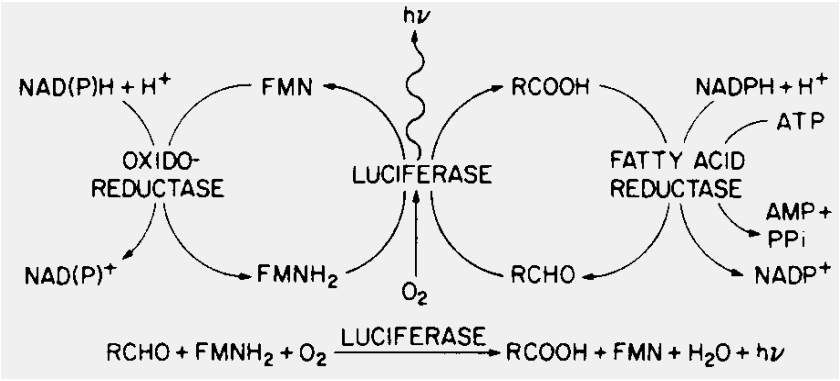
Experiment	**Bioluminescence of *Vibrio harveyi*** (demonstration)
Advisor	Munti Yuhana myuhana@botinst.unizh.ch
Reading	Chapter 7.6 (Fig. 7.21), 13.11 in BBOM 9th Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 9th Edition, (BBOM, International Edition), Prentice Hall, 1999. ISBN: 0-13-085264-3
Objectives	• To understand bacterial bioluminescence • To observe changes in luminescence activity of *Vibrio harveyi* in the dark • To understand that luciferase is involved in luminescence expression • To illustrate that bacterial luminescence is oxygen-dependent
Background	Luminescent bacteria (e.g., species of the genus ***Photobacterium*** and ***Vibrio***) are wide-spread in the marine environment where they exist as planktonic forms and are involved in symbioses. Some physiological characteristics of luminescent bacteria are: Gram-negative, motile rods, aerobic (many of them are facultative anaerobes), able to emit light. They are associated with fish, squid, shrimp and other animals, as **parasites** or as **saprophytes**. Luminescence can be used by the host organism for a variety of purposes, such as attraction of prey, intraspecies communication, or escape from predators. All luminous bacteria encode biochemically similar luminescence systems. Light production is catalyzed by the enzyme **luciferase**, a **mixed function oxidase** consisting of two different subunits, i.e., an alpha subunit of approx. 42,000 Dalton, and a beta subunit of approx. 37,000 Dalton. The *in vitro* light emitting reaction is coupled to **aerobic oxidations**:  **blue green light,** λ ≈ 490 nm Fig. 1. Substrates, products, and pathways involved in the bacterial bio-luminescence reaction (Engebrecht et al., 1983). Luciferase catalyzes the oxidation of a reduced flavin and a long-chain aldehyde, producing oxidized flavin and the corresponding long chain fatty acid. A fatty acid reductase has been implicated in the recycling of the fatty acid to the aldehyde. Expression of the **genes for luciferase (*lux*)** occurs in late log phase (Nealson, 1977) and has been shown to be induced by a small sensory molecule called **autoinducer**. An autoinducer is a diffusable compound produced by the bacteria. It can accumulate in the environment during growth. This autoinduction system allows luminous bacteria to monitor their own population density.

Literature	Engebrecht, J., K. Nealson, and M. Silverman. 1983. Bacterial Bioluminescence: Isolation and Genetic Analysis of Functions from *Vibrio fischeri*. Cell (32): 773-781. Nealson, K. 1977. Autoinduction of Bacterial Luciferase: Occurrence, Mechanism, and Significance. Arch. Microbiol. (112): 73-79.
Practical Work	The students will observe the dependence of luminescence by *V. harveyi* on oxygen.
Materials and Demonstration Protocols	Bacterial strain: luminous *Vibrio harveyi* isolated from brackish water shrimp (*Penaeus monodon* Fab.) off the coast of Java, Indonesia. We will partially fill a long glass tube with a well grown culture of *V. harveyi,* go to a dark room, close our eyes for several minutes to get adapted to the darkness and observe the luminescence. We will turn the tube upside down several times to allow the oxygen present in the air bubble to diffuse into the medium.
Laboratory Rules & Precautions	There is no risk associated with this demonstration, but be careful in the dark, and do not drop the fragile glass tube containing the liquid culture.
Goals & Experiences gained	Familiarity with one of the more unusual phenotypic characteristics of a prokaryote: bacterial bioluminescence.
Timing	15 minutes
Reporting	Note observations and explanations in your lab book
Questions to be answered	Why are some of the bacteria able to emit light in the dark? Why does luminescence fade so quickly?