# Next-generation optogenetic tools

**Development of new-generation microbial rhodopsins with enhanced voltage-sensitivity and ion selectivity using molecular dynamics simulations**

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## In Short

- Molecular dynamics simulations applying membrane potentials
- Investigation of the effect of mutations in the chromophore binding pocket in color tuning
- Investigation of ion permeation in channelrhodopsins
- Rational design of microbial rhodopsin-derived voltage sensors for optogenetic application

The application of light-sensitive proteins has revolutionized neurosciences since 2005. Thus far, the primary players in this field have been light-gated ion channels that facilitate ion transport across membranes.[1] Channelrhodopsins, known for their high temporary precision, have become indispensable optogenetic tools for manipulating neurons and neural circuits.[1,2] Moreover, beyond their application in basic research, channelrhodopsins have shown promise in disease treatment.[3,4] Channelrhodopsin-1 and -2 (ChR1, ChR2) were the first two identified natural channelrhodopsins that allow for non-selective cation conduction.[5,6] Over time, tremendous efforts in channel engineering and genomic screening have led to the identification of new members of channelrhodopsins with enhanced ion selectivity.

In 2014, the first enzyme rhodopsin, rhodopsinguanylyl-cyclase (RGC) of *Blastocladiella emersonii* was discovered.[7] These RGCs not only represent a completely new and uncharacterized family of proteins, but also possess remarkable potential for optogenetic applications. They can be employed to optically trigger cGMP-dependent signalling processes and/or open cGMP-gated ion channels in various cell types. Hegemann and collaborators have identified and characterized different RGCs, including a heterodimeric RGC with green rhodopsin serving as the light sensor and NeoR as the light modulator.[8] NeoR, with its remarkable near-infrared (NIR) sensitivity, is particularly interesting for in vivo applications. However, its unique properties require thorough characterization to harness its potential for NIR optogenetics.

This project encompasses two main objectives. The first objective aims to unravel the NIR sensitivity of NeoR and develop voltage-sensitive NeoRs using atomistic MD simulations. The simulations will allow us to quantify the hydrogen bonding network and water occupancy in the chromophore binding pocket, which will be correlated with the experimental maximum absorption of the retinal. The second objective is to investigate the ion permeation mechanism and ion selectivity using atomistic MD simulations. The simulations will be performed using the Computational Electrophysiology method (Figure 1A), enabling the simulation of inward and outward ion flow within a simulation box[9]. With this approach, we aim to explore cation and anion permeation in various naturally occurring and engineered channelrhodopsins.

## WWW

https://leibniz-fmp.de/research/research-section/chemical-biology/han-sun

## More Information

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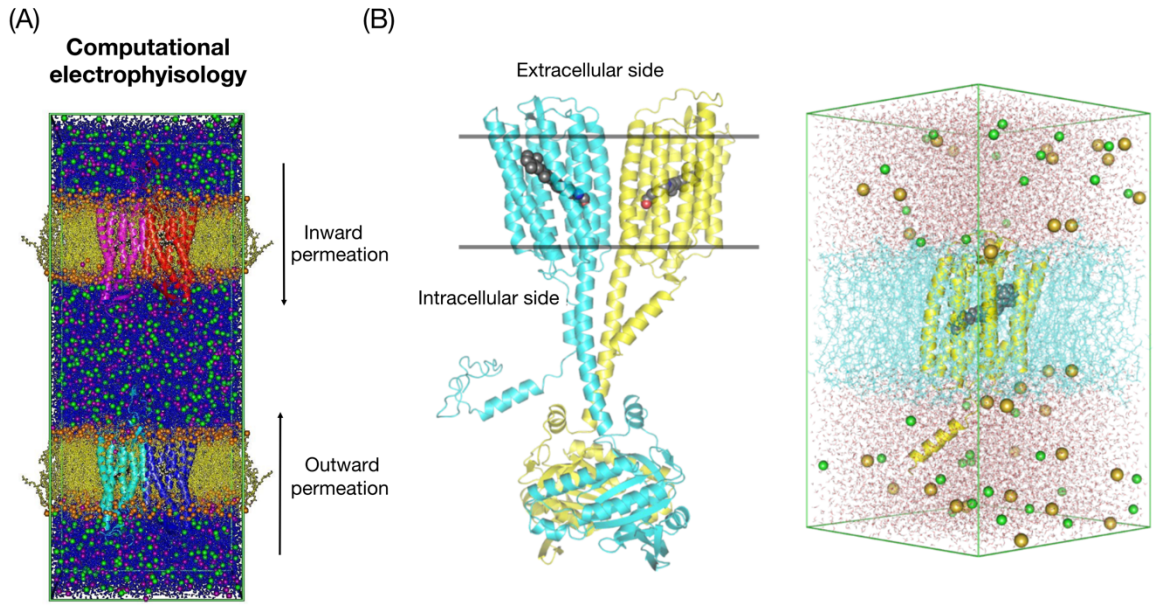


***Figure 1:*** *(A) Computational electrophysiology simulation setup of channelrhodopsin C1C2 for simultaneous simulations of inward and outward ion permeation. (B) Left: heterodimeric model of RgRGC1 (cyan cartoon) and RgNeoR (yellow cartoon) calculated by AlphaFold-Multimer[10]; Right: simulation setup of RgNeoR (residues 1 to 279) shown as yellow cartoon. Lipid bilayers and water molecules are depicted as cyan and red stick models, respectively. Sodium and chloride ions are represented as yellow and green spheres, respectively. All-trans retinal is represented as grey sphere model.*

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## Project Partners

(1) Experimental Biophysics Group, Humboldt-Universität zu Berlin

(2) Institute of Chemistry, The Hebrew University of Jerusalem

## Funding

Project funding: DFG supported Cluster of Excellence "Unifying Systems in Catalysis", project number: 390540038

## DFG Subject Area

201-02