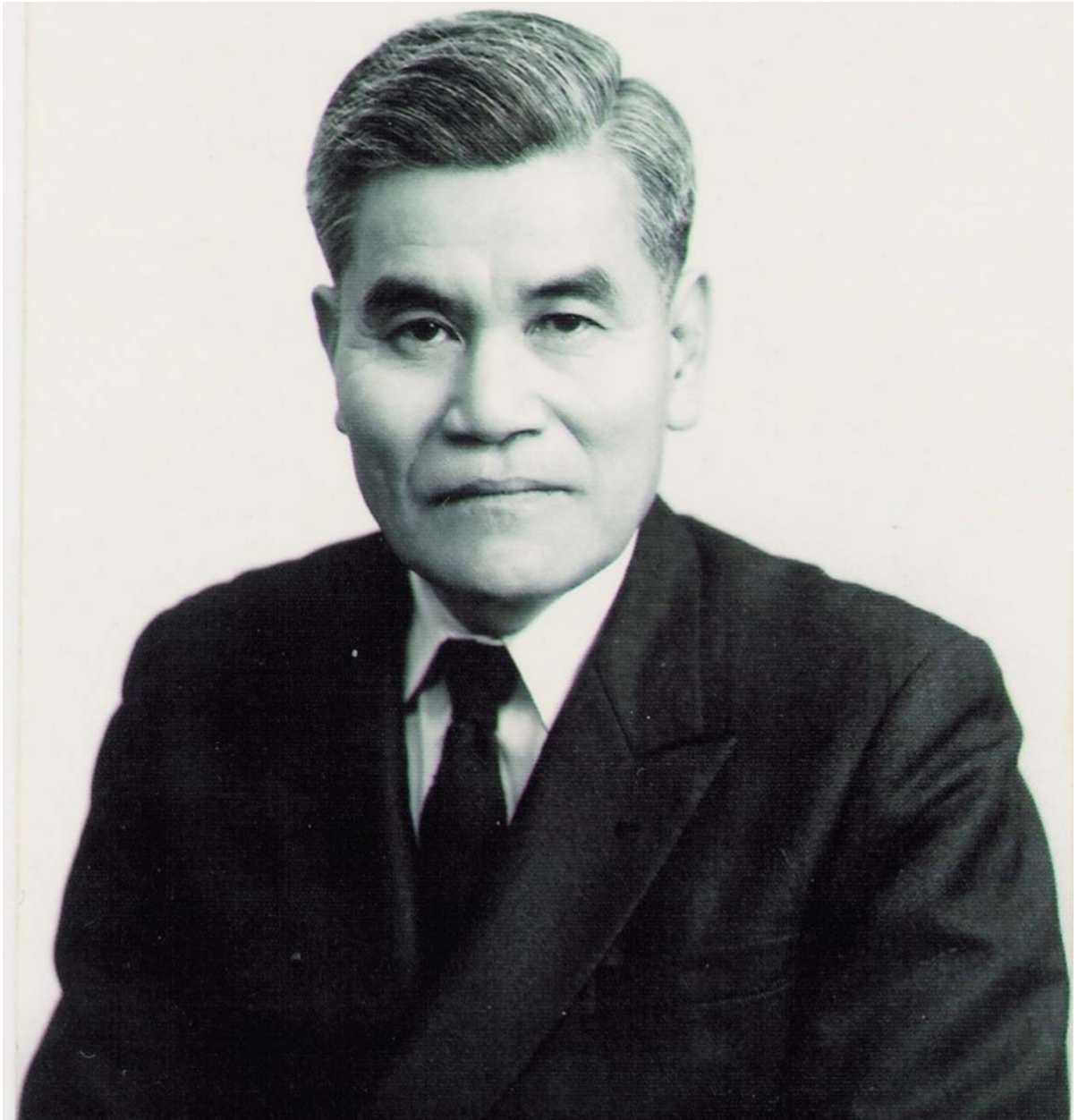
Discovery of Green Fluorescent Protein, GFP

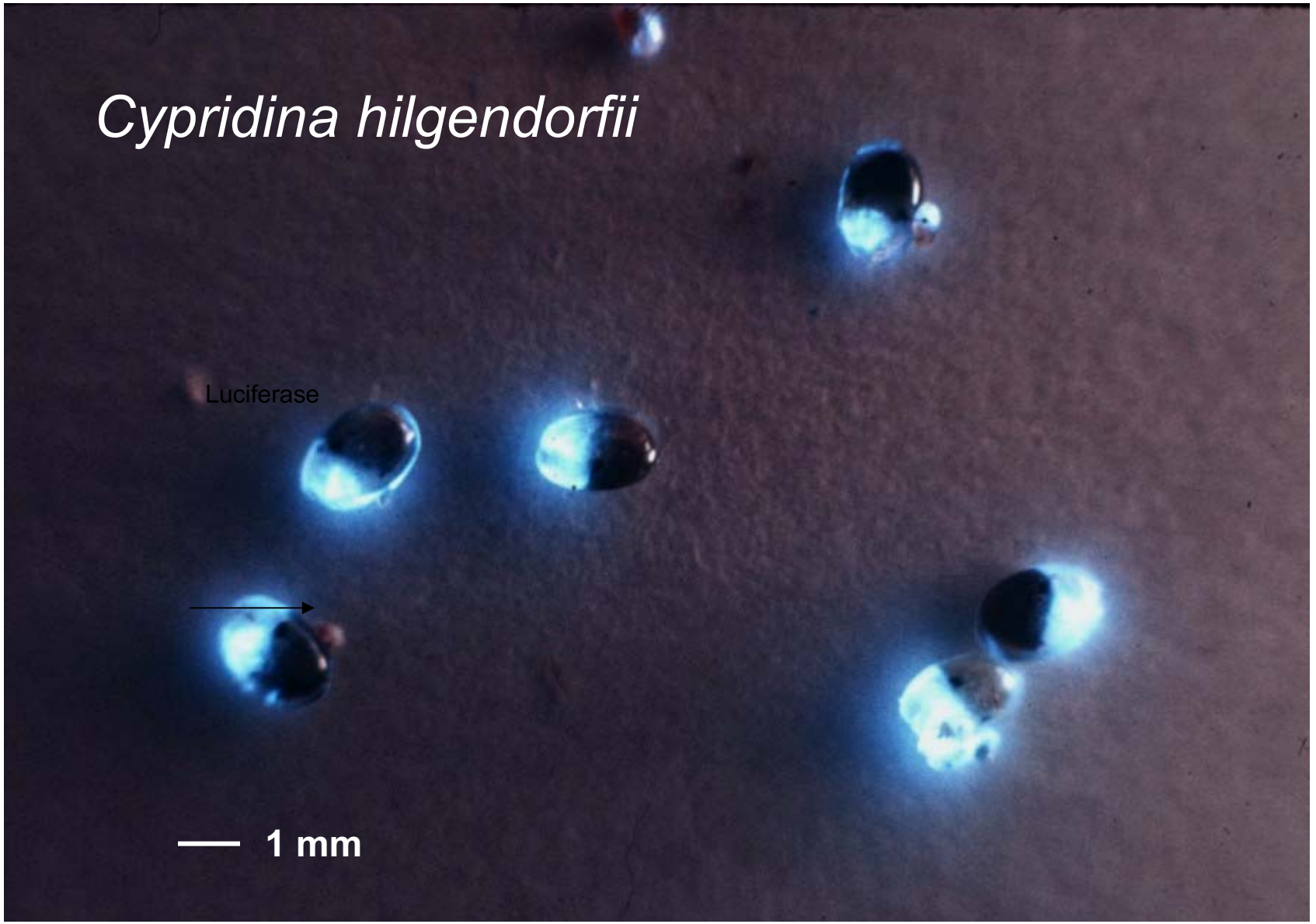
Osamu Shimomura

Ruins of the Medical College of Nagasaki, 1945

Prof. Shungo Yasunaga (1911-1959)
Nagasaki University

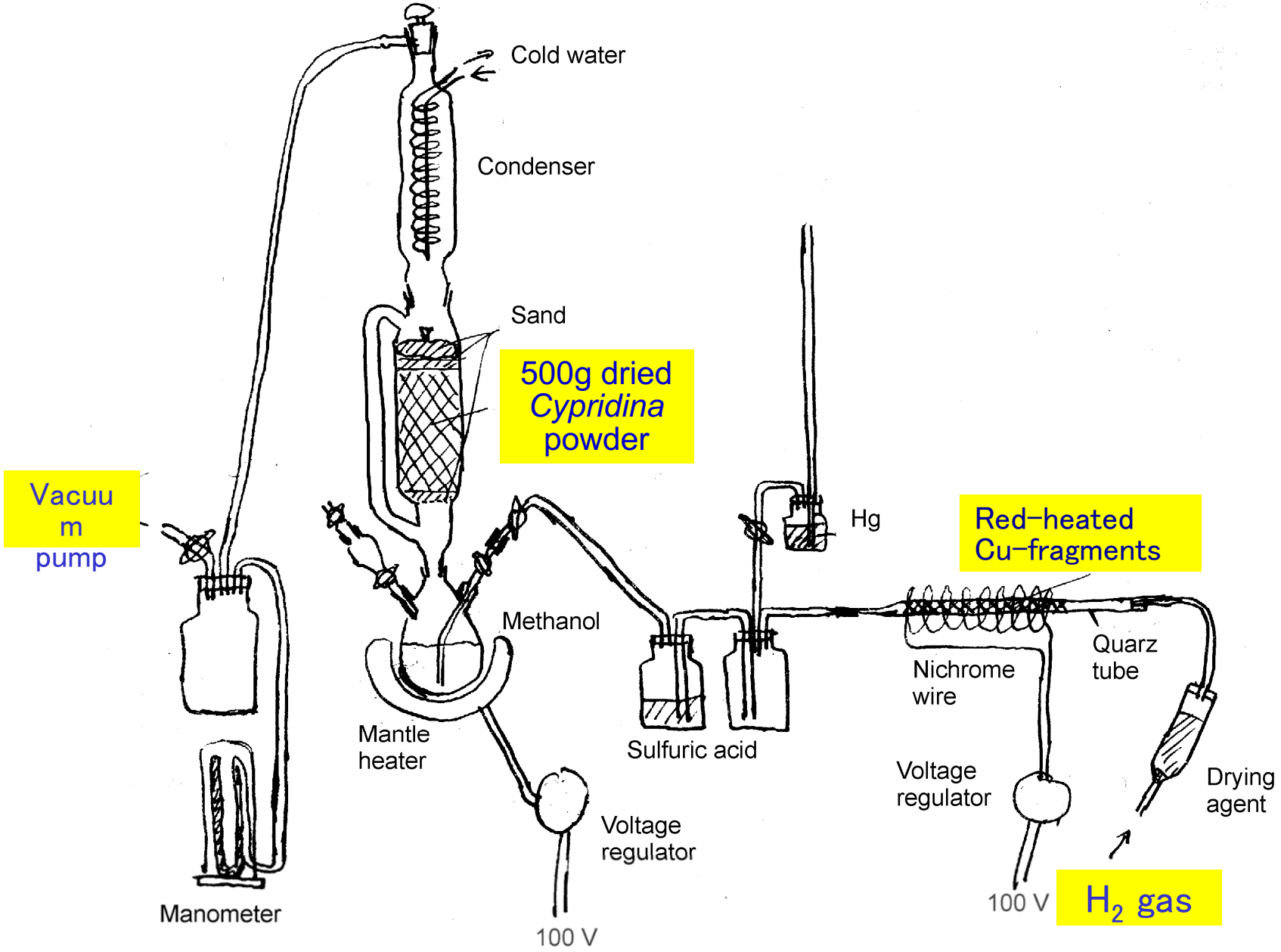
Prof. Yoshimasa Hirata (1915-2000)
Nagoya University



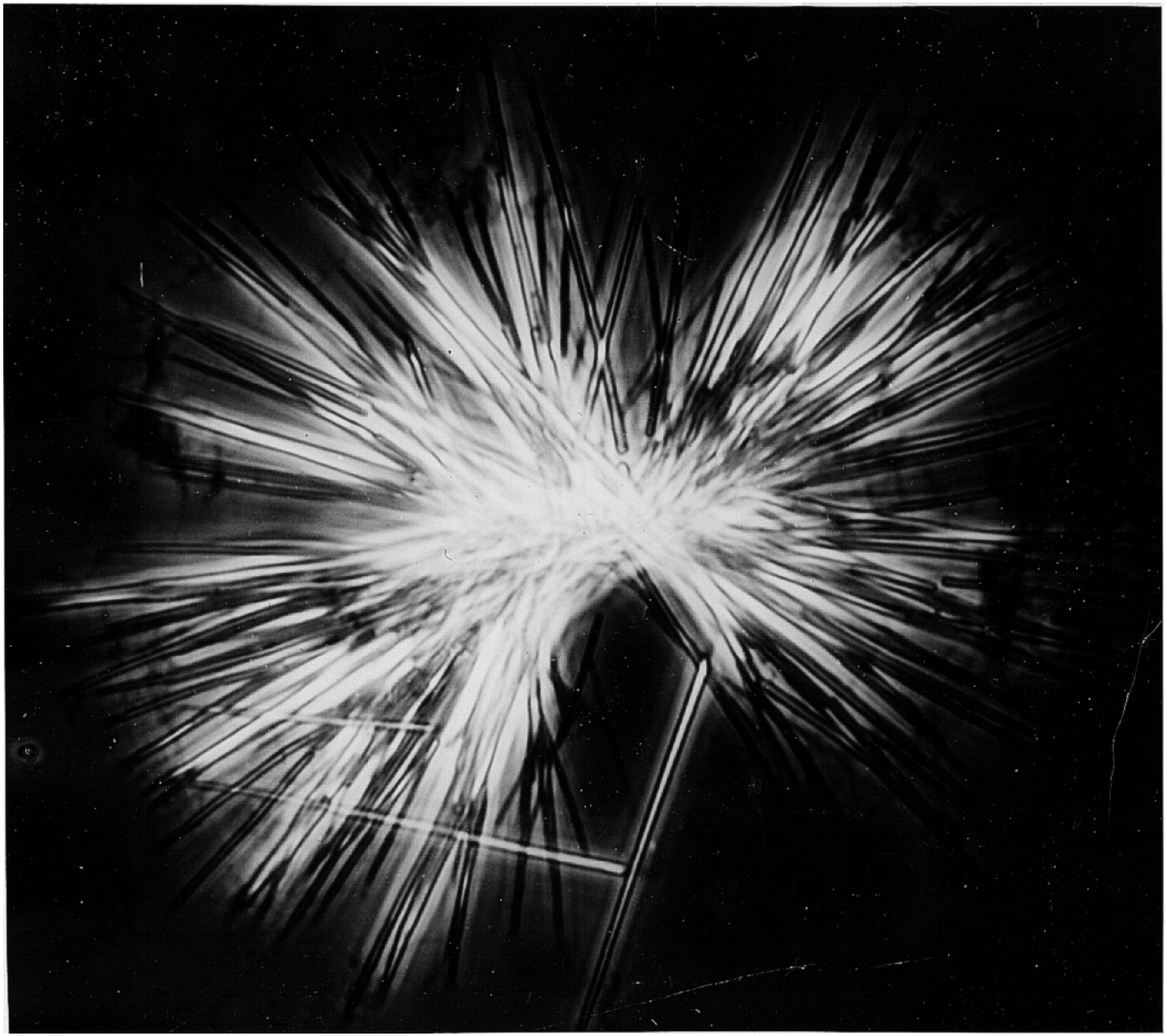


$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Light} + \text{Oxyluciferin}$$

Extraction of *Cypridina* luciferin

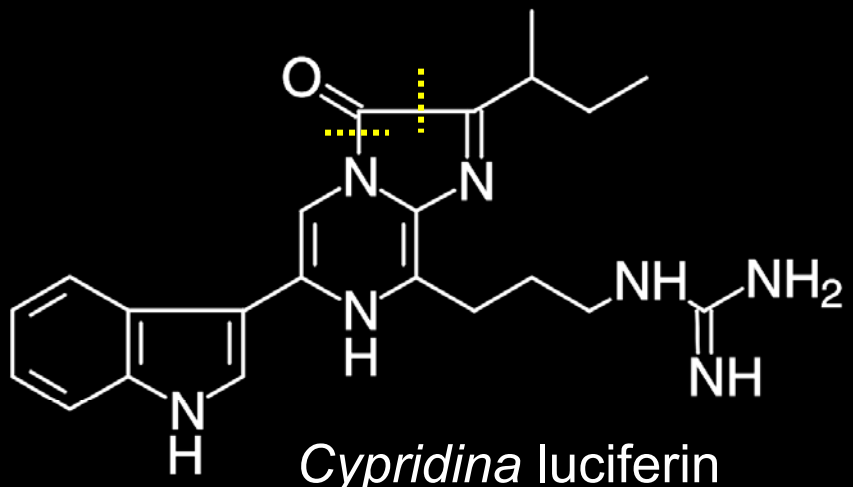


Crystals of *Cypridina* luciferin (1956)



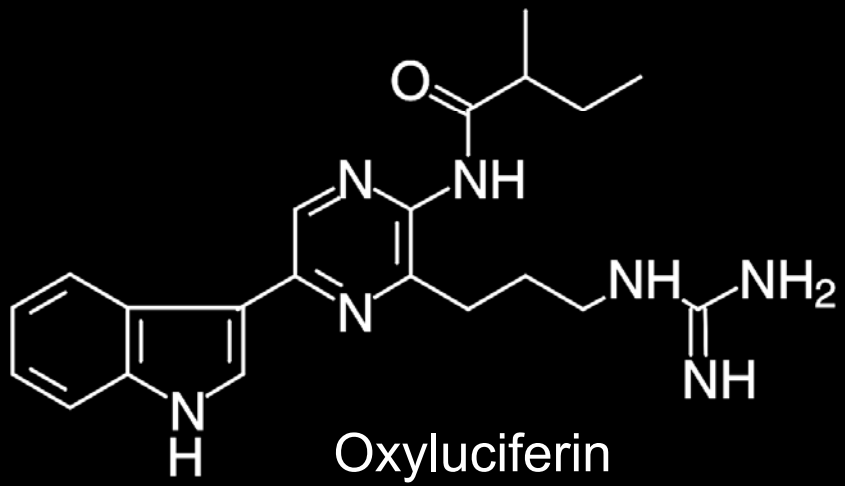
Luminescence Reaction of *Cypridina* Luciferin

Kishi et al., 1966



Cypridina luciferin

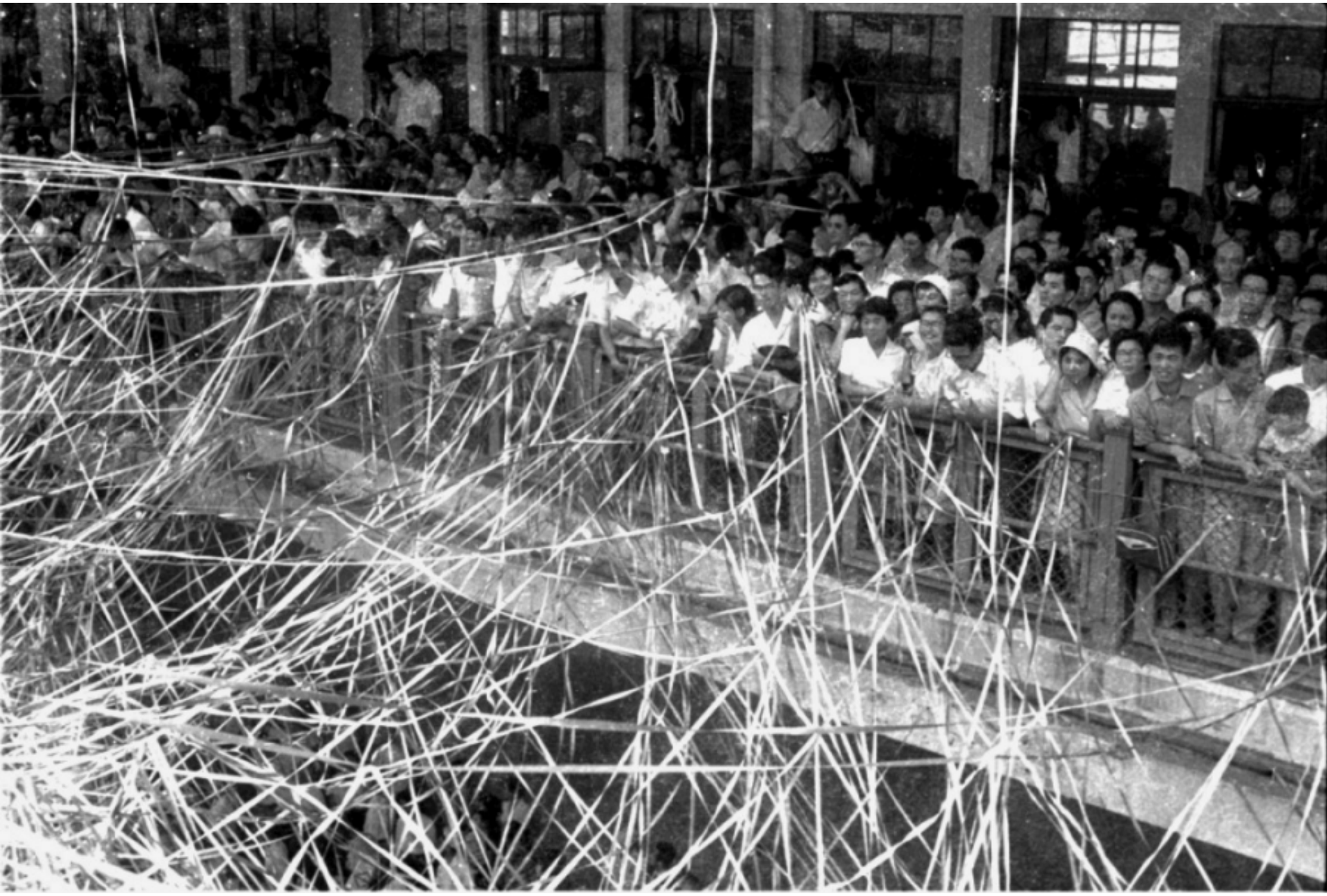
Luciferase and O_2



Oxyluciferin

+ CO_2 + **Light**

Hikawa-Maru leaving Yokohama, Aug. 1960

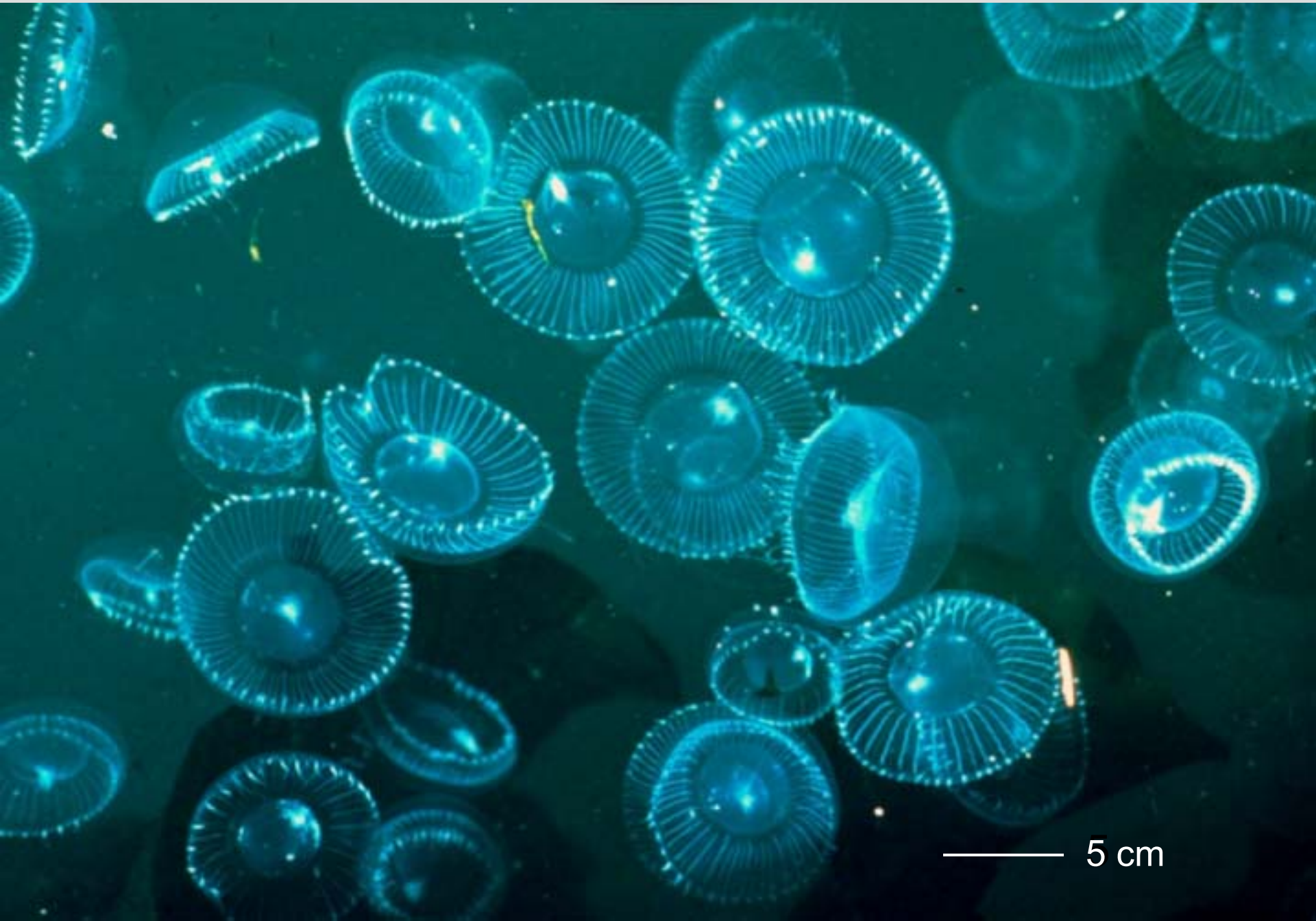


Hikawa-Maru leaving Yokohama, Aug. 1960

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

Friday Harbor, 1961

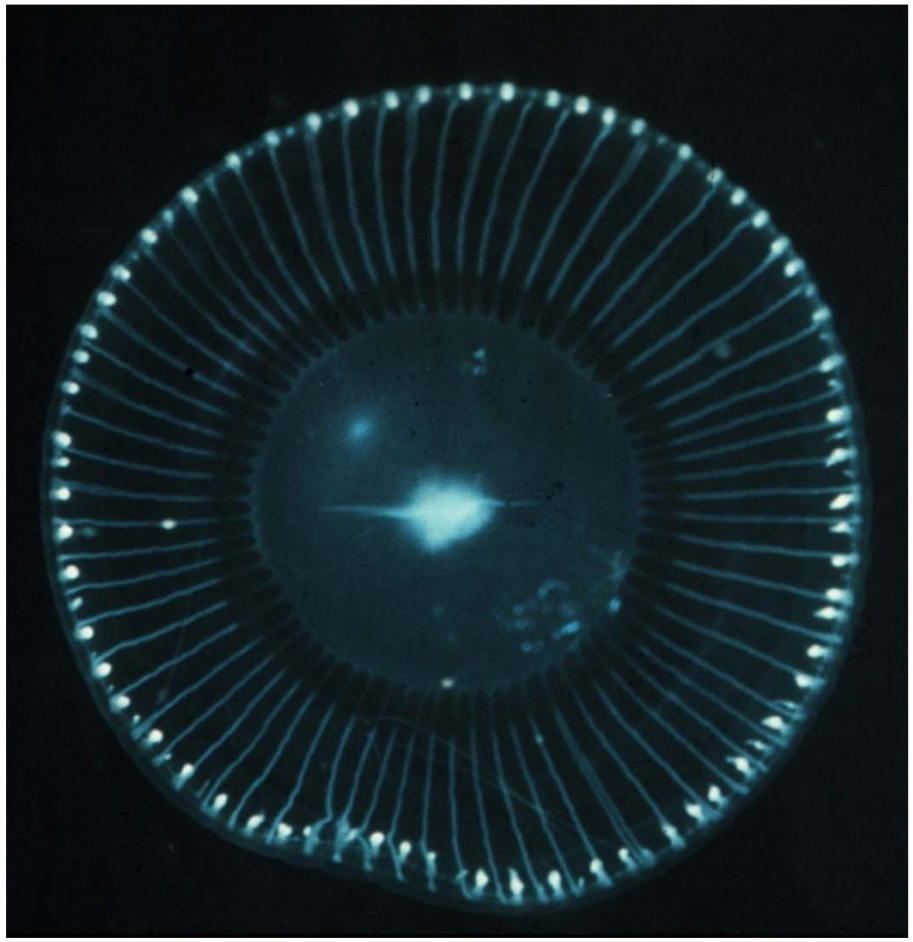
Aequorea aequorea



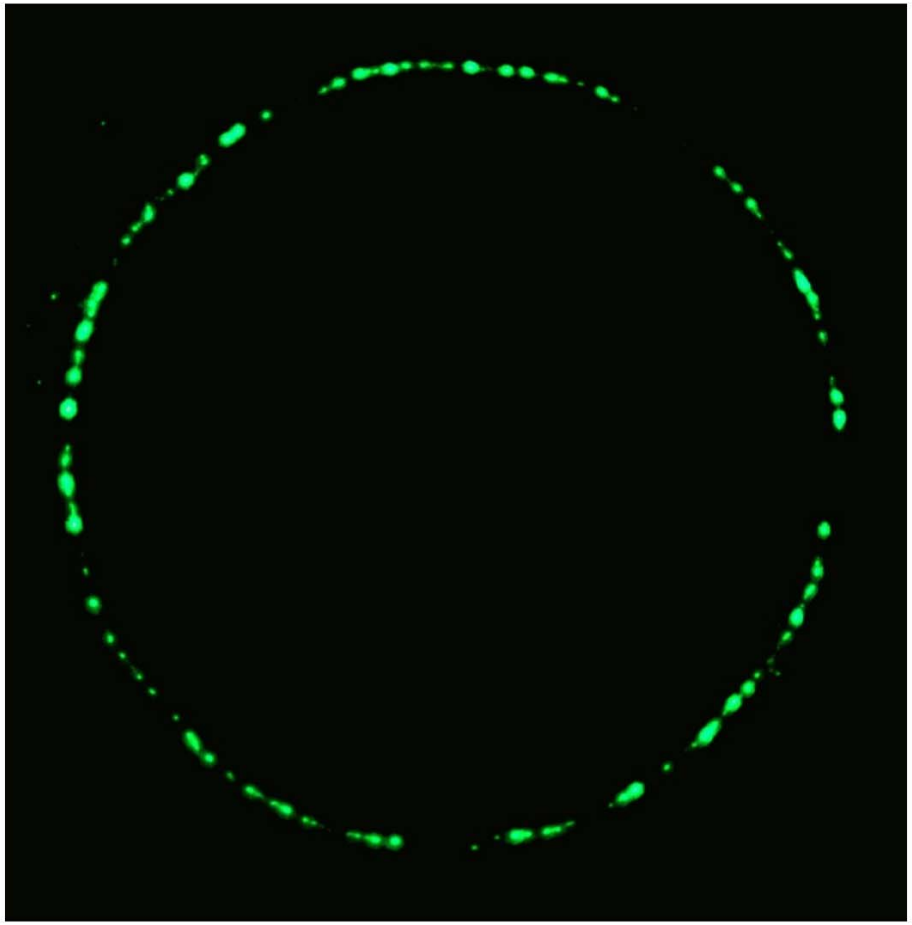
Univ. of Washington, Friday Harbor Laboratories, 1961

Aequorea aequorea

In Daylight

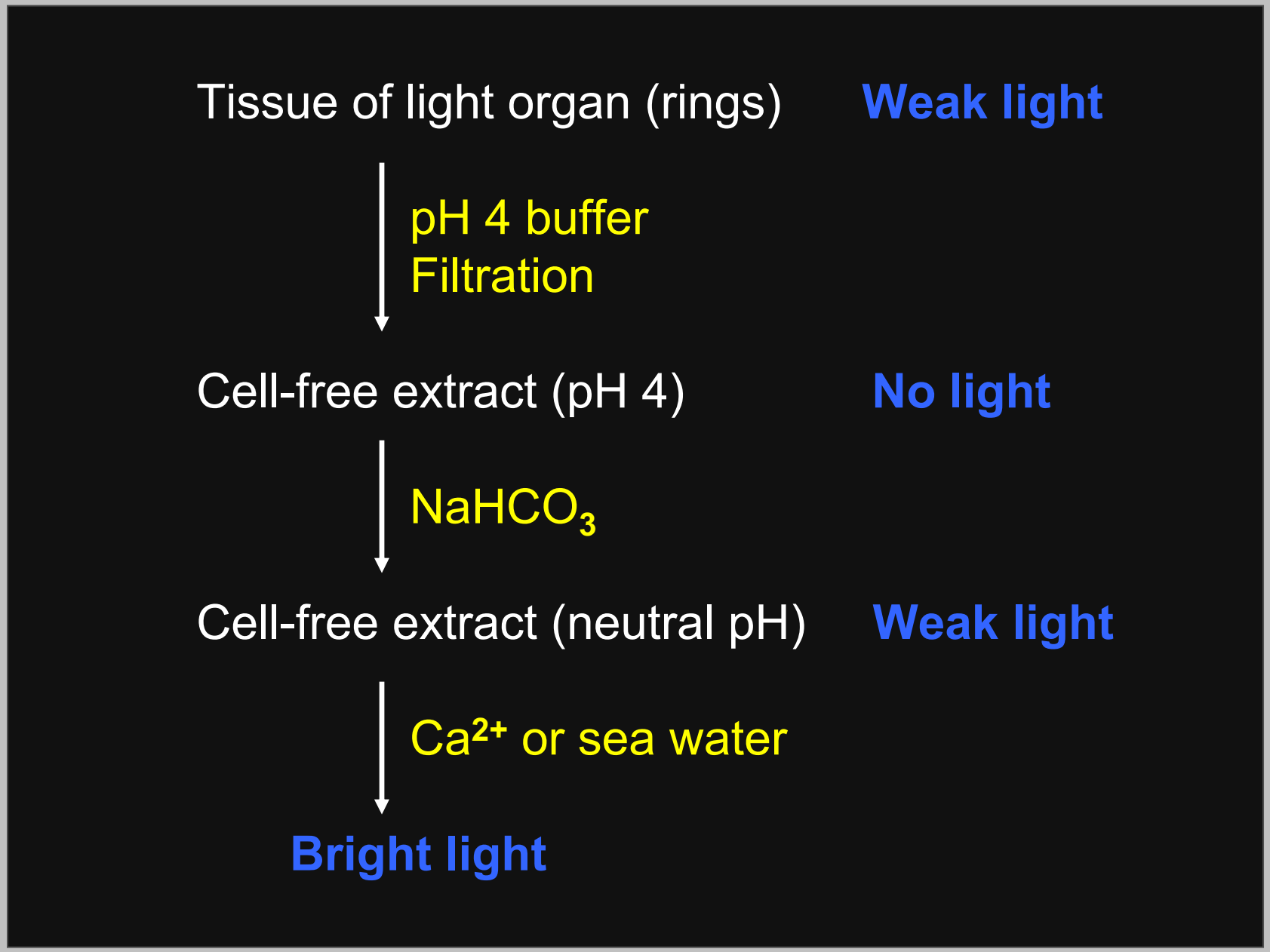


Luminescence in Dark

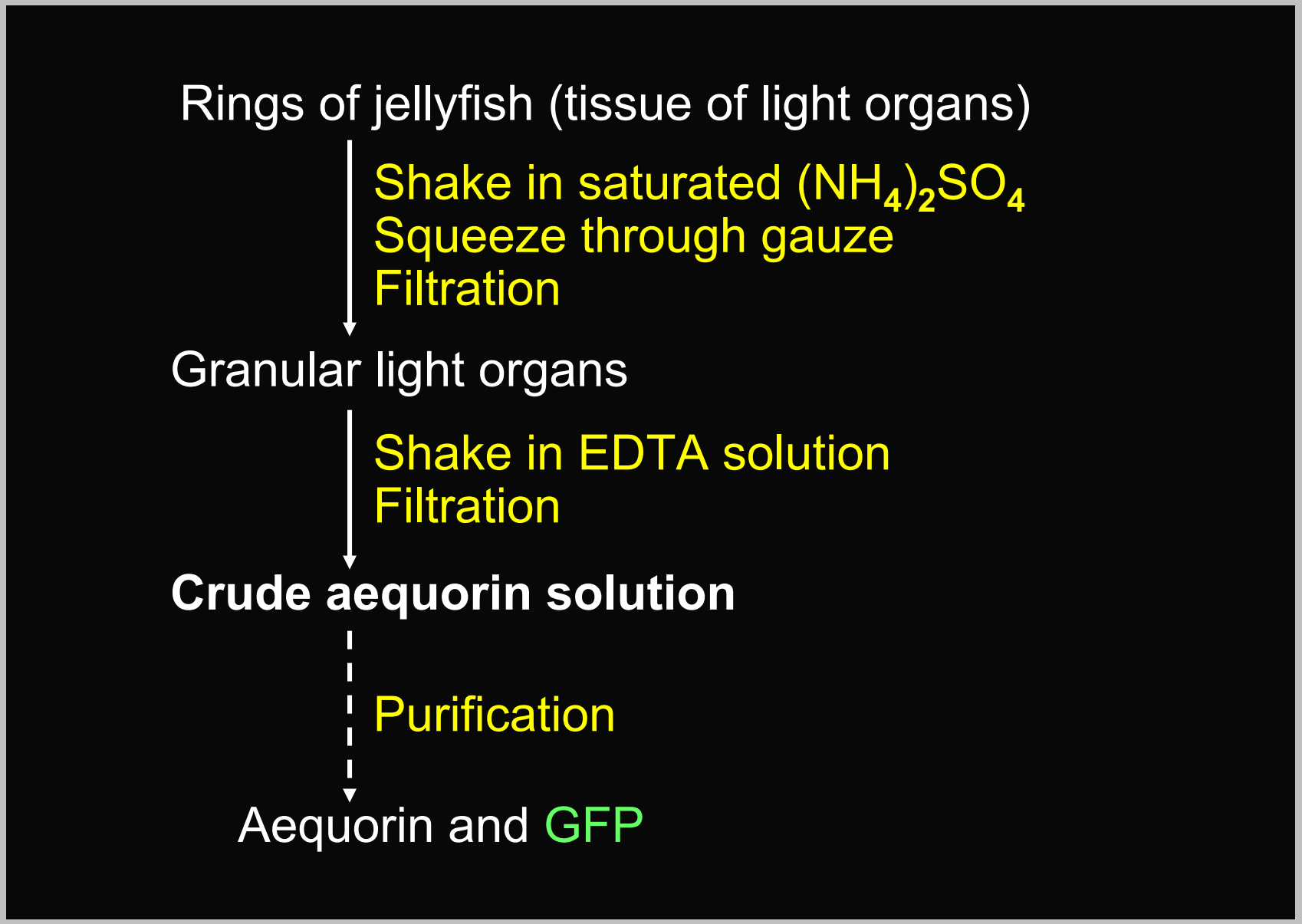


Basic Strategy for the Extraction of Bioluminescent Substances

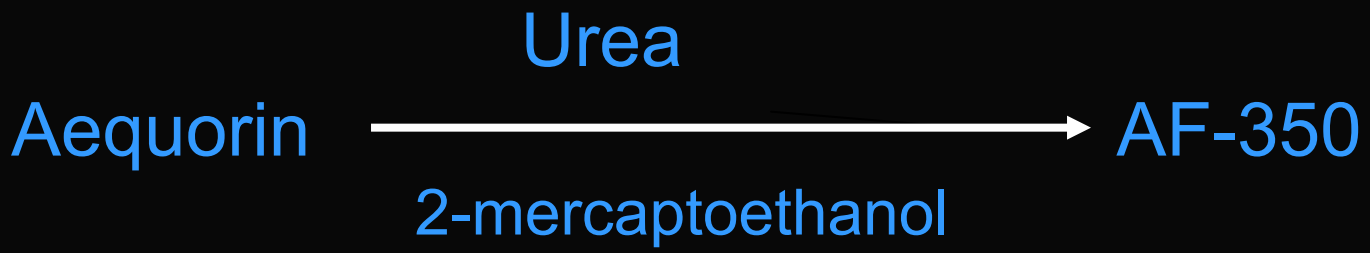
A bioluminescent substance in light organs must be solubilized and extracted under a condition that reversibly inhibits the emission of light.


Tissue of light organ (rings)
Weak light
pH 4 buffer
Filtration
Cell-free extract (pH 4)
No light
NaHCO3
Cell-free extract (neutral pH)
Weak light
Ca2+ or sea water
Bright light

Extraction of Aequorin and GFP



Blue-fluorescent compound AF-350



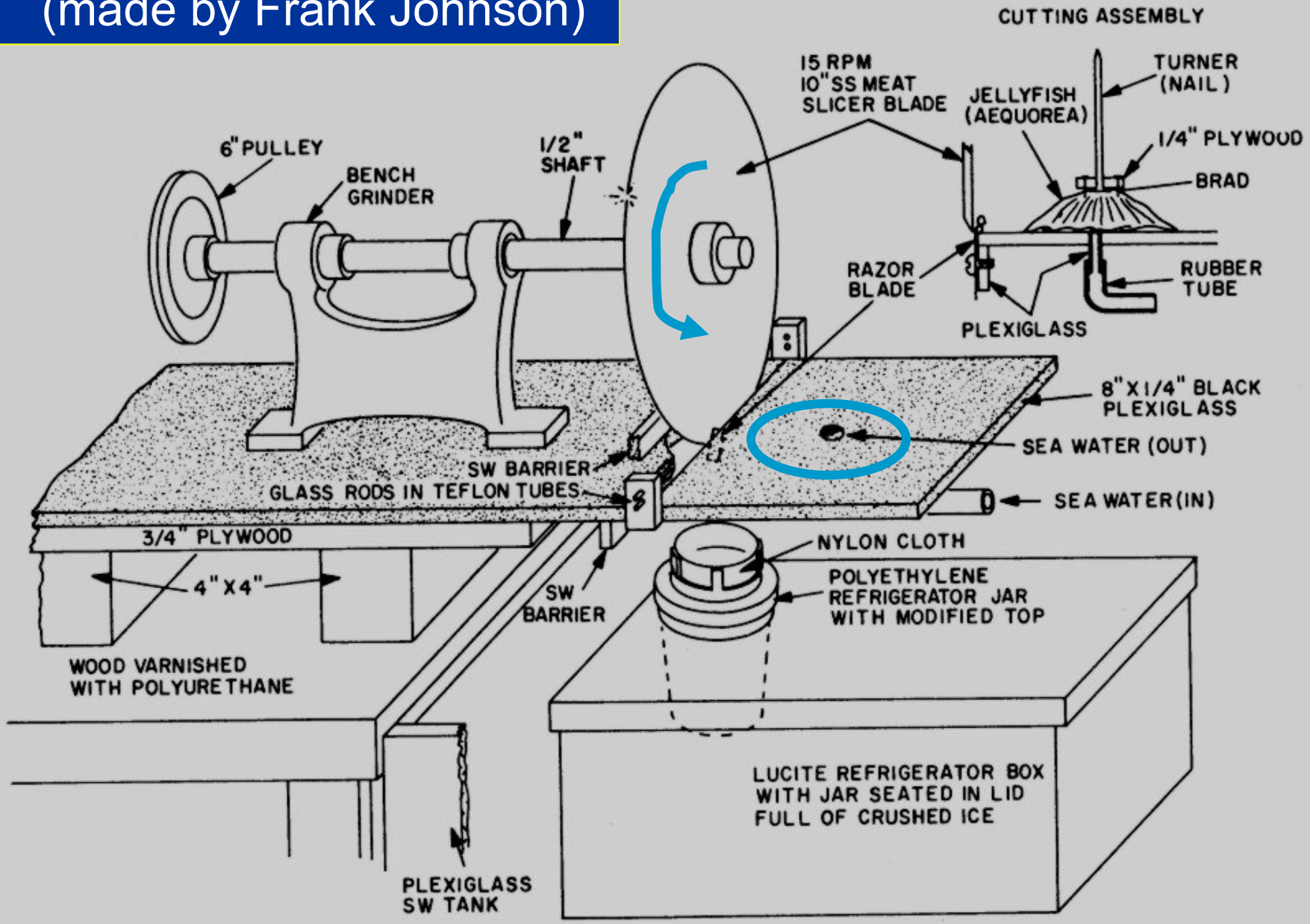
To obtain 1 mg of AF-350, about 150 mg of aequorin is needed, requiring to collect and process 50,000 jellyfish (2.5 tons) in one summer (2,000-3,000 jellyfish per day).

Jellyfish Collectors (1974)

Dr. Johnson collecting jellyfish

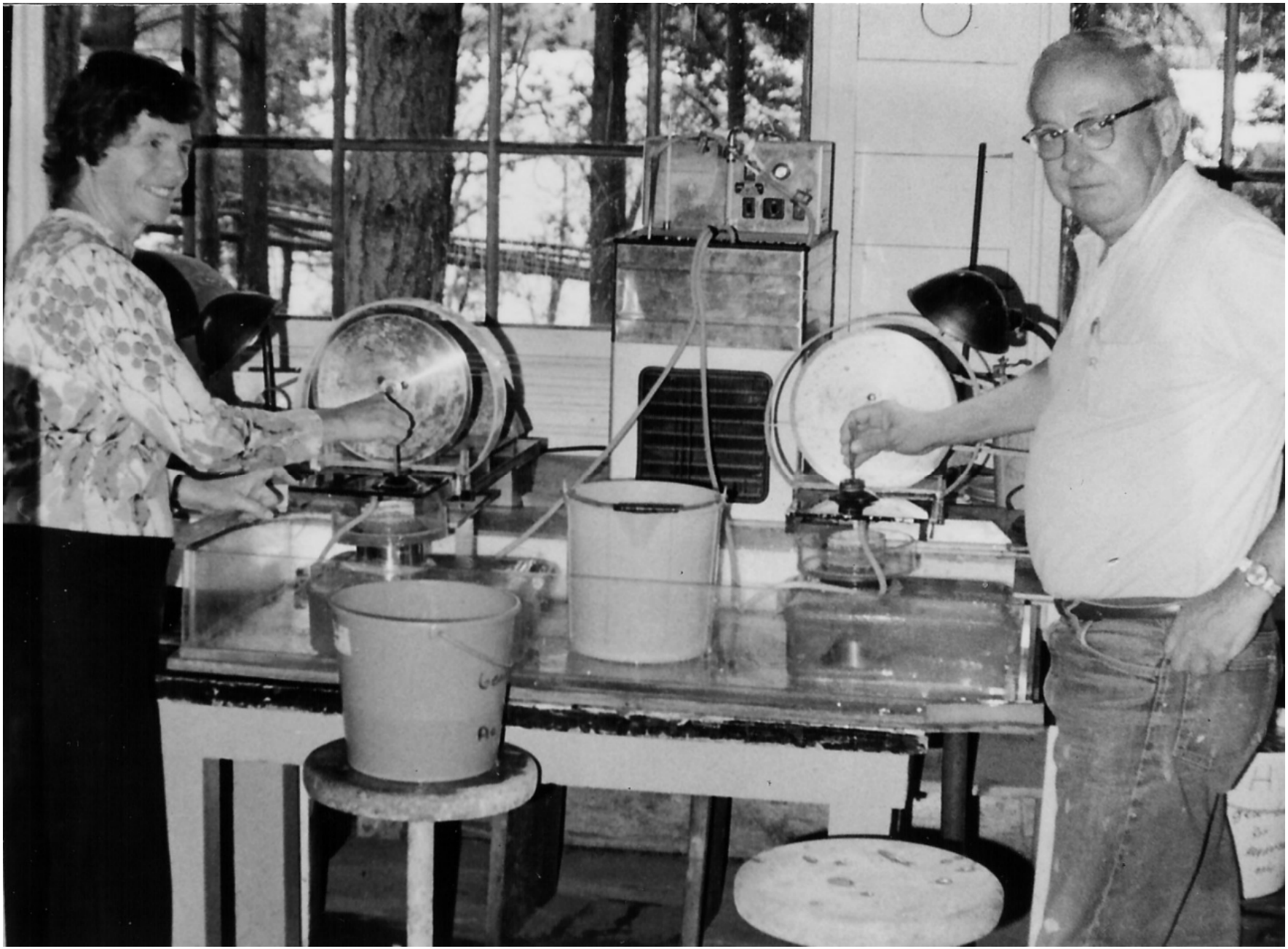

My family collecting jellyfish

Jellyfish Ring-cutter (made by Frank Johnson)



1969

Test-run of ring-cutters by Johnsons,1968

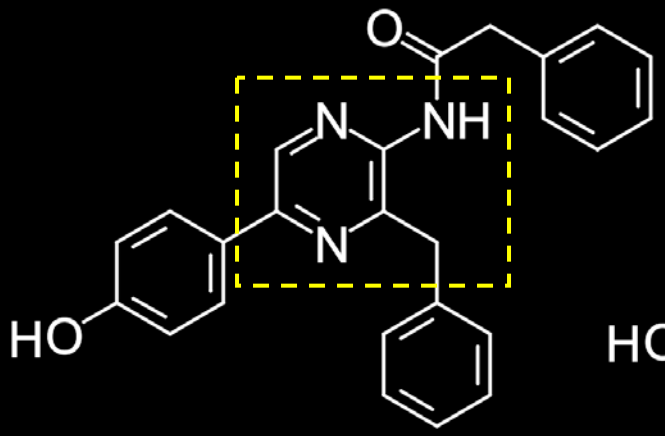


Cutting rings

Extraction of aequorin

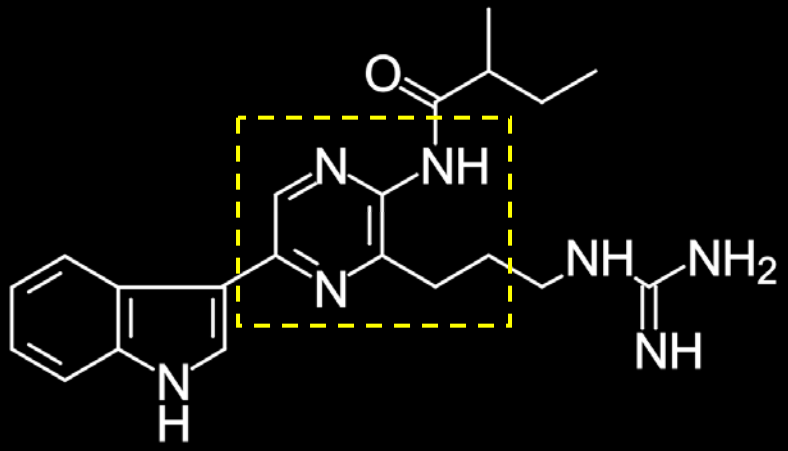
Structure Elucidation of Coelenterazine

AF-350
(Coelentramine)

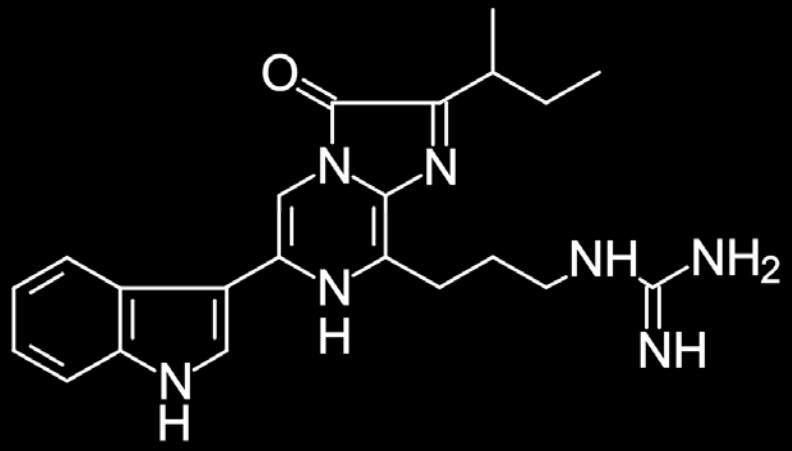


Coelenteramide

Coelenterazine

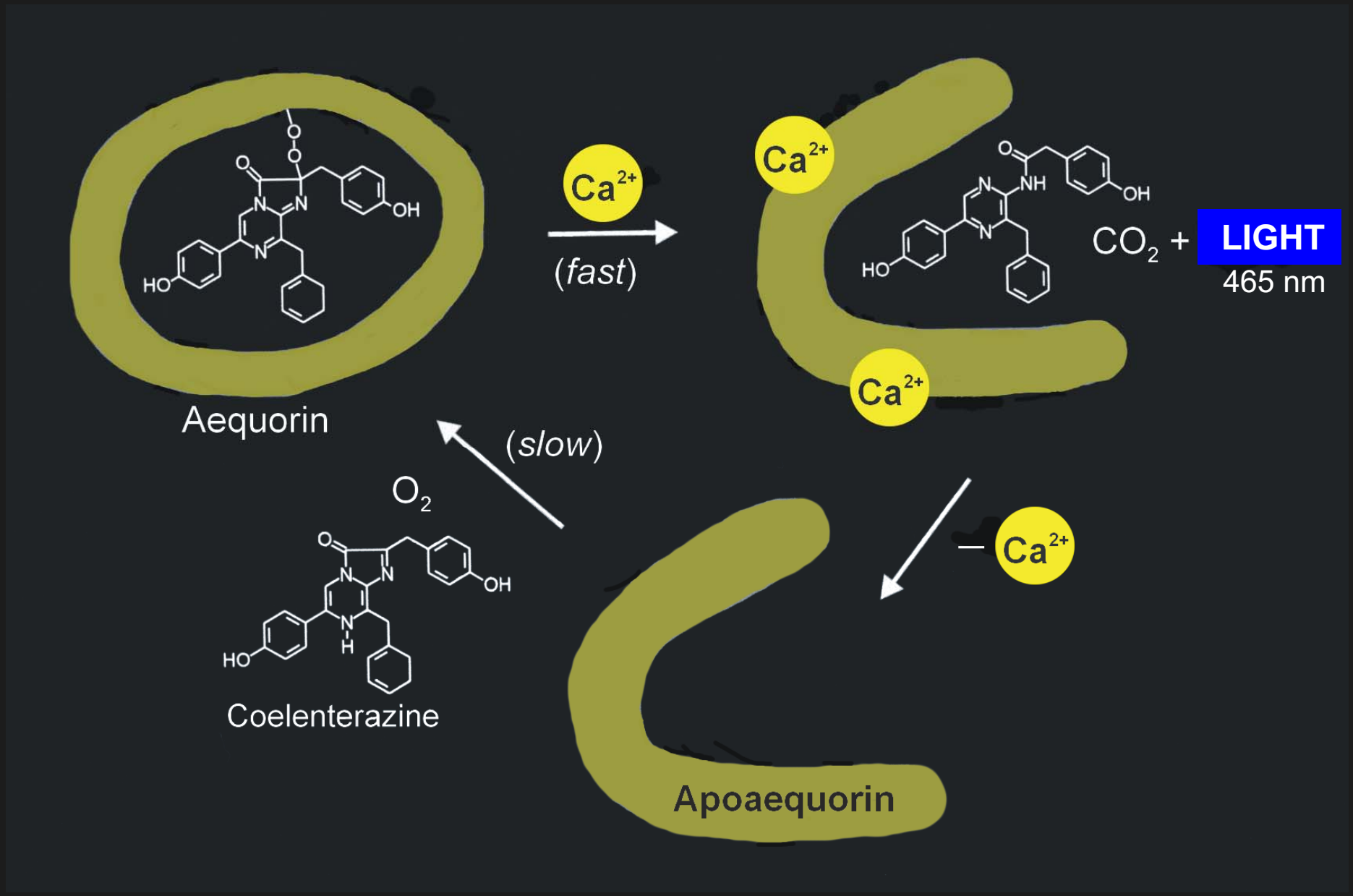


Cypridina oxyluciferin

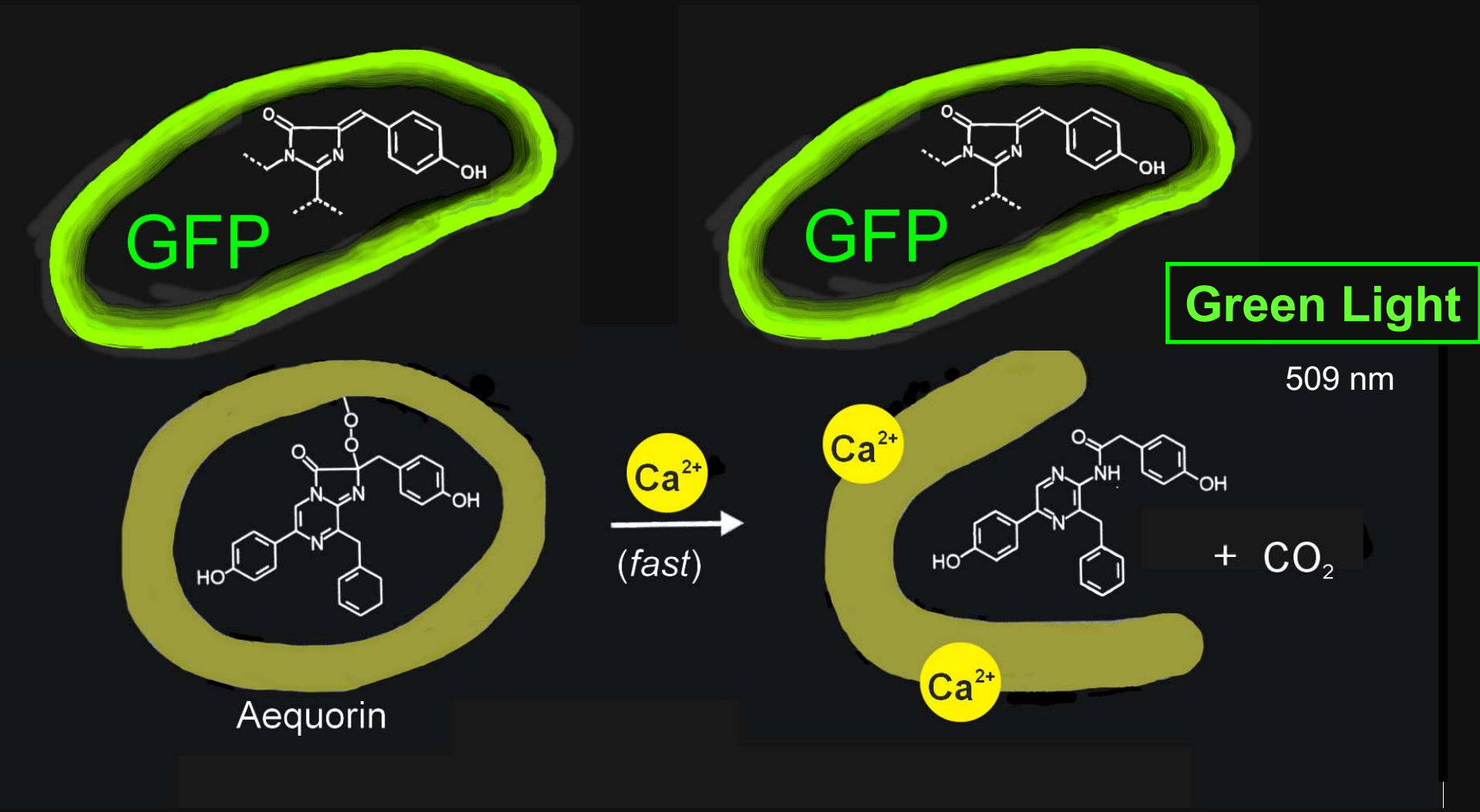


Cypridina luciferin

Luminescence and Regeneration of Aequorin

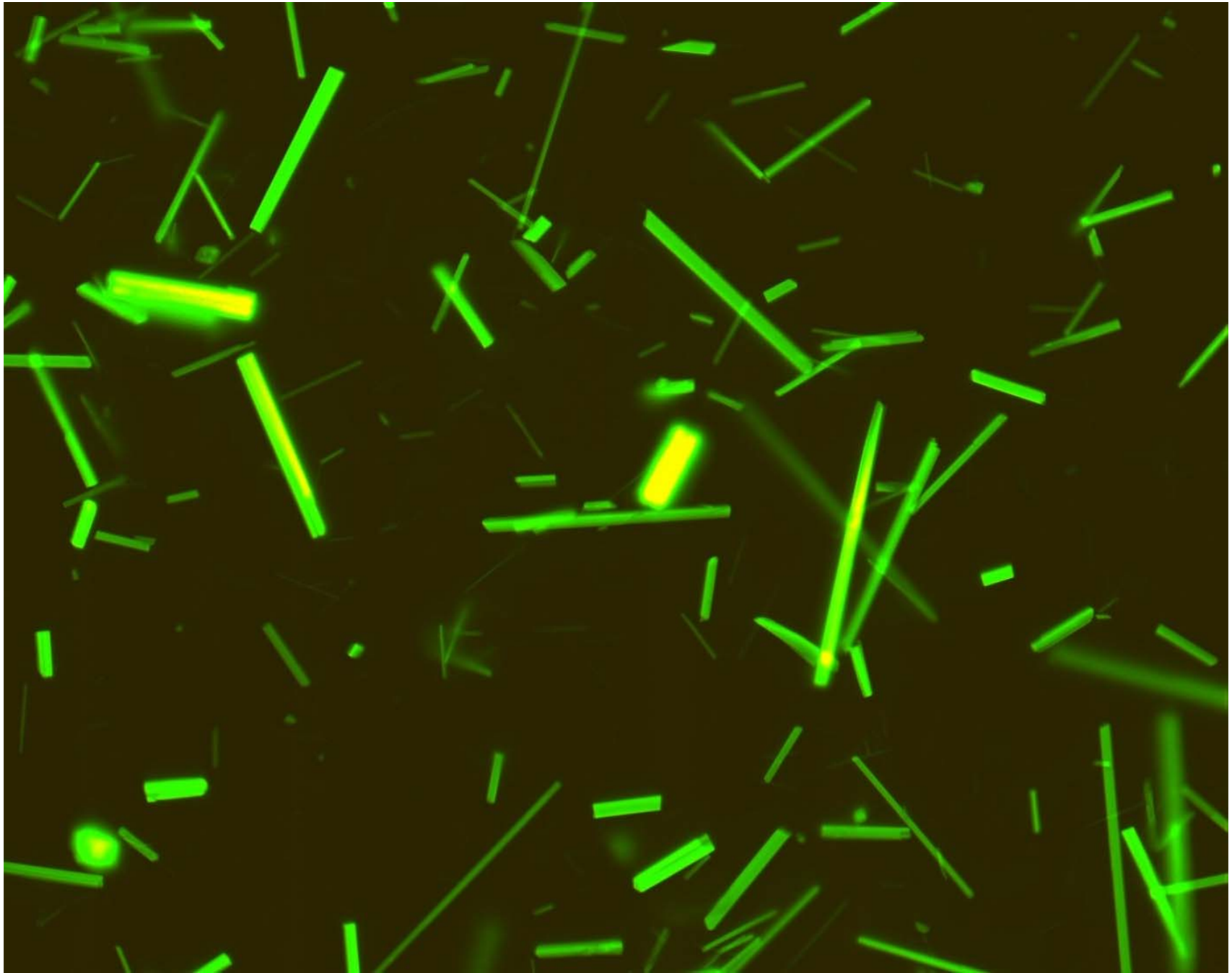


Aequorin Luminescence with GFP



GFP Crystals

Photo by Dr. Shinya Inoue



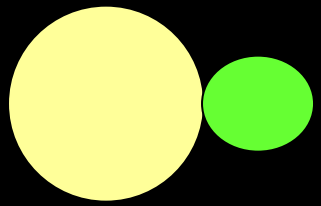
Isolation of GFP Chromophore

GFP (100 mg)

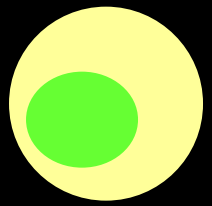
Denature at 90 °C
Digest with papain
Extraction with butanol at pH 1
TLC purification

Isolated chromophore
(0.1 mg)

FEBS Lett. 104, 220-2 (1979)

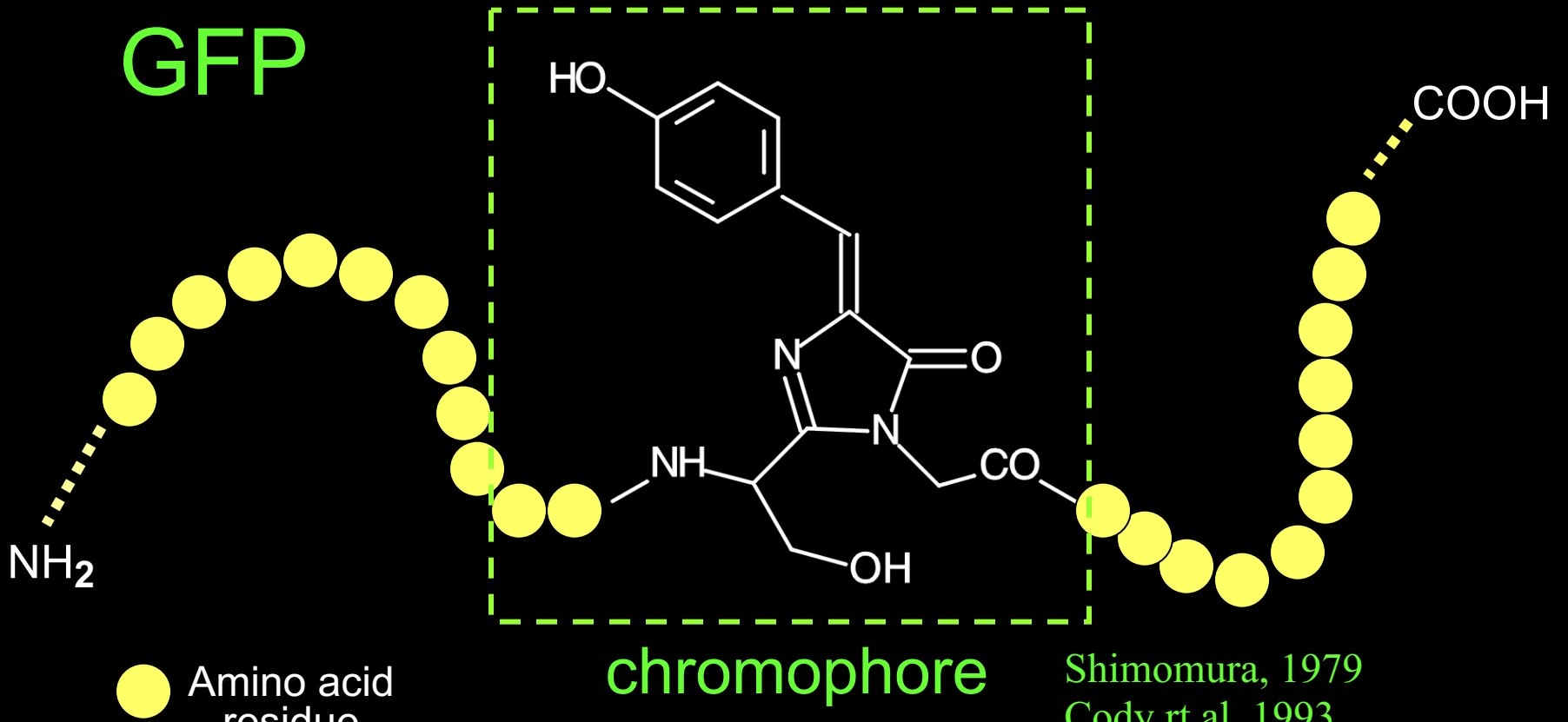


Ordinary Fluorescent Proteins



GFP

GFP



Shimomura, 1979
Cody rt al, 1993

Acknowledgments

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