Exploring Bioluminescent Proteins in Arctic Waters

Application Note

Keywords

- Under The Pole expeditions
- Protein families
- QE *Pro* spectrometer

Techniques

- Bioluminescence
- Fluorescence

Applications

- Biological research
- Medical research and diagnostics

Under The Pole Expedition Dives Deep

Marcel Koken (mhmkoken@gmail.com) is a Ph.D.-level research scientist and molecular biologist from CNRS-LABOCEA who has spent a large part of his career working on characteristics of bioluminescence and fluorescence in proteins and organisms. In a recent conversation, Dr. Koken described his research and the use of an Ocean Optics spectrometer as part of the Under The Pole (UTP) undersea expeditions, a series of research ventures begun in 2010 to "learn exactly what's happening in our oceans," as its director, Ghislain Bardout, explains on the organization's website.

The following material has been edited for clarity. All images shown here are courtesy of Under The Pole. We thank UTP, Dr. Koken and our representative in France, IDIL Fibres Optiques, for their assistance.

Ocean Optics (OO): Tell us about your work, which combines elements of molecular biology, biochemistry and cell biology to solve various challenges.

Marcel Koken (MK): My research tries to isolate and characterize bioluminescent and fluorescent protein families that are complementary or even better than the well-known GFP (Green Fluorescent Protein) and GFP-like proteins. The idea

is to understand the mechanisms of these molecules and how they function, and in that way, to prepare additional and better tools to monitor, for instance, in situ protein localization or interactions for basic biological and medical research and diagnostics.

Today, these fluorescent and bioluminescent molecules are of utmost importance in many domains, as diverse as quick and sensitive screening for disease treatment and detection of military land mines and tank-cloaking.

Bioluminescence is a biological phenomenon that allows living animals to emit their proper light for one of three functions: communication, self-defense or predation. In all known systems, an enzyme (luciferase) oxidizes a substrate (luciferin) with or without the help of additional molecules (ATP, ions, etc.). As there is no sequence similarity between the luciferases of the different systems (they all evolved independently; a nice example of convergent evolution), it is worthwhile isolating and characterizing these proteins from newly identified organisms as the chance is big to detect new and interesting properties.

On the other hand, fluorescence is a purely physical phenomenon not needing any living entity! Light is emitted from a sample after being excited with photons of higher energy (and thus lower wavelength). On living animals harboring fluorescent patterns these are mainly used for intra-species communication and signaling toward predators and prey. For instance, in common budgies [parakeet species native to Australia], it isn't the green color that attracts the females to the male coat, but the violet and yellow fluorescent spots (invisible to the naked human eye) that are rendered visible to the female by the sun's deep blue and ultraviolet light.

Figure 1. Dr. Marcel Koken's work as part of the Under The Pole III expedition allowed him to characterize bioluminescent and fluorescent proteins in Arctic Ocean organisms.

OO: What motivated you to participate in the Under The Pole mission?

MK: Three years ago, a Norwegian group [led by Dr. Jørgen Berge from The Arctic University of Norway, Department of Artic and Marine Biology] detected for the first time bioluminescence under the polar ice in the middle of the Arctic winter. This part of the world has the particularity to switch from six months of light to six months of darkness. This long absence of light (and the cold waters) makes Arctic seas of special interest for bioluminescence and natural fluorescence research; complete darkness is ideal for bioluminescence, whereas absence of excitation light seems unfavorable for fluorescence.

OO: How did you prepare for this expedition?

MK: Prior to this expedition, I had for quite a long time made the hypothesis that lots of bioluminescence may be detected in this part of the world. Also, that fluorescence would be much less abundant during half a year, as no excitation light penetrates these waters.

After I was invited to take part in the UTP III expedition (**https://www.underthepole.com/**), I decided to purchase a portable fluorescence spectrometer to be able to measure and characterize these light signals. In the past, I used an Ocean Optics **HR4000 spectrometer**, and naturally I wanted the most sensitive portable spectrometer in the UV-Vis range I could find. Through the French Ocean Optics representative, **IDIL Fibres Optiques**, I acquired the back-thinned detector-based **QE *Pro* spectrometer**, which offered a perfect compromise in terms of weight, footprint, price and performance.

OO: What are the challenges of working in such a harsh environment?

MK: Scientific researchers normally do not dive deeper than 30-40 meters, but several of the UTP divers use recyclers and heated dive-suits and therefore, can securely go down to more than 100 meters even in these icy waters. This allowed us to collect rare samples at great depth, in a non-destructive and very careful way. This contrasts with the classical way of using nets and drags that quite destroy some samples, and most importantly, that launch bioluminescence reactions, often making it impossible to detect light emission in the animals when they arrive

on the ship's deck. Collection by divers often keeps the bioluminescent properties of the animals intact, and thus allows more representative measurements. Moreover, it provides us with better source material for biochemical analysis.

So, samples were collected delicately by hand and brought aboard for photography and measurements. It is very important to take live measurements directly after sample collection, and subsequently to freeze them quickly in liquid nitrogen or, as in our case, in a -80 °C freezer.

Underwater, organisms were illuminated with Keldan® LED dive lamps emitting either at 405 nm (LED module: UV) or at 450 nm (LED module: Blue). Excitation light was blocked with a yellow filter (Altuglas PMMA polycarbonate) in front of the divers' masks or the camera lenses to easily discern the fluorescence and to not be hindered by the blue halo of the lamps.

Figure 2. Under The Pole's divers collected specimens from as deep as 100 meters, without adversely affecting the bioluminescent properties of the animals.

OO: Why is this research and exploration important?

MK: The first leg of the UTP III expedition tried to find organisms that fluoresce or bioluminesce in the icy Arctic waters. As the environment was very different from the commonly prospected ones, we hoped to stumble upon new organisms with completely new fluorescent or luminescent proteins.

Bioluminescence and natural fluorescence research are important for two reasons. One concerns, of course, the basic understanding of why animals are developing these types of machinery and signals and how they use them to communicate, hunt or defend themselves.

The second is that understanding these mechanisms will provide us, humans, with very nice tools that are currently already widely used in biology, medicine and industry. Just to give an example that I like a lot: tuberculosis detection.

Tuberculosis is a severe illness killing about 1.7 x 10^6 people [1.7 million] each year (data 2016), so even a bit more than AIDS. The classical way of detecting the bacteria causing the disease was plating a sputum sample on agar plates containing the different antibiotics that had to be tested. After a month, tiny colonies formed on plates containing inefficient antibiotics. Often patients died during this incubation timespan.

Today, sputum specimens are infected with a bacteriophage harboring a luciferase gene and the sample is again plated on the antibiotics plates. Now, after only two days, the luciferin substrate is poured on the plates and light production is registered. Absence of light indicates the efficient antibiotics' action and the patient can immediately and effectively be treated.

These tools are extensively used in many other applications of basic and applied research, but they are suboptimal for others. Therefore, a large demand exists for additional and/or better tools, especially for auto-fluorescent proteins emitting in the near infrared part of the spectrum.

Figure 3. Specimens observed during the expedition revealed characteristics of bioluminescence and fluorescence.

OO: What other research in this area are you planning that involves spectroscopy?

MK: The type of analyses I performed during the Northwest Passage will, of course, continue in the

future, as screening for new fluorescent and bioluminescent organisms in little-explored regions is one of the ways I have chosen to discover interesting new molecules. So, I am trying to organize and/or to participate in future expeditions, especially on the African continent. This part of the world is for the moment completely under-prospected for both phenomena, and it is probably a goldmine for finding new fluorescent and bioluminescent organisms.

Also, the next leg of the UTP III expedition will continue looking for natural fluorescence and is an integrative part of one of the programs that concerns the understudied coral species living in the twilight zone below 50 meters in the Pacific Ocean. Of course, other fluorescent and bioluminescent organisms will be photographed and preserved in case a happy encounter should take place.

Also, the QE *Pro* spectrometer will serve this summer for the characterization of the light emission of several French glowworm species. One of the projects I am developing within the framework of the "French National Glowworm and Firefly Observatory," a citizen science project we launched three years ago, wants to compare glowworm specimens living on the islands south of Brittany with their mainland counterparts. These populations are probably genetically separated for 4,500 and 12,000 years since the last Ice age. We would like to know whether they changed morphologically and physiologically, especially for their light emission properties.

OO: What is your dream for a potential future UTP expedition?

MK: There is a publication dating back more than a hundred years about potential bioluminescence in the **Greenland shark**. During UTP III, we wanted to verify whether this is indeed the case, but unluckily we didn't manage to find one of these huge sharks. So, in the future, I really would like to confirm if these Arctic sharks that can grow up to 6.5 meters and are thought to be the longest living vertebrates (between 200 and 400 years!) are indeed bioluminescent and perhaps even fluorescent.

Although many scientists doubt that this animal as well as the "parasitic" copepods that often lives in its eyes are both bioluminescent, it would be an ideal means to attract its food. I could well imagine that this shark is prowling the depths of the Arctic Ocean in search of its prey, the deep-diving seals. And that being luminescent in the pitch dark that reigns at 1,000 meters could help it to attract the seals to its big mouth. Moreover, this could justify why seals have been found harboring spiral scars on their bodies. To explain this observation, I could envisage that a seal made the mistake of entering the shark's mouth, realized that it wasn't a hospitable place and tried to escape by turning around, with the shark's teeth scraping a scar in the form of a spiral.