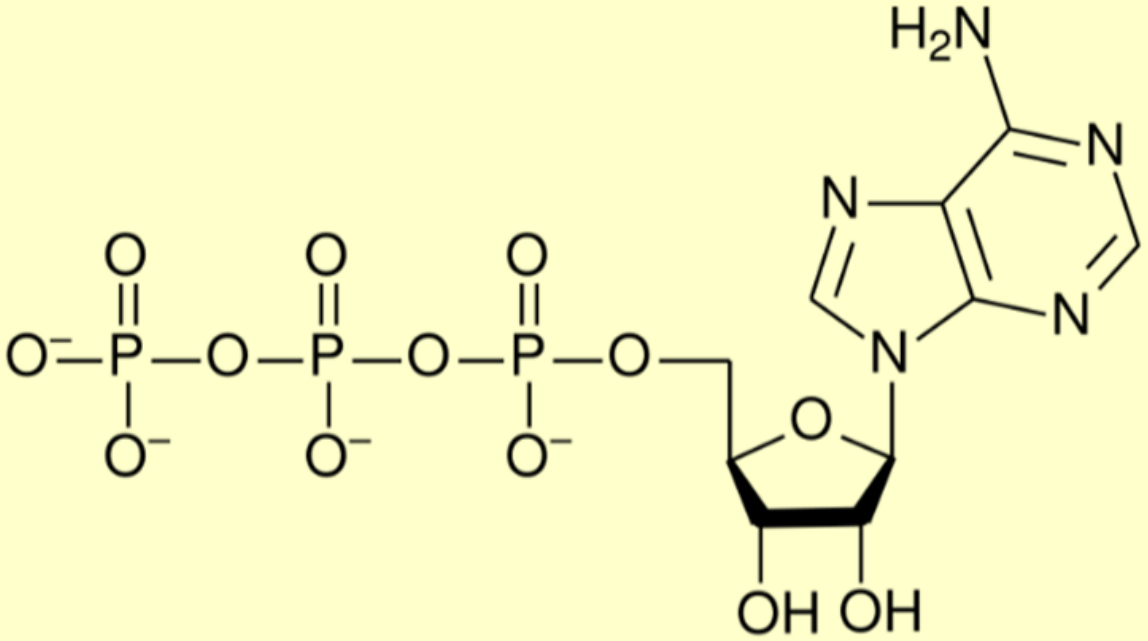
Adenosine Triphosphate

• **W.D.McElroy** (1947) discovered **ATP** to be an essential requirement for bioluminescence of firefly extracts.

• ATP is more stable than luciferin

• Dubois's hot water extracts were probably **ATP, not luciferin**.

ADENOSINE TRIPHOSPHATE



Firefly Luciferin
FF-LH_2

1. Yellow photon energy, $E = hc/\lambda$ ~ 55 kCal/mol

2. ATP hydrolysis $\longrightarrow$ 7 kCal/mol (insufficient)

3. Oxygen was also necessary and this suggested the chemical energy could be supplied by oxidation of some reduced substrate, the **genuine** luciferin:

$FF\text{-}LH_2 + O_2 \longrightarrow FF\text{-}L{=}O + H_2O + CO_2$
+ > 60 kCal/mol?

Crystallization of Firefly Luciferin
Bitler and McElroy, 1957

1. **15000** fireflies; dry; make acetone powder.
2. Extract with boiling water.
3. Discard precipitate and extract with ethyl acetate.
4. Column purify: Celite-Fullers Earth, then partitioning with chloroform-butanol.
5. Concentrate and crystallize from acetone.
6. Yield **9 mg**. Yellow-green fluorescence, MW 308

→ bioluminescence with pure firefly luciferase

W. D. McElroy

“ Firefly Mountain” (1955)

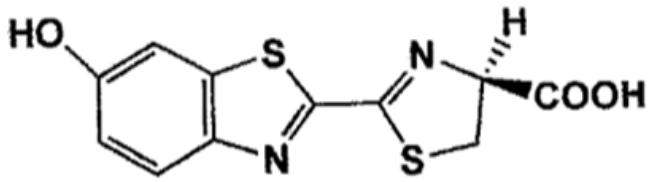
Firefly Bioluminescence 1960

1. Luciferase, luciferin, oxygen, ATP, Mg^{2+}
2. FF-Lase mass 100 kDa (now 60) with no visible absorption.
3. FF-LH_2 MW 308 structure solved
4. Reaction is **specific** for ATP
5. FF-Lase has **two** enzyme activities:
    1. Adenylation STEP 1
    2. oxygen bioluminescence STEP 2
6. Seliger (1959) measured **quantum yield**: photons/luciferin = 0.88 ± 0.25 (now 0.5)

Firefly Luciferin

• 1950's, firefly luciferase (FF-Lase) was purified as a ~100 kDa protein.

• 1961. Firefly luciferin (FF-LH_2) structure determined.



D-Firefly Luciferin (LH_2)

Beetle Luciferin

Beetle Biochemistry

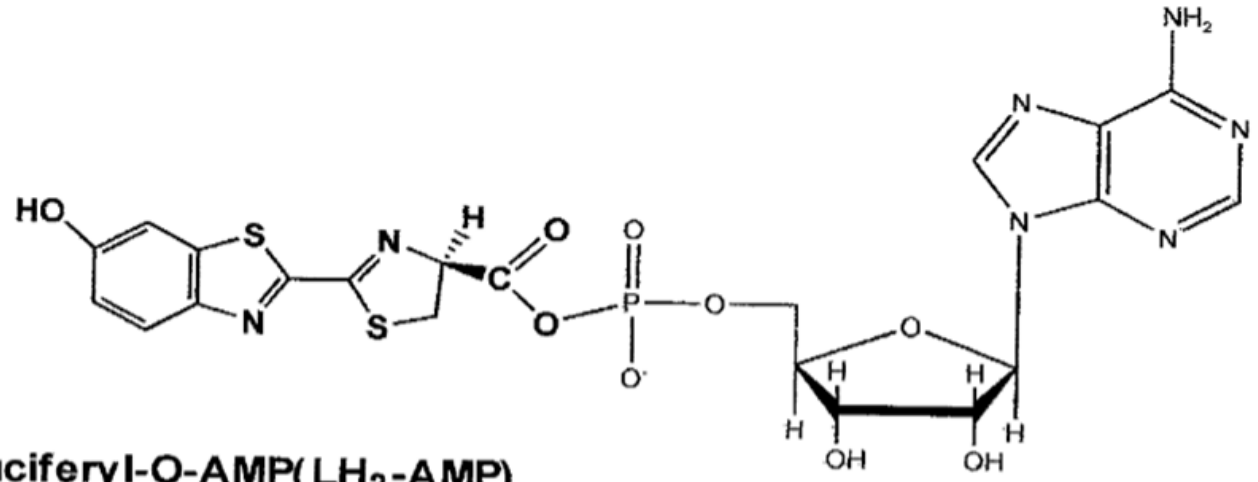
Step 1: Activation of Luciferin

Two steps are catalyzed by FF-Lase and the Mg^{2+} cofactor, the first with either **D**- or **L**-firefly luciferin

$FF\text{-}LH_2 + ATP \rightarrow FF\text{-}LH_2\text{-}AMP + PP_i + H_2O$

Luciferyl-adenylate

D-Luciferyl Adenylate



D-Luciferyl-O-AMP(LH_2-AMP)

Note the asymmetric carbon (**..\\\\\\\H**) in the structure. Only the **D optical** isomer is light-active, as it has to fit into the luciferase active site (the "lock and key" hypothesis).

Beetle light

Step 2: The Light Reaction

Catalyzed by the FF-Lase

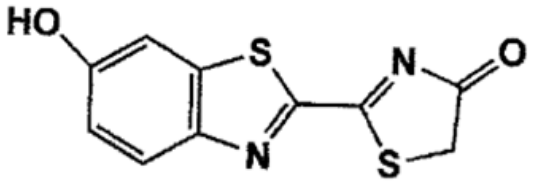
D-LH_2-AMP + O_2 $\longrightarrow\!\!\longrightarrow$ L=O* + CO_2 + AMP
AMP = adenosine monophosphate

L=O* is oxy-FF-Luciferin in its excited (fluorescent) state.

$\longrightarrow\!\!\longrightarrow$ means two (or several) steps

Product: Oxyluciferin

LH_2-AMP + O_2 → **L=O*** + CO_2 + AMP



Oxyluciferin

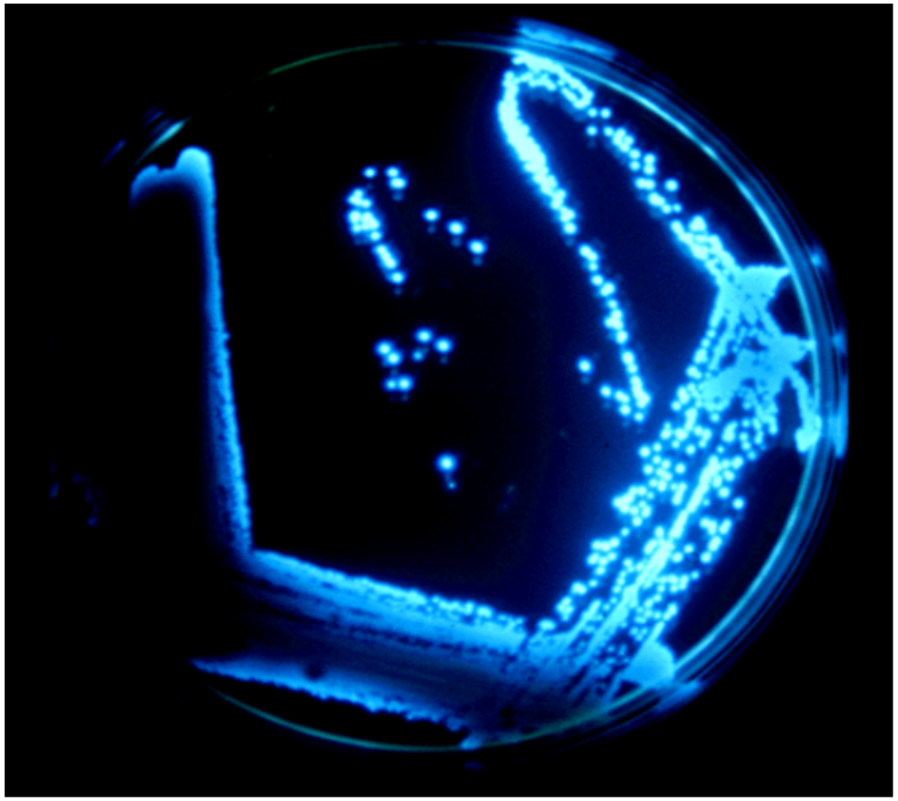
The **free energy** of this reaction is obtained by the decarboxylation step, 60 kcal/mol, sufficient to populate the excited state.

Bioluminescent Bacteria

Worldwide distribution, mostly marine.

• Occurrence:
Parasitic – infection on living animals (amphipods, insects, shrimp).
Saprophytic – dead fish or flesh
Symbiotic – luminous organs of many squid and fish.
• Classification:
mainly 3 genera- *Vibrio, Photobacterium, Xenorhabdus.*

BIOLUMINESCENT BACTERIA PLATE



Bacterial Luciferase (B-Lase)

1953-55

McElroy and coworkers purified bacterial luciferase by acid precipitation and ammonium sulfate precipitation and reported some enzyme kinetics based on bioluminescence intensity.

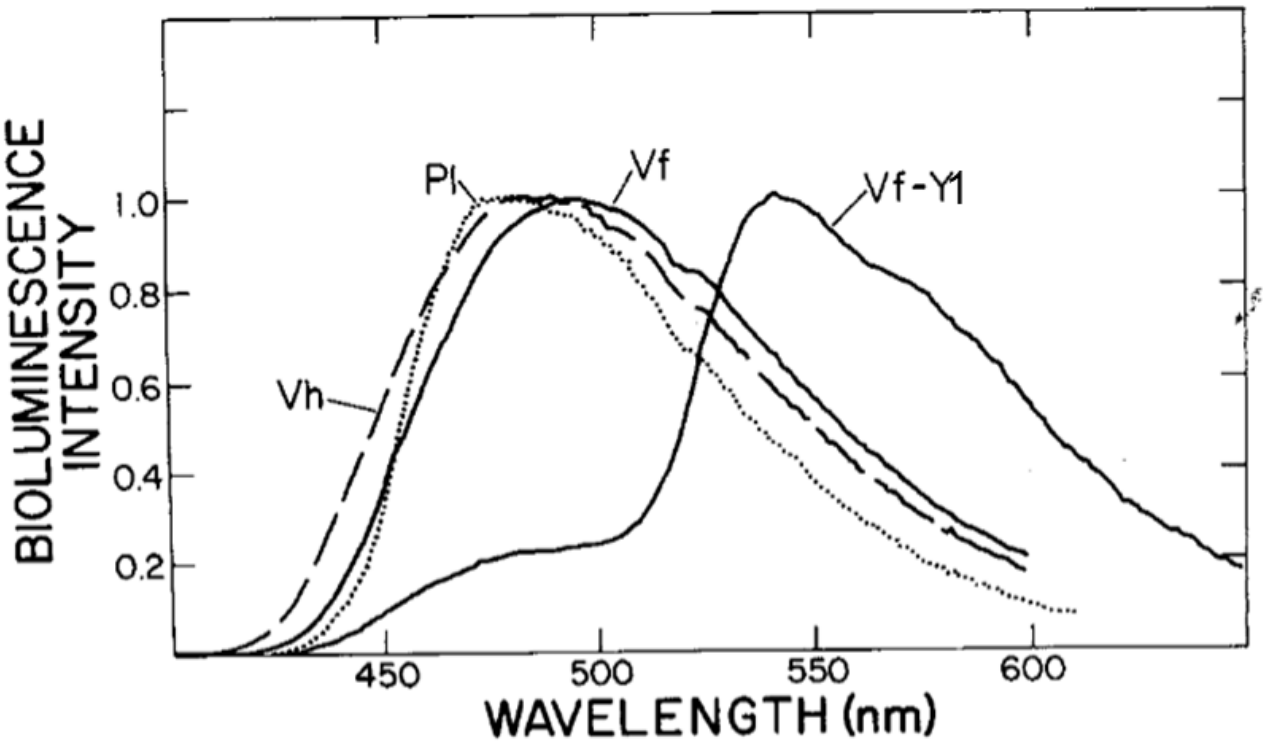
$E + S \rightleftharpoons E.S \rightarrow P^* \rightarrow$ light

bioluminescence $\propto$ $[P^*] \propto [ES]$

Quantitative Spectroscopy

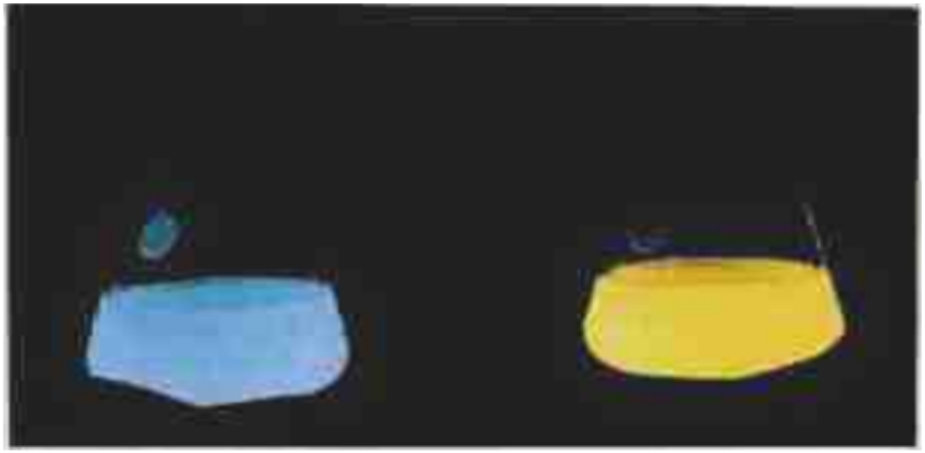
• 1935, the **photoelectric** detection device (the "light meter") was invented.
• 1938, broad spectrum of a deep-sea fish symbiont had a **yellow** maximum (520 nm).
• 1943, Giese isolated a variant with brilliant luminescence producing much yellow pigment (probably **flavin**).
• 1938-1950, accurate spectral scans showed broad spectra with maxima 460-490 nm.

In Vivo Spectra Type Dependent



Bacterial Bioluminescence Colors

Most types show a max around 490 nm.
Photobacterium species are often more blue and Y1 strain of *Vibrio fischeri* is yellow.
The color shift originate from the fluorescence of an "antenna protein", Lumazine Protein **LumP**, or its yellow variant, yellow fluorescence protein, **YFP.**

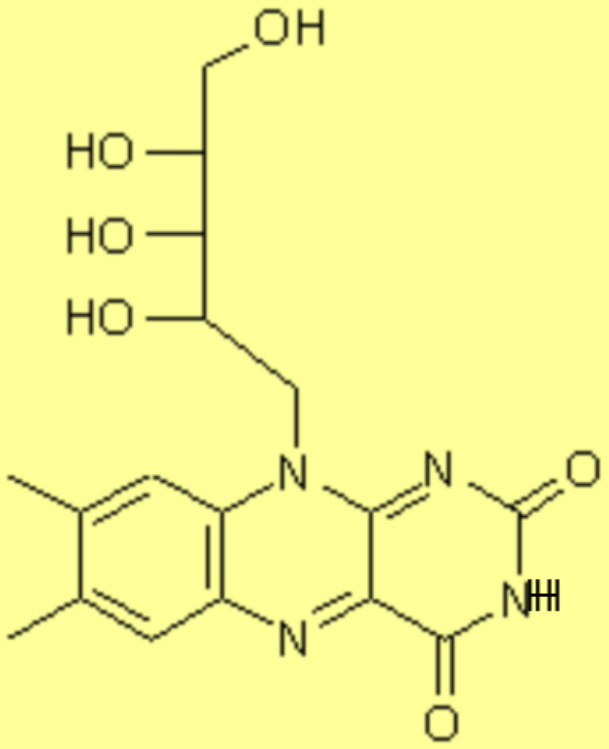


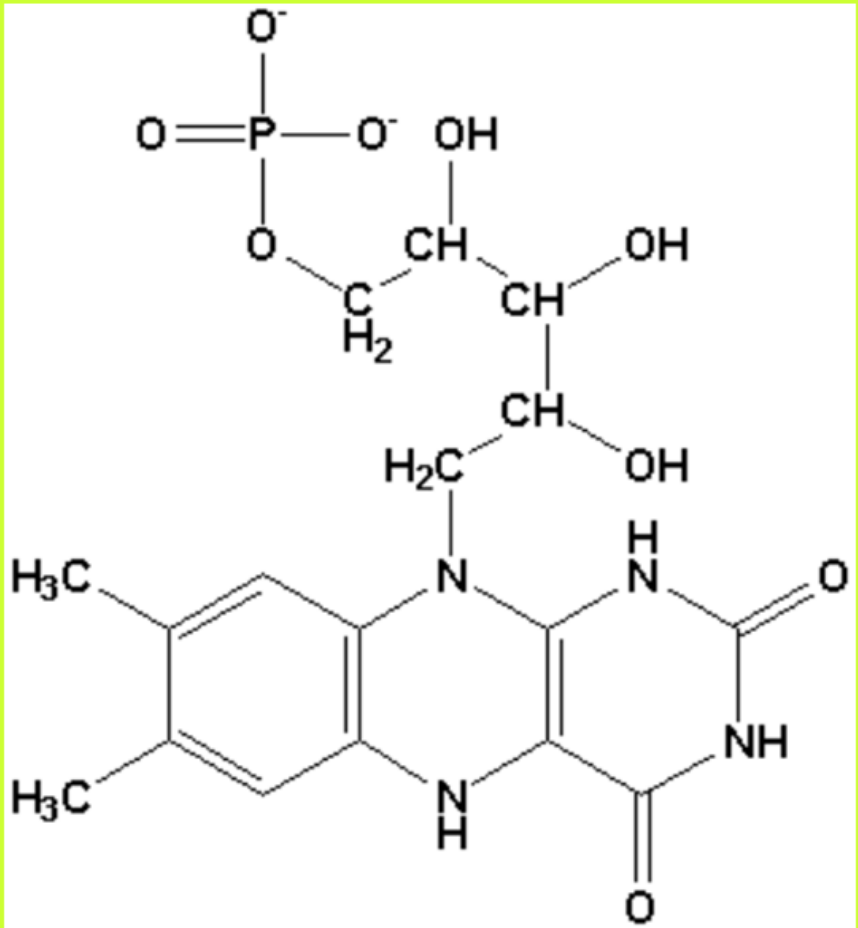
Two Bacterial Luciferins ?

• 1950's, McElroy and Hastings (Johns Hopkins University), **$FMNH_2$**, reduced flavin mononucleotide.

• Strehler and Cormier (Oak Ridge National Laboratory) long-chain **aldehyde**,

RIBOFLAVIN
(Vitamin B2)

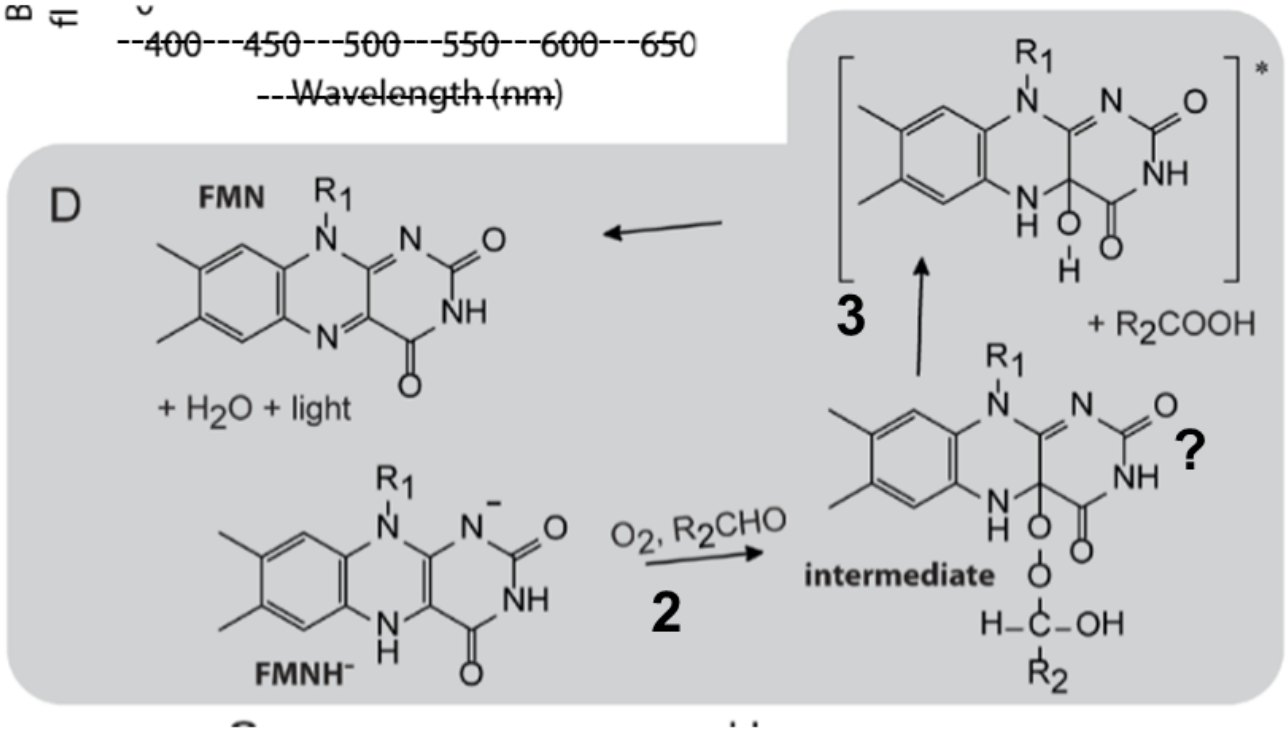




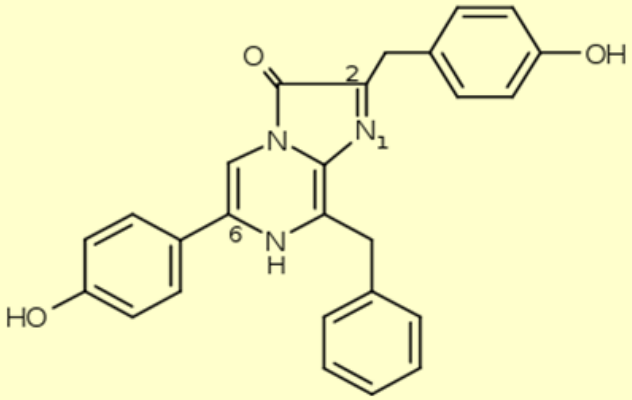
Flavin Mononucleotide reduced (FMNH2)

Bacterial biochemistry

1. Bacterial luciferase + $FMNH_2$ □ Lase-$FMNH^-$



Coelenterazine Bioluminescences



Coelenterazine is named because it was identified as the luciferin in many **coelenterates** (jellyfish, etc.). It is common to many marine systems. Two well-studied systems are:

- Ca^{2+}- regulated photoproteins
- Renilla luciferase reaction

Three Biochemical Pathways

• Coelenterazine (**CZ**) bioluminescence falls into three biochemical categories.

• Chemical mechanism is the **same** in all the imidazopyrazinone bioluminescence systems.

• Variants are modifications to suit the animal's purpose for the light emission.

1. Extra-cellular Bioluminescence

The coelenterazine (**CZ)** and luciferase (Lase) are squirted into the surrounding sea-water. The Lase contains a **secretion sequence** allowing its transport across the cell's membrane.

Cypridina (a crustacean; "sea fireflies")

Cyp-luciferin + O_2 + Cyp-Lase → 453 nm + …

Metridia and *Gaussia* (copepods)

CZ + M-Lase + O_2 → 480 nm + …

2. Luciferin & R-Lase

- *Renilla* "Sea Pansy" (soft coral): classical enzyme-substrate reaction.
- Photophore (light organ)- several proteins involved.
- **CZ** contained in a "binding protein" (**BP**).

BP- CZ + Ca^{2+} $\rightarrow$ BP + **CZ**

CZ + R-Lase + O_2 $\rightarrow$ **480** nm + …

3. Photoproteins

• *Aequorea, Obelia*, *Clytia,* (jellies),etc., contain calcium-regulated photoproteins.
• Bioluminescence is intracellular.
• Photoproteins are luciferases with a stabilized peroxy intermediate,**CZ-OOH.**
• **O_2** is **not** required for the light reaction, just Ca^{2+}.

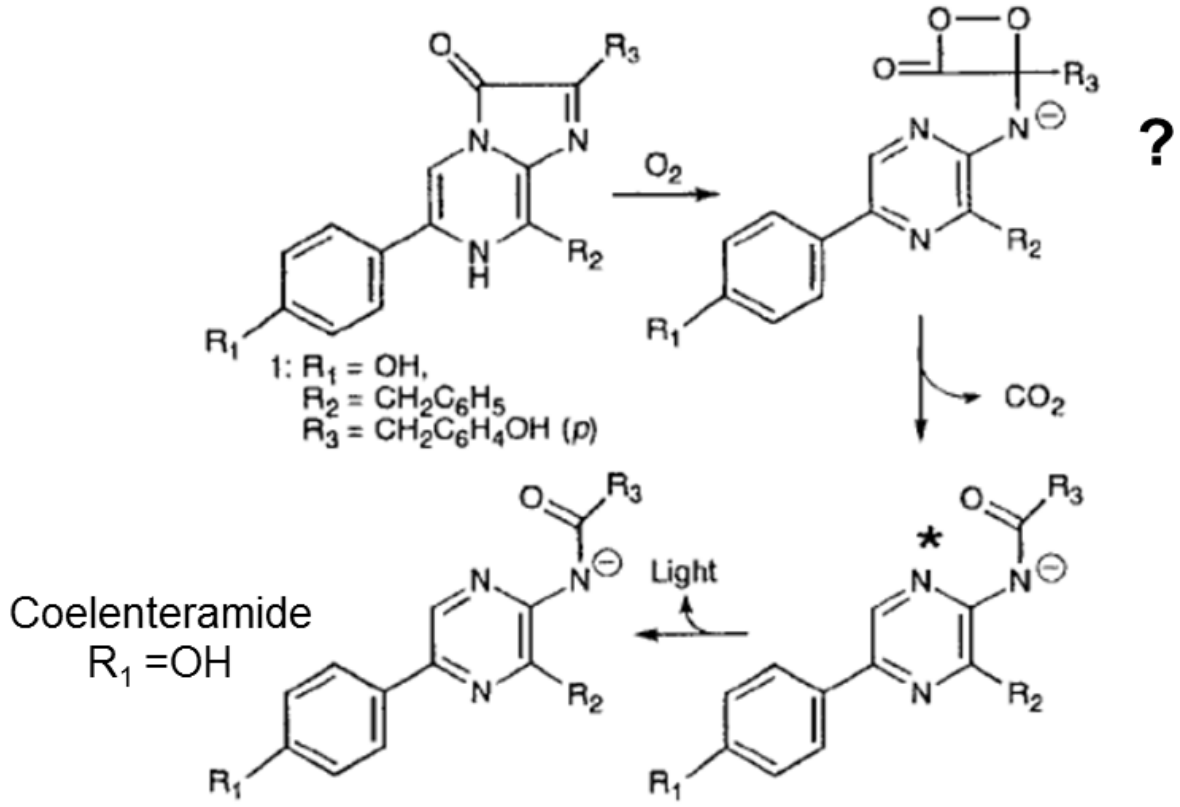
aequorin □ ⟶ **465 nm** (spectral maximum)

obelin □ ⟶ **475 - 495 nm**

clytin □ ⟶ **470 nm**

• + *cognate* **GFP** ⟶ **500 – 510 nm**

CZ Chemiluminescence model



Tuning via Antenna Proteins

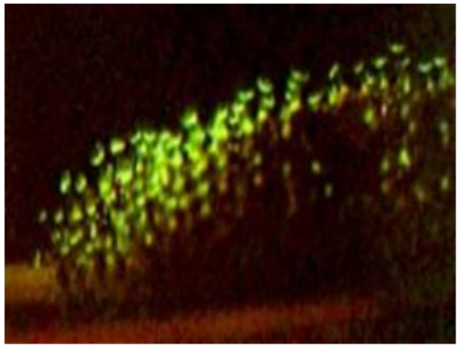
Protein-protein complexation occurs enabling excitation coupling via Förster Resonance Energy Transfer (FRET)

Coelenterazine systems: Green-fluorescent Protein **GFP**

Bacterial bioluminescence:
Lumazine Protein **LumP**

Green-fluorescent Protein

Most coelenterazine systems in vivo utilize **GFP**, so that the in vivo color is shifted to the **green** rather than the **blue** observed for the in vitro reaction.



Aequorea: room light and dark *Renilla*

Literature

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