RUHR UNIVERSITÄT BOCHUM RUB

**CSC-RUB PhD Project Proposal**

**Title:** Comparative characterization of the function and optogenetic potential of bioluminescence light-reception of two species of flashlight fish

**Sector of research:** Cellular neuroscience, optogenetics, biophysics, neuroimaging

**Degree awarded**: PhD in Neuroscience

**Keywords**: optogenetics, bioluminescence, flashlight fish, photoreceptors, GPCRs

**Supervisors of PhD project:**

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**Research focus of supervisor:**

**Bioluminescence and fluorescence – from marine animals to mouse brain.** Bioluminescence and fluorescence are fascinating phenomena in marine animals but their physiological and behavioral function is not very well characterizeds. Therefore, we started to investigate the role of bioluminescence and fluorescence for animal behavior in the laboratory and also in the native environment in Egypt and Indonesia. We are in particular interested in the neuronal processing of bioluminescent signals in flashlight fish and how these signals are used for intra- and interspecific communication. In addition, we started to investigate the role of fluorescence in various types of fish (i.e. lizardfish and mandarin fish) as well as in nudibranchs. Besides our interest in understanding the physiological and behavioral function of bioluminescence and fluorescence, we will investigate the newly identified luciferases, fluorescent proteins and photoreceptors for their potential application in neuronal circuits in mice.

**Publications and H-Index in the last 5 years:** Publications 30 H-Index 9

**Publications:**


- Hellinger, J., P. Jägers, M. Donner, F. Sutt, M.D.M.D. Mark, B. Senen, R. Tollrian, S. Herlitze, D.M. Mark, B. Senen, R. Tollrian, and S. Herlitze. 2017. The flashlight fish anomalops katoptron uses bioluminescent light to detect prey in the dark. *PLoS One*. 12:1–18. doi:10.1371/journal.pone.0170489.
- Hellinger, J., P. Jägers, K. Spoida, L.C. Weiss, M.D. Mark, and S. Herlitze. 2020. Analysis of the Territorial Aggressive Behavior of the Bioluminescent Flashlight Fish Photoblepharon steinitzi in the Red Sea. *Front. Mar. Sci.* doi:10.3389/fmars.2020.00078.
- Jägers, P., L. Wagner, R. Schütz, M. Mucke, B. Senen, G. V. Limmon, S. Herlitze, and J. Hellinger. 2021. Social signaling via bioluminescent blinks determines nearest neighbor distance in schools of flashlight fish Anomalops katoptron. *Sci. Rep.* 11:6431. doi:10.1038/s41598-021-85770-w.
- Karapinar, R., J.C. Schwitalla, D. Eickelbeck, J. Pakusch, B. Mücher, M. Grömmke, T. Surdin, T. Knöpfel, M.D. Mark, I. Siveke, and S. Herlitze. 2021. Reverse optogenetics of G protein signaling by zebrafish non-visual opsin Opn7b for synchronization of neuronal networks. *Nat. Commun.* 12:4488. doi:10.1038/S41467-021-24718-0.
- Li, X., D. V Gutierrez, M.G.G. Hanson, J. Han, M.D.M.D.M.D. Mark, H. Chiel, P. Hegemann, L.T.L.T. Landmesser, and S. Herlitze. 2005. Fast noninvasive activation and inhibition of neural and network activity by vertebrate rhodopsin and green algae channelrhodopsin. *Proc Natl Acad Sci U S A*. 102:17816–17821. doi:0509030102 [pii]10.1073/pnas.0509030102.
- Mark, M.D., M. Donner, D. Eickelbeck, J. Stepien, M. Nowrousian, U. Kück, F. Paris, J. Hellinger, and S. Herlitze. 2018. Visual tuning in the flashlight fish anomalops katoptron to detect blue, bioluminescent light. *PLoS One*. 13. doi:10.1371/journal.pone.0198765.
- Masseck, O.A., K. Spoida, D. Dalkara, T. Maejima, J.M. Rubelowski, L. Wallhorn, E.S. Deneris, and S. Herlitze. 2014. Vertebrate cone opsins enable sustained and highly sensitive rapid control of gi/o signaling in anxiety circuitry. *Neuron*. 81:1263–1273. doi:10.1016/j.neuron.2014.01.041.

**Summary of research plan**

**Background:** Bioluminescence is the ability of a living organism to emit light. The emission of light is caused by a chemical reaction involving various enzymes and complex molecules, which are described as luciferin-luciferase-systems.

Bioluminescence often occurs in the marine environment, where the light is used in particular by fish to detect prey and most likely to communicate. Bioluminescence is found in at least 42 families of fish, where it is produced intrinsically or by bacterial symbionts kept in specialized organs. The fact that bioluminescence is mainly found in deep sea fish makes it difficult to study the species-specific function of bioluminescence over long periods of time and under semi-controlled conditions (i.e. in coral reef tanks). Exceptions are the flashlight fishes (Anomalopidae). The family Anomalopidae encloses various genera including *Anomalops (A. katoptron)* and *Photoblepharon (P.steinitzii)* .

In the last years we studied in particular how bioluminescent blinks in P. steinitzii and A. katoptron determine behavior in the laboratory and in the native environment in Egypt and Indonesia (Mark et al., 2018; Hellinger et al., 2017, 2020; Jägers et al., 2021). Both species have bacteria filled light organs under their eyes, where the bacteria produce the bioluminescent signal. Their photoreception is specialized to detect these bioluminescent signals.

**Therefore, the goal of this PhD project is to characterize and compare these two bioluminescent and visual systems and to explore their *optogenetic* potential for controlling neuronal signals in living animals.**

***Optogenetics*** has developed in the last decade into an important research area in particular in neuroscience to control single neurons and neuronal networks in a cell-type specific manner by light. Light activated proteins from bacteria, plants and animals are used to control the firing and intracellular signaling pathways of the cell type of choice (Karapinar et al., 2021; Li et al., 2005; Masseck et al., 2014).

**Study objective:**

1. Functional comparison between the bioluminescent systems of *Anomalops katoptron* and *Photoblepharon steinitzii*
2. Comparison and evolutionary relationship between the expression and biophysical properties of the visual and non-visual (extraocular) photoreceptors of *Anomalops katoptron* and *Photoblepharon steinitzii*
3. Exploring the optogenetic potential of photoreceptors of Anomalops katoptron and Photoblepharon steinitzii in mouse brain

**Expected Results:** We expect that we will find functional differences between the bioluminescent systems and visual perception between the two species of flashlight fish.

**Methods:** In this study we will use expression of recombinant proteins in heterologous expression systems, the biophysical characterization of recombinant photoreceptors using electrophysiological and imaging techniques, and in vivo and in vitro imaging methods in mouse brain and brain slices.

**Candidate Requirements:** Candidates should have studied neuroscience, neurobiology, marine biology or a related discipline**.** Experience with data acquisition and data analysis, molecular biology and cell culture techniques and rodent handling would be helpful. Good English language skills are required.

**Motivation for CSC application**: The successful candidate will be trained in all methods mentioned above. These methods are well established within our laboratory. The candidate will intensively interact with graduate students and experienced scientist, who will aid in teaching the techniques. Prof. Dr. Stefan Herlitze is one of the pioneers in the field of optogenetics and will explore new dimensions to visualize and control neuronal brain function.