Abstract:

Title of Project: Far-red sensitive Shrimp Rhodopsins

Co-supervisor 1: Prof. Peter Hegemann, Humboldt-Universität zu Berlin

Co-supervisor 2: Prof. Peter Hildebrandt, Technische Universität zu Berlin

Co-supervisor 2 (alternative): Han Sun, FMP Berlin Buch & FU-Berlin

Due to the deeper tissue penetration of far red light compered to visible light, and the demand for the signal amplification for multicomponent catalysis, we will express and characterize far red sensitive rhodopsins of Mantid Shrimp and other crustaceans with a focus on bistable rhodopsins that can be switches on and off by using light of different quality.

We already began with the expression of 14 crustacean rhodopsins from Shrimp, lobster and other Crustaceans in HEK cells and Pichia pastoris. We are looking for a PhD student who is interested in an extended spectroscopic and biochemical characterization of these Crustacean rhodopsins. The project will include optimization of expression and membrane targeting, studies on the spectral dynamics on time scales from femtoseconds to seconds by using UV-Vis, FTIR- Raman spectroscopy, and functional cell assays. Further adaptation of the spectral properties will be based on molecular dynamics calculations including QM/MM.

Title of Project: “Far red sensitive Shrimp rhodopsins”

Co-supervisor 1: Prof. Peter Hegemann, HU Berlin, UniSysCat, Neurocure

Co-supervisor 2: Prof. Peter Hildebrandt, TU Berlin, UniSysCat

. **1. Overall goal of the project**

- The key objective of the project is to establish far red sensitive bidirectional switchable rhodopsins that activate G-protein signaling cascades, to be used for optogenetic applications in brain research and other scientific fields.

2. State of the art and starting point of the PhD project

During the past two years, Dr. Alina Pushkarev in our group began to work on the expression of Shrimp rhodopsins in collaboration with the laboratory of Sonja Kleinlogel in Bern, who studied Shrimp vision during her PhD >18 years ago. We originally concentrated on the Shrimp *Neogonodactylus oerstedii*, because this Shrimp contains up to 15 rhodopsins with spectral sensitivity maxima up to 700 nm (Franklin, Marshall et al. 2016).

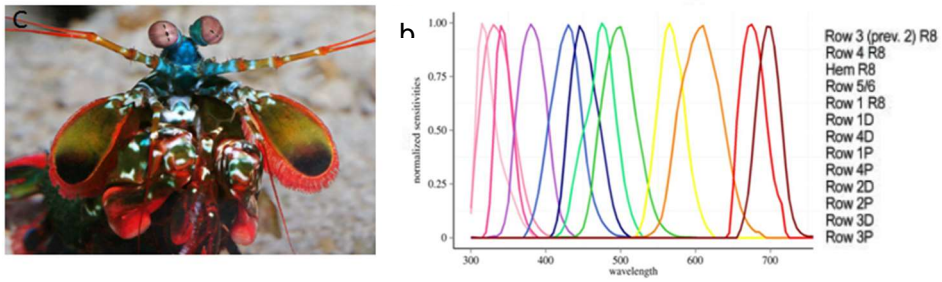

Fig.1. The mantis shrimp’s color vision system. a. The multiple eyes of *Odontodactylus scyllarus,* a member of the Gonodacylodiea family, known for possessing far-red absorbing receptors. b. The spectral sensitivities of mid-band rows 1-4 of *Neogonodactylus oersterdii*. a. Courtesy of Sonja Kleinlogel, PhD Thesis 2004 and b. is taken from (Franklin, Marshall et al. 2016).

The expression turned out to be difficult but Alina successfully expressed 4 rhodopsins and one showed bistability with maxima of 540 nm and 430 nm for the dark state and the signaling state respectively that could be of high interest and will be studied further. In preliminary cell assays Camille Brouillon demonstrated that this rhodopsins is coupling to Gi-type trimeric G-protein further activating the cAMP-signaling pathway. Camille will continue her work with us in Berlin since Sonja Kleinlogel accepted an attractive Industry job and is no longer part of the project.

Recently we received another 200 sequences of crustaceans from our collaborator Magan Porter (Porter, Awata et al. 2020, Cronin, Porter et al. 2022) and we are including 14 other rhodopsins in our expression studies.

- include selected representative references which help interested applicants to get familiar with the topic

Broser, M., A. Spreen, P. E. Konold, E. Peter, S. Adam, V. Borin, I. Schapiro, R. Seifert, J. T. Kennis, Y. A. B. Sierra and P. Hegemann (2020). "NeoR, a near-infrared absorbing rhodopsin." Nature communications **11**(1): 1-10.

Cronin, T. W., M. L. Porter, M. J. Bok, R. L. Caldwell and J. Marshall (2022). "Colour vision in stomatopod crustaceans." Philos Trans R Soc Lond B Biol Sci **377**(1862): 20210278.

Franklin, A. M., N. J. Marshall and S. M. Lewis (2016). "Multimodal signals: ultraviolet reflectance and chemical cues in stomatopod agonistic encounters." R Soc Open Sci **3**(8): 160329.
Porter, M. L., H. Awata, M. J. Bok and T. W. Cronin (2020). "Exceptional diversity of opsin expression patterns in Neogonodactylus oerstedii (Stomatopoda) retinas." Proceedings of the National Academy of Sciences **117**(16): 8948-8957.
Rozenberg, A., …Hegemann, O. Beja and M. Shalev-Benami (2022). "Rhodopsin-bestrophin fusion proteins from unicellular algae form gigantic pentameric ion channels." Nat Struct Mol Biol **29**(6): 592-603.

➢ 3. Specific aims and how they may be reached:

- ➢ Objectives: The objective is to identify and characterize bistable far-red sensitive animal rhodopsins that can be switched on and off with light of different wavelength to trigger specific G-protein signaling systems preferentially Gi or Gq for optogenetic applications.
- ➢ sketch potential experimental strategies: Identification of potentially interesting crustacean rhodopsins with far red sensitivity, screening for expression in HEK-cells, upscaling of expression in *Pichia*, testing G-protein signaling in cell assays, spectroscopic analysis and further engineering with the support of theoreticians.
- ➢ work packages **Work package 1**: Expression of far red sensitive crustacean rhodopsins in HEK-cells for survey and in *Pichia pastoris* for purification in large amounts. For optimization of expression the rhodopsins will be fused to fluorescent marker proteins as YFP or mCherry and a number of expression-, membrane targeting-, and ER-release-signals will be tested. Expression and targeting will be tested by confocal microscopy. In case of still bad targeting, larger fragments of well expressing rhodopsins will be integrated/exchanged into the Shrimp rhodopsins. The customized rhodopsin will be purified by double affinity purification and tested for functionally and G-protein activation in HEK and ND cells.

  Work package 2: Purified rhodopsins will be analyzed by in house flash photolysis and FTIR spectroscopy. Steady state Resonance Raman spectroscopy will be carried out in collaboration with Peter Hildebrandt, Ultrafast UV-Vis and FTIR spectroscopy will be done with Henrike Müller-Werkmeister in Potsdam, and possibly Ultrafast UV-Vis/Raman spectroscopy will be done with Miroslav Kloz in Praque.

  Work package 3: engineering of the protein with respect to absorption of the dark and signaling state, protonation and deprotonation of the dark and signaling states, photochemical dynamics as well as the modulation of the voltage sensitivity will be designed in collaboration with Han Sun (FMP) and tested by the PhD student.
- ➢ relevant specific references: we have some experience far red sensitive microbial rhodopsins originating from fungi and marine algae and we developed models to explain the far red absorption and experience on voltage sensing modulation with Han Sun. (Broser, Spreen et al. 2020),(Rozenberg, Kaczmarczyk et al. 2022, Silapetere, Hwang et al. 2022)

Broser, M., A. Spreen, P. E. Konold, E. Peter, S. Adam, V. Borin, I. Schapiro, R. Seifert, J. T. Kennis, Y. A. B. Sierra and P. Hegemann (2020). "NeoR, a near-infrared absorbing rhodopsin." Nature communications **11**(1): 1-10.
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Silapetere, A., S. Hwang, Y. Hontani, R. G. Fernandez Lahore, J. Balke, F. V. Escobar, M. Tros, P. E. Konold, R. Matis, R. Croce, P. J. Walla, P. Hildebrandt, U. Alexiev, J. T. M. Kennis, H. Sun, T. Utesch and P. Hegemann (2022). "QuasAr Odyssey: the origin of fluorescence and its voltage sensitivity in microbial rhodopsins." Nat Commun **13**(1): 5501

- short statement on facilities / specific equipment / other resources available if applicable
  Our lab is fully equipped for biochemisty, molecular biology, culturing of Pichia pastoris and animal cell culture systems, time resolved UV-Vis and FTIR from 10 microseconds to minutes, and electrophysiology on human cells and Xenopus oocytes.
- possible further collaborations.The only collaboration outside the UniSysCat is Miroslav Kloz in Praque, who currently runs the best ultrafast UV-Vis/Raman set up in Europe.