

Discovery of Green Fluorescent Protein, GFP

Osamu Shimomura

Ruins of the Medical College of Nagasaki, 1945



Shigeo Hayashi, 1945

Prof. Shungo Yasunaga (1911-1959)
Nagasaki University



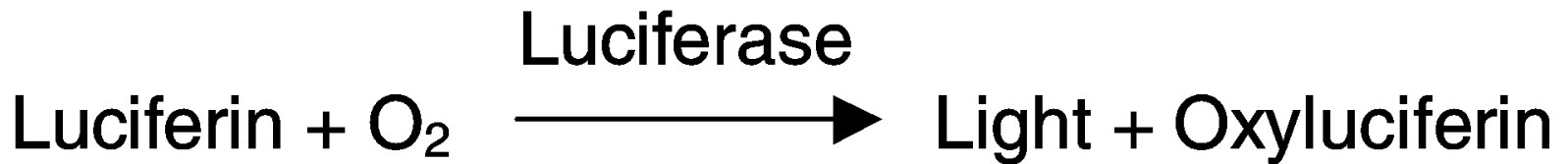
Prof. Yoshimasa Hirata (1915-2000)
Nagoya University



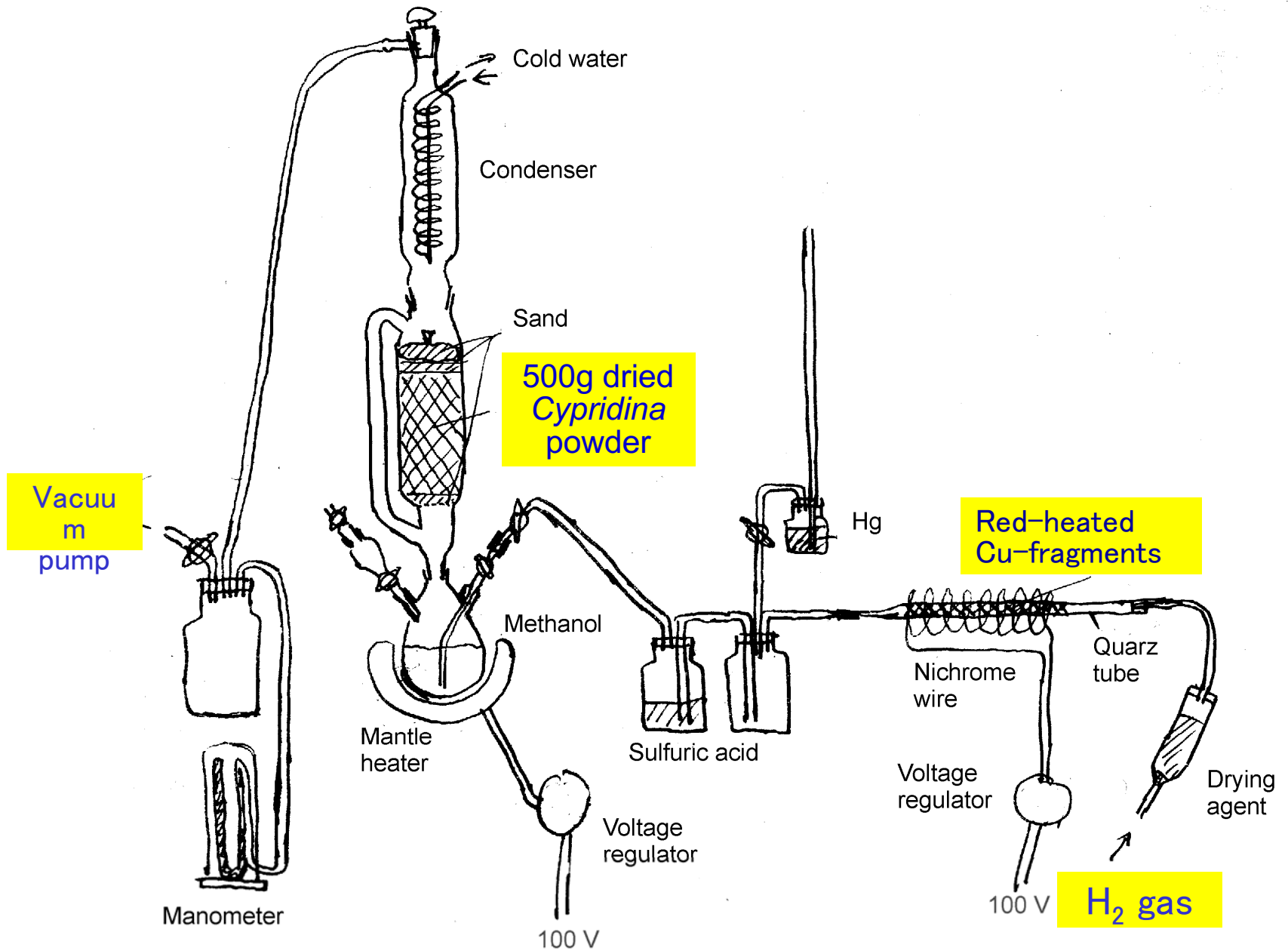
Cypridina hilgendorffii

Luciferase

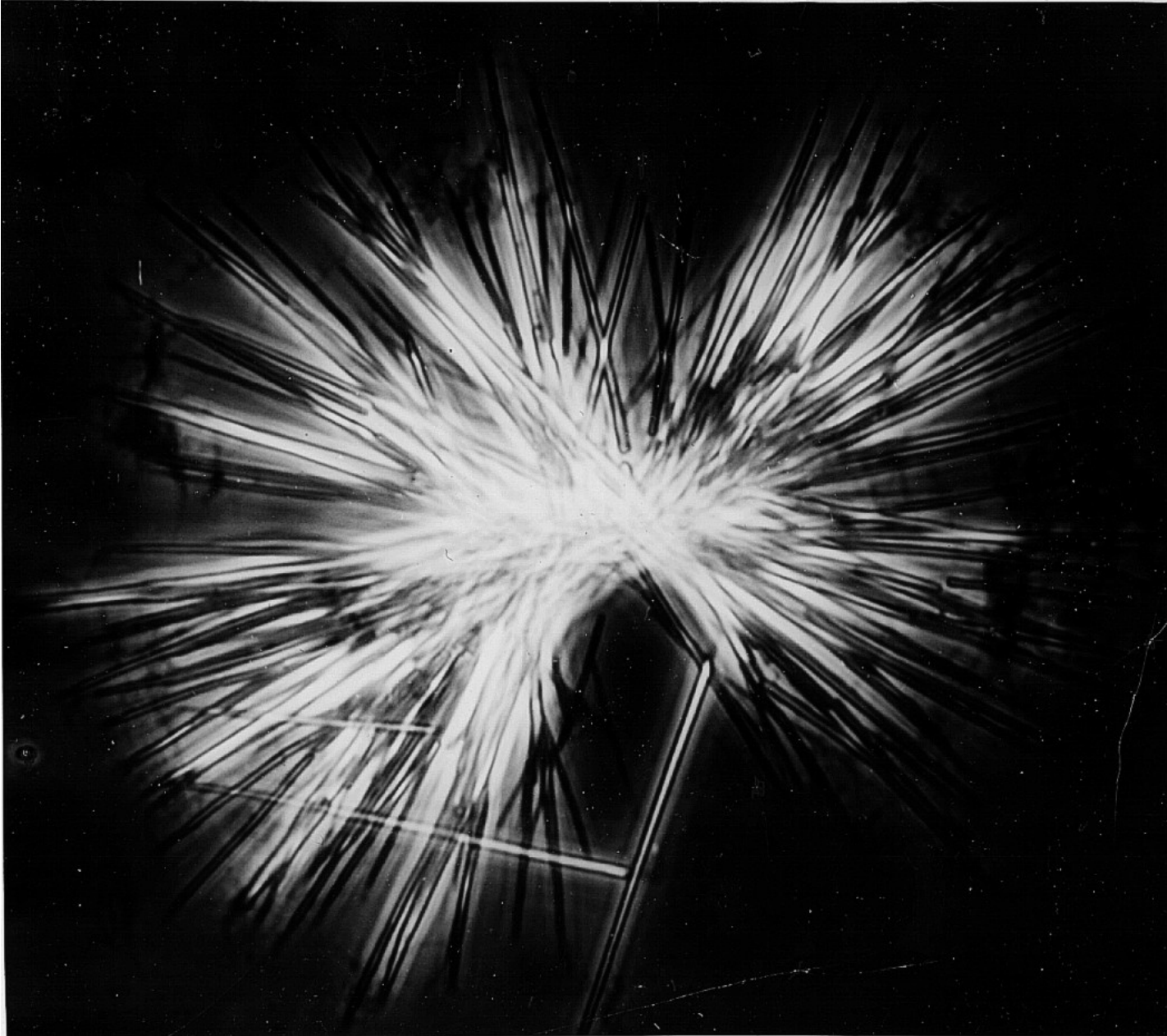
— 1 mm



Extraction of *Cypridina* luciferin

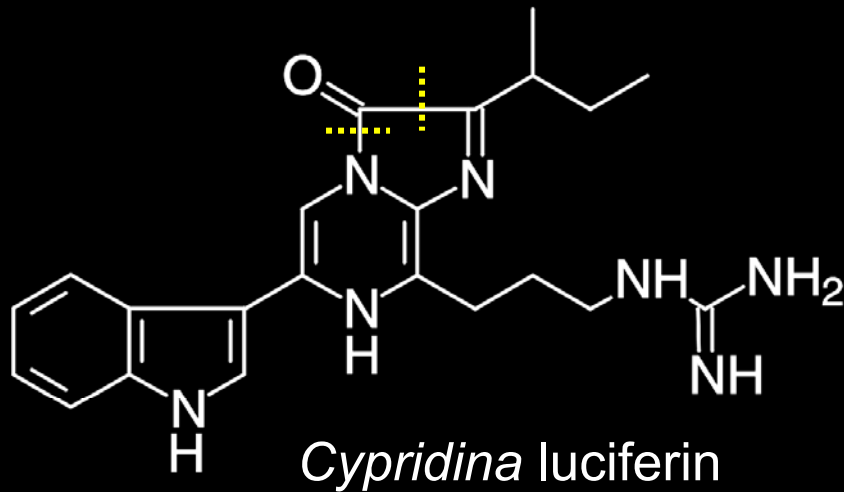


Crystals of *Cypridina* luciferin (1956)

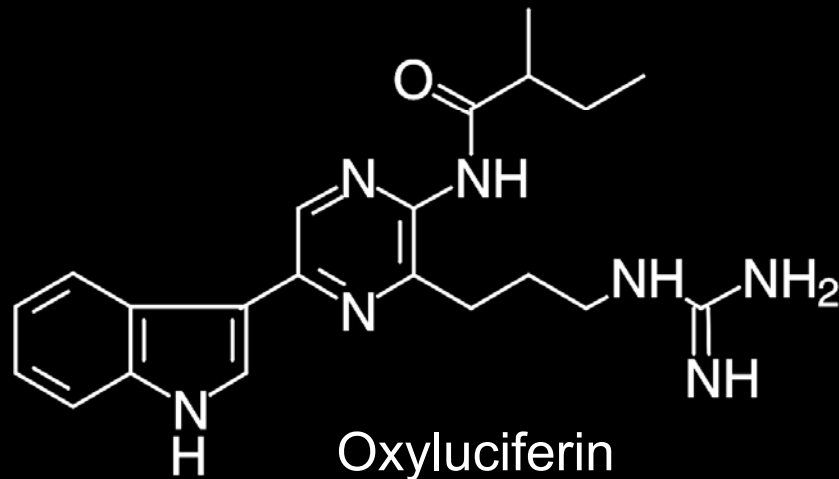


Luminescence Reaction of *Cypridina* Luciferin

Kishi et al., 1966

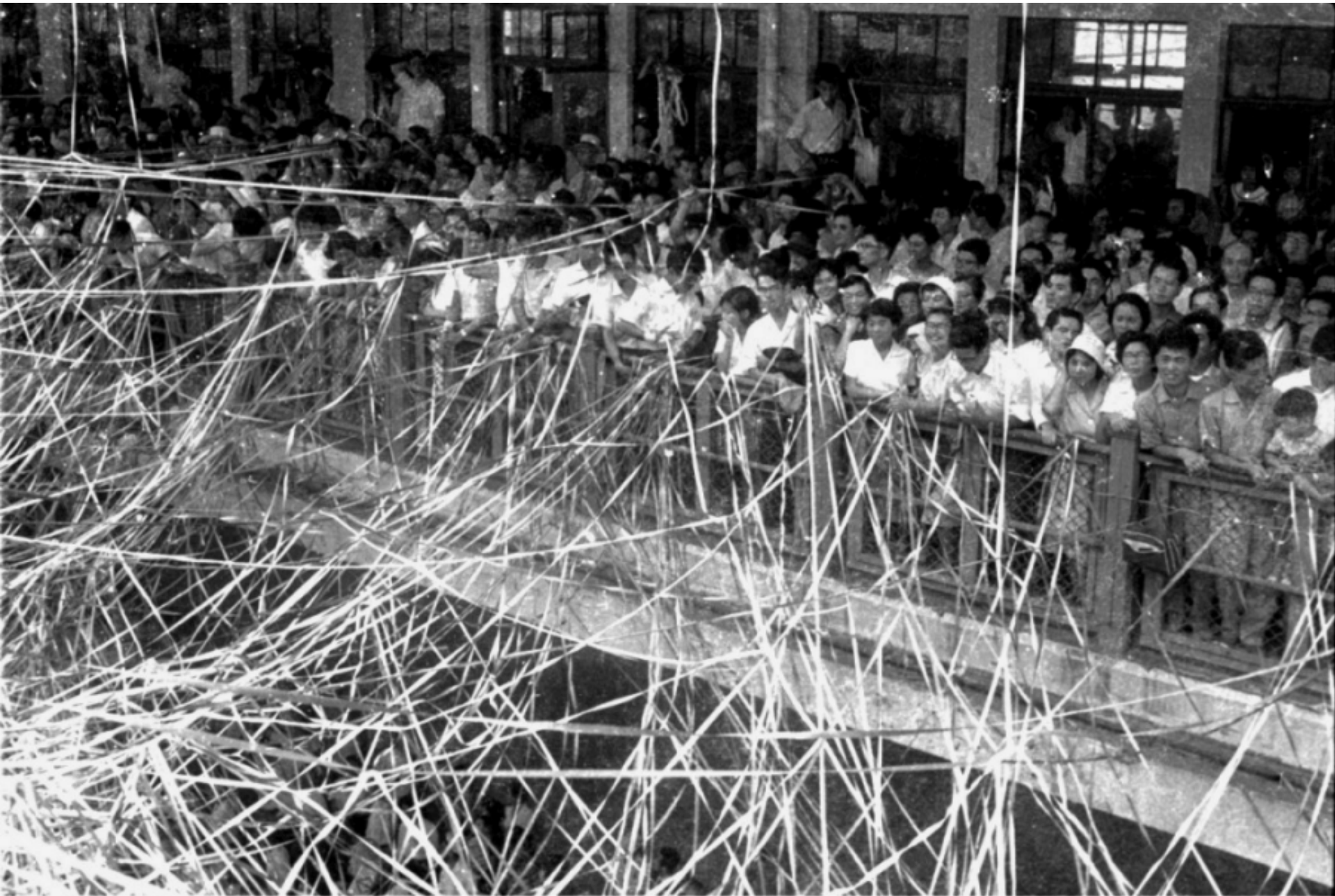


Luciferase and O₂



+ CO₂ + Light

Hikawa-Marū leaving Yokohama, Aug. 1960



Hikawa-Maruru leaving Yokohama, Aug. 1960



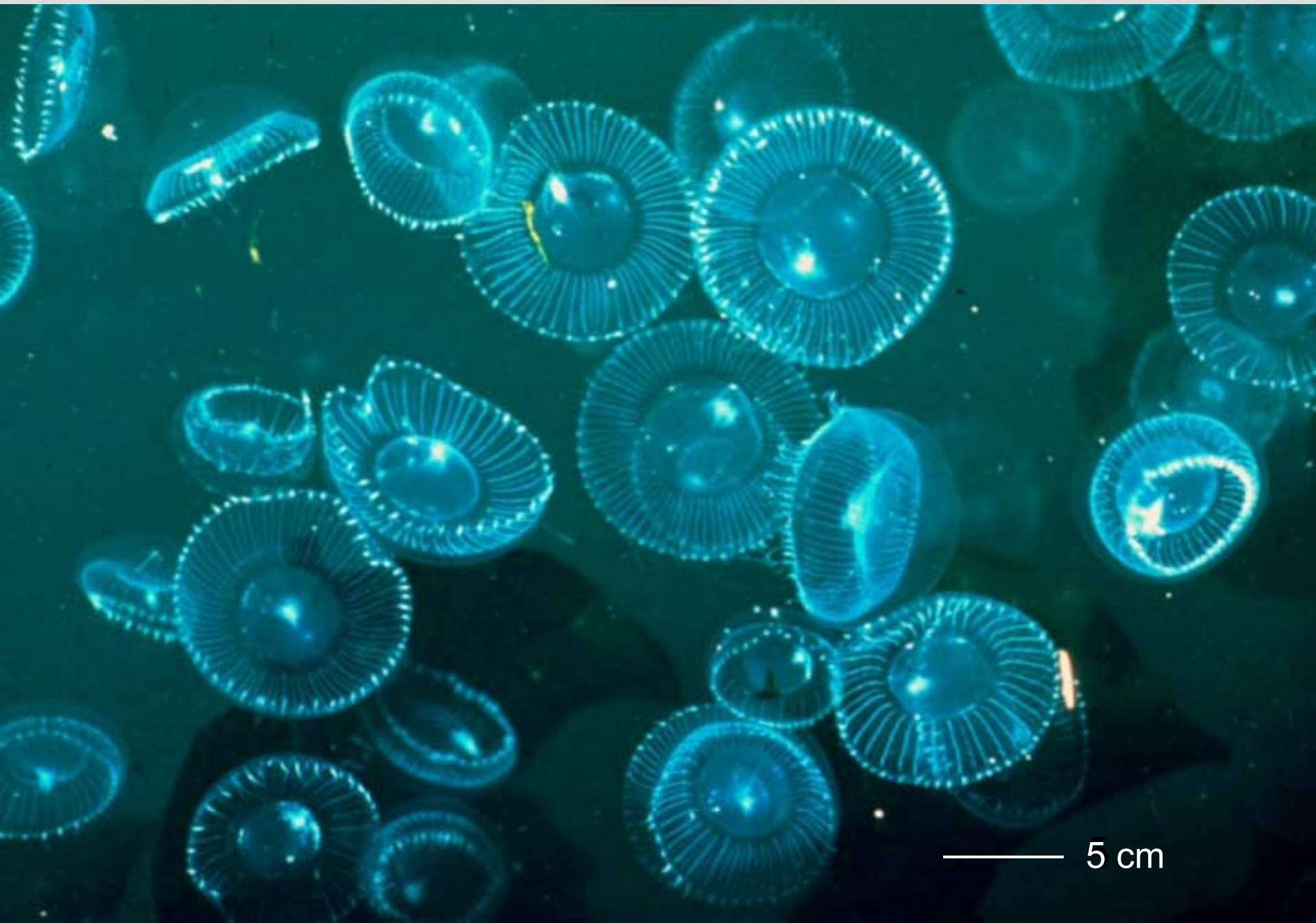
Prof. Frank H. Johnson (1908-1990)
Princeton University



Friday Harbor, 1961



Aequorea aequorea

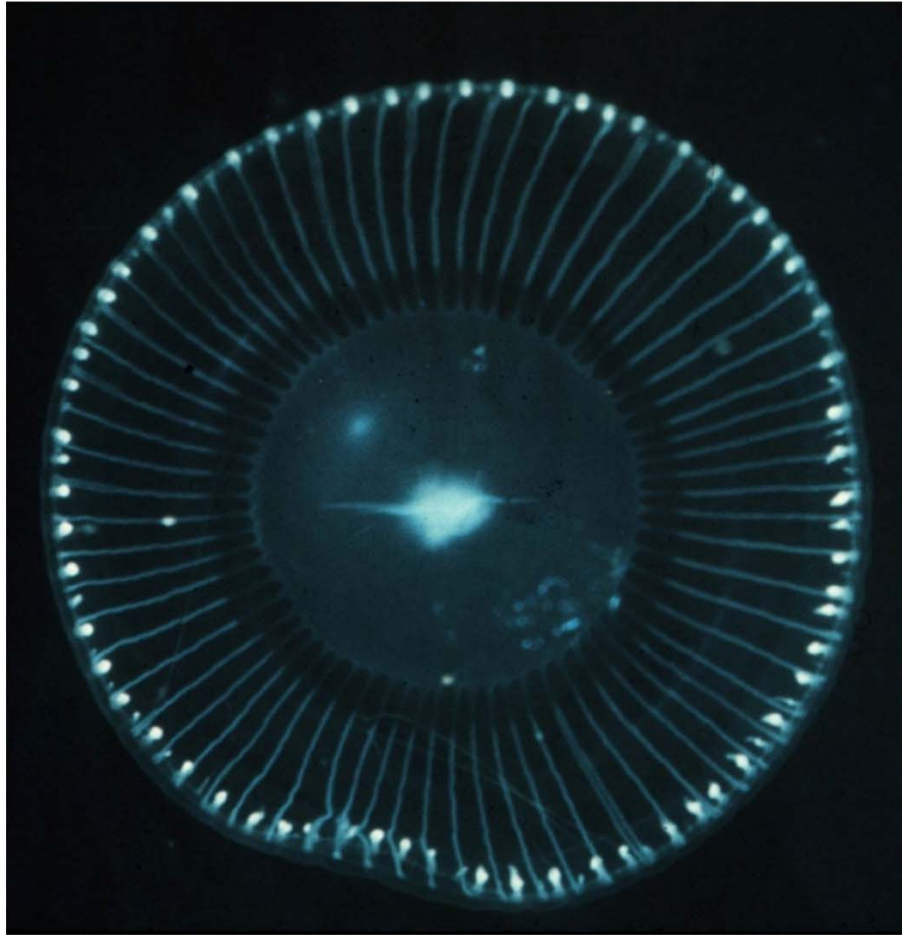


Univ. of Washington, Friday Harbor Laboratories, 1961

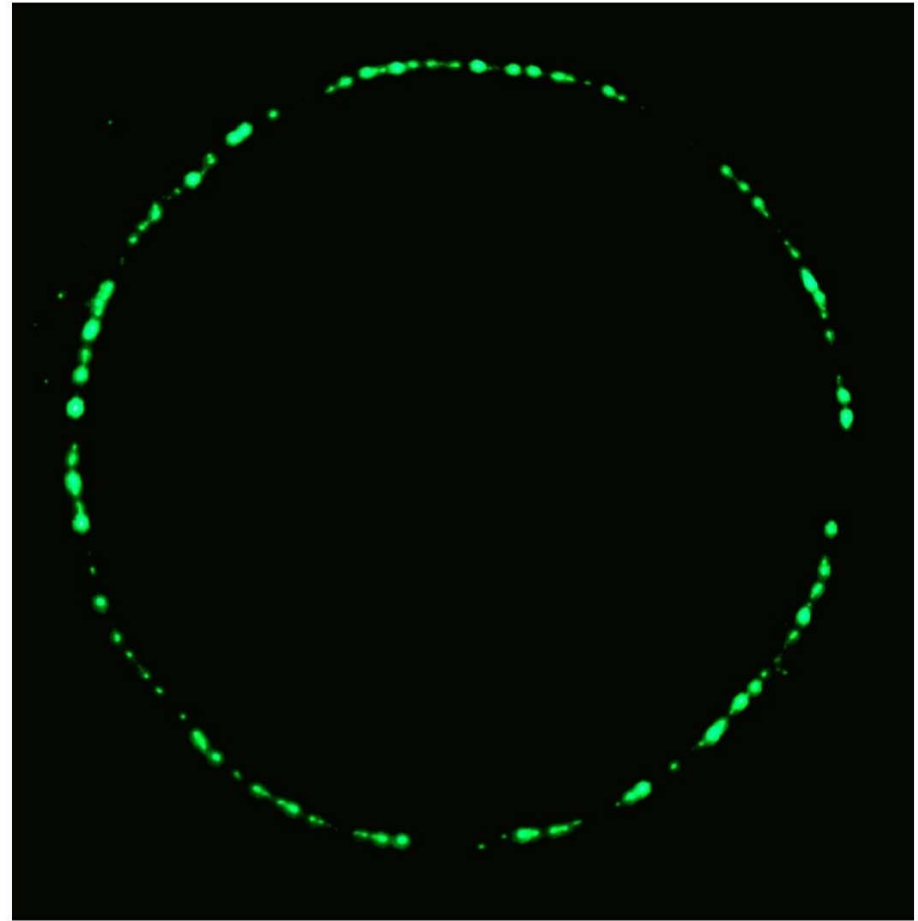


Aequorea aequorea

In Daylight

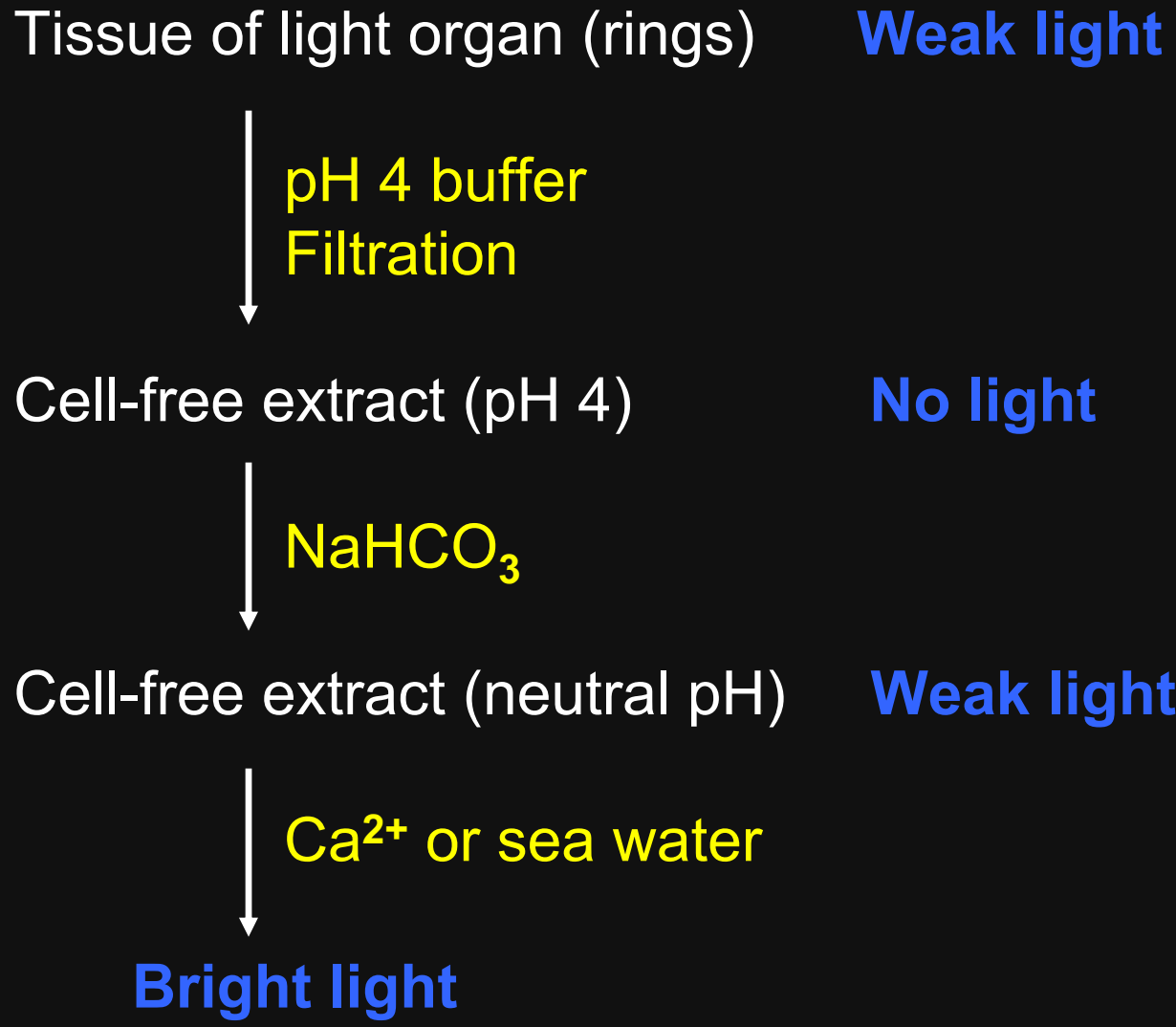


Luminescence in Dark



Basic Strategy for the Extraction of Bioluminescent Substances

A bioluminescent substance in light organs must be solubilized and extracted under a condition that reversibly inhibits the emission of light.



Extraction of Aequorin and GFP

Rings of jellyfish (tissue of light organs)

Shake in saturated $(\text{NH}_4)_2\text{SO}_4$
Squeeze through gauze
Filtration

Granular light organs

Shake in EDTA solution
Filtration

Crude aequorin solution

Purification

Aequorin and GFP

Blue-fluorescent compound AF-350



To obtain 1 mg of AF-350, about 150 mg of aequorin is needed, requiring to collect and process 50,000 jellyfish (2.5 tons) in one summer (2,000-3,000 jellyfish per day).

Jellyfish Collectors (1974)



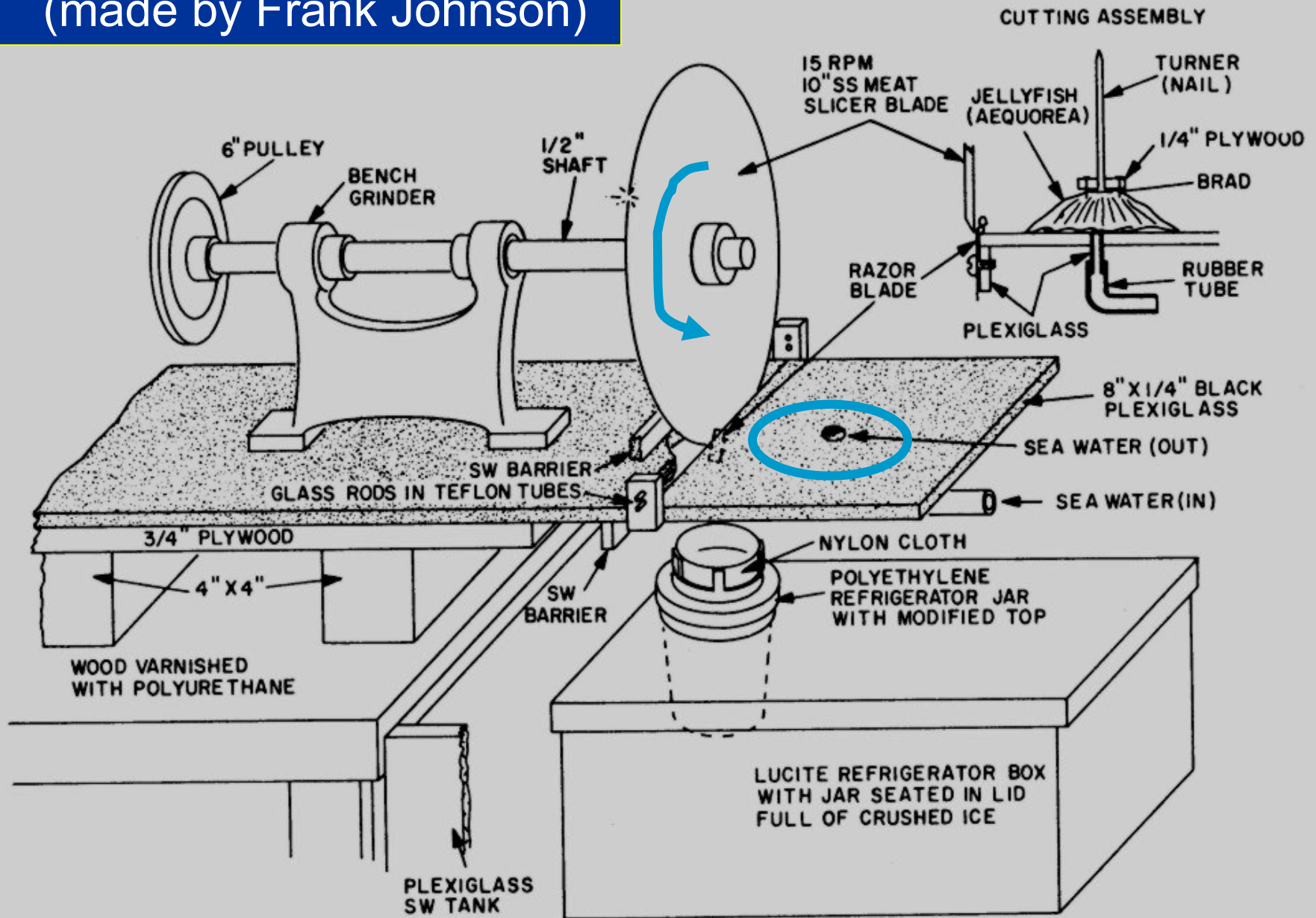
Dr. Johnson collecting jellyfish



My family collecting jellyfish



Jellyfish Ring-cutter (made by Frank Johnson)



Test-run of ring-cutters by Johnsons, 1968



Cutting rings



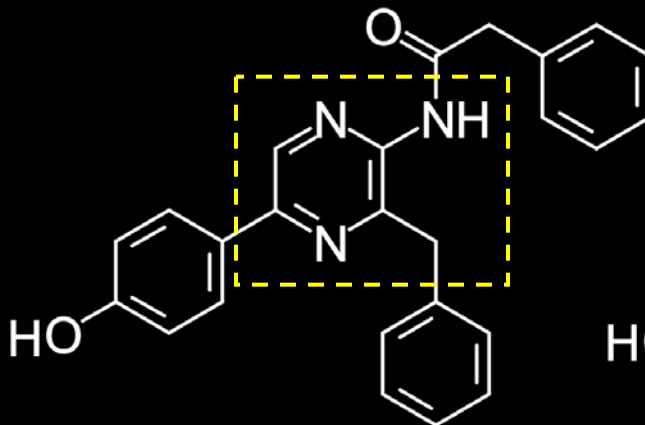
Extraction of aequorin



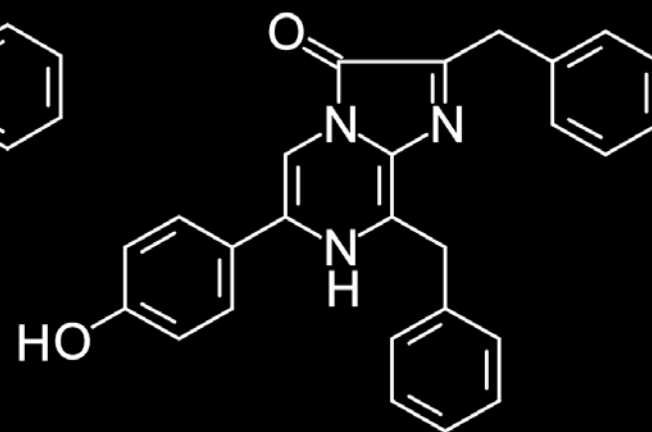
Structure Elucidation of Coelenterazine



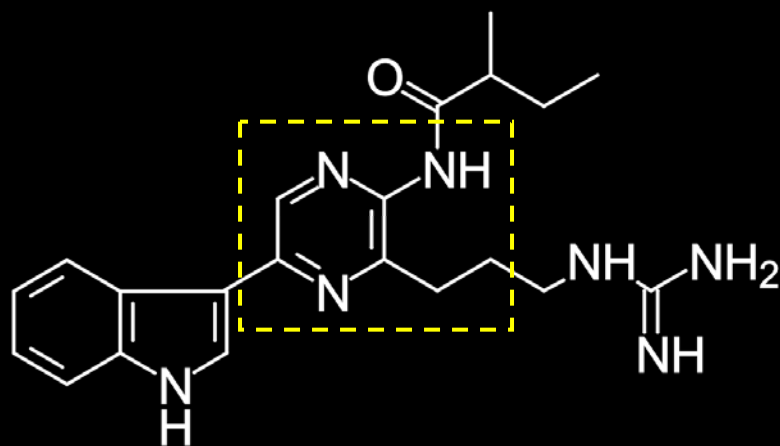
AF-350
(Coelentramine)



Coelenteramide



Coelenterazine

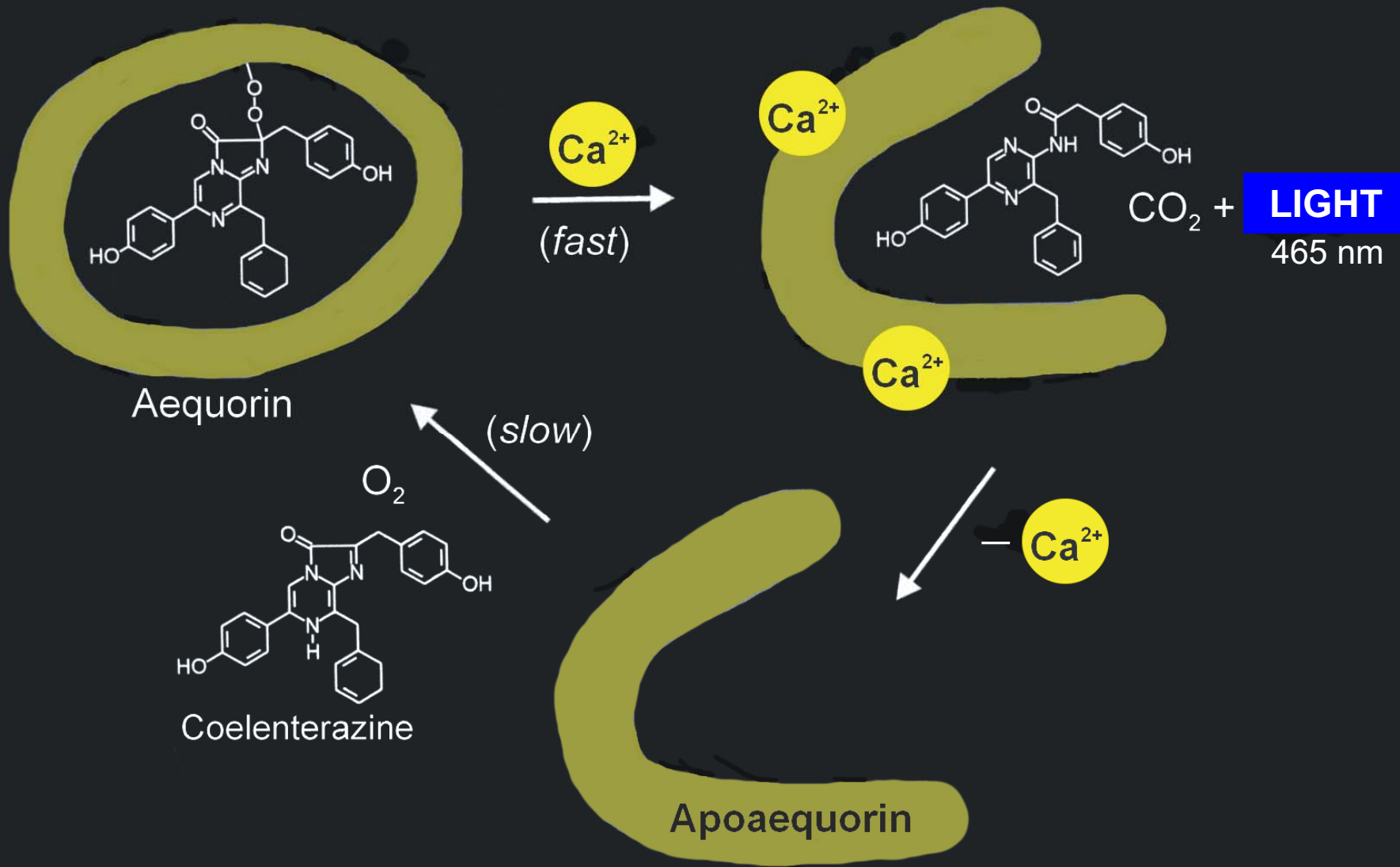


Cypridina oxyluciferin



Cypridina luciferin

Luminescence and Regeneration of Aequorin

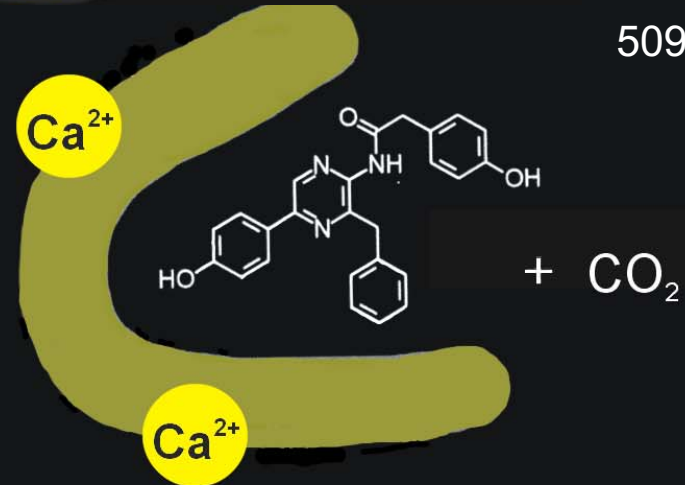
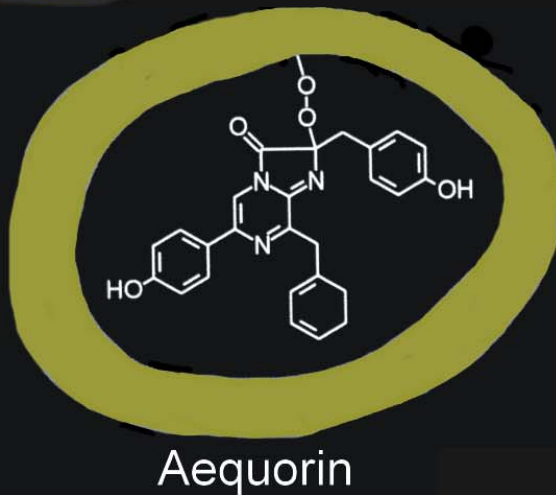


Aequorin Luminescence with GFP



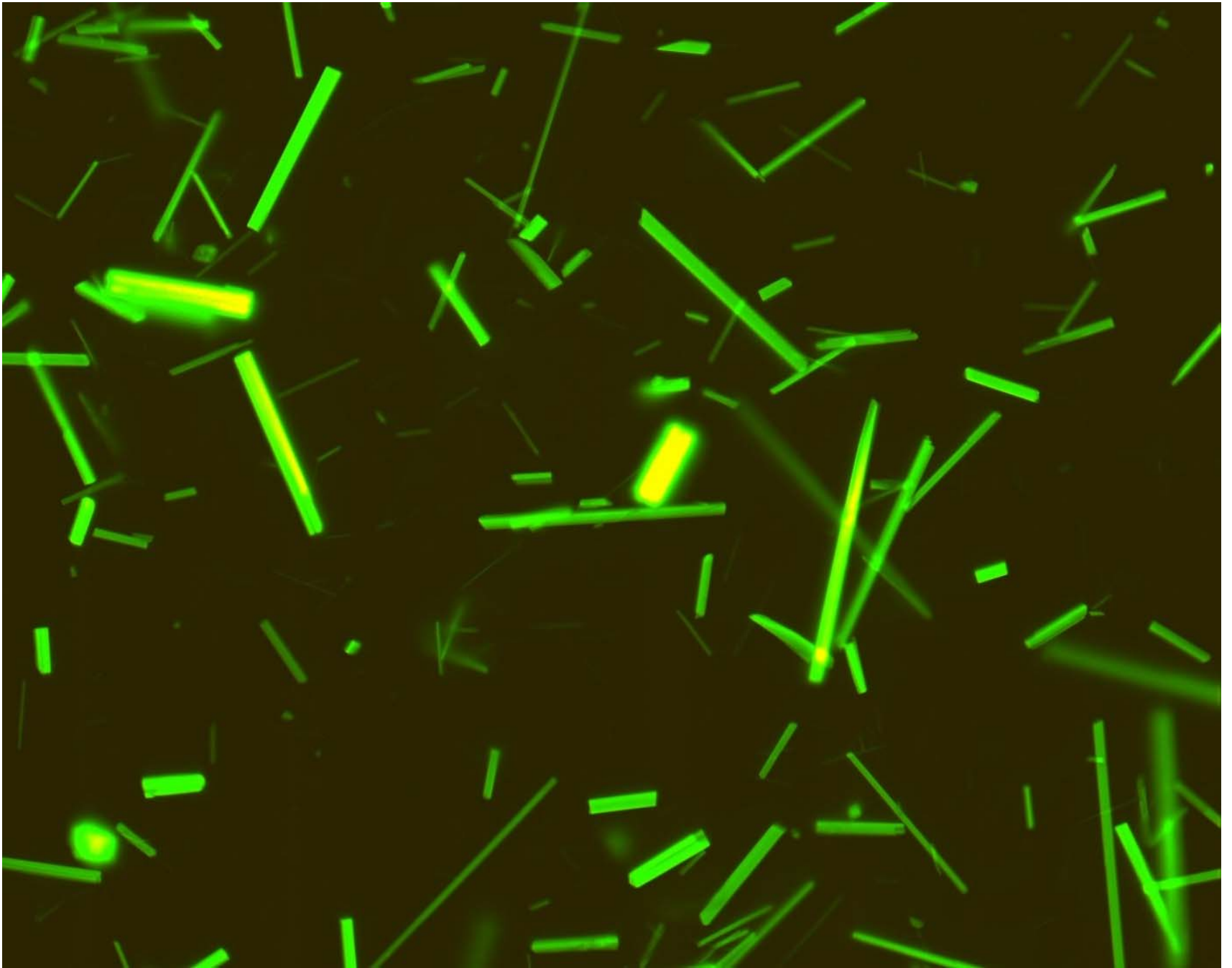
Green Light

509 nm



GFP Crystals

Photo by Dr. Shinya Inoue



Isolation of GFP Chromophore

GFP (100 mg)



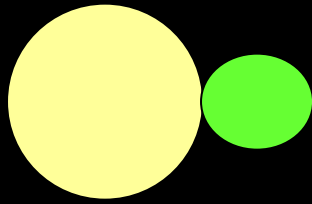
Denature at 90 °C

Digest with papain

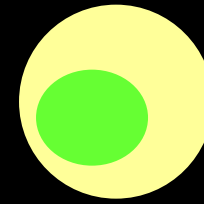
Extraction with butanol at pH 1

TLC purification

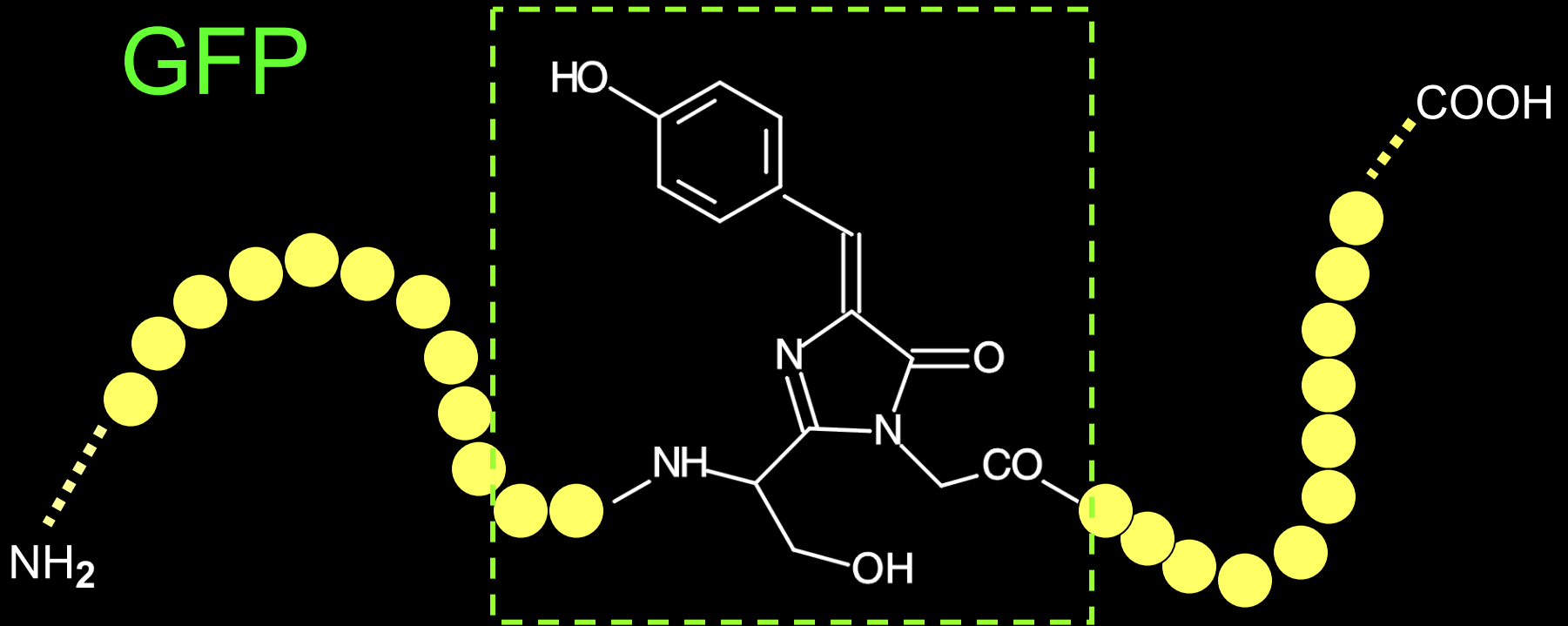
Isolated chromophore
(0.1 mg)



Ordinary Fluorescent Proteins



GFP



● Amino acid residue

Shimomura, 1979
Cody et al, 1993

The background of the slide is a dark, deep blue-green color, filled with numerous glowing blue jellyfish. The jellyfish are of various sizes and orientations, some appearing as bright, circular discs with radial lines, while others are more elongated or partially obscured. The overall effect is a dense, ethereal field of bioluminescent organisms.

Acknowledgments

I thank the many collaborators and colleagues who helped our study of aequorin and GFP, and the NSF and NIH for financial support